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Original Research Paper

PHYTOCHEMICAL ANALYSIS OF LIBYA *ORIGANUM MARJORANA* L. LEAVES USING TLC, AND FTIR

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ABSTRACT:
Origanummajorana L. (marjoram) fundamental oil having a place to the family Lamiaceae has antimicrobial properties against nourishment deterioration and foodborne microscopic organisms and so, it may have the most prominent potential for utilize in mechanical applications. This study designed phytochemicals of Libya Origanum marjorana L. using preliminary test for secondary metabolites, thin layer chromatography (TLC), Fourier Transform Infrared spectroscopy (FTIR). The active ingredients in the leaves were extracted using methanol as a solvent. The phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, saponins, phenols, terpenes, amino acids, Glycosides and carbohydrates. TLC profiling shows different bands of chemical compounds with different Rf. The FTIR spectrum confirmed the presence of important functional group such as hydroxyl, alkene, carbonyl groups.

Keywords: Phytochemical screening, Origanum marjorana L, Spectroscopy

INTRODUCTION:

Medicinal plants are a source of many potent and powerful drugs. According to World Health Organization, 80% of the people rely primarily on traditional health care system and mostly on herbal medicines. herbal medicines have a long history of human interactions with the environment. Plants used for traditional medicine contain, a wide range of substances that can be used to treat chronic as well as infectious diseases (1,2). Origanum vulgare L. is a perennial aromatic herb belonging to the family Lamiaceae (3) used for thousands of years as spices and as local medicines in traditional medicine (4). O. vulgare L. is the most wide spread among all the species within the genus. It is distributed all over Europe, West and Central Asia up to Taiwan (5). Origanum majorana L. (Lamiaceae), also known as marjoram is an herbaceous, perennial plant that commonly grows (6) in Mediterranean regions Given its broad variety of chemical characteristics and aroma, this species has been traditionally used in popular medicine to treat many illnesses as a spasmodic, antimicrobial, digestive, expectorant and aromatic ingredient for whooping and convulsive coughs (7). Investigations on phytochemistry of Origanum majorana L. essential oils originating from different area in the world and their antimicrobial activity were previously reported (8). Additionally, several studies have demonstrated that different solvent extracts of marjoram leaves possess antioxidant, antimicrobial, and anti-inflammatory effects (9). Methanol extracts of plant samples were found to inhibit the growth of microorganism's more than aqueous extracts (10). Moreover, in others plant species methanol extract presents considerable antioxidant and antimicrobial activity(11,12). Sweet marjoram is characterized by a strong, spicy and pleasant odour and flavor (13). Analysis of herb reported presence of especially volatile oil as major constituents, due to its aromatic nature. Various phytochemical tests revealed the presence of terpenoids, flavonoids and tannins in ethanol extract whereas saponins and carbohydrates were present in stem and root water extract, respectively. Alkaloids, glycosides and proteins were absent in both of the extracts (root and stem)18. Essential oil from O. majorana contains terpinen-4-ol (31.15 %), cis-sabinene hydrate (15.76 %), p-cymene (6.83 %), sabinene (6.91 %), trans-sabinene hydrate (3.86 %) and α-terpineol (3.71 %) as the main constituent 20. The most prominent

components of O. majorana20 were carvacrol (65 %) and thymol (4 %). Fig. 1 shows structures of p-cymene, thymol, sabinene and carvacrol, respectively.

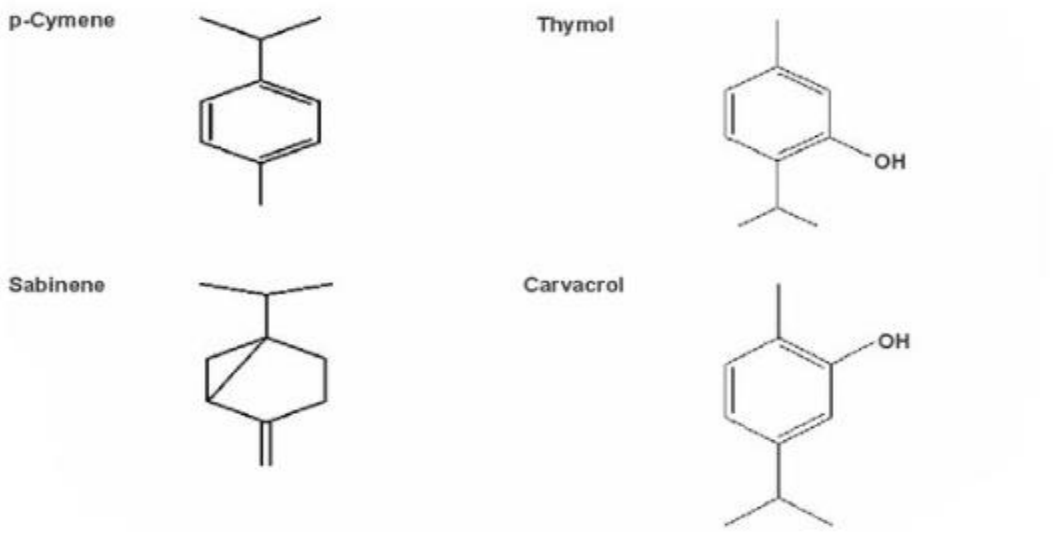

Fig 1. Major phytoconstituents reported in Origanum majorana L. essential oil

MATERIAL AND METHODS:

This study designed Phytochemical Analysis of *Origanum marjorana* L. by test for secondary metabolites, and by techniques of; thin layer chromatography (TLC), Fourier Transform Infrared spectroscopy (FTIR).

Collection and authentication of plant material:

Fresh leave of *Origanum majorana L* were collect from west of Misrata city- Libya in summer 2021 and authentication in research laboratory in Faculty of Medical Technology, Misurata–Libya then washed with running water and drained for a week.

Preparation of Plant Extracts:

The stored *Origanum majorana L* powder (1 g) was extracted with 20 mL 99% methanol for 24 hours. After the extraction process, the solvents were removed by air drying using vacuum in a rotary-evaporator at 40°C to obtain crude extract and stored at 18°C in refrigerator.

Fig (2): Origanum majorana L

Phytochemical Screening of the Plant Extract:
Phytochemical test was conducted to identify the presence or absence of secondary metabolites namely; alkaloids, flavonoids, steroids, saponins, phenols, terpenes, amino acids, Glycosides carbohydrates, according to the method described by (14).

Qualitative Analyses:
Following standard protocols were used for qualitative analysis of samples to check for the presence of Alkaloids, Flavonoids, Steroids, Saponins, amino acids, Carbohydrates, glycosides, Phenols and Terpenoids (15,16).

Test for Alkaloids:
One mL of the peel extract was added to 2 mL conc. HCl. Then, few drops of Mayer's reagent was added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for Flavanoids:
2 ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Steroids:
1 ml of extract was treated with few drops of chloroform, acetic anhydride and conc. H_2SO_4 and formation of dark pink or red colour indicated the presence of steroids.

Test for Saponins:
22 ml of extract was added to 6 ml of distilled water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of Saponins.

Test for free amino acids:
2 ml of extract was heated with 0.2 % solution of Ninhydrin which result in the formation of purple colour, suggesting the presence of free amino acid.

Test for Carbohydrate:
1 ml of plant extract was mixed with alpha napthol solution and then to the sides of the test tube conc. H_2SO_4 is added. Appearance of violet ring indicates the presence of carbohydrates

Test for Glycoside:
One mL of the plant extract was added to 3 mL chloroform and 10% ammonium solution. Formation of pink color indicates the presence of glycosides.

Test for Phenols:
One mL of the plant extract was added to 2 mL distilled water followed by few drops of 10% ferric chloride. Formation of blue/green color indicates the presence of phenols.

Terpenes Test for:
One mL of the plant extract was added to 2 mL chloroform along with conc. Sulphuric acid. Formation of red brown color at the interface indicates the presence of terpenoids.

Quantitative Analyses:
The presence of secondary metabolites from the leaves, roots and stem bark of the test plants were quantitatively determined by adopting standard protocols. Total Alkaloids compounds were determined using the method described by (17), Saponins by the method of (18), Flavonoids by the methods of (19).

Alkaloids:
520 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Saponins:
The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Flavonoids:
About 15 grams of each part of dried plant samples separately were ground then extracted recurrently with 50 ml of 80% aqueous methanol, at room temperature. The complete solution was filtered through Whatman filter paper No 42. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath; the dry content was weighed to a constant weight.

Thin Layer Chromatograph:
Extracts of *Origanum majorana L* were spotted on TLC plates 2cm above its bottom coated with silica gel with different solvent solution and the different spots developed were observed by means of UV light at l max 254 -360 nm and the retention time (R_f) were correspondingly calculated and recorded (20).

FTIR Spectroscopic Analysis:
The extracts were examined under FTIR spectrophotometer and they were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The peak values of the FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation (21).

RESULTS AND DISCUSSION:
Qualitative phytochemical analyses:
The preliminary phytochemical qualitative screening of the leaves of *Origanum majorana L* revealed the presence of all the chemical constituents such as Alkaloids, Flavonoids, Steroids, Saponins, amino acids, Carbohydrates, glycosides, Phenols and Terpenoids. the result is summarized in Table (1).

phytochemical test	Alkaloids	Flavonoids	steroids	saponins	amino acids	carbohydrates	Glycosides	Phenols	Terpenes
Methanol extract	+	+	-	+	+	+	-	+	-

+ = Present, - = Absent

Quantitative phytochemical Analyses:
The results of various quantitative phytochemical analysis of an methanolic extract of *Origanum majorana L* leaves shown in table 2. The percentage yield of Flavonoids was found higher in the extract 65% and lower was for alkaloids 14 %, while, 61% obtained for the saponins.

Table (2) .Quantitative Analysis of Phytochemicals present in *Origanum majorana* L leaves

phytochemical test	Alkaloids	Saponins	Flavonoids
Percentage Yields (%)	10	30	41

Thin Layer Chromatography (TLC):
TLC profiling of *Origanum majorana L* extracts gives an impressive result that shows a different bands of chemical compounds, with R_f value of between a minimum of 0.20 to a maximum of 0.90. The eluted compounds showed different colorations such as yellow, green, orange. The retention factors (R_f) for each band was recorded and presented in Table (3).

Table (3) : TLC profiles of *Origanum majorana L* leaves extract

Solvent system	**No of spot**	***R_f* value**
Chloroform:Benzene (10:90)	3	0.4 0.3 0.2

Chloroform:Benzene (40:60)	2	0.8 0.7
Chloroform:Methanol (10:90)	4	0.7 : 0.5 : 0.4 : 0.2
Chloroform:Methanol (20:80)	3	0.5 : 0.4 : 0.2
Ethyl acetate:Methanol (10:90)	-	-
Benzene:Ether:Formic acid (50:40:10)	2	0.9 : 0.7

FTIR Analysis:

FTIR spectroscopy is a strong instrument for identification of functional groups present in extracted compounds from plants. The FTIR spectra of *Origanum majorana* L leaves extraction were described in Figure (3), Table (4). The broad band obtained at 3311 cm^{-1} assigned to the O–H stretching, are illustrated by a strong and weak peaks at 2927 cm^{-1} attributed to the C–H bond stretching. The peak at 1509 cm^{-1} are assigned to the C=C stretching and bending vibrations in the aromatic rings, the carbonyl groups in *Origanum majorana* L are associated with a small band at 1599 cm^{-1}. Cm-1 was assigned to N=O 1364The vibrational absorption band at A notable band at 1250cm^{-1} and 776cm^{-1} can be assigned to C-O and C-X stretching (22)

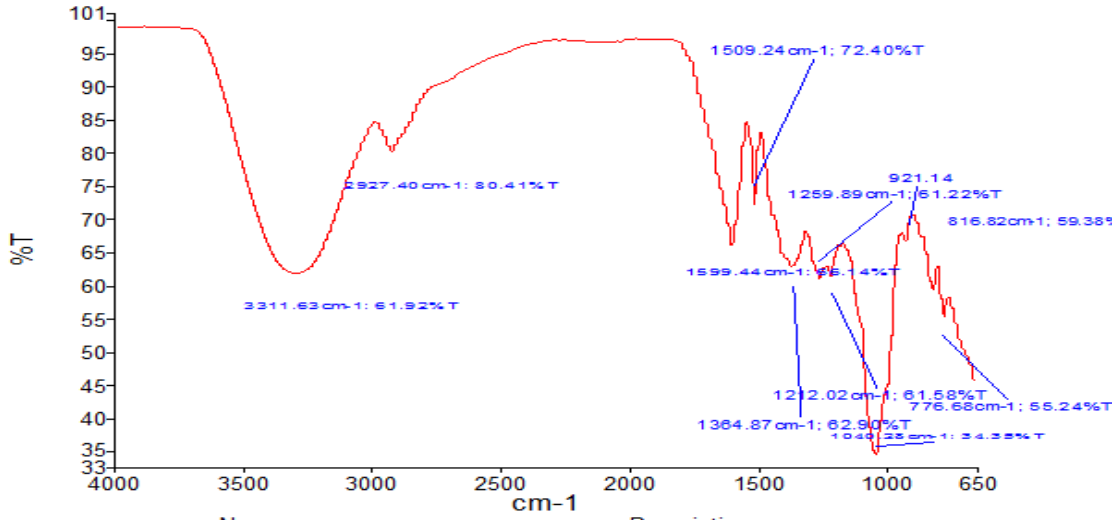

Figure (3): The FTIR spectra of *Origanum majorana L*

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