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CUPMAP is inviting papers for Volume 4 Issue 2, which is scheduled to be published on December, 2021. Last date of submission: December 15, 2021. However, an early submission will get preference in case of review and publication process. Please submit your manuscripts according to instructions for authors by the Journal online submission system.

Sincerely,
Prof. Dr. Nazım ŞEKEROĞLU
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Materials and methods should be clearly presented to allow the reproduction of the experiments.

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Effect of a Tropical Plant (*Hunteria umbellata*) in the Management of Streptozotocin Induced Diabetes Mellitus and other Physiological and Biochemical Functions in Wistar Rats

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Abstract

Hunteria umbellata seeds, leaves and root are used as decoctions in Western and Southern part of Nigeria for the treatment of blood related ailments. The antidiabetic effect of *Hunteria umbellata* extracts (aqueous and methanol) on hematological, lipid profile and biochemical parameters in streptozotocin-induced diabetes in female Wistar rats was studied. The qualitative phytochemical screening of the seed extracts was determined using standard chemical methods. Twenty five Wistar rats weighing between 117 g and 170 g were shared into five groups of five animals per group for the physiological studies. All treatment groups were administered 500 mg/kg per body weight of *H. umbellata* extracts. Group I; normal control, Group II; negative or diabetic control, Group III; metformin + streptozotocin (STZ), Group IV; aqueous extract of *H. umbellata* + STZ and Group V; methanol extract of H. umbellata + STZ. Diabetes was induced with 55 mg/kg body weight STZ. Qualitative phytochemical screening of aqueous and methanol seed extracts of *H. umbellata* revealed the presence of secondary metabolites such as saponins, phytate, oxalate, anthraquinones, cyanogenic glycoside, phenols and alkaloids were recorded highest concentrations in both extracts. The hypoglycaemic studies of methanol extract revealed significant ($p<0.005$) reduction on the fasting blood glucose levels of experimental animals for Group V compared to moderate reduction in Group III. Group IV recorded increase in the blood glucose levels of Wistar rats. The haematological parameters were not significant ($p>0.05$) for all treatment groups. Group V recorded significance ($p<0.05$) amount of high density lipoproteins (HDL), total cholesterol (CHOL), triglyceride (TRIG) and low density lipoproteins (LDL) while CHOL was significant ($p<0.05$) for Group III. The biochemical parameters were within safe limit. The methanol extract of H. umbellata possesses the potentials for the managements of blood glucose levels and other physiological and biochemical functions compared to conventional drug (Metformin).

Key Words: Antidiabetic, Phytochemical screening, Streptozotocin, Wistar rats, *Hunteria umbellata*

1. Introduction

Diabetes mellitus (DM) is a chronic and endocrine disorder caused by inherited and/or acquired deficiency in the production of insulin by the pancreas (Selvaraju et al., 2013). The prevalence of DM is increasing across the world. In 2011, it was estimated that over 346 million people lived with DM worldwide (WHO, 2011). Nearly 80% of deaths due to DM occur in low and middle income countries

particularly in sub-Saharan Africa. Medicinal plants are known to possess bioactive components that may be used in the treatment and management of illness and disease condition in traditional medicine practice as a result of high cost of orthodox drugs and low potency of some orthodox drugs. *Hunteria umbellata* K. Schum, is a tree with a height of about 15 – 22 m belonging to the family Apocynaceae. The leaves of *H. umbellata* are broad, abruptly acuminate and broadly lineate (Gurib-Fakim, 2006). The plant is grown mostly in Southern parts of Nigeria, Ghana and the rainforest regions of Cameroon (Aderele et al., 2020). Many dialects in Nigeria have different names for the plant such as Osu (Edo), Erinor abeere (Yoruba, Southwest) and Nkpokiri (Igbo, Southeast). H. umbellata have been reported to be used for traditional medicine for the treatment of various ailments (Reynolds and Sofowora, 1984; Keay et al., 1989). The trees of *H. umbellata* are used as ornamental plants in Southern part of Nigeria where it is planted for shades and decoration and guarded jealously by owners from human scavengers. Localities in Western and Southern part of Nigeria apply the leaves, seeds and root of *H. umbellata* with other plants mixed with coconut water as decoctions for the treatment of fever, blood pressure, piles, headache and other body related ailments. The stem of the plant are used as chewing stick, due to the cleansing effect derived from its use as an alternative to tooth brush. Many researchers have reported the medicinal uses of *H. umbellata* such as treatment of ulcers, diabetes mellitus and dysmenorrhoea (Oboh et al., 2017).

This study examines the antidiabetic effect of aqueous and methanol seed extracts of H. umbellata and its effect on hematological, lipid profile and biochemical parameters in streptozotocin-induced diabetes in female Wistar rats.

2. Material and Methods

2.1. Sample Collection and Identification

The seeds of *Hunteria umbellata* were purchased from Oshodi Markets in Oshodin Local Government Area, Lagos State in April 2015. The seeds were air dried for three weeks, after which the epicarps were removed and further air dried for another three weeks to obtain constant weight. The air drying was carried out to protect the bioactive components of the plant seed. The identification of the plant was carried out by Dr. E. I. Aigbokhan, of the Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

2.2. Preparation of Sample

The dried plant seeds of *H. umbellata* were pulverized using electronic grinder Lab. Mill (Model: Serial NO. 4745, Christy and Norris Ltd, England). The pulverized seeds were stored in air tight plastic container for experimental analyses.

2.3. Extraction of Plant Materials

400 g of the seeds were blended using a sterile grinder. The blended seeds were transferred into Pyrex flask containing 1.5 litres of aqueous (sterile distilled water) and non-aqueous solvent (methanol) and allowed to soak for 24 h for the aqueous and 72 h for the non-aqueous mixture. After 24 hr the mixture was macerated using a sterile cotton wool. At the end of extraction, the homogenate was filtered through a Whatman Filter Paper No. 1 using a glass funnel. The filtrates were labeled accordingly for subsequent use. The filtrates were concentrated using Water Bath at temperature of 80^{o}C to dryness. The crude extract was obtained and stored for further analyses.

2.4. Percentage Yield

The percentage yield of *H. umbellata* was obtained using the methods of Kao (2018). The percentage yield was computed using the mathematical formula below:

$$\text{Yield } \% = \frac{W_2 - W_1}{W_0}$$

W_2 = Weight of extract + container W_1 = Weight of container
W_1 = Weight of container
W_0 = Initial weight of sample before extraction

2.5. Qualitative Phytochemical components of *Hunteria umbellata*

The qualitative phytochemical analyses of the selected plant seeds were determined using the methods of Price (1985) and Thirumalai et al. (2011). All determinations were carried out in triplicates.

2.6. *In-vivo* Diabetic Study

Twenty five female Wistar rats weighing between 117 g and 170 g were shared into five groups of five animals per group. The animals were allowed to acclimatize for three weeks before administration of drugs. Freshly prepared solution of streptozotocin (STZ) (Sigma, USA), 55 mg/kg body weight in 0.1 mol/L of cold citrate buffer pH 4.1 was introduced into the overnight fasted animals by a single intra-peritoneal injection (Igbe et al., 2009). The control experimental animals were injected with distilled water. The animals were considered diabetic at the blood glucose level values above 250 mg/dL on the third day after STZ injection using a glucometer and acute test strip. The diabetic animals were treated for three weeks, while blood glucose levels were taken on a weekly basis.

2.7. Experimental Animal Grouping

Twenty five experimental animals of five animals per group were shared for experimentation as follows:

Group I- Normal control (non-diabetic rats)

Group II- Negative control (diabetic without treatment)

Group III- Positive control (diabetic + 500 mg/kg body weight Metformin)

Group IV- Diabetic + 500 mg/kg body weight of aqueous seed extract of *H. umbellata.*

Group V- Diabetic + 500 mg/kg body weight of methanol seed extract of *H. umbellata.*

2.8. Extract Administration and Observation

Three days after induction of diabetes, experimental animal Groups III, IV, and V were treated with 500 mg/kg body weight standard drug (Metformin), 500 mg/kg body weight aqueous seed extract of *H. umbellata* and 500 mg/kg body weight methanol crude extract of *H. umbellata* respectively.

Group I (control) was administered distilled water at each treatment and Group II (negative control) was left untreated. Treatments (Aqueous and methanol seed extract of *H. umbellata*) were administered orally to the respective groups at a dose of 500 mg/kg.

2.9. Collection of Blood Samples and Plasma Preparation for Analysis

The experimental animals were sacrificed by anesthetizing the rats in enclosed container with chloroform. Blood samples were collected by ocular punctures into the abdominal aorta and the heart (Adeneye and Adeyemi, 2009). The blood was further centrifuged for 10 min at 3000 rpm using the centrifuge. The clear supernatants (plasma) collected were used for the

estimation of lipid profiles and liver function tests.

2.10. Determination of Animal Body Weight

The initial weights of all the experimental animals in the different experimental groups were determined at the start of the experiment. Weekly weight measurements of the experimental animals were taken in all the groups and values recorded. The final weight measurement was carried out on the last day of the experiment. The differences in the weight in the various groups recorded accordingly (Bain et al., 2017).

2.11. Determination of Blood Glucose Level

The initial blood glucose of fasting Wistar rats before STZ induction was taken using a glucometer with acute test strip. The weekly blood glucose was taken in all groups of animals by pricking the tail of the rats and blood dropped on a strip fastened to the glucometer; this was carried out weekly for the three weeks study (Bain et al., 2017).

2.12. Haematological Study of Blood Samples of Experimental Animals

The serum obtained from the blood of the experimental animals were analysed for haematological parameter at the Department of Haematology, University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. Blood samples was analysed for packed cell volume (PCV), haemoglobin level (Hb), total white blood cells count (TWBC) and differential white blood cells counts (DWBC) according to the methods of Trinder (1969).

2.13. Determination of Plasma Lipid Profiles

The plasma total protein (TP), total cholesterol (TG), triglyceride (TG) and HDL-cholesterol (HDL-Chol) were determined according to the methods of Friedewald et al. (1972) using Randox Diagnostic kit. Low density lipoprotein-cholesterol (LDLChol) was calculated using formula adopted by Xing-Jiu et al. (2006).

2.14. Biochemical Analysis (Liver function test)

The liver function test was carried by digesting the plasma in Teco diagnostic (TC) kits and measured using the spectrophotometer. Plasma obtained from clotted blood samples from experimental animals were analyzed for alkaline phosphatase (ALP). Aspartate transaminase (AST) and alanine transaminase (ALT) was determined following the methods of Reitman et al. (1957) and Azwanida (2015).

2.15. Statistical Analyses

Data obtained were analyzed by one-way analysis of variance (ANOVA) using Student T-test to determine the significance differences in group results.

3. Results and Discussion

Medicinal plants have repeatedly been used as non-prescription medication for type 2 diabetes in rural areas in sub-Saharan Africa. Their potency may be due to several factors that are yet to be completely studied. *H. umbellata* is one medicinal plant that has been explored for its antidiabetic properties. The percentage yield of aqueous and methanol seed extracts of *H. umbellata* revealed highest solubility in aqueous (48.73 %) solvent compared to methanol (3.765%) (Table 1).

Table 1. Percentage yield profile of aqueous and methanol crude extracts of *H. umbellate*

Solvent	W2 (g)	W1 (g)	W0 (g)	Yield %
Aqueous	493.41	298.5	400	48.73
Methanol	156.06	141.0	400	3.765

W_0: Initial weight of sample before extraction,
W_1: Weight of container,
W_2: Weight of extract + weight of container

The result showed that the seed of *H. umbellata* was more soluble in polar solvent compared to non-polar solvent. The high percentage yield, with preserved integrities of the extracts is an indication that the method of extraction can be adopted as a standard method of extract preparation, using the common available equipment in our laboratories as alternatives to other standard equipment that are not readily available (Niranjan and Kanaki, 2008). Qualitative phytochemical screening of aqueous and methanol seed extracts of *H. umbellata* indicated the presence of secondary metabolites such as alkaloids, phenols, saponins, phytate, oxalate, anthraquinones (Table 2). It was reported that medicinal plants with hypoglycemic and antidiabetic effect usually contain high concentration of alkaloids and flavonoids (Murray et al., 1994). The result of Qualitative phytochemicals of *H. umbellata* in this study does not completely agree with this assertion. Alkaloids and phenol was highest compared to other secondary metabolites for aqueous and methanol extracts.

Table 2. Qualitative phytochemical screening of aqueous and methanol crude extracts of *H. umbellata*.

Parameters	mg/kg	
	Aqueous	Methanol
Oxalate	9.9	3.7
Phytate	6.6	2.3
Tannins	0.2	0.3
Flavonoids	0.0	0.3
Saponins	12.8	6.4
Alkaloids	20.8	19.0
Phenols	8.1	26.7
Cyanogenicglycoside	3.6	3.9
Anthraquinones	8.4	7.7

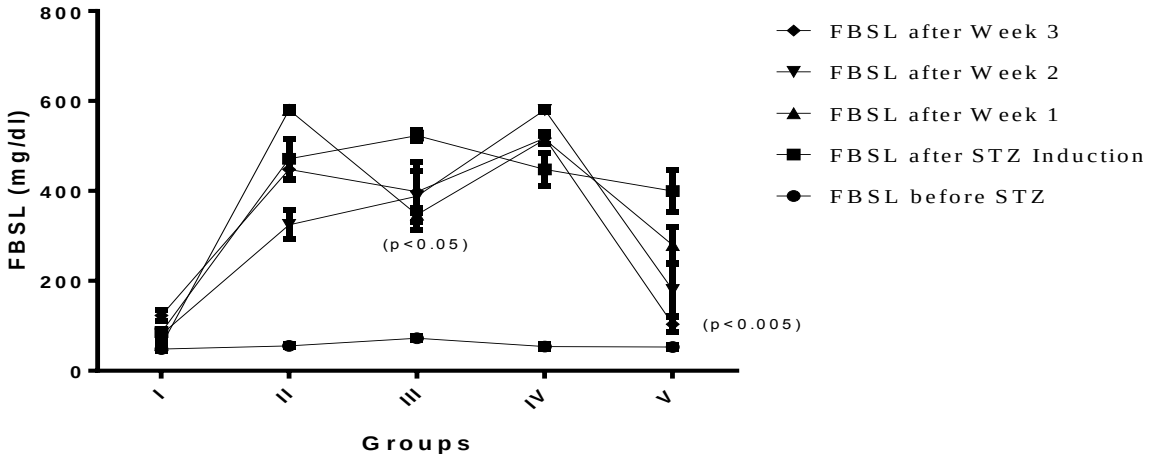
Experimental animals induced with STZ have been reported to produce a diabetic state that is characterized by loss of weight, polydipsia, polyuria, glucosuria, polyphagia, hypoinsulinaemia and hyperglycemia (Subash-Babu et al., 2007). The effect of *H. umbellata* extracts on the fasting body weight of experimental animals revealed weight changes in Group I, II and Group IV (Table 3). Group I recorded significant ($p<0.002$, $p<0.001$) increase in body weight compared to other treatment groups that received 55 mg/kg body weight STZ. Group II recorded significant ($p<0.05$) decrease for week 1 and 2. Group IV showed significant ($p<0.05$) decrease in weight for week 1 and week 2. Group III and V had no significant ($p>0.05$) difference in the body weight of experimental animals for 21 days treatment period. The decrease in body weight for Groups II and IV may be attributed to the underutilization of glucose due to the hyperglycemic action in both groups. This result is in agreement with the work of Muhd et al. (2012), who reported that the decrease in the body weight of diabetic rats may be due to catabolism of fats and protein.

Diabetes mellitus is a metabolic endocrine disorder which affects multiple organs, causing severe complications such as the destruction of 68 to 80 % beta cell of the islet of Langerhans (Adeneye et al., 2013). These destruction results in the increase in the blood glucose level (hyperglycemia). The fasting blood glucose level after STZ induction compared to fasting blood sugar level after three weeks treatments revealed normal blood glucose level for group 1 and hyperglycemia in Group II. Group III treated with standard drug (Metformin) recorded significant ($p<0.05$) decrease at week one and week three, while Group V (treated with methanol seed extracts of *H. umbellata*) recorded significant ($p<0.05$) decrease in blood sugar levels for week one and two and high significant ($p<0.005$) decrease for week three (Figure 1).

Table 3. Effects of *H. umbellata* seed extracts on the fasting body weight (FBW) of experimental animals

Group	FBW before STZ (g)	FBW after Week 1 (g)	FBW after Week 2 (g)	FBW after Week 3 (g)
I	147.7±6.86	164.7±8.09***	168.3±8.19**	175.4±8.03***
II	134.0±6.15	123.6±4.38*	121.6±3.94*	122.8±2.96
III	139.5±5.62	133.3±6.64	135.3±6.73	138.4±10.8
IV	160.4±5.81	136.7±6.03*	133.3±5.81*	128.6±12.4
V	164.2±4.31	177.0±9.65	178.8±8.96	182.9±7.65

Body weight in Mean±SEM, n=5, Significance at *p<0.05, **p<0.002, ***p<0.001 compared to FBW before STZ induction, FBW: fasting body weight
Group I: Normal control (no diabetes)
Group II: Negative control (induced 55mg/kg BW STZ)
Group III: Positive control (55mg/kg BW STZ + 500mg/kg BW Metformin) Group IV:
55mg/kg BW STZ + 500mg/kg BW Aqueous extract of *H. umbellata* Group V: 55mg/kg BW
STZ +500mg/kg BW Methanol extract of *H. umbellata*



FBSL in Mean±SEM, n=5, Significance at *p<0.05, **p<0.005 compared to FBSL after STZ induction, FBSL: fasting blood sugar level
Group I: Normal control (no diabetes)
Group II: Negative control (induced 55mg/kg BW STZ)
Group III: Positive control (55mg/kg BW STZ + 500mg/kg BW Metformin)
Group IV: 55mg/kg BW STZ + 500mg/kg BW Aqueous extract of *H. umbellata*
Group V: 55mg/kg BW STZ +500mg/kg BW Methanol extract of *H. umbellata*

Figure 1. Effect of *H. umbellata* seed extracts on the fasting blood sugar level (FBSL) of experimental animals

Group IV recorded increase blood sugar levels for the three weeks treatment period, indicating that the aqueous extract of the plants does not possess antidiabetic properties. The levels of blood glucose for Group IV treated with methanol seed extracts of *H. umbellata* were more statistically significant compared to Group III treated with standard drug due to the reduction in blood glucose levels. The result of this study confirmed that methanol extract of *H. umbellata* may best be used in the management of diabetic condition as a result of its reducing tendencies of blood sugar level of diabetic experimental animals compared to the Metformin. The result of this study correlates with several authors' findings (Adeneye and Adeyemi. 2009a; Adeneye and Adeyemi, 2009b; Adeneye and Crooks, 2015, Kakade et al., 1972) who reported significant reduction of blood glucose levels of streptozotocin-induced diabetes mellitus in experimental animals treated with methanol extract of *H. umbellata*.

The examination of blood serum is a good way of assessing the health status as it plays a vital role in physiological, nutritional and pathological status of individuals (Ayinde et al., 2010; Ashafa et al., 2009). Hematological parameters are used to determine the extent of deleterious effect on blood constituents of animals (Rees, 2007; Adesokan and Akanji, 2010). It is used to explain blood related functions of chemical compounds/plant extract (Onoja et al., 2018). The mean hematological parameters for all groups showed no significance ($p>0.05$) (Table 4).

Three weeks observation may not be enough to allow for hemolysis of blood. Therefore, longer weeks of monitoring may be encouraged to ascertain the actual effect of the plant extract on hematological parameters. This result contradicts the report on the significant reduction of hematological parameters of alloxan-induced diabetics (Ogbera et al., 2009; Dave et al., 2019). Diabetes mellitus (DM) has been reported to be associated with cardiovascular morbidity and this may partly be explained by the abnormal lipid profile which is sometimes a feature of DM (Han et al., 2012). The study of the effect of *H. umbellata* on the lipid profile of streptozotocin-induced experimental animals showed lipid profile parameters such as HDL, CHOL and LDL in all experimental groups (Table 5). Group V recorded significance ($p<0.05$) amount of HDL, CHOL, TRIG and LDL while CHOL was significant ($p<0.05$) for Group III. Other lipid profile parameters in this study showed no statistical significance ($p>0.05$). The high cholesterol level may be attributed to the presence of hypertension for diabetic conditions which however may be contributory factor in the prevalence of dyslipidemia in type 2 DM. This result agrees with the reports of Harris (2005) and Kyoungho et al. (2014), who reported that high cholesterol presence in plasma of diabetic experimental animals treated with plant extract confirms the rise in blood pressure for animals that are diabetic.

Table 4. Effects of *H. umbellata* seed extracts on the hematological parameters of experimental animals

Parameters	Unit of measurement	Group I	Group II	Group III	Group IV	Group V
WBC	($\times10^3$/ul)	12.83±4.64	6.240±0.44	7.140±1.01	17.63±8.01	8.600±0.45
LY	%	50.50±10.6	45.26±9.25	51.32±3.91	48.80±14.1	49.00±2.35
MO	%	6.533±0.81	9.700±1.62	9.540±1.07	7.533±0.49	7.367±0.58
GR	%	42.97±10.8	45.02±8.06	39.14±3.11	43.67±15.4	43.63±1.78
RBC	($\times10^6$/ul)	5.873±0.44	6.398±0.164	6.504±0.33	4.933±0.71	5.803±0.27
Hgb	g/dl	12.97±0.94	13.40±0.47	15.20±0.36	11.00±1.19	13.00±0.65
PCV	%	38.67±1.37	40.70±1.18	41.46±1.97	34.97±2.64	38.43±1.87
PLT	(×103/ul)	797.3±63.2	300.8±106	422.8±148	539.0±27.5	752.7±55.1

Hematological parameters in Mean±SEM, n=5, not Significance at $p>0.05$, WBC: White blood cells, LY: Lymphocytes, MO: Monocytes/Macrophages, GR: Granulocytes, RBC: Red blood cells, Hgb: Hemoglobin , PCV: Packed cell volume, PLT: Platelets,

Group I: Normal control (no diabetes), Group II: Negative control (induced 55mg/kg BW STZ), Group III: Positive control (55mg/kg BW STZ + 500mg/kg BW Metformin) Group IV: 55mg/kg BW STZ + 500mg/kg, BW Aqueous extract of *H. umbellata* Group V: 55mg/kg BW STZ +500mg/kg BW Methanol extract of *H. umbellata*

Table 5. Effect of *H. umbellata* on the Lipid profile of streptozotocin-induced diabetic experimental animals

	mg/dL			
Group	**HDL**	**CHOL**	**TRIG**	**LDL**
I	138.68±15.01	155.89±14.69	66.667±12.13	36.720±16.35
II	158.83±9.058	140.75±9.948	92.436±18.87	38.854±14.84
III	140.75±21.74	*199.50±18.66a*	107.56±28.07	39.150±17.89
IV	124.24±24.32	184.46±4.780	91.877±37.61	49.250±19.39
V	103.39±17.00a*	194.49±12.29*	46.497±32.51a*	81.803±19.19a*

Lipid profile in Mean±SEM, n=5, Significance at a*p<0.05 with respect to normal control, *p<0.05 with respect to negative control, HDL: High density lipoproteins, CHOL: Total cholesterol, TRIG: Triglyceride, LDL: Low density lipoproteins Group I: Normal control (no diabetes), Group II: Negative control (induced 55mg/kg BW STZ) Group III: Positive control (55mg/kg BW STZ + 500mg/kg BW Metformin) Group IV: 55mg/kg BW STZ + 500mg/kg BW Aqueous extract of *H. umbellata* Group V: 55mg/kg BW STZ +500mg/kg BW Methanol extract of *H. umbellata*

The liver plays a major role in the regulation of carbohydrate homeostasis as a result of the hepatocellular glycogen accumulation which leads to hepatomegaly and liver enzyme abnormalities in poorly controlled diabetes patients (Han et al., 2012). Liver function tests (LFTs) are used in clinical practice to screen potential damage to liver, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs (Harris, 2005). Type 2 DM have been reported to be associated with higher incidence of abnormal LFT compared to the individuals without diabetes, elevated ALT being the most common abnormality (Harris, 2005). The study on the effect of *H. umbellata* on the liver function test of streptozotocin-induced diabetic animals revealed the mean liver enzymes in this study such as ALT, AST, ALP for all experimental groups (Table 6). ALT showed significance ($p<0.05$) decrease for Group V while AST and ALP were not statistically significant for Group I to group IV. The result of ALT, ALP and ALP for Group I to Group V were within safe limits (Kyoungho *et al*., 2014; Harris, 2005).

Table 6. Effect of *H. umbellata* on the liver function test of Streptozotocin-induced diabetic experimental animals

	U/L		
Group	**ALT**	**AST**	**ALP**
I	20.617±2.70	24.633±3.87	80.820±12.3
II	22.137±0.95	29.460±5.22	119.59±29.9
III	29.419±3.46	25.800±2.56	74.794±15.2
IV	25.264±3.39	19.980±2.05	64.610±2.97
V	13.373±4.25*	28.333±2.11	42.467±6.09

Liver enzymes in Mean±SEM, n=5, Significance at *p<0.05 with respect to normal control, *p<0.05 with respect to negative control, ALT: Alanine transaminase, AST: Aspartate Aminotransferase, ALP: Alkaline phosphatase,

Group I: Normal control (no diabetes), Group II: Negative control (induced 55mg/kg BW STZ), Group III: Positive control (55mg/kg BW STZ + 500mg/kg BW Metformin) Group IV: 55mg/kg BW STZ + 500mg/kg BW Aqueous extract of *H. umbellata* Group V: 55mg/kg BW STZ +500mg/kg BW Methanol extract of *H. Umbellate*

4. Conclusion

Failure of over the counter drugs in the management of patients with high blood sugar level (diabetes) has continued to draw attention to research into medicine plants as alternative to orthodox drugs. Medicinal plants possess phytochemical compounds depending of the extracting solvent used. The result of this study showed positive antidiabetic properties, significant physiological and biochemical functions on Wistar rats administered with methanol seed extract of *H. umbellate.* The methanol extract of *H. umbellata* possesses the potentials for the managements of blood glucose levels in experimental animals compared to Metformin. Attention should be drawn to proper utilization of medicinal plants in the management of diabetes condition in sub Saharan Africa.

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Author Contribution

Fidelis Ifeakachukwu OKOLAFOR carried out the research and wrote the manuacript

Frederick Osaro EKHAISE suppervised the project, did the editing of manuscript

Conflicts of Interest

There were no conflicts of interest in the course of this research.

References

1. Adeneye, A.A. and Crooks, P.A., 2015. Weight losing, antihyperlipidemic and cardioprotective effects of the alkaloid fraction of *Hunteria umbellata* seed extract on normal and triton-induced hyperlipidemic rats. Asian Pacific Journal of Tropical Biomedicine, 5(5):387–394. http://dx.doi.org/10.1016/s2221-1691(15)30374-9
2. Adeneye, A.A., Crooks, P. A., Fadhel-Albayati, Z., Miller, A., Zito, S.W., Adeyemi, O.O. and Agbaje, E.O., 2013. The novel antihyperglycaemic action of *Hunteria Umbellata* seed fractions mediated via intestinal glucose uptake inhibition. African Journal of Traditional, Complementarily and Alternative Medicine,10(2):189-202. http://dx.doi.org/10.4314/ajtcam.v10i2.3
3. Adeneye, A.A. and Adeyemi, O.O., 2009a. Hypoglycaemic effects of the aqueous seed extract of *Hunteria umbellata* in normoglycaemic and glucose and nicotine-induced hyperglycaemic rats. International Journal of Natural Product Resources, 2(1):9-18. http://doi:10.1055/s-0028-1084243
4. Adeneye, A.A. and Adeyemi, O.O., 2009b. Further evaluation of antihyperglycaemic activity of *Hunteria umbellata* K. Schum Hallier F. seed extract in experimental diabetes. Journal of Ethnopharmacology, 126(2): 238-243. htpps://doi: 10.1016/j.jep.2009.08.037
5. Adeneye, A.A., Crooks, P. A., Fadhel-Albayati, Z., Miller, A., Zito, S.W., Adeyemi, E.O. Agbaje, 2013. The novel antihyperglycaemic action of *Hunteria Umbellata* seed fractions Mediated via intestinal glucose uptake inhibition. African Journal of Traditional, Complement and Alternative Medicine, 10(2):189-202. https://doi.org/10.1055/s-0030-1265859
6. Aderele, O.R. Rasaq, A.K. and Momoh, J.O., 2020. Phytochemical screening, mathematical analysis and antimicrobial activity of methanolic seed extract of *Hunteria Umbellata*. European Journal of Medicinal Plants, 1–17. http://dx.doi.org/10.9734/ejmp/2020/v31i1630325
7. Adesokan, A. and Akanji, M. 2010. Haematological changes following chronic administration of aqueous extract of *Enantia chlorantha* to albino rats. Tropical Journal of Health and Science, 17(1). http:// dx.doi.org/10.4314/tjhc.v17i1.52791
8. Ashafa, O.T., Yakubu, M.T., Grierson, D.S. and Afolayan, A.J., 2009. Toxicological evaluation of the aqueous extract of *Felicia muricata* Thunb. leaves in Wistar rats. African Journal of Biotechnology, 8: 949-54.

https://doi.org/10.3109/13880200903373664
9. Ayinde, O.E., Ojo, V., Adeyina, A.A. and Adesoye, O., 2010. Economics of using Cocoa bean shell as feed supplement for rabbits. Pakistan Journal of Nutrition, 9:195-197. https://doi: 10.3923/pgn.2010.195.197
10. Azwanida, N.N., 2015. A Review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal and Aromatic Plants, 4: 196. https://doi:10.4172/2167-0412.1000196
11. Bain, B.J., Bates, I. and Laffan, M.A., 2017. Dacie and Lewis Practical Haematology (12th edition), Elsevier,London,652p. https://doi.org/10.1111/bjh.14872
12. Dave, M., Gupta, A.K. and Heernath, P.P., 2019. Correlation between fasting blood sugar level, HbA1C level and serum lipid levels in Type 2 diabetes mellitus patients. International Journal of Contemporarily Medical Research, 6(7). http://dx.doi.org/10.21276/ijcmr.2019.6.7.13
13. Friedewald, W.T., Levy, R.I. and Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry,18:499-502. https://doi.org/10.1093/clinchem/18.6.499
14. Gurib-Fakim, A., 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine, 27(1): 1-9. https://doi.org/10.1016/j.mam.2005.07.008
15. Han, N., Htoo, H.K.S. and Aung, H., 2012. Determinants of abnormal liver function tests in diabetes patients in Myanmar. International Journal of Diabetes Research, 1(3):36-41. http://dx.doi.org/10.5923/j.diabetes.20120103.02
16. Harris, E.H., 2005. Elevated liver function tests in type 2 diabetes. Clinical Diabetes, 23(3):115- 119. http://dx.doi.org/10.2337/diaclin.23.3.115
17. Igbe, I., Omogbai, E.K.I. and Ozolua, R.I., 2009. Hypoglycaemic activity of aqueous seed extract of *Hunteria umbellata* in normal and streptozotocin-induced diabetic rats. Pharmaceutical Biology, 47(10):1011-1016. https://doi.org/10.1080/13880200902973803
18. Kakade, M.L., Simons, N.R., Liener, I.E. and Lambert, J.W., 1972. Biochemical and nutritional assessment of different varieties of soybeans. Journal of Agricultural and Food Chemistry, 20:87-90. htpps://doi: 10.1021/jf60179a024
19. Kao, F.J., 2018. Effect of extracting solvents on antioxidant activities from fruit of *Phyllanthus emblica* Linn. Medicinal and Aromatic Plants, 07. http://dx.doi.org/10.4172/2167-0412-c1- 018
20. Keay, R.W.J., Onochie, C.F.A. and Stanfield, D.P., 1989. Tree of Nigeria (formerly Nigerian trees, 1960, 1964), Oxford University, Oxford, UK 234pp.
21. Kyoungho, L., Joohee, H. and Soo-Geun, K., 2014. Increasing risk of diabetes mellitus according to liver function alterations in electronic workers. Journal of Diabetes Investigation, 5:671–676. http://dx.doi.org/10.1111/jdi.12202.
22. Muhd, H.M.I, Siti, B.B., Mohamad, O. and Jamaludin, M., 2012. Protective role of *Hibiscus sabdariffa* calyx extract against streptozotocin induced sperm damage in diabetic rats. Experimental and Clinical Psychopharmacology, 11:659-669. https:// doi:10.17877/DE290R-5145
23. Murray, P., Pitt, B. and Webb, R.C., 1994. Ramipril prevents hypersensitivity to phenylephrine in aorta from streptozotocin-induced diabetic rats. Diabetologia, 37(7): 664–670. http://dx.doi.org/10.1007/s001250050161
24. Niranjan, R.M. and Kanaki, S., 2008. Phytochemical standardization of herbal drugs and polyherbal formulations. Bioactive Molecules and Medicinal Plants, UK, pp. 349-369. https://doi:10.1007/978-3-540-74603-4_19
25. Oboh, G., Adebayo, A.A. and Ademosun, A.O., 2017. Erection-stimulating, anti-diabetic and antioxidant properties of Hunteria *umbellata* and *Cylicodiscus gabunensis* water extractable phytochemicals. Journal of Complementary and Integrated Medicine, 15(1). http://dx.doi.org/10.1515/jcim-2016-0164
26. Ogbera, A.O., Fasanmade, O.A., Chinenye, S. and Akinlade. A., 2009. Characterization of lipidparameters in diabetes mellitus - a Nigerian report. International Archives of Medicine, 2(19):1-7. http://dx.doi.org/10.1186/1755-7682-2-19
27. Onoja, S.O., Udem, S.C. and Anaga, A.O. 2018. Ameliorative effects of *Helianthus annuus* against nephrotoxic, cardiac, and haematological disorders in alloxan-induced hyperglycae mia in albino rats. Journal of Veterinary Research, 62(3): 371–377. http://dx.doi.org/10.2478/jvetres-2018-005
28. Price, R., 1985. Phytochemical methods - a guide to modern techniques of plant analysis. Physiological Plant Pathology, 27(2):255–256.

http://dx.doi.org/10.1016/0048-4059(85)90073-6

29. Rees, A.J., 2007. A comparison of the haematological effects of pregnancy on the Han Wistar ra. Toxicology, 240(3): 165–166. http://dx.doi.org/10.1016/j.tox.2007.06.044
30. Reitman, S. and Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28(1):56-63. http://doi: 10.1093/ajcp/28.1.56
31. Reynolds, T. and Sofowora, A., 1984. Medicinal plants and traditional medicine in Africa. Kew Bulletin,39(3):667. http://dx.doi.org/10.2307/4108615
32. Selvaraju, K, Narayanaswamy, T. Thirunavukkarasu and T. Gangaipillai, A., 2013. Anti diabetic effect of methanolic leaf extract of *Pongamia pinnata* on streptozotocin induced diabetic rats. Journal of Coast Life Medicine, 1(2):113-117. https://doi:10.12980/JCLM.1.2013J9
33. Subash-Babu, P., Prabuseenivasan, S. and Ignacimuthu, S.C., 2007. A potential antidiabetic agent. Phytomedicine, 14: 15–22. https://doi: 10.1016/j.phymed.2006.11.005
34. Thirumalai,T., Theresa, V.S., Elumalai, E.K. and David, E., 2011. Hypoglycemic effect of *Brassica juncea* (seeds) on streptozotocin induced diabetic male albino rat. Asian Pacific Journal of Tropical Biomedicine, 4:323-325. http://doi: 10.1016/S2221-1691(11)60052-X
35. Trinder, P., 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. Journal of Clinical Pathology,22(2):158-161. https://dx.doi.org/10.1136/jcp.22.2.158
36. World Health Organization (WHO). World health statistics. 2011;170. ISBN ISBN: 978- 92-4-256419-8
37. Xing-Jiu, H., Yang-Kyu, C., Hyung-Soon, I., Oktay, Y., Euisik, Y. and Hak-Sung, K., 2006. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. MDPI, 6: 756-

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In-vitro Comparison Release Study of Novel Liposome and Conventional Formulation Containing *Rosmarinus officinalis* Extract

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Abstract

The purpose of this research article was to examine the impact of conventional Rosmarinus officinalis (without delivery systems) extract and extract incorporated liposomal delivery system on skin permeation velocity in vitro conditions was to determine whether there was a significant difference (in terms of duration) over the determined parameters.

The environment condition was 47 mm diameter with a capacity of 20 ml in a water bath applied to the membrane surface in 37 ° C and using a double walled franz diffusion cell containing a syntetic polycarbonate membrane filter. 1., 3., 5., 10., 20., 40. in minutes and upto 160 minutes 50 rpm rotation speed (mimicking the application speed obtained from the skin) with classical methods and delivery named by calculating the transition rates of Rosmarinus officinalis extracts obtained by the alternative method through the diffusion cell.

As a result of the study, it was clearly determined that the mixture of Rosemary (Rosmarinus officinalis) extract in the liposomal carrier system at a volume ratio of 1: 1 had an in vitro release rate of around 30% compared to 100% in 40 minutes, respectively, according to the traditional formula.

Key Words: Phytochemicals, Herbal liposomes, Rosmarinus officinalis, In-vitro release, Franz diffusion cell

1. Introduction

Herbal actives and plant sourced raw materials have been widely used against many health problem and cosmetic industry since centuries (Aslan, 2007). *Rosmarinus officinalis L.* is taxonomically included in the Lamiaceae family and has a pleasant scent, evergreen, perennial, branches reaching 50-110 cm in length, with leaves 1-3 cm in length. The upper surface of the leaves is dark green, soft and long hairy, the lower surface is light colored, with a dense soft hair, flowers in the form of a white star, densely hairy, and the fruit is brown in color (Mill, 1982; Baytop,

1999). Rosemary is considered to be one of hundreds of species in the genus Salvia, native to the Mediterranean and Asia, but highly resistant in cool climates, evergreen (Drew, 2017). It is a plant of Mediterranean origin, loves the seaside and grows very well. The Latin meaning of the name Rosmarinus is the flower of the sea and is associated with the spread of the plant. Especially Turkey Adana, Hatay, Mersin, Antalya frequently seen in the area. Today, culture is carried out in many countries, some of them are in European countries such as France, Spain, Portugal, Italy, Greece, neighboring European countries, the Balkans, the USA and Mexico (Sönmez, 2008). It is a plant that spread around the world in time, it was brought to England by the Romans. It is very popular in the UK and has been used as a spice in meals, benefited from its medicinal properties, and used as a garden herb for its fragrance. In the field of pharmacy, Hippocrates, Galen, Dioscarides prescribed for stomach, flu and liver problems. It was used a lot in French pharmacies especially during the Renaissance and became the most prescribed herb.

When we look at the naming of the plant, Rosmarinus' name is the old name of the plant in classical Latin. The historical background of rosemary is included in cuneiform stone tablets from 5000 BC and is mentioned in Materia Medica (Ambrose, 2016). When we look at the historical adventure of the plant, it is seen that it has records in the Chinese dynasty in 220 AD, and it is estimated that later the Romans came to European lands with the eastern expeditions (Madison, 2017). One of the first alcohol-based perfumes described as Hungarian water, Hungarian water was made from Rosemary. Leaves, branches and flowering tips are removed for use (Centre for Agriculture and Bioscience International, 2018). Rosemary leaves are used as flavouring in traditional Mediterranean cuisine and herbal teas can be made (Database USDA Nutrient, 2014). Another important point in terms of use with foods is that omega 3-rich oils that are prone to rancidity have been shown to increase shelf life and heat stability (Daniells, 2017).

In traditional medicine, it has been used orally as a tonic, in the case of constipation, anti-inflammatory, abdominal pain, headache, antispasmolytic, gout treatment, biliary therapy, diuretic, antirheumatic, antidepressant, external wound healing, anti-scalp capping, hair loss (Baytop, 1999; González-Trujano et al., 2007). The dried leaves of the rosemary plant are maserated in olive oil and massaged into the hair roots, and the infusion prepared from its branches is used for diabetes. It is also recorded that it is used as a sedative as an infusion tea prepared from a mixture containing the leaves of the plant (Sarıkan, 2007).

The phenolic compounds responsible for the pharmacological activity of *Rosmarinus officnalis* are rosmarinic acid, carnosic acid and carnosol. Carnosic acid is the main component of the phenolic diterpenes in the rosemary plant (Kuhlmann and Röhl, 2006). The structure of carnosic acid is $C_{20}H_{28}O_4$ and it comes from the terpenoids class, also known as isoprenoids or terpenes, it is one of the main polyphenolic diterpenes in the plant. In addition, many biological effects are seen; Anti-inflammatory and antiviral effects, nervous protective, anti-aging, antimicrobial, antiangiogenic, liver protective, antiadipogenic, antihyperglycemic and

anticarcinogenic properties have also been reported (Bozic et al,, 2015; Jung et al, 2015).

Carnosic acid and carnosol are responsible for the antioxidant properties of the Rosmarinus officinalis plant and possibly protect chloroplasts and chloroplastic and perhaps other organelle membranes from oxidative stress. It has been reported that it has more antioxidant effect than butylhydroxytoluene and butylhydroxyanisole, which are generally used synthetic antioxidants.

Another important phenolic compound of *Rosmarinus officinalis* is Rosmarinic acid, which is the ester of caffeic acid and 3,4-dihydroxyphenyllactic acid. Liver protective, anti-inflammatory, antiangiogenic, antitumor, antidepressant, anti-neurodegenerative and HIV-1 inhibitory properties are known (Furtado et al, 2010). It has been shown in clinical studies that the diterpenes found in *Rosmarinus officinalis* plant induce various factors and also inhibit neuronal cell death (Habtemariam et al., 2016). In a study with *Rosmarinus officinalis* extract, the effects on mRNA expressions in the hippocampus and frontal lobe of rats were examined. It shows that the plant extract causes long-term memory improvement, which can be explained in part by the inhibition of AChE activity in the rat brain. (Ozarowski et al., 2013). In a study conducted with rosemary plant, it was stated that it can limit the weight gain that develops due to fatty onset and prevent metabolic disorders related to obesity. (Sedighi, 2014). It is thought to reduce body weight and fat ratio by inducing the pancreas by inhibition of lipase activity. (Harach et al., 2010). In a research on colon cancer cells, the apoptosis of the cells increased thanks to Rosmarin extract and carnosic acid. It significantly increased the expression of Nrf2 and inhibited HCT116 heterograft tumor formation (Yan et al., 2015). In another cytotoxic study with rosemary extract, its effect on cancers in different organs was examined. In a study conducted with lung cancer, colon cancer, breast cancer and cervical cancer, rosemary was found to have the highest cytotoxic activity on lung cancer (Alanazi, 2016). In an *in vivo* study on the potency of R. officinalis in gastric ulcer, it was observed that gastric ulcerations were prevented in male rats whose stomach was stimulated with 70% alcohol. (Amaral et al., 2013). Its use as a fragrance, the essential oil of Salvia Rosmarinus is burned as incense to spread fragrant body perfumes or aroma in a room, and is used in shampoo and cleaning products. Phytochemically, Rosemary essential oil contains 10-20% camphor and contains a number of phytochemicals such as rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid, and carnosol (Vallverdu, 2014).

The epidermis, which is the upper level of the skin structure, consists of the stratum corneum (SC) followed by the lucidum, granulosum, spinosum and basal membrane, followed by the dermis and hypodermis layer (Biju et al., 2006). Transdermal delivery systems must overcome the inherent properties of the skin structure. Transdermal delivery systems must overcome the natural properties of the stratum corneum as a skin barrier. The stratum corneum acts as a protective layer that prevents the penetration of foreign compounds from the external environment into the skin and also prevents the skin's moisture loss (Atmakuri and Dathi, 2010).

Liposomes are a new generation carrier system with both a hydrophilic head and a lipophilic chain part, which increases their absorption from the skin by trapping different active ingredients in their structure (Aslan et al., 2020; Duman et al., 2014). Thanks to their properties that mimic the skin and cell membranes, they are preferred in many areas ranging from cosmetics to biotechnology, biochemistry and physiology to food supplements (Ethemoğlu et al., 2017; Yazıcı et al., 2011; Günal et al., 2019). A liposome is a useful tool for transporting herbal active ingredients to the inner layers of the skin by passing through the skin barrier due to its double-layered structure obtained from phospholipids similar to the skin structure (Uhumwangho and Okor, 2005) Phospholipids, which are components of liposomes, have many advantages as a carrier of active ingredients such as strong tissue affinity, biodegradability and low toxicity (Yadav et al., 2011). It is advantageous for liposomal delivery systems to provide the effect of increasing the bioavailability while minimizing the toxicity of the active ingredient in the delivery of plant origin active ingredients at an optimum rate. Advantages of liposomal delivery systems are;

- Liposomes are used in active ingredient encapsulation systems because of their advanced features.
- The liposome can entrapped both lipophilic and water soluble active ingredients. For that reason, as an active ingredient transporter, the liposome can indiscriminately carry active ingredients via cellular structure.
- The liposomal formulation can provide a sustained and controlled formulation release and increase active ingredient solubility.

Table 1. Herbal liposomal formulations

Herbal Source	*Active materials*	Biological Activity	Applications of liposome formulations	Year	References
Pueraria lobota	*Puerarin*	Anti-arrhythmia Activity	These formulations modify their surface charge and membrane integrity	2007	Rong and JuQun, 2007
Nux vomica	*Strychnine, Brucine*	Anti-tumour, analgesic and anti-inflammatory	Activities Increase stability of formulations	2010	Chen et al., 2010
Myrtus communis	*1,8-cineole, linalool, myrtenyl acetate, myrtenol*	Antioxidant and antimicrobial activity	Increase in its activities	2008	Gortzi et al., 2008
Diospyros montana Roxb	*Diospyrin*	Anti-cancer activity	Enhancement of its anti-tumour effect	2005	Hazra et al., 2005
Artemisia arborescens L.	*Artemisia arborescens*	Antiviral activity	Increase in antiviral activity and stability	2005	Fadda at al., 2005
Magnolia officinalis	*Magnolol*	Inhibiting vascular smooth muscle cells (VSMCs) proliferation	Enhance the therapeutic efficacy	2008	Chen 2008
Purchased From Sigma Chemicals	*Quercetin*	Antioxidant activity	Enhance therapeutic efficacy	2010	Ghosh et al., 2010

Franz diffusion cells have become very important in research studies of the passage of drugs and cosmetics through the skin. Although there are question marks in terms of analytical validation, studies conducted in recent years, it can be said that transition through the skin in in vitro conditions without the need for animal studies provides an advantage for researchers in all respects. Synthetic polycarbonate membrane filters reduce variability and increase reproducibility (Fern et al., 2010, Andrews et al., 2013).

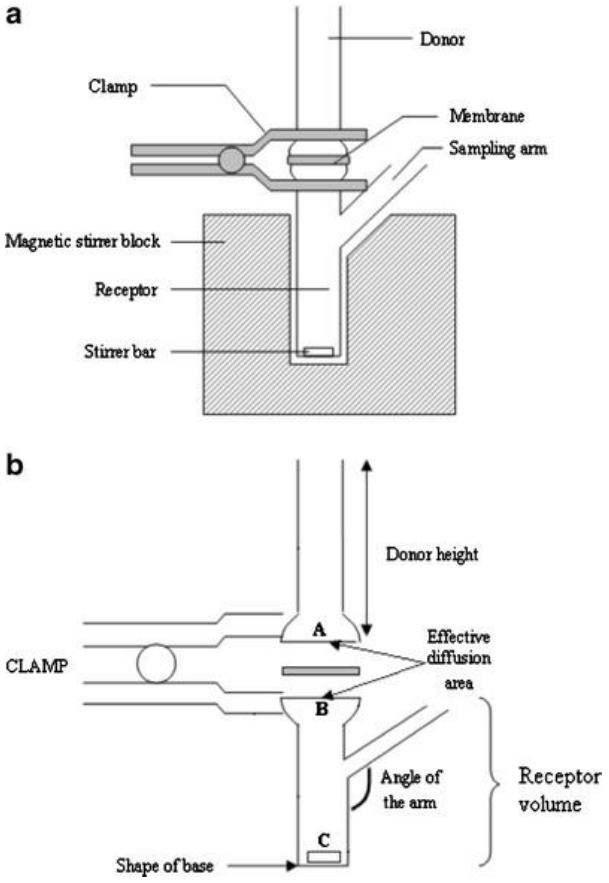

Figure 1. Shematic Representation of a Franz Diffusion Cell System for *In Vitro* Release Studies (Andrews et al., 2013)

In this research paper was to indicate the effect of conventional *Rosmarinus officinalis* (without delivery systems) extract and liposomal delivery system incorporated extract on skin permeation velocity *in vitro* conditions was to determine whether there was a significant difference in terms of duration over the determined parameters.

2. Material and Methods

2.1. Materials

Hydrogenated Soybean Phoshatidylcholine (HSP) and phospholipids were provided from LIPOID (Germany). Cholesterol (CHOL) was obtained from Sigma (UK). Rosmarinus officinalis extract was provided from İnan Tarım (Turkey). All other chemical reagents were of analytical purity. Franz diffusion cell was used in order to determine the absorbtion rate of delivery system (Permegear, USA).

2.2. Preparation of Herbal Liposome Formulations

Liposome carrier systems were designed by thin film preparation method (Gunal et al., 2019). Firstly, liposome dispersions (HSP and Cholesterol) were designed by dissolving the 40 µmol mL^{-1} of phosphatidylcholine and lipophilic components in 30 mL chloroform in a round flask. Chloroform solvent was evaporated using a rotary evaporator under 0-100 mbar pressure to form a thin film over the wall of the flask. Also, ethanol was accurately evaporated using rotary evaporator from plant extract. The thin film was then solved over a water bath with buffer *Rosmarinus officinalis* extract as 1:1 v/v ratio. Optimized liposome formulation was selected according to previous experimental study (Aslan et al., 2020)

2.3. *In vitro* Release Study

All permeation experiments have been done using an endless dosage regimen. This test was a single application and covers the application starting from 1 minute to 40 minutes and then the following results. 50 rpm of the new formulations obtained by conventional method and liposomal formulations (imitating the application speed to the skin), 1.3.5.10.20. 40. and 160 minutes, respectively.

The permeation values were compared by calculating the velocities of passage through the diffusion cell in minutes and up to 160 minutes. Briefly, the working principle is based on the application of the tested product approximately 1 ml once to the jacketed Franz diffusion cell in 37°C, phosphate buffer solution at pH:7.4 . This product simulates keeping in contact with the skin for up to 1 minute and up to 40 minutes. Afterwards, samples were taken at specified times and the results were recorded with the measurements in the UV spectrophotometer. Agilent 8453 (USA) was used for the determination of absorbance in UV spectrophotometer and branded analyzer, which is widely preferred in UV analysis in many research studies. Since the skin is known to have tight intercellular connections around 220 nm and also a polycarbonate membrane filter was preferred because of inert material. Permegear branded equipment (Standard 25mm Jacketed Franz Cell) was used for the diffusion cell. The amount of test material applied to each cell was 1 ml, the receptor volume was 20 ml and the amount taken at certain times was 0.5 ml. The standard calibration curve of rosmarinic acid has been extracted. By taking samples, their absorbance was measured at 272 nm. Bypassing from absorbances to % concentration, the concentration at which 100% of the product releases was determined. Herbal liposome formulation (HLF) and conventional formulation containing *Rosmarinus officinalis* extract (CFR) were compared according to *in vitro* release study via Franz diffusion cell.

3. Results and Discussion

3.1. Calibration Curve Results of Rosmarinic acid

Rosmarinic acid was a major component of Rosmarinus officinalis extract to determine UV Spectral analyze. For that reason, calibration curve has been presented and calculated between 0.05 mM and 0.25 mM according to the results in Figure 2 at 323nm.

3.2. *In vitro* Release Study Results

In vitro release is an important parameter of absorbtion for topical herbal liposomal carrier models. According to in vitro release study results, HLF formulation presented better penetration behaviour compare to CFR at the end of the 160 minutes in vitro release study.

All data were given Table 2 and Figure 3.

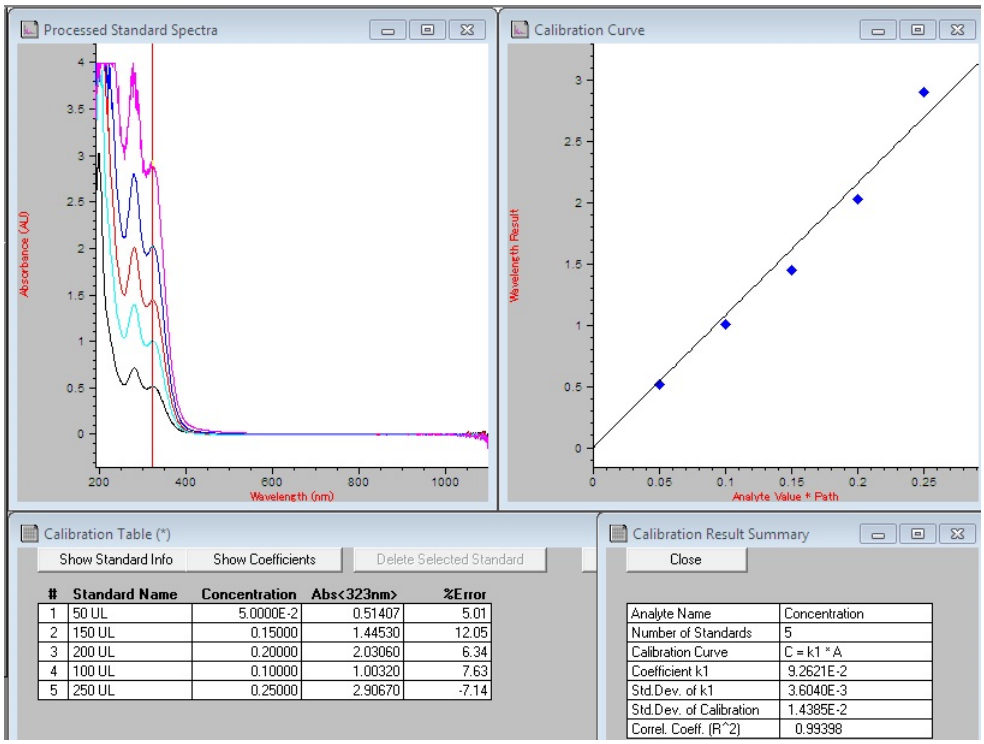

Figure 2. Calibration curve of Rosmarinic acid at 323 nm.

Table 2. Cumulative release table of HLP and CFR formulation (n=6).

Time (min.)	HLP (Herbal Liposome)						CFR (Conventional Rosemary Extract)					
0	0	0	0	0	0	0	0	0	0	0	0	0
1	3	3,22	2,33	4,96	3,96	5,13	1,02	1,35	0,88	1,08	1,04	1,12
3	15,06	15,22	14,76	15,28	14,86	15,88	2,12	2,06	2,16	2,22	2,1	2,24
5	37,44	36,76	35,98	36,52	37,96	37,82	4,94	5,52	5,38	5,2	5,14	5,28
10	55,26	54,88	55,63	55,74	54,9	54,58	10,04	9,92	10,16	10,02	9,98	9,84
20	87,98	88,54	88,44	88,7	87,66	89,02	21,63	20,74	20,48	21,02	20,96	20,8
40	97,66	98,88	99,66	97,84	98,55	99,24	34,3	36,54	35,68	35,4	35,34	35,28
160	100,98	99,8	98,9	99,96	100,02	99,62	60,82	60,9	58,28	57,6	61,29	59,06

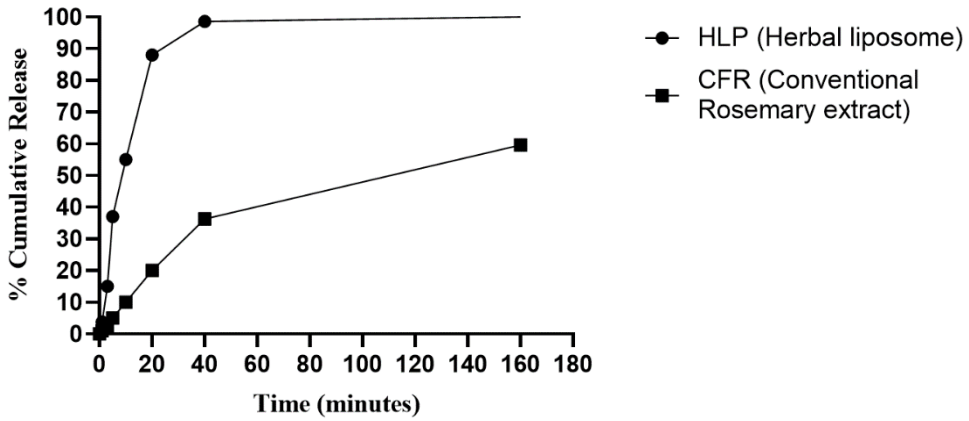

Figure 2. Cumulative *in vitro* release study result of HLF and CFR formulations (n=6).

4. Conclusion

In this research; spectroscopic analysis and in vitro release studies were considered to select the better carrier system for Rosemary extract formulation. For that reason, HLF formulation was selected depending on the release behaviour (Table2 and Figure 3). Size controlled liposome formulation showed that it was better penetration enhancer for the topical delivery systems compare to the conventional extract systems.

The objective of this study is to develop a topical delivery system which is containing herbal actives, with innovative formulation. As a result of the study, it was clearly determined that the mixture of rosemary (Rosmarinus officinalis) extract in the liposomal carrier system at a volume ratio of 1: 1 compared with the conventional formula has a very high in vitro release rate of 160 minutes, even absorbed within minutes. Ex vivo and cell culture studies will be planned in near future.

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Author Contributions

İsmail ASLAN devised the project, the main conceptual ideas and proof outline.

Ahmet Arif KURT worked out almost all of the technical details, and performed the numerical calculations for the suggested experiment. All authors discussed the results and commented on the manuscript.

Conflicts of Interest

We declare that there are no matters of conflict of interest. No funding was received for this article; we did not receive any sponsorship.

References

1. Alanazi, A. M. (2016). The cytotoxicity effect of rosemary and myrrh extracts on cancer cell lines (Doctoral dissertation, Tennessee State University).
2. Aslan, İ , Kurt, A . (2020). Characterization and Optimization of Phytosome Formulation Containing Alcohol-free Umckalin from Pelargonium sidoides . Current Perspectives on Medicinal and Aromatic Plants (CUPMAP), 3 (1) , 49-53 . https://doi.org/10.38093/cupmap.737878
3. Aslan, I. (2007) Plants and Cosmetics. Fitomed, 3, 49-51.
4. Atmakuri LR, Dathi S. (2010), Current trends in herbal medicines. J Pharm Res.; 3(1):109-113.
5. Biju SS, Talegaonkar S, Mishra PR, Khar RK. (2006) Vesicular system: an overview. Indian J Pharm Sci.; 68(2): 141-153.
6. Božić, D., Papaefthimiou, D., Brückner, K., de Vos, R.C., Tsoleridis, C.A., Katsarou, D., Papanikolaou, A., Pateraki, I., Chatzopoulou, F.M., Dimitriadou, E., Kostas, S., Manzano, D., Scheler, U., Ferrer, A., Tissier, A., Makris, A.M., Kampranis, S.C. and Kanellis, A.K. (2015). Towards elucidating carnosic acid biosynthesis in Lamiaceae: functional characterization of the three first steps of the pathway in *Salvia fruticosa* and *Rosmarinus officinalis*. PLoS One, 10(5), e0124106.
7. Centre for Agriculture and Bioscience International.,(2018), "Rosmarinus officinalis (rosemary)".
8. Chen C. (2008), Inhibiting the vascular smooth muscle cells proliferation By EPC and DPPC liposome encapsulated magnalol. J Chin Inst Chem Eng.;39:407-411.
9. Chen J, Chen Z, Wang W. (2010), Ammonium sulphate gradient loading of brucine into liposome: effect of phospholipid composition on entrapment efficiency and physicochemical properties in vitro. Drug Dev Ind Pharm.;36:245-253.
10. Daniells, Stephen (2017). "Oregano, rosemary extracts promise omega-3 preservation". Food Navigator.
11. Database USDA Nutrient, (2014),"Nutrition Facts – Dried rosemary, one teaspoon (1 g)". nutritiondata.com. Conde Nast, , version SR-21..
12. Dawn C P Ambrose,(2016), Leafy Medicinal Herbs: Botany, Chemistry, Postharvest Technology and Uses by Dawn Ambrose, 216, 210-11
13. Drew, Bryan T.; González-Gallegos, Jesús Guadalupe; Xiang, Chun-Lei; Kriebel, Ricardo; Drummond, Chloe P.; Walker, Jay B.; Sytsma, Kenneth J. (2017). "Salvia united: The greatest good for the greatest number". Taxon. 66(1): 133–145.

14. Duman, G., Aslan, İ., Özer, A. Y., İnanç, İ., Taralp, A. (2014). Liposome, gel and lipogelosome formulations containing sodium hyaluronate. Journal of liposome research, 24(4), 259–269. https://doi.org/10.3109/08982104.2014.907305.
15. Ethemoglu, M. S., Seker, F. B., Akkaya, H., Kilic, E., Aslan, I., Erdogan, C. S., Yilmaz, B. (2017). Anticonvulsant activity of resveratrol-loaded liposomes in vivo. Neuroscience, 357, 12–19. https://doi.org/10.1016/j.neuroscience.2017.05.026.
16. Fadda AM, Sinico C, Lai F, Logu AD. (2005), Liposomal incorporation of artimisia arborescenceL. Essential oil and in vitro antiviral activity. Eur J Pharma Biopharma.;59:161-8.
17. Furtado, R.A., De Araujo, F.R.R., Resende, F.A., Cunha, W.R. and Tavares, D.C. (2010). Protective effect of rosmarinic acid on V79 cells evaluated by the micronucleus and comet assays. Journal of Applied Toxicology, 30(3), 254-259
18. Ghosh D, Ghosh S, Sarkar S, Ghosh A, Das N, Das Saha K, et al. (2010) Quercetin in vesicular delivery systems: evaluation in combating arsenic-induced acute liver toxicity associated gene expression in rat model. Chem Biol Interact.;186:61-71.
19. Gortzi O, Lalas S, Chinou L. (2008), Re-evaluation of bioactivity and antioxidant activity of myrtuscommunis extract before and after encapsulation in liposome. Eur Food Res Technol.;226:583-90
20. Gunal, M. Y., Ayla, Ş., Bedri, N., Beker, M. Ç., Çağlayan, A. B., Aslan, İ. ... Kılıç, Ü. (2019). The effects of topical liposomal resveratrol on incisional and excisional wound healing process. Turkderm-Turkish Archives of Dermatology and Venerology, 53(4), 128-134. http://doi.org/10.4274/turkderm.galenos.2019.82612.
21. Habtemariam, S. (2016). The therapeutic potential of rosemary (Rosmarinus officinalis) diterpenes for Alzheimer's disease. Evidence-Based Complementary and Alternative Medicine, 1-15.
22. Harach, T., Aprikian, O., Monnard, I., Moulin, J., Membrez, M., Béolor, J. C., ... ve Darimont, C. (2010). Rosemary (Rosmarinus officinalis L.) leaf extract limits weight gain and liver steatosis in mice fed
23. Hazra B, Kumar B, Biswas S, Pandey BN, Mishra KP. (2005) Enhancement of the tumour inhibitory activity, in vivo, of diospyrin, a plantderived quinonoid, through liposomal encapsulation. Toxicol Lett.;157:109-17
24. Jung, K.J., Min, K. J., Bae, J.H. and Kwon, T.K. (2015). Carnosic acid sensitized TRAIL-mediated apoptosis through down-regulation of c-FLIP and Bcl-2 expression at the post translational levels and CHOP-dependent up-regulation of DR5, Bim, and PUMA expression in human carcinoma caki cells. Oncotarget, 6(3), 1556).
25. Kuhlmann, A. and Röhl, C. (2006). Phenolic antioxidant compounds produced by in vitro. Cultures of Rosemary (Rosmarinus officinalis.) and their antiinflammatory effect on lipopolysaccharide-activated microglia. Pharmaceutical Biology, 44(6), 401-410.) .
26. Madison D. (2017)The Illustrated Encyclopedia of Fruits, Vegetables, and Herbs: History, Botany by Deborah Madison, p.266
27. Ng, SF., Rouse, J.J., Sanderson, F.D. et al. Validation of a Static Franz Diffusion Cell System for In Vitro Permeation Studies. AAPS PharmSciTech 11, 1432–1441 (2010). https://doi.org/10.1208/s12249-010-9522-9
28. Ozarowski, M., Mikolajczak, P. L., Bogacz, A., Gryszczynska, A., Kujawska, M., Jodynis-Liebert, J., ... ve Bartkowiak-Wieczorek, J. (2013). Rosmarinus officinalis L. leaf extract improves memory impairment and affects acetylcholinesterase and butyrylcholinesterase activities in rat brain. Fitoterapia, 91, 261-271.
29. Rong G, JuQun X. (2007) Studies on molecular interection between puerarin and PC liposomes. Chinese Sci Bull.;52:2612-7
30. Sedighi, R. (2014). Carnosic Acid Enriched Rosemary Extract Prevents Obesity and Metabolic Syndrome in High-Fat Diet-Fed Mice (Doctoral dissertation, North Carolina Agricultural and Technical State University).
31. Uhumwangho MU, Okor RS. 2005 Current trends in the production and biomedical applications of liposomes: a review. J Biomed Sci.; 4: 9-21. http://doi.org/10.4314/jmbr.v4i1.10663
32. Vallverdú-Queralt, Anna; Regueiro, Jorge; Martínez-Huélamo, Miriam; Rinaldi Alvarenga, José Fernando; Leal, Leonel Neto; Lamuela-Raventos, Rosa M. (2014). "A comprehensive study on the phenolic profile of widely used culinary herbs and spices: Rosemary, thyme, oregano, cinnamon, cumin and bay". Food Chemistry. 154: 299–307.
33. Yadav D, Suri S, Choudhary AA, Sikender M, Hemant, Beg NM, et al. (2011), Novel approach: Herbal remedies and natural products in pharmaceutical science as nano drug delivery systems. Int J Pharm Tech.;3:3092-116.
34. Yazici, M., Duman, G., Aslan, I., Durucan, A., & Sahin, F. (2011). The antifungal activity of liposomal ointment formulation of essential oil of Satureja hortensis. Current Opinion in Biotechnology, (22), S108. http://doi.org/10.1016/j.copbio.2011.05.345

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Phytochemical Screening, Free Radical Scavenging, and In vitro Anti-bacterial Activity Studies of Various extracts of Selected Medicinal Plants of Nepal

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Abstract

Bacteria are unique prokaryotic agents which carry genetic information in double stranded DNA matrix and even reside in the normal flora of human. Such bacteria sometime become the cause of human infection and disease by evading bodies protective mechanisms. Antibacterial agents are the group of materials that fight against such pathogenic bacteria, killing or reducing their metabolic activity. Meanwhile, naturally occurring plant phytochemicals are being used as antimicrobial and antibacterial agents by various group of indigenous and ethnic populations. This study was carried out to extra plot the safety, efficacy and therapeutic potency of the selected medicinal plants which are used traditionally as a source of anti-microbial, anti-bacterial and free radical scavenger. Quantitative phytochemical screening revealed that methanolic extract of *Crassocephalum cripidiodes* showed maximum Total Phenolic Content (TPC) of 322.16±0.01 µg GAE/mg of extract and acetone extract of *Ficus semicordata* showed maximum Total Flavonoid Content (TFC) of 500.35±0.045 µg QE/mg of extract. Among the selected plant extracts, the most potent antioxidant activity was revealed by methanolic extract of *Crassocephalum crepidiodes* with IC_{50} of 6.95µg which is close to that of standard ascorbic acid having IC_{50} 4.21µg. Bacterial susceptibility assay was carried out against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Among the selected plants, greater zone of inhibition was shown by acetone extract of *Ficus semicordata* against *Pseudomonas aeruginosa*. In conclusion, the study revealed that *Ficus semicordata*, *Cirsium argyracanthum* and *Ficus hispida* have anti-bacterial property against the selected bacterial strain which provide evidence-based scientific proof towards the traditional use of these plant samples in curing bacterial diseases.

Key Words: Ethnomedicinal plants, phenols, flavonoids, free radical scavenging activity, antibacterial activity

1. Introduction

Bacteria are prokaryotic organisms which carry their genetic information in a double-stranded circular molecule of DNA. Ribosome is present in the cell cytoplasm of bacteria and except Mycoplasma, all other bacteria have both cell wall and cell membrane. Bacteria a unique prokaryote which even resides on normal flora of human may sometime become a cause of

human infection and disease. Such pathogenic bacteria evade the body's protective mechanisms, use its resources and cause several diseases (Doron, 2008). Antibacterial agents are a group of materials that fight against these pathogenic bacteria, killing or reducing their metabolic activity. With this, their pathogenic effect on the biological environment will be minimized making anti-bacterial agents suitable to be used for both pathogenic and prophylactic treatment(Pasquale et al., 2005).

However, the efficacy of such synthesized anti-bacterial agents is being endangered these days because of the rapid emergence of bacterial resistance worldwide (Ventola, 2015). The major reasons behind such bacterial resistance are enzymatic degradation of antibacterial drugs, alteration of bacterial proteins that are antimicrobial targets and changes in membrane permeability to antibiotics (Dever & Dermody, 1991). On the other hand, market available anti-bacterial agents also bear several side effects such as diarrhea, weakness, blood disorder, fungal infection of mouth and digestive tract, joint swelling, dehydration etc. (Labu et al., 2013). Meanwhile, naturally occurring plant phytochemicals are gaining interest as antimicrobial, antibacterial and in other ailments as traditional sources of therapeutic supplement but evidence-based scientific evaluation of these medicinal plants are still not sufficient to justify their efficacy and safety (Taylor, 2013). Therefore, this study was carried out to extra plot the safety, efficacy and therapeutic potency of the few medicinal plants which are used traditionally as a source of anti-microbial, anti-bacterial and free radical scavenger.

Followingly, several biochemical reactions in our body generates reactive oxygen species (ROS) which may damage the crucial biomolecules required for body functioning (Kumaran, 2006). Active natural products and phytochemical components especially polyphenols, flavonoids, phenyl propanoids, phenolic acids, tannins are known for their free radical scavenging and anti-oxidant activities of plants (Nickavar et al., 2007). Meanwhile, various studies revealed that different extracts of plants with free radical scavenging activity even showed anti-bacterial and anti-microbial effect revealing the relation between anti-oxidant and anti-bacterial properties of plant samples (Chanda et al., 2010: Safari et al., 2019).

For many reasons, herbal products and crude plant parts or their bioactive compounds are gaining interest in the treatment of diseases from ancient time till to the modern generation. Due to the increasing diseases, the development of resistance to existing drugs and the demands for lesser side effect drugs, researcher groups are more concerned to explore the best medicine raw material from plants with modern scientific and technological ideas (Chandran et al., 2020). In this study, six different medicinal plants were selected from local area of Kaski district, Nepal based on their traditional implication as an antimicrobial and anti-bacterial agent. The major reason for selection of only these medicinal plants aside from other is due to their less scientific studies to prove their anti-bacterial properties compare to other though they are widely utilized for their anti-bacterial purpose. Those selected medicinal plants are *Cirsium argyracanthum Candolle* sp., *Crassocephalum crepidioides* (Benth) S.

Moore, *Ficus hispida* L.f, *Ficus semicordata* Buch. -Ham.ex Sm, *Impatiens balsamina* L. and *Prunus persica* L. *C. argyracanthum* a biennial short lived monocarpic thistle is being locally used as poultice in sore jaw. A hot infusion of the whole plant has been used as a herbal steam in the treatment of rheumatic joints while decoction is being used in the treatment of bleeding piles (Zia et al., 2011). In a study by Nazaruk, et al., (2008), the methanolic extracts of *C. argyracanthum* inflorescences showed antioxidant properties which depend on phenolic compounds. *C. crepidioides* also called thickhead, red flower rag leaf is being used traditionally to treat indigestion and leaf lotion or decoction is being used to treat upset stomach and headaches. In another study by Omotayo et al. (2015), it has been shown that *C. crepidioides* possess antioxidant, chemo preventive and anti-inflammatory properties (Omotayo, et al., (2015).

F. hispida is an evergreen tree about 6 m high with smooth, pale, and colored horizontally wrinkled bark (Ali et al., 2011). Traditionally, the juice of these plants is taken for liver problems, twigs for earache, while seed, fruit and bark are used for emetic and purgative purpose (Kunwar et al., 2006). *F. semicordata* is a small to medium size tree upto 15 m tall. Traditionally the plant is being used for fever, menstrual disorders, while root is even applied to headache. The bark of the plant is even used to treat gastric troubles and peptic ulcers while immature fruit is used to treat constipation and latex is used to treat children with fever (Kaur et al., 2016).

I. balsamina is a succulent erect herb about 1 m high. The different parts of plants were used as traditional remedies for disease and skin afflictions. The flower is applied to burns and even use for rheumatism, fractures, and ailments. It is also used in the treatment of constipation and gastritis (Akiyama et al., 2015). Decoction of flower is an effective emetic and laxative and it promotes the flow of urine (Meenu et al., 2015).

P. persica is a perennial plant which grows upto 7 m tall and wide. Locally, the leaves of this plant are used as astringent, demulcent, diuretics, expectorant, laxative, and mild sedative. The leaves are used internally in the treatment of gastritis, whooping cough, and bronchitis. The dried and powdered leaves have sometime been used for wound healing purposes (Kant et al., 2018).

This study was conceived to figure out the scientific background of these selected medicinal plants by taking their traditional and local uses as a reference basis for selection of these plants. In this study, qualitative and quantitative phytochemical screening, free radical scavenging activity and in-vitro anti-bacterial activity of the crude extracts of these selected medicinal plants were evaluated.

2. Material and Methods

2.1. Chemicals, Reagents, and Test Organisms

1,1 Diphenyl-2 picryl hydrazyl radical (DPPH) was purchased from Tokyo Chemical Industry, Japan. The test organisms *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were provided as generous gift from Manipal

Teaching Hospital, Kaski, Nepal. Standard drugs Gentamycin, Vancomycin and Ascorbic acid were provided as a generous gift from Asian Pharmaceuticals Pvt. Ltd, Bhairahawa, Nepal. All chemicals and reagents were used as analytical reagent grade.

2.2. Collection and Identification of Plants Samples

Selected medicinal plants (as shown in Table 1) were collected from different area of Kaski district, Nepal. The herbaria were prepared and identified with the help of taxonomist from National Herbarium and Plant Laboratories, Godawari, Kathmandu, Nepal. The voucher specimen of each collected plants was deposited in the crude drug museum of School of Health and Allied Sciences, Pokhara University.

The collected samples were chopped into small pieces and were shaded dried. The samples were incubated in hot air oven at 40°C for complete removal of moisture, which is detected by weight variation test at different time intervals. After the samples were completely dried, they were powdered with the help of grinder.

2.3. Sample Extraction

Dried sample of selected plants were extracted with different solvents by using maceration process. One hundred gram of each plant materials were macerated first with chloroform for 24 hours with intermittent shaking and the extract was filtered using Whatmann no. 1 filter paper to obtain chloroform extract. The residue was again subjected to second successive maceration with chloroform for another 24 hours with intermittent shaking followed by filtration. The obtained residue was subjected to acetone for double maceration and the same procedure is repeated for methanol as well. The extracts of all plants were concentrated in rotary evaporator.

Table 1. Phytochemical screening and biological activity of the selected plants

Scientific Name	Family	Common Name	Used Part	Crude Drug Voucher No.
C. argyracanthum	Asteraceae	Thakailo	Root	PUCD-2019-20
C. crepidiodes	Asteraceae	Salaha	Leaves	PUCD-2019-21
F. hispida	Moraceae	Tote	Leaves	PUCD-2019-22
F. semicordata	Moraceae	Khanayo	Leaves	PUCD-2019-23
I. balsamania	Balsaminaceae	Tiuri	Leaves	PUCD-2019-24
P. persica	Rosaceae	Aaru	Seeds	PUCD-2019-25

2.4. Phytochemical Screening

2.4.1. Qualitative Phytochemical Screening

Qualitative phytochemical screening was performed as per the method given by Auwal et al. (2014) and Tepal et al. (2016) for the determination of active phytoconstituents present in plant samples. Determination of the presence of compounds such as alkaloids, carbohydrates, glycosides, saponins, phenols, flavonoids, tannins and terpenoids were analyzed using screening procedure.

2.4.2.Quantitative Phytochemical Screening

2.4.2.1. Total Phenolic Content (TPC)

The TPC was determined by the Folin-Ciocalteau (FC) method as given by

Ainsworth, et al., (2007). 1 mL of 2 N Folin-Ciocalteau reagent was added to 1 mL of 1mg/mL plant extract followed by the addition of 5mL distilled water. After 5 minutes of incubation, 1 mL of 10% Na_2CO_3 was added and incubated for one hour in the dark at room temperature. The absorbance was measured at 725 nm using UV-visible spectrophotometer. Each assay was performed in triplicates. Total phenolic content was expressed as µg of gallic acid equivalent per mg (GAE/mg) of plants extract.

2.4.2.2. Total Flavonoid Content (TFC)

The TFC were determined by the aluminum chloride method as given by Li, et al., (2007). 4 mL of RO water was added into 1 mL of 1 mg/mL plant extract followed by addition of 0.3 mL of 5% sodium nitrite solution which was allowed to stand for 5 minutes. Then, 0.3 mL of 10% of aluminum chloride was added followed by addition of 2 mL of 1 M sodium hydroxide. Quercetin was used as standard. The absorbance was taken at 510 nm using UV-visible spectrophotometer. Each assay was performed in triplicates. Total flavonoid was expressed as µg of quercetin equivalent per mg (QE/mg) of the plants extract.

2.5. Antioxidant Activity Analysis

2.5.1. DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity was performed as per the method given by Villano, et al., (2006) with few modifications. 2 mL extract solution of three different concentrations (1, 10, and 100µg/mL) were mixed with 2mL of DPPH solution. Then, it was incubated for 30 minutes at room temperature and the absorbance values were measured at 517 nm. Each assay was performed in triplicates.

Radical scavenging activity was calculated by using following equation:

$$\%\ DPPH\ Scavenging\ activity\ = \frac{Abs_{\ sample}\ - Abs_{control}}{Abs_{\ control}} \times\ 100\%$$

Where,
Abscontrol = Absorbance of control
Abssample = Absorbance of sample

2.6. Anti-bacterial Activity Study

Bacterial susceptibility screenings of all extracts were performed against *S. aureus*, *P. aeruginosa* and *K. pneumoniae*.

2.6.1. Well Diffusion Method

Anti-bacterial activity of those plant extracts was determined by the well diffusion method given by Vijayakumar et al. (2013). Wells of 6 mm diameter were prepared by using sterilized borer. Then the agar plates were swabbed with selected bacterial strains under sterile conditions. Finally, the wells were impregnated with 100µL of 10mg/mL, 50mg/mL, and 100mg/mL solution. Gentamycin and Vancomycin were used as the standard antibiotics. All the MHA agar plates with extracts were incubated for 18-24 hours at 37°C. The diameters of zone of inhibition (mm) were measured with the help of ruler after 18-24 hrs. of incubation period.

2.6.1.1. Determination of MIC and MBC

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the plant extracts against the given bacterial strain were determined as per the method given by Mohammed et al. (2017). MIC was

determined by broth dilution method. From the stock solution, different concentrations (10, 30, 50, 80 and 100 mg/mL) were prepared by diluting with MH broth medium and 100 µL bacterial suspension of selected bacteria to make the final volume of 4 mL. Tube containing bacteria serves as positive control and tube containing broth only serves as negative control. Each test tube was covered and incubated for 24 hrs. at 37°C. After incubation, it was difficult to interpret MIC and MBC visually due to the presence of colored compounds of the plant extracts. Therefore, MIC and MBC were determined by sub-culturing the test dilutions on to a fresh MH agar medium and incubated further for 18-24 hrs. The lowest concentration able to inhibit any visible growth was taken as MIC and the lowest concentration at which no growth of bacteria was observed was taken as MBC.

2.7. Data Analysis

Data were collected and presented in suitable tables and bar diagrams. The experimental data were expressed as mean ± SD and were analyzed by linear regression analysis with Microsoft office excel 2007 for both antioxidant and antibacterial activity.

3. Results and Discussion

3.1. Phytochemical Screening

3.1.1. Qualitative Phytochemical Screening

Phytochemical screening of the plant extracts in different solvents showed presence of alkaloids, flavonoids, glycosides, saponins, tannins, carbohydrates, and terpenoids. The details of the phytochemical screening were shown in Table 2.

Table 2. Phytochemical screening of different extracts of the plant samples

Plants		*C. argyracanthum*			*C. cripidiodes*			*F. hispida*			*F. semicordata*			*I. balsamina*			*P. persica*		
Phytochemicals		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Alkaloids	Mayer test	+		+	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+
	Wagner test	-	+	+	-	-	-	+	-	+	-	+	+	-	+	+	-	+	+
Carbohydrates	Molish test	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
	Benedict test	-	+	+	-	-	+	-	-	+	-	+	+	-	-	+	-	-	-
Glycosides	Borntrager's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	Foam test	-	-	-	-	+	+	-	+	-	-	+	-	-	+	+	-	-	-
Phenols	Ferric chloride test	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Flavonoids	Alkaline reagent test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Tannins	Gelatin test	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	+	+	-
Terpenoids	Salkowski's test	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+

Where, 1: Chloroform, 2: Acetone, 3: Methanol, (+): determined, (-): Not determined.

From the results, it is seen that most of the plant extracts were rich in glycosides, phenols, flavonoids, tannins and terpenoids. Alkaloids were present in methanolic extracts of all plant samples as shown by Mayer test. Molish test showed that carbohydrates were present in almost all the plant extracts. According to the results, it is seen that all three extracts of I. balsamina are rich in almost all the phytoconstituents.

3.1.2. Quantitative Phytochemical Screening

3.1.2.1. Total Phenolic Content

Total phenolic content was quantified for all plant extracts by FC method using gallic acid as standard. Results were expressed as µg gallic acid equivalent per mg of extracts. Among the selected plants, methanolic extract of C. cripidiodes (322.16±0.01 µg GAE/mg of extract) showed maximum and chloroform extract of Prunus persica (6.03±0.02 µg GAE/mg of extract) showed the minimum phenolic content. The calibration curve of different concentration of gallic acid is shown in Figure 1 and the phenolic content of each plant samples is given in Table 3.

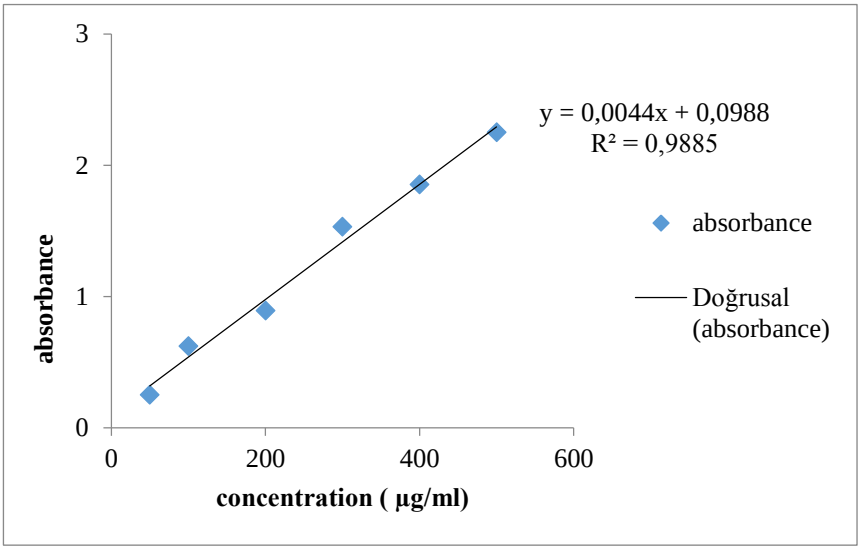

Figure 1. Calibration curve of gallic acid for total phenolic content

Table 3. Total phenolic expressed as µg GAE/mg extract

Plants	Total phenolic content (µg GAE/ mg ± SD)		
	Chloroform	Acetone	Methanol
Cirsium argyracanthum	55.121±0.03	76.78±0.01	124.28±0.03
Crassocephalum crepidiodes	124.212±0.08	131.33±0.06	322.16±0.01
Ficus hispida	82.01±0.03	160.42±0.09	180.57±0.05
Ficus semicordata	119.28±0.08	145.19±0.01	164.28±0.09
Impatiens balsamina	8.83±0.03	135.19±0.02	176.56±0.01
Prunus persica	6.03±0.02	87.47±0.08	96.25±0.02

Data are expressed as mean ± standard deviation(n=3)

3.1.2.2. Total flavonoid content (TFC)

Results were expressed as µg quercetin equivalent per mg of extract. Among the selected plants acetone extract of F. semicordata (500.35±0.05 µg QE/mg of extract) showed maximum and methanolic extract of P. persica (49.76±0.01 µg QE/mg of extract) showed minimum flavonoid content. Calibration curve of different concentration of quercetin (50, 100, 200, 300, 400 and 500 µg/mL) is shown in Figure 2 and total flavonoid content of all plant extracts is shown in Table 4.

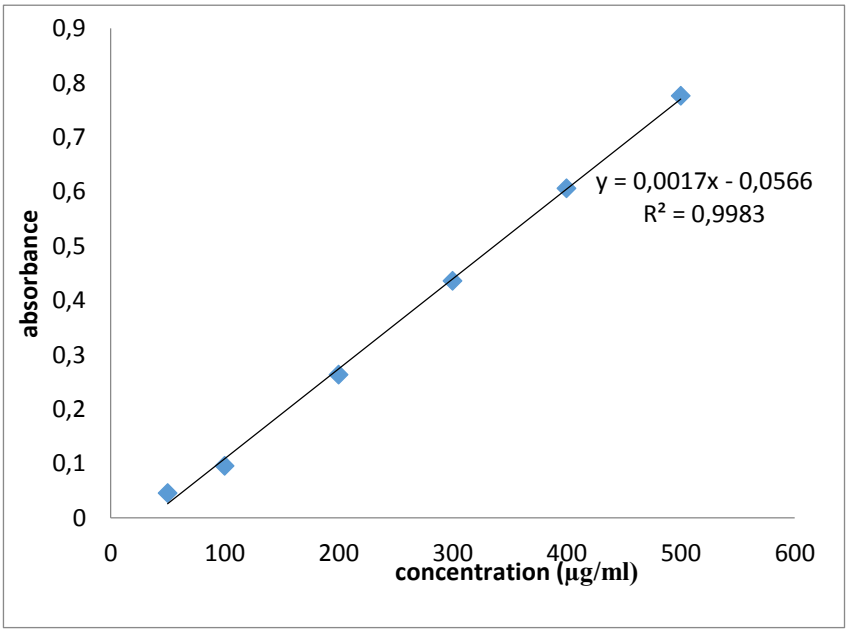

Figure 2: Calibration curve of quercetin for total flavonoid content

Table 4: Total flavonoid content expressed as µg QE/mg extract weight.

Plants	Total flavonoid content (µg QE/ mg ± SD)		
	Chloroform	Acetone	Methanol
Cirsium argyracanthum	202.90±0.04	308.19±0.12	91.72±0.01
Crassocephalum crepidiodes	361.52±0.04	337.21±0.02	348.78±0.02
Ficus hispida	234.47±0.06	315.64±0.09	97.21±0.03
Ficus semicordata	144.27±0.07	500.35±0.04	240.15±0.04
Impatiens balsamina	126.23±0.04	165.84±0.05	99.17±0.04
Prunus persica	215.84±0.03	88.98±0.03	49.76±0.01

Data are expressed as mean ± standard deviation(n=3)

3.2. Antioxidant Activity Analysis

Among the selected plant extracts, the most potent antioxidant activity was revealed by methanolic extract of C. crepidiodes with IC50 of 6.95µg which is close to that of standard ascorbic acid having IC50 4.21µg. The result of antioxidant activity is represented in Table 5.

Table 5: Percentage free radical scavenging activity of ascorbic acid and plants extracts

Plant extracts	Solvents	% DPPH scavenging activity			
		1µg/mL	10µg/mL	100µg/mL	IC_{50} µg
Cirsium argyracanthum	Chloroform	20.50±2.39	30.18±0.69	38.70±1.01	>100
	Acetone	34.56±0.94	52.76±1.05	63.36±1.82	8.63
	Methanol	13.13±0.75	23.96±2.76	38.94±0.88	>100
Crassocephalum crepidiodes	Chloroform	13.13±2.11	24.65±2.32	26.26±0.34	>100
	Acetone	32.71±1.72	33.87±2.60	37.78±0.69	>100
	Methanol	37.32±2.91	56.52±1.65	67.26±1.05	6.95
Ficus hispida	Chloroform	23.73±1.05	31.79±0.79	41.24±2.76	>100
	Acetone	29.49±2.56	34.33±2.01	41.47±2.22	>100
	Methanol	18.66±2.42	35.94±2.95	45.85±2.36	>100
Ficus semicordata	Chloroform	11.98±2.11	14.06±1.05	15.2±2.73	>100
	Acetone	41.24±1.23	50.00±0.59	55.29±2.73	10
	Methanol	18.89±1.23	35.02±0.91	44.092±0.69	>100
Impatiens balsamina	Chloroform	11.98±1.25	14.28±0.98	22.59±1.80	>100
	Acetone	28.34±0.78	32.25±2.07	34.56±2.11	>100
	Methanol	15.20±2.32	20.27±2.61	26.26±0.98	>100
Prunus persica	Chloroform	20.73±2.16	23.04±1.98	30.64±0.94	>100
	Acetone	28.57±1.65	33.87±2.60	41.01±0.56	>100
	Methanol	19.81±2.03	22.12±1.26	25.11±1.35	>100
	Ascorbic acid	25.02±0.07	95.05±0.16	97.00±0.31	4.21

Data are expressed as mean ± standard deviation(n=3)

3.3. Antibacterial Assay

The potency of plants extracts, and standard antibiotic discs were assessed by measuring zone of inhibition (mm) which is given in Table 6.

Among the selected plants, greater zone of inhibition was shown by acetone extract of F. semicordata against P. aeruginosa. The positive controls used were Vancomycin and Amikacin. 25% DMSO was used as negative control for this study. According to Table 6 extracts displayed a variable degree of antibacterial activity on different tested strains. All determinants were carried out in triplicates and expressed as mean±SD. None of the extract showed zone of inhibition in chloroform.

3.4.1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC is the lowest concentration of the compound at which the growth of microorganism is reduced. MBC is the minimum concentration of extract that inhibit all bacteria and no growth is observed by naked eyes. The results of MIC and MBC are represented in Table 7.

Table 6. Zone of inhibition of plants extracts and antibiotic discs (mm)

Plant extract		*S. aureus*			*K. pneumonia*			*P. aeruginosa*		
		10mg/mL	50mg/mL	100mg/mL	10mg/mL	50mg/mL	100mg/mL	10mg/mL	50mg/mL	100mg/mL
C. argyracanthum	Acetone	-	12mm	15mm	-	-	-	10mm	12mm	14mm
	Methanol	-	-	14mm	-	-	-	-	-	-
C. crepidiodes	Acetone		11mm	17mm	-	-	-	-	-	-
	Methanol	-	15mm	21mm	-	14mm	19mm	-	12mm	14mm
F. hispida	Acetone	-	-	-	-	-	-	-	-	-
	Methanol		11mm	14mm	11mm	12mm	15mm	11mm	13mm	16mm
F. semicordata	Acetone	-	10mm	14mm	10mm	13mm	19mm	11mm	16mm	23mm
	Methanol	11mm	14mm	21mm	-	12mm	15mm	12mm	15mm	20mm
I. balsamina	Acetone	-	14mm	19mm	-	-	-	-	-	-
	Methanol	-	13mm	19mm	-	12mm	13mm	-	12mm	14mm
P. persica	Acetone		-	-	-	-	-	-	-	-
	Methanol		-	-	-	-	-	-	-	-
Amikacin (30µg)						21mm			24mm	
Vancomycin (30µg)			20mm			10mm			10mm	

Table 7. MIC and MBC of plant extracts against different gm (+ve) and gm (-ve) bacteria

		S. aureus		*K. pneumoniae*		*P. aeruginosa*	
	Sample	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Acetone	*C. argyracanthum*	30	>100			10	100
	C.crepidiodes	50	>100				
	F. semicordata	50	>100	10	100	10	10
	I. balsamina	30	80				
Methanol	*C.crepidiodes*	30	30	30	100	50	>100
	F. hispida	50	>100	10	>100	10	100
	F. semicordata	10	30	30	>100	10	80
	I.balsamina	30	80	30	>100	50	>100

There is global resurgence of interest towards natural herbal species, medicinal plants as well as towards traditional complementary and alternative source of medicine for treatment of various ailments (Bodeker et al., 2005). Various plants and plant parts are being utilized by various indigenous group of people while dealing with numerous disease (Tolossa et al., 2013). Based on these facts, the selected species of plants used in this study were also being utilized by various ethnic community of Kaski district, Nepal as anti-septic, disinfectant, and antimicrobial and other disease related to Reactive Oxygen Species (ROS) and free radicals.

From the mechanism behind the ability of plant species to cure such diseases, are secondary metabolites found in plants such as alkaloids, flavonoids, glycosides, saponins, tannins, carbohydrates, terpenoids and others (Vaishnav et al., 2011). In a study by Nadka et al. (2017), showed the antioxidant property of natural phenolic compounds in bio-polyesters. The study revealed that the potential of several naturally occurring phenolic compounds such as vanillic acid, vitamin E, and quercetin as stabilizers against the photo-oxidative degradation of poly lactic acid (Dintcheva et al., 2017). Other phenolic groups showing antioxidant characteristics are polyphenols, flavonoids and flavan-3-ols. The characteristics are due to the hydrogen of the phenoxyl group which could be donated to a radical. Three major criteria as defined by Bors to be the best free radical scavenger are addition of the following group in the structure shown in Figure 4: i) the presence of two hydroxy groups in the 3',4' position on the B ring resulting in stability to the radical formed mainly in the 3' position; ii) a double bond in the 2,3-position providing higher conjugation with other double bonds; iii) 3- and 5- hydroxyl groups with a 4-oxo function. These criteria are fulfilled by quercetin. Followingly, flavan-3-ols include monomeric units such as epicatechin and catechin; gallate derivatives of the monomeric flavon-3-ols such as epigallocatechin, epicatechin gallate and epigallocatechin gallate and oligomers of the monomeric flavan-3-ols (Fraga, 2007). These are the compounds which act as best free radical scavengers. In our study, from the quantitative phytochemical screening it was revealed that acetone extracts of *F. semicordata* have highest flavonoid content which is 500.35±0.04 µgGAE/mg which could be related to the potent antioxidant activity of acetone extracts of *F. semicordata* with IC50 value of 10 µg/ml. On the other hand, methanolic extracts of *C. crepidiodes* showed high TPC which is 322.16±0.01 µgGAE/mg and TFC of 348.78±0.02 µgGAE/mg. This result of *C. crepidiodes* of high TPC and TFC could be correlated with the potent free radical scavenging property of methanolic extracts of *C. crepidiodes* with IC50 value of 6.95 µg/mL. On the other hand, methanolic extracts of *C. argyracanthum* showed potent free radical scavenging property with IC_{50} value of 8.63 µg/mL which could also be coincide with the presence of most of the phytoconstituents as well as high presence of TPC.

Similarly, secondary metabolites of the plants are also known for their anti-bacterial and anti-microbial activity. Alkaloids, an organic heterocyclic nitrogen containing compounds are basic forming-water soluble salt. The amino acid derived nitrogen is present in it. Some alkaloids are classified as phenylalkylamines, pyrrolidines, tropane, pyrrolizidines and purine alkaloids (Compean et al., 2014). In a study by Abukakar et al. (2008), *Tamarindus indica* aqueous pulp extract was analyzed antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa* and tested for presence of phytoconstituents. The phytochemical screening revealed the presence of alkaloids as main phytoconstituents (4.32%) while the extract showed anti-bacterial activity against all

tested microorganisms except *Salmonella typhi*. This showed the correlation between alkaloids and anti-bacterial property of plant constituents. Meanwhile, in another study by Mariita et al. (2011), the methanolic extracts of *Scadoxus multiflorus* was tested for their anti-bacterial property against *Mycobacterium fortuitum*, *Staphylococcus aureus and Salmonella typhi* and the result revealed better MIC and MBC. Further study of phytoconstituents in this plant showed the high presence of flavonoids which shows the correlation between flavonoid compounds with their anti-bacterial property. Followingly, cavacrol, thymol and eugenol, which belongs to naturally presenting phenols with a ten-carbon unit are also known for anti-microbial, antioxidant and anti-bacterial property (Rajput et al., 2018).

In our study, acetone extract of *F. semicordata* showed potent MIC of 10mg/mL with *K. pneumoniae*. Both acetone and methanol extracts showed potent MIC of 10mg/mL towards *P. aeruginosa.* The phytochemical screening showed that both acetone and methanol extracts of *F. semicordata* have positive alkaloid test and the TFC and TPC are also significantly high in these plant extracts which could be correlated for their anti-bacterial property. Similarly, acetone extracts of *C. argyracanthum* showed MIC of 10mg/mL against *P. aeruginosa* while methanol extract of *F. hispida* showed MIC of 10mg/mL against both *K. pneumoniae* and *P. aeruginosa.* The phytochemical screening results revealed that methanolic extracts of *F. hispida* is rich in almost all the phytoconstituents as shown by positive result of qualitative phytochemical screening.

Antioxidant is all about the reduction or removal of free radicals and nascent oxygen from an environment (Velioglu et al., 1998). On the other hand, many bacteria could also depend on these nascent oxygens for survival in any given environment (Mujovo, 2010). Thus, by reduction or removal of free radicals also, the plant phytoconstituents could show the anti-microbial activity. In our study as well, the most potent antioxidant activities among selected plants were shown by *F. semicordata*, *C. crepidiodes* and *C. argyracanthum*. These plants also showed potent antibacterial activity with zone of inhibition exceedingly more than 20mm. This clearly defines the correlation between antioxidant and anti-bacterial property of the plant extracts.

4. **Conclusion**

The study revealed that *F. semicordata*, *C. argyracanthum* and *F. hispida* have anti-bacterial property against the selected bacterial strain. This result coincides with the local and traditional use of these plant materials and provides scientific evidence for their anti-microbial property. This study even paved marvelous pathway for further study of these plant materials. Further isolation of the active phytoconstituent from the plant material could be done for the preparation of lead compound which could serve as active antibacterial agents.

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Conflicts of Interest

The author declares no conflict of interest.

Author Contribution Statements

Rishiram BARAL, Amrit KARKI and Saurav KARKI conceived and designed the experiments. Amrit KARKI, Saurav KARKI, Bhuvan NEUPANE, Pratigya KOIRALA,

Seema BARAL and Rishiram BARAL performed the experiments. Sushil PANT supervised the research activity and setup methodology of experiment. Amrit KARKI, Saurav KARKI and Bhuvan NEUPANE analyzed the data. Rishiram Baral wrote the paper.

References

1. Abukakar, M.G., et al. 2008. Phytochemical screening and antibacterial activity of Tamarindus indica pulp extract.Asian Journal of Biochemistry, 3(2), 134-138.
2. Ainsworth, E.A. & Gillespie, K.M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature protocols, 2(4), 875-877. DOI: 10.1038/nprot.2007.102
3. Akiyama, S. & Ohba, H. 2015. Studies of Impatiens (Balsaminaceae) of Nepal 2. Impatiens jurpia, I. urticifolia, and Allied Species. Natl. Mus. Nat. Sci., Ser. B, 41(4), 161-178.
4. Ali, M. & Chaudhary, N. 2011. Ficus hispida Linn.: A review of its pharmacognostic and ethnomedicinal properties. Pharmacognosy Reviews, 5(9), 96-102 DOI: 10.4103/0973-7847.79104
5. Chanda, S., Dudhatra,S., & Kaneria, M. 2010. Antioxidative and antibacterial effects of seeds and fruit rind of nutraceutical plants belonging to the Fabaceae family. Journal of Food and Function, 1, 308-315. DOI: 10.1039/c0fo00028k
6. Chandran, R. & Abrahamse, H. 2020. Identifying plant-based natural medicine against oxidative stress and neurodegenerative disorders.Journal of Oxidative Medicine and Cellular Longetivity, 9 pages. DOI: https://doi.org/10.1155/2020/8648742
7. Compean, K.L. & Ynalvez, R.A. 2014. Antimicrobial activity of plant secondary metabolites: A review. Research Journal of Medicinal Plant, 8(5), 204-213. DOI: 10.3923/rjmp.2014.201.213
8. Dever, L.A. & Dermody, T.S. 1991. Mechanisms of bacterial resistance to antibiotics. Archives of Internal Medicine, 151(5), 886-895. DOI:10.1001/archinte.1991.00400050040010
9. Dintcheva, N.T., Arrigo R., Baiamonte M., Rizzarelli P. & Curcuruto G. 2017. Concentration-dependent anti-/pro-oxidant activity of natural phenolic compounds in bio-polyesters. Journal of Polymer Degradation and stability, 142, 21-28. DOI: https://doi.org/10.1016/j.polymdegradstab.2017.05.022
10. Doron, S. & Gorbach S. L. 2008 Bacterial infections: overview. International Encyclopedia of Public Health, 273-282. DOI: https://dx.doi.org/10.1016%2FB978-012373960-5.00596-7
11. Fraga, C. G. (2007) Plant polyphenols: how to translate their in vitro antioxidant actions to in vivo conditions. IUBMB Life, 59(4-5), 308-315. DOI: 10.1080/15216540701230529
12. Kant, R., Shukla R. K. & Shukla. A. 2018. A review on Peach (Prunus persica): an asset of medicinal phytochemicals. International Journal of Research Applied Science Engineering and Technology, 6(1), 2186-2200.
13. Kaur, V., Kumar, T. & Upadhyaya K. (2016) An overview on the phytomedicinal approaches of Ficus semicordata.World Journal of Pharmacy and Pharmaceutical Sciences, 5, 606-616.
14. Kumaran, A. and Karunakaran, R.J. 2006. Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus.Journal of Food chemistry, 97(1), 109-114. DOI: https://doi.org/10.1016/j.foodchem.2005.03.032
15. Kunwar, R.M. & Bussmann, R.W. 2006. Ficus (Fig) species in Nepal: a review of diversity and indigenous uses. Lyonia, A Journal of Ecology and Application, 11(1), 85-97.
16. Labu, Z.K., Jahan, K. & Afrin, A. 2013. Glimpse of Usage of Antimicrobial Agents and its Unwanted Effects. International Journal, 4(4), 768-774.
17. Li, J.W., Fan, L.P., Ding, S.D.,& Ding, X.L. 2007. Nutritional composition of five cultivars of Chinese jujube. Journal of Food Chemistry, 103, 454-460. DOI: https://doi.org/10.1016/j.foodchem.2006.08.016
18. Mariita, R.M., Ogol, C.K.P.O., Oguge N.O. & Okemo, P.O. 2011. Methanol extract of three medicinal plants from Samburu in Northern Kenya show significant antimycobacterial, antibacterial and antifungal properties. Research Journal of Medicinal Plant, 5(1), 54-64. DOI : 10.3923/rjmp.2011.54.64
19. Meenu, B et al. (2015) Impatiens balsamina: An overview. Journal of Chemical Pharmaceutical Research, 7, 16-21.
20. Nazaruk, J., (2007) Antioxidant activity and total phenolic content in Cirsium five species from north–east region of Poland. Fitoterapia, 79, 194-196. DOI: http://dx.doi.org/10.1016/j.fitote.2007.11.008
21. Nickavar, B., Kamalinejad, M. & Izadpanah, H. (2007) In vitro free radical scavenging activity of five Salvia species. Pakistan Journal of Pharmaceutical Science, 20(4), 291-294.
22. Omotayo M.A., Avungbeto O., Sokefun, O. & Eleyowo, O. 2015. Antibacterial activity of

Crassocephalum crepidioides (fireweed) and Chromolaena odorata (siam weed) hot aqueous leaf extract. International Journal of Pharmaceutical and Biological Science, 5, 114-122.
23. Pasquale, T. R. & Tan, J. S. 2005. Nonantimicrobial effects of antibacterial agents. Journal of Clinical Infectious Diseases, 40, 127-135.
24. Rajput, J.D. et al., 2018. Perspectives on medicinal properties of natural phenolic monoterpenoids and their hybrids. Journal of Molecular diversity, 22, 225-245. DOI: https://doi.org/10.1007/s11030-017-9787-y
25. Safari, M. & Ahmady-Asbchin S. 2019. Evaluation of antioxidant and antibacterial activities of methanolic extract of medlar (Mespilus germanica L.) leaves. Journal of Biotechnology and Biotechnological Equipment, 33(1), 372-378. DOI: 10.1080/13102818.2019.1577701
26. Halayel, M.H.F. et al., 2017. Anti-bacterial activity of peppermint (Mentha piperita) extracts against some emerging multi-drug resistant human bacterial pathogens. Journal of Herbal Medicine, 7, 27-30. DOI: https://doi.org/10.1016/j.hermed.2016.08.003
27. Taylor, P.W. 2013. Alternative natural sources for a new generation of antibacterial agents. International Journal of Antimicrobial Agents, 42(3), 195-201. DOI: https://doi.org/10.1016/j.ijantimicag.2013.05.004
28. Tepal, P. 2016. Phytochemical screening, total flavonoid and phenolic content assays of various solvent extracts of tepal of Musa paradisiaca. Malaysian Journal of Analytical Sciences, 20, 1181-1190.
29. Tolossa, K. et al. 2013. Ethno-medicinal study of plants used for treatment of human and livestock ailments by traditional healers in South Omo, Southern Ethiopia. Journal of Ethnobiology and Ethnomedicine, 9, 1-15. DOI: http://www.ethnobiomed.com/content/9/1/32
30. Vaishnav, P. & Demain A.L. 2011. Unexpected applications of secondary metabolites. Journal of Biotechnology Advances, 29(2), 223-229. DOI: https://doi.org/10.1016/j.biotechadv.2010.11.006
31. Velioglu, Y.S., Mazza, G., Gao, L. & Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry, 46, 4113-4117.
32. Ventola, C.L. 2015. The antibiotic resistance crisis: part 1: causes and threats. Journal of Pharmacy and Therapeutics, 40(4), 277-283
33. Vijayakumar, M., Priya, K., Nancy, F.T., Noorlidah, A., & Ahmed, A.B.A. 2013. Biosynthesis, characterisation and anti-bacterial effect of plant-mediated silver nanoparticles using Artemisia nilagirica. Journal of Industrial Crops and Products, 41, 235-240. DOI: https://doi.org/10.1016/j.indcrop.2012.04.017
34. Villano, D. et al. 2007. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Journal of Talanta, 71, 230-235. DOI: https://doi.org/10.1016/j.talanta.2006.03.050
35. Khan, Z.U.H., Ali, F., Khan, S.U. & Ali, I. 2011. Phytochemical study on the constituents from Cirsium arvense. Mediterranean Journal of Chemistry, 2, 64-69.

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Effect of the Physalis peruviana and Linum usitatissimum Extracts Against Toluene-Induced Oxidative Damages in Kidney and Liver Tissues of Rats

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Abstract

Toluene causes a great environmental problem, because it is widely used in the industry. This study was intended to examine the effect of golden berry (Physalis peruviana, PP) and flaxseed (*Linum usitatissimum*, LU) extracts on the malondialdehyde (MDA), reduced glutathione (GSH) fatty acid and lipophilic vitamin content in the kidney and liver tissues of Sprague Dawley male rats, which were oxidative stressed by toluene. PP and LU were extracted and then subjected to vitamin and flavonoid analyses. The rats were divided into four groups as control (C), toluene (T), toluene + *P. peruviana* (T + PP), and toluene + *L. usitatissimum* (T + LU). T was injected intraperitoneally at a concentration of 0.3 mL/kg. In addition, PP and LU were also injected intraperitoneally at a concentration of 0.5 mL/kg. The treatments were maintained for two months. Afterwards, the rats were decapitated and tissues were removed. Tissue samples were homogenized using buffer solutions. MDA, GSH, lipophilic vitamin, cholesterol and fatty acid content was also determined. In conclusion, MDA concentration level significantly increased and GSH concentration level significantly decreased in the toluene group ($p<0.001$; $p<0.001$). GSH level increased and the MDA concentration decreased in T+PP and T+LU treated groups ($p<0.01$; $p<0.01$). Palmitic (C16:0), stearic (C18:0), oleic (C18:n-9), docosahexaenoic (C22:6n-3) and arachidonic (C20:4n-6) acid concentrations were mainly higher in the kidney and liver tissues which were collected from the T+PP and T+LU treated groups than those collected from the control group. The K1, delta-tocopherol and alfa–tocopherol acetate vitamin concentrations were higher in T+PP and T+LU treated groups than those at group treated with the toxin. Determined concentrations were similar to those measured in the control group. T+PP and T+LU decreased the cholesterol concentration in the kidney and liver tissues ($p<0.05$ and 0.01, respectively). According to results; while antioxidant defense systems of toluene groups have decreased, T+PP and T+LU possessed a protective feature against the oxidative stress caused by toluene.

Key Words: *Physalis peruviana*, *Linum usitatissimum*, toluene, malondialdehyde, antioxidant, rat

1. Introduction

The interest in including herbal products in diet for their health benefits have increased considerably in the recent years (Jamshidi-Kia et al., 2018; Trovato and Ballabio, 2018). Antioxidants of plants origin have high free radical removal and lipid peroxidation

inhibitor capacities. Most botanical products perform antioxidant activity (Kris-Etherton et al., 2004). Aromatic herbs antioxidants can cure oxidative-induced kidney damage by decreasing lipid peroxidation and increasing the scavenging ability of the antioxidant defense system (Hendawi et al., 2016).

Flaxseed (*Linum usitatissimum*, LU), a member of the Linaceae family is an important medicinal plant in terms of alpha tocopherol and omega-3 fatty acids. Studies have revealed that flaxseed has anticancer, antiviral, antibacterial, anti-inflammatory and antioxidant properties. LU has also been stated to have a positive effect on heart health due to the intense omega-3 fatty acid and alpha tocopherol in its composition (Parich et al., 2019; Kaur et al., 2017; Gholaminejhad et al., 2017). *Physalis peruviana* (PP) is a plant belonging to *Physalis* genus of the Solanaceae family. PP is used in folk medicine as an antispasmodic, diuretic, antiseptic, sedative and analgesic. PP has been detected exhibit antimicrobial, anti-inflammatory, immunomodulatory and anti-hypercholesterolemic properties (Khalaf-Allah et al.,2016).

The toxicity of many xenobiotics for example, toluene is related to its free radical production. Free radicals are composed of various chemical structures comprising various Reactive Oxygen Species (ROS) containing hydroxyls, superoxides, nitric oxide and lipid peroxides (Arvindkumar, 2011; Arun and Asha, 2007). ROS has a significant impact on the pathogenicity course for many illnesses such as neurodegenerative diseases, cancer, cardiovascular diseases, cataract and rheumatism. Histological analysis of ROS caused by toxic materials points at their effect on the kidney and liver tissue. Liver and kidney are significant organs in all living Mammalia groups the removal of toxins (Aylward et al., 2008; Arvindkumar, 2011). Toluene is an aromatic solvent used in many industrial and commercial practices (Abdel-Salam et al., 2020). It is used as a solvent in adhesive, fabric, dye, ink, plastic industries and cleaning products (Bayil et al., 2008). Human beings contact with toluene both as a result of occupational exposure during its commercial production, and its use as a result of bad habits.

In the study, the aims were to determine the effect of PP and LU extracts used to avoid formation of lipid peroxidation (LPO, MDA) resulting in significant damage on cell membrane structure due to oxidative stress caused by toluene, on the MDA and fatty acid composition, lipophilic vitamin content, total cholesterol and several other sterol levels in the kidney and liver tissue of rats. The effect of these extract on the protein and glutathione levels was also investigated in this study.

2. Material and Methods

2.1. Herbal Materials

Fruits of PP(*Physalis peruviana*) and seeds of LU (*Linum usitatissimum*) were used as herbal materials, and they were obtained from herbal shop in Elazig province of Turkey. PP purchased from herbal shop was grown at greenhouses in Antalya and LU was grown in Diyarbakir, Turkey. The fruits of PP and the seeds of LU were extracted with 85% methanol. Evaporation process for the methanol phase of the extract was performed via the rotatory evaporator equipment under vacuum, and the

temperature was increased to 55°C. Afterwards, the dried extracts were dissolved in dimethylsulfoxide (DMSO) and then injected to rats in the groups every other day via intraperitoneal route, and this administration was done throughout 60 days.

2.2. Flavonoid Analysis of the Extracts via HPLC Device

The methanolic extracts of the flavonoids were analysed by PREVAIL C18 (15x4.6 mm, 5 µm) HPLC column tool. Methanol/water/acetonitrile mix (46/46/8, v/v/v) including 1% acetic acid was used as the mobile phase (Zu et al., 2006). The obtained results were presented as µg/g.

2.3. Extraction and Analysis of ADEK Vitamins with Phytosterols

Plant samples were homogenized by mixing n-hexane/isopropyl at 3/2 (v/v) ratio (Hara and Radin, 1978). And then, the hydrolysis was performed with 5% KOH at 85°C and the extraction of phytosterols was obtained by adding hexane. The quantity of ADEK vitamins with phytosterols were analyzed at 202 nm and 326 nm by a UV detector on HPLC equipment (Katsanidis and Addis, 1999).

2.4. Experimental Animals

Spraque Dawley male rats used in experimental research were obtained from the Experimental Research Center at School of Medicine, Fırat University (22.05.2012/66). The experimental processes were performed at the same institute. Four groups were formed with the rats. Substance concentrations intraperitoneally for these groups were as noted below:

1.C (n=7): 0.3 mL/kg at olive oil
2.T (n=7): a mix of 0.3 mL/kg of 20% T with olive oil
3.T+PP (n=7): 0.3 mL/kg of T+0.5 mL/kg of extract PP
4.T+LU (n=7): 0.3 mL/kg of T + 0.5 mL/kg of extract LU

As far as ethical committee approved, the specimens anaesthetized to sacrifice by withdrawing blood from the animal body, and after animals were decapitated. Shortly after this process, kidney and liver tissue samples were divided. These samples were shortly cleaned with 0.9% serum physiologic, and they were stored at -80°C in order to use biochemical analyses.

2.5. ADEK and Cholesterol Levels Determination via HPLC Device

Firstly, 5 mL of supernatant and 5% KOH solution were put in 25-mL test tubes with caps. After that the tubes were vortexed, they were incubated at 85°C for 15 minutes. Later, the tubes were taken from incubator and, 5 mL of distilled water was added and mixed. Evaporation of the hexane phase was performed by nitrogen stream in order to obtain dried extract. Then, this extract was dissolved in 1 mL of acetonitrile/ methanol (50% + 50%, v/v) mix. They were placed into the vials in order to analyze ADEK vitamins (Katsanidis and Addis, 1999). The analyses were done on Shimadzu fully equipment HPLC equipment. Computations were performed by Class VP 6.27 program (Shimadzu, Kyoto Japan).

2.6. Total Protein Levels and GSH Determination

In order to investigate the glutathione and total protein levels in the tissues, 28 different samples collected from the specimens were homogenized in 0.1m TrisHCl and 20 mM EDTA (pH:7) buffer, later divided into tissue pellet by centrifugation at 9000 rpm for 5 min at

+4°C. After this process, the supernatant was separated from two aliquots, one of which was used for GSH test. Therefore, the proteins at supernatant were precipitated by using 1 ml of 5% metaphosphoric acid reactive. This mix was centrifuged at 4500 rpm to 4 min, thus the pellet was settled and the supernatant was removed into another tube. The supernatant was suplementated 1 mL 150 µl DTNB with 2 mL of 0.3 M Na2HPO4 solution, the color altered to yellow was read at 412 nm against blank (Teare et al., 1993). The total protein analyzes were carried out with the supernatant obtained from the glutathione analyzes. Then, 50µl the supernatant was separated with subjected to Lowry (1951) method.

2.7. HPLC Analyzes of the Quantity of LPO

One ml sample was obtained from animal group; 0.6% 2-thiobarbituric acid (TBA) chemical materials and 2 ml distilled water was put into the mixture. Later, the mixed samples were kept at 90°C for 60 minutes. Then, 3 ml butanol was included into the samples and consequently the pink color formed after the chemical reaction. The samples were centrifuged and the intensity of the supernatant section was kept into the vials.

LPO measurements were performed via a fluorescence detector on the HPLC tool, which is Inertsil ODS-3 C18 HPLC column (150x4.6 µm). The mobile phase was formed using 75% ACN / 30 mM KH2PO4 (pH=5) mixture. 5 minutes was determined as analyzes time. As a standard of LPO, 1,1,3,3-tetraethoxypropane (TEP) was utilized (Ohkawa et al., 1979). The analysis results were given with nmol/µl.

2.8. Isolation of Fatty Acids by Gas Chromatography

Fatty acids were isolated by adding 10 ml 3/2 (v/v) hexane / isopropanol mixture (Hara and Radin, 1978) on liquid phase of the samples remaining after LPO. Then, hexane phase was placed into different test tubes and 5 ml 2% methanolic sulfuric acid was added into the tube. Then, the mixture was kept in the incubator at 55°C to 12 hours. Hence, 5 ml of 5% sodium chloride was added into tube and then the fatty acid methyl esters were extracted to 5 ml of hexane. Furthermore, 5 ml of 2% KHCO3 solution was placed into the mixture, and the hexane phase was evaporated by nitrogen stream (Christie, 1990). Finally, fatty acid methyl ester residues were dissolved in 1 ml heptane and placed into vials. Fatty acid methyl ester was determined by Shimadzu GC 17 device equipment (Kyoto, Japan).

2.9. Data Analyses

SPSS 15.0 Software was utilized to analyze results. The control and experimental groups was compared with the analyzes of ANOVA method with LSD tests. The obtained data were reported as mean ± SEM. The difference between the groups, $p > 0.05$, $p<0.05$, $p<0.01$, and $p<0.001$ values were used to determine meaning of the data.

3. Results and Discussion

The reduced GSH, total protein with MDA concentrations determined at kidney tissue were displayed in Table 1. The GSH concentration in the kidney tissue in group T was greatly less than that of the control group ($p<0.001$). The GSH concentration was statistically higher in groups T+LU and T+PP than that of the control group ($p<0.01$). Total protein content of the kidney tissue in groups T, T+PP and T+LU were all

partially statistically higher than that in the control group (p<0.05). After all, there was no noticeable change among toluene treated groups (p>0.05).

Table 1. GSH, Total protein, MDA concentrations at kidney tissue of the rats*

Groups	GSH (µmol/g)	Total Protein (mg/g)	MDA (nmol/g)
C	2.19 ± 0.11	146.13±2.54	29.00±1.18
T	1.13±0.5^{d}	126.26±1.76^{b}	45.40±0.81^{d}
T+LU	2.73±0.13 ^{c}	123.52±2.88^{b}	37.31±1.48^{c}
T+PP	2.50±0.21 ^{c}	122.73±2.83^{b}	39.62±1.00^{c}

*Each value is the mean ± S.E. (standard error) of 7 repetitions. Superscripts after values in a same line with different letters represent significant difference. a: p>0.05, b: p<0.05, c: p<0.01, d: p<0.001.
a: Values of p>0.05 is not statistically significant.
b: Values of p<0.05 is statistically significant.
c: Values of p<0.01 is statistically more significant
d: Values of p<0.001 is statistically most significant
C: Control, T: Toluene, LU: *Linum usitatissimum*, PP: *Pysalis peruviana*, T+LU: Toluene+ *Linum usitatissimum*, , T+PP: Toluene+ *Physalis peruviana* GSH: Reduced glutathione, MDA: Malondialdehyde.

The reduced GSH, total protein with MDA concentrations determined at liver tissue were presented in Table 2. The GSH concentration at liver tissue in group T was much lower than control group (p<0,001). Besides, GSH concentration in the T+PP and T+LU groups was lower than the control group (p<0.01). The amount of MDA concentration was present in the T groups while it was not found at control group (p<0.001). This concentration was found at T+LU group, and it was also significantly high at T+PP group (p<0.01; p<0.01.

Table 2. GSH, Total protein, MDA concentrations at liver tissue of the rats*

Groups	GSH (µmol/g)	Total Protein (mg/g)	MDA (nmol/g)
C	8.05±4.08	180.71±13.54	42.39±2.72
T	1.91±0.04^{d}	171.40±8.72^{b}	50.94±2.25^{c}
T+LU	4.17±0.21^{c}	197.24±17.98^{b}	45.21±4.61^{b}
T+PP	4.27±0.21^{c}	194.81±12.80^{b}	43.68±2.02

*The meaning of the symbols is given under Table 1.

The analyses that were performed using the HPLC device showed the methanol extract of PP and LU (Tables 3,4). When analyzed in terms of LU lipophilic vitamins and phytosterols α-tokoferol, stigmasterol, ergosterol high amounts were determined as 70.92, 34.7 and 21.85, respectively. In the analysis lipophilic vitamins and phytosterols of PP α-tokoferol, stigmasterol, ergosterol high amounts were determined as 85.29, 38,10 and 21,54 respectively.

Table 3. Flavonoid contents of herbal materials in methanolic extracts (µg/0.6g). *

Flavonoids	PP	LU
Cathechin	2.33	3.26
Rutin	1.27	2.86
Resveratrol	0.02	0.01
Myricetin	0.26	0.20
Morin	0.05	0.02
Naringenin	0.02	0.01
Quercetin	0.01	0.01

*LU: *Linum usitatissimum*, PP: *Pysalis peruviana*

High amounts of catechin, rutin and myricetin were detected in PP and LU in terms of flavonoid content.

Table 4. Lipofilik vitamin and phytosterol of herbal materials in methanolic extracts (µg/0.6g)*

Lipophilic Vitamin and Phytosterol	PP	LU
K_1	3.63	4.06
K_2	2.92	3.54
α-Tokoferol	70.92	85.29
R-Tokoferol	18.33	14.96
D_3	1.05	1.14
Retinol	0.06	0.02
Ergosterol	21.85	21.54
Stigmasterol	34.77	38.10
β-sitosterol	20.88	22.34

*LU: *Linum usitatissimum*, PP: *Pysalis peruviana*

D2 concentration was very significantly lower in group T by comparison with the control group ($p<0.001$). δ tocopherol concentration was partially higher in groups T+PP and T+LU in comparison to that of control group ($p<0.05$) with the α tocopherol acetate concentration was importantly higher with at groups T+PP and T+LU by comparison with the control group ($p<0.01$). The retinol concentration was importantly higher at group T+PP in comparison with the control group ($p<0.01$). Retinol acetate concentration at T group was very importantly lower than that of the control group ($p<0.001$), with the value of at group T+PP was not importantly vary from the control group ($p>0.05$). The cholesterol level in group T was partially higher than that of the control group ($p<0.05$) (Table 5).

A comparison of the ADEK vitamin and cholesterol content in the liver tissue of the T, T+LU and T+PP groups with the control group designated that the vitamin K1 concentration at T and T+PP groups ($p<0.01$).The vitamin K2 was not significantly the T+PP groups whereas K2 was significantly lower the T groups relatively to that of the control group ($p<0.01$).

Table 5. Lipophilic vitamins and cholesterol concentrations in the kidney tissue of rats*

Parameters	C	T	T+PP	T+LU
Vitamin K_1 µg/g tissue	1.72±0.13	1.58±0.07	2.20±0.12^b	2.07±0.12^b
Vitamin K_2 µg/g tissue	4.23±0.20	3.79±0.18	5.75±0.26^b	4.56±0.14
Vitamin D_2 µg/g tissue	1.50±0.11	0.33±0.13^d	0.61±0.171	0.84±0.44
Vitamin D_3 µg/g tissue	1.30±0.16	1.48±0.85	1.89±0.10^b	1.54±0.76
δ–Tocopherol µg/g tissue	1.44±0.10	1.06±0.08	1.85±0.11^b	1.71±0.16^b
α–Tocopherol µg/g issue	16.22±0.52	13.39±0.30	13.09±0.23	15.21±0.50
α–Tocopherol acetate µg/g tissue	7.49±0.21	5.77±0.20	9.06±0.20^c	7.76±0.13^b
Retinol µg/g tissue	3.77±0.16	2.12±0.17	4.58±0.23^c	3.64±0.19
Cholesterol µmol/g tissue	3.78±0.15	4.42±0.15^b	3.18±0.15	3.47±0.20

*The meaning of the symbols is given under Table 1.

D_2 concentration was importantly lower the T group in comparison to that of the control group ($p > 0.05$). Vitamin D_3 concentration at T+PP groups was not importantly varied from the control group ($p>0.05$). δ tocopherol concentration was significantly higher in groups T with T+PP while it was importantly lower as regards the control group ($p<0.01$) and ($p<0.001$). Alpha tocopherol concentration was partially remained group by with to the control group whereas the values of T+LU with T groups were significantly lower ($p>0.05$) ($p<0.01$) and ($p<0.001$). Retinol concentration in T with T+LU groups was not important difference determined ($p<0.05$) and ($p>0.05$), (Table 6).

The C16:0 (palmitic acid) concentration was importantly lower at the T group in comparison to the control group ($p<0.01$) and was partially higher at T+PP group ($p<0.05$). The C16:1 (palmiteolic acid) concentration was importantly lower at T group in comparison to control group ($p<0.01$). The C18:0 (stearic acid) concentration was partially higher at T+PP group in comparison to the control group ($p<0.05$). The C16:0 (palmitic acid) concentration was importantly lower at the T group in comparison to the control group ($p<0.01$) and was partially higher at T+PP group ($p<0.05$). The C16:1 (palmiteolic acid) concentration was importantly lower at T group in comparison to control group ($p<0.01$).

Table 6. Lipophilic vitamins and cholesterol concentrations in the liver tissue of the rats*

Parameters	C	T	T+PP	T+LU
Vitamin K_1 µg/g tissue	6.79±0.66	2.41±1.05^c	5.47±0.23^b	9.06±1.46^c
Vitamin K_2 µg/g tissue	4.39±0.38	2.31±0.26^c	3.86±0.22	4.36±0.25^a
Vitamin D_2 µg/g tissue	3.61±0.18	1.59±1.87^c	2.56±0.11	2.45±0.62
Vitamin D_3 µg/g tissue	2.44±0.59	1.73±0.59	3.33±0.16	2.93±0.74^a
δ –Tocopherol µg/g tissue	2.23±0.55	0.94±0.21^d	2.55±0.26	4.21±0.50^c
α –Tocopherol µg/g tissue	9.63±1.42	3.21±1.00^d	7.21±0.56^c	10.19±1.07^b
α –Tocopherol acetate µg/g tissue	8.46±0.21	5.77±0.20	9.72±0.20^c	7.76±0.13^b
Retinol µg/g tissue	342.05±22.6	310.01±11.7^b	329.55±19.2^b	342.41±11.6^a
Cholesterol µmol/g tissue	2.90±0.41	3.62±0.18^b	2.53±0.16	2.61±0.58

*The meaning of the symbols is given under Table 1.

Table 7. Fatty acid concentrations in the kidney tissue of the rats (mg/g)*

Fatty acids	C	T	T+PP	T+LU
C16:0	2.51±0.04	**1.65±0.01^c**	2.08±0.04	2.81±0.03^b
C16:1	0.39±0.01	**0.19±0.01^c**	0.25±0.01	0.30±0.01
C18:0	1.93±0.04	1.69±0.04	1.71±0.06	2.34±0.06^b
C18:1n-9	1.01±0.08	0.93±0.02^a	0.91±0.03^a	1.33±0.02^b
C18:1n-7	0.40±0.01	0.27±0.01	0.33±0.01	0.48±0.01^b
C18:2n-6t	0.07±0.01	0.03±0.01	0.05±0.01	0.08±0.01^a
C18:2n-6c	2.02±0.07	**1.29±0.05^c**	1.64±0.04^b	2.20±0.06^b
C20:3n-6	0.10±0.01	0.08±0.01	0.10±0.01^a	0.12±0.01
C20:4n-6	3.77±0.11	3.15±0.07	3.47±0.06	4.33±0.04^b
C22:6n-3	0.16±0.01	0.14±0.01	0.16±0.01^a	0.20±0.01^b
Others	0.78±0.03	0.52±0.03	0.53±0.01	0.75±0.01^a
ΣSFA	4.44±0.03	**3.34±0.05^c**	3.79±0.10	**5.15±0.09^c**
ΣMUFA	1.80±0.10	**1.39±0.04^c**	1.49±0.05	**2.11±0.04^b**
ΣPUFA	6.12±0.21	**4.69±0.14^c**	5.42±0.12	**6.93±0.13^c**
ΣFatty Acid	13.14±0.37	**9.94±0.27^c**	11.23±0.29	**14.94±0.2^b**

*The meaning of the symbols is given under Table 1.
Σ: Total.
ΣSFA: Total Saturated Fatty Acid.
ΣMUFA: Total Monounsaturated Fatty Acid.
ΣPUFA: Total Polyunsaturated Fatty Acid.

The C18:0 (stearic acid) concentration was partially higher at T+PP group in comparison to the control group ($p<0.05$). C18:1n9 (oleic acid) concentration of the T and T+LU groups was similar to that of the control group ($p>0.05$), and the

concentration at T+PP group was partially higher ($p<0.05$). The C18:1n7 (Vaccenic acid) concentration was partially higher at T+PP group in comparison to the control group ($p<0.05$) whereas the C18:2n6t (linoleic acid) concentration was statistically like to that of the control group (Table 7). C20:3n6 (dihomo - gamma linoleic acid) concentration of the T+LU group was similar to that of the control group ($p>0.05$) whereas C20:4n6 (arachidonic acid) concentration was significantly increased in the T+PP group in comparing to that in the control group ($p<0.05$). C22:6n3 (docosohexoeonic acid) concentration partially increased in the T+PP group in comparing to the control group ($p<0.05$) whereas the gradient at concentrations between that at T+LU group with the control group was determined as insignificant ($p>0.05$) (Table 7). A comparison of the T, T+LU and T+PP groups with the control group indicated that the ΣSFA (saturated fatty acid) and ΣPUFA (total unsaturated fatty acid) concentration was significantly lower within group T with higher in T+PP ($p<0.01$). The ΣMUFA (total mono unsaturated fatty acid) and total fatty acid concentration was significantly lower in group T ($p<0.01$) and partially higher in T+PP ($p<0.05$) (Table 7).

A comparison of the fatty acid content in liver tissue C16:0, C18:0, C18:2n6c and other fatty acid were significantly lower T group in comparison to the mean value of the control group ($p<0.001$). The C18:1n9 concentration was significantly lower in T with T+LU groups ($p<0.001$). C18:1n7 concentration in the T+PP groups was not statistically similar to that of the control group ($p > 0.05$). In addition, C20:4n6 concentration was determined to be significantly different in the T+LU and T+PP groups from the values of the control group ($p>0.05$), (Table 8).

Table 8. Fatty acid concentrations in the liver tissue of the rats (mg/g)*

Fatty acids	C	T	T+LU	T+PP
C16:0	1.69±0.17	**1.00±0.15^{c}**	1.21±0.02	1.11±0.14
C16:1	0.21±0.01	0.11±0.01	0.12±0.01	0.13±0.01
C18:0	2.56±1.53	**1.05±0.19^{c}**	1.21±0.02	1.28±0.17
C18:1n-9	0.69±0.04	**0.19±0.08^{c}**	**0.16±0.01^{c}**	0.25±0.03
C18:1n-7	0.21±0.03	0.10±0.04	0.12±0.01	0.21±0.03^{a}
C18:2n-6t	1.44±0.20	**0.90±0.20^{c}**	1.19±0.06	1.01±0.09
C18:2n-6c	0.09±0.01	0.07±0.01	0.08±0.01^{a}	0.08±0.01^{a}
C20:3n-6	0.18±0.07	**0.02±0.01^{d}**	**0.03±0.01^{d}**	0.09±0.01
C20:4n-6	2.45±0.47	2.14±0.36	2.42±0.09^{a}	2.36±0.01^{a}
C22:6n-3	0.90±0.10	0.49±0.14	0.69±0.04	0.50±0.08
Others	0.63±0.30	**0.23±0.05^{c}**	0.40±0.02	0.32±0.05
ΣSFA	4.25±1.70	**2.05±0.34^{c}**	2.42±0.04	2.39±0.31
ΣMUFA	1.11±0.08	**0.40±0.13^{c}**	**0.40±0.03^{c}**	0.59±0.07
ΣPUFA	4.97±0.84	**3.55±0.71^{c}**	4.33±0.20	3.96±0.19
Σ Fatty Acid	10.33±2.92	**6.23±1.23^{c}**	7.55±0.29	7.26±0.62

*The meaning of the symbols is given under Table 1.
Σ: Total.
ΣSFA: Total Saturated Fatty Acid.
ΣMUFA: Total Monounsaturated Fatty Acid.
ΣPUFA: Total Polyunsaturated Fatty Acid.

Discussion: The biological observation of exposure to toxic chemicals in work environment is important for evaluating the risks that threaten human health and the provision of occupational safety (Gerin et al., 1998; EPA, 1959). Toluene and its metabolites cause damage in many different types of tissue through oxidative stress (Gotohda et al., 2009). During the toxicity research of toluene, many rats died due to nephrosis which was common among them. Also, there was damage to the tubular epithelia of the kidney because of a terminal disease in rats. The researchers examined the rats' kidneys by taking parts to determine any sign of hyaline droplets to proximal tubules. The findings were negative. In addition, there were histopathologic lesions in liver which consisted of hepatocellular hypertrophy (Huff , 2003; Hendawi et al., 2016). The genotoxic outcome of such DNA oxidation acts a part of the pathogenesis of several illnesses Costa et al., (2005) as well as the emergence of cancer and neurodegenerative disorders (Kayhan et al., 2009).

Our results indicated that the MDA concentration in the kidney tissue of the rats that were treated with toluene was significantly high, but MDA concentration was the highest in the liver tissue (Table 1), since toluene that is absorbed by the blood is diffused through the living Mammalia groups. The increase in MDA concentration is generally accepted to be a predictive factor of the lipid peroxidation initiating as a result of the oxidative stress, which was, in this case, caused by toluene. The toxic effects of toluene as indicated by high MDA levels are associated with free radicals, and ROS encountered in house painters, who were exposed to toluene, causes damage in the biological membranes (Kris-Etherton et al., 2004). A study conducted by Moro compared the blood samples of male workers from several industrial sectors, who were exposed to low concentrations of toluene, with that of males who were not associated with any type of occupation. The blood MDA level of the industrial workers was reported to be twice more than the level found in non-workers [Moro et al., 2010].House painters, who are exposed to low concentrations of toluene constantly, were determined to be affected more from oxidative damage (Moro et al., 2012). This study indicated that the increase in the MDA concentration of the group treated with *P. peruviana* extract together with toluene was relatively lower than that of the group treated with the chemical alone. A comparison of the results of the experiments conducted with the control group and the toluene-treated group indicated that the MDA concentration was considerably higher in the group T whereas this level was limited in groups supplemented with the extract (Table 1). Moreover, ROS production increase leads oxidative stress induced kidney damage. Oxidative stress induced kidney damage is significantly reduced by antioxidants (Nasri, 2013).

Several plant species and their oil extracts and phytochemicals have widely been used as antioxidants in the therapeutics and prevention of several diseases in traditional medicine (Scalzo et al., 2005). Therefore, the researchers of this study also investigated the effect of *P. peruviana* and flaxseed extracts on several parameters used as a measure against toluene toxicity. The aqueous extract of *P. peruviana* was reported to comprise of phenol and saponin flavonoids as the major constituents by a

phytochemical study. These compounds possessed antioxidant activity (Ramadan and Moersel, 2003). Another study also reported *P. peruviana* to possess antioxidant activity (Wu et al., 2005).

Arvindkumar et al. (2011) performed nephrectomy on male Wistar rats with the aim of determining the protective role of LU (flaxseed) ethanol extracts on rat kidneys. The experimental results indicated that the extract was effective on the protection of the kidneys elevating GSH and MDA concentrations, thereby reducing necrosis (Abdel-Moneim et al., 2011a). Abdel-Moneim et al. (2011b) treated male rates with lead acetate, a toxic metal ion (20mg/kg), and examined the effect of flaxseed oil (1000 mg/kg) at kidney cytotoxicity. The results of the study indicated that the rats that were given flaxseed oil along with lead acetate had similar GSH concentration and antioxidant enzyme levels as those of the control group. Furthermore, a considerable improvement was observed in the histopathology of the kidneys; i.e. its function and other factors including the antioxidant levels. This affirmative effect of flaxseed was reported to be associated with its lignan content (Arvindkumar et al., 2011).

The results of the experiments comparing the control group to that of the toluene treated group indicated that the MDA concentration was significantly elevated in group T whereas this level was limited in groups supplemented with the extract. The decrease in GSH concentration was noticeable in group T. The GSH concentration to the control group with to the extract-supplemented groups was statistically similar. The present findings are consistent with previously reported studies (Table 1). Many of those botanical-based studies supporting as a results of this study reported protective features of phytochemicals against oxidative stress occurring in the liver and kidney tissue, which was at the tissue under investigation in the present analysis as well. Moreover, another study presents the impact of ROS on the antioxidant defense mechanisms by reducing glutathione concentration in the intracellular (Tapiero et al., 2002).

Another study reported a variety of levels of lipid peroxidation with endogenous antioxidant levels to workers who were exposed to paint (Meek and Chan, 1994). Intraperitoneal injection of toluene results in a similar damage to the body of inhalation. This study indicated that GSH concentration with the protein content of the toluene-treated groups was importantly lower than the control group (Tables 1, 2). The GSH level of control group and that of the *P. peruviana*-treated group were statistically similar (Tables 1, 2). This affirmative effect of *P. peruviana* was thought to be caused by the phytochemicals and phenolic compounds in its composition (Tables 3, 4). In this context, 28-hydroxywithanolide, withanolides, phygrine, kaempferol, and quercetin di-andtriglycosides in *Physalis spp* are investigated (Abdel Moneim, 2016; Abdel-Moneim et al., 2014; Fang et al., 2012; Arun and Asha, 2007).

The vitamin and cholesterol compositions were various owing to the phytochemical content of the plant extracts. In this study, the analyzes were done on HPLC equipment showed that in methanol extract of PP with LU, there were α-tocopherol, δ-tocopherol,

vitamins D_3, E with K, stigmasterol, β-sitosterol, retinol molecules with flavonoids (e.g. cathechin, rutin, myricetin, morin with naringenin) (Tables 3, 4). Vitamin concentration in groups supplemented with PP and LU being similar to those in the control group and this value being higher than the one measured for group T would indicate that this fruit would have a rich vitamin and mineral content (Tables 5, 6).

Plant sterols are compounds that have similar chemical structures and function in a similar way with cholesterol. Stigmasterol is the most common plant sterol. In contemporary diet, the daily uptake of plant sterols is estimated to be in the range of 160-400 mg whereas this number was thought to reach or exceed one gram in ancient cultures. Nuts, cereals and seeds are good sources of plant sterols. β-sitosterol, campesterol and stigmasterol are the most common varieties of sterols found to the plants. The absorption of plant sterols in humans is less than that of cholesterol. The cholesterol absorption inhibition characteristic of plants sterols was investigated primarily due to the structural similarities between plant sterols and cholesterol (Scalzo et al., 2005).The high polyunsaturated fatty acid content and the presence of some fatty acid compounds make *P. peruviana*, which was used in this study, an ideal nutrient (Ramadan and Moersel, 2003). The investigation of the vitamin concentrations to this study indicated that to concentrations of some vitamins were considerably higher in the extract supplemented LU and PP groups by comparison that of the group T (Tables 5, 6). Furthermore, the concentration of many vitamins in groups T+LU and T+PP was very similar to their values in the control group, and some were reported to be higher than those measured in the tissue samples of the control group. The results of the presently conducted experiments indicate that the cholesterol concentration was higher in group T, which was exposed to toxic materials. Cholesterol concentration in groups T+LU and T+PP, which were supplemented with extracts, was lower and similar to the value of the control group (Tables 5, 6).

The results of the fatty acid analysis indicated an elevation in the concentration of some fatty acids and the phyto extract in T+LU and T+PP groups compared to the values measured for group T samples. This situation might have stemmed from the supplemented chemicals and plant extracts affecting the fatty acid metabolism. The C16:0 concentration in the kidney tissue of group T rats was significantly less than that of the control group (Table 7). This situation might have been caused by chemicals for example toluene, affecting the activity of enzymes such as acetyl-CoA carboxylase and fatty acid synthase that play a role in lipid biosynthesis since C16:0 is the final step in fatty acid biosynthesis; it is synthesized by fatty acid synthase and released through a series of enzymatic reactions. Therefore, a decrease in the C16:0 concentrations might be explained by a decrease in fatty acid synthesis. On the other hand, the C16:1 concentration in group T was considerably lower than the control group (Table 7). This decrease was assumed to have been caused by the inhibition of SCD activity. The C18:2n-6c (linoleic acid) concentration in the kidney and liver tissues of the toluene treated rats was much lower than the control group's value. The decrease in C18:2n-6c concentration would be an indicator of a decrease in the activity to the

delta 6 and delta 5 desaturase enzymes that belong to the delta 6 desaturation metabolic pathway. This would possibly be the reason for the change in the linoleic acid concentration in the tissue (Table 8).

Toluene results in a severe environmental pollution since it is commonly preferred by the industrial organizations. The impacts of toluene on animals and human were examined by various studies. The experimental studies show that antioxidants have constructive abilities against toluene's destructive effects. In this work, investigation of the antioxidant effects for PP and LU was performed through the animal experiments by inducing oxidative stress with toluene. The plant extracts used in the present study were proved to be effective in the prevention of LPO in vivo. The fatty acid and vitamin profiles and GSH with total protein concentrations were determined in Sprague Dawley rats. The results show that oxidative stress induced by toluen is eliminated with PP and LU compounds. We hope the results of this study will shed light on further studies focusing on the utilization of natural plant antioxidants against several xenobiotics which we are frequently exposed to.

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Conflicts of Interest

The author declares no conflict of interest.

Author Contribution Statements

Zehra GÖKÇE and Ökkeş YILMAZ conceived and designed the experiments. Zehra GÖKÇE performed the experiments. Ökkeş YILMAZ supervised the research activity and setup methodology of experiment. Zehra GÖKÇE wrote the paper and Hatayi ZENGİN contributed to writing the paper.

References

1. Abdel Moneim A.E., Prevention of carbon tetrachloride (CCl4) induced toxicity in testes of rats treated with Physalis peruviana L. fruit,.Toxicology and Industrial Health., 32-6 (2016) 1064–1073.
2. Abdel-Moneim A.E., Bauomy A.A., Diab M.M., Shata M.T., Al-Olayan E.M. and El-Khadragy M.F.,The Protective Effect of Physalis peruviana L. Against Cadmium-Induced Neurotoxicity in Rats,Biological Trace Element Research.,160-3 (2014) 392-399.
3. Abdel-Moneim A.E., Dkhil M.A. and Al-Quraishy S., The protective effect of flaxseed oil on lead acetateinduced renal toxicity in rats, Journal of Hazardous Materials., 194-1 (2011a) 250–255.
4. Abdel-Moneim A.E., Dkhil M.A. and Al-Quraishy S., The redox status in rats treated with flaxseed oil and lead-induced hepatotoxicity, Biological Trace Element Research143-1 (2011b) 457-467.
5. Abdel-Salam, O.M., Sleem, A. A., Khadrawy, Y.A., and Morsy, F.A. Prevention of toluene- induced brain neurodegeneration by atropine and neostigmine. Journal of Basic Pharmacology and Toxicology, 4(1), (2020).1-9.
6. Arun M. and Asha V.V., Preliminary studies on antihepatotoxic effect of Physalis peruviana Linn.against carbon tetrachloride induced acute liver injury in rats, The Journal of Ethnopharmacology 111-1 (2007) 110–114.
7. Arvindkumar E.G., Suresh S. and Jadhav S.L., Renoprotective effect of *Linum usitatissimum* seeds through haemodynamic changes and conservation of antioxidant enzymes in renal ischaemia reperfusion injury in rats, Arab Journal of Urology, 9-3 (2011) 215-221.
8. Aylward L.L., Barton H.A. and Hays S.M., Biomonitoring equivalents (BE) dossier for toluene (CAS No. 108 88-3), Regulatory Toxicology and Pharmacology., 51-3 (2008) 27–36.
9. Bayil S., Cicek H., Cimenci I.G. and Hazar M., How volatile organic compounds affect free radical and antioxidant enzyme activity in textile workers, Journal of industrial hygiene and toxicology., 59-4 (2008) 283–287.

10. Christie W.W., Gas chromatography and lipids, The Oil Pres, Glaskow (1990).
11. Costa C., Pasquale R., Silvari V., Barbaro M. and Catania S., In vitro evaluation of oxidative damage from organic solvent vapors on human skin, Toxicology in Vitro ., 20-3 (2005) 324–331.
12. EPA., USA., Toxicological Review of Toluene, EPA/635/R-05/004 (1959).
13. Fang S.T., Liu J.K. and Li B., Ten new withanolides from Physalis peruviana, Steroids., 77-1 (2012) 36–44.
14. Gerin M., Siemiatychi J., Desy M. and Krewski D., Associations between several sites of cancer and occupational exposure to benzene, toluene, xylene, and styrene: results of a case-control study in Montreal,American Journal of Industrial Medicine., 34-2 (1998) 144–156.
15. Gholaminejhad M., Arabzadeh S., Akbari M., Mohamadi Y. and Hassanzadeh G., Anti-oxidative and neuroprotective effects of flaxseed on experimental unilateral spinal cord injury in rat. Journal of Contemporary Medicine 3-10 (2017) 213–217.
16. Gotohda T., Nishimura A. and Morita K., Immuno histochemical studies on early stage of hepatic damage induced by subacute inhalation of toluene vapor in rats, Journal of Applied Toxicology, 29-6 (2009) 505-509.
17. Hara A. and Radin N.S., Lipid extraction of tissues with a low-toxicity solvent, Journal of Biochemistry and Analytical Biochemistry., 90-1 (1978) 420-426.
18. Hendawi M.Y., Alam R.T. and Abdellatief S.A., Ameliorative effect of flaxseed oil against thiacloprid-induced toxicity in rats: hematological, biochemical, and histopathological study, Environmental Science And Pollution Research İnternational., 23-12 (2016) 11855-11863.
19. Huff J., Absence of carcinogenic activity in Fischer rats and B6C3F1 mice following 103-week inhalation exposures to toluene, International Journal of Occupational Safety and Health., 9-2 (2003) 138- 146.
20. Jamshidi-Kia F., Lorigooini Z. and Amini-Khoei H. . Medicinal plants: past history and future perspective, Journal of Herbmed Pharmacology 7(1) (2018) 1-7.
21. Katsanidis E. and Addis P.B., Novel HPLC analysis of tocopherols and cholesterol in tissue, Free Radical Biology and Medicine., 27 – 1 (11-12) (1999) 1137-1140.
22. Kaur N., Kishore L. and Singh R., Therapeutic effect of *Linum usitatissimum* L. in STZ-nicotinamide induced diabetic nephropathy via inhibition of AGE's and oxidative stress. The Journal of Food Science and Technology., 54-2 (2017) 408-421.
23. Kayhan F.E.B., Koç N.D., Contuk G., Muşlu M.N. and Sesal N.C., Sıçan Böbrek Dokusunda Endosulfan ve Malathion'un Oluşturduğu Yapısal Değişiklikler, Journal of Science and Arts J., 12-1 (2009) 43-51.
24. Khalaf-Allah A.E.R.M., El-Gengaihi S.E., Hamed M.A., Zahran H.G. and Mohammed M.A., Chemical composition of goldenberry leaves against hepato-renal fibrosis. Journal of dietary supplements., 13-4 (2016) 378-392.
25. Kris-Etherton P.M., Lefevre M., Beecher G.R., Gross M.D., Keen C.L. and Etherton T.D., Bioactivecompounds in nutrition and health-research methodologies for establishing biological funtion: the antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis, The Annual Review of Nutrition., 24-1 (2004) 511-538.
26. Lowry O.H., Rosenbrough N.J., Farr A.L. and Randall R.J., Protein measurement with the folin-phenolreagent, Turkish Journal of Biochemistry., 193-1 (1951) 265-277.
27. Meek M. and Chan P., Toluene: evaluation of risks to human health from environmental exposure in Canada, Environmental Carcinogenesis and Ecotoxicology Reviews., 12-1 (1994) 507–515.
28. Moro A.M., Charao M., Brucker N., Bulcao R., Freitas F., Guerreiro G., Baierle M., Nascimento S., Waechter F., Hirakata V., Linden R., Thiesen F.V. and Garcia S.C., Evaluation of genotoxicity and oxidative damage in painters exposed to low levels of tolüene, Mutation Research., 746-1 (2012) 42– 48.
29. Moro A.M., Charão M., Brucker N., Bulcão R., Freitas F., Guerreiro G., Baierle M., Nascimento S., Waechter F., Hirakata V., Linden R., Thiesen F.V. and Garcia S.C., Effects of low-level exposure to xenobiotics present in paints on oxidative stress in workers., Science of the Total Environment., 408-20 (2010) 4461–4467.
30. Nasri H., World kidney day: acute kidney injury; a public health aware, Iranian Journal of Public Health., 42-1 (2013) 338-40.
31. Ohkawa H., Ohishi N. and Yagi K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Analytical Biochemistry., 95-2 (1979) 351-358.
32. Parikh M., Maddaford T.G., Austri, J.A., Aliani M., Netticadan T. and Pierce G.N., Dietary Flaxseed as a Strategy for Improving Human Health. Nutrients., 11-5 (2019) 1171.
33. Ramadan M.F. and Moersel J., Oil goldenberry (Physalis peruviana L.), The Journal of Agricultural and Food Chemistry, 51-4 (2003) 969–974.
34. Scalzo J., Politi A., Pellegrini N., Mezzetti B. and Battino M., Plant genotype affects total antioxidant capacity and phenolic contents in fruit, Nutrition, 2-1 (2005) 207–213.
35. Tapiero H., Tew K.D., Ba G.N. and Mathe G., Do they play a role in the prevention of humanpathologies, Biomedicine and Pharmacotherapy, 56-4 (2002) 200–207.

36. Teare, J.P., Punchard, N.A., Powell, J.J., Lumb, P.J., Mitchel, W.D. and Thompson, R.P.H. Automatedspectrophotometric method for determining oxidized and reduced glutathione in liver. Clinical Chemistry, 39-4 (1993) 686-689.
37. Trovato M. and Ballabio C., Botanical products: general aspects. In: Food supplements containing botanicals: benefits, side effects and regulatory aspects, Cham: Springer., (2018) 3–26.
38. Wu S.J., Ng L.T., Huang Y.M., Lin D.L., Wang S.S., Huang S.N. and Lin C.C. Antioxidant activities of *Physalis peruviana*, Biological and Pharmaceutical Bulletin., 28-6 (2005) 963–966.
39. Zu Y., Li C., Fu Y. and Zhao C., Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD, Journal of pharmaceutical and biomedical analysis., 41-3 (2006) 714–719.

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Current Perspectives on Medicinal and Aromatic Plants

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Antimicrobial, Antibiofilm-forming Properties of Equisetum arvense L. Shoot Extracts

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Abstract

Under the current conditions of growing antibiotic resistance of microorganisms, studies of antimicrobial properties of natural substances, including those obtained from medicinal plants, acquire special interest. The future outlook of such studies is caused by the fact that the resistance of microorganisms to vegetable-based substances may develop much slower or may not develop at all. This work is devoted to investigation into antimicrobial, antibiofilm-forming and some phytochemical properties of *Equisetum arvense* L. extracts. The results of the study showed high antibiofilm-forming activity of *Equisetum arvense* L. extracts exemplified by Staphylococcus biofilm. Antimicrobial properties of the reviewed extracts were also ascertained. Antibacterial activity was identified against typical and clinical antibiotic-resistant bacterial strains isolated from the mouth cavity of patients suffering from inflammatory processes. High antioxidant activity of the extracts was shown. A set of properties, in particular the antimicrobial and antibiofilm-forming activity, high content of tannins and antioxidant activity, shows good prospects to include horsetail (Equisetum) extracts in the oral cavity care plan.

Key Words: Extracts of medicinal plants, antimicrobial effect, antibiofilm-forming activity

1. Introduction

Under the present-day conditions of growing antibiotic resistance of microorganisms, studies aimed at searching for natural, including plant-based, substances with antimicrobial activity acquire special importance. This trend is related to the variety of biologically active compounds having a broad spectrum of pharmacological effect, antioxidant, anti-inflammatory and even antitumour activities (Gezici & Sekeroglu, 2019). Plant-based substances have been widely used in conventional and folk medicine, food, pharmaceutical and beauty industries. Studies aimed at searching for the antimicrobial active substances that are at the same time able to destroy bacterial biofilm have become of

special interest. The microorganisms of the biofilm are known to be more resistant to antimicrobial preparations and an additional pathogenicity factor (O'Toole et al., 2000; Kalemba&Kunicka, 2003). This issue is especially important for oral cavity diseases, where most pathogens of inflammatory diseases are contained in the form of biofilm which complicates the treatment of persisting inflammatory diseases (Shunmugaperumal, 2010). In our previous works, we indicated to high percentage of antibiotic-resistant bacterial strains in oral cavity microbial associations affected by chronic inflammatory process (Kryvtsova & Kostenko, 2018; 2019). Against the background of complicated clinical course of the inflammatory processes, S*taphylococcus* spp. genus bacteria and *Staphylococcus* spp.+Candida spp.; *Staphylococcus* spp. + *Enterobcteriacese* spp. associations were the dominating associants (Kryvtsova & Kostenko, 2018; Kryvtsova, 2019). In [Sidashenko, 2015], it was also shown that biofilm microorganisms are characterized by a higher level of resistance to antimicrobial preparations. Therefore, searching for substances with complex antimicrobial and antibiofilm-forming effects has become of particular interest (Kryvtsova at al., 2019; Piegerová, 2019; Rhos & Recio, 2005).

The objective of this work was to investigate into the antimicrobial, antibiofilm-forming, antioxidant and certain biochemical properties of *Equisetum arvense* L. shoot extracts.

2. Material and Methods

2.1. Collection of Plant Material

The plant material was collected in the vicinity of the village of Luta, Zakarpatska oblast (Trancarpathia), dried at the temperature of 30-35°C in shadow, then ground and placed in tightly closed containers.

2.2. Preparation of Plant Extracts

Ethyl and methyl extracts of of *Equisetum arvense* L. were made. A 10 g batch of the dry plant material was pulverized to powdery mass. In an Erlenmeyer flask, 10 g of the plant material were blended with 200 ml of or 96º ethyl or methyl alcohol (Sigma, Germany). The opening was closed with a food wrap to avoid evaporation. Following a 30-minute-long incubation in the ultrasonic bath (Kraintek) at 35° C, the blend was filtered through Whatman No. 1 filter paper. The clear solution was placed in an evaporative device (16-17/32" x 34-59/64" G5B, Coated Dry Ice Condenser Rotary Evaporator) to obtain pure alcoholic extract at 50 ˚C, 82 rpm. Then, extracts were exposed to evaporation under reduced pressure at 40 °C in order to remove ethyl or methyl.

2.3. Antimicrobial Activity

The antibacterial activity of the studied extracts *was assessed* by the minimum inhibitory concentration (MIC) coefficient [Rhos & Recio, 2005]. To study the MICs of the plant extracts, the following solutions in beef-extract broth were produced: 100; 50; 25; 22.5; 20; 17.5; 15; 12.5; 10; 7.5; 5; 3.5; 2.5; and 2.25 mg/ml. The bacterial suspension was introduced into each test-tube in the amount of 100 µl, which corresponded to 0.5 McFarland standard (1.5×10^8 CFU/ml) from a 24-hour culture of microorganisms in sterile physiological solution. The test-tube was incubated for 24 hours at 37 °C, whereupon part of the contents of each test-tube was inoculated

into the beef-extract broth. The last test-tube, whose inoculations did not show any growth of the microbial culture, was taken as the MIC. The negative controls were the following: bacterial suspension + dimethyl sulfocide; bacterial suspension + alcohol.

As test cultures, the following bacteria and yeasts from the American Type Culture Collection were used: *Candida albicans* ATCC 885-653; *Staphylococcus aureus* ATCC 25923*; Escherichia coli* ATCC 25922; *Enterococcus faecalis* ATCC 29212; *Streptococcus pyogenes* ATCC 19615; and as reference – *S. aureus CCM* CCM 4223 biofilm-forming strain. We also used clinical strains of bacteria and yeasts (*S. aureus, E. coli, S. pyogenes*, *E. faecalis, C. albicans*) isolated from the oral cavities of patients suffering from inflammatory periodontium and pharynx diseases. We chose clinical strains with multiple resistance to at least two classes of antibiotics. As a positive control, the following were used: gentamicin (10 mg/disk) for Gram-negative bacteria, ampicillin (10 mg/disk) for Gram-positive bacteria, and nystatin (100 UI) for *Candida*. As negative control, DMSO were used.

2.4. Determination of Antibiofilm Activity

With the purpose of studying the antibiofilm formation activity, an 18-hour culture of the reference *S. aureus* CCM 4223 grown at 37 °C was used. Into the wells, 180 µl of bacterial suspension, McFarland in broth (Tryptic soy broth (TSB), Himedia, India) were introduced. The *Vaccinium vitis-idaea L.* leaves and berries extracts were adjusted to the concentrations of 1%, 5% and 10% in DMSO (Sigma-Aldrich, USA) and introduced into the wells in the amount of 20 µl per well. Upon the addition of the bacterial suspension, the concentrations of plant extracts in the broth were equal to 0.1%, 0.05% and 0.01%, respectively. The wells with only 180 µl of broth and 20 µl of 10% DMSO served as control. Following a 24-hour incubation in the thermostat at 37 °C, the supernatant was withdrawn and washed 3 to 5 times with distilled water. Following a 30-minute incubation, it was dyed with 200 µl of 0.1% solution of crystal violet; then the dye was withdrawn, and the supernatant was washed 3 to 5 times with distilled water. Into every well, 200 µl of 30% acetic acid were added and incubated for 10 minutes. Optical density was measured on the Synergy HT (Biotek, USA) spectrophotometer at 550 nm. The mean absorbance ($OD_{550\ nm}$) of the samples was determined, and the percentage inhibition obtained using Eq.1. (Sandasi *et. al.*, 2011).

Negative controls: 180 µl of bacterial suspension + 20 µl of alcohol (ethyl or merthyl, respectively); 180 µl of suspension + 20 µl of dimethyl sulfocide. When a 50% reduction in absorbance was observed, it was considered as significant inhibition.

2.5. Antioxidant Activity

The antioxidant activity of extracts of medicinal plants and essential oils was identified by means of spectrophotometric 2-diphenyl-1-picrylhydrazyl free radical (DPPH•) scavenging method (Blois, 1958). The antioxidant activity was expressed as percentage (%) of the scavenging activity. Trolox was used for comparison. The optical density of the mixture was identified spectrophotometrically with the use of a Spectrophotometer Beckman Coulter DU 530 following 30 min. of incubation at the wavelength of 515 nm. The percentage of DPPH radical scavenging activity was calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs (control)} - \text{Abs(sample)}}{\text{Abs (control)}} \times 100$$

where Abs (control): Absorbance of DPPH radical + methanol; Abs (sample): Absorbance of DPPH radical + extract.

2.6. Determination of Tannins

Tannins were determined spectrophotometrically (Galavo et. al., 2018) with the use of a Folin-Ciocalteu reagent. The optical density was measured at 750 nm (A), using the Beckman Coulter DU 530v spectrophotometer (USA); water was used as the solution for comparison. The percentage of tannins was expressed compared with the activity of pyrogallol [Medini et al., 2014].

2.7. Determination of the Total Amount of Flavonoids

The flavonoid content was determined by absorption spectrophotometry. For quantitative determination, spectrophotometric methods based on the measurement of absorption of the aluminium chloride and flavonoids complex was used. The quantitative content was recounted into rutin, and simultaneously the absorption of the standard rutin solution (the comparison solution) was measured. The total amount of flavonoids was determined by aluminium chloride spectrophotometric method [Medini et al., 2014]. The optical density was determined on the Beckman Coulter DU 530 spectrophotometer.

2.8. Laboratory Base for Research

The microorganisms from the oral cavities of patients with chronic periodontium inflammatory processes were isolated on the basis of the Dental Polyclinics, Uzhhorod National University; the extracts were manufactured and their antioxidative activity and contents of tannins and flavonoids were determined on the basis of the Department of Pharmacognosy and Botany, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; the antimicrobial activity of plant extracts was studied at the Microbiological Laboratory of the Department of Genetics, Plant Physiology and Microbiology, Uzhhorod National University, and Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice.

2.9. Statistical Analysis

The obtained data were expressed as mean ± standard deviation (SD) of three measurements. The Tukey's test was applied for comparisons of means; the differences were considered significant if $p < 0.05$.

3. Results and Discussion

3.1.Antibiofilm-Forming and Antimicrobial Effects of Extracts

Equisetum arvense L. extracts demonstrated high destruction ability against the biofilm formed by *S.aureus*. In case of 0.1% concentration, the ethyl extract reduced the process of biofilm formation by 95.90%; the methyl extract – by 69.86% (Fig. 1-2). In case of 0.05% concentration, the reductions were 77.8% for the ethyl extract, and 69.38% for the methyl extract. A substantial antibiofilm-forming effect was ascertained even for 0.01% extracts: the ethyl extracts reduced the biofilm-forming process by 63.0%, and the methyl extract – by 48.72%. The studies showed the antimicrobial activity of horsetail extracts against Gram-

positive and Gram-negative microorganisms. The extracts demonstrated higher activity against reference strains than clinical ones (see Table 1).

E. arvense has been well known for high contents of bioactive components, like phenolic compounds, saponins, aconite, oxalic and malic acids, tars, tannins, pectin, flavones, vitamin C, carotenoids and mineral substances [Jackson 1995; Pallag et al., 2018]. There are data about antimicrobial properties of *Equisetum arvense* extracts. In [Pallag et al., 2018], it was shown that *Equisetum arvense* L. demonstrated antibacterial effect upon pathogenic Gram-positive cocci, though it did not affect Gram-negative bacteria and *C. albicans*. Literary sources provide information on antimicrobial activity of 1 g/ml concentrations of horsetail methyl extract against *S. epidermidis* та *E. coli*, but no effect upon C*. albicans* was observed. This extract also showed antimicrobial activity against *K. pneumoniae*, *P. aeruginosa* and *S. enteritidis*. The antimycotic effect of horsetail extract was ascertained against *A. niger*. [Aldaas, 2011; Yoshinobu, 1992]. In [Wojnicz et al., 2012], an antimicrobial effect of *E. arvense* extracts upon *E.coli* was established. *E. arvense* extracts that had an antimicrobial effect upon coliform bacterium had three flavonoids and phenolic acids (protocatechuic, ferulic and caffeic acids). Our studies ascertained the anti-staphylococcus and antibiofilm-forming activities of field horsetail extracts. For the first time, the antibiofilm-forming effect of horsetail extracts upon the biofilm formed by *Staphylococcus aureus* was shown. This ability may be used for biofilm destruction to improve the bioavailability of antimicrobial drugs.

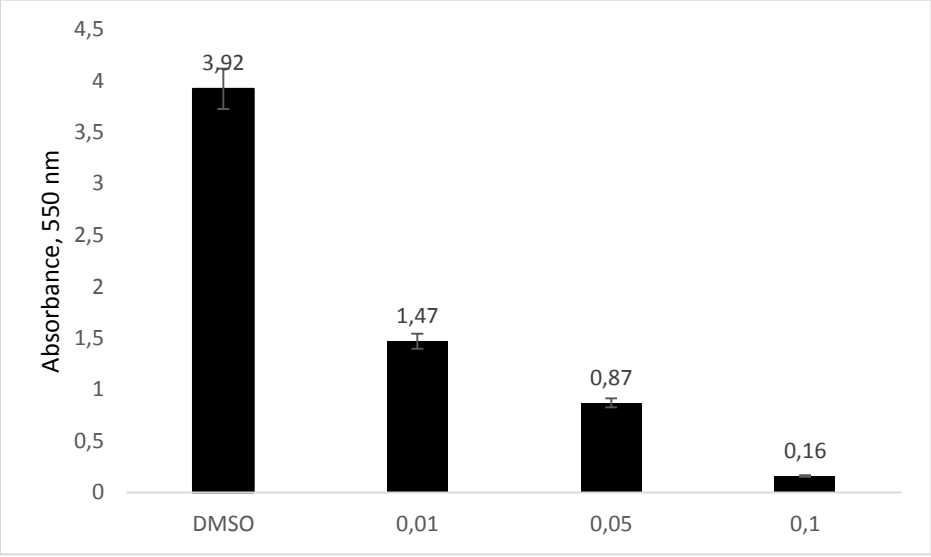

Figure 1. Impact of *Equisetum arvense* L. ethyl extract upon the formed *S. aureus* biofilm. Control: *S. aureus* suspension in broth + dimethylsulfoxide were taken as 100% and used as control (OD = 3.82 ± 0.2), n = 3

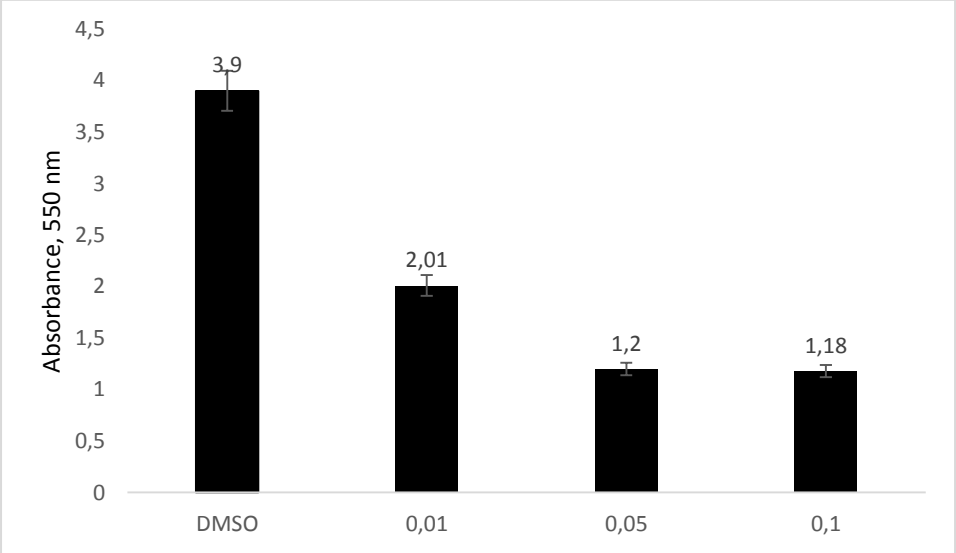

Figure 2. Impact of *Equisetum arvense L. methyl* extract upon the formed *S. aureus* biofilm Control: *S. aureus* suspension in broth + dimethylsulfoxide were taken as 100% and used as control (OD = 3.82 ± 0.2), n = 3

Table 1. Antimicrobial effect of ethyl and methyl extracts of *Equisetum arvense* L. shoots against reference and clinical bacterial strains, mg/ml, $\bar{x} \pm SD$

Test cultures	Minimum inhibit concentration	
	Ethyl extract	Methyl extract
S. aureus ATCC 25923	7,33±0,13^{d}	15,5±0,5^{c}
S. aureus clinical strains	15,5±0,5^{b}	20,58±0,8^{a}
E. coli ATCC 25922	15,5±0,25^{b}	15,5±0,25^{c}
E. coli clinical strains	12,58±0,8^{c}	15,41±0,52^{c}
E. faecalis ATCC 29212	12,67±0,29^{c}	12,67±0,29^{d}
E. faecalis clinical strains	5,42±0,38^{e}	5,25±0,43^{e}
S.pyogenes ATCC 19615	17,5 ± 0,5^{a}	17,5 ± 0,5^{b}
S.pyogenes clinical strains	16,00 ±1,30^{b}	17,5±0,76^{b}

The control: 1) extracting solvent (ethanol) – no inhibition zone; 2) solvent (dimethylsulfocide) – no inhibition zone; the data differ statistically significantly as compared with the control – ethanol and dimethylsulfocide

3.2. Phytochemical Screening of Antioxidant Activity

The extracts were shown to be characterized by a high level of tannins and antioxidant activity (Table 2). The high antioxidant activities combined with the antimicrobial activity and high antibiofilm-forming effects lay behind the prospects of the use of horsetail extracts as part of oral cavity care plans. The literature mentions the modulating effect of *Equisetum arvense* L. extract upon endothelial cells that submit to the influence of the hypertonic environment. The experimental data have proved that if applied in low doses, *Equisetum arvense* L. may become a new therapeutic approach to lower the heightened oxidative stress and hypertonicity-related apoptosis (Pallag, 2018).

Table 2. The level of tannins and flavonoids, and the antioxidant activity of ethyl and methyl extracts of *Equisetum arvense* L. shoots, %

Ethyl extracts	Methyl extracts
tannins	
2.89±0.04	2.85±0.3
flavonoids	
0.95±0.06	0.70±0.05
antioxidant activity	
78.10±0.5	74.88±1.0

Conclusions

Our research has shown the antimicrobial activity of *Equisetum arvense L.* ethyl and methyl extracts upon antibiotic resistant strains of *Staphylococcus* genus bacteria. These trends were shown both on typical and clinical strains that were isolated from the oral cavities of patients suffering from chronic diseases of oral cavity and characterized by high antibiotic resistance. High antibiofilm-forming activity of *Equisetum arvense* L. ethyl extract was established. A significant antioxidant activity of the reviewed extracts was shown. The obtained results indicated to good prospects for further research aimed at development of horsetail-based preparations for oral cavity care, because they, unlike chemical preparations, as a rule, have no side effects but have an astringent effect and antioxidant properties. *Equisetum arvense* L. is an especially valuable vegetative material as it has for a long time been used in ethnic pharma medicine.

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Conflicts of Interest

The author declares no conflict of interest.

Author Contribution Statements

Marina KRYVTSOVA conceived and designed the experiments. Jana KOŠČOVÁ performed the experiments. Tanya KOHUCH supervised the research activity and setup methodology of experiment. Marianna SAVENKO wrote the paper and Nokolay SPIVAK contributed to writing the paper.

References

1. Aldaas, S., (2011). Cytotoxic and antibacterial activity of an extract from a Saudi traditional medicinal plant *Equisetum arvense*. MSc thesis, King Abdullah University of Science and Technology, Thuwal, 45-46. DOI:10.25781/KAUST-85V93
2. Blois, M. S., 1958. Antioxidant Determinations by the Use of a Stable Free Radical. Nature, 181:1199-1200
3. Djeridane, A., Yous, M., Nadjemi, B., Boutassouna, D., Stocker, P., *et al*. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry, 97 (4), 654-660. DOI:10.1016/j.foodchem.2005.04.028
4. Galvão, M. A., Arruda A. O, Bezerra, I. C., Ferreira, M. R., Soares, L. A. (2018). Evaluation of the Folin-Ciocalteu method and quantification of total tannins in stem barks and pods from libidibiaferrea (Mart. ex Tul) L. P. Queiroz. Brazilian Archives of Biology and Technology, 61, e1817058. DOI:10.1590/1678-4324-2018170586
5. Gezici, S., Şekeroğlu, N. (2019). Current Perspectives in the Application of Medicinal Plants Against Cancer: Novel Therapeutic Agents. Anti-cancer agents in medicinal chemistry, 19(1), 101–111. DOI:10.2174/1871520619666181224121004
6. Jackson, B., P. (1995). Herbal drugs and phytopharmaceuticals: A handbook for practice on a scientific basis, edited and translated from the second German edition by Norman Grainger Bisset, London; Medpharm Scientific Publishers, Stuttgart, 10(5), 337.
7. Kalemba, D., Kunicka, A. (2003). Antibacterial and Antifungal Properties of Essential Oils. Current Medicinal Chemistry, 10(10), 813–829. DOI:10.2174/0929867033457719
8. Kryvtsova, M., V., Kostenko, Ye.,Ya. (2020). Dominant microbial associations of the oral cavity in the conditions of generalized periodontitis and features of there sensitivity to antibacterial drugs.

Studia Biologica, 14(1), 51–62. DOI:10.30970/sbi.1401.613
9. Kryvtsova, M., V., Kostenko, Ye.,Ya., Salamon, I. (2018). Compositions of essential oils with antimicrobial properties against isolates from oral cavities of patients with inflammatory diseases of parodentium. Regulatory Mechanisms in Biosystems, 9(4), 491-494. DOI: 10.15421/021873
10. Kryvtsova, M., V., Trush, K., Eftimova, J., Koščová, J., Spivak, M.J. (2019). Antimicrobial, antioxidant and some biochemical properties of Vaccinium vitis-idea L. Mikrobiolohichnyi Zhurnal, 81 (3), 40-52. DOI:10.15407/microbiolj81.03.040
11. Kryvtsova, M.V. (2019). Microscopoc Candida genus fungi in the structure of microbial associations in the condition of generalized periodontitis and their sensitivity to antibiotics and essential oils. Bulletin of Problems Biology and Medicine, 1(2), 263-266. DOI:10.29254/2077
12. Medini, F., Fellah, H., Ksouri, R., Abdelly, C. (2014). Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant Limonium delicatulum. Journal of Taibah University for Science, 8(3), 216-224. DOI:10.1016/j.jtusci.2014.01.003
13. O'Toole, G.A. (2011). Microtiter dish biofilm formation assay. Journal of vsualized experiments, 47. DOI: 10.3791/2437
14. O'Toole, G., Kaplan, H., B., Kolter, R. (2000). Biofilm formation as microbial development. Annual Reviews in Microbiology, 54, 49-79. DOI:10.1146/annurev.micro.54.1.49
15. Pallag, A., Filip, G.,A., Olteanu, D., Clichici, S., Baldea, I., *et a*l. (2018). *Equisetum arvense* L. Extract Induces Antibacterial Activity and Modulates Oxidative Stress, Inflammation, and Apoptosis in Endothelial Vascular Cells Exposed to Hyperosmotic Stress. xidative Medicine and Cellular Longevity. Hindawi Limited, 3060525. DOI:10.1155/2018/3060525
16. Piegerová, A., Koščová, J., Schusterová, P., Nemcová, R., Kryvtsova, M. (2019). In vitro inhibition of biofilm formation by Staphylococcus aureus under the action of selected plant extracts. Folia Veterinaria, 63(1), 48–53. DOI:10.2478/fv-2019-0007
17. Ríos, J. L., Recio, M. C. (2005). Medicinal plants and antimicrobial activity. Journal of ethnopharmacology, 100(1-2), 80–84. DOI:10.1016/j.jep.2005.04.025
18. Shunmugaperumal, T., 2010. Biofilm-Related Infections in the Oral Cavity. Biofilm Eradication and Prevention, 184–225. DOI:10.1002/9780470640463.ch7.
19. Sandasi, M., Leonard, C. M., Van Vuuren, S. F., & Viljoen, A. M. (2011). Peppermint (Mentha piperita) inhibits microbial biofilms in vitro. South African Journal of Botany, 77(1), 80–85. DOI:10.1016/j.sajb.2010.05.011
20. Sidashenko, O., I., Voronkova, O.,S., Sirokvasha, O., A., Vinnikov, A. I. (2015). Exhibition pathogenicity factors in biofilm-forming and nobiofilm-forming strains of *Staphylococcus epidermidis.* Mikrobiolohichnyi Zhurnal, 77(2), 33–37. DOI:10.15407/microbiolj77.02.033
21. Wojnicz, D., Kucharska, A.,Z., SokolLetowska, A. (2012). Medicinal plants extracts affect virulence factors expression and biofilm formation by the uropathogenic *Escherichia coli.* Urological Research, 40(6), 683-697. DOI: 10.1007/s00240-012-0499-6
22. Yoshinobu, Y. (1992). Antitumor activity of crude protein extracted from *Equisetum arvense* LINN'E. Journal of Analytical Bio-Science, 22, 421-424.

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Can Medicinal Plants Help in the Treatment of the New Coronavirus? Some R & D Aspects in Slovak Republic

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Abstract

At the end of February 2020, the World Health Organization published the final report: Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19), which directly presents the findings of a joint mission of experts from around the world in China in the period after the pandemic. Researchers have been searching for anything that could help other countries fight the new type of 2019-nCoV virus; among other things, how to prevent it`s spread or how to treat it as effectively as possible. In China, doctors, pharmacists and ordinary citizens use the phytotherapy of traditional Chinese medicine on a daily basis to prevent and treat COVID-19. As part of this report, it is recommended to carry out research on natural substances isolated from medicinal plants, the aim of which should be to evaluate their real effectiveness against this disease. Experts agree that we are using vaccines or medicament that would be effective in the fight against the new type of coronavirus SARS CoV-2. While we look forward to it, let us remember which medicinal plants and their natural substances can bring us relief from its manifestations during COVID-19 disease. It is important to know, that relevant herbal drugs and supplements standardized to the active antiviral natural substance. They can play an important role in the treatment and management of the coronavirus pandemic and its subsequent recurrences.

Key Words: COVID-19 pandemic, herbs, immune effect, natural components

1. Introduction

All over the world medicinal plants fulfill their role at the intersection between the traditional medicine and modern medicine, which uses mainly synthetic drugs. The success of traditional Chinese medicine in the treatment of civilization diseases lies in its overlap with conventional methods of treatment used by general practitioners. One of the latest examples of the significant contribution of traditional Chinese phytotherapy is the use of the artemisinin from wormwood (*Artemisia annua* L.) in treating the malaria. In 2015, the Nobel Prize in Physiology and Medicine was attributed to Chinese scientist Dr. Youyou Tu for her contribution to artemisinin discovery (Karkalik et al., 2018). The pandemic of coronavirus disease 2019 (COVID-19) is changing the world like never before. This crisis is unlikely contained in the absence of effective therapeutics or vaccine. It is the duty of every researcher and his institution to

contribute to solving this unfavorable global situation. The purpose of the paper is to present some aspects of the use of medicinal plants with antiviral activities and supporting research conducted in Slovakia at the University of Presov in Presov.

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3. World differences in the use of medicinal plants

In Europe, the use of medicinal plants is relatively widespread, but their therapeutic effect is not significantly and sufficiently appreciated. On the contrary, conventional medicine dominates in the United States, and is completely separate from the traditional medicine and herbal medicine. U.S. general practitioners use and prescribe only synthetic drugs and the population is being vaccinated.

It is true that the course of the spread of coronavirus 2019-nCoV in individual parts of the world (China - Europe - USA - South America) depends on the discipline of the population in the implementation of all measures to slow down the virus spread. However, somewhere in the background is the knowledge and use of medicinal plants and isolated natural substances that act and treat COVID-19 comprehensively without any side effect. The number of people who were infected, cured or who died of the disease on various continents of the world clearly indicate that medicinal plants and their secondary metabolites (natural substances) can play a role in managing of the infection (Benarda and Pandiella, 2020).

4. University of Presov and R & D in Slovakia

For more than 15 years at the University of Presov, we have been producing drugs (dry plant parts) of medicinal plants with high contents of therapeutically active natural substances for plant-derived products of several collaborating pharmaceutical companies at home and abroad. Mass-produced medicinal products require special attention in connection with its definition, specification, testing and proof of quality, which we have mastered. A thorough evaluation is mandatory not only for medicinal products originating from individual plant species, but for each herbal

product and also for marketed products such as food (teas), food supplements and cosmetics.

The knowledge of the main active constituents, their identification, the knowledge of the chemical composition (chemical profile/fingerprint) and determination of the biological activity is required when using medicinal plants. Quantitative contents of individual natural substances determine the value of the plant, also in terms of its commercial use.

A significant success at the international level and unique in the Slovak Republic is the use of bred varieties of medicinal plants with a high content of therapeutically active substances, either their own (legally protected throughout the EU) or introduced into cultivation from neighboring countries (Salamon et al., 2018). Within the identification of natural substances and their qualitative and quantitative composition, we have introduced a number of spectrophotometric methods and chromatographic methods with methodology for each plant species separately.

As part of international cooperation with several institutions, for example: Institute of Microbiology and Virology of Zobolotny, UAV, Kiev, Ukraine and Institute of Epidemiology and Infection Diseases of L.V. Gromashevsky, NAMN, Kiev, Ukraine, we also focus on testing the antiviral activity of isolated plant extracts, essential oils and natural components.

5. Coronavirus SARS CoV-2

The new strain of 2019-nCoV virus belongs to droplet infections of the respiratory tract and lungs (Andersen et al., 2020), the estimated incubation time of the disease is 2 to 14 days, but it can be longer. Symptoms of COVID 19 include fever above 38 ° C, cough, difficulty breathing, muscle and headache, tiredness and malaise. However, some individuals may transmit the virus without feeling the symptoms of the disease. The replication cycle of the virus (also 2019-nCoV) in the host cell (respiratory system) has its sequence (Helmy-Yosra et al., 2020): - early phase of infection: recognition, attachment, penetration; - introduction into the cell: stripping, release of the genome into the nucleus; - late phase: genome replication, macromolecules, fusion and release; - eclipse phase (period from genome stripping): loss of infectivity after fusion and emergence of new virions; and - latent period - the period of eclipse until the release of a number of virions and their attack on a significant number of host cells (Coutarda et al., 2020).

6. Inhibition of hemagglutinin protein and viral neuraminidase enzyme

The hemagglutinin protein, together with the enzyme neuramidase, helps the virus to multiply in the host cell. Several scientific experiments (Wilks et al., 2012; Zakay-Rones et al., 1995) have shown that natural components (anthocyanin flavonoids) extracted from the fruits of the elderberry (*Sambucus nigra* L.) prevent the spread of swine flu infection by preventing its entry into the H_1N_1 virus from entering the lung host cell. The effects of these substances are comparable to the chemical structure of Oseltamivir® (trade name: Tamiflu, manufactured by Roche, Basel, Switzerland). In vitro experiments have shown that these flavonoids are able to prevent the entry of various parasitic

strains of influenza viruses into human cells (Ishiguro et al., 2018).

The very interesting news with the use of elderberry extracts came from the US, where experienced the second-strongest growth in the mainstream channel in 2019, and sales surged in the first half of 2020 due to the COVID-19 pandemic. This is the second consecutive year of mainstream sales growth of more than 100 % for this herb, which is commonly used for immune support and cold and flu symptoms (Smith et al., 2019). A meta-analysis of elderberry for cold- and flu-related respiratory symptoms published in February 2019, for example, found that the herb "substantially reduce upper respiratory symptoms." The authors of the analysis concluded that elderberry supplementation could help reduce "antibiotic misuse for upper respiratory symptoms due to viral infections, and [could be] a potentially safer alternative to prescription drugs for routine cases of the common cold and influenza (Hawkins et al., 2019).

7. Substances that prevent the virus from progressing

Another natural substance that prevents the virus from entering the cell is rosmarinic acid. It can be extracted from several species of medicinal plants, such as balm (*Melissa officinalis* L.), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.), origanum (*Origanum vulgare* L.) and peppermint (*Mentha × piperita* L.). However, the problem with this plant is the large variability of the content of the natural substance (from 8.5 to 28.5 $mg.kg^{-1}$), which depends not only on the chemotype, but mainly on the soil and climatic conditions for cultivation. Rosmarinic acid is a naturally occurring polyphenolic compound. The qualitative and quantitative compositions of the main aromatic and polyphenolic constituents were examined and compared in lemon balm leaves from two years cultivation. The results show total hydroxycinnamic compounds 6.2±0.5 %, contents of rosmarinic acid 1.4 ± 0.1% (in 2010) and 1.7 ± 0.1% (in 2014) and total flavonoid compounds 0.5% (Friedman, 2015). In our research, we focused on the extraction of balm leaves of a new variety of 'Citronella' and the determination of rosemarinic acid quantity. High contents of this natural substance have been confirmed. It is possibly to recommend the use of this balm variety for the production of high quality raw material for the pharmaceutical industry (Salamon et al., 2019).

The enzyme neuraminidase is located on the surface of the virus and allows it to exit the host cells. This enzyme is blocked by other natural substances, including flavonoid anthocyanin (cyanidine-3-sambubiocide, identified amount: 8200 $ng.ml^{-1}$) isolated from the elderberry fruits (*Sambucus nigra* L.) (Wu et al., 2015; Swaminathan et al., 2013). The drug product with antiviral effect may be lyophilisate of anthocyanins, isolated from elderberry fruits using freeze-drying (lyophilization). Anthocyanins are instable in different conditions and our process of freeze-drying stabilizes them. Lyophilization is used in cases where it is necessary to remove water or occasionally other components from solutions (anthocyanins) sensitive to temperature, pH, light or change in structure, usually of biological origin without changing the original properties and their long-term stabilization. The qualitative and quantitative characteristics of these

flavonoids were determined by LCMS IT TOF (Salamon et al., 2021). The final product of anthocyanins in our R&D could be used as food supplement, gelatin tablets, but also injections. They have been suggested to be beneficial for cardiovascular and neurodegenerative disease, as well as eye, muscle disorders and sarcopenia, even for possible dietary treatment of Duchenne muscular dystrophy (Wicks et al., 2018).

8. Defense ability of the organism (immunity)

In their viral infection, human cells are prevented from producing interferons into tissues and blood. These are special proteins (called IFN-β) that serve as information about their involvement and a signal to trigger the defense mechanisms, i.e. the formation of cells killing the intruder (immune response). At that moment, an inflammatory process takes place in the body, which in our case is accompanied by fever. It is not clear how raising the body temperature helps to suppress the infection. On the one hand, it can help increase the activity of defense mechanisms, for example by making antibodies. Sometimes, however, there is a situation, where the body responds incorrectly to its own cells and considers them foreign. He defends itself. Then we are talking about the auto immune diseases. In the case of COVID-19, severe pneumonia, and as a result of its complications, death can occur. It is therefore necessary to eliminate the body's high temperature by all possible means. Anthocyanin flavonoids extracted from the fruits of elderberry (*Sambucus nigra* L.) have, in addition to suppressing the replication of the virus in the host cell, also the ability to increase the production of interferons (IFN-β). In this context, there has already been a discussion as to whether the use of a standardized extract of these natural substances can elicit an auto-immune response and thus human death. The claim that these phenomena are unrelated is based primarily on a long history of using basal extracts to support immunity (Frokiaer et al., 2021; Barak et al., 2001).

It refers to Sambucol® (manufactured by Pharma Care Inc., San Diego, CA, USA), which has a special and unique extraction process and has undergone more than 20 years of scientific research and is currently distributed in 50 countries around the world (Chrubasik et al., 2015; Knudsen et al., 2015). Another example of the healing power of natural substances is *Echinacea angustifolia* DC. It is well known for its immunostimulatory substances (echinacoside, echinacin, rutoside, isobutylamides and others), which have the ability to increase the production of interferons (IFN-β) (Wu et al., 2009). Their antiviral activity is comparable to the already mentioned preparation of Oseltamivir®. For the treatment of viral infections in children, Echinacin® (producer: MEDA Pharma GmbH & Co. KG, Radebeul, Germany) is recommended, for which the species *Echinacea purpurea* (L.) MOENCH is used. It has been shown that such a method of treatment can significantly shorten the duration of infection compared to other methods of treatment (Rauš et al., 2015).

Further scientific research points to the ability of mass-produced preparations from species of the genus *Echinacea* to strengthen and stabilize the immune system in diseases of viral respiratory

origin. Echinacea (*Echinacea* spp.) sales increased by 4.9% from 2018, making it the second top-selling ingredient, and ivy leaf (*Hedera helix* L.) sales increased by 14%, making it the sixth top-selling supplement in this channel (Smith et al., 2020).

9. PLpro inhibitory effects

The papain-like protease (PLpro) of severe acute respiratory syndrome SARS-CoV-2 plays essential roles in virus replication and immune evasion (Gaoa et al., 2021). PLpro is an attractive target because it plays an essential role in cleavage and maturation of viral polyproteins, assembly of the replicase-transcriptase complex, and disruption of host responses. It was determined collection of structures details inhibitors recognition and interactions providing fundamental molecular and mechanistic insight into PLpro. All compounds inhibit the peptidase activity of PLpro *in vitro*, some block SARS-CoV-2 replication in cell culture assays. These findings will accelerate structure-based drug design efforts targeting PLpro to identify high-affinity inhibitors of clinical value (Osipiuk et al., 2021).

Several natural compounds were found to possess promising PLpro inhibitory effects. Indeed, Song et al. (2014) demonstrated that six cinnamic amides extracted from *Tribulus terrestris* L. fruits were able to inhibit SARS-CoV PLpro. Terrestrimine showed the best inhibitory activity of SARS-CoV PLpro. The first contribution from Slovakia presents the information about an occurrence of this plant species and quantity determination of the specific constituents and heavy metal pollution in this plant material. The expeditions for general collection were organized several times a year (1999 – 2001) in chosen localities of Western Slovakia / Nesvady, Nove Zamky / and a small area cultivation was carried out in Nitra and Streda nad Bodrogom (Eastern Slovakia). The quantity of furostanol saponins is various in different plant parts and was modified by growing conditions (Salamon et al., 2006). The high interest on the production of puncture vine (*Tribulus terrestris* L.) for different purposes led scientists in Slovakia to start its cultivation, but climatic conditions greatly influence the cultivation of the plant. The Slovakian study aimed to the introduction of puncture vine into large cultivation scale was carried out. Two different methods of cultivation were compared and production of biomass and evaluation of the content of furostanol saponins were done. Transplantation of seedlings raised in the greenhouse into the open field was more effective for the production of high amount of biomass and active components than using plants that are directly sown. The transplantation of seedlings into open field is suitable for the puncture vine cultivation as it increases biomass production (Salamon et al., 2016).

10. Help with the fever

Fever (*febris*, *pyrexia*) is a common pathological symptom in acute inflammatory diseases of the respiratory system. Fever is not a disease, but a non-specific symptom of a possible disease. In addition to bed rest and a rich supply of fluids, fever-reducing drugs (antipyretic action) containing natural substances and sweating agents (diaphoretic effect) can prove effective. For this purpose chamomile (*Matricaria recutita* L.) is very well suited, because of a high content of sesquiterpenes -/-/-α-bisabolol and chamazulene in essential oil, or the application of dry extracts standardized to

a high content of apigenins (Salamon, 2019), which have an effect on several physiological functions of the human body and has anti-inflammatory, antioxidant, antibacterial and antiviral activities.

11. Conclusion: Herbs – good remedies in the treatment of viral diseases

Return to natural healing substances is basically not a return, because many of them are used every day in medicines. However, in the light of events, it is even more important today (than ever before) to explore the unexplored possibilities of medicinal plants and to maximally support the search for ways to their therapeutically active substances in all available ways.

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Conflicts of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contribution Statements

Ivan salamon designed and wrote the paper.

References

1. Andersen, K.G., Rambaut, A., Lipkin, W.I., Holmes, E.C., Garry, R.F. 2020. The Proximal Origin of SARS-CoV-2. Nature medicine, 26 (4), 450-452.
2. Barak V, Halperin T, Kalickman I. 2001. The effect of Sambucol, a Black Elderberry-based, Natural Product, on the Production of Human Cytokines: I. inflammatory cytokines. European Cytokine Network. 12(2), 290–296
3. Benarda, B., Pandiella, A. 2020. Medicinal Plants as Sources of Active Molecules against COVID-19 (Review). Frontiers in Pharmacology. 11(1189), 1-16
4. Chrubasik, S. 2015. Review of Pharmacological Effects and Clinical Efficacy of Fruit Products and Functional Foods from Elderberry (Sambucus spp.) as Compared with Chokeberry (Aronia melanocarpa L.). Acta Horticulturae. 1061, 89-92
5. Coutarda, B., Valle, C., De Lamballerie, X., Canard, B., Seidahc, N.G., Decroly, E. 2020. The Spike Glycoprotein of the New Coronavirus 2019-nCoV Contains a Furin-like Cleavage Site Absent in CoV of the Same Clade. Antiviral research. 176(2), 104-112.
6. Friedman, T. 2015. The Effect of Rosmarinic Acid on Immunological and Neurological Systems: A Basic Science and Clinical Review. Journal of Restorative Medicine. 4(1), 50-59
7. Frokiaer, H., Henningsen, L., Metzdorff, S.B., Weiss, G., Roller, M., Flanagan, J. 2012. Astragalus Root and Elderberry Fruit Extracts Enhance the IFN-beta Stimulatory Effects of Lactobacillus acidophilus in Murine-derived Dendritic Cells. PLoS One. 7(10), 478-482
8. Gaoa, X., Qina, B., Chena, P., Zhua, K., Houa, P., Wojdylab, J.A., Wang, M., Cuia, S. 2021. Crystal Structure of SARS-CoV-2 Papain-like Protease. Acta Pharmaceutica Sinica B, 11(4), 237-245
9. Hawkins, J,, Baker, C., Cherry, L., Dunne, E. 2019. Black Elderberry (Sambucus nigra) Supplementation Effectively Treats upper Respiratory Symptoms: a Meta-analysis of Randomized, Controlled Clinical Trials. Complementary Therapies in Medicine. 42(6), 361-365.
10. Helmy, Y.A., Fawzy, M., Elaswad, A., Sobieh, A., Kenney, S.P., Shehata, A.A. 2020. The COVID-19 Pandemic: A Comprehensive Review of Taxonomy, Genetics, Epidemiology, Diagnosis, Treatment, and Control. Journal of Clinical Medicine 9(4), 1225
11. Ishiguro, N., Koseki, N., Kaiho, M. et al. 2018. Clinical Effectiveness of Four Neuraminidase Inhibitors (Oseltamivir, Zanamivir, Laninamivir, and Peramivir) for Children with Influenza A and B in the 2014-2015 to 2016-2017 Influenza Seasons in Japan. Journal of Infection Chemotheraphy. 24(6), 449-457
12. Karkalík, Š., Rudá-Kučerová, J. 2018. Malária, jej liečba a profylaxia (in Slovak). Klinická farmakologia a farmácia. 32(2), 20-27
13. Knudsen, B.F., Kaack, K.V. 2015. Review of Human Health and Disease Claims for Elderberry

(Sambucus nigra) Fruit. Acta Horticulturae. (1061), 121-131
14. Osipiuk, J., Azizi, S.A., Dvorkin, S., Endres, M., Jedrzejczak, R., Jones, K. A., Kang, S., Kathayat, R.S., Youngchang, K., Lisnyak, V.G., Maki, S.L., Nicolaescu, V., Taylor, C.A., Tesar, C., Zhang, Y.A., Zhou, Z., Randall, G., Michalska, K., Snyder, S.A., Bryan C. Dickinson, B.C., Joachimiak, A. 2021. Structure of Papain-like Protease from SARS-CoV-2 and its Complexes with Non-covalent Inhibitors. Nature Communications. 12(1), 1-17
15. Rauš, K., Klein, P., Schoop, R., Fisher, P. 2015. Effect of an Echinacea-based Hot Drink Versus Oseltamivir in Influenza Treatment: a Randomized, Double-blind, Double-dummy, Multicenter, Noninferiority. Current Therapeutic Research – Clinical and Experimental. 77(2), 66–72
16. Salamon, I., Sezer, E.N.S., Kryvtsova, M., Labun, P. 2021. Antiproliferative and Antimicrobial Activity of Anthocyanins from Berry Fruits after their Isolation and Freeze-Drying. Applied Sciences – MDPI, (11):1-15
17. Salamon, I., Kryvtsova, M.V., Trush, K.I., Fandalyuk, A.I., Spivak, M.J. 2019. Agro-ecological Cultivation, Secondary Metabolite Characteristics and Microbiological Tests of Lemon Balm (Melissa officinalis L.) – the Variety Citronella. Regulatory Mechanisms in Biosystems, 10(3), 265-269
18. Salamon, I. 2019. Slovak Chamomile Varieties and their Comparison of Natural Components. Current Perspective on Medicinal and Aromatic Plants (CUPMAP). 2(2), 59-65
19. Salamon, I., Haban, M., Otepka, P., Habanova, M. 2018. Perspectives of Small- and Large-Cultivation of Medicinal, Aromatic and Spice Plants in Slovakia. Medicinal Plants – International Journal of Phytomedicines and Related Industries, 9(4), 261-267
20. Salamon, I., Grulova, D., DeFeo, V. 2016. Comparison of Two Methods for Field Grow of Puncture Vine (Tribulus terrestris L.) in Slovakia. Acta Agriculturae Scandinavica, Section B – Soil & Plant Science. 66(3), 267-271
21. Salamon, I., Haban, M., Baranec, T., Habanova, M., Knoll, M. 2006. The Occurrence of Puncture Vine (Tribulus terrestris L.) and its Metabolic Characteristics in Slovakia. Biologia, 61(1), 25-30
22. Smith, T., May, G., Eckl, V., Reynolds, C.M. 2020. US Sales of Herbal Supplements Increase by 8.6 % in 2019. Herbalgram, 127(1), 54-67
23. Song , Y.H., Kim, D.W., Curtis-Long, M.J., Yuk, H.J., Wang, Y., Zhuang, N., Lee, K.H, Jeon, K.S., Park, K.H. 2014. Papain-like protease (PLpro) inhibitory effects of cinnamic amides from Tribulus terrestris fruits. Biological and Pharmaceutical, 37(6), 1021-1028
24. Swaminathan, K, Dyason, J, Maggioni, A., von Itzstein, M., Kevin, M. Downard, K.M. 2013. Binding of a Natural Anthocyanin Inhibitor to Influenza Neuraminidase by Mass Spectrometry. Analytical and Bioanalytical Chemistry. (405), 6563–6572
25. Wicks, S.M., Salamon, I., Calderon, A.I., de Blanco, E.J.C., Mahady, G.B., 2018. Sarcopenia, Diabetes, and Nutritional Intervention. In Nutritional and Therapeutic Interventions for Diabetes and Metabolic Syndrome; Elsevier Science Publishing Co. Inc.: San Diego, United States, 279–292, ISBN 978-0-12-812019-4.
26. Wilks, S,, de Graaf, M., Smith, D.J., Burke, D.F. 2012. A Review of Influenza Hemagglutinin Receptor Binding as it Relates to Pandemic Properties. Vaccine. 30(29), 4369-4376
27. Wu, H., Johnson, M.C., Lu, C.H., Fritsche, A.L., Thomas, Z.C., Greenlief, C.M. 2015. Determination of Anthocyanins and Total Polyphenols in a Variety of Elderberry Juices by UPLC-MS/MS and other Methods. Acta Horticulturae, 1061. 43-51
28. Wu, H., Nardone, A., Lacetera, N. 2009. Effects of a Standardized Purified Dry Extract from Echinacea angustifolia on Proliferation and Interferon Gamma Secretion of Peripheral Blood Mononuclear Cells in Dairy Heifers. Research in Veterinary Science. 87(3), 396-398
29. Zakay-Rones, Z., Varsano, N., Zlotnik, M., Manor, O., Regev, L., Schlesinger, M., Mumcuoglu, M. 1995. Inhibition of Several Strains of Influenza Virus in vitro and Reduction of Symptoms by an Elderberry Extract (Sambucus nigra L.) during an Outbreak of Influenza B Panama. Journal of Alternative Complementary Medicine. 1(4), 361-36

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Prevention of Viral Effect and Enhancement of Immune System with the help of Herbal Plants and Himalayan Crude Drugs in SAR-COV-2 Patient: A Review

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Abstract

After December 2019, Severe Acute Respiratory Syndrome (SAR-COV-2) become a life-threatening issue to the entire human society when it started to spread exponentially all over the world from Wuhan city of China. The virus directly hits the upper and lower respiratory tract of human airways and causes severe damage to the human lung, leading to multiorgan failure, hypoxemia, and dyspnea. Similarly, studies revealed that SAR-COV-2 severely hit the younger and aged population in which the immune system is seriously compromised. The cell of the immune system such as T-cells, B-cells, NK cells, etc. helps to fight against such viral antigen and resist critical viral damage. Therefore, enhancement of the immune system could also be an effective approach to prevent viral infection and even aid in the reduction of the death count. Nowadays, dietary, and herbal remedies are being integrated into the mainstream of the healthcare systems because of their multi-ingredient character, and some of them are known to render efficacy comparable to that of synthetic drug substances. Several studies have also revealed the immune enhancement, the immunomodulatory and antiviral activity of these herbal products in SAR-COV-2 patients. This study was carried out by using Google Scholar, Web of Science, PubMed, Science direct to search the literature related to the use of the herbal product and their actively isolated compound to fight against SAR-COV-2. The study has highlighted the role of several active phytoconstituents present in Himalayan crude drugs that helps to reduce the viral titer in the respiratory system as well as aid in immune enhancement.

Key Words: Severe Acute Respiratory Syndrome (SAR-COV-2), Immune enhancement, Human respiratory system, Herbal medicine, and Himalayan crude Drugs, Phytoconstituents

1. Introduction

After the first diagnosis of coronavirus patient in Wuhan China in December 2019, Severe Respiratory Syndrome Coronavirus 2 (SAR-COV-2) has spread exponentially all over the world and became a life-threatening issue to entire human society. Though the disease was expected to spread from the bat at the beginning, the intermediate host for the disease is still unclear. WHO declared the disease a pandemic on 11th March 2020. The clinical sign and symptoms of the patient suffering from SAR-COV-2 range from asymptomatic

cases to severe acute respiratory distress syndrome, multi-organ failure (MOF), hypoxemia, dyspnea, severe pneumonia, etc. (Infusino et al., 2020). SAR-COV-2 is a single-stranded positive-sense RNA virus that shares a genomic sequence similarity of 96% with bat coronavirus and 79.6% sequence identity to SAR-COV. Upon entry into the human cells through Angiotensin Converting Enzyme (ACE2), and less likely by TMPRSS2, SAR-COV-2 takeover the protein synthesis pathway of human cells, start synthesizing viral protein, and assemble the protein for subsequent viral replication (Chen et al.). This virus directly hits the upper and lower respiratory tract of human airways and causes severe damage to the human lung. Damage to the alveolar cell causes inefficient exchange of oxygen and carbon dioxide between blood and lungs leading towards the shortage of oxygen in human blood. This is the main reason behind hypoxemia, dyspnea, MOF as well as death in SAR-COV-2 patients (Chilvers et al., 2001).

Currently, many nations are showing their best effort for the implementation of appropriate preventive and control measures. Neither vaccines nor direct anti-viral drugs are well established still now for the treatment of SAR-COV-2 in humans and animals (Wang et al., 2020). Though few attempts have been made by different American Pharmaceutical and biotechnological companies like Pfizer (Pfizer vaccine), Oxford University (AstraZeneca), their efficacy cannot last for long, shows multiple side effects, and patient even after vaccination also caught by coronavirus disease (Hunter et al., 2021, (Chagla et al., 2021). Similarly, in the late 2020s, after the recognition of mutant type SAR-COV-2 virus in the UK, the development of pharmaceutical remedies for treating COVID19 is becoming more challenging (Wise et al., 2020). Therefore, until the discovery of effective drugs and vaccines against coronavirus, complementary and alternative therapeutic approach with the use of herbal product, Himalayan crude drug, dietary remedies etc. should be encouraged for the prevention and control of disease. Traditional herbal remedies help to enhance the person's immunity, keep in check the symptoms of COVID19, and sometimes even showed curative effects as well. Government of Pakistan have urged their citizen to use garlic, turmeric, ginger, cinnamon, black pepper, and honey as home remedies. On the other hand, Bangladesh news suggested the public consume warm water with ginger and clove extracts, black cumin seeds, honey and fruit with vitamin C, etc. (Azam et al., 2020). Various primary and secondary metabolic products from plants are well known for different pharmacological actions. Phenol, flavonoid, saponin, terpenoids, glycosides, tannins, polysaccharides, alkaloids, etc. are some secondary metabolites that can be used effectively in the prevention and cure of various bodily ailment. Based on these, the pandemic world due to COVID-19 is even encouraged towards the use of herbal and dietary products. This review provides a brief overview of the use of the traditional herbal product and Himalayan crude drugs to fight against SAR-COV-2. The review is more focused on highlighting the mechanism of action of some isolated active chemical constituents against SAR-COV-2 from Himalayan crude drugs.

2. Methods

Data related to medicinal plants, their active constituents, mechanism of action were

obtained using different search engines including Web of Science, PubMed, Google Scholar, and Science direct. The reference article includes only those that were published in English-language journals and meet required quality standards concerning the information. During the study, only those medicinal plants which are being commonly used as local and traditional medicine for the treatment of various ailments were selected. Different keywords which were used for data search include, 'herbal and dietary remedies', 'COVID-19', 'immunomodulatory action', 'anti-viral activity, and 'active phytoconstituents'. The data were organized and analyzed using Microsoft Excel (2010) software and then summarized into tables and figures.

Severe Acute Respiratory Syndrome Coronavirus 2 (SAR-COV-2)**:** *Structure and Genomic*: Coronaviruses have four genera: alpha(α), beta(β), gamma (γ), and delta (δ) of which SAR-COV-2 belong to the β-coronavirus family. This novel coronavirus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SAR-COV-2) is the cause of Coronavirus disease 2019 (COVID-2019) and declared as a pandemic by World Health Organization (WHO) on March 11, 2020 (Astuti et al., 2020). SAR-COV-2 are enveloped, single-stranded RNA viruses whose surface is covered by protein spikes. The genome sequence identity of SAR-COV-2 is 79% identical with SAR-COV, 50% identical with MERS-COV, and genome organization is shared with other beta coronaviruses as well (Lu et al., 2020). Open reading frames (ORFs) having positive viral RNA genome organization in SAR-COV-2 code for structural and non-structural proteins. The structural protein is a spike (S), nucleocapsid (N), membrane (M), and envelop (E) proteins. The S1 subunit of S protein facilitates the attachment of the SAR-COV-2 virus with the host cell through Angiotensin Converting Enzyme (ACE2) receptor. The amino acid sequence of the S1 subunit involved in the viral attachment is glutamine, asparagine, leucine, phenylalanine, and serine. The S2 subunit promotes membrane fusion (Satarker et al., 2020). On the other hand, the viral genome of SAR-COV-2 is encapsulated by the N protein. The main role of N protein in SAR-COV-2 is to enhance the efficiency of viral RNA transcription and is even essential for viral replication. The nucleocapsid protein of human coronavirus is produced at a high level in the infected cell and is critically important for virion assembly (Savastano et al., 2020). Followingly, the M glycoprotein is the most abundant structural protein of SAR-COV-2. M protein binds to all other structural proteins and this binding helps in the stabilization of N proteins and promotes completion of viral assembly by stabilizing the N-protein RNA complex, inside the internal virion. Even the host cell entry of virus with the aid of S protein is mediated by the M protein (Bianchi et al., 2020 & Thomas et al., 2020).

Meanwhile, upon the viral entry into the host cell, the virus translates its replicase gene (ORF1) which is further divided into ORF1a and ORF1ab. At the end of ORF1a, a ribosome frame-shifting sequence consisting of two large polyprotein precursors of different lengths, pp1a and pp1ab are present. Polyprotein pp1a consists of non-structural proteins (Nsp) 1 -11, and polyprotein pp1ab comprises the complete translated coding region Nsp 1-16 (Ziebuhr et al., 2000 & Lee et al., 1991).

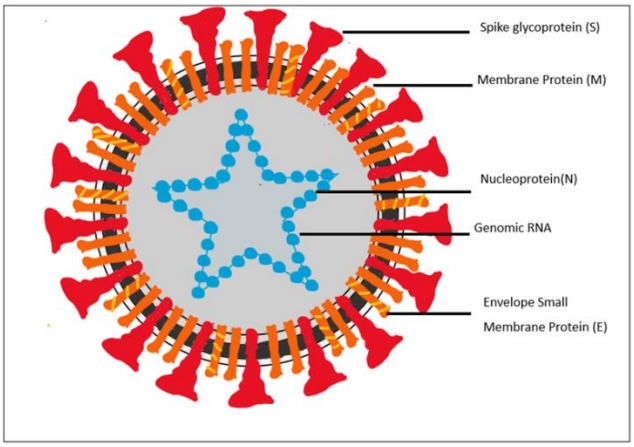

Figure 1. Severe Acute Respiratory Syndrome Coronavirus 2 (SAR-COV-2). (Astuti et al., 2020)

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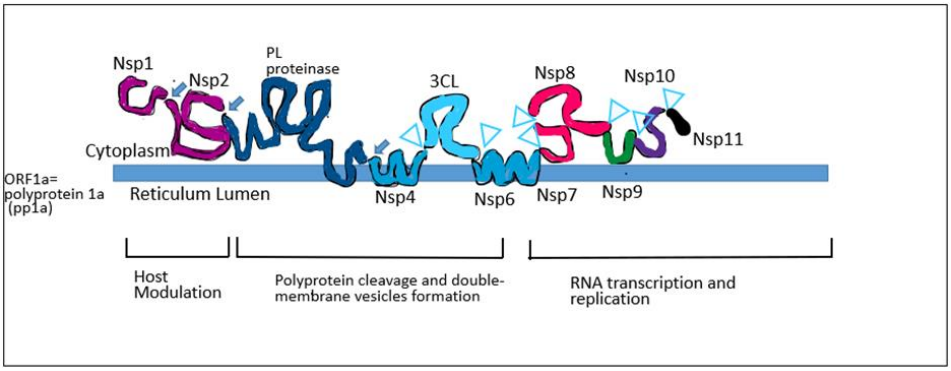


Figure 2: SAR-COV-2 polyproteins ORF1a. (Roe et al., 2021)

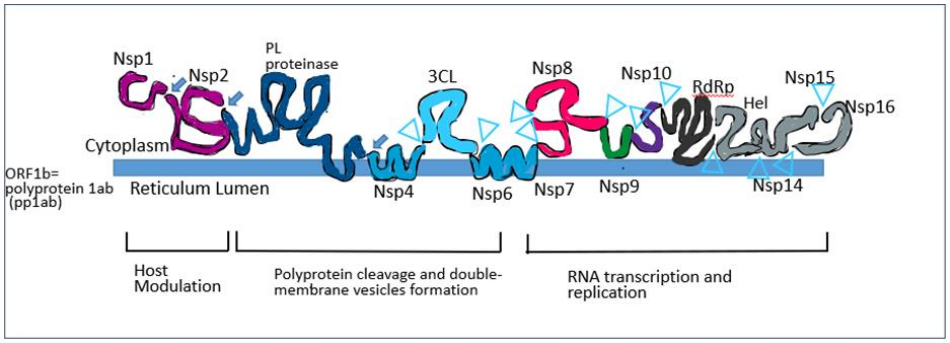


Figure 3: SAR-COV-2 polyproteins ORF1b (Roe et al., 2021)

The complete set of 16 Nsp of pp1ab is mainly divided into three categories. Nsp1 and Nsp2 are categorized for Host modulatory function. Nsp3 to Nsp6 are categorized for their polyprotein cleavage and double-membrane vesicle formation role. Lastly, Nsp7 to Nsp16 are categorized for their RNA transcription and replication function. papain-like proteinase (Nsp3), 3C-like proteinase (Nsp5), and helicase(Nsp13) play a major role in viral transcription and replication as these proteins help in the cleavage of the viral polyprotein (Roe et al., 2021).

Figure 2 shows that the Open reading Frame (ORF1a) in SAR-COV-2 is proteolytically cleaved into 11 putative non-structural proteins (Nsps). Nsp1 and Nsp2 are Hosts

modulating genomic components, while Nsp3 to Nsp6 are polyprotein cleavage and double-membrane vesicles forming components. Similarly, Nsp7 to Nsp11 are RNA transcription and replication components. And Figure 3 shows that the Open reading Frame (ORF1b) in SAR-COV-2 is proteolytically cleaved into 16 putative non-structural proteins (Nsps). Nsp1 and Nsp2 are Hosts modulating genomic components, while Nsp3 to Nsp6 are polyprotein cleavage and double-membrane vesicles forming components. Similarly, Nsp7 to Nsp16 are RNA transcription and replication components.

Animal host and spillover: In December 2019, SAR-COV-2 was firstly recognized in Wuhan city, China. Those patients were found to be exposed to live animals such as poultry, bats, snakes, frogs, rabbits, marmots, hedgehogs, etc. during their work in the Huanan seafood wholesale market suggesting these as possible zoonotic spillover (Malik et al., 2020, Tiwari et al., 2020). SAR-COV-2 firstly reported to be originated from bats but still different animal species such as snake, pangolin, and turtle were suggested as potential intermediate hosts, however, the pangolin is even highly suggested among them. The exponential mutation rates of RNA viruses ease them to get adapted to a wide range of hosts. Though a bat is taken as the natural host of the SAR-COV-2 virus, the intermediate host is still unclear, and to interrupt the transmission chain, it is of utmost importance to identify the potential intermediate host. Few studies demonstrate Pangolin as an intermediate host while recently SAR-COV-2 infection is reported in cats, dogs, minks, tigers lions, mice, non-human primates, and tree shrews (Zhao et al., 2020 & Oreshkova et al., 2020).

Receptor and Pathogenesis: Among four structural proteins in the SAR-COV-2 structure, the attachment and entry inside the host cell are mediated by Spike protein (S). The cellular furin-like proteases such as transmembrane protease serine 2 (TMPRESS 2), furin, and cathepsins cleave the S protein into two distinct polypeptides S1 and S2 which is also known as S protein priming (Hoffmann et al., 2020). S1 is known to attach to the cellular receptor ACE2 and S2 leads to the fusion of viral and cellular membrane (Robson et al., 2020). Upon receptor binding, spike protein triggers the fusion of viral and cellular membranes through proteolytic cleavage (Glowacka et al., 2011). Polyprotein pp1a and polyprotein pp1ab are proteolytically processed by virus-encoded proteases which aid in the achievement of mature and functionally active replication machinery of the virus (Ziebuhr, Snijder, et al., 2000). Followingly, the modulation of host cell factors and preparation of cells for viral RNA synthesis could be achieved through the proteolytically processed polyprotein. Meanwhile, C-terminal translation products of pp1ab largely catalyze and or regulate the processes of RNA replication and transcription which is mediated by the virus RNA-dependent RNA polymerases, RdRp (Nsp12) (Fehr et al., 2015, (De Wilde et al., 2017). On the other hand, Nsp3, 4, and 6 mediate the configuration of replication complexes which help in the formation of membrane structures such as double-membrane vesicles and convoluted membrane vesicles. The active replication complex even promotes the continuous and discontinuous synthesis of negative-sense

RNA templates which subsequently ease the formation of genomic copies and a nested set of subgenomic RNA (Sawicki et al., 2007). Replication of genomic and subgenomic RNA on double-membraned vesicles leads to the formation of structural protein of SAR-COV-2 such as S protein, E protein, M protein is glycosylated before localizing to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and are assembled into virions (Vennema et al., 1990, Krijnse-Locker et al., 1994 & Haan et al., 2005). The N protein which encapsidates the newly made RNA will be localized within the cytoplasm. As the completion of RNA synthesis, genomic RNA and N protein move to the ERGIC and assimilate into budding virions (Siu et al., 2008). Followingly, S protein which is expressed on the cell surface triggers cell-cell fusion between infected and nearby uninfected cells. Consequently, massive, multinucleated cell complexes called syncytia are often formed which facilitate the spread of the virus. This is how the pathogenesis of SAR-COV-2 is often expressed (Gallagher et al., 1992).

Effect of SAR-COV-2 in human Respiratory system: The respiratory system which consists of a network of organs easing in breathing includes mainly airways, lungs, and blood vessels. These parts work in coordination actually to move oxygen throughout the body and clean out waste gases like carbon dioxide (Sharma et al., 2006). On the other hand, the respiratory system is classified roughly as the upper and lower respiratory tract. The upper respiratory tract includes the nose, pharynx, and larynx while the lower respiratory tract includes the trachea, bronchial tree, and lungs. Both tracts are open to the outside and are lined with mucous membranes (Krause et al., 2017). SAR-COV-2, Severe acute respiratory syndrome by its name directly hit the respiratory system of the human body. The effect of SAR-COV-2 on the human respiratory system is well known and highly concerned fact in this pandemic era. The major target of this virus is in the epithelial cell of the respiratory system which finally leads to acute respiratory distress syndrome (ARDS). In most cases, the disease occurs as fever and without any respiratory symptoms at the beginning. However, as the disease progress, various degree of pulmonary abnormalities later appear in all patients due to severe damage in the lung tissue and alveoli (Gu et al., 2007). In the respiratory tract, epithelial stem cells are divided into epithelial cells in the distal airway and pulmonary alveolar epithelial cells. Two types of differentiated epithelial cells made the pulmonary alveolar epithelial cells: alveolar type 1 (AT1) cells, which mediate gas exchange, and alveolar type 2 (AT2) cells which secret surfactant. On the other hand, basal cells in the proximal airways give rise to multiple cell types such as secretory club cells and ciliated cells (Zacharias et al., 2018 & Nabhan et al., 2018). Other different cells present in the airway are bronchioalveolar stem cells, club cell-like stem cells, and p63+ basal cells, etc. Infection in this alveolar and distal epithelial cell leads to a defect in lung regeneration capacity and the hit in a ciliated and secretory epithelial cell leads to the death in the lungs cell due to the scarcity of mucus (Basil et al., 2020, (Wang et al., 2020). Though studies demonstrate that ACE2 and TMPRSS2 are present in nasal and bronchial epithelium cells by immunohistochemistry, gene expression of ACE2 and TMPRSS2 have been reported to occur largely in alveolar epithelial type II cells. Therefore, the lungs

are the central part of SAR-COV-2 pathogenesis as compare to the upper respiratory tract (Wu et al., 2020 & Lin et al., 2020).

Role of Human Immune System to Fight against SAR-COV-2: The immune system of our body is developed in such a way that it protects us against diseases, harmful substances, germs, and cell changes. The key player white blood cells travel throughout the body through the blood vessel. The cell of the immune system can be classified as lymphocytes (which contain T-cells, B-cells, and NK cells), neutrophils, and monocytes/macrophages (Notarangelo et al., 2004). For examining invading microbes, the interchange of cells and fluids between blood and lymphatic vessels persist in which the lymphatic system as well in the immune response plays a significant role. Specialized compartments present in the lymph nodes encounter antigens and when such antigens are present in the bloodstream, they are transported to tissues throughout the body. But the immune cells gather and serve to dispose of such antigen and contribute to the prevention of the body from various diseases and ailments (Chowdhury et al., 2020). The three major types of immunity are innate immunity with rapid response, adaptive immunity with slow response, and passive immunity.

As the SAR-COV-2 virus gain access inside the target cell, the whole virus or its surface epitopes is recognized which causes innate or adaptive immunity to respond. Toll-like receptors 3, 7, and 8 firstly recognize the virus inducing the higher production of Interferon (INF) (Le Bon et al., 2002 & Thiel et al., 2008). With the invasion of the host cell by SAR-COV-2, the early response against the N protein is shown by the B cell, while antibodies against S protein could be detected after 4-8 days from the appearance of initial symptoms. The study demonstrated that SAR-COV-2 specific IgA, IgG, and IgM antibodies were detected after the onset of symptoms at different time points in infected patients. IgG level was revealed to persist for a longer period while IgM level is assumed to decline after 3 months (Li et al., 2008). On the other hand, Memory T cells are also a part of the immune system which are generated and designed to fight against re-infection. $CD4^+$ memory T cells ease in restimulation of B cells and other immune cells by cytokine production while $CD8^+$ cytotoxic memory T cells destroy the infected cell during subsequent infection (Stockinger et al., 2006). Meanwhile, $CD4^+$ helper T cells (Th) have two subtypes: Type 1 helper T cells (Th1) and Type 2 helper T cells (Th2). The pro-inflammatory cytokines may stimulate the Th1 cell response while specific generations of related cytokines like IL-4 and IL-10 divert the response towards the Th2 response (Velazquez-Salinas et al., 2019). Other parts of immunity system which are involved to fight against SAR-COV-2 are dendritic cell, phagocytes, NK cells, macrophages, Neutrophils, Eosinophils, Basophils, Monocytes, Lymphocytes, etc. (Chowdhury et al., 2020). Therefore, above all, it is necessary to boost our immune system and strengthen it to make it able to fight against SAR-COV-2 by preventing its entry into the host cell or minimizing invasion or destruction of host cell after its entry inside the cell.

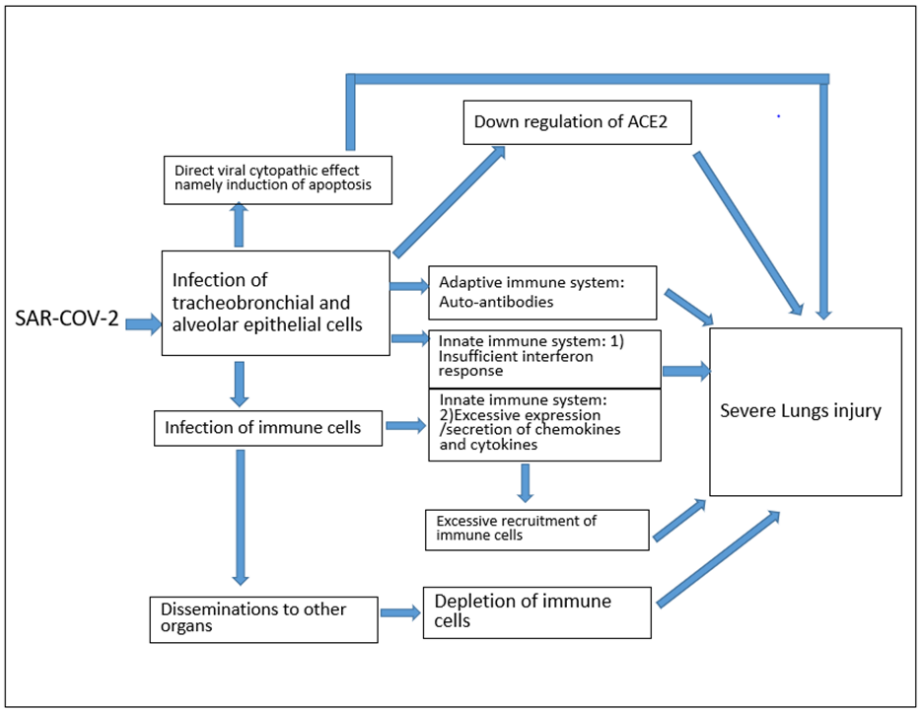


Figure 4: Flow chart showing the effect of SAR-COV-2 in the human respiratory system and human immune system. (Gu et al., 2007, Chowdhury et al., 2020).

Dietary and Herbal medicine: Recently dietary and herbal medicine is gaining widespread popularity all over the world and moving to get integrated into the mainstream of the healthcare systems (Bent et al., 2008). The major reason for the increasing interest in herbal therapy is low cost, wide acceptance of public for being included in natural product which claims itself as low toxic, flexibility in its accessibility, preparation, and use (Builders et al., 2019). Medicine from herbal products usually contains a variety of pharmacologically active compounds and this multi-component character of herbal medicines can render efficacy sometimes comparable to that of synthetic drug substances (Mosihuzzaman et al., 2012). Plants and herbs produce various chemicals for their metabolic activities and to protect themselves from various diseases and predators. Primary metabolites produced by them are carbohydrates, fats, amino acids, vitamins, hormones, etc. are utilized by plants for growth, development, stress adaptation, defense. On the other hand, herbal and medicinal plants even produce many secondary metabolites which can be utilized for therapeutic efficacy to treat various sort of ailments. The major class of secondary metabolites which could be utilized to treat disease and ailments are alkaloids, phenolics, flavonoids, terpenoids, and glycosides (Izhaki et al., 2002). Use of herbal medicine is taken for a wide range of marked advantages, such as energy and memory improvement, treatment of a specific condition such as glucose-lowering effect, antibacterial, anticancer, anticonvulsant, anti-inflammatory, antifungal, antiviral, and for various other diseases and ailments (Tyagi et al., 2003). Recently, SAR-COV-2 hit the global world destructively but medicine and vaccine for the treatment of viruses are not well defined and still in the phase of development. In this pandemic world, dietary and herbal remedies are nowadays being taken as

appropriate options to boost the immune system as well as to fight against the effect of SAR-COV-2 in the human respiratory system (Panyod et al., 2020).

Dietary and Herbal Remedy for Immune Modulatory activity against SAR-COV-2: Several studies demonstrated the efficacy of herbal medicine as immunomodulatory and antiviral activities for influenza virus as well as SAR-COV-2. Food and herbs aid as a dietary or complementary therapy to stave off septicemia and reinforce immunity such as antiviral agents for masks, as disinfectants to curb aerosol transmission, or as sanitizing agents to disinfect surfaces (Panyod, Ho et al., 2020). Studies demonstrated that various natural compounds can be utilized to suppress viral-mediated cytokine production, tissue destruction as well as production of excessive inflammatory infiltrates. Of them, phenolics, polysaccharides, quinones, alkaloids, and terpenoids are shown as promising immunomodulatory phytochemicals (Wang et al., 2008 & Albulescu et al., 2020).

Phenolics: A study by Traboulsi et al. 2015, demonstrated that liquiritigenin (ILG), a phenolic compound retards the pronouncement of inflammatory cytokines which was induced after the infection of a cell with influenza virus. The study even demonstrates that the anti-inflammatory activity of ILG is dependent more on the stimulation of the peroxisome proliferator-activated receptor-gamma pathway (Traboulsi et al., 2015). In another study by Zu et al., 2012, it was demonstrated that theaflavins fractions derived from black tea has been found to exhibit a potent inhibitory effect against the influenza virus in vitro. The study revealed that among the various mechanisms for the inhibition against influenza virus, decrement in the expression level of the inflammatory cytokine Intraluekin-6 by theaflavin fraction is a potent cause (Zu et al., 2012). Similarly, quercetin, kaempferol, and some other derivatives obtain from bee pollen aid to fight against COVID-19 as an immune enhancer (Rzepecka-Stojko et al., 2015).

Polysaccharides: Several natural foods are being explored to obtain polysaccharides which help in immune-boosting and immune modulation as well as in reducing the damage caused by SAR-COV-2. The use of food rich in polysaccharides is a possible option to upsurge immunity and reduce the risk accompanied by SAR-COV-2 contamination. Still, the use of polysaccharides as a therapeutic strategy is not yet well established but application as dietary and food supplements is a rational possibility (Wang et al., 2020). peng et al. 2019, performed a study about the immunostimulatory activity of water-soluble polysaccharides which are derived from *Citrus medica L.var. sarcodactyis* and the study demonstrated that the active polysaccharide component isolated from the plant extract showed immune-enhancing ability (Peng et al., 2019). The immune system of our body is known to be stimulated through a bond between functional groups and molecular groups on the cell surface. The initiation of the signaling pathway is from the moment polysaccharides bind to the membrane receptors in the defense cells. This causes the cycle of biochemical reaction to getting activated regulating gene expression in the ribosomes initiating protein production (Barbosa et al., 2020). Similarly,

polysaccharides even modulate the activity of gene expression. Some polysaccharides aid in the assembly and expression of messenger RNA during nitric oxide production and pro-inflammatory cytokines (Barbosa et al., 2020). In a study conducted by Shen et al. 2017, wheat obtained polysaccharides show that they can promote cytokine expression through the MAP38 p38 signaling pathway which is negotiated by Toll-like receptor 4 (Shen et al., 2017). In another study by Li et al. 2020, a fungus Ganoderma lucidum was studied for immune-modulatory function and the structure-activity relationship was studied with two characterized fractions i.e., GLP-1 and GLP-2 of Ganoderma lucidum polysaccharides. Among two fractions GLP-1 was proved to be potent with better immune response and promotion of production of Immunoglobulin A (Li et al., 2020).

Alkaloids: Another group of secondary metabolites which shows antiviral potential against coronaviruses is the alkaloids. In a study by Dong et al. 2019, the antiviral property of Stephania tetrandra was studied and from the study, it was revealed that benzylisoquinoline alkaloids tetrandrine, fangchinoline, and cepharanthine are proved to be potent antiviral agents for the prevention and treatment of human coronavirus infection as well as showed beneficial immunomodulation (Dong et al., 2019). In another study by Abdulrahman et al. 2020, *Nigella sativa* L was studied to explore the potency of this plant for hitting SAR-COV-2 targets and from the insilico study, it was demonstrated that few compounds like nigelledine, hederagenin, thymohydroquinone, and thymoquinone had high to moderate affinity with SAR-COV-2 enzymes and proteins. Coupling of SAR-COV-2 to host cell receptors and duplication is dramatically inhibited by these compounds (Koshak et al., 2020).

***Terpenoids*:** The Terpenoid group of compounds are also known as an immune booster and enhances protection from viral replication and lowers levels of lung viral titers. In a study conducted by Yoo et al. 2012, Ginsenosides, triterpenic saponins, and other active compounds with different pharmacological effects were studied. From the study, it was demonstrated that red ginseng extracts containing a terpenoid group of compounds significantly enhanced protection, lessen the level of viral load in the lungs (Yoo et al., 2012). Similarly in another study by Goswami et al. 2018, methanolic extract of *Boswellia serrata* was examined to explore the antiviral activity. From the study, B-boswellic acid (BA), a pentacyclic terpenoid potently inhibits wild-type and a clinically isolated type of Herpes Simplex Virus. From the study, the mechanism of inhibition is significant downregulation of NF-kB and p38 MAP kinase which are directly related to the immune response of the human system (Goswami et al., 2018). Few other studies have also displayed the modulation of the NF-Kb pathway of virus due to plant extract. Pterodontic acid, a type of sesquiterpene compound isolated from *Laggera periodontal* was also exemplified to have a broad-spectrum effect against different types of influenza viruses like a human (H1N1) and avian (H9N2) influenza virus. The major mechanism for such inhibition is the reduction of the inflammatory response by retarding activation of the NF-Kb pathway (Guan et al., 2017).

Dietary and herbal remedies for prevention and cure of SAR-COV-2 patients by acting in the viral enzymatic system: In the recent COVID-19 pandemic, still, the scientific world is struggling to find specific drugs and vaccines to overcome the threat to the global population. When such actual allopathic remedies are still under development, the promotion of the application of crude herbal remedies to fight against COVID-19 is of utmost importance. Meanwhile, many studies have even reported that foods and herbs could be used as complementary and dietary therapy to prevent infection, strengthen immunity and some of them still have strong antiviral properties as well (Panyod, Ho et al., 2020). Two viral proteases of SAR-COV-2, a chymotrypsin-like protease ($3CL^{pro}$) and papain-like protease (PL^{pro}) are attractive drug targets for the development of herbal remedies as an antiviral agent.

***Chymotrypsin-like protease ($3CL^{pro}$) and papain-like protease (PL^{pro}) Phenolics*:** In a study by Jo-young et al. 2016, polyphenols obtained from *Broussonetia papyrifera* were evaluated to determine the inhibitory activity against 3CLpro. Phenolic compounds like broussochalone B, broussochaloneA, 4-hydroxyisolonchocarpin, papyriflavonol A, kazinol J, etc. were isolated for the study. The study revealed that all extract is potent against 3CLpro and PLpro, though activity against PLpro was found more potent. Among the isolated compounds, 3' -(3-methyl but-2-enyl)-3',4,7-trihydroxyflavane was found to be more potent with an IC50 value of 3.7 μM (Park et al., 2017). In another study (Jo et al., 2020)., three flavonoids were studied for their inhibitory activity against 3CLpro by using the tryptophan-based fluorescence method, and from the study, it was determined that three flavonoids Herbacetin, rhoifolin, and pectolinarin are effective to block the enzymatic activity of SAR-COV-2 3CLpro Meanwhile, Ananta et al. 2020, have performed a study to determine phytochemicals as potent inhibitors against 3CLpro and PLpro. The study determined that out of 32 phytochemicals used in the study, amentoflavone and gallocatechin gallate exhibit the best binding affinity to 3CLpro and PLpro (Swargiary et al., 2020). The study performed by Nguyen et al. 2012, demonstrated that quercetin, epigallocatechin gallate, and gallocatechin gallate resemble good inhibition towards 3CLpro with IC50 values of 73, 73, and 47 μM respectively (Nguyen et al., 2012).

***Terpenes*:** Gideon et al. 2020, performed a study to determine potential terpenoids and alkaloids from the African medicinal plants which inhibit 3CLpro. The study revealed that it 6-oxoisoiguesterin and 22-hydroxyhopan-3-one group of terpenoid binds to the receptor tie-up locus and the catalytic duo of SAR-COV-2 $3CL^{pro}$ inhibiting the enzyme activity (Gideon et al., 2020). Meanwhile, in another study by Ji-Young et al. 2012, the experiment was performed to determine the inhibitory activity of Tanshinones, a diterpenoid compound derived from Salvia miltiorrhiza, and the study, determined tanshinones as good inhibitors of both 3CLpro and PLpro. Additionally, the inhibitory activity was found selective since the compound showed no inhibitory activity against other proteases (Park et al., 2012). Following, Wen et al. 2007, experimented to investigate the SAR-COV-2 inhibitory activity of specific plant terpenoids and liquids. The experiment revealed that betulinic acid and

savinin, abietane-type diterpenoids are competitive inhibitors of SAR-COV-2 3CL with K_i values of 8.2 mM and 9.1 mM (Wen et al., 2007).

***Hydroxanthracene derivatives*:** Luo et al. 2009, experimented with the extract of *Rheum palmatum* L. to determine the 3CLpro inhibitory activity of the plant sample and from the study, it was revealed that emodin, an anthracene derivative have 3CLpro inhibitory activity along with activity against S protein and ACE2 receptor protein (Luo et al., 2009).

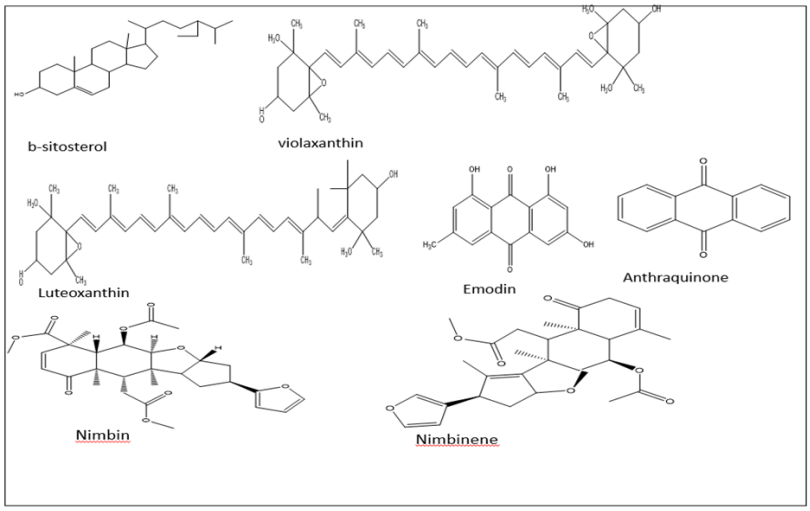
Table 1: List of Himalayan Crude drugs with their possible mechanism of action against SAR-COV-2

Herbs	Parts used	Active phytoconstituent	Mode of action	References
Lentinula edodes	Mycelium	α-glucan and β-glucan	α-glucan and β-glucan derived from this mushroom help inactivation of natural killer (NK) and T cells	(Dietologica et al., 2020)
Asparagus racemosus	Root	asparoside-C, asparoside-D and asparoside-F	An in-silico study showed that active constituents asparoside-C, asparoside-D, and asparoside-F showed potent inhibition against spike receptor-binding domain and NSP15 Endoribonuclease	(Chikhale et al., 2020)
Acorus calamus	Usually root		Help in the treatment of atopic dermatitis unusually seen in SAR-COV-2 patients	(Das et al., 2020)
***Aloe vera* L.**	Leaves	Aloin, aloe-emodin, acemannan	Aloin, aloe-emodin, acemannan interact with the viral enzyme and help in the breakdown of viral envelop protein	(Mpiana et al., 2020)
Azadirachta indica	Leaves and bark	Nimbin, nimbinene, nimbolide, nimbolinin, nimbandiol	Nimbin, nimbinene, Nimbolide, Nimbolinin, Nimbandiol from Azadirachta indica inhibits SAR-COV-2 main protease	(Umar et al., 2021)
Bauhinia variegata	Roots, bark, buds	Flavonoids, beta-sitosterol, lupeol	Flavonoids, beta-sitosterol, lupeol can be used as an immunomodulator and immune stimulant	(Mahalwar et al., 2021)
Camellia sinensis	Leaves	3-Isotheaflavin-3 gallate	3-Isotheaflavin-3 gallate inhibits Chymotrypin-like $3CL^{pro,}$ viral protein	(Boozari et al., 2020)
Canavalia ensiformis	Seed		Binds to the glycosylated membrane proteins and prevent target cell recognition and viral entry	(Greig et al., 1977)
Cannabis sativa	Leaves, seed, bark		Use in the modulation of expression of host ACE2 receptor protein and downregulate serine protease TMPRSS2	(Wang et al., 2020)
Cassia fistula	Fruit	Procyanidin B2	Procyanidin B2 isolated from this plant extract is identified as a protease inhibitor of SAR-COV-2	(Ravi et al., 2020)
Cordyceps Sinensis	Whole animal and fungal part		Alcoholic extract enhance immunity and shows immunomodulatory effect by inducing IFN-c and regulating T lymphocyte.	(Le et al., 2020)
Ganoderma lucidum	Mycelium		Enhance the immune system by upsurging the level of virus-specific antibodies and their neutralizing activities	(Panyod et al., 2020)
Houttunia cordata	Whole Plant		Increase the proportion of CD4+ and CD8+ T-cells. Increase the secretion of IL-2 and IL-10. Inhibitory effect in RNA-dependent RNA polymerase (RdRp) and SAR-COV 3C-like protease($3CL^{pro}$)	(Lau et al., 2008)
Justicia adhatoda	Leaves, flower buds	Vasicolinone and anisotine	Vasicolinone and anisotine from this plant helps in the inhibition of SAR-COV-2 by binding in the RNA dependent RNA polymerase	(Gowrishankar et al., 2021)
Momordica charantia	Fruit		Helps to minimize binding of spike protein to ACE2 receptor as well as improve immunity	(Desai et al., 2020)

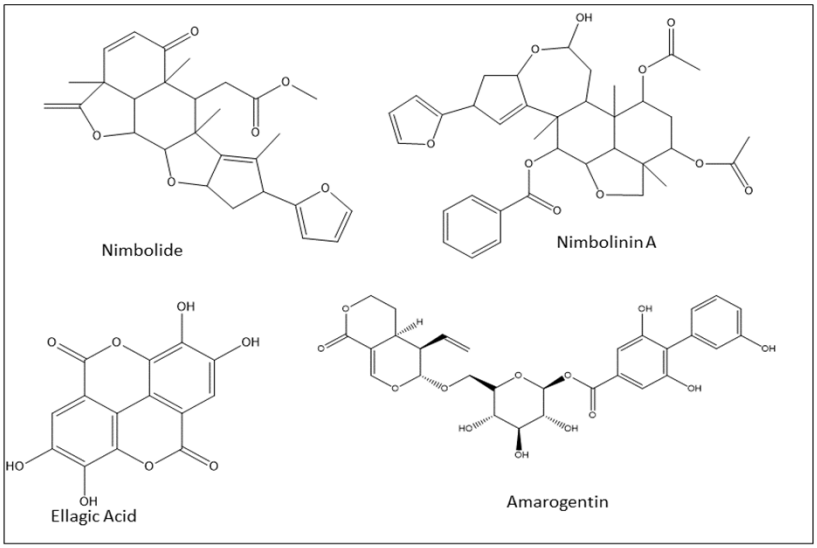
Table 2. List of Himalayan Crude drugs with their possible mechanism of action against SAR-COV-2

Herbs	Parts used	Active phytoconstituent	Mode of action	References
Ocimum sanctum	Leaves, small branches	Apigenin and Ursolic acid	Apigenin and Ursolic acid from this plant are known to improve respiratory parameter, enhance the level of Helper T cell and Natural Killer (NK) cells	(Srivastava et al., 2020)
Oroxylum indicum	Root, bark, and fruit	Oroxindin and scutellarein	Oroxindin and scutellarein help in SAR-COV-2 viral inhibition by acting in the Main protease (Mpro)	(Shah et al., 2021)
Phyllanthus Emblica	fruit	Ellagic acid	Ellagic acid stimulates the immune function and some other constituents are known for their inhibition of Spike receptor binding domain	(Arokiyaraj et al., 2020)
Picrorhiza scrophuulariiflora	Rhizome	Scrocaffeside A	Scrocaffeside A obtained from this plant significantly enhance the activity of peritoneal macrophages and natural killer cell boosting innate immunity	(Dongre et al.)
***Polygonum multiflorum* Thunb**	Root tubers and vines	Emodin and anthraquinone	Emodin and anthraquinone compounds derived from these plants significantly block the S protein and ACE2 interaction	(Ho et al., 2007)
Rauvolfia serpentina	Roots	Reserpine	Reserpine from Rauvolfia serpentina help in the inhibition of $3CL^{pro}$	(Krishna et al., 2020)
***Rheum officinale* Baill**	Root tubers	Emodin and anthraquinone	Emodin and anthraquinone compounds derived from these plants significantly block the S protein and ACE2 interaction	(Ho, Wu et al., 2007)
Rheum palmatum	Root, stem		Beneficially neutralize the binding of spike protein to the host ACE2 receptor. Subside the release of inflammatory mediators reducing lung injury.	(Hu et al., 2021)
Shilajit	Rock	Fulvic acid, humic acid, hippuric acid, and benzopyrones	Fulvic acid, humic acid, hippuric acid, and benzopyrones have immune-boosting as well as antiviral activity	(Saharan et al., 2021)
Swertia chirayata	Whole plant	Amarogentin	Amarogentin from this plant binds with the SAR-COV-2 spike glycoprotein and ACE2	(Maurya et al., 2020)
Terminalia chebula* and *Terminalia bellerica	Fruit		Highly use in strengthening immunity and improving cough in combination with *Phyllanthus emlica* as *Triphala churna*.	(Shankar et al., 2021)
Tinospora cordifolia	stem		Inhibition of SAR-COV-2 main protease as well as play role in immune enhancement	(Shree et al., 2020), (Kumar et al., 2020)
Urtica *dioica*	Rhizome, root, and leaves	B-sitosterol, luteoxanthin, and violaxanthin	B-sitosterol, luteoxanthin, and violaxanthin act as antiviral agent by binding to ACE2 receptor inhibitor	(Upreti et al., 2021)

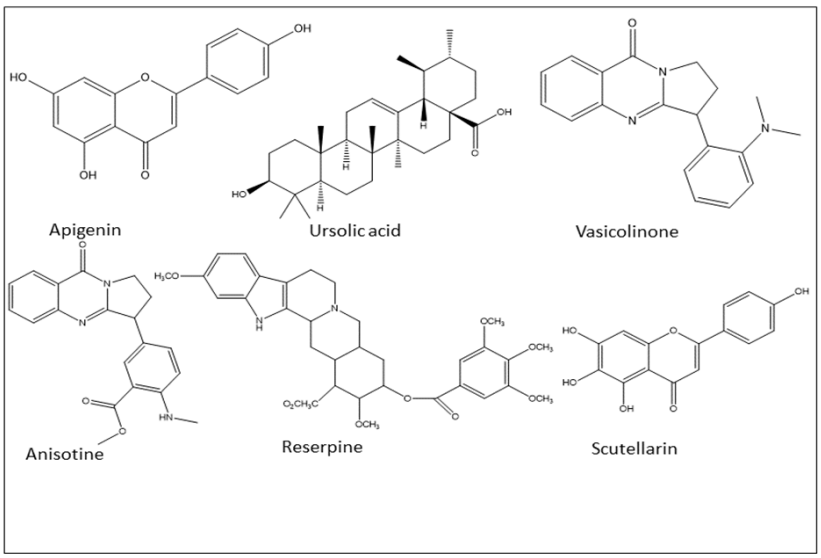
a.


b-sitosterol
violaxanthin
Luteoxanthin
Emodin
Anthraquinone
Nimbin
Nimbinene

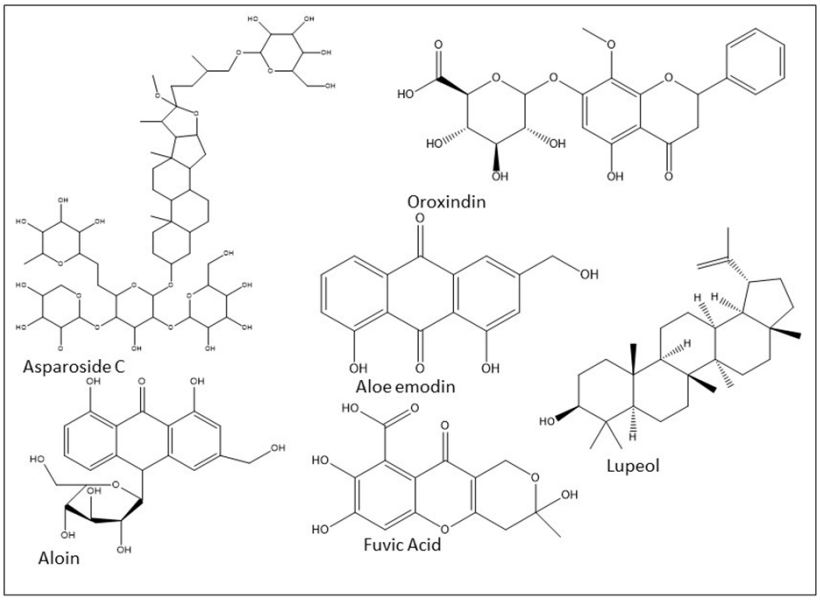
b.


Nimbolide
Nimbolinin A
Ellagic Acid
Amarogentin

c.


Apigenin
Ursolic acid
Vasicolinone
Anisotine
Reserpine
Scutellarin

d.



e.

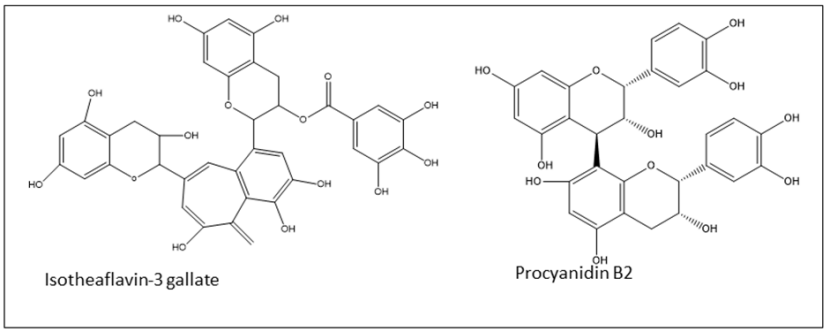


Figure 5. a. b. c. d. e. Chemical structures of active phytoconstituent against SAR-COV-2

Conclusion

A big population of indigenous and ethnic groups in different parts of the world is using Herbal medicine and Himalayan crude drug for the treatment of various bodily ailments and diseases. The use of these natural phytoconstituents is either for prevention and cure of disease or it plays the role of the enhancement of the human immune system to fight against such ailments. During the current situation of the SAR-COV-2 pandemic, herbal medicine could be consumed either as a diet or supplement to subside septicemia and upsurge immune power or it is applied as a supportive therapy along with known anti-COVID drugs to reduce viral titers in the upper and lower respiratory tract. Many active secondary metabolites present in these medicinal plants could be isolated and utilized against COVID-19 in various ways such as an inhibitor of the binding of spike protein to host cell ACE2 receptor, an inhibitor of virus PLpro, or 3CLpro, and many others. Similarly, SAR-COV-2 is proved to be deadly especially for the younger and older population whose immunity is compromised seriously. In such a population, Himalayan crude drug and herbal medicine could be utilized as an immune booster. In this regard, this review could be a mediator between modern allopathic therapy and traditional crude drug consumption which shows a strong impact on the capacity for the prevention and treatment of COVID-19.

Acknowledgments

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Conflict of Interest

The author declares no conflict of interest.

Author Contribution

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References

1. Infusino, F. et al. 2020. Diet supplementation, probiotics, and nutraceuticals in SARS-CoV-2 infection: a scoping review. Nutrients 12(6), 1-21. https://doi.org/10.1002/jmv.25681
2. Chen, Y.L. & Guo, D. 2020. Emerging coronaviruses: Genome structure, replication, parthenogenesis. Journal of Virology, 92(418423), 418-423. https://doi.org/10.1002/jmv.25681
3. Chilvers, M., et al. 2001. The effects of coronavirus on human nasal ciliated respiratory epithelium. European Respiratory Journal 18(6), 965-970. DOI: 10.1183/09031936.01.00093001
4. Wang, Z., Chen, X., Lu, Y., Chen, F. & Zhang, W. 2020. Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment. Bioscience trends. 14(2020), 64-68 https://doi.org/10.5582/bst.2020.01030
5. Hunter, P.R. & Brainard, J.S. 2021. Estimating the effectiveness of the Pfizer COVID-19 BNT162b2 vaccine after a single dose. A reanalysis of a study of'real-world'vaccination outcomes from Israel. Medrxiv doi: https://doi.org/10.1101/2021.02.01.21250957
6. Chagla, Z. 2021. The BNT162b2 (BioNTech/Pfizer) vaccine had 95% efficacy against COVID-19≥ 7 days after the 2nd dose. Journal of Internal Medicine, 174(2), JC15. https://doi.org/10.7326/ACPJ202102160-015
7. Wise, J. Covid-19: New coronavirus variant is identified in UK. British Medical Journal Publishing Group; 2020. https://doi.org/10.1136/bmj.m4857
8. Azam, M.N.K, Al Mahamud R., Hasan, A., Jahan. R., & Rahmatullah, M. 2020. Some home remedies used for treatment of COVID-19 in Bangladesh. 8(4), 27-32.
9. Astuti, I.J.D. 2020. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Journal of Diabetes, 14(4), 407-412. https://doi.org/10.1016/j.dsx.2020.04.020
10. Lu, R., et al. 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. 395(10224), 565-574. https://doi.org/10.1016/S0140-6736(20)30251-8
11. Satarker, S. & Nampoothiri, M. 2020. Structural proteins in severe acute respiratory syndrome coronavirus-2. Journal of Archives of medical research, 51(6), 482-491. https://doi.org/10.1016/j.arcmed.2020.05.012
12. Savastano, A., Opakua, A.I., Rankovic, M. & Zweckstetter, M., 2020. Nucleocapsid protein of SARS-CoV-2 phase separates into RNA-rich polymerase-containing condensates. Nature

communications 11(1), 1-10. https://doi.org/10.1038/s41467-020-19843-1
13. Bianchi, M., et al. 2020. Sars-CoV-2 Envelope and Membrane proteins: differences from closely related proteins linked to cross-species transmission. BioMed Research International 2020 (4389089), 1-6. https://doi.org/ 10.1155/2020/4389089
14. Thomas, S. 2020. The structure of the membrane protein of SARS-CoV-2 resembles the sugar transporter semiSWEET. Journal of Pathogens andImmunity, 5(1), 342-363. https://dx.doi.org/10.20411%2Fpai.v5i1.377
15. Ziebuhr, J., Snijder, E.J. & Gorbalenya, A.E. 2000. Virus-encoded proteinases and proteolytic processing in the Nidovirales. Journal of Microbiology, 81(4), 853-879.
16. Lee, HJ., et al. 1991. The complete sequence (22 kilobases) of murine coronavirus gene 1 encoding the putative proteases and RNA polymerase. Journal of Virology, 180(2), 567-582. https://doi.org/10.1016/0042-6822(91)90071-I
17. Roe, M.K, Junod, N.A., Young, A.R., Beachboard DC & Stobart CC. 2021. Targeting novel structural and functional features of coronavirus protease nsp5 (3CLpro, Mpro) in the age of COVID-19. Journal of General Virology 102(001558), 1-14. https://doi.org/10.1099/jgv.0.001558
18. 2019. A Novel Coronavirus from Patients with Pneumonia in China. The New England journal of medicine, 382, 727-733. DOI: 10.1056/NEJMoa2001017
19. Malik YS, et al. 2020. Emerging coronavirus disease (COVID-19), a pandemic public health emergency with animal linkages: current status update. doi: 10.20944/preprints202003.0343.v1
20. Tiwari R, et al. 2020. COVID-19: animals, veterinary and zoonotic links. Journal of Veterinary 40(1), 169-182. https://doi.org/10.1080/01652176.2020.1766725
21. Zhao J, Cui W & Tian B-p. 2020. The potential intermediate hosts for SARS-CoV-2. Frontiers in microbiology 11, 602-611 DOI: 10.1002/ jmv.25731
22. Oreshkova N, et al. 2020. van der; Stegeman, J. SARS-CoV2 Infection in Farmed Mink, Netherlands doi: https://doi.org/10.1101/2020.05.18.101493
23. Hoffmann M, et al. 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell, 181(2), 271-280. e278. https://doi.org/10.1016/ j.cell.2020.02.052
24. Robson B. 2020. COVID-19 Coronavirus spike protein analysis for synthetic vaccines, a peptidomimetic antagonist, and therapeutic drugs, and analysis of a proposed achilles' heel conserved region to minimize probability of escape mutations and drug resistance. Computers in biology medicine, 121(103749) 1-27. https://doi.org/10.1016/j.compbiomed.2020.103749
25. Glowacka I, et al. 2011. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. Journal of virology, 85(9), 4122-4134. https://dx.doi.org/10.1128%2FJVI.02232-10
26. Fehr AR & Perlman S. 2015. Coronaviruses: an overview of their replication and pathogenesis. Coronaviruses 1-23.
27. De Wilde AH, Snijder EJ, Kikkert M & van Hemert MJ. 2017. Host factors in coronavirus replication. Roles of host gene non-coding RNA expression in virus infection1-42.
28. Sawicki SG, Sawicki DL & Siddell SG. 2007. A contemporary view of coronavirus transcription. Journal of virology, 81(1), 20-29. https://dx.doi.org/10.1128%2FJVI.01358-06
29. Vennema H, Heijnen L, Zijderveld A, Horzinek M & Spaan W. 1990. Intracellular transport of recombinant coronavirus spike proteins: implications for virus assembly. Journal of virology, 64(1), 339-346.
30. Krijnse-Locker J, Ericsson M, Rottier P & Griffiths G. 1994. Characterization of the budding compartment of mouse hepatitis virus: evidence that transport from the RER to the Golgi complex requires only one vesicular transport step. The Journal of cell biology,124(1), 55-70. https://doi.org/10.1083/jcb.124.1.55
31. de Haan CA & Rottier PJ. 2005. Molecular interactions in the assembly of coronaviruses. Advances in virus research, 64, 165-230. https://doi.org/10.1016/S0065-3527(05)64006-7
32. Siu Y, et al. 2008. The M, E, and N structural proteins of the severe acute respiratory syndrome coronavirus are required for efficient assembly, trafficking, and release of virus-like particles. Journal of virology, 82(22), 11318-11330. https://dx.doi.org/10.1128%2FJVI.01052-08
33. Gallagher TM, Buchmeier MJ & Perlman S. 1992. Cell receptor-independent infection by a neurotropic murine coronavirus. Journal of Virology, 191(1), 517-522. https://doi.org/ 10.1016/0042-6822(92)90223-C
34. Sharma G & Goodwin J. 2006. Effect of aging on respiratory system physiology and immunology. Clinical interventions in aging, 1(3), 253-260. https://dx.doi.org/10.2147%2Fciia.2006.1.3.253
35. Krause R, et al. 2017. Mycobiome in the lower respiratory tract–a clinical perspective. 7(2169) 1-9. https://doi.org/10.3389/fmicb.2016.02169
36. Gu J & Korteweg C. 2007. Pathology and pathogenesis of severe acute respiratory syndrome. The American journal of pathology,

170(4), 1136-1147. https://doi.org/10.2353/ajpath.2007.061088
37. Zacharias WJ, et al. 2018. Regeneration of the lung alveolus by an evolutionarily conserved epithelial progenitor. Natural Product Communications, 555(7695), 251-255. http://www.nature.com/doifinder/10.1038/nature25786
38. Nabhan AN, Brownfield DG, Harbury PB, Krasnow MA & Desai TJ. 2018. Single-cell Wnt signaling niches maintain stemness of alveolar type 2 cells. Journal of Science, 359(6380), 1118-1123. DOI: 10.1126/science.aam6603
39. Basil MC, et al. 2020. The cellular and physiological basis for lung repair and regeneration: past, present, and future. Cell, 26(4), 482-502. https://doi.org/10.1016/ j.stem.2020.03.009
40. Wang Q, et al. 2020. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell, 181(4), 894-904. https://doi.org/10.1016/j.cell.2020.03.045
41. Wu C, Zheng S, Chen Y & Zheng M. 2020. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCoV, in the nasal tissue. MedRxiv. https://doi.org/10.1101/2020.02.11.20022228
42. Lin W, et al. 2020. Single-cell analysis of ACE2 expression in human kidneys and bladders reveals a potential route of 2019-nCoV infection. BioRxiv, doi: https://doi.org/10.1101/2020.02.08.939892
43. Notarangelo L, et al. 2004. Primary immunodeficiency diseases: an update. Journal of Allergy and Clinical Immunology, 114(3), 677-687. https://doi.org/10.1016/j.jaci.2004.06.044
44. Chowdhury MA, Hossain N, Kashem MA, Shahid MA & Alam A. 2020. Immune response in COVID-19: A review. Journal of Infection in Public Health, 13(11) https://doi.org/10.1016/j.jiph.2020.07.001
45. Le Bon A & Tough DF. 2002. Links between innate and adaptive immunity via type I interferon. Current opinion in immunology 14(4), 432-436. https://doi.org/10.1016/j.jiph.2020.07.001
46. Thiel V & Weber F. 2008. Interferon and cytokine responses to SARS-coronavirus infection. J Cytokine growth factor reviews, 19(2), 121-132. https://doi.org/10.1016/j.cytogfr.2008.01.001
47. Li CK-f, et al. 2008. T cell responses to whole SARS coronavirus in humans. The Journal of Immunology, 181(8), 5490-5500. DOI: https://doi.org/10.4049/jimmunol.181.8.5490
48. Stockinger B, Bourgeois C & Kassiotis G. 2006. CD4+ memory T cells: functional differentiation and homeostasis. Immunological reviews 211(1), 39-48. https://doi.org/10.1111/j.0105-2896.2006.00381.x
49. Velazquez-Salinas L, Verdugo-Rodriguez A, Rodriguez LL & Borca MV. 2019. The role of interleukin 6 during viral infections. Frontiers in microbiology, 10(1057).
50. Chowdhury MA, Hossain N, Kashem MA, Shahid MA & Alam A. 2020. Immune response in COVID-19: A review. Journal of Infection Public Health, https://doi.org/10.1016/j.jiph.2020.07.001
51. Bent S. 2008. Herbal medicine in the United States: review of efficacy, safety, and regulation. Journal of general internal medicine 23(6), 854-859.
52. Builders PF, Builders, F., Ed. & IntechOpen: London U. 2019. Introductory chapter: Introduction to herbal medicine. Journal of Herbal Medicine 1-9.
53. Mosihuzzaman M. 2012. Herbal medicine in healthcare-an overview. Natural Product Communications, 7(6), 807-812. https://doi.org/10.1177%2F1934578X1200700628
54. Izhaki I. 2002. Emodin–a secondary metabolite with multiple ecological functions in higher plants. Journal of New Phytologist, 155(2), 205-217. https://doi.org/10.1046/j.1469-8137.2002.00459.x
55. Tyagi A & Delanty N. 2003. Herbal remedies, dietary supplements, and seizures. Journal of Epilepsia, 44(2), 228-235. https://doi.org/10.1046/j.1528-1157.2003.19902.x
56. Panyod S, Ho C-T & Sheen L-Y. 2020. Dietary therapy and herbal medicine for COVID-19 prevention: A review and perspective. Journal of traditional complementary medicine, 10(4), 420-427. https://doi.org/10.1016/j.jtcme.2020.05.004
57. Wang H, et al. 2008. The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. J Emerg Med, 26(711-715. https://doi.org/10.1016/j.ajem.2007.10.031
58. Albulescu R, et al. 2020. COVID-19 and diabetes mellitus: Unraveling the hypotheses that worsen the prognosis. Journal of Experimental Therapeutic Medicine, 20(6), 1-1. https://doi.org/10.3892/ etm.2020.9324
59. Traboulsi H, et al. 2015. The flavonoid isoliquiritigenin reduces lung inflammation and mouse morbidity during influenza virus infection. Journal of Antimicrobial agents and chemotherapy, 59(10), 6317-6327.
60. Zu M, et al. 2012. In vitro anti-influenza virus and anti-inflammatory activities of theaflavin derivatives. Journal of Antiviral research 94(3), 217-224. https://doi.org/10.1016/j.antiviral.2012.04.001
61. Rzepecka-Stojko A, et al. 2015. Polyphenols from bee pollen: structure, absorption, metabolism and biological activity. Molecules, 20(12), 21732-

21749. https://doi.org/10.3390/molecules201219800
62. Wang N, et al. 2020. Structural characterisation and immunomodulatory activity of polysaccharides from white asparagus skin. J Carbohydrate polymers, 227(115314). https://doi.org/10.1016/j.carbpol.2019.115314
63. Peng B, et al. 2019. Isolation, structural characterization, and immunostimulatory activity of a new water-soluble polysaccharide and its sulfated derivative from Citrus medica L. var. sarcodactylis. International journal of biological macromolecules, 123, 500-511. https://doi.org/10.1016/j.ijbiomac.2018.11.113
64. Barbosa JR, dos Santos Freitas MM, da Silva Martins LH & de Carvalho Junior RN. 2020. Polysaccharides of mushroom Pleurotus spp.: New extraction techniques, biological activities and development of new technologies. Journal of Carbohydrate polymers, 229(115550), https://doi.org/10.1016/ j.carbpol.2019.115550
65. Barbosa JR, et al. 2020. Obtaining extracts rich in antioxidant polysaccharides from the edible mushroom Pleurotus ostreatus using binary system with hot water and supercritical CO2. Journal of Food chemistry, 330(127173). https://doi.org/10.1016/j.foodchem.2020.127173
66. Shen T, et al. 2017. Polysaccharide from wheat bran induces cytokine expression via the toll-like receptor 4-mediated p38 MAPK signaling pathway and prevents cyclophosphamide-induced immunosuppression in mice. Journal of Food Nutrition Research, 61(1), 1344523. https://doi.org/10.1080/16546628.2017.1344523
67. Li J, et al. 2020. Purification, structural characterization, and immunomodulatory activity of the polysaccharides from Ganoderma lucidum. International journal of biological macromolecules 143, 806-813. https://doi.org/10.1016/j.ijbiomac.2019.09.141
68. Kim DE, et al. 2019. Natural bis-benzylisoquinoline alkaloids-tetrandrine, fangchinoline, and cepharanthine, inhibit human coronavirus OC43 infection of MRC-5 human lung cells. Biomolecules, 9(11), 696. https://doi.org/10.3390/biom9110696
69. Koshak AE & Koshak EA. 2020. Nigella sativa l. as a potential phytotherapy for covid-19: A mini-review of in-silico studies. Current Therapeutic Research, 93 (100602). https://doi.org/10.1016/j.curtheres.2020.100602
70. Yoo D-G, et al. 2012. Protective effect of Korean red ginseng extract on the infections by H1N1 and H3N2 influenza viruses in mice. Journal of medicinal food, 15(10), 855-862. https://doi.org/10.1089/jmf.2012.0017
71. Goswami D, et al. 2018. Boswellia serrata oleo-gum-resin and β-boswellic acid inhibits HSV-1 infection in vitro through modulation of NF-κB and p38 MAP kinase signaling. Journal of Phytomedicine, 51, 94-103. https://doi.org/10.1016/j.phymed.2018.10.016
72. Guan W, et al. 2017. Pterodontic acid isolated from Laggera pterodonta inhibits viral replication and inflammation induced by influenza a virus. Molecules, 22(10), 1-14. https://doi.org/10.3390/molecules22101738
73. Park J-Y, et al. 2017. Evaluation of polyphenols from Broussonetia papyrifera as coronavirus protease inhibitors. Journal of enzyme inhibition medicinal chemistry, 32(1), 504-512. https://doi.org/10.1080/14756366.2016.1265519
74. Jo S, Kim S, Shin DH & Kim M-SJ. 2020. Inhibition of SARS-CoV 3CL protease by flavonoids. Journal of enzyme inhibition medicinal chemistry, 35(1), 145-151. https://doi.org/10.1080/14756366.2019.1690480
75. Swargiary A, Mahmud S & Saleh MA. 2020. Screening of phytochemicals as potent inhibitor of 3-chymotrypsin and papain-like proteases of SARS-CoV2: an in silico approach to combat COVID-19. Journal of Biomolecular Structure Dynamics 1-15. https://doi.org/10.1080/07391102.2020.1835729
76. Nguyen TTH, et al. 2012. Flavonoid-mediated inhibition of SARS coronavirus 3C-like protease expressed in Pichia pastoris. Biotechnology letters 4(5), 831-838. DOI 10.1007/s10529-011-0845-8
77. Gyebi GA, Ogunro OB, Adegunloye AP, Ogunyemi OM & Afolabi SO. 2020. Potential inhibitors of coronavirus 3-chymotrypsin-like protease (3CLpro): An in silico screening of alkaloids and terpenoids from African medicinal plants. Journal of Biomolecular StructureDynamics, 39(9), 1-13. https://doi.org/10.1080/07391102.2020.1764868
78. Park J-Y, et al. 2012. Tanshinones as selective and slow-binding inhibitors for SARS-CoV cysteine proteases. Journal of Bioorganic medicinal chemistry, 20(19), 5928-5935. https://doi.org/10.1016/j.bmc.2012.07.038
79. Wen C-C, et al. 2007. Specific plant terpenoids and lignoids possess potent antiviral activities against severe acute respiratory syndrome coronavirus. Journal of medicinal chemistry, 50(17), 4087-4095. https://doi.org/10.1021/jm070295s
80. Luo W, et al. 2009. Anti-SARS coronavirus 3C-like protease effects of Rheum palmatum L. extracts. Journal of Bioscience trends 3(4).
81. Dietologica MG. 2020. Possible therapeutic role of a highly standardized mixture of active compounds derived from cultured Lentinula edodes mycelia (AHCC) in patients infected with

2019 novel coronavirus. J Minerva gastroenterologica e dietologica.
82. Chikhale RV, et al. 2020. In-silico investigation of phytochemicals from Asparagus racemosus as plausible antiviral agent in COVID-19. Journal of Biomolecular Structure Dynamics, 1-15. https://doi.org/10.1080/07391102.2020.1784289
83. Das K. 2020. Application of Indian medicinal herbs for skin problems following safety measures against COVID-19. Iranian Journal of Dermatology, 23, 24-37. https://dx.doi.org/10.22034/ijd.2020.120510
84. Mpiana PT, et al. 2020. Aloe vera (L.) burm. F. as a potential Anti-COVID-19 plant: a mini-review of its antiviral activity. European Journal of Medicinal Plants, 86-93. https://doi.org/10.9734/ejmp/2020/v31i830261
85. Umar HI, et al. 2021. In-silico analysis of the inhibition of the SARS-CoV-2 main protease by some active compounds from selected African plants. Journal of Taibah University Medical Sciences, 16(2), 162-176. https://doi.org/10.1016/j.jtumed.2020.12.005
86. Mahalwar A & Mahalwar V. 2021. Preventive Measures against COVID-19: Indian System of Medicine. International Journal of Alternative Complementary Medicine, 2(1), 01-12.
87. Boozari M & Hosseinzadeh H. 2020. Natural products for COVID-19 prevention and treatment regarding to previous coronavirus infections and novel studies. Journal of Phytotherapy Research, 35, 864-878. https://doi.org/10.1002/ptr.6873
88. Greig A & Bouillant A. 1977. Binding effects of concanavalin A on a coronavirus. Canadian Journal of Comparative Medicine, 41(1), 122.
89. Wang B, et al. 2020. In search of preventative strategies: novel anti-inflammatory high-CBD cannabis sativa extracts modulate ACE2 expression in COVID-19 gateway tissues. Preprints 2020, 2020040315. doi: 10.20944/preprints202004.0315.v1
90. Ravi L. 2020. Procyanidin B2 of Cassia fistula a Potent Inhibitor of COVID19 Protease: A Molecular Dynamic Simulation Analysis. Asian Journal of Pharmaceutics, 14(2), 274-281.
91. Le Q & Lay H. 2020. Whether Herbal Medicines Play an Important Role in the COVID-19 Therapeutics and Boosting Immune as One of the Preventive Solutions: A Science Opinion. Journal of Ayurvedic Herb Med, 6(1), 1-3.
92. Panyod S, Ho C-T, Sheen L-YJJot & medicine c. 2020. Dietary therapy and herbal medicine for COVID-19 prevention: A review and perspective. 10(4) https://doi.org/10.1016/j.jtcme.2020.05.004
93. Lau K-M, et al. 2008. Immunomodulatory and anti-SARS activities of Houttuynia cordata. Journal of Ethnopharmacology, 118(1), 79-85.
94. Gowrishankar S, et al. 2021. Promising phytochemicals of traditional Indian herbal steam inhalation therapy to combat COVID-19–An in silico study. Journal of Food and Chemical Toxicology, 148(111966), https://doi.org/10.1016/j.fct.2020.111966
95. Desai A, Desai C, Desai H, Mansuri A & Desai J. 2020. Possible Role of Medicinal Plants in COVID-19-a Brief Review. International Journal of Scientific Development Research, 5(4), 205-209.
96. Srivastava A, Chaurasia J, Khan R, Dhand C & Verma S. 2020. Role of Medicinal plants of Traditional Use in Recuperating Devastating COVID-19 Situation. Journal of Medicinal and Aromatic Plants, 9(359), 2167-0412.
97. Shah S, et al. 2021. Exploring the active constituents of Oroxylum indicum in intervention of novel coronavirus (COVID-19) based on molecular docking method. Network Modeling Analysis in Health Informatics Bioinformatics, 10(1), 1-12. https://doi.org/10.1007/s13721-020-00279-y
98. Arokiyaraj S, Stalin A, Kannan BS & Shin H. 2020. Geranii Herba as a Potential Inhibitor of SARS-CoV-2 Main 3CLpro, Spike RBD, and Regulation of Unfolded Protein Response: An In Silico Approach. Journal of Antibiotics, 9(12), 863. https://doi.org/10.3390/antibiotics9120863
99. Dongre K. Herbal Immunity Boosters in Respect to COVID-19 Outbreak.
100. Ho T-Y, Wu S-L, Chen J-C, Li C-C & Hsiang C-Y. 2007. Emodin blocks the SARS coronavirus spike protein and angiotensin-converting enzyme 2 interaction. J Antiviral research, 74(2), 92-101.
101. Krishna G, Pillai VS & Veettil MV. 2020. Approaches and advances in the development of potential therapeutic targets and antiviral agents for the management of SARS-CoV-2 infection. European Journal of Pharmacology, 885(173450). https://doi.org/10.1016/j.ejphar.2020.173450
102. Hu K, et al. 2021. Efficacy and safety of Lianhuaqingwen capsules, a repurposed Chinese herb, in patients with coronavirus disease 2019: A multicenter, prospective, randomized controlled trial. Phytomedicine, 85(153242). https://doi.org/10.1016/j.phymed.2020.153242
103. Saharan A, Wilson K, Mittal P, Kapoor R & Nazmi AK. 2021. THE PANDEMIC-COVID 19-A RACE OF PHARMACEUTICALS. Plant Archives, 21(1), 2098-2104. https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.346
104. Maurya VK, Kumar S, Bhatt ML & Saxena SK. 2020. Antiviral activity of traditional medicinal plants from Ayurveda against SARS-CoV-2 infection. Journal of Biomolecular Structure Dynamics, 1-17. https://doi.org/10.1080/07391102.2020.1832577
105. Shankar R, Balkrishna A, Srivastava A, Joshi B & Mishra RK. 2021. Role of traditional medicines as

a challenge for Coronavirus (Covid-19). Indian Journal of Traditional Knowledge,19. 118-123.
106. Shree P, et al. 2020. Targeting COVID-19 (SARS-CoV-2) main protease through active phytochemicals of ayurvedic medicinal plants–Withania somnifera (Ashwagandha), Tinospora cordifolia (Giloy) and Ocimum sanctum (Tulsi)–a molecular docking study. Journal of Biomolecular Structure Dynamics, 1-14. https://doi.org/10.1080/07391102.2020.1810778
107. Kumar G & Chander H. 2020. Scope for utilization of Curcuma longa, Embelica officinales and Tinospora cordifolia for human health in COVID-19 like scenario in Himachal Pradesh, India. Epidemiology Transmission of Infectious Diseases, 199-210.
108. Upreti S, Prusty JS, Pandey SC, Kumar A & Samant M. 2021. Identification of novel inhibitors of angiotensin-converting enzyme 2 (ACE-2) receptor from Urtica dioica to combat coronavirus disease 2019 (COVID-19). Journal of Molecular diversity, 1-15. https://doi.org/ 10.1007/s11030-020-10159-2