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Research Article

Phytochemical Comparative Studies, Antioxidant and Antimicrobial of Artemisia and Star Anise

Khadeeja Y. Abid[1,*], Faris T. Abachi[2]

ABSTRACT

The tradition of investigating plants and extensively scrutinizing their biologically or pharmacologically active compounds has markedly increased due to their fewer adverse effects compared to synthetic drugs. Through this study, we aim to establish a phytochemical screening and analyze and compare the antioxidant and antimicrobial properties of Artemisia and star anise. The antioxidant characteristics of plant extracts we assessed, the chemical bonds and components composition were analyzed in the sample and further experiments were done to assess the antimicrobial activities. On the results, we found that all the extracts of both the species were very impactful in the inhibition of E. *coli*, C. *Albicans*, and S. *aureus*. However, S. *aureus* was more sensitive to star anise and E. *coli* and C. *albicans* were inhibited better through the alcoholic extracts of *Artemisia vulgaris*. Star anise has a higher and more effective antioxidant activity against DPPH with a calculated value of 78.3 % at a concentration of 750 ppm, at a concentration of 500 ppm it was 65.3% and in the least concentration of 250 ppm, it was 23.3% compared to *Artemisia vulgaris*. Additionally, both extracts possessed marked antifungal action.

Key words: Artemisia, Anise, Antioxidant, Phytochemical, Antibacterial.

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INTRODUCTION

In recent times, the tradition of investigating plants and extensively scrutinizing their biologically or pharmacologically active compounds has markedly increased due to their fewer adverse effects compared to synthetic drugs.[1] Through this approach, many naturally found elements of plants have been used as an alternative to medicines. It has been reported that around 60% of the total world population uses herbal remedies for health care.[2]

A diverse genus of kingdom Plantae known as genus Artemisia is widely known for its therapeutic and biologically beneficial components, it is composed of approximately 500 species[3] They are broadly used for their therapeutic effects and in food industries to avoid deterioration caused by oxidation and also as bio-pesticide.[4]

A study was held to chemically analyze the therapeutic properties of Artemisia Negrei, which is generally used in the Mountain areas of Morocco for the treatment of dermatologic diseases, digestive tract, and genital tract infections. The essential oil was prepared through a fresh plant with the help of the Clevenger apparatus. On the results, the study revealed that the oil consisted of 34 components that played a noteworthy antibacterial role in both positive and negative bacteria with MIC values and maximum zone of inhibition values of approximately 3.25 to 12.5 mg/ml and 18–37 mm correspondingly.[5]

Another species that is prominently known for its antimicrobial and antioxidant function is *Illicium verum*, it's typically known as star anise and is commonly found in China.[6] Studies have shown that *Illicium verum* has a major antiviral effect too, its important constituent shikimic acid is a molecule used in drugs for the treatment of influenza, both type A and B.[7]

Apart from the microbial phenomenon, due to lipid peroxidation, free radicals are generated in food causing its quality to worsen which is an oxidative fashion of decline in food quality.[8] A known and effective yet convenient way to control the proliferation of microbes and the toxicity-causing agents in food is the use of essential oil.[9] Compounds such as estragole, trans-anethole, and limonene in the essential oil of star anise enable it to function against microorganisms and preserve food. Another molecule presents in the dried fruit of *Illicium verum* called anethole contributes to boosting its antifungal, antibacterial, and antiviral.[10]

Through this study, we aim to establish a phytochemical screening and analyze and compare the antioxidant and antimicrobial properties of Artemisia and star anise.

MATERIALS AND METHODS

Fourier Transform Infrared Spectroscopy (FTIR) Bruker FTIR apparatus, (Germany) over a range of 4000-500 cm^{-1}, used to identify the chemical the oil and ethanol extract of studied plants extracts (Star anise and Artemisia Vulgaris) were tested by GC–MS) with a quadruple detector and a capillary column (30 m×0.25 mm innerdiameter×0.25µm film thickness).

Preparation of extract of studied plants: The dried plants purchased from local markets were converted into powder with the help of a grinder, then (10g) of plants were immersed with 100ml of distilling water with continued stirring for 15 min $55C^0$ and the mixtures were filtered with cheese clothes and the resulting filtrates (crude extracts) were filtered with filter paper then the stock solution stored in a sealed

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storage tube at $4C^0$ in the refrigerator. Another (10g) dried plants were converted into powder with the help of a grinder, then extracted with 100ml of Absolut ethanol the extraction process was done using soxhlet at ($55C^0$) and the raw crude extract was stored at $4C^0$ in the refrigerator.

Screening for phytochemical properties of Star Anise plant and *Artemisia Vulgaris*: Phytochemical screening of Star Anise and *Artemisia Vulgaris* extract was assessed by standard methods, which included detection tests of alkaloids, terpenoids, phenol, carbohydrate, saponins, coumarin glycosides, flavonoids, quinine, protein, steroids, tannin, anthracyanins, and betacyanins.

Radical scavenging activity: The results obtained from the scavenging activity of ethanolic extract of a serial dilution of Star anise extract and *Artemisia vulgaris* extract against DPPH activity were compared with the standard antioxidant as ascorbic acid.

Antimicrobial activity of extracts: It was analyzed using the agar disk diffusion method. S. *aureus*, E. *coli*, and C. *albicans*, as a model for bacteria and yeast were used. Different concentrations of each Star Anise and Artemisia vulgaris extract were prepared.

Determination of Inhibitory Concentration: Following standard procedure, determination of MIC in ethanolic Extract of Star Anise and *Artemisia Vulgaris* using serial dilutions were done with brain heart infusion broth microdilution assay. Characterization of bioactive compounds in the aqueous and ethanolic liquid (table 1).

Identification of the compound

The dried plant Star Anise and Artemisia Vulgaris extract was analyzed by using FT.IR spectrophotometer.

Fourier Transform Infrared (FTIR) spectroscopy analysis

FT-IR spectroscopy was utilized to confirm the star anise extract and *artemisia Vulgaris* extract as shown in figure 1 and table 2.

Experiments were carried out to test the antimicrobial activity of various concentrations of Artemisia and star anise plant extract solution. The antibacterial activity of their aqueous (maceration), alcoholic (soxhlet) extracts against S. *aureus*, E. *coli*, and C. *albicans* were assessed using the diffusion method. For each pathogen, the mean zone of inhibition (ZOI) of three replicate antimicrobial experiments was recorded as represented in table 3.

The outcome confirmed that the compounds of all alcoholic extracts of Artemisia and stare anise were effective in inhibiting the studied microorganisms were found to be more susceptible to Artemisia alcoholic extract than Staphylococcus aureus, which is more sensitive to anise. Furthermore, their microbial activity was found to increase

Table 1: Characterization of phytoconstituents of Star anise and *Artemisia vulgaris* using aqueous and ethanoic extract.

Phytoconstituents Test	Star anise		Artemisia Vulgaris	
	Aqueous extract	Ethanolic extract	Aqueousextract	Ethanolic extract
Alkaloids	_	+	+	+
Phenols	_	+	+	+
Terpenoids	_	_	_	_
Flavonoids	+	+	+	+
Steroids	+	_	+	+
Quinine	_	_	_	_
Saponins	_	_	_	_
Coumarine Glycoside	+	+	+	+
Protein	_	+	+	+
Carbohydrate	+	_	_	_
Tannin	+	+	+ Pseudotanine	+ Pseudotanine
Betacynine or Anothocynin	+ -	+ -	+ -	+ -

Table 2: Results of the FTIR test.

	Stretching vibration band (cm^{-1}). 1	Band shape	The band refer to	Expected Functional group
Artemisia Vulgaris	3534,3399	broad	OH GROUP	alcohol
	3010	sharp	Aromatic CH	Aromatic system
	2921-2853	Medium	CH,CH2 ,CH3	Aliphatic system
	1740	sharp	C=O	Stretching C=O of ester
	1242	sharp	C-O	Ester
Star Anise	3325.19	broad	OH GROUP	alcohol
	2971-2917	Medium	Aliphatic CH, CH, CH2CH3	Aliphatic system
	2319		S-H or aldehyde	system
	1640	sharp	C=O	Stretching C=O of ester
	1380-1269	sharp	Symmetric, asymmetric	Ester

Table 3: The antimicrobial activity of different concentrations of studied plants on the growth of microorganisms.

Extracts Concentrations (mg/ml)	Inhibition zone (mean (mm) ±SD)														
	Staphylococcus aureus					Escherichia coli					Candida albicans				
	100	50	25	12.5	6.25	100	50	25	12.5	6.25	100	50	25	12.5	6.25
Artemisia	23	21	21	18	17	26	23	23	22	21	20	20	17	16	15
Star anise	26	24	22	21	19	22	20	18	18	15	19	18	18	18	17

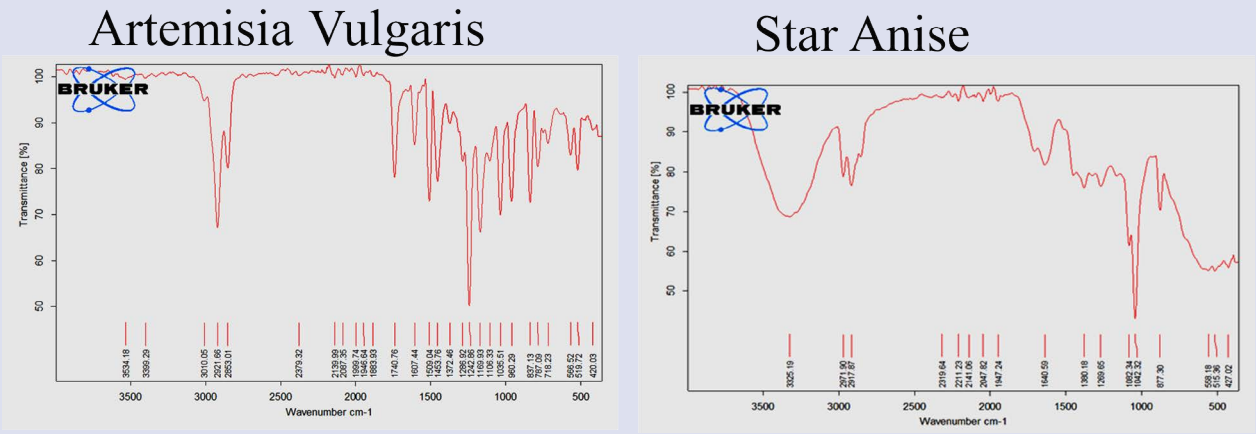

Figure 1: Representative result of FTIR.

Figure 2: The inhibitory zones of the used pathogen.

with increasing dosage as shown graphically in (figure 2). The antimicrobial efficacy of common antibiotics was evaluated, and ZOIs of Ciprofloxacin for Staph. aureus and E. coli, and NY statin for *Candida albicans* were 40, 37, and 25 mm, respectively.

DISCUSSION

To efficiently assess the antioxidant characteristics of plant extracts "2, 2-diphenyl-1-picrylhydrazyl free radical scavenging activity (DPPH RSA)" is proven to be a reliable method.[11] On the results of our screening it has been noted that star anise has a higher and more effective antioxidant activity against DPPH with a calculated value of 78.3 % in a concentration of 750 ppm, in a concentration of 500 ppm it was 65.3% and in the least concentration of 250 ppm, it was 23.3%. Previous studies have highlighted similar results which support our conclusion in a comparative study between the antioxidant activities of star anise and *Carum nigrum* commonly known as black caraway, the study presented that the volatile oil prepared by the extracts of both species had an antioxidant impact however the oil from extracts of star anise showed 25 compounds with relatively more antioxidant activity while in black caraway there were 22 compounds and comparatively lower activity.[12]

A detailed study was held to observe the biological activities of star anise in the combination of four different types of solvents including water, chloroform, methanol, and ethanol. The highest number of phenols were found in methanol. The nitrite and hydroxyl radical scavenging activity revealed that both methanol and ethanol extracts were highly active. Meanwhile, in the case of DPPH free radical scavenging methanol was proved to be the most effective solvent compared to the rest three and lastly, in their activity against Bacillus subtilis and Micrococcus luteus, water was the best-proven solvent.[13]

To evaluate the chemical constituents of the Star anise GC-MS technique was used, the evaluation revealed a total of above 40 components in which trans-anethole was in the largest quantity making up to 81%, followed by 6.5% of limonene and small quantities of anisaldehyde and another compound chavicol with 1.8% and 2% respectively.[14]

Apart from star anise, for the DPPH evaluation of Artemisia, the species used in this study was *Artemisia vulgaris* and the results stated its activity as follows; in the concentration of 250ppm, it was 30%, 64% in the concentration of 500ppm and lastly 87.8% in 750 ppm. The results proved it to be an antioxidant however its activity was relatively less in comparison to star anise. It has been reported that the antioxidant activity of Artemisia depends on the number of extracts.[15]

As a naturally occurring antioxidant, the leaves of Artemisia vulgaris are descriptively studied. Research suggests methanol is the best solvent concerning its activity.[16] A total of twenty-two compounds were recognized in *Artemisia vulgaris* out of which fifteen compounds were phenolic, most of which were the derivatives of chlorogenic acid and flavonoids. The study further adds that the extracts of *Artemisia vulgaris* possess the potential of working as a strong reducing agent and an antioxidant with great radical-scavenging capability.

In a study in Brazil, the components of essential oil from the leaves of *Artemisia vulgaris* were examined through chromatography technique. The examination resulted in three major components which were 13.66% humulene, 16.17% germacrene, and the largest proportion of Caryophyllene with 37.45%.[17]

FTIR - Fourier Transform Infrared Spectroscopy is a dependable and widely used method run to deeply analyze the chemical bonds and components in a sample by studying the infrared absorption spectrum. Multiple studies have carried out this method in plant extracts, specifically in star anise and Artemisia vulgaris too, to confirm the existence of biologically beneficial bonds.[18,19] It has been studied that the extracts are extremely useful yet highly prone to several side reactions with food and other drugs which limit their indication, to address this issue microcapsules are used.[20] Fourier Transform Infrared Spectroscopy has also been used to confirm the microencapsulation approach and proved it to be efficient. Through our study, we found the expected bonds in *Artemisia vulgaris* are as follows; Aromatic system, alcohol functional group, Aliphatic system, ester, and Stretching C=O of ester. While in star anise there were Aliphatic systems, alcohol, ester, and Stretching C=O of ester.

To examine the antimicrobial activities of star anise and *Artemisia vulgaris*, various experiments were conducted in this study. Different concentrations of extracts were taken at different points. The impact of their alcoholic and aqueous extracts was recorded for studied bacteria as well as fungi. The studied sample include, S. *aureus*, E. *coli*, and C. *albicans*. Our results revealed that all the extracts of both species were very impactful in the inhibition of studied microorganisms. Staphylococcus was more sensitive to star anise and E. *coli* and *Candida albicans* were reduced better through the alcoholic extracts of *Artemisia vulgaris*.

On the evaluation of the antimicrobial activity of the essential oil of *Artemisia vulgaris*, an advanced study was conducted which established the comparison between the dried and fresh extracts of Artemisia on 1199 strains of 113 species. 23% of the bacteria were more sensitive to fresh herbs and 21% to dried herbs while on a general analysis 30% of fungal species and 25.9% of bacterial species were sensitive to the essential oil of *Artemisia vulgaris*.[21] Another comparison was done between two species of Artemisia; *Artemisia vulgaris* and *Artemisia Campestris* which stated that the extracts gained from Campestris had a higher proportion of the compound called quercetin as compared to the extracts of Vulgaris and also the antimicrobial activities were seen more in Artemisia campestris. Additionally, the activity of both extracts was more in yeast than in bacteria.[22]

As an antioxidant, extract of these plants could find an application in a wide range of chronic diseases which are associated with oxidative stress, such as, diabetes,[23-26] hyperlipidemia,[27,28] metabolic syndromes,[29] ulcerative diseases,[30-33] and thyroid disorders.[34] The potency of these plant extracts could be comparable to commonly used antioxidant vitamins in terms of potential therapy.[35]

CONCLUSION

The results from our study are supported by multiple trials and experiments that had been held previously. Both the extracts from star anise and Artemisia vulgaris play an important role in the inhibition of the growth of microorganisms and have a significant antioxidant role. Furthermore, it has also been mentioned that the antifungal activities of both species are remarkable.

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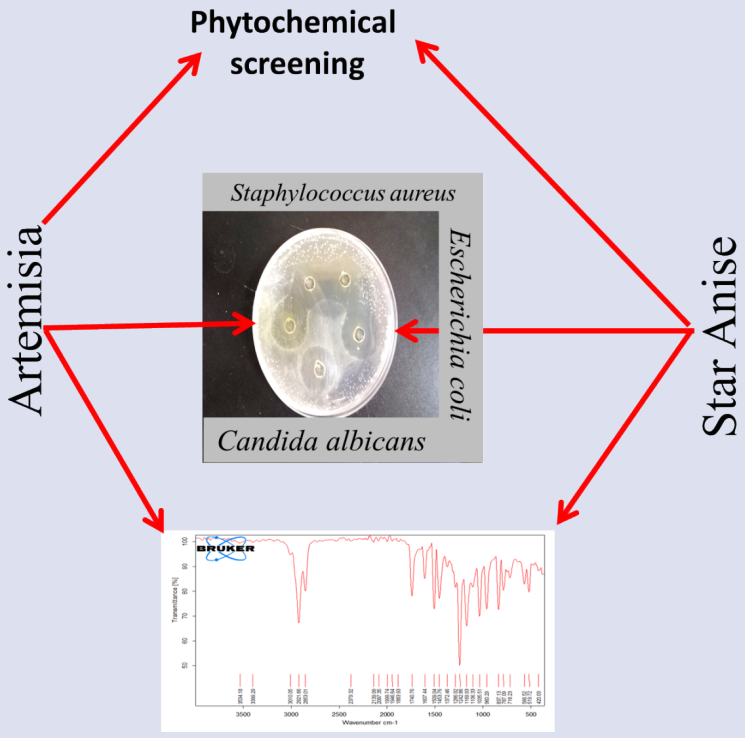
GRAPHICAL ABSTRACT



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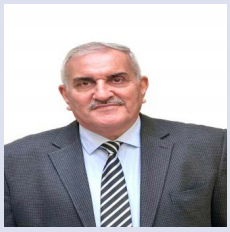

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