Volume: 4, Issue: 2, December 2021
ISSN: 2619- 9645 (Print)
e-ISSN: 2667- 5722 (On-line, Pdf)

CUPMAP

Current Perspectives on Medicinal and Aromatic Plants
An International Scientific Journal
http://dergipark.gov.tr/cupmap - www.cupmap.org

Current Perspectives on Medicinal and Aromatic Plants

(CUPMAP)

Curr. Pers. MAPs

ISSN: 2619- 9645 (Print) **e-ISSN:** 2667-5722

This journal is international peer-reviewed and published two issues per year.

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Volume 4 **Issue 2** **December, 2021**

Current Perspectives on Medicinal and Aromatic Plants
Curr. Pers. MAPs
An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

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Journal Name	Current Perspectives on Medicinal and Aromatic Plants
Journal Abbreviation	*Curr. Pers. MAPs*
Scope & Subjects	Agriculture, Biology, Molecular Biology & Genetics, Chemistry, Biochemistry, Botany, Ethnobotany, Environmental Science, Forestry, Horticulture, Health Care & Public Health, Nutrition & Food Science, Pharmaceutical Sciences
ISSN	ISSN: 2619-9645 | e-ISSN: 2667-5722
Publisher	Nazım ŞEKEROĞLU
Language	English
Frequency	Biannually (June and December)
Type of Publication	Peer Review Double-Blinded
Publication Fee	Any Submission or Processing Charges
Access type	Open Access Policy
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ISSN: 2619-9645 | e-ISSN: 2667-5722

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ISSN: 2619- 9645 (Print) e-ISSN: 2667-5722

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Sincerely,

Prof. Dr. Nazım ŞEKEROĞLU
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Curr. Pers. MAPs
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ISSN: 2619-9645 | e-ISSN: 2667-5722

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Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

CURRENT PERSPECTIVES ON MEDICINAL AND AROMATIC PLANTS (CUPMAP)

An International Scientific Journal - ISSN: 2619- 9645 (Print)

Volume: 4, Issue: 2, June 2021

Current Perspectives on Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

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December, 2021 - KİLİS

Research article
Curr. Pers. MAPs, (2021); 4(2): 87-92

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Antimicrobial Activity of Leaf, Fruit, and Gall Extract of *Pistacia terebinthus* Growing in Tessala

Bellifa NAZIM [1*], Ferkous HOUSSEM [2], Benhaddou ISMAIL [3], Merad YASSINE [4], Matmour DEROUICHA[5]

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https://doi.org/10.38093/cupmap.1004210

Received : 04/09/2021
Accepted : 05/12/2021

Abstract

Nature is a large deposit of active molecules of plant origin, and the resources of the flora are far from being fully inventoried. Today, the world continues to search for plants that can be used as the basis for new and relatively new treatments. In vitro antibacterial activity was evaluated on the MeOH extract of leaves, fruit galls, and essential oil mastic gum of *Pistacia terebinthus* from Tessala (Western Algeria) against four human pathogenic microorganisms (*Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) using a disc diffusion method. The gall extract revealed a remarkable antimicrobial effect against the tested microorganisms. Strong activity was observed for samples of gall extract against *Staphylococcus aureus* with inhibition zones of 20 mm. These results suggested that the samples of gall extract of *P. terebinthus* tested for antimicrobial activity can be listed as bactericides.

Key Words: Antimicrobial activity, Galls, *Pistacia terebinthus,* Tessala

1. Introduction

Nature is a huge deposit of active molecules of plant origin, and the resources of the flora are far from being fully inventoried. Today, the world continues to search for plants that can be used as the basis for new and relatively new treatments, this systematic search for the therapeutic resources of aromatic and medicinal plants opens up extremely promising prospects for the pharmaceutical industry (Guedira, 2008).

The terabinth is a shrub from 2 to 3 meters, deciduous, imparipinnate giving red drupes where it is generally found at rocky sites with open vegetation; it avoids the driest and coldest locations and distribution It is found in all parts of the West from Portugal to Turkey and Morocco to Libya, it penetrates quite deeply in the southern Alps. In Algeria, the genus Pistacia is represented by four species, namely *Pistacia terebinthus Pistacia vera, Pistacia atlantica,* and *Pistacia lentiscus.* (Quezel, 1962; Lapie and Maige, 1914).

Galls are remarkable plant structures that have been observed, studied, and used since antiquity. They are abnormal growths in plants, induced by viruses, bacteria, fungi, nematodes, and arthropods, in a wide variety of plant families. Pistacia common plant galls with therapeutic applications, some aphids species induce the formation of different galls in *Pistacia terebinthus, Forda formicaria, F. marginata, Paracletus cimiciformis, Geoica utricularia,* and *Baizongia pistacia,* most of them are irregular, globose, chili, coral, cauliflower, curved, but another one gets the shape of fruits, identification of them in their geographical range is based on gall characteristics as well as on aphid morphology (Blackman and Eastop, 1994; Piras et al., 2017).

The terebinth pistachio tree is particularly sensitive to this type of insects that transform leaflets to reddish the ecological niche with distinctive architecture, the artwork of aphids which hem the leaflets. This phenomenon attracts the attention of ecologists and biologists for a long time. Gall-formers are parasitic organisms that manipulate plant traits for their benefit. Galls have been shown to protect their inhabitants from natural enemies such as predators and parasitoids by various chemical and mechanical means. Much less attention, however, has been given to the possibility of defense against microbial pathogens in the humid and nutrient-rich gall environment. (Álvarez et al., 2016).

A few research on the chemical composition and biological activities of *P. terebinthus* were made tree. Because the chemical composition of leaves fruit differs significantly from those of galls, the goal of this study is to determine the antimicrobial activity focused on the leaves, fruits, galls, and essential oil of mastic gum (Piras et al., 2017).

2. Material and Methods

2.1. Vegetal material

Leaves, fruits, and galls of *P. terebinthus* were collected from Tessala mountain (Western Algeria). Samples were taken in September and November at sites in the west of the province of Sidi Bel Abbes (Algeria). Samples were collected from the last stages of development of the galls (Fig 1).

Fig 1. Morphological aspects of fruit and gall leaves of *Pistacia terebinthus*

2.2. Extraction

The routine extraction method that we use is maceration by solvents of increasing polarity. The MeOH extract of the aerial part of *Pistacia terebinthus* is prepared from 20 g of grinding of the leaves, fruits, and galls, which are macerated in 500 ml of methanol at room temperature and protected from light for 24 hours, with maximum stirring. The mixture is then filtered on a paper filter. The operation is repeated a second time on the mark. The filtrates obtained are added and evaporated to dryness with the aid of a rotary evaporator «BÜCHI» at a temperature of 40-50°C, then the dry extract is kept in the refrigerator

2.3. Antibacterial activity

The evaluation of the antibacterial activity was carried out by the method described by (Pfaller and Herwaldt, 1997) and (Selka et al., 2016) by diffusion on disks in an agar medium, this method allows an estimation of the inhibition of the growth of the microorganisms which is estimated in terms of the diameter of the zone of inhibition of the microbial growth around the disks containing the samples to be tested after 24 h of incubation at an adequate temperature of 37°C. The MeOH extract of the galls, the MeOH extract of the leaves, fruit, and essential oil mastic gum of *P. terebinthus* are dissolved in DMSO dimethylsulfoxide. We used 4 bacterial strains of the ATCC type from the Institut Pasteur in Algiers: *Escherichia coli* ATCC 25922, *Staphyloccocus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778 *Pseudomonas aereginosa* ATCC 9027).

The culture medium consists of Muller-Hinton and 20 g of Agar-agar adjusted to a pH of 7.4, the agar is poured into Petri dishes 90 mm in diameter. The preparation of bacterial inoculum is usually carried out after several steps. Initially, the samples kept refrigerated must be activated in a liquid MH medium after 24 hours at 35°C, 1 ml of standardized bacterial inoculum (108 CFU/ml) is aseptically deposited and spread on the surface of the medium with the aid of a stall, the excess liquid is sucked with a sterile pasture pipette. The disks (Ø 0.5 cm) are impregnated with a variable quantity (between 1 and 10 µl) of the selected product and placed on the inoculated agar. Negative controls are prepared using DMSO. Ampicillin is used as a positive reference to determine the sensitivity of each bacterium. The Petri dishes are then incubated for 24 hours at 37°C. The antibacterial activity is evaluated by measuring the diameter of the inhibition zone. The categorization of bacterial strains concerning the extracts tested is as follows: susceptible strains S (Ø≥11mm); intermediate strains I (5mm<Ø<11 mm) and resistant strains R(Ø≤5mm). Ø: diameter of the inhibition zone, according to the European committee on Antimicrobial Susceptibility.

3. Results and Discussion

The results of the antibacterial activity of the various extracts are presented in Table 1. *In vitro* antibacterial activity was evaluated on the MeOH extract of leaves, fruit galls, and essential oil mastic gum of *P. terebinthus* in the presence of positive control of ampicillin. The essential oil of galls has greater antibacterial activity than that of other extracts with an inhibition diameter ranging from 5 to 20 mm.

The results show that the extracts have antimicrobial activities of varying degrees according to the strains of the different microorganisms tested, it is observed that the large inhibition zones appear with the extract of essential oil mastic gum HE (3) and the extract of the gall (4) on Staphylococcus aureus. For the MeOH extract of the leaves (2), there is minimal inhibition against the three strains: *Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis* (low activity), and Pseudomonas aeruginosa show a negative result (no inhibition). The extract of the fruit

(1) exhibits moderate activity, the latter showing zones of inhibition with the four strains. This analysis shows that the most important inhibitory effect is obtained by the MeOH extract of galls with *Staphylococcus aureus* (Gram-positive), where a large inhibition zone is observed.

Table1. The results of the antibacterial activity of the various extracts

Produits	***E. coli*** **D C**	***B. subtilis*** **D C**	***P. aereginosa*** **D C**	***S. aureus*** **D C**
1/ fruit extract	**9 I**	**14 S**	**12 S**	**13 S**
2/ leave extract	**7 I**	**9 I**	**5 R**	**9 I**
3/ mastic	**5 R**	**12 S**	**11 S**	**13 S**
4/ galls extract	**12 S**	**11 S**	**9 I**	**20 S**

D: diameter of the inhibition zone; C: categorization of strains, S: sensitive; I: intermediary; R: resistant.

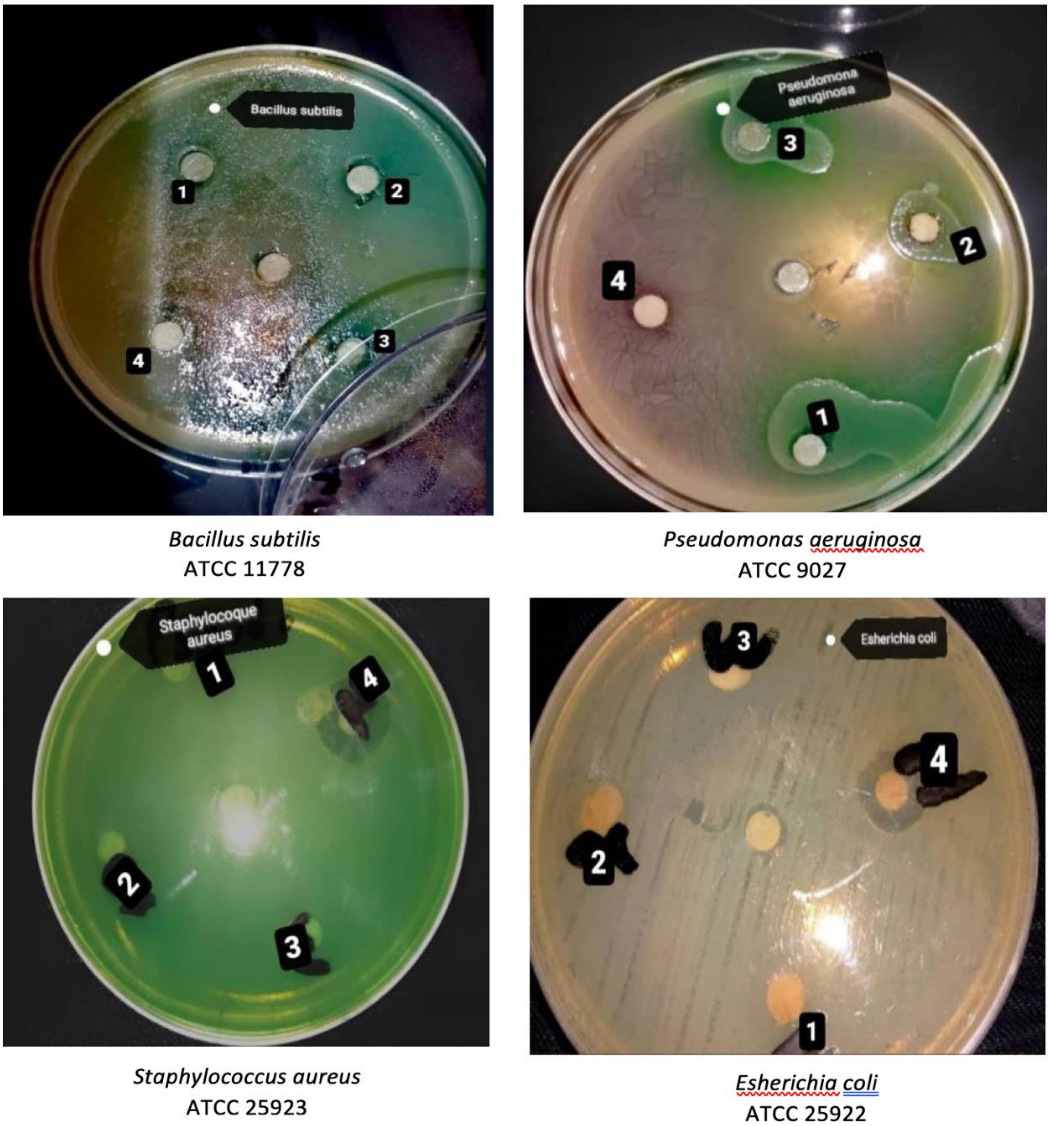

Fig 2. Antibacterial activity of different parts of *Pistacia terebinthus*
MeOH fruit extract (1); MeOH extract of leaves (2); Essential oil of mastic gum extract (3), MeOH galls extract (4)

The various extracts of *P. terebinthus* showed significant antibacterial activity against various Gram-positive and Gram-negative bacteria, as has already been specified. The antibacterial activity in vitro is estimated on the methanolic extract of the leaves, fruit, galls of *P. terebinthus* , essential oil mastic gum of *P. terebinthus* extract in the presence of positive control of ampicillin. The essential oil of galls has greater antibacterial activity than that of other extracts with an inhibition diameter ranging from 9 to 20 mm. The antibacterial activity depends on the nature of the active secondary metabolites but especially on the nature of the Gram+ or Gram- bacteria and also on the extraction method carried out. Moreover, it has been known since antiquity that essential oils exhibit a non-negligible antiseptic activity (Pulaj et al., 2016; Piras et al., 2017).

The high activity of Pistacia's essential oils is mainly due to its richness with active compounds such as terpenes and essentially phenols. the essential oil of this plant has a very high antibacterial power on several bacterial strains. In addition, other works show the value of Pistacia essential oils. These results are consistent with the work of Ibrahim SIFI et al., who studied the antimicrobial activity of essential oils of *Pistacia atlantica* galls from three southern Algerian regions on six microbial strains, the study revealed a significant action against Gram-positive bacterial strains with a MIC of 0.44 for *Bacillus cereus*. Several studies show that Gram-positive bacteria are highly sensitive to Gram-negative bacteria, which can be attributed to the difference in the outer layers of Gram-negative bacteria compared to Gram-positive bacteria (Alma et al., 2004; SIFI, 2020).

The difference in antibacterial activity found between the methanolic extract of leaves, fruits of galls, and putty could be explained by the fact that the essential oil of *Pistacia terebinthus* contains 90% of the monoterpenes including alpha-pinene, Limonene reputed by their antiseptic power, while the methanolic extract is more concentrated in phenolic compounds to 80% of the tannins and flavonoids of high molecular weight catechin which influence their passage through the bacterial membrane. One study indicated that the antibacterial activity of *P. terebinthus* essential oil can be attributed to the combination of several compounds. Alpha pinene, terpineol, and linalol showed high antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, comparable to that of mastic oil (Kivcak et al., 2004; Ulukanli et al., 2014).

4. Conclusion

This work is a contribution to the valorization of aromatic and medicinal plants in Algeria in the region of Tessala. The gall extract revealed a remarkable antimicrobial effect against the tested microorganisms. Strong activity was observed for samples of gall extract against Staphylococcus aureus with inhibition zones of 20 mm (for 50 µl). These results suggested that the samples of gall extract of *P. terebinthus* tested for antimicrobial activity can be classified as bactericides.

Acknowledgements

The authors thank Prof. Dr. Chelghoum for his assistance and all the members of pharmacognosy Laboratory. Thanks, are also due to Prof. Dr. Yebous for his contribution and assistance and Prof. Dr. Achouri.

Author Contribution

B.N.: Drafting the research protocol, Identification of plants, Manuscript writing, F.H.: Identification of plants, M.D.: Discussion, Corrections. B.I.: extraction, control. M.Y.: Conducting the antibacterial activity. All authors reviewed, commented, and approved the final manuscript.

Conflicts of Interest

The authors have no conflicts of interest to declare and disclose any financial field.

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Research article
Curr. Pers. MAPs, (2021); 4(2): 93-102

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Relationship Between Essential Oil Content, Fruit Yield and Yield Component in *Foeniculum vulgare* Mill.

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https://doi.org/10.38093/cupmap.991656
Received: 06/09/2021
Accepted: 05/12/2021

Abstract

Foeniculum vulgare Mill. naturally spread in Turkey flora, and it's also cultivated. Its cultivation is widely carried out in Burdur and Denizli region. Especially, it is used as a milk enhancer when drunk as tea for breastfeeding mothers and as a carminative and relaxing for babies. The aim of the study was to determine the relationship between yield and quality characteristics of *F. vulgare* L. is cultivated and used in Turkey. For this purpose, correlation analysis and path analysis were applied to essential oil content (EOC) and fruit yield. EOC was significantly and positively associated with the number of umbellet (0.684 **), number of fruit (0.589*), Thousand Fruit Weight (TFW) (0.563*) and plant height (0.530*). The highest positive correlation was obtained among with fruit yield and biological yield (r=0.929**). Fruit yield has a significant correlation also between fruit number (0.805**), TFW (0.726**), plant height (0.696**) and umbellet number (0.609**). Branch number has a negative and unsignificant correlation between all the characteristics, but also has a negative and significant correlation among plant height. According to the path analysis results, the highest direct influence on EOC were the biological yield (-33.02%), but negative and fruit yield (30.23%) parameters. The maximum positive effects on fruit yield in fennel belongs to biological yield (59.88%), TFW (29.41%) and plant height (20.16%). In addition, fruit number, plant height and TFW showed the maximal indirect effects on fruit yield through biological yield. The same traits also affected the essential oil content and indirectly and positively. Therefore, these traits can be evaluated as selection criterion to attain higher essential content and fruit yield in *F. vulgare* L.

Key Words: Fennel, Umbel, Umbellet, Correlation, Path, Thousand Grain Yield.

1. Introduction

Three main varieties of fennel, which is an element of the *Apiaceae* family, have been described by Seidemann (2005); *F. vulgare* Mill. var. *piperitum* (bitter fennel), *F. vulgare* Mill. var. *dulce* (sweet fennel) and *F. vulgare* Mill. var. *azoricum* (Florence fennel). Sweet fennel is a native of southern Europe, the Mediterranean region. It has also been naturalized in many parts of northern Europe, Cyprus, the USA, southern Canada and Asia, the Far East and Australia (Malhotra, 2012). *Foeniculum vulgare* Mill. grows naturally spread in Northern, Western and Eastern Anatolia (Avci, 2013). It's also cultivated various provinces such as Antalya, Gaziantep, Manisa, Bursa, Denizli, and the most produced around Burdur province (Baydar, 2009). Fennel is cultivated especially for its fruits and for the essential oil taken from fruits, as well as for its bulbs and foliage. Essential oil content, components and their ratios vary according to ecological and growing conditions such as sowing time and density, irrigation, fertilization. Sweet fennel fruits contain 2-5.8% essential oil, the components are trans-anethole, fenchone, limonene, methyl chavicol, α- phellandrene and anisaldehyde.

The purpose of breeding in medicinal and aromatic plants, as in other plants is high yield, resistance to biotic and abiotic stress; however, the amount and quality of the active ingredient are also important. Obtaining varieties with high grain yield, essential oil content and component ratios is the main goal of fennel breeding. The yield and essential oil content are polygenic characters affected by environmental conditions. For this reason, it will be more advantageous to carry out breeding studies on yield components that affect essential oil content and fruit yield. Path coefficient analysis determines the direct and indirect contribution of various characters to yield. Correlation analysis supply knowledge about the relationships between yield components. In breeding studies, selection is successful when there is a strong relationship among essential oil content, composition, and morphological and agronomic characteristics. Therefore, it is considered to be more advantageous to investigate the morphological and agronomical properties that affect the content of essential oil, rather than directly increasing the content of essential oil in medicinal plants. (Avcı and Giachino, 2019). For all these reasons, in this study, it was aimed to determine the relationship between yield and quality characteristics of fennel cultivated and used in Turkey.

2. Material and Methods

Sweet fennel (*Foeniculum vulgare* Mill.) seeds used as study material were obtained from producers of Burdur province. The experiment was planned as two vegetation years with three replications accordingly the randomized block design in Isparta, Turkey. The plots were designed in six rows of 300 x 40 cm. Irrigation, weed control and fertilization were carried out regularly.

The characteristics examined in the study are plant height (cm), number of branches per plant, number of umbels per plant, number of umbellets per umbel, number of fruits per umbel, fruit yield (kg ha^{-1}), biological yield (kg ha^{-1}), thousand fruit weight (g) and essential oil content (%). EOC was obtained by hydro distillation with Neo-Clevenger apparatus (Wichtl, 1971). Path analysis was used to determine the meaningful traits influencing essential oil content and fruit yield and their direct and indirect effects. Correlation and path analysis were conducted according to method followed by Singh & Chaudhary (1979) and Dewey & Lu (1959), respectively. Evaluation of the obtained data was made utilization of the TARIST software (Açıkgöz et al., 2004).

3. Results and Discussion

The correlations between the studied traits of fennel (*Foeniculum vulgare* Mill.) are given in Table 1. When Table 1 is examined; the highest positive correlation revealed fruit yield and biological yield with the correlation coefficient *r = 0.929***. The fruit yield also, was considerably and positively associated with number of fruits (*r = 0.805 ***), Thousand fruit yield (TFW) (*r = 0.726 ***), plant height (*r = 0.696***) and number of umbellets (*r = 0.609***). These traits are considered major components for fruit yield. This result is compatible with those obtained by Al-korddy (2000), Singh and Mittal (2003), Bahmani et al (2012) and Singh et al. (2014) who reported significant and positive relationship between seed yield and plant height. Similarly, Dashora and Sastry (2011), found a favorable and important correlation between seed yield and number of umbellets (0.43**), seed number (0.46**), biological yield (0.84**) and harvest index (0.45**).

Biological yield showed a very high correlation with fruit yield as cited above (*r=0.929***). Biological yield, also related with number of fruit (*r=0.667***) at the P < 0.01 level. Furthermore, it exhibited significant positive correlations with plant height (*r=0.498**) and TWF (*r=0.468**) at the P < 0.05 level. This is in accord with the results of Cosge et al. (2009) who found a very high positive relationship among biological yield and single plant yield (r=0.915).

Plant height demonstrated a notable positive relationship with number of umbellets (r = *0.748***), number of fruits (*r = 0.771***), fruit yield (*r = 0.696***) and TWF (*r = 0.671***) at the P < 0.01 level, and with biological yield (*r = 0.498**) and essential oil content (*r = 0.530**) at the P <0.05 level. Similarly, Singh and Mittal (2003) showed that there is a positive and important relationship between seed yield and plant height, umbels number, TWF in sweet fennel. Conversely, Patidar et al. (2017) found that plant height was importantly and negatively related with only the number of umbellate per umbel (-0.29*). In this study, it was determined that there is a significant but negative correlation at P <0.05 level between plant height and branch number (*r = -0.570**). There was no statistically significant relationship between plant height and the number of umbels. The fact that plant height, which contributes indirectly to seed yield, shows a positive correlation with other traits except for these two, indicating that it may be important for genotype selection.

The significant and positive correlations were detected among the number of umbellets with TWF (*r=0.825***), number of fruits (*r=0.750***), plant height (*r=0.748***), EOC (*r = 0.684 ***) and fruit yield (*r =0.609***) at the P <0.01 level. These results are consistent with the results of Kalleli et al. (2019) for number of fruits per umbel and TFW.

The number of fruits was remarkably and positively associated with fruit yield (*r = 0.805***), plant height (*r=0.771***), number of umbellets (*r=0.750***), TFW (*r = 0.670***) and biological yield (*r = 0.667***) at the P <0.01 level and with EOC (*r = 0.589**) at the P <0.05 level.

A significant positive correlation was observed between TFW and number of umbellets (*r=0.825***), fruit yield (*r= 0.726***), plant height (*r= 0.671***), number of fruits (*r=0.670***) at the P <0.01 level and EOC (*r=0.563 **) and biological yield (*r= 0.468 **) at the P <0.05 level. It displayed negative insignificant correlation with the remaining two traits.

The number of branches showed a negative but statistically non-significant relationship with all other traits examined except the plant height (*r= -0.570**). The number of umbels could not show a meaningful and significant relationship with all the other properties examined. In contrast, Yadav et al. (2013) found a positive relationship between seed yield and the number of umbels and branches. Again, Kalleli et al. (2019) also found a positive association between seed yield plant height and number of branches, considered these characteristics as the main components for seed yield. This situation may be due to ecological conditions and sowing frequency.

Essential oil content was markedly associated with number of umbellets (*r=0.684***) at the P <0.01 level, and with number of fruits, TFW and plant height at P <0.05 level (*r=0.589*, 0.563** and *0.530**, respectively). Further, it had a positive but insignificant correlation with fruit yield, number of umbels and biological yield, and a negative non-significant relationship with the number of branches. Meena and Dhakar (2017) in fennel observed significant positive relationships between essential oil ratio and parcel seed yield, days to germination, king umbel anthesis, number of branches secondary, number of umbellets per umbel while they found a significant but negative correlation with number of umbels per plant. Lal (2007), emphasizing that seed yield is in a significant and positive association with essential oil and t-anethole content at both genotypic and phenotypic levels, revealed that these two characteristics are good criteria for selection.

Table 1: Correlation coefficients and significance between traits studied in fennel.

	Plant height	Number of branches	Number of umbels	Number of umbellets	Number of fruits	Fruit yield	Biological yield	TFW
Number of branches	**-0.570***	1.000						
Number of umbels	0.121ns	-0.170ns	1.000					
Number of umbellets	**0.748****	-0.251ns	-0.029ns	1.000				
Number of fruits	**0.771****	-0.404ns	0.373ns	**0.750****	1.000			
Fruit yield	**0.696****	-0.299ns	0.041ns	**0.609****	**0.805****	1.000		
Biological yield	**0.498***	-0.186ns	0.062ns	0.371ns	**0.667****	**0.929****	1.000	
TFW	**0.671****	-0.274ns	-0.220ns	**0.825****	**0.670****	**0.726****	**0.468***	1.000
Essential oil content	**0.530***	-0.289ns	0.205ns	**0.684****	**0.589***	0.357ns	0.086ns	**0.563***

**: significant at the 0.01 probability level; *: significant at the 0.05 probability level; ns: non-significant

In order to identify the direct and indirect effects of examined traits on essential oil content of *Foeniculum vulgare* Mill., path analysis was applied based on the average of two vegetation years and are presented in Table 2. Path analysis demonstrated that only fruit yield (30.23 %) had strong direct effect on the EOC while number of umbellets (1.05%) had negligible positive direct effects. The correlation coefficients of this traits on EOC were similarly positive, but fruit yield (r= 0.357ns) was non-significant whereas number of umbellets (r=0.684**) was statistically significant, unlike path analysis. In addition, the relationship of these two characters with each other was significant and positive (r=0.609**) (Table 1). Fruit yield displayed the supreme direct influence (30.23 %), also positive indirect efficacy on EOC via plant height (25.54%), number of umbels (8.27%), number of umbellets (24.11%), number of fruits (27.40%), biological yields (32.28%) and thousand fruit weight (25.37%). However, the positive direct influence of fruit yield on EOC was equilibrated by relatively high minus indirect effect through the number of branches (-23.90%). Coşge et al. (2009), similar to ours, stated that the highest direct influence on essential oil percentage was the single plant yield (32.95%), and unlike us, the plant height (24.60%) and the number of branches (15.11%) had the highest direct effect. According to Faravani et al. (2018), plant biomass, number of umbellate in umbel, canopy cover and 1000-seed weight had the most direct effect on essential oil yield of cumin.

Table 2. Path coefficient and percentages of the direct and indirect effects of investigated properties on the essential oil content of *Foeniculum vulgare* Mill.

	DIRECT EFFECTS		INDIRECT EFFECTS							
	Essential oil content (EOC)		Plant height		Number of branches		Number of umbels		Number of umbellets	
	Path coefficient	Path %	Path coeff.	Path %	Path coeff.	Path %	Path coeff.	Path %	Path coeff.	Path %
Plant height	-0.56	8.29	-	-	0.05	0.79	-0.03	0.43	0.05	0.73
Number of branches	-0.09	2.99	0.32	10.29	-	-	0.04	1.31	- 0.02	0.53
Number of umbels	-0.24	19.39	- 0.07	5.52	0.02	1.29	-	-	- 0.002	0.16
Number of umbellets	0.07	1.05	- 0.42	6.70	0.02	0.37	0.01	0.11	-	-
Number of fruits	-0.10	1.41	- 0.44	5.93	0.04	0.52	- 0.09	1.22	0.05	0.68
Fruit yield	2.50	30.23	- 0.39	4.76	0.03	0.34	- 0.01	0.12	0.04	0.49
Biological yield	-2.37	33.02	- 028	3.91	0.02	0.24	- 0.02	0.21	0.03	0.34
TFW	-1.73	24.26	- 0.38	5.29	0.03	0.36	0.05	0.74	0.06	0.76
	DIRECT EFFECTS		INDIRECT EFFECTS							
	Essential oil content (EOC)		Number of fruits		Fruit yield		Biological yield		Thousand fruit weight	
	Path coefficient	Path %	Path coeff.	Path %	Path coeff.	Path %	Path coeff.	Path %	Path coeff.	Path %
Plant height	-0.56	8.29	-0.08	1.17	1.74	25.54	-1.18	17.35	-1.16	17.08
Number of branches	-0.09	2.99	0.04	1.34	-0.75	23.90	0.44	14.09	0.47	15.18
Number of umbels	-0.24	19.39	-0.04	3.13	0.10	8.27	-0.15	11.96	0.38	30.81
Number of umbellets	0.07	1.05	-0.08	1.23	1.52	24.11	-0.88	13.96	-1.43	22.69
Number of fruits	-0.10	1.41	-	-	2.01	27.40	-1.58	21.57	-1.16	15.83
Fruit yield	2.50	30.23	-0.08	1.01	-	-	-2.20	26.70	-1.26	15.25
Biological yield	-2.37	33.02	-0.07	0.96	2.32	32.28	-	-	-1.81	11.30
TFW	-1.73	24.26	-0.07	0.97	1.81	25.37	-1.11	15.54	-	-

Table 3. Path coefficient and percentages of the direct and indirect effects of investigated properties on the fruit yield of *Foeniculum vulgare* Mill.

	DIRECT EFFECTS		**INDIRECT EFFECTS**							
	Fruit yield		Plant height		Number of branches		Number of umbels		Number of umbellets	
	Path coeff.	Path %	Path coeff.	Path %	Path coeff.	Path %	Path coeff.	Path %	Path coeff.	Path %
Plant height	0.25	20.16	-	-	-0.02	1.39	0.004	0.34	-0.08	6.28
Number of branches	0.03	5.23	-0.14	24.62	-	-	-0.01	1.03	0.03	4.52
Number of umbels	0.04	13.09	0.03	11.32	-0.01	1.93	-	-	0.03	1.14
Number of umbellets	-0.11	8.96	0.19	16.10	-0.01	0.65	-0.001	0.09	-	-
Number of fruits	0.04	3.18	0.19	14.60	-0.01	0.93	0.01	0.99	-0.08	5.92
Biological yield	0.75	59.88	0.13	10.02	-0.01	0.45	0.02	0.18	-0.04	3.11
TFW	0.37	29.41	0.17	13.30	-0.01	0.66	-0.01	0.61	-0.09	6.81
Essential oil content	0.12	15.14	0.13	17.37	-0.01	1.15	0.01	0.94	-0.07	9.33
	DIRECT EFFECTS		**INDIRECT EFFECTS**							
	Fruit yield		Number of fruits		Biological yield		Thousand fruit weight		Essential oil content	
	Path coeff.	Path %	Path coeff.	Path %	Path coeff.	Path %	Path coeff.	Path %	Path coeff.	Path %
Plant height	0.25	20.16	0.03	2.61	0.37	29.86	0.25	20.05	0.06	4.94
Number of branches	0.03	5.23	-0.02	2.93	-0.14	23.86	-0.10	17.53	-0.03	5.78
Number of umbels	0.04	13.09	0.02	5.87	0.05	17.36	-0.08	30.50	0.02	8.86
Number of umbellets	-0.11	8.96	0.03	2.71	0.28	23.76	0.31	26.33	0.08	6.80
Number of fruits	0.04	3.18	-	-	0.50	37.57	0.25	18.81	0.07	5.15
Biological yield	0.75	59.88	0.03	2.25	-	-	0.17	13.98	0.01	0.81
TFW	0.37	29.41	0.03	2.23	0.35	27.63	-	-	0.07	5.16
Essential oil content	0.12	15.14	0.03	3.23	0.07	8.43	0.21	27.34	-	-

The most negative and direct influence on EOC was by biological yield, TFW and number of umbels (-33.02, -24.26, -19.39%, respectively). In addition, plant height (-8.29%), number of branches (-2.99%) and number of fruit (-1.41%) also had a negative direct but low-level effect on EOC. Aharizad et al. (2013) reported that citral content, leaf width and total dry weight had an almost positive direct effects on essential oil percentage in their study on lemon balm populations. In contrast, they reported that the number of tillers, leaf length and dry weight of leaves had a direct negative effect on essential oil percentage.

Biological yield had the highest negative direct influence on EOC (-33.0.2%). Our findings show similarity with the Cosge et al. (2009)'s outcomes (35.16 %). Only the number of branches (14.09 %) had a positive indirect effect on EOC via biological yield. It also gave high indirect negative effects via plant height (-17.35 %), number of umbels (-11.96 %), number of umbellets (-13.96 %), number of fruits (-21.57 %), fruit yield (-26.70 %) and thousand fruit weight (-15.54 %).

TFW indicated that the second most negative direct influence (-24.26%) on EOC. Cosge et al., (2009) were determined the most negative direct influence on essential oil ratio as the one thousand seed weight (68.74%). It had high positive indirect influence on EOC through number of umbels (30.81%) and number of branches (15.18%). However, also exhibited negative indirect effects on EOC through plant height (-17.08%), number of umbellets (-22.69%), number of fruits (-15.83%), fruit yield (-15.25%) and biological yields (-11.30%).

The plant height, number of umbels, number of fruits, biological yield and TFW were positively correlated with EOC, but only plant height, number of fruits and TFW showed a significant association with EOC. In addition, the direct effects of these three traits on EOC were negative. This indicates that the observed positive correlations of these traits with EOC were due to their indirect positive effects on EOC through number of branches (10.29%, 1.34% and 15.18%, respectively) and number of umbel (30.81%). In other words, number of fruits (27.40%), plant height (25.54%) and TFW (25.37%) showed high indirect positive effects on EOC via fruit yield. Prominence should be given to the indirect influences when a character has a positively correlated and considerably positive indirect influence, but a direct negative effect on economic characteristics such as grain yield or essential oil content (Singh and Chaudhary 1979). This shows the importance of identifying genotypes with considerable EOC by indirect selection for the above-mentioned traits in the breeding program.

The most indirect positive effects of plant height, number of umbellets, number of fruits, biological yield and TFW were on EOC through fruit yield while number of branches and number of umbels has provided the most indirect positive effects on EOC through TFW. Also, fruit yield exhibited most positive indirect effect via number of umbellets but insignificant. According to Bahmani et al. (2012), the highest indirect influences on EOC were obtained for dry biomass weight over leaf number (0.3949). Interestingly, all traits affected EOC negatively indirect via plant height, number of fruits and biological yield, except number of branches. That is, the indirect effects of the number of branches on the EOC were positive and low to moderate. The highest negative indirect effect was revealed by fruit yield (-26.70%) through biological yield. This was followed by the number of branches (-23.90%) through fruit yield and the number of umbellets (-22.69%) via TFW.

The path coefficients related to the direct and indirect effects of the investigated characters on fruit yield are given in Table 3. All traits, viz plant height, number of branches, number of umbels, number of fruits, biological yield, TFW and EOC displayed a positive direct effect on fruit yield, just number of umbellets with a -8.96 % contribution ratio had a direct negative effect on fruit yield. Biological yield (59.88 %) provided the highest positive direct contribution. The direct effect of biological yield on seed yield of fennel was affirmed by Bahmani et al. (2015). Whereas, opposite to our result, biological yield has negative influence on seed yield in sweet fennel (Coşge et al., 2009). TFW (29.41 %), plant height (20.16 %), EOC (15.14 %), number of umbels (13.09 %), number of branches (5.23 %) and number of fruits (3.18 %) followed the biological yield. These results are consistent with the results of Patel and Patel (2015) for number of branches per plant, number of umbels per plant and test weight and Singh et al. (2020) for umbels per plant, seeds per umbels and plant height in normal environment and for umbels per plant, test weight and plant height in drought environment. Majority of these traits also exerted positive and significant correlation with fruit yield. Biological yield (r = 929**), number of fruit (r = 0.805**), thousand fruit weight (r=0.726**), plant height (r=0.696**) and number of umbellets (r=0.609**) had positive and highly significant ($p < 0.01$) correlation with fruit yield. It showed that, the positive and significant correlations of traits with fruit yield were due to the direct effects. Therefore, it is possible to propose that the characters could be used for indirect selection of genotypes for the yield. When we examine the indirect effects of biological yield, all traits have a highly positive indirect influence on fruit yield except the branches number (-23.86%). The maximal indirect positive effect on fruit yield through biological yield was shown by the number of fruits (37.57%), followed by plant height (29.86 %), thousand fruit weight (27.63%), number of umbellets (23.76%), number of umbels (17.36%) and EOC (8.43%).

Thousand fruit weight (29.41%) revealed the next highest positive direct effect on fruit yield. It also exhibited high indirect effects on fruit yield through essential oil content (27.34%), number of umbellets (26.33%), plant height (20.05%), number of fruit (18.81%) and biological yield (13.98%). But the positive direct impact of TFW on fruit yield was stabilized by the rather elevated indirect inverse influence via the number of umbels (-30.50%) and number of branches (-17.53%). This agrees with earlier reports of Cosge et al. (2009) whereas, Sefidan et al. (2014) reported positive and low direct effect of the TFW on fruit yield and negative indirect effect of the TFW on yield via number of umbel and number of fruits per umbel. In addition, Kumavat (2010) informed that TFW had a weak and positive direct effect on fruit yield.

Another character that has a high direct effect on fruit yield is plant height (20.16 %). When the indirect effects of plant height on fruit yield were examined, it was noticed that all traits had a positive indirect effect on fruit yield, of which EOC (17.37 %), number of umbellets (16.10 %), number of fruit (14.60 %), thousand fruit weight (13.30 %), number of umbels (11.32 %) and biological yield (10.02 %) were balanced by the relatively high negative indirect influence of the number of branches (-24.62%).

The number of umbellets (-8.96%) was the only trait that had a negative direct effect on fruit yield. However, this trait positively and significantly correlated with fruit yield at the 0.01 probability level (r=0.609**) (Table 1). In this case that is required to examine the indirect effects of this trait. Here, it is seen that the number of branches and the number of umbels have positive indirect effects on fruit yield via the number of umbellets. When the correlation coefficient is considerably positive and the direct influence is negative, a limited concurrent selection pattern will be applied, that is, constraints will be enforced to override the unwanted effect to profit the direct effect. (Sing and Kakar 1977; Rashid et al., 2010).

The most indirect positive effects of plant height, number of umbels, number of fruits, and TFW were on fruit yield through biological yield while number of umbellets, biological yield, and EOC has provided the most indirect positive effects on fruit yield through TFW. Among the traits, just the number of branches had the positive and small indirect influence on fruit yield via the number of umbellets, while it had a negative indirect effect through all other traits. In addition, while the simple correlation between the number of branches and fruit yield was negative and insignificant, the number of branches directly affected the fruit yield positively but low. Therefore, it is clear that indirect effect of this trait through other variables is negative. Similar situation was reported by Sefidan et al. (2014) for the relationship between 1000 grain weight and grain yield. Further, the most indirect effect was related to number of fruits (37.57 %), plant height (29.86 %), and thousand fruit weight (27.63 %) which was applied on fruit yield via biological yield. Bahmani et al. (2012) reported that the most indirect influence on seed yield were detected for number of leaves through weight of dry biomass (0.4135). All traits affected fruit yield negatively indirect and at a negligible level via number of branches. The most indirect negative effects on fruit yield were related to number of umbels (-30.50%) via TFW and number of branches (-24.62 %) via plant height and number of branches (-23.86 %) via biological yield.

4. Conclusion

When the results were evaluated in general, the correlation of EOC with umbrella number, fruit number, TFW and plant height was positive and quite significant. All these characters are the advantageously traits which are important to coordinate selection stage to improve the EOC in fennel. Path coefficient analysis showed that among the causal (independent) traits; fruit yield and umbellet number had positive direct effect on EOC. In addition, plant height, number of fruits and TFW also affected the essential oil content indirectly and positively. Therefore, these properties can be used as efficient selection criterions to achieve high yields in improvement programs to increase the EOC of fennel.

The correlation of fruit yield with biological yield, number of fruits, TFW, plant height and number of umbellet was positive and highly significant. In addition, all these traits exhibited positive and significant relationships with each other. Therefore, these characters can be taken as selection criteria to obtain higher fruit yield in fennel. In addition, all traits except the number of umbellets had a direct positive effect on fruit yield. The most important positive influence was determined on fruit yield through biological yield, TFW and plant height. In addition, fruit number, plant height and TFW showed the highest indirect effects on fruit yield thought biological yield. Therefore, these traits can be evaluated as selection criterion to attain higher seed yield in fennel.

Author Contribution

R.R.A.G and A.B.A contributed equally to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Conflicts of Interest

The authors declares that they have no conflict of interest.

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Research article
Curr. Pers. MAPs, (2021); 4(2): 103-107

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

The Effects of GA_3 Treatments and Nutrient Media on *In Vitro* Seed Germination of *Allium tuncelianum* (Kollman), Özhatay, Matthew, Şiraneci

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https://doi.org/10.38093/cupmap.1034719

Received: 09/12/2021
Accepted: 30/12/2021

Abstract

In this study, the effects of GA_3 and nutrient media (BDS and MS) on *in vitro* seed germination of *Allium tuncelianum* (Kollman), N. Özhatay, D. Matthew, Şiraneci, which is one of the endemic plants in Turkey, were investigated. In order to promote seed germination, GA_3 was applied to seeds under sterile conditions for 48 hours at different concentrations (2 mg L^{-1}, 3 mg L^{-1} and 4 mg L^{-1}). Then the seeds were cultured in BDS and MS medium. The results showed that the germination rate decreased with the increase of the applied concentrations of GA_3. The highest germination rate (17.73%) was obtained from seeds treated with 2 mg L^{-1} GA_3. It was determined that the effects of nutrient media on the germination of *Allium tuncelianum* seeds were important, and the highest germination values were obtained from the BDS medium.

Key Words: *Allium tuncelianum*, BDS, GA_3, Germination, MS

1. Introduction

Allium tuncelianum (Kollman), Özhatay, Matthew, Şiraneci, which is locally called as "Tunceli garlic" or "Ovacık garlic", is an endemic species unique to Turkey. It is widely found in especially in Ovacık and Pulümür districts of Tunceli province located at the foot of Munzur Mountain. *Allium tuncelianum* has single cloved, cream-white bulbs and also has the ability to flower and give fertile black seeds different from common garlic (Yanmaz and Ermiş, 2005; Yanmaz et al., 2010; Yarali and Yanmaz, 2016; Kizil et al., 2017; Yarali Karakan, 2019).

It has been reported that consumption of *Allium tuncelianum* stimulates the body's immune system, lowers blood sugar and cholesterol levels, improves blood circulation and thus reduces the risk of heart attack (Agbas et al., 2013; Atila et al., 2017; Yarali Karakan, 2019). In addition, Sehitoglu et al. (2018) stated that *Allium tuncelianum* has a stronger antioxidant, antiradical activity and effective essential omega acid levels than common garlic in terms of fatty acid compositions.

Allium tuncelianum is usually propagated by seeds and cloves. However, there is a problem of germination in the seeds due to dormancy that occurs after the seeds mature.

This situation makes it difficult to propagate the plant by seed (Alper, 2005; Kizil et al., 2017; Yarali Karakan, 2019). Therefore, extensive studies are needed to develop tissue culture techniques for *in vitro* propagation and seed germination of *Allium tuncelianum* (Kizil et al., 2014; Yarali Karakan, 2019). In this study, the effect of GA_3 as a germination stimulant and nutrient media were investigated *in vitro* conditions in order to ensure the propagation of *Allium tuncelianum* by seed.

2. Material and Methods

Allium tuncelianum seeds were kept in sodium hypochlorite solution (containing 5% NaOCl) for 20 minutes to ensure surface sterilization, and then washed three times for three minutes with distilled water (Kizil et al., 2017). In order to promote germination, different concentrations of GA_3 (2 mg L^{-1}, 3 mg L^{-1} and 4 mg L^{-1}) were applied to the seeds for 48 hours under sterile conditions. Then the seeds were cultured on BDS (Dunstan and Short, 1977) and MS (Murashige and Skoog, 1962) medium supplemented with 2.0% sucrose at 4 °C (Kizil et al., 2017), with 15 replications from each application and 15 seeds in each replication under *in vitro* conditions (Figure 1). 2 mm rootlets were accepted as germination criterion (Anonymous, 1985; Figure 2).

Figure 1. a) Sterilization of seeds, b) Filter sterilization of GA_3 solutions, c) Seeds treated at different GA_3 concentrations for 48 hours, d) Cultured seeds.

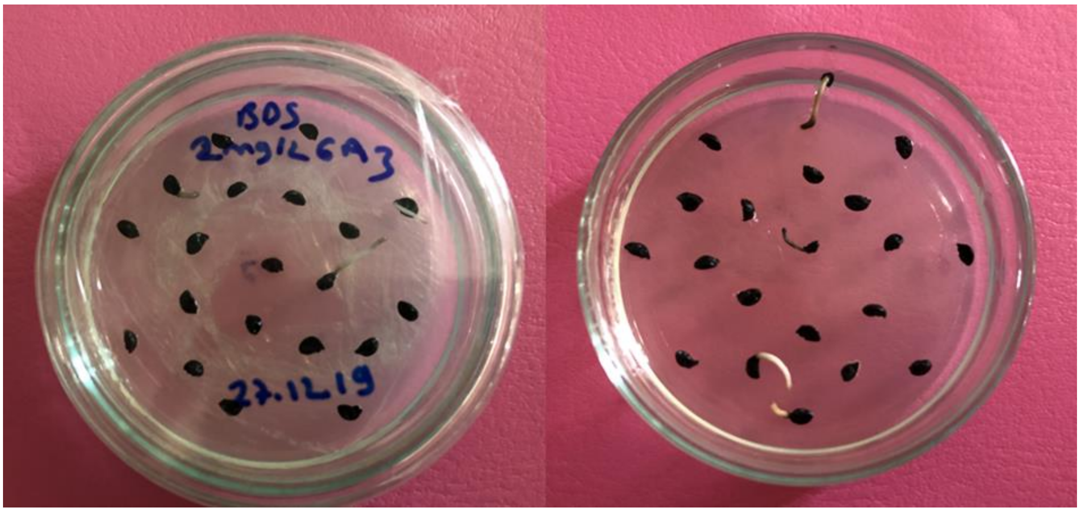

Figure 2. Germinated seeds in BDS medium treated with 2 mg L^{-1} GA_3

2.1. Statistical Analysis

The experiment was established according to completely randomized design (CRD) with 15 replications. Tukey test was used to identify different groups after analysis of variance.The level of statistical significance was taken as 5% and calculations were made with JMP software package version 5.0.1.

3. Results and Discussion

In this research, in order to reveal the effects of plant growth regulator application, the seeds were treated with gibberellic acid solution at different concentrations (2 mg L^{-1}, 3 mg L^{-1} and 4 mg L^{-1} GA_3) prepared under sterile conditions for 48 hours. Then, all seeds were cultured in BDS and MS medium at 4 °C. The effects of different concentrations of GA_3 applications and nutrient media on the germination of *Allium tuncelianum* seeds are shown in Table 1. When Table 1 was examined, it was determined that the seeds applied with 2 mg L^{-1} GA_3 had the highest germination rate of 17.73%. The germination rate decreased with the increase of the applied dose. Thus, the lowest germination rate was obtained from the application of 4 mg L^{-1} GA_3 in both nutrient media. It has been reported that storage at 4°C results in hydrolysis of seed starch resulting in mobility of carbohydrates in *Allium* species. Thus, macro-carbohydrate molecules are converted into sucrose, glucose and fructose, which are utilized during cell metabolism and energy required for the growth of plants. These have significant effects to break seed dormancy of garlic (Kizil et al., 2017). Similarly, it was reported that garlic seeds treated with low temperature at 4°C and gibberellic acid (GA_3) were helpful in seed dormancy break (Arguello et al. 2001). Yanmaz and Ermis (2005) used 0.5, 1.0, 2.0 mg L^{-1} doses of GA_3 for 24 and 48 hours in their study aiming to solve the germination problem in *Allium tuncelianum* seeds. They stated that GA_3 application for 48 hours was more effective on the germination and also it has been determined that 1.0 mg L^{-1} and 2.0 mg L^{-1} doses of GA_3 stimulate germination and increase the germination rate up to 18-20% depending on the low temperature period. In the study, it was determined that the effects of nutrient media on the germination of *Allium tuncelianum* seeds were also important. When BDS and MS media were compared, the highest germination values were obtained from BDS medium (Table 1). On the other hand, in their study to break dormancy in *Allium tuncelianum* seeds, Kizil et al., (2017) reported that it was higher in MS medium.

Table 1. Effects of GA_3 treatments and different nutrient media on germination of *Allium tuncelianum* seeds

GA_3 treatments	Nutrient media			
	BDS		MS	
	Germinated seeds (number/petri)	Germination rate (%)	Germinated seeds (number/petri)	Germination rate (%)
Control	0,80b	5,33	1,13 ab	7,53
2 mg L^{-1}	2,66a	17,73	1,33ab	0,86
3 mg L^{-1}	1,26ab	8,40	1,53ab	10,20
4 mg L^{-1}	0,40b	2,66	0,46b	3,06

Significant at P = 0.05 level

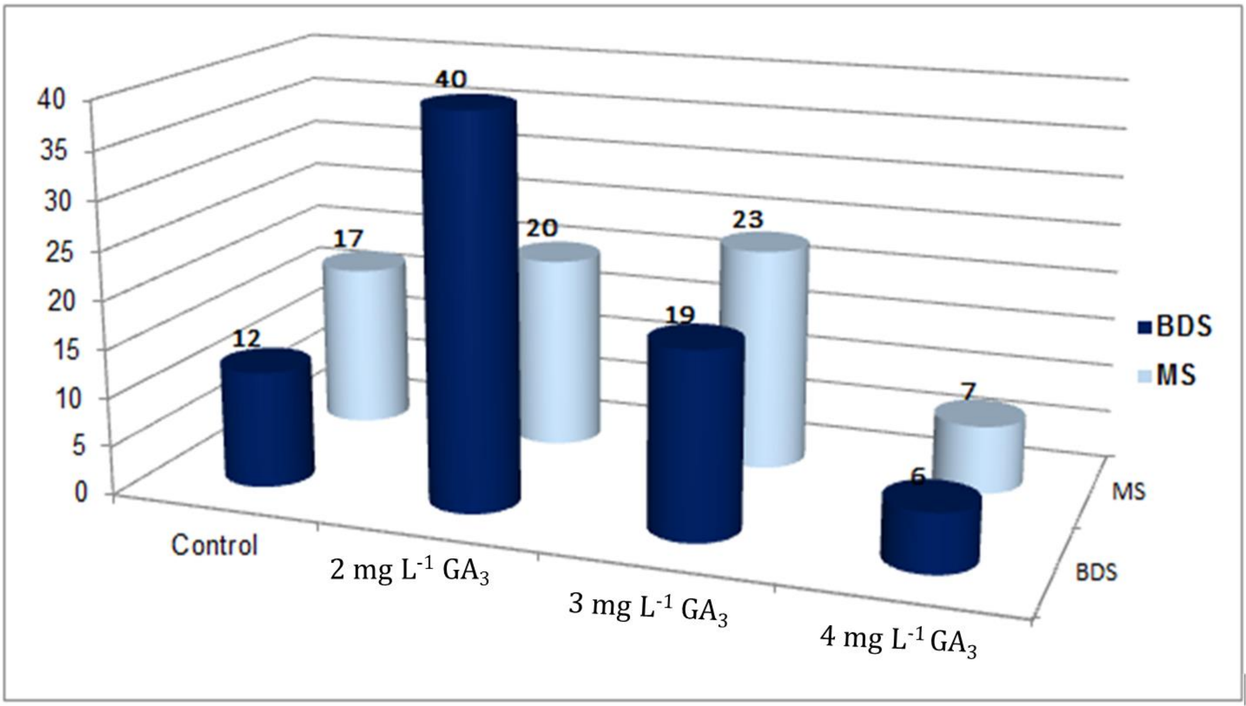

Figure 3. Number of germinated seeds in different GA_3 treatments and nutrient media.

4. Conclusion

The effects of GA_3 treatments and nutrient media on seed germination were found to be significant. The seed germination rate was higher at 2 mg L^{-1} GA_3 treatment than the other treatments. And also it was determined that the effects of nutrient media on the germination of *Allium tuncelianum* seeds were also important and the highest germination values were obtained from BDS medium. The results obtained from this research will contribute to future research for protection and propagation of *Allium tuncelianum.*

Author Contribution

Faika YARALI KARAKAN designed the experiments and wrote the paper.

Acknowledgements

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Conflicts of Interest

The author declares no conflict of interest.

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Research article
Curr. Pers. MAPs, (2021); 4(2): 108-120

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Plant-Based Milk Alternative: Nutritional Profiling, Physical Characterization and Sensorial Assessment

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https://doi.org/10.38093/cupmap.1037118
Received: 15/12/2021
Accepted: 30/12/2021

Abstract

The aim of this work was the characterization of four plant-based milks: oat, almond, hemp, and quinoa's milk. The nutritional composition of each milk, as well as its physico-chemical characteristics, microbiologic stability, and antioxidant potential, were determined in order to investigate the effect of the first ingredient on the overall quality of the plant-based milk. Finally, a hedonic sensory analysis was conducted in order to determine the acceptability of these products. In terms of nutritional value, obtained results highlighted that oat's milk has the highest carbohydrate, protein and fiber contents (23g/100ml, 5g/100ml and 3g/100ml, respectively). However, hemp's milk has the highest fat and calcium content (7g/100ml and 37.5 mg/ 100 ml, respectively). The pH of tested plant-based milk, were all similar and close to neutrality. The Withening Index (WI) values found were 21.46 for almond's milk, 9.48 for oat's milk, 34.97 for hemp's milk, and 0.1 for quinoa's milk. The antioxidant capacity assessment demonstrated that tested plant-based milk have an interesting potential. Measured inhibition capacities ranged from 78.34 % (quinoa's milk) to 63.14 % (oat's milk), and their total polyphenol content ranged from 0.310 mg EAG/g DM (oat's milk) to 0.836 mg EAG/g DM (quinoa's milk). The microbial stability results showed that quinoa's milk had the best bacterial stability, with a maximum denomination of 56 *104 UFC/ml for the first 8 days of storage, while almond and oat's milks had better fungal stability and were free of contaminants up to the eighth day of storage. During the hedonic sensory analysis, the almond's milk was the most appreciated by the panelist. Gathered results suggest that plant-based milks are high in nutrients and phenolic compounds, and they deserve to be considered as "healthy aliment".

Key Words: Plant-based milks; Nutritional composition; Microbiologic stability; Sensory analysis

Introduction

Milk and dairy products are ranked fourth on the food pyramid, and it is undoubtedly their nutritional value that has earned them a place among the essential foods for human health. Apart from its high content of macronutrients such as proteins (3.25g/100g), carbohydrates (4.85g/100g), and lipids (3.63g/100g), as well as micronutrients such as vitamins (vitamin A, B, and D) and minerals (calcium, phosphorus), milk is a multi-beneficial food. In fact, milk proteins help to maintain the balance of lean mass, osseous mass, and water, which accounts for 89.4g/100g of hydric mass (Wu et al., 2016). Furthermore, its balanced macronutrient and micronutrient provides them with high biodisponibility. Moreover, milk has a protective effect on the cardiovascular system by lowering the risk of arterial hypertension, as well as a protective effect against metabolic syndrome, childhood obesity, type 2 diabetes, certain cancers, nervous system disorders, and immune or digestive system disorders (Thorning et al., 2016).

Since several years, milk has been the target of an anti-milk movement that denies all of its health benefits. It has been also accused of being linked to a variety of diseases, including hormone-dependent cancers (due to the presence of hormones in milk), cardiovascular disease and obesity (due to its high content of saturated fats and cholesterol (Siri-Tarino et al., 2010), chronic gastrointestinal inflammation (McNees et al., 2015) and even questioning its place in the diet. The activists behind this movement support their cause by pointing out that milk is directly linked to lactose intolerance, which has reached a global prevalence of 75%, as well as allergies to milk proteins, which rank first among allergies in children and infants.
On a national level, in addition to the health issues mentioned above, the availability of milk to consumers is extremely heterogeneous in some Tunisian regions. Several factors are involved, such as the rise in industrial milk prices, the lack of access to pasteurized milk, or other socioeconomic or demographic factors. On the other hand, milk has long been thought to be a carrier of microorganisms that cause a variety of infectious diseases. In fact, according to the study, milk is a vector of the Mycobacterium bovis, which causes extra-pulmonary tuberculosis, which is still prevalent in Tunisia.

Another major reason for which proponents of the movement argue is that the manufacturing of milk and dairy products is responsible for the emission of greenhouse gases with harmful effects on the environment. This production was estimated to be in the range of 1 969 million tonnes of CO_2 equivalent (26 %) for global milk production in 2007 (about 553 million tonnes), which equates to 4% of total global greenhouse gas emissions. Reliant on his mother nature, and facing the alarming rates of diseases linked to milk consumption, man must develop animal-free alternatives to dairy products, such as the development of plant-based milks.

Vegetable milks are obtained by boiling and filtering plant matter (Sethi et al., 2016), which we may divide into five major categories: cereal-based lattes (oat, rice, etc.), legumes (soy, nut, etc.), nuts (almond, coco, hazelnut, pistachio, etc.), seeds (sesame, linen, hemp, etc.), and milks made from pseudo-cereal (quinoa, amaranth, etc.). Each form of milk has a different nutritional makeup depending on the first material used. Quinoa milk, for example, is known for its high levels of protein and polyunsaturated fatty acids of the omega 3 and omega 6 kinds. While hemp grain milk is high in monounsaturated fatty acids, almond milk is high in fibers, vitamins, and minerals (Silva et al., 2020), antioxidants and phytoestrogens. As a result, these gluten-free lattes would be a good option for those who have health issues related to milk and dairy products, as well as those who consume gluten-free products. When it comes to environmental effect, plant-based milks have a less impact than cow's milk. In fact, a study published in (Smedman et al., 2010) found that the CO_2 emissions from animal-derived milk are in the range of 99 g CO_2 equivalent/100 g of product, compared to 30 and 21 g CO_2 equivalent/100 g of product for soy and oat, respectively. The water footprint (the amount of water required for the manufacture of a product) is a second indicator that researchers use to estimate the impact of food on the environment. In this context, it was estimated that producing a litre of cow's milk necessitated 628 liters of water, compared to 371 liters for almond, 270 liters for rice, 48 liters for oat, and 28 liters for soy milk (Poore and Nemecek, 2018) .

In this context, our research has focused on plant-based milks as a nutritional, economic, and environmental alternative to cow's milk and dairy products. As a result, we attempted to the preparation of four plant-based milks: oat, almond, hemp, and quinoa's milk. The nutritional composition of each milk, as well as its physico-chemical characteristics, microbiologic stability, and antioxidant potential, were determined in order to investigate the effect of the first ingredient on the overall quality of the plant milk. Finally, a sensory analysis was conducted with a group of Tunisians in order to determine the acceptability of these products that are still unfamiliar to many.

2. Material and Methods

2.1. Plant sampling: During this research, we attempted to make four different types of plant milks: almond (made from nuts), oats (made from seeds), hemp (made from grains), and quinoa (made from pseudo-cereals). Seeds were purchased from Tunisan local market.

2.2. Milk preparation: The first step is to soak vegetable materials in tap water for 12 hours in a refrigerator set at 4 degrees Celsius. After that, 200 gr of each vegetable material were rinsed and mixed for 5 minutes in a mixer (Moulinex Power Blendy 400 W) with mineral water in a 1:5 ratio. Finally, the vegetable milk were obtained by filtering the mixture through a vegetal milk bag. The obtained filtrate was kept in the refrigerator until analysis.

2.3. Determination of the nutritional composition of vegetable milks

Protein content: The kjeldhal technique was used to determine the total protein content of each milk sample. In summary, the first step is to demineralize the organic matter using sulfuric acid, followed by a second step of alkalinizing the reaction products. The coefficient of conversion of organic azote to proteins is 6.25 (Damasceno et al., 2020).

Lipid content: It was determined by dissolving milk in a solvent, which in this case is ether diethylique, and then evaporating the solvent (Damasceno et al., 2020).
Sugar content. The sugar content was determined using spectrophotometry (Damasceno et al., 2020).

Fiber content: The fiber content was determined using AOAC 2015 standards. A portion of 2 g lyophilized milk is placed in 200ml of 1,25 $H2SO4$ and boiled for 30 minutes. The solution was then placed in a funnel bucher with a muslin fabric. The residue was filtered and then washed with hot water to remove the acid. The residue was then placed in 200 ml of 1.25 % NaOH and boiled for 30 minutes before being filtered. The residue is then placed in a humidity extraction oven until it reaches a constant weight.

Calcium content: The calcium content was determined using spectrophotometry (Damasceno et al., 2020).

2.4. Determination of the antioxidant activity and the total polyphenol content

Extraction: For the extraction step, a total of 25 mL of an 80 % methanol solution was added to 5g of each lyophilized sample, followed by agitation for 24 hours at room temperature. The tubes were then centrifuged for 5 minutes at 6000 tr / min. The surnageant (extract) was collected after centrifugation for analysis (Yilmaz-Ersan et al., 2018).

The capacity to neutralize the DPPH radical measurement: The anti-oxidant activity were assessed in vitro using the DPPH° method (BenJemaa et al., 2021). A sample of 1 ml of each plant extract (20 mg/ml) is placed in the presence of 250 µl of DPPH (0.22 mM in methanol). The mixture is placed in the dark for 30 minutes to react, and the absorbance is measured at 517 nm against a negative control. The results were expressed as a percentage of inhibition, which is calculated as the intensity of the coloring of the mixture decreases, according to the formula:

IC = (DO control – DO sample / DO control)*100

IC: inhibition capacity; DO control: control absorbance; DO sample: the absorbance of investigated milk extract.

2.5. Physico-chemical properties of vegetal milk pH: The pH of the four milks was determined by diluting them 1:10 in pure water and measuring them using a pH meter. For each studied sample, all measurements were taken at ambient temperature (Aziz et al., 2018). The color was measured with a colorimeter, and the results were expressed in terms of the chromatic space L * a * b * as defined by the International Commission on Lighting (CIE) in 1976. The three coordinates represent the color's clarity (L *), its location between red and green (a *), and its position between yellow and blue (b *) (Tapsell et al., 2006).

The whiteness index was calculated using the following formula:

$$WI = 100 - \sqrt{((100 - L)^2 + a^2 + b^2)}$$

2.6. Evaluation of the microbiologic stability of plant milks: The microbiologic stability of the studied milks was assessed by counting sample's living flore stored at 4°C for 12 days. To do so, 100 µl of each milk were pre-mixed at j=1; 4; 8; 12 in order to do a series of dilutions (1:10) up to 10^{-3} (BenJemaa et al., 2017). 100 mL of each of the last two dilutions were plated on agar plates (Muller Hinton for Bacteria and Sabouraud for yeast and fungi) and viable colonies were counted after incubation for 24 hours at 37°C for Bacteria and 5 days at 20°C for yeast and fungi. The following formula (Ałtyn et al., 2020) was used to calculate the Colony Formant Unit (CFU):

$$N\ (CFU/ml) = \frac{\sum c}{V\ (n1 + n2 * 0,1)\ d}$$

$\sum c$ = Total number of colonies counted in boxes with a number of colonies ranging from 20 to 300; V: inoculum volume in each bottle; n1: the number of Petri dishes counted in the first dilution; n2: the number of Petri dishes counted in the second dilution; d: dilution factor, based on which the first counts were made.

2.7. Sensory analysis of vegetal milks: The sensory analysis took place over five hours at the University Mahmoud El Materi, with 70 students. The samples were kept at 15°C throughout the experiment and served in 20 ml pots filled halfway. A hedonic test to determine the acceptability of products in terms of color, odor, taste, and aftertaste by panels (BenJemaa et al., 2021).

2.8. *Statistical analysis:* The data from the sensory analysis were treated with Excel 2007 program. The statistical analysis was carried out with the help of the statistical software SPPSS version 23, and a margin of error of 5% was taken into account. The number of participants considered for the hedonistic test is 60.

3. Results

With a similar appearance and color to cow's milk but a different flavor, can plant-based milks have a nutritious composition and interesting physico-chemical properties to replace cow's milk in people with intolerances, allergies, or other health issues?

3.1. *Nutritional composition of vegetable milks determination:* In terms of carbohydrates, oat milk has the highest content (23g/100ml), compared to other vegetable milks (6g for almond, 7g for hemp, and 3.7g for quinoa milk). The comparison of observed values with those of cow's milk reveals that, with the exception of quinoa's milk, the vegetal milks studied are richer in carbohydrates. In fact, the carbohydrate content of almond, oat, and hemp's milk is significantly higher than that of cow's milk (4.85g/100g).

In terms of protein content, the results showed that different tested milk varieties had different levels of content. In comparison to other plant-based milk (3g for hemp, 2g for almond, 1.5 for quinoa), oat milk has a higher content (5g/100ml). The protein content of hemp milk is comparable to that of cow's milk (3 g/100g), however the protein content of almond and quinoa milks is lower. It is worth noting that oat's milk is higher in protein than cow's milk. Hemp's milk has the highest lipid content (7 g/100 g) when compared to other vegetal milk (2.7 g/100 g for almond and oat's milk, and 1.8 g/100 g for the quinoa's milk). This content is much higher than that of cow's milk (3.63g/100 g).

In terms of fibers, it is worth noting that almond, oat, and quinoa milks have high fiber content (1; 3; and 1.5 g/100g, respectively).

The focus on the calcium content of studied plant-based milks showed that the four samples have higher levels (375 mg/100g for hemp, 360 mg/100g for oat, 341 mg/100g for quinoa milk, and 225 mg/100g for almond milk) than cow's milk (120 mg/100g).

Table 1. Nutritional comparison of the four tested vegetable milk and cow's milk.

Milk's type	Carbohydrates (g/100 g)	Proteins (g/100 g)	Lipids (g/100 g)	Fibers (g/100 g)	Calcium (mg/100 g)
Almond	6±2.03**c**	2±0.05**b**	2.7±0.9**b**	1±0.06**b**	225±23**b**
Oat	23±1.5**e**	5±0.7**d**	2.7±0.65**b**	3±0.36**d**	360±10**d**
Hemp	7±0.6**d**	3±1.2**c**	7±1.41	0±0.01**a**	375±75**e**
Quinoa	3.7±0.02**a**	1.5±0.01**a**	1.8±0.02**a**	1.5±0.07**c**	341±6**c**
Cow	4.85±1.01**b**	3.32±1.01**c**	3.63±1.3**c**	0±0.02**a**	120±11**a**

*The values that are followed by the same letter are not statistically different at $p<0.05$.

3.2. Physicochemical propreties of vegetable milks

pH measurement: The acidity of the four plant-based milks studied is represented in Table 2. The results show that the pH values measured are statistically similar and close to neutrality.

Table 2. pH determination of almond, oat, hemp, and quinoa's milk.

	Almond	Oat	Hemp	Quinoa
pH	6,72 ± 0.02**a**	6,61 ±0.9**a**	6,90 ±0.84**a**	6,46 ±0.6**a**

The values that are followed by the same letter are not statistically different at $p<0.05$.

Colorimetric proprieties of vegetable milks

The colorimetric properties of almond, oat, hemp, and quinoa's milk were expressed as a function of the Whitening Index WI, which was calculated using the chromatic space L * a * b * of each milk. The data in Table 3 represent the obtained results.

Table 3. Colorimetric properties of plant milks expressed as a function of the chromatic space L * a * b * and the Whitening Index

	L*	a*	b*	WI
Almond	21,54 **c**	0,43 **d**	3,32 **b**	21,46 **c**
Oat	9,56 **b**	-0,84 **b**	3,51 **b**	9,48 **b**
Hemp	34,99 **d**	-0,05 **c**	1,53 **a**	34,97 **d**
Quinoa	0,19 **a**	-1,25 **a**	3,98 **b**	0,10 **a**

The values that are followed by the same letter are not statistically different at $p<0.05$.

It is worth noting that, despite their white hue, the four studied vegetal milks had distinct colorimetric properties. In fact, the calculation of each milk WI yielded statistically unique values. Furthermore, the highest WI was found in hemp's milk, which was in the range of 34.97, followed by almond's milk (WI= 21.46), and then oat's milk (WI=9.48). Quinoa's milk, on the other hand, has the lowest WI (WI=0.1).

3.3. Total polyphenol measurement and antioxidant activity determination

Total polyphenol measurement: The quantitative assessments of the total phenol content were determined using the equation of linear regression of the calibration curve, which was traced using Gallic acid as a reference. The results are expressed in milligrams of EAG per milligram of DM (Figure 1).

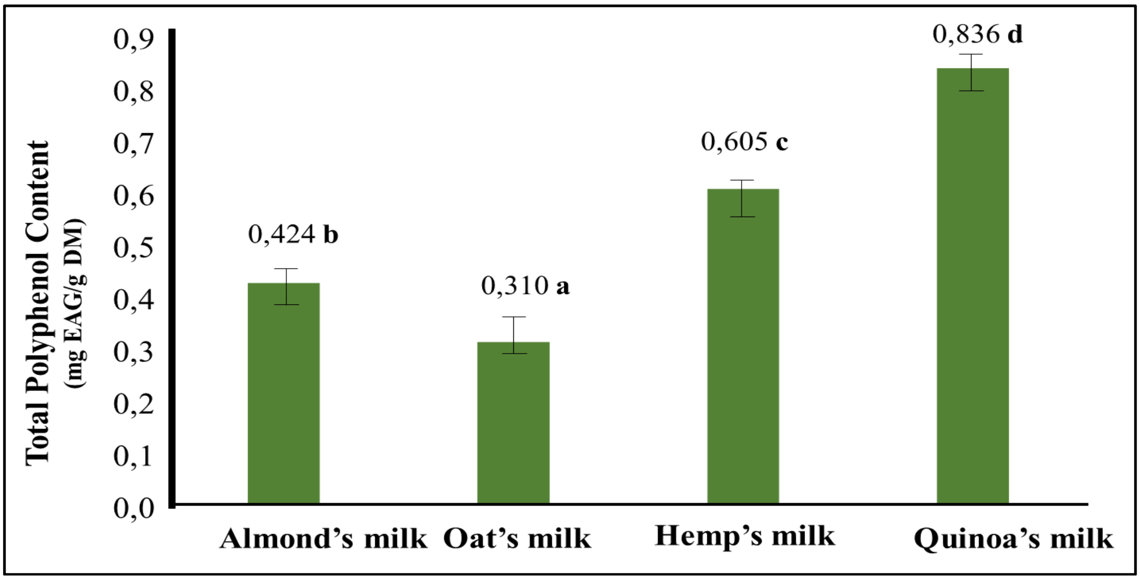

Figure 1. Total polyphenol content of almond, oat, hemp, and quinoa's milks at a concentration of 200 mg/ml. The results are expressed in mg EAG/g DM. The values that are followed by the same letter are not statistically different at $p<0.05$.

The findings showed that the four plant-based milks have an interesting total polyphenol content, with significant variation depending on the species. In fact, measured content ranged from 0.31 mg EAG/g DM (oat milk) to 0.836 mg EAG/g DM (quinoa milk).

The capacity to neutralize the DPPH radical measurement: The DPPH method is based on determining the amount of DPPH radical neutralised by an antioxidant, which is in this case a bioactive compound found in plant milk samples (Figure 2).

The findings showed that the studied plant-based milks have an antioxidant potential, as measured by their ability to absorb the free radical $DPPH^{+}$. In this context, oat and hemp milks had similar capacities (PI = 63%), however almond and quinoa milks had higher potential (73.12 et 78.34 % , respectively).

3.4. Evaluation of the microbiologic stability of plant milks:

The microbiologic stability of the four studied plant-based milks was assessed by bacterial and fungal enumeration of milk samples stored at 4°C for 12 days. The obtained results are shown in Figures 3A and 3B.

The results of Figure 3A showed that the bacterial contamination of hemp's milk developed quickly. In fact, after four days of storage at 4°C, the number of the living bacteria in the hemp's milk had surpassed 200 *10⁴ CFU/ml. However, the rate of bacterial growth in almond and oat's milk was slower, and the enumeration did not reach 200 *10⁴ CFU/ml until after 8 days of storage at 4°C. It is worth noting that quinoa's milk had the best bacterial stability, with a maximum denomination of 56 *10⁴ UFC/ml for the first 8 days of storage. Even though, a substantial increase in this charge (300 *10⁴ CFU/ml) was seen towards the end of the study.

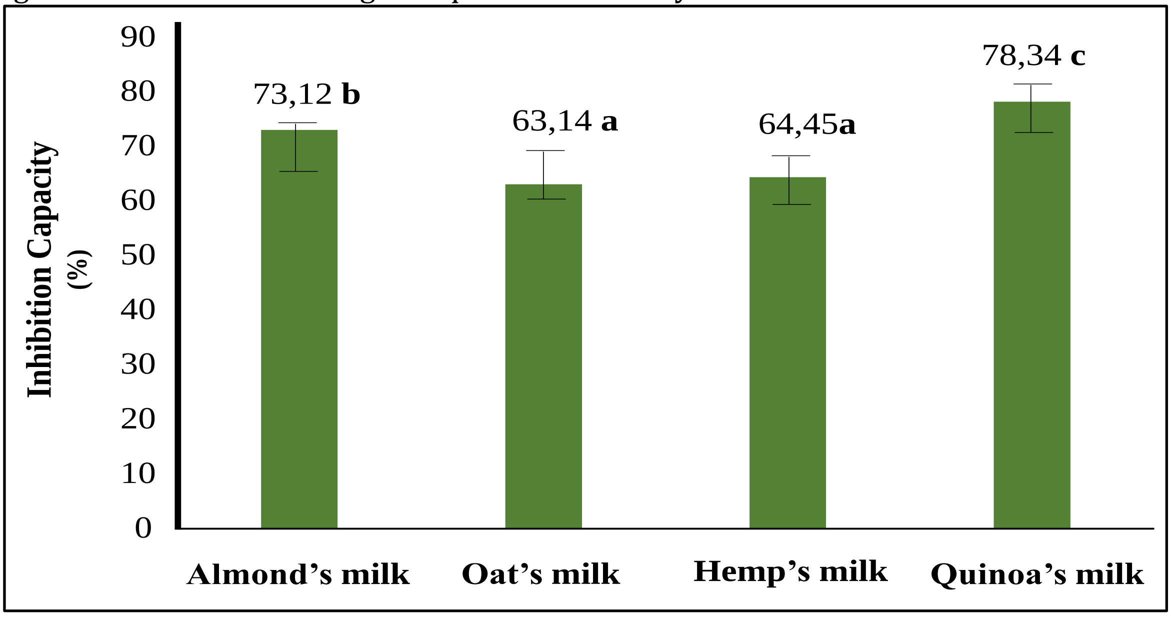

Figure 2. The radical DPPH inhibition capacity measurement of almond, oat, hemp and quinoa's milk (20 mg/ml). The results are expressed as a percentage of inhibition capacity. The values that are followed by the same letter are not statistically different at $p<0.05$.

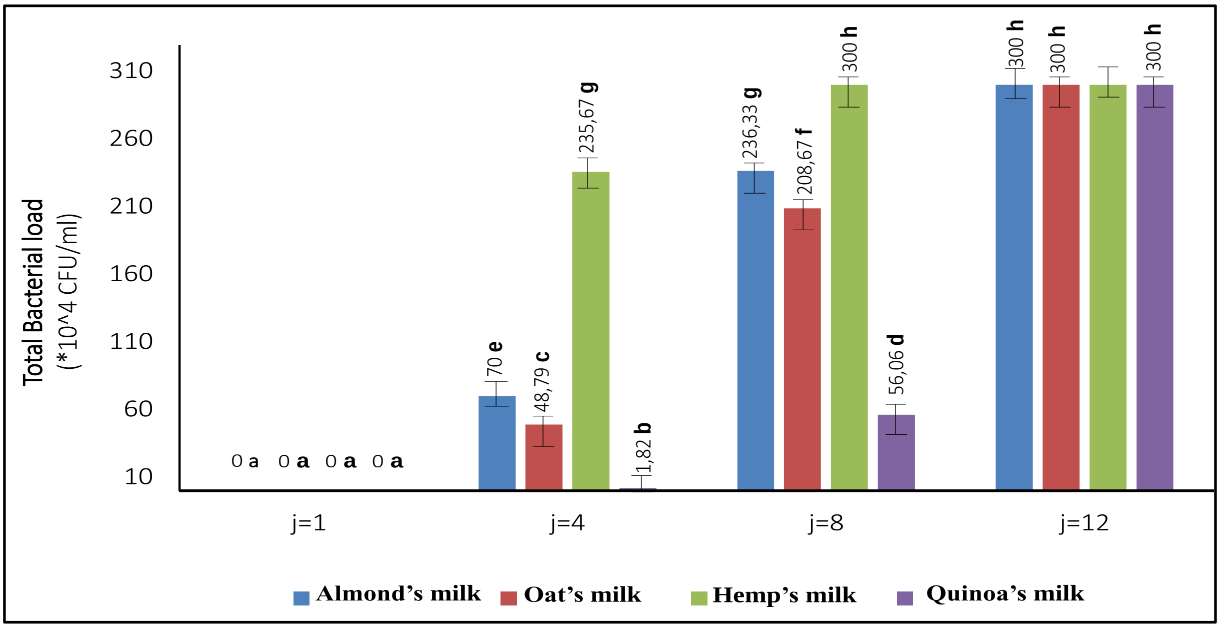

Figure 3A. Total bacterial load of almond, oat, hemp, and quinoa's milk, stored at 4°C for 12 days. The results are expressed in *10⁴ CFU/ml. The values that are followed by the same letter are not statistically different at $p<0.05$

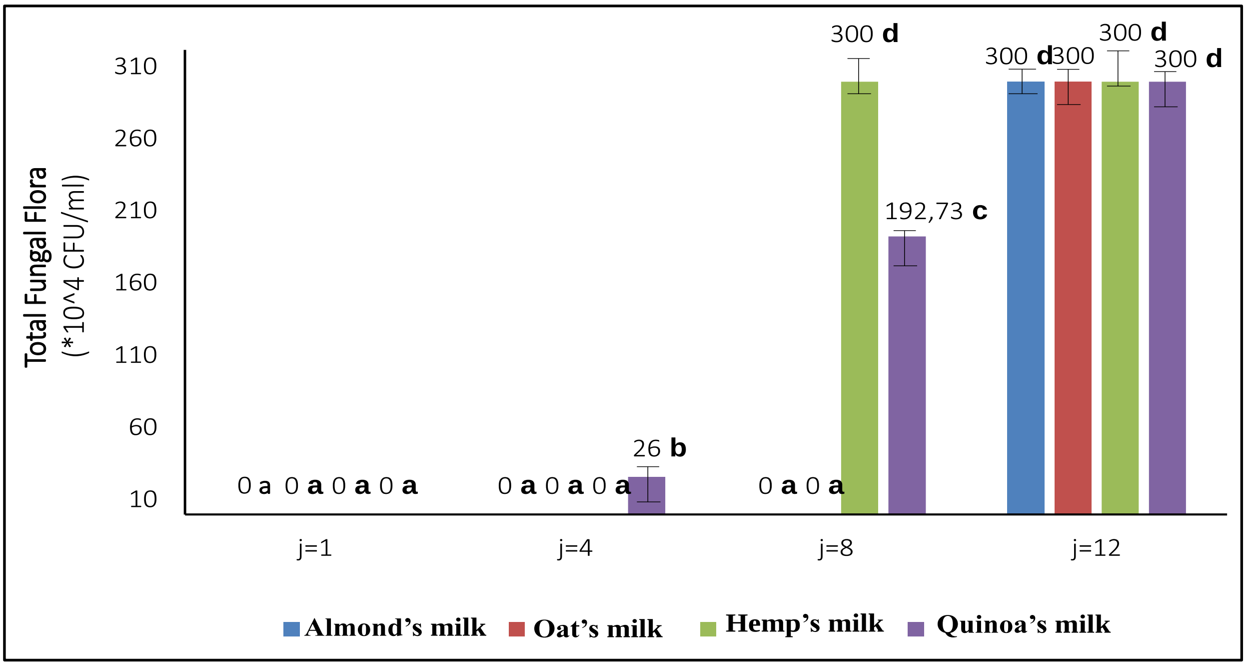

Figure 3B. Total fungal load of almond, oat, hemp, and quinoa's milk, stored at 4°C for 12 days. The results are expressed in *104 CFU/ml. The values that are followed by the same letter are not statistically different at $p<0.05$.

Figure 3B showed that the four studied vegetal milks were able to maintain their fungal stability for up to four days with charges less than $26 *10^4$ CFU/ml. However, at the end of the eighth day of storage at 4°C, the charges in yeast and fungi of the hemp and quinoa's milks had rapidly developed and reached $200 *10^4$ CFU/ml. It is worth noting that almond and oat's milks had better fungal stability and were free of contaminants up to the eighth day of storage.

3.5. Sensorial hedonic analysis: The hedonic test of the four tested varieties (shown in Figure 4) showed that the evaluation of organoleptic attributes (color, odor, taste, after taste, and the overall appreciation) varies depending on the milk's type.

In terms of color, it is worth noting that almond's milk was the most appreciated, with a score of 3.76. On the other hand, oat's milk was the least liked, receiving a score of 3.02. For other milks, their overall ratings are 3.41 and 3.06 for quinoa and hemp's milk, respectively.

In terms of odor perception, the ratings assigned to the four milks were similar, ranging from 3 to 4, with a score of 3.86 for quinoa's milk, 3.68 for hemp's milk, 3.45 for almond's milk, and 3.27 for oat's milk. It can be concluded that quinoa's milk has the best odor compared to oat's milk, which has the worst odor.

The almond's milk was the most well-liked sample, with a score of 3.86, while the quinoa's milk was far less well-liked, with a score of 2.47. For the other varieties of vegetal milks, a score of 3.21 was given to oat's milk and a score of 2.62 was given to hemp's milk.

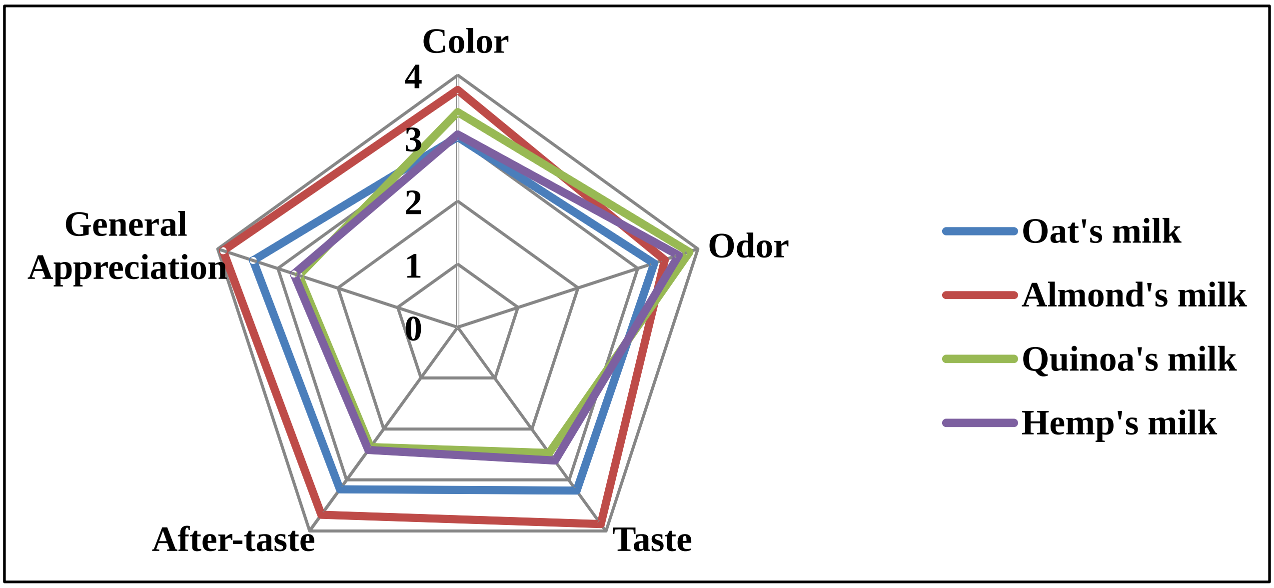

Figure 4. Hedonic sensory analysis of almond, oat, hemp, and quinoa's milk. The results are expressed on a five-point scale (1 being the least liked and 5 being the most liked). The values that are followed by the same letter are not statistically different at $p<0.05$.

4. Discussion

Milk substitutes of plant origin, or plant extracts, are preparations extracted from legumes, oleaginous fruits, cereals or pseudo-cereals, and are known for their beneficial effects on human health. All plant-based beverages are defined as fluids resulting from the maceration, grinding, and filtering processes, which include extracting nutritional components from water (representing approximately 80% of the final product) (Munekata et al., 2020).

Their white color and somewhat thicker texture, similar to that of cow's milk, is the result of decomposition and the size reduction of the raw materials and their homogenization, earning them the mark "plant-based milk". Even if others argue that this word should not be used to describe these products and that we should instead refer to them as "milk substitutes," as suggested by the Codex Alimentarius Commission in 1994, which defines a "subtitution aliment" as "an aliment designed to resemble another aliment".

Although "plant-based milk" are commonly known as low-caloric and low-fat foods, but this is not valid for all types of milk.
We came to the conclusion that plant-based milks are not necessarily lower in calories than cow's milk (60 kcal/100g of product) (Vanga et al., 2018). Their nutritional profiles, on the other hand, are more interesting. Although they do not include cholesterol or saturated fats, such as goat milk, some of them do contain polyunsaturated fatty acids of the omega 3 and omega 6 types (Munekata et al., 2020).

The literature has revealed a more interesting aspect on the nutritional front: their richness in micronutrients and bioactive compounds contained in the primary material, and which are known for their health benefits. For example, almond's milk contain significant amounts of vitamins E and C, known for their anti-oxidant properties (Sethi et al., 2016). Legumes are a good source of mono- and polyunsaturated fatty acids, minerals (Fe^{2+}, Zn^{2+}, Mg^{2+}), and phytoestrogens (isoflavones), which are known for their effects in the fight against osteoporosis, cardiovascular disease, gastrointestinal cancer, and menopause symptoms (Patisaul et al., 2010).

This research focused on determining the nutritional profiling of four different varieties of plant-based milks (almond, oat, hemp, and quinoa's milk). The selection of milks was made based on their nutritional profiles and the importance of their consumption across the world. In order to maximize the nutritional value of these plant-based beverages, we compared their compositions to a standard beverage and discovered that their macronutrient and micronutrient content is now far more interesting and significant than nutritionist recommendations.

In this study, differences have been detected in the nutritional compositions of tested milks, and other studies' milks, which may be explained, in part, by the differences in the nutritional content of the original used materials, as demonstrated by the study for almonds (Yada et al., 2011). Indeed, environmental factors such as cultivation region, cultivation methods, climate conditions that vary between harvest years and grain maturity, and interactions between these factors all influence the nutritional value of almonds. Furthermore, because almonds are natural products, variation in their composition might be expected even within a single cultivar. This research has highlighted the importance of genotype as a primary factor in determining nutritional content.

Furthermore, the manufacturing method for plant-based milks differs. Our research focused on artisanal vegetable milks due to a lack of commercially available products, whereas other studies focused on commercial plant-based milks, implying that conservation, enrichment, and improvement processes were carried out because these products present a significant challenge in achieving a similar appearance to the cow's milk to which the consumer is accustomed.

Calcium is the micronutrient whose presence in plant-based milks is the most concerning. While our research revealed some interesting calcium levels for various types of plant milk, it remains that it has a relatively low biodisponibility when compared to calcium found in cow's milk. This may explain why, while the majority of plant-based milk substitutes have calcium levels similar to cow's milk, their biodisponibility varies depending on the added source, the most common of which being calcium carbonate and tricalcium phosphate (Silva et al., 2020).

The stability of the final product, according to the study (Silva et al., 2020), depend on the size of the obtained particles after the maceration and the grindin as well as the filtering of raw material. The plant-based milk substitute is made up of colloidal systems made up of large dispersed particles such as solid primary materials, proteins, fat globules, and amidon granules that easily settle (Sethi et al., 2016). As a result, creating a stable finished product that can be stored for a long time without leaving a sand, granule, chalky gold taste in the mouth is difficult. The use of stabilizers such as hydrocolloids to reduce the size of solid particles has been proposed as a solution to the problem of stability (Mäkinen et al., 2016).

We concluded that plant-based milks may be supplemented with nutritional additives in order to bring their nutritional profile closer to that of cow's milk, therefore altering their nutritional composition. Plant-based beverages are typically subjected to an ultra-high temperature (UHT) treatment before being packaged to ensure food quality and safety while in storage. However, conventional high-temperature processing might accelerate the degradation of sensitive components such as vitamins and minerals. This could justify the fact that the nutritional profile might be harmed throughout the manufacturing process (Mäkinen et al., 2016). Given that the ability of plant-based milk to reduce the DPPH radical is dependent on the amount of polyphenol it contains, the DPPH and total polyphenol content must be positively correlated. Interestingly, this statement was confirmed because the highest DPPH inhibition (78.34%) corresponds to the highest polyphenol content (0.167 mg EAG/ml) and the lowest DPPH inhibition (63.14%) corresponds to the lowest polyphenol totality.

The majority of the pH values found in this study are similar to those found in many literature-based comparison studies. If the pH of the milk differs from the one found in our study, it could be due to a difference in the pH of the water or the addition of other compounds that can change the pH of the milk. For instance, we noted that the quinoa milk in our study had a slightly lower pH (6.46) than the milk in the study of Jeske et al., 2018. Actually, the addition of enzymes: to the latter (to improve its emulsifying properties), may not be optimal due to quinoa proteins. Alpha amylase and protease both require an acidic pH to perform their proteolysis properly (Jakopović et al., 2019), which could explain the pH difference between different quinoa's milks.

In terms of DLC determination, we attempted to compare our results to microbiologic standards related to raw milk, which must be less than $40*10^4$ CFU/ml, owing to a lack of scientific studies highlighting the microbiologic properties of raw milk. We have seen that plant-based milks are more conducive to microorganism development from the start, making them unfit for consumption, which highlights the value of a thermal treatment to extend their shelf life (Munekata et al., 2020). In the study (Paul et al., 2020), the almond and oat's milk had a lifespan of 170 days and 28 days, respectively, at 4 C°, highlighting the necessity of thermal treatment.

However, even after thermal treatment, the duration of life of the hemp's milk was found to be 3 days, leading us to conclude that the methods used to extend the lifespan of the hemp's milk are ineffective. Alternative treatment technologies such as high hydrostatic pressure, high-pressure homogenization, ultra-sons, and pulsing electrical fields have been proposed to reduce these drawbacks (Sethi et al., 2016). Other methods, such as the use of microwaves, high pressure, ultraviolet sterilization, pulsing electric fields, and ultra-sons, can be used as alternatives to thermal treatments. A scientific evaluation of these novel treatment technologies revealed promising results in terms of extending the shelf life, preserving nutritional properties, and reducing the loss of bioactive compounds in plant-based foods (Silva et al., 2020).

There are few studies on the comparison of sensory analysis of plant milks in the literature. Nonetheless, the study (Jeske et al., 2018), the most similar to ours, compared the sensory properties of a variety of plant-based milks, including almond, oat, rice, soy, hemp, and lentils. Consumers ranked appearance, aroma, flavor, mouthfeel, and overall sensation as the most important sensory properties. The variability of the vegetable milk hedonic appreciation could be related to the industrial process used to improve one or more aspects of the plant milk. All of these processes have an effect on the organoleptic qualities of plant milks.

5. Conclusion

As a result of this research on plant-based milks, it could be concluded that these products have an interesting nutritional composition and physico-chemical properties, and that they could partially replace milk and milk products. Furthermore, these plant extracts include anti-oxidant properties that are known to be beneficial to health. These products, which do not have a place on the market with a local production, should attract the attention of industrialists in order to find a position among the other imported products, and why not among the dairy products.

Author Contribution

M.B.J., R.G., H.F., R.K. and R.S.B. contributed equally to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Acknowledgements

The Tunisian Ministry of Higher Education, Scientific Research, Information and Communication Technologies supported this study.

Conflict of Interest

The authors declare no conflict of interest.

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Research article
Curr. Pers. MAPs, (2021); 4(2): 121-127

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Harvesting time substantially affects the squalene content in olive oils of Gemlik and Kilis Yaglik varietes

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https://doi.org/10.38093/cupmap.1031125
Received: 01/12/2021
Accepted: 30/12/2021

Abstract

Olive (Olea europaea L.) is a significant crop especially in Mediterranean countries. In Turkey olives and olive oil have a major agricultural importance. Olives and olive oil have been used not only nutrient and as a flavor enhancer in Mediterranean dishes, but also a medicine because of having essential roles for human health. The pharmaceutical value and uses are related to biochemical content and compounds available. Mainly four chemical sources: mono-unsatured fatty acids, polyphenols, carotenoids and squalene are functional bioactives in olive oil particularly for pharmaceutical affects. The present study was designed to examine changes of squalene contents of olive oil gained Kilis Yağlık and Gemlik olive cultivars' fruits harvested in tree different time along the two years. Squalene content in olive oils varied according to harvest time, cultivar and cultivation year. Accordingly, the variety affected the squalene content, in Gemlik cultivar squalene was more than Kilis Yaglık cultivar. In terms of its cultivated year, in the high yield year, an increase in squalene content was observed. In olive oil, squalene content decreased first then increased by the fruit maturation.Therefore, harvest time effected biochemical content of oil by the fruit ripening stages.

Key Words: Olea europaea L., olive oil, squalene, harvest time, olive varietes

1. Introduction

Olive is an important agricultural plant grown in the Mediterranean basin.Turkey is also one of the important olive producing countries. According to 2019 data, Turkey ranks 4th in the world olive production with 1525000 tons. Spain is in the first rank with 5965080 tons, followed by Italy (2194110 tons) and Morocco (1912238 tons) in the second and third ranks, respectively (Faostat, 2019). The olive plant has been used as food and medicine in this region for centuries. The olive plant was distributed to Mediterranean countries by eastern civilizations (Sanchez-Rodriguez et al., 2019). Olive oil, which is used by humans in diets, has positive effects on physiological and cellular activities, has been produced since ancient times. (Martakos et al., 2020). Olive oil is acquired by crushing olive fruits and is a useful and valuable component of the Mediterranean diet. In recent years, due to the increasing importance of the Mediterranean diet in terms of health and nutrition, studies on olive and olive oil have increased.

It is considered one of the healthiest plant-based oils by consumers around the world, as there are many arguments for the role of olive oil, particularly in preventing heart and blood vessel ailments. (Fernández-Cuesta et al., 2013). Olive oil includes of fatty acids with a carboxyl group (COOH) and a carbon atom in this carboxyl group. The main fatty acid with beneficial health effects in olive oil is oleic acid, which is an unsaturated fatty acid and create more than half of the total fatty acids (Alowaiesh et al., 2018). Oleic acid is accompanied by numerous biologically active minor components, including a wide variety of sterols, squalene, phenolic compounds and tocopherols (Fernandez-Cuesta et al., 2013).

Squalene is a inherently consisting terpenoid aliphatic hydrocarbon that is produced by all animals and plants, including humans, and represents almost all of the hydrocarbons, and represents almost all of the hydrocarbons (Martakos et al. 2020, Cayuela and Garcia., 2018). Squalene is mainly derived from shark liver oil, but squalene, which is produced from plants, has become more important over time (Fernández-Cuesta et al., 2013). Squalene is found in nature, primarily in olive oil, and in vegetable oils such as rice bran oil, wheat germ oil, palm oil, or amaranth oil. On the other hand, squalene (C30H50), known as microalgae oil, which can also be obtained from microalgae, is one of the oils obtained from squalene algae with a double hydrogen bonded isoprene frame (Figure 1.). It has been stated that it can be designed to be used as a fuel for jets while being used as a biofuel, as it creates a hydrocarbon source equivalent to heavy oil (Oya, et al., 2015, Kimura, et al., 2018). Olive oil is one of the sources that contain the most squalene, which makes up more than half of the non-soap-forming substance. It has been reported that the concentrations of pigment, tocopherol and squalene in olive oil differ based on location, olive fruit varieties, growing parameters, ripening stage and oil production parameters (Martakos et al., 2020).

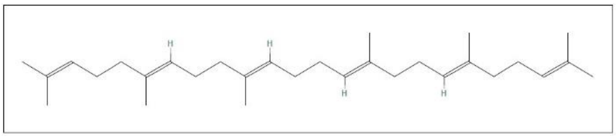

Figure 1. Chemical Structure of Squalene (Tansey, T. R. and Shechter, I. 2000).

Squalene plays an significant role in the quality of olive oil. Many scientific articles have been written to reveal its benefits to human health and its anti-cancer properties (Newmark, 1999, Rodriguez-Rodriguez and Simonsen, 2012). In extra virgin olive oil, squalene, the main component of small compound fractions, is a known biological response modifier related to oxygen, immune system and steroid synthesis providing anti-aging, anti-inflammatory and hypocholesterolemic activities. The scavenging and repairing ability against foreign products, together with the results in experiments performed in vitro associated with cardiovascular diseases, support a possible protective role of squalene against oxidative stress and free radicals, indicating the high potential of this molecule for use in medicine, cosmetics and nutrition (Mastralexi and Tsimidou, 2021).

For olive oil stability the role of squalene was investigated for various concentrations and experimental conditions (Psomiadou and Tsimidou, 1999). Olive oil has proven moderate antioxidant activity due to the higher concentration. It has been stated that the moderate antioxidant activity of squalene in olive oil can be explained by the different existing lipids competing with each other, leading to a decrease in the oxidation rate.

Warleta et al., (2010) studied cell proliferation, scavenging properties, apoptosis, cellular cycle profile, reactive oxygen species (ROS) level and oxidative effect of squalene on DNA damage using human breast cells. According to the results obtained, squalene exhibited antitumor properties against different types of cancer and a protective effect on breast cancer.

In this study, the relationship of squalene content, an important compound that determines the quality of olive oil, were investigated with olive harvest time, as well as the effect of cultivars and crop load.

2. Material and Methods

In the research olive fruits of Kilis Yaglik and Gemlik cultivars grown in Kilis region were used. Kilis Yaglik variety was obtained from Kilis Dogancay area, and Gemlik variety was obtained from Kilis 7 Aralik University Faculty of Agriculture Mercidabık Campus. While the crop load of trees was high in 2018, it was low in 2019.

2.1. Harvest and Oil Extraction: The fruits of the selected olive trees were harvested in mid-October, November and December in 2018 and 2019. Olives were extracted without waiting (1 day after harvest) in an oil extraction device (Olio Mio Mini, Italy) with a two-phase decanter system. The olives were first crushed in the mill section, and the resulting olive paste was kneaded in a malaxer at 30 °C for 1 hour and then separated into oil and pulp (pomace+black water) in the decanter. Obtained oils were stored in dark glass containers in a dark, dry and cool environment until analysis.

2.2. Analysis of Oils: According to the European Standard NF EN ISO 12966-2, about 0.1 g of olive oil in 10 ml of hexane was trans-methylated with a 0.5 ml solution of KOH in methanol (2 M). Fatty acid methyl esters (FAMEs) were analyzed in accordance with European Standard NF EN ISO 5508. Analyzes were performed using a Shimadzu brand GC-2010 Plus instrument equipped with a flame ionization detector (GC-FID) (T 320° C) using a capillary.

2.3. Statistical Analysis: JUMP7 statistical program was used to determine statistical significance levels by employing the Tukey's multiple comparison test and the differences between individual averages were considered to be statistically important at $p< 0.05$. The results were expressed as mean.

3. Results and Discussion

The squalene content of the olive oils obtained from Kilis Yaglik and Gemlik olive varieties harvested at different times are shown in Table 1 and Figure 2. Accordingly, while there was more squalene in Gemlik cultivar, it first decreased and then increased in both cultivars periodically. While the highest squalene was obtained from Gemlik variety as 2.76% in December, it was obtained from Gemlik variety at least in November.

While the squalene content was higher in 2018, less squalene was obtained in 2019 when the yield was low. According to the results obtained, it was seen that the variety affected the amount of squalene, and fluctuations in the squalene content occurred with the periodical maturation. The product load also affected the squalene content, and more squalene accumulation occurred in the year when the yield was high. The amount of squalene in olive oil is affected by the variety, environmental and climatic data, year and crop load. Olive varieties found around the world have varied widely in squalene content (Martínez-Beamonte et al., 2019) According to the study conducted in Spanish cultivars, large differences were observed between 'Arbequina' and 'Picual' cultivars (Beltrán et al., 2016).

In another study, cultivars 'Drobnica', 'Nocellara de Belice', 'Oblica' and 'Souri' were found to have the highest squalene content (Fernández-Cuesta et al., 2013). In addition, it was observed that there were differences even within the same cultivars. This wide range has also been demonstrated by studies conducted in different countries (Manzi et al., 1998; Baaccouri et al., 2008; Ambra et al., 2017; Queslati et al.; 2009; Uluata et al., 2016).

Depending on the variety, maturity levels are important for obtaining high oil yields (Baccouri et al., 2007; Anastasopoulos et al., 2011; Bodoira et al., 2015; Laroussi-Mazghani et al., 2016). Besides this, the ripeness of the fruit is affected by the ırrigation, climate, growing area and conditions, temperature and harvest time.

Accordingly, the biochemical content of the oil also changes (Beltran et al., 2016; Manzi et al., 1998). Due to limited information about the 'Koroneiki' variety, it could not be determined that the squalene content of olive oil is a change from traditional or organic production of the applied agricultural regime (Anastasopoulos et al., 2011). Baccouri et al. (2007), in their study, compared the groups fed with rain water and fed with irrigation water in the cultivar 'Chetoui Tunisia'. It was determined that the squalene content in the oils obtained from olive plants fed with irrigation water was 1/3 lower than those fed with rain water.

Table 1. Changes squalene content of olive oil obtained Kilis Yağlık and Gemlik varietes by the harvest time and years.

	Years							
	2018 (1,74 a)				**2019** (1,50 b)			
Harvest Time	**Gemlik**		**Kilis Yaglık**		**Gemlik**		**Kilis Yağlık**	
October	1,42	def	1,79	bc	1,86	b	1,38	ef
November	1,21	f	1,35	ef	1,49	de	1,88	b
December	2,76	a	1,88	b	1,61	cd	0,78	g
Cultivars Average	**Gemlik** 1,73 a				**Kilis Yağlık** 1,51 b			

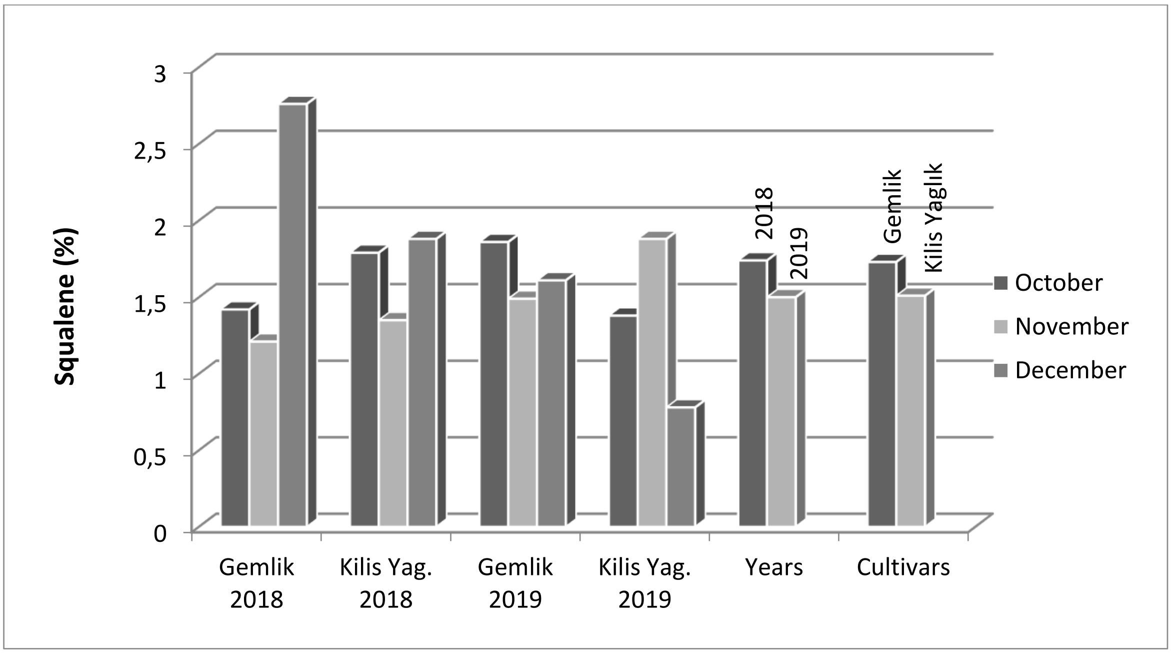

Figure 2. Effect of harvest time on squalene content of oils obtained from Kilis Yağlık and Gemlik varietes

In many studies revealed that the main reason for the variability in the biochemical structure of the oil is the maturity level, as well as the region where it grows, soil structure, harvest time, irrigation status and harvest years affect the content. (Ben Mansour et al., 2015; Çetinkaya, 2017; Lazzez et al., 2011). The content of squelen may be changed by the maturity that was found that the squele first increased and then decreased in Tunusian varietes (Sakouhi et al., 2011).

4. Conclusion

Major and minor components in olive oil are affected by variety, harvest time and crop load. In the study, Gemlik cultivar synthesized more squalene than Kilis Yaglik cultivar. Squalene content also changes with harvest time. More squalene was synthesized in the year when the yield was high. In order to obtain a stable oil with optimum quality characteristics in olive oil, it is of great importance to determine the appropriate variety and harvest time for the purpose. In addition, considering the effect of variety and harvest time on other components and each other, optimum conditions for squalene synthesis should be provided.

Author Contribution

Hakan CETİNKAYA conceived and designed the experiments. İbrahim Samet GOKCEN performed the experiments. Hakan CETİNKAYA wrote the paper and İbrahim Samet GOKCEN contributed to writing the paper.

Acknowledgements

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Conflict of Interest

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

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Review article
Curr. Pers. MAPs, (2021); 4(2): 128-150

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Natural Substances and Coronavirus: Review and Potential for the Inhibition of SARS-CoV-2

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https://doi.org/10.38093/cupmap.1029572

Received : 28/11/2021
Accepted : 30/12/2021

Abstract

Coronaviruses are responsible for an increasing economic, social and mortality burden, as the causative agent of diseases such as the severe acute respiratory syndrome (SARS), the Middle East Respiratory Syndrome (MERS) and recently the COVID-19. Existing natural compounds, especially those known for their antiviral activity, may be useful as therapeutic agents against coronavirus infections. This study aims to review the currently available scientific literature on natural substances of plant origin with promising antiviral effects against coronaviruses. PubMed, Science Direct and Biomed Central databases were searched for articles including the keywords "*Coronavirus*", "*SARS-CoV-2*" as well as "*Alkaloids*", "*Polyphenols*", "*Terpenes*" and "*Secondary metabolites*". 145 research articles published between 2003 and 2020 were selected. The majority of the studies on natural substances acting against coronaviruses were performed in the last two years: 2020 (31,72%) and 2021 (60,69%) coinciding with the emergence of the new coronavirus SARS-CoV-2. Most studies were performed by *in silico* methods with a percentage of 66,67%, 25,45% by *in vitro* methods and only 7,88% by *in vivo* tests. Our research resulted in a list of 963 natural substances of plant origin tested against Coronavirus. Polyphenols represent the most tested secondary metabolites against Coronavirus, followed by terpenes and then alkaloids. Quercetin, Catechin, Glycyrrhizin, Kaempferol, Rutin, Curcumin, Myrecitin, Apigenin and Hesperidin were the most cited substances. In the future, we hope that the active ingredients of medicinal plants can be used to treat SARS-CoV-2 infection in humans.

Key Words: SARS-CoV-2, COVID-19, Natural compounds, Secondary metabolites, Review.

1. Introduction

Coronaviruses were first identified in humans in the 1960s. They are viruses that cause emerging diseases, i.e., new infections due to changes or mutations in viruses. Human coronaviruses mainly cause respiratory infections, ranging from the common cold to severe and sometimes lethal pneumonia (Vabret, Dina, Brison, Brouard, & Freymuth, 2009).

Three coronaviruses cause infections that can be severe or even fatal: severe acute respiratory syndrome (SARS-CoV) that appeared in China and caused the 2003 epidemic, Middle East Respiratory Syndrome (MERS) that caused the 2012 epidemic, and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that causes coronavirus-2019 (COVID-19) disease (Asrani, Hasan, Sohal, & Hassan, 2020).

In late December 2019, China reported the emergence of a new infectious disease, caused by a virus named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), initially transmitted from animals to humans and then from humans to humans. Within a short time, SARS-CoV-2 spread to other countries, killing thousands of people. As a result, the World Health Organization (WHO) has declared the Coronavirus-2019 (COVID-19) disease a pandemic and currently it is considered the second leading cause of death after cardiovascular disease.

The WHO states that no effective therapy has been approved to date for the prevention or treatment of this disease. Although vaccines have now been launched, evidence of their safety and efficacy in the population is still awaited. This suggests the need to broaden the scope of research for effective treatments. Among other therapeutic options, natural products and derivatives constitute a vast source of potential drug molecules. Nature provides a huge well of active ingredients that remain to be discovered to treat diseases.

Historically, 80% of clinically important drug developments are still inspired by these nature-derived entities. Therefore, products of natural origin or phytochemicals have continuously served humanity as a noble source of therapeutically important elements. And these products are of considerable importance in the event of a global health crisis and represent one of the most practical and promising approaches to reduce the intensity of pandemics through their therapeutic potential.

The main objective of this study is to review the currently available scientific literature on natural substances of plant origin with promising antiviral effects against Coronavirus.

2. Material and Methods

2.1. Bibliographic research of the data

The databases used were: PubMed, Science Direct and Biomed Central. The bibliographic search and the downloading of articles were carried out during the period from January 1, 2003 to May 31, 2021.

2.2. Search strategy

First, the keywords of the search equation were entered in the search engines of the databases used. We used English keywords related to the virus such as: "***Coronavirus***", "***SARS-CoV-2***", and related to phytotherapy and natural compounds sush as: "***Alkaloids***", "***Polyphenols***", "***Terpenes***" and "***Secondary metabolites***". Afterwards, we used the following filters: between 2000 and 2020, academic articles, in English. The articles we included in the research project met the following criteria:

- Any article design: *in silico* study, *in vitro* trial, *ex vivo* trial, *in vivo* trial, randomized controlled trials, clinical trials and meta-analyses with full text available in Open Access or downloadable;
- Articles written in English or French;
- Articles published during the period from January 1, 2003 to May 31, 2021.

2.3. Data analysis and exploitation

After downloading the considered articles, a deep reading was necessary to classify the articles and note the following informations:

- Year of publication;
- Country;
- Type of study;
- Substance's name;
- Substance's Chemical structure;
- Substance's plant origin;
- Plant's scientific name and botanical family;
- Substance's chemical class;
- Antiviral mechanism of action.

The data collected was then reported on Excel® and Google Sheets® softwares to convert the results into graphs and facilitate the analysis task.

3. Results and Discussion

A total of 5330 articles were identified by electronic search on PubMed, Science Direct, and BioMed Central. A summary of the study selection process is shown in Figure 1. 1881 articles were selected based on title and abstract, and 1368 after duplicate removal. At this stage, full texts were assessed and 1220 articles were excluded for ineligibility. Finally, 145 studies were included in our study. The search yielded a list of 963 natural substances of plant origin tested against Coronaviruses.

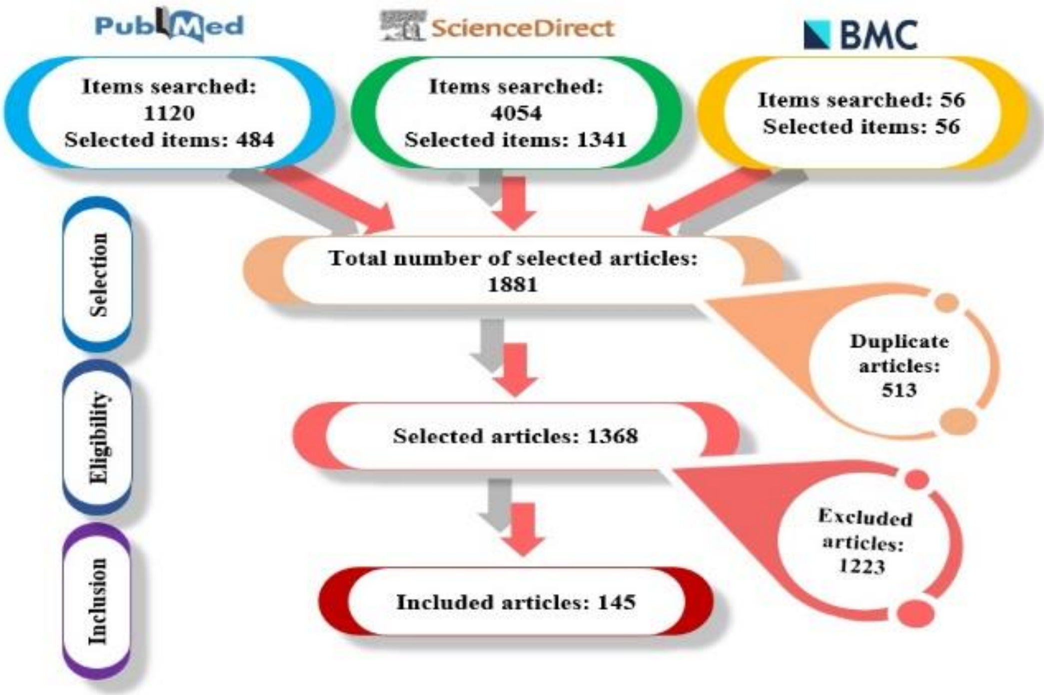

Figure 1. Articles search and selection's flowchart.

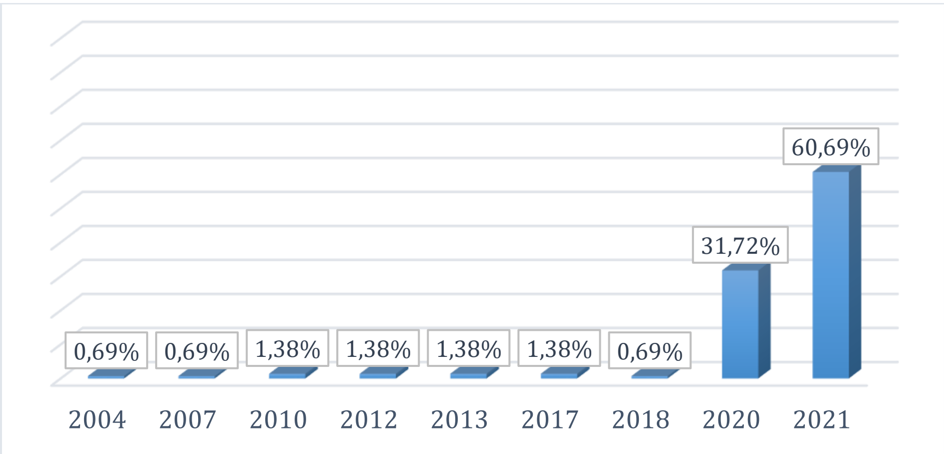

Figure 2. Articles distribution by publication's date.

3.1. Year of publication

The majority of the studies on natural substances acting against Coronaviruses were carried out in the last two years: 2020 (31.72%) and 2021 (60.69%), 2004 represents the year of the first publication and the number of articles published between 2004 and 2020 did not change significantly Figure 2.

This finding can be clearly justified by the emergence of the new coronavirus SARS-CoV-2 and the universal scope and severity of the pandemic caused by it compared to the last two epidemics due to SARS-CoV-1 and MERS-CoV.

3.2. Country

The states producing the most publications are the Asian countries mainly India with a percentage of 35.2% followed by China (16.6%) then South Korea (9.7%). The other countries: Egypt, Germany, Nigeria, USA, Brazil, Taiwan published between 6 and 3 articles and the rest including Algeria published less than 3 articles Figure 3.

Figure 3. Articles distribution by country.

3.3. Type of study

Most of the studies on Coronaviruses and natural substances were carried out by the *in silico* method with a percentage of 66.67%, 25.45% by *in vitro* methods and only 7.88% by *in vivo* tests Figure 4.

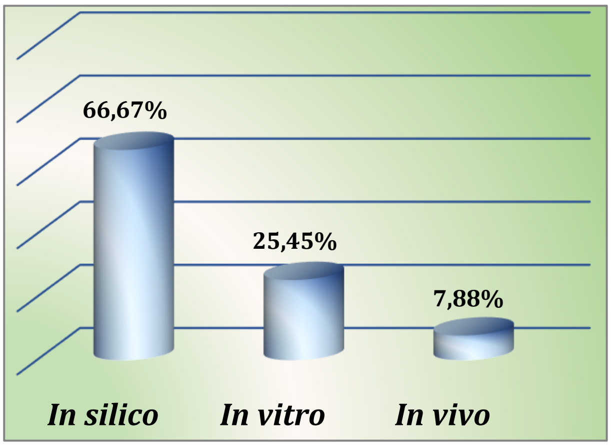

Figure 4. Articles distribution by study's type.

In silico methods are virtual screening approaches or methods based on algorithms developed for screening a large number of molecules in a shorter time and identifying a potential drug candidate (Hariprasad Puttaswamy et al., 2020). The use of these approaches has increased in the last two years, which is expected due to:

- The rapid result provided by these methods;
- The possibility of anticipation and prediction without the need for raw materials, extraction or purification;
- The ethical approach that does not require the use of animals;
- The lower costs and risks compared to traditional methods.

3.4. Substance's chemical classes

Of the 963 natural substances mentioned in the articles studied, polyphenols represent the most tested secondary metabolites against Coronavirus with a percentage of 51%, followed by terpenes (29%) and then alkaloids (18%). The other compounds such as some primary metabolites constitute only 2% of the studied substances Figure 5.

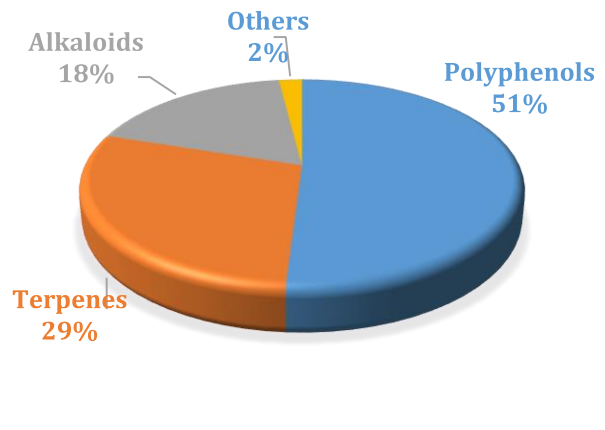

Figure 5. Molecules distribution by chemical classes.

Polyphenols are natural molecules known to have antiviral activity against a wide range of viruses, including HIV-1, HIV-2, HSV-1, HSV-2, influenza virus, dengue virus, HBV, HCV, infectious bronchitis virus (IBV), Murbarg virus, Ebola virus, Newcastle disease virus (NDV), polio virus-1, lentivirus and coronavirus.

In the case of the latter, polyphenols act against coronaviruses using various mechanisms, including activation or inhibition of cell signaling pathways or arrest of the papain-like protease (PL pro) and the enzyme 3-chymotripsin-like protease (3CLpro)(Shin, Oh, & Jeong, 2021).

3.5. Botanical family

Of the 963 natural substances mentioned in the articles studied, the Menispermaceae represents the most studied botanical family against Coronavirus with a percentage of 6.4% followed by the Asteraceae (6.3%) then the Rosaceae (5.6%) and Lamiaceae (5.3%). The other botanical families present a percentage lower than 4.5% Figure 6.

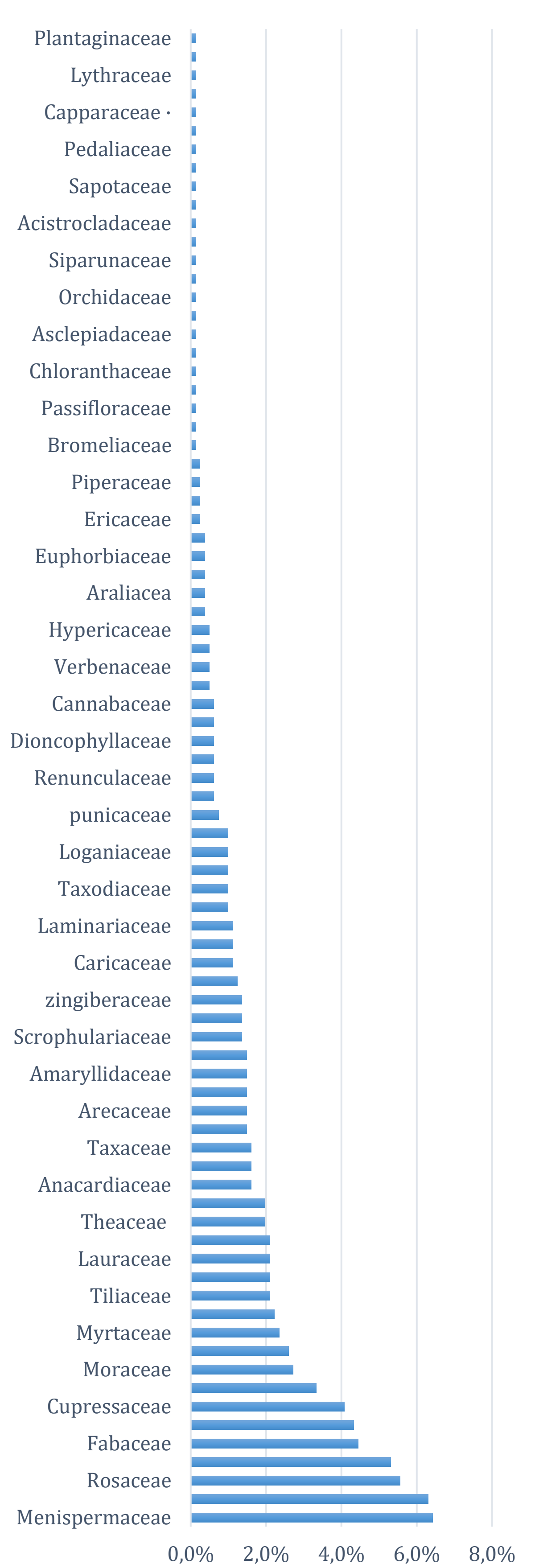

Figure 6. Molecules distribution by origin specie's botanical family.

Table 1. The 10 most quoted natural substances.

Natural substance	Chemical class	Virus	Action mechanism	Study design	References
Catechin and analogs *Camellia sinensis* *Carica papaya* *Mangifera indica* *Moringa oleifera* *Acacia nilotica* *Psidium guajava* *Rosa hybrida*	Polyphenol	SARS-CoV SARS-CoV-2	M^{pro},PL^{pro}, Helicase, protein N, protein S, RBD, ACE2, RdRp, CTSL, Nsp6, Nsp15 and furin inhibitor.Proteine S interaction with GRP78 and replication inhibitor Reduce cytokine storm Antioxidant agent	*In silico* *In vitro* *In vivo*	(Allam et al., 2020; Arokiyaraj, Stalin, Kannan, & Shin, 2020; Attia et al., 2021; Du et al., 2021; Elsbaey, Ibrahim, Bar, & Elgazar, 2021; R. Ghosh, Chakraborty, Biswas, & Chowdhuri, 2020b; Gogoi et al., 2021; Gupta et al., 2020; Halder et al., 2021; Hariyono, Patramurti, Candrasari, & Hariono, 2021; Jang et al., 2021; Jang et al., 2020; Jena, Kanungo, Nayak, Chainy, & Dandapat, 2021; Kumar et al., 2021; Liu, Raghuvanshi, Ceylan, & Bolling, 2020; Meyer-Almes, 2020; Mhatre, Naik, & Patravale, 2021; Mishra et al., 2021; Natesh et al., 2021; Nguyen et al., 2021; Pitsillou, Liang, Hung, & Karagiannis, 2021; Pitsillou, Liang, Ververis, Hung, & Karagiannis, 2021; Roh, 2012; Shin et al., 2021; Singh, Sk, Sonawane, Kar, & Sadhukhan, 2020; Umar et al., 2021; Vardhan & Sahoo, 2021; Yañez et al., 2021; M. Zhao et al., 2021)

Quercetin and analogs *Geranii Herba* *Ephedra sp* *Crocus sativus* *Allium cepa* *Broussonetia papyrifera* *Moringa oleifera* *Psidium guajava* *Camellia sinensis* *Rosa hybrida* *Azadirachta indica* *Mangifera indica* *Ginkgo biloba* *Corchorus olitorius* *Justicia adhatoda* *Psidium guyava* *Lespedeza cuneata* *Polygonum aviculare* *Rhododendron aureum* *Taxillus kaempferi*	Polyphenol	SARS-CoV MERS-CoV SARS-CoV-2	Inhibit SARS-CoV-2 cell entry via ACE2 receptor Inhibit proteolytic process, 3CLpro, PLpro, RdRp and interaction with HR2 domain Decrease inflammation factors Reduces cytokine storm Antioxidant agent	*In silico* *In vitro* *In vivo*	(Arokiyaraj et al., 2020; Attia et al., 2021; Azim et al., 2020; Du et al., 2021; Gao, Song, & Song, 2020; Gheware et al., 2021; K. Ghosh, Amin, Gayen, & Jha, 2021; Ibrahim et al., 2020; Kumar Verma et al., 2021; Kushwaha et al., 2021; Liu et al., 2020; Mesli, Ghalem, Daoud, & Ghalem, 2021; Meyer-Almes, 2020; Nguyen et al., 2021; Niu et al., 2021; Park et al., 2017; H. Puttaswamy et al., 2020; Shaji et al., 2021; P. Sharma & Shanavas, 2020; Shin et al., 2021; Singh et al., 2020; Umar et al., 2021; Xiong et al., 2021; Xu, Gao, Liang, & Chen, 2021; Yañez et al., 2021)
Glycyrrhizin and analogs *Glycyrrhiza uralensis* *Glycyrrhiza glabra*	Terpene	SARS-CoV MERS-CoV SARS-CoV-2	Inhibitor of the inflammatory response / prevents the development of a cytokine storm Inhibitor of 3CLpro, PLpro, RdRp, TMPRSS2, protein S, RBD, ACE2, Nsp1, furin and Endoribonuclease	*In silico* *In vitro* *In vivo*	(F. Chen et al., 2004; L. Chen et al., 2020; Ding et al., 2020; Diniz, Perez-Castillo, Elshabrawy, Filho, & de Sousa, 2021; Gowda, Patrick, Joshi, Kumawat, & Sen, 2021; Gurung, Ali, Lee, Farah, & Al-Anazi, 2021; Hejazi, Beg, Imam, Athar, & Islam, 2021; Luo et al., 2020; Muhseen, Hameed, Al-Hasani, Tahir ul Qamar, & Li, 2020; Patil et al., 2021; H. Puttaswamy et al., 2020; A.

					Sharma, Tiwari, & Sowdhamini, 2020; Toor, Banerjee, Lipsa Rath, & Darji, 2021; van de Sand et al., 2021; Vardhan & Sahoo, 2020, 2021; Yu et al., 2021; Z. Zhao et al., 2021; Zígolo, Goytia, Poma, Rajal, & Irazusta, 2021)
Kaempferol and analogs *Geranii Herba* *Carica papaya* *Ephedra sp* *Crocus sativus* *Senna alexandrina* *Broussonetia papyrifera* *Moringa oleifera* *Mangifera indica* *Ginkgo biloba* *Justicia adhatoda*	Polyphenol	SARS-CoV MERS-CoV SARS-CoV-2	Inhibits SARS-CoV-2 cell entry via ACE2 receptor Inhibits proteolytic process, 3CLpro, PLpro, RdRp, Nsp14, Nsp16 and protein S	*In silico* *In vitro*	(Arokiyaraj et al., 2020; Du et al., 2021; Gao et al., 2020; Gheware et al., 2021; Hariyono et al., 2021; Ibrahim et al., 2020; Mehmood et al., 2021; Natesh et al., 2021; Nguyen et al., 2021; Park et al., 2017; H. Puttaswamy et al., 2020; Shaji et al., 2021; Singh et al., 2020; Umar et al., 2021; Xiong et al., 2021; Yañez et al., 2021)
Rutin *Withania somnifera* *Passiflora incarnata Theobroma cacao*	Polyphenol	SARS-CoV SARS-CoV-2	3CLpro, PLpro, protein E, Nsp 15, ACE2, Endoribonuclease inhibitor Reduce cytokines	*In silico* *In vitro*	(Attia et al., 2021; Bhowmik et al., 2020; A. Ghosh, Chakraborty, Chandra, & Alam, 2021; K. Ghosh et al., 2021; Kumar et al., 2021; Kushwaha et al., 2021; Liu et al., 2020; Meyer-Almes, 2020; Nguyen et al., 2021; Niu et al., 2021; Patil et al., 2021; Pitsillou, Liang, Hung, et al., 2021; Pitsillou, Liang,

					Ververis, et al., 2021; Yañez et al., 2021; Zígolo et al., 2021)
Curcumin and analogs *Curcuma longa*	Polyphenol	SARS-CoV-2 SARS-CoV MERS-CoV	Inhibitor of interaction of protein S with GRP78, Mpro, ACE2, proteinS, RdRp Decrease inflammation factors and cytokines Increase the number of reg T cells	*In silico* *In vitro* *In vivo*	(Allam et al., 2020; L. Chen et al., 2020; A. Ghosh et al., 2021; Gupta et al., 2020; Halder et al., 2021; Ibrahim et al., 2020; Jena et al., 2021; Kodchakorn, Poovorawan, Suwannakarn, & Kongtawelert, 2020; Kumar Verma et al., 2021; Nguyen et al., 2021; Singh et al., 2020; Tahmasebi et al., 2021; Valizadeh et al., 2020; Wen et al., 2007)
Myricetin and analogs *Citrus sinensis* *Camellia sinensis* *Withania somnifera* *Myrica penssylvanica* *Isatis indigotica* *Torreya nucifera* *Moringa oleifera*	Polyphenol	SARS-CoV-2	Inhibitor of Mpro, Nsp 15, TMPRSS2, RdRp Reduce cytokines	*In silico* *In vitro*	(Attia et al., 2021; A. Ghosh et al., 2021; K. Ghosh et al., 2021; Gogoi et al., 2021; Kumar et al., 2021; Nguyen et al., 2021; Niu et al., 2021; Patil et al., 2021; H. Puttaswamy et al., 2020; Singh et al., 2020; Umar et al., 2021; Zígolo et al., 2021)
Apigenin and analogs *Carica papaya* *Hypericum perforatum* *Cocos nucifera*	Polyphenol	SARS-CoV-2	3CLpro, PLpro, RdRp, Nsp 15, ACE2, protein S inhibitor	*In silico* *In vitro*	(Elsbaey et al., 2021; Fayed et al., 2021; Gowrishankar et al., 2021; Hariyono et al., 2021; Kumar et al., 2021; Messaoudi et al., 2021; Natesh et al., 2021; Nguyen et al., 2021; Ryu et

					al., 2010; Singh et al., 2020; Xiong et al., 2021; Yañez et al., 2021)
Luteolin *Ephedra sp* *Ginkgo biloba* *Justicia adhatoda*	Polyphenol	SARS-CoV-2	Inhibitor of 3CLpro, PL pro, ACE2, protein S, Nsp14, Nsp15, RdRp, TMPRSS2 Reduces cytokines	*In silico* *In vitro*	(Du et al., 2021; Gao et al., 2020; Gheware et al., 2021; Kumar et al., 2021; Liu et al., 2020; Nguyen et al., 2021; Niu et al., 2021; Singh et al., 2020; Xiong et al., 2021; Xu et al., 2021; Yañez et al., 2021)
Hesperidin and analogs *Citrus sinensis* *Withania somnifera* *Isatis indigotica*	Polyphenol	SARS-CoV-2	Mpro, Nsp 1, Endoribonuclease, RdRp, TMPRSS2, S protein inhibitor Blocks the 3a channel protein Reduces ARDS	*In silico* *In vitro*	(Attia et al., 2021; A. Ghosh et al., 2021; K. Ghosh et al., 2021; R. Ghosh, Chakraborty, Biswas, & Chowdhuri, 2020a; Gupta et al., 2020; Kodchakorn et al., 2020; Nguyen et al., 2021; Patil et al., 2021; Singh et al., 2020; Vardhan & Sahoo, 2021; Zígolo et al., 2021)

3.6. Antiviral mechanism of action

Most of the studies on natural substances acting against coronaviruses showed that several metabolites acted by inhibition of the main protease (Mpro) with a percentage of 43.71%, others by inhibition of the viral protein RNA-dependent RNA polymerase (RdRp), the angiotensin converting enzyme (ACE2), the surface protein (protein S) or by the inhibition of the papain-like protein (PLpro). Other studies do not show by which mechanisms these secondary metabolites acted against coronavirus.

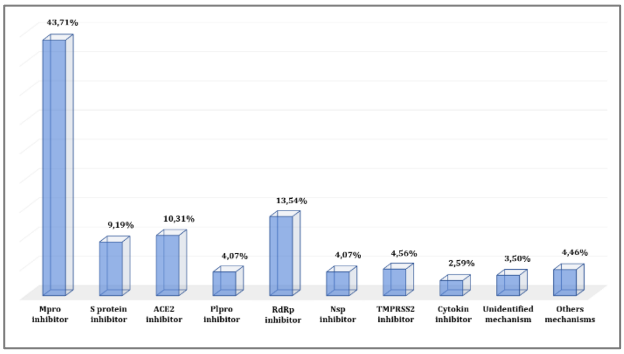

Figure 7. Molecules distribution by action's mechanism.

3.7. Most cited substances

Taking into account the citation's frequency in the articles, we ranked the 10 most cited substances (mentionned in more than 3 studies) in descending order (Table 1). Out of these 963 substances, catechins are the most cited compounds (29), followed by quercetin (25), glycyrrhizin (19) and kaempferol (16). The other substances had a lower mention's frequency.

3.7.1. Polyphenols

- **Quercetin**

Quercetin is a flavonoid widely present in the plant kingdom, found in grapefruit, onions, apples and black tea. A lesser amount exists in green leafy vegetables and beans. Quercetin has a range of pharmacological activities as an antioxidant and anti-inflammatory agent. An experiment confirmed that quercetin could enhance ligand-induced apoptosis of senescent idiopathic pulmonary fibrosis fibroblasts and reduce pulmonary fibrosis *in vivo* (Xu et al., 2021). In 2017, an *in vitro* study showed that quercetin and quercetin-β-galactoside can inhibit the activity of viral proteases (3C-like protease=3CL pro and PL pro) of SARS-CoV and MERS-CoV (Park et al., 2017). In addition, recent studies (*in silico* and *in vitro*) have reported the potential inhibitory effects of quercetin and these analogues on the main protease of SARS-CoV-2 (Attia et al., 2021; Kushwaha et al., 2021; Meyer-Almes, 2020; Nguyen et al., 2021; Umar et al., 2021; Xiong et al., 2021).

Another docking technique was also used to further define the inhibitory activity of the glycosides of this flavonol. This technique revealed that quercetin 3,5-digalactoside recorded the lowest binding energy with Mpro. It was observed that flavonols with two glucose moieties recorded lower binding energy (LE) than flavonols with one or three glucose moieties (H. Puttaswamy et al., 2020).

Quercetagenin, another flavonol could effectively inhibit SARS-CoV-2 replication *in vitro* by 58% with an IC50 of 145 µM. It has also been reported that quercetin and quercetin 3-(6-malonylglucoside) could reduce SARS-CoV-2 entry by blocking ACE2 activity (Mesli et al., 2021; Xu et al., 2021). Quercetin analogs can also bind to RdRp and PL pro (Gheware et al., 2021; Singh et al., 2020).

- **Rutin**

Rutin flavonoid isolated from the extract of different medicinal plants such as *Withania somnifera*, Passion flower and *Theobroma cacao* (A. Ghosh et al., 2021; Yañez et al., 2021) has various biological activities, including anti-inflammatory and antiviral activities. Studies have shown that rutin has antimicrobial activity and, through *in silico* studies, possible inhibitory activity of several proteins essential for SARS-CoV-2 to complete its viral cycle. However, its antiviral spectrum is broader and it is experimentally tested as an antiviral agent against retroviruses, orthomyxoviruses, herpes viruses, hepatitis B and C viruses and H1N1 influenza virus.

Other experiments performed by different researchers mention that rutin can be used as a potential inhibitor of Mpro and ACE2 of COVID-19(Bhowmik et al., 2020; A. Ghosh et al., 2021; Kumar et al., 2021; Meyer-Almes, 2020; Patil et al., 2021; Yañez et al., 2021). In addition, *in vitro* enzyme inhibition assays also showed that rutin had inhibitory activity against SARS-CoV 3CLpro since the 3CLpro sequence of SARS-CoV-2 is very similar to that of SARS-CoV (Liu et al., 2020; Nguyen et al., 2021).

- **Kaempferol**

Kaempferol is a flavonol that can be extracted from several plants namely *Moringa oleifera*, *Carica papaya* and *Ephedra sp* (Gao et al., 2020; Hariyono et al., 2021; Umar et al., 2021). In an *in vitro* study, kaempferol extracted from *Broussonetia papyrifera* was identified as an inhibitor of viral proteases (PL pro and 3CL pro) in both SARS-CoV and MERS-CoV (86). Studies have recently begun to focus on SARS-CoV-2 infection, kaempferol and its analogues have been tested against various coronavirus target proteins such as 3CL pro, RdRp, ACE2 and protein S both *in silico* and *in vitro* (Du et al., 2021; Gao et al., 2020; Ibrahim et al., 2020; Mehmood et al., 2021; Natesh et al., 2021; Nguyen et al., 2021; Umar et al., 2021; Xiong et al., 2021).

- **Myricetin**

Myricetin is a flavonol that can be extracted from *Isatis indigotica*, *Torreya nucifera* or *Moringa oleifera* (M, Reddy, Hema, Dodoala, & Koganti, 2021; Umar et al., 2021). Myricetin and its analogues have been tested against Mpro, RdRp, Transmembrane serine protease 2 (TMPRSS2), endoribonuclease and IL-6 both *in silico* and *in vitro*. They showed good docking scores especially against TMPRSS2 and RdRp (A. Ghosh et al., 2021; Kumar et al., 2021; M. Reddy et al., 2021; Nguyen et al., 2021; Niu et al., 2021; H. Puttaswamy et al., 2020; Singh et al., 2020; Umar et al., 2021). According to an *in vitro* study, the absence of hydroxyl groups in the C3 and C4 B-ring was the reason for the lower inhibitory activity of kaempferol and quercetin than myricetin (Nguyen et al., 2021).

- **Naringenin**

Naringenin is a flavonone extracted mainly from *Citrus* fruits, *Isatis indigotica*. It has been reported as an antiviral agent against Zika virus (Attia et al., 2021; R. Ghosh et al., 2020a; M. Reddy et al., 2021). Naringenin showed no inhibitory effect against Mpro of SARS-CoV (R. Ghosh et al., 2020a). In contrast, it scored good docking against Mpro, TMPRSS2 and RdRp of SARS-CoV-2(M. Reddy et al., 2021; Singh et al., 2020; Yañez et al., 2021). Naringin, a heteroside of naringenin, also showed good scores with Mpro and non-structural protein 15 (Nsp 15) (Kumar et al., 2021; Yañez et al., 2021).

- **Hesperidin**

Hesperidin and its aglycone hesperitin are flavonones extracted mainly from *Citrus* fruits. Hesperidin has antimicrobial, anti-inflammatory, cardiovascular and antidiabetic (Type II) effects (K. Ghosh et al., 2021). Several studies have noted the possible inhibitory activity of hesperidin and hesperitin against various proteins target namely Mpro, TMPRSS2 and RdRp (R. Ghosh et al., 2020a; Kodchakorn et al., 2020; M. Reddy et al., 2021; Nguyen et al., 2021; Singh et al., 2020; Varghese et al., 2021).

In the flavanones group, the order of M pro inhibitory activity was naringin < hesperidin < naringenin. Naringenin is glycosylated naringin. However, its inhibitory activity was 3.2 times higher than that of naringin. Hesperidin, which contained 7-OH glycosylation at the A ring like naringenin and the methoxy group at the 5′ position of the B ring, was found to have higher inhibitory activity than naringin but lower inhibitory activity than naringenin, indicating that glycosylation at the C7 position enhanced the inhibitory effect of M pro. In contrast, the methoxy group at C5′ in the B ring reduced its inhibitory activity (Nguyen et al., 2021).

- **Catechins and gallate catechins**

Catechins are polyphenols, more precisely they are part of the flavonoid family; the subclass of flavanols, present in some foods, including green tea (*Camellia sinensis*) and *Carica papaya*. The effect of catechin and its analogues in the inhibition of SARS-CoV-2 associated coronavirus replication has been recently studied and various mechanisms of action have been attributed to the antiviral activities of catechin, such as inhibition of protein S, RdRp, ACE2 and Mpro (R. Ghosh et al., 2020b; Halder et al., 2021; Hariyono et al., 2021; Jena et al., 2021; Meyer-Almes, 2020; Singh et al., 2020).

In an *in silico* study, catechins extracted from *Mangifera indica* and *Moringa oleifera* were identified as potent inhibitors of Mpro with a very high docking score (Umar et al., 2021). In addition, epicatechin is a structural analogue of catechin that exhibits in addition to other inhibitory effects, the ability to inhibit furin, protein N and Nsp6 (Mishra et al., 2021; Vardhan & Sahoo, 2021).

Epigallocatechin gallate (EGCG), the main catechin in green tea, is known to exert antiviral activity against several types of viruses including herpes virus, hepatitis virus and influenza A virus. *In silico* studies to test antiviral activity against SARS-CoV-2 showed that epigallocatechin gallate bound well to key targets including Spike, 3CLpro, PLpro and RdRp (R. Ghosh et al., 2020b; Mhatre et al., 2021). In addition, *in vitro* studies have confirmed its efficacy in inhibiting replication and reducing cytokine storm (Shin et al., 2021; M. Zhao et al., 2021).

- **Curcumin**

Curcumin and its analogues are the main constituents of turmeric (*Curcuma longa L.)* and other Curcuma spp. which are widely used around the world as a culinary spice, popular dietary supplement ingredient as well as in traditional medicine due to its wide range of health benefits including anti-inflammatory, anti-cancer, cardiovascular, respiratory and immune benefits. In addition, the suppression of several cytokines by curcumin has suggested that it may be a useful approach in treating Ebola patients against the cytokine storm. Curcumin has a variety of antiviral activities against dengue virus, herpes simplex virus, Zika virus and chikungunya virus. CC also inhibits aminopeptidase N (APN) which has been identified as a cellular receptor for alpha CoV (L. Chen et al., 2020; Halder et al., 2021).

Another study showed that curcumin could effectively inhibit the major protease of SARS-CoV in Vero E6 cells *in vitro* (Wen et al., 2007). Recently numerous researches have shown its potential inhibitory power against SARS-CoV-2 Mpro both *in silico* and *in vitro* (A. Ghosh et al., 2021; Halder et al., 2021). In an *in vitro* study with curcumin and its analogues, the order of inhibitory effects was bisdemethoxycurcumin < curcumin < dimethylcurcumin. In this group, curcumin contained two methoxy groups (C2′ and C4") and showed higher inhibitory activity on M pro than did bisdemethoxycurcumin, which did not have the methoxy group. However, its inhibitory activity was lower than that of dimethylcurcumin, which contained a C2′ methoxy group(Nguyen et al., 2021). Two double-blind randomized controlled trials showed a significant increase in regulatory T cells and a decrease in cytokine levels (IL-6, IL-1β) as well as an attenuation in the mortality rate in severe patients. Curcumin has major problems of water solubility, high metabolism and rapid excretion from the body. This is solved by the nanoscale formulation namely nanocurcumin (Tahmasebi et al., 2021; Valizadeh et al., 2020). Further molecular docking studies revealed that curcumin can also bind to RdRp, ACE2 and protein S (Jena et al., 2021; S. Singh et al., 2020).

- **Luteolin**

Luteolin is a flavonoid and more specifically a flavone. It has multiple biological activities, including anti-inflammatory, anti-cancer, anti-oxidant, antiviral and cardiac protective. It has been reported that luteolin can interfere with the virus at the beginning of its life cycle, to some extent blocking the absorption and internalization of the flu virus. In addition, various studies have confirmed that luteolin inhibits the NS2B/NS3 protease activity of the dengue virus. It was also documented that luteolin has an anti-Epstein-Barr virus (EBV) effect.
An *in silico* study showed that luteolin extracted from *Ephedra sp* and *Ginkgo biloba* is an inhibitor of SARS-CoV-2 3CLpro. Other studies have also shown that this compound is a potent inhibitor of ACE2 and RdRp. Overall, luteolin has a good antiviral effect, suggesting that luteolin may be a potential drug for the treatment of COVID-19 and their actual effect in the treatment of this disease needs to be verified by further studies (Du et al., 2021; Gao et al., 2020; Kumar et al., 2021; Liu et al., 2020; Singh et al., 2020; Xiong et al., 2021; Yañez et al., 2021).

Luteolin-7-glucoside (Yañez et al., 2021), Luteolin-6-C-arabinosid, Luteolin-6-C-glucoside, Luteolin-6C-glucoside-8C-arabinoside and Luteolin-6-8-di-C-arabinoside are luteolin analogues isolated from *Justicia adhatoda* extract (Gheware et al., 2021). They also exhibit antiviral activity against SARS-CoV-2.

- **Apigenin**

Apigenin is a compound of the flavonoids family, which has anti-inflammatory properties. An *in silico* study done by Indonesian researchers on *Carica papaya* showed the antiviral activity of this natural substance such as the inhbition of Mpro, PLpro and RdRp. In addition, several studies on different apigenin analogues such as Apigeninidin 5-O-glucoside, apigenidin and 6,6'-biapigenin have shown their antiviral efficacy against SARS-CoV-2 via inhibition of Mpro and RdRp (Elsbaey et al., 2021; Ryu et al., 2010).

- **Caffeic acid**

Caffeic acid is a polyphenol, naturally present in all plants as it is a key intermediate in lignin biosynthesis. An *in silico* study by Indonesian researchers showed that caffeic acid extracted from *Carica papaya* decreases the inflammatory factors of SARS-CoV-2 (Hariyono et al., 2021). Other *in silico* studies have confirmed that this compound acts as an inhibitor of Mpro and RdRp (Adem et al., 2021; Bhowmik et al., 2020; Kumar et al., 2021; Nguyen et al., 2021; Singh et al., 2020; Umar et al., 2021; Yañez et al., 2021).

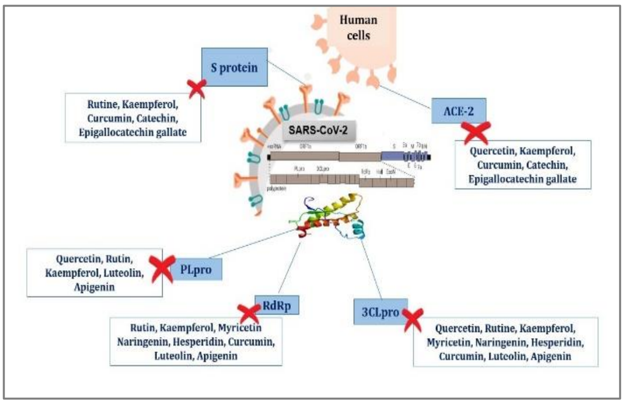

Figure 8. Polyphenols and Anti-SARS-CoV-2 inhbition's mechanisms

3.7.2. Terpenes

- **Glycyrrhizin**

Glycyrrhizin also called glycyrrhizic acid is a triterpene saponin extracted mainly from the root of *Glycyrrhiza glabra* (licorice) and *Glycyrrhiza uralensis*. A large number of studies have shown that licorice and its components have a protective effect on inflammation and lung damage and it is a promising medicinal plant for the treatment of SARS infections. In addition, glycyrrhizin was active against other viruses such as varicella-zoster virus, herpes simplex virus and dengue virus. It is an effective hepato-protective substance in patients with chronic hepatitis C and can protect against a variety of liver diseases such as chronic viral hepatitis, drug-induced and chemical-induced liver damage, non-alcoholic fatty liver, autoimmune hepatitis and hepatocellular carcinoma. It is also used for the treatment of skin inflammation.

The effect of glycyrrhizin in inhibiting the replication of SARS-CoV-2 associated coronavirus has been recently studied and various mechanisms of action have been attributed to the antiviral activities of glycyrrhizin, such as inhibition of endoribonuclease (Patil et al., 2021), inhibition of Mpro, PLpro, RBD, RdRp and ACE2, inhibition of protein S and accessory protein Nsp1 and inhibition of TMPRSS2 (Muhseen et al., 2020; H. Puttaswamy et al., 2020; A. Sharma et al., 2020; Toor et al., 2021; van de Sand et al., 2021; Vardhan & Sahoo, 2020; Yu et al., 2021). In addition, glycyrrhyzin plays an important role in inhibiting immune hyperactivation and the development of cytokine storm factors (Yu et al., 2021).

18β-glycyrrhetinic acid is active against SARS-CoV, SARS-CoV-2 and MERS-CoV by acting on the inhibition of Mpro (Diniz et al., 2021; Vardhan & Sahoo, 2021; Zígolo et al., 2021). Glycyrrhetinic acid is the aglycone of glycyrrhizin (Luo et al., 2020).

- **Andrographolide**

Andrographolide is the major active component isolated from the extract of the herb *Andrographis paniculata* (Banerjee et al., 2021; Sa-Ngiamsuntorn et al., 2021). This labdane-type diterpene lactone possesses a wide range of biological activities, including antiviral, antibacterial, antiparasitic, antitumor, and promotive antidiabetic potential. Previous studies have shown that andrographolide possesses a broad spectrum of antiviral properties, which inhibits various viral infections, including influenza A virus, human immunodeficiency virus (HIV), Chikungunya virus (CHIKV), dengue virus (DENV) by acting on GRP78 and Enterovirus D68 (EV-D68. Andrographolide induces endoplasmic reticulum (ER) stress leading to cancer cell death by apoptosis via induction of increased levels of reactive oxygen species (ROS) that can inhibit virus-induced carcinogenesis (Shi et al., 2020). Additional inhibitory effects of andrographolide include those of cell migration, invasion, matrix metalloproteinase expression, anti-angiogenesis, autophagy, and pathway dysregulation have been reported for inflammatory disorders, including cancer.

In addition, *in silico* studies to test antiviral activity against SARS-CoV-2 also showed that andrographolide bound well to key targets, including Spike protein, 3CLpro, and PLpro, indicating that andrographolide has potential efficacy against SARS-CoV-2 (Banerjee et al., 2021; Kodchakorn et al., 2020; Sa-Ngiamsuntorn et al., 2021; Shi et al., 2020; Verma et al., 2021). Overall, as a plant-derived compound, andrographolide is widely distributed with low cytotoxicity, but its potent antiviral activity against a variety of viruses requires further investigation.

- **Artemisinin**

Artemisinin is the main active component of *Artemisia annua*. It is a sesquiterpenic lactone. Artemisinin is an ancient antimalarial drug, has saved millions of lives, and has been reported to have multiple pharmacological activities, including anticancer, antiviral and immune modulation.

In an *in vitro* study, artemisinin was chosen to test their anti-SARS-CoV-2 potential using African green monkey kidney Vero E6 cells. Cytotoxicity assays were performed prior to the antiviral assay to determine the cytotoxicity of the selected compounds, and viral RNA copies in the supernatants were determined by quantitative real-time PCR (qRT-PCR) to determine the antiviral effects of the compounds. The results of this study show that artemisinin and its derivatives: Arteannuine B showed the highest anti-SARS-CoV-2 potential with an EC50 of 10.28 ± 1.12 M.

Artesunate and dihydroartemisinin showed similar EC50 values of 12.98 ± 5.30 M and 13.31 ± 1.24 M, respectively, which could be achieved clinically in plasma after intravenous administration. Further mode of action analysis revealed that arteannuine B and lumefantrine acted in the post-entry stage of SARS-CoV-2 infection. This research highlights the anti-SARS-CoV-2 potential of artemisinin and provides leading candidates for anti-SARS-CoV-2 drug research and development (Cao et al., 2020). Other *in silico* studies have shown that this chemical compound is a potent inhibitor of BRD2 and the accessory protein Nsp1(Aydın, Altınel, Erdoğmuş, & Son Ç, 2021; Gupta et al., 2020; Li et al., 2021).

3.7.3. Alkaloids

Since the discovery of this class of natural products, several biological activities associated with alkaloids have been reported, including analgesic, antibacterial, antifungal, anti-inflammatory, anticancer and antiviral. Among the alkaloids that have antiviral activity is berberine (Fielding, da Silva Maia Bezerra Filho, Ismail, & Sousa, 2020).

- **Berberin**

Berberin is an isoquinoline alkaloid derived from the Chinese herb *Coptis chinensis* and plants of the genus *Berberis*. Its broad biological properties identified in preclinical studies include anti-inflammatory, anti-arrhythmic, antimicrobial and cholesterol-lowering activity. Berberin has broad-spectrum antiviral activity *in vitro* against viruses from several different families, including influenza A virus, enterovirus, chikungunya virus, hepatitis B and C viruses, HIV, respiratory syncytial virus, human cytomegalovirus, herpes simplex virus, and human papilloma virus (Varghese et al., 2021).

In one study, Berberin showed good binding activities to the S1 subunit of SARS-CoV-2. Then, to determine whether this compound may be a candidate for broad-spectrum anti-coronavirus activity, they performed further evaluation on the S1 subunits of MERS-CoV and SARS-CoV. The results showed similar binding activity with the S1 subunit of MERS-CoV but reduced affinity for SARS-CoV(Yu et al., 2021). In addition, Berberin showed good docking scores against Mpro and Nsp 15 (Kumar et al., 2021; Zígolo et al., 2021).

Another study identified potential therapeutic targets for berberine against SARS-CoV and SARS-CoV-2 using computer modeling. The most important targets for berberine include NF-κB and MAPK, which are cytokine storm-regulating proteins, and CASP and BAX, which are relevant targets for preventing tissue damage by suppressing cell death signaling pathways. Thus, they demonstrated for the first time that berberin significantly reduces viral replication, suppresses viral entry of the host receptor

ACE2 and TMPRSS2, and decreases inflammatory markers, including IL-6, IL-8, IL-1α, and CCL2 in SARS-CoV-2-infected Calu3 cells (Wang et al., 2021). Berberin has also shown efficacy against SARS-CoV-2 at low micromolar concentrations in vitro in Vero E6 cells (Varghese et al., 2021). In a randomized controlled trial of 39 hospitalized patients with severe COVID-19 grouped into 2 groups, the first received berberin plus routine treatment within 14 days of admission and the control group received only routine treatment. No significant differences were observed between the 2 groups in the trend of IL-6, TNF-α, CRP, procalcitonin, and white blood cell levels within 14 days. In subgroup analyses of patients with diarrhea, berberine significantly improved changes in IL-6, TNF-α, and CRP levels. They hypothesized that berberin may reduce serum levels of inflammatory mediators through the protection and maintenance of gastrointestinal function (Zhang et al., 2021).

4. Conclusion

Natural products have long been used as a treasure trove of drug discovery. These structurally diverse molecules exert a wide range of pharmacological activities, including exceptional antiviral activity. Considerable effort has been devoted to the development of anti-coronavirus drugs from natural products, particularly in the context of global public health challenges such as the SARS-CoV outbreaks in 2003 and the current SARS-CoV-2. In order to provide a more systematic understanding of the research on anti-coronavirus activity of natural products, we have reviewed the relevant studies to date, and summarized the properties of many natural bioactive molecules according to their chemical family, mechanism of action... Most of these natural products are listed as inhibitors against SARS-CoV and SARS-CoV-2 and some molecules act on MERS-CoV.

This study compiled data on different types of phytoconstituents with antiviral activity against coronaviruses as well as phytoconstituents with affinities against SARS-CoV-2 therapeutic targets such as RdRP, 3CLpro, PLpro and host cell targets such as ACE-2, mainly based on computational screening methods. Among these substances, polyphenols, terpenes and alkaloids showed very encouraging anti-coronavirus activity, which could provide a large number of promising candidates for anti-coronavirus drug development and offer potential weapons against SARS-CoV-2 in the current dilemma.

However, further *in vivo* and *in vitro* studies are needed to confirm the bioactivity of these compounds against COVID-19.

Overall, the development of phytopharmaceuticals as an alternative approach could be considered a viable therapeutic option against SARS-CoV-2 in the current COVID-19 pandemic.

Acknowledgements

This work was supported by the Departement of pharmacy, Faculty of Medecine, ABOU BEKR BELKAID University, Tlemcen, Algeria.

Author Contribution

Amal HELALI and Khadidja BENCHACHOU conceived and designed the study. Meryem Wafa HAMMADI and Manel HOUALEF performed the data research and exploitation. Amal HELALI wrote the paper.

Conflicts of Interest

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

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