Nat. Volatiles & Essent. Oils, 2021; 8(6): 5214-5221

NVEO
Natural Volatiles & Essential Oils

Phytochemical Analysis, Heavy Metals Content And Antimicrobial Activity Of Selected Iraqi Medical Plants

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Abstract

For decades, one of the most popular ideas in human health literature is the idea that using of the medicinal plants as antibacterial source. The presents study was carried to estimate: Firstly, the levels of heavy metals (Mg , Zn, Cu , Mn and Fe) in four plants (Laurus nobilis L. , Zingiber officinale , Origanum majorana L. and Trigonella foenum graecum L.) were determined by flame atomic absorption spectrophotometry. Mg has the highest level in Zingiber officinale with 250 µg/ ml , while the level of Cu in Zingiber officinale was dip down to 0.8 µg /ml .

Secondly: the antimicrobial activity of Laurus nobilis L. , Zingiber officinale , Origanum majorana L. and Trigonella foenum graecum L. against reference strain of Staphylococcus aureus , Bacillus subtilis , Escherichia coli and Pseudomonas aeruginosa . We performed the agar diffusion method e and indicated the bactericidal action. Laurus nobilis L. extract showed strong antimicrobial action against Pseudomonas aeruginosa with 23mm, while Zingiber officinale extract gave the maximum inhibition against Escherichia coli with 25.5 mm. Both extracts of Origanum majorana L. and Trigonella foenum graecum L. experience intense antimicrobial action against Staphylococcus aureus with (22.5, 21 mm) respectively.

The results of the current study indicate that the levels of the heavy metals in the studying plants were in the allowed range and the extracts derived from the plants using in this study might be the active source of antimicrobial compounds.

Key words: phytochemical, heavy metals, antimicrobial, laurus nobilis , zingiber officinale

Introduction:

Recent researches have revealed that synthetic drugs utilized currently are costly and inconvenient for the treatment of diseases [1]. According to the World Health Organization medical plants are used by 80% of the world population. There are growing appeals for using medical plants as a source of antimicrobial molecules [2]. One primary problem is expansion of antimicrobial resistance by microorganisms. One approach to solve this problem involves the use of alternative antimicrobial agents of herbal origin [3]. Human require a several mineral elements in different amounts for adequate growth and health protection. Minerals work as cofactor in the many of enzyme controlled reactions and dominance the activity of muscles and nerves [4].

Laurus nobilis L. belongs to the family Lauraceae , Aqueous extracts of Laurus nobilis L. have been used as anti-inflammatory, anti-fungal and antibacterial [5].

Zingiber officinale belongs to Zingiberaceae family, the part of the plant utilize is rhizome [6]. The Zingiberaceous plants have medicinal properties as antibacterial, anti-fungal and antioxidant [7].

Origanum majorana L. is a perennial herb belonging to the family Labiatae, researchers reported the existence of large number of phytochemicals in several parts of the plant such as phenols, flavonoids, vitamins and fatty acids [8].

Fenugreek (Trigonella foenum graecum L.) belongs to the Leguminosae family. The herb is an aromatic herbaceous annual [9]. Fenugreek being rich in phytochemicals such as alkaloids, saponins, amino acids and fatty acids [10].

This research was performed to estimate the effective chemical compounds and the concentrations of some heavy metals for four types of medical plants in Iraq and to throw the light on their antimicrobial activity.

Experimental:

2.1 Medicinal plants

Four medicinal plants, namely Laurus nobilis L. , Zingiber officinale , Origanum majorana L .and Trigonella foenum graecum L. were purchased from local market.

2.2 Preparation of different plants extracts

The leaves of Laurus nobilis L. and Origanum majorana L ., rhizomes of Zingiber officinale and seeds of Trigonella foenum graecum L. were separately grounded into fine powder. 100 g of each grounded tested plant were separately soaked in 500 ml ethanol 96 % at room temperature, then blending it by incubator shaker for 48 hours. The solvents were discarded using a rotary vacuum evaporator at 40° C to yield concentrated extracts and refrigeration until used [11].

2.3 Chemical detection of active compounds

Detection of Tannins: Used the method in [12].

Detection of Phenols: Used the method in[13] .

Detection of Alkaloides: Used the methods in [13].

Detection of Saponines : Used the method in[14].

Detection of Flavonoides : Used the method in[12].

Detection of Glycosides: By used Fehling reagent.

2.4 Preparation of standard solution for Atomic Absorption Spectrophotometer (A.A.S) measurement

Tow gram powder of each plant sample was dissolved in 10 ml of aquaregia and heated for 5-10 min and up to marked 25 ml by adding deionized water. Determination of heavy metals in samples was performed using atomic absorption spectroscopy (AAS) model AA-7000 (Shimadzu – Japan).

2.5 Microorganisms strains

The microorganisms that used in this study were to type Grame-positive bacteria (Staphylococcus aureus and Bacillus subtilis) and Gram –negative bacteria (Escherichia coli and Pseudomonas aeruginosa). The microorganisms were obtained from wond , food poisoning , buns and stock cultures of the Laboratory of Microbiology College of Science , University of Al- Mustansiriyah .

2.6 Antibacterial activity

Antibacterial activity was estimated by using agar –well diffusion method according to [15]. Petri plates containing 20 ml of Nutrient agar medium were seeded with a 24 h culture of bacterial strains. Well (6 mm) diameter were cut into the ager and 50 µl of the plant extracts were tested in a concentration of (50, 75,100) mg/ml. The inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 colony-forming units CFU/ml .Incubation was performed at 37 ^{0}C for 24 h. The determined of antibacterial activity was based on measurement of diameter of the inhibition zone formed around the well. Minimum inhibitory concentration (MIC) was estimated by the micro-dilution method using serially diluted (2-fold) plant extracts according to [15].

Results and Discussion

The present study confirmed the findings about phytochemical analysis in the Laurus nobilis L. , Zingiber officinale , Origanum majorana L .and Trigonella foenum graecum L. and the results summarized in Table (1).

Table (1) Chemical detection of phytochemicals in Laurus nobilis L , Zingiber officinale , Origanum majorana L. and Trigonella foenum graecum L.

Active compounds	**Laurus nobilis** L.	**Zingiber officinal**e	**Origanum majorana** L	**Trigonella foenum graecum L.**
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Resins	+	+	+	+
Saponines	-	+	-	+
Tannins	+	+	+	+
Phenols	+	+	+	+
Glycoside	+	+	+	+

The results from Table (1) showed the presence of active compounds such as Flavonoids, Tannins, Alkaloids, Resins, Phenols and Glycoside in all the plants studied, On the other hand Saponines was lack in both Laurus nobilis L. and Origanum majorana L. These results tie well with previous studies [8], while disagreement with other studies found the absence of Saponins in Zingiber officinale and it is existence in Laurus nobilis L. [16].

The values for the concentration s of heavy metals in the studied plants are given in Table (2).

Table (2) Concentration (µg / ml) of heavy metals in Laurus nobilis L, Zingiber officinale, Origanum majorana L. and Trigonella foenum graecum L.

Elements	Laurus nobilis L.	Zingiber officinale	Origanum majorana L.	Trigonella foenum graecum L
Mg	56	250	23	120
Zn	15	4.50	30	25
Cu	13	0.80	11	50
Mn	10	17.00	15	8
Fe	36	2	75	112

From the results, it is clear that, the highest concentration of Mg (250 µg / ml) was found in the Zingiber officinale . Followed by Trigonella foenum graecum L. with (120 µg/ ml). The higher level of Mg in Zingiber officinale is may be due to the type of the soil which have been used for cultivating the plant are highly dressing manure and organic residues, as a results Mg are highly obtainable for plant uptake . So, the plant has extremely amount of these metal [17].

Our findings on the concentration of the heavy metals in the studied plants at least hint that the higher level of Zn was in Origanum majorana L. (30 µg / ml) , while the lower level was in Zingiber officinale (4.50 µg / ml). Trigonella foenum graecum L. has the higher level of Cu (50 µg / ml) , while the concentration of Cu in Zingiber officinale was (0.80 µg / ml) only.

Zingiber officinale was exhibiting the most accumulated Mn levels (17.00 µg / ml), on the other hand the less levels of Mn was found in Trigonella foenum graecum L. (8 µg / ml) . This suggests that higher Mn levels in Zingiber officinale may be attributed to the existence of this metal in relatively acidic soils. However , in the line with the ideas of acidic soil , it can be concluded that the chemical forms of Mn existent in acidic soil was Mn^{+2} released from soil by H^{+} , which is generated from NH_4^{-} , can be easily accumulated in the Zingiber officinale .[17]. The levels of Fe in the samples of studied plants showed that Trigonella foenum graecum L. contained higher amount of Fe (112 µg / ml) , while the less level of Fe was found in Zingiber officinale (2 µg / ml).

In this section, we will illustrate the antimicrobial activity of alcoholic extracts of four different plants at multiple concentrations against two Gram (+) and two Gram (-) bacteria and the results showed in tables (3, 4, 5 and 6).

Table (3) Inhibitory properties (inhibition zone diameter in mm) of Laurus nobilis L. alcoholic extract on different type of bacteria.

Inhibitory effects of alcoholic extract of Laurus nobilis L. against bacteria				Concentration of alcoholic extract of Laurus nobilis L. (mg/ml)
Staphylococcus aureus	Bacillus subtilis	Escherichia coli	Pseudomonas aeruginosa	
Inhibition zone diameter in mm				
14	8	12.5	14	25
16	11	14	17	50
18	14.5	16	19	100
21	17	22	23	200

Table (4) Inhibitory properties (inhibition zone diameter in mm) of Zingiber officinale alcoholic extract on different type of bacteria.

Inhibitory effects of alcoholic extract of Zingiber officinale against bacteria				Concentration of alcoholic extract of Zingiber officinale (mg/ml)
Staphylococcus aureus	Bacillus subtilis	Escherichia coli	Pseudomonas aeruginosa	
Inhibition zone diameter in mm				
9	11.5	16	12	25
17	15	19	16	50
20	18	22	19.5	100
22	20.5	25.5	21	200

Table (5) Inhibitory properties (inhibition zone diameter in mm) of Origanum majorana L. alcoholic extract on different type of bacteria.

Inhibitory effects of alcoholic extract of Origanum majorana L. against bacteria	Concentration of alcoholic

Staphylococcus aureus	Bacillus subtilis	Escherichia coli	Pseudomonas aeruginosa	extract of Origanum majorana L. (mg/ml)
Inhibition zone diameter in mm				
10.5	-	12	11	25
17	12	15.5	15	50
19	16.5	18	18.5	100
22.5	19	22	20	200

Table (6) Inhibitory properties (inhibition zone diameter in mm) of Trigonella foenum graecum L. alcoholic extract on different type of bacteria.

Inhibitory effects of alcoholic extract of Trigonella foenum graecum L. against bacteria				Concentration of alcoholic extract of Trigonella foenum graecum L. (mg/ml)
Staphylococcus aureus	Bacillus subtilis	Escherichia coli	Pseudomonas aeruginosa	
Inhibition zone diameter in mm				
8	-	-	-	25
12	11.5	11.5	9.5	50
17	15	16	15.5	100
21	18	20	18	200

From the tables above, key findings emerge: The antimicrobial activities of divers plants extracts as found in this work were contingent on the concentrations of the extracts. For instance, 25 mg/ml of alcoholic Laurus nobilis L .extract inhibited Staphylococcus aureu , Bacillus subtilis , Escherichia coli and Pseudomonas aeruginosa respectively with inhibition zone range from (8 to 14 mm) , and it is gradually rise until peak to 23 mm in 200 mg/ml with Pseudomonas aeruginosa , while 25 mg/ml of Trigonella foenum graecum L. extract showed non inhibition effect with Bacillus subtilis , Escherichia coli and Pseudomonas aeruginosa . The inhibition effects appeared at 50 mg/ml and experiences significant increased until reached to 18 mm for both Bacillus subtilis and Pseudomonas aeruginosa , whereas the inhibition zone surge to 20 mm for Escherichia coli .

From tables 4 and 5 results demonstrated that the extracts of both Zingiber officinale and Origanum majorana L. screened showed various inhibitory effects against all bacterial species. The

Zingiber officinale extract exhibited the largest zone of inhibition against Escherichia coli with the concentration 200 mg/ml , while the smallest zone of inhibition was 9 mm with concentration 25 mg/ml against Staphylococcus aureu , on the other hand , the largest zone of inhibition for Origanum majorana L. extract was showed against Staphylococcus aureu with concentration 200 mg/ml , while at 25 mg/ml from the same extract showed non inhibitory effect with Bacillus subtilis .

Our findings on antimicrobial activities of various plants extracts at least hint that the presences of chemical active compounds like phenols, flavonoids, alkaloids, glycoside and Tannins have an essential role in antimicrobial activity of the plants studied [18].

Conclusions

In conclusion, this study would appear that promising antimicrobial activities for the extracts of different plants were studied in this work. The antimicrobial activities of the extracts are expected perhaps duo to the present of phytochemicals as well as the plants may be a good source of minerals (Mg, Zn, Cu, Mn, Fe). The present study has provided the justification for medicinal properties of the plants and also utilized as a dietary supplement.

Further research on phytochemical studies might extend the explanations of the components responsible for antibacterial activity of these extracts against bacteria.

Acknowledgments

The authors wish to acknowledge to staff of the Laboratory of Microbiology College of Science, University of Al- Mustansiriyah for samples provision and use of laboratory facilities.

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