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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.

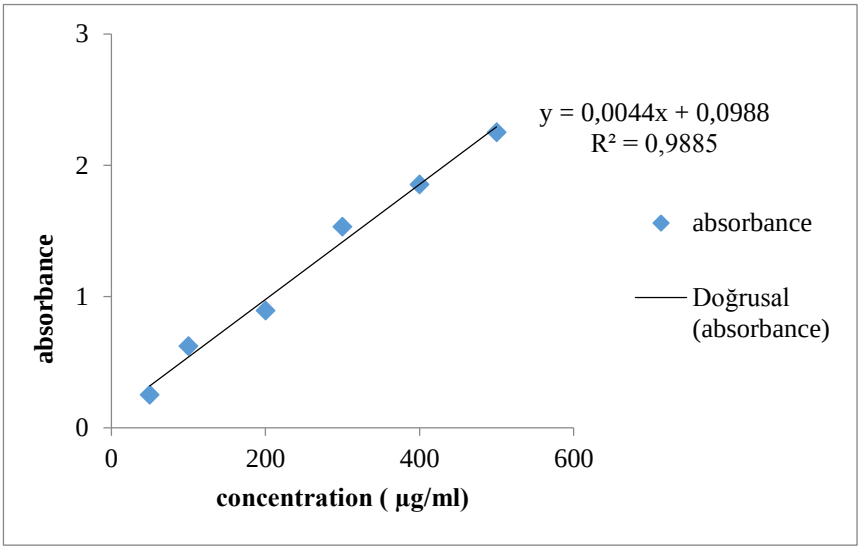

Figure 1. Calibration curve of gallic acid for total phenolic content

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.

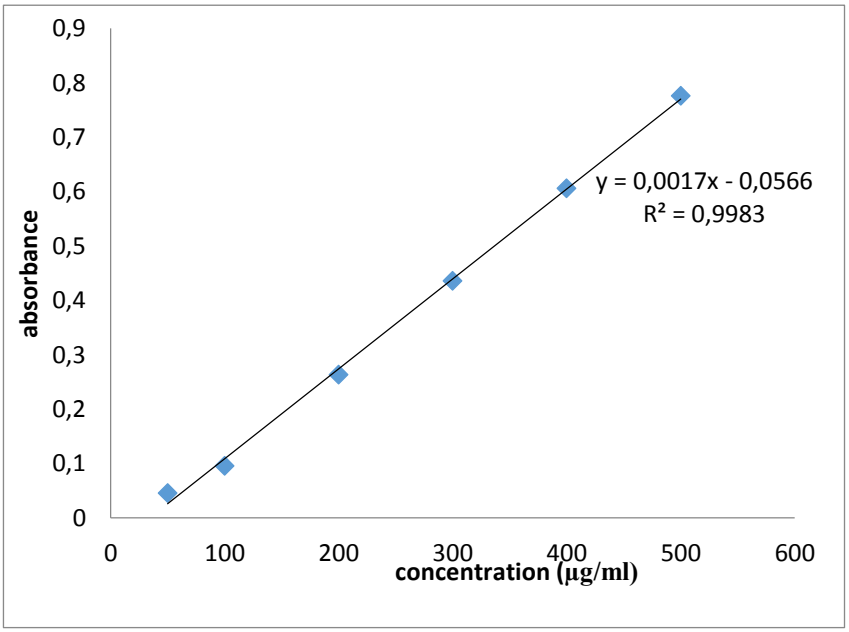

Figure 2: Calibration curve of quercetin for total flavonoid content

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.

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