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Pharmacognostical and Phytochemical Study of Calendula Officinalis L Leaves Cultivated in Baghdad

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Abstract. *Calendula officinalis* L. is Mediterranean plant known for its clinical and medicinal application. It is yellow to orange flower grown up in spring usually. Two different extracts were obtained from the leaves of this plant, polar (methanolic) and non-polar (hexanic). GC-MS analysis for these extracts results of total 27 components. Fifteen of them were in methanolic extract with the superior of *n*-hexadecanoic acid and oleic acid at the percentage of 32.22 and 31.48 %, respectively. Also, hexanic extract showed twelve different compounds with the majority of *n*-hexanoic acid and cis-vaccenic acid at the percentage of 35.38 and 20.15 %, respectively. Phytochemical study of this plant exhibited the presence of saponins, tannins, flavonoids, and terpenoids. In addition, glandular multicellular multiseriate stalk trichomes and anomocytic stomata were depicted in fresh leaves microscopical analysis. While the analysis of dry leaves revealed the presence of stone cell, fiber, starch, and pitting vessels.

Keywords: Pharmacognostical, Phytochemical, Calendula officinalis

INTRODUCTION

C. officinalis (commonly known as Marigold or Pot Marigold) is a native plant to United State of America (USA) and west Asia. It is grown in the gardens of Arabs, Greeks, Indians, and South Europeans since the twelfth century for medicinal purposes [1-3]. Flowers and leaves of *C. officinalis* are the main parts that used for medications. Many previous studies were performed on flowers of this plants and the results revealed that its extract was used for treatment of small pox, suppression of menstrual flow, measles, and jaundice [2]. It has many satisfactory dermatological applications to eliminate acne and pimples, treat skin rashes wound healing, and first-degree burns [4-7]. It is widely used as a cream or an ointment in cosmetics [2], [8]. The extract is used to reduce pain, as nourishing, diuretic, and antiseptic as well [3], [9].

These flowers were proved to have multi pharmacological activities like anti-bacterial, anti-inflammatory, anti-oxidant, anti-diuretic, anti-pyretic, anti-viral, anti-fungal, anti-HIV, hepatoprotective, and tumor reducing potential activities [10-15]. On the other hand, plants' leaves results in anti-phlogistic, anti-septic, anti-spasmodic, astringent, aperients, diaphoretic, resolvent, stimulant, and skin cure effects [3], [16], [7].

In addition, review of the literatures revealed that Marigold contains a wide range of chemical constituents such as steroids, coumarins, tannins, flavonoids, saponins, triterpendiol esters, quinines, carotenoids, polysaccharides, triterpens,amino acids, volatile and essential oils, and many further compounds [1],[2],[3],[9], [16],[17],[11],[12],[18].To the best of our knowledge, there is neither microscopical study for fresh leaves nor phytochemical study for hexanic extract for dry leaves of *C. officinalis* cultivated in Iraq. Therefore, this project is designed to compare the chemical constituents of different extracts, by different solvent, of dry leaves by GC-Mass. It is aimed to study the microscopical properties for fresh leaves of the same plant as well.

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MATERIALS AND METHODS

Materials

All used solvents were commercially available and used directly without extra purification. Methanol was ordered from Scharlab. S.L./ Spain and hexan was purchased from Sigma Aldrich/ China.

Instrumentation

The microscopical study was done by light microscope from Olympus/ Philippines. GC-MS analysis was performed by Agilent GC-MS/ USA.

Leaves Sample Collection

Fresh leaves were collected from botanical garden, College of Pharmacy, Mustansiriyah University, Baghdad, on March 2019. The species was identified by the national herbarium in Abu-Graib, Baghdad. The leaves were well washed and shaded dry at room temperature. Then, they were grinded by mechanical grinder into powder, and collected in dry clean glass jar till use.

Extract Preparation

Non polar (hexanic) extract was extracted by soxhlet using *n*-hexane as a solvent. Then, the remained mark was extracted with methanol to produce the polar one. Both extracts were dried under vacuum and collected separately in clean glass vials at room temperature till use for further analysis.

Phytochemical Study

Many standard procedures were carried out to detect the bioactive compounds present in both extracts following the methods that mentioned in the previous studies [19],[20],[21],[22],[23]. All procedure details are mentioned in the following sections.

Saponins

Foam index method was performed to identify saponins. 2.5 mL of each sample was added to 10 mL of distilled water and mixed in a test tube for 30 seconds. Honey comb forth was formed after approximately 15 minutes. After standing for one min., the presence of this forth confirm the existence of saponins [19],[20].

Tannins

Few drops of 10% ferric chloride were added to few milliliters of each extract to detect gallic and catecholic tannins. Blue color was observed when there was gallic tannins and green color was seen when there was catecholic tannins [19],[20], [21].

Flavonoids

The extract sample (4 mL) was mixed with 1.5 mL of 50% methanol. The mixture was warmed with magnesium metal. Then, 5 to 6 drops of concentrated hydrochloric acid were added until red or orange color was formed. The formation of orange color meant the presence of flavones, and red color meant the presence of flavonoids [19], [20].

Terpenoids

Both of chloroform and acetic anhydride (0.5 mL) were added to 4 mL of each extract in a separated test tubes. The presence of terpenoids was detected by the presence of red-violet color which formed after adding concentrated H_2SO_4 [19],[20].

Alkaloids

Methanolic and hexanic extracts were dissolved separately in diluted hydrochloric acid. Then, filter the precipitate. The following reagents were added separately into each filtrate to test the presence of alkaloids [19], [20],[21].

Wagner's Test

Formation of reddish brown precipitate after adding Wagner reagent to the collected filtrate indicated the presence of alkaloid derivatives in the sample. Wagner reagent was prepared by mixing of iodine solution with potassium iodide [23].

Dragendroff's Test

Formation of orange precipitate after adding Dragendroff's reagent to the sample tube indicated the presence of alkaloid compounds. This reagent was a solution of potassium bismuth iodide [23].

Coumarins

Ammonium hydroxide (0.5 mL of 10% solution concentration) was added to 5 mL of each extract. Two drops of the mixture were spotted on a filter paper and tested under UV-light. Coumarins confirmed to be present if intense fluorescence light was detected [23].

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS (GC-MS)

GC-MS study was performed using Agilent GC-MS/ USA to analyse the gas chromatography for each sample. The analysis was done at Tehran University, Tehran, Iran.

Microscopical Examination

Microscopical examination for *C. officinalis* leaves was performed at laboratory of Pharmacognocy and medicinal plants, Dept. of Pharmacognocy and Medicinal Plants, College of Pharmacy, Mustansiriyah University, Baghdad.

Powdered Leaves Microscopy

A small quantity of finely powdered shady dried leaves was placed on a slide and covered with two drops of chloral hydrate. After that, it was covered with a cover slip and examined under the microscope. Different cell components were observed and documented by shooting photographs using digital camera.

Fresh Leaves Microscopy

Fragments of the outer epidermal layer of fresh leaves were covered with chloral hydrate and examined under the microscope at room temperature. Trichomes and stomata type were diagnosed in this examination.

RESULTS AND DISCUSSION

Phytochemical Study

To determine the secondary metabolite in C. officinalis leaves, a qualitative phytochemical study was performed. From the study results, saponins, tannins, and flavonoids were found in methanolic extract and were not in hexanic. Whereas, terpenoids were available in hexanic and were not in methanolic extract. In addition, both of alkaloids and coumarins compounds were not found in both extracts. Table 1 displays phytochemical study of Iraqi cultivated Marigold leaves extracts. These results are in agreement with previous study for methanolic extract of C. officinalis flowers (24). In another study, all of saponins, tannins, flavonoids, terpenoids, alkaloids, and volatile oils were available in 80% methanolic and 70% ethanolic extracts of C. officinalis leaves (3, 12). Furthermore, carbohydrates and amino acids were found in methanolic extract of these leaves (11).

TABLE 1. Qualitative phytochemical study of *C. officinalis* extracts.

Sq.	Compounds	Hexanic extract	Methanolic extract
1	Saponins	-	+
2	Tannins	-	+
3	Flavonoids	-	+
4	Terpenoids	+	-
5	Alkaloids	-	-
6	Coumarins	-	-

GC-Mass Chromatography Analysis

The gas chromatography and mass spectrometry analysis of polar extract (methanolic) of *C. officinalis* leaves shows the presence of 5-octadecene, valencene, β-selinene, α-gurjunene, 1-heptadecene, carbonic acid, tetradecyl 2,2,2-trichloroethyl ester, 1-nonadecene, pentadecyl pentafluoropropanoate, octadecyl chloroacetate, pentadecyl trifluoroacetate, (-)-trans-pinane, methyl palmitate, n-hexadecanoic acid, oleic acid, octadec-9-enoic acid as major constituents at 14.04, 16.23, 17.56, 20.76, 21.48, 22.80, 23.80, 26.21, 26.45 min., respectively. **Table 2** and **Fig. 1** display all compounds profile. *n*-Hexadecanoic acid was the major component in this study of 32.22 % area. These results are not in agreement with the previous Iraqi study of Abdul Jalill R. Dh. (2014) except of α-gurjunene and methyl palmitate compounds which appear in her study (24). Moreover, methyl palmitate, methyl cis-vaccenate, and methyl eladate are available in 50% methanolic extract of Iraqi flowers (17)

TABLE 2: Main compounds profile in methanolic extract of marigold leaves.

Sq.	Compounds	Retention time (min.)	Area (%)	Quality
1	5-Octadecene	14.04	2.30	90
2	Valencene	16.23	2.50	98
3	β-Selinene	16.23	2.50	97
4	α-Gurjunene	16.23	2.50	97
5	1-Heptadecene	17.56	3.01	91
6	Carbonic acid, tetradecyl 2,2,2-trichloroethyl ester	17.56	3.01	90
7	pentadecyl pentafluoropropanoate	20.76	1.68	93
8	1-Nonadecene	17.56	3.01	90
9	Octadecyl chloroacetate	20.76	1.68	93
10	Pentadecyl trifluoroacetate	20.76	1.68	93
11	(-)-trans-Pinane	21.48	3.59	90
12	Methyl palmitate	22.80	2.85	95
13	*n*-Hexadecanoic acid	23.80	32.22	99
14	Oleic Acid	26.21	31.48	99
15	Octadec-9-enoic acid	26.45	6.66	99

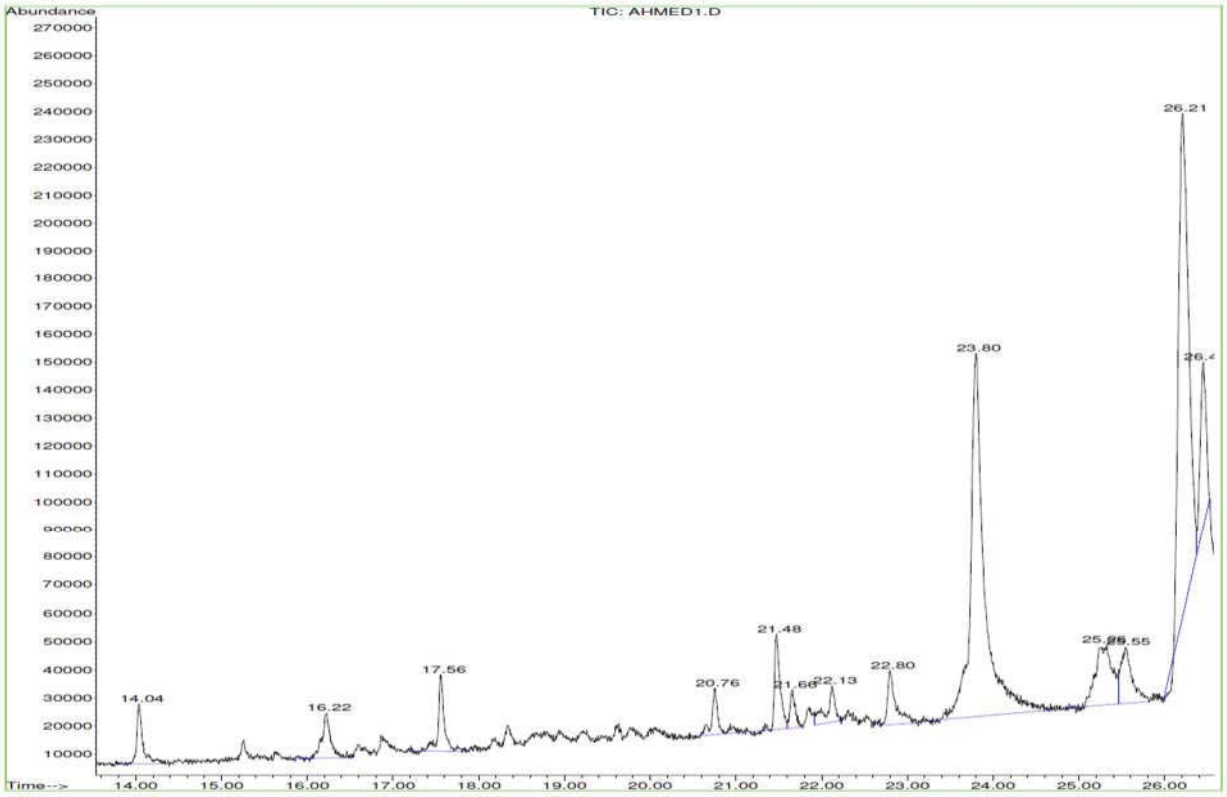

FIGURE 1. GC-MS analysis of methanolic extract of marigold leaves.

The gas chromatography and mass spectroscopy analysis of non-polar extract (hexanic) of C. officinalis leaves shows the presence of lemnalol, β-cubebene, cedrene, (+)-ledene, (R)-gamma-cadinene, oplopenone, oplopane, 7-acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane, 1,3,6-triazahomoadamantane, n-hexanoic acid, cis-vaccenic acid, octadecanoic acid as majour components at 14.25, 16.25, 16.61, 18.36, 20.67, 23.81, 26.22, 26.46 min., respectively as shown in Table 3 and Fig.. 2. n-Hexanoic acid was the major component in hexanic extract of 35.38% area. There is no previous Iraqi GC-MS analysis for hexanic extract of C. officinalis.

TABLE 3. Main compounds profile in hexanic extract of marigold leaves.

Sq.	Compounds	Retention time (min.)	Area (%)	Quality
1	Lemnalol	14.25	2.53	94
2	β-Cubebene	14.25	2.53	90
3	Cedrene	14.25	2.53	90
4	(+)-Ledene	16.25	5.83	99
5	(R)-gamma-cadinene	16.61	1.95	97
6	Oplopenone	18.36	2.67	96
7	Oplopane	20.67	5.75	98
8	7-Acetyl-2-hydroxy-2-methyl-5-opropylbicyclo[4.3.0]nonane	20.67	5.75	90
9	1,3,6-Triazahomoadamantane	20.67	5.75	90
10	*n*-Hexanoic acid	23.81	35.38	99
11	Cis-Vaccenic acid	26.22	20.15	99
12	Octadecanoic acid	26.46	6.34	98

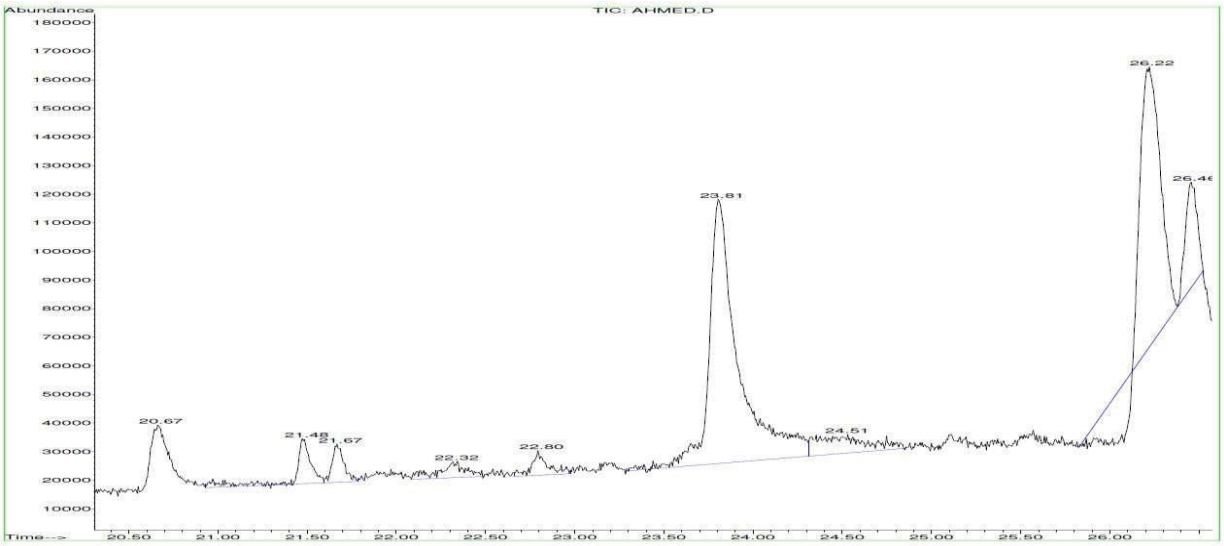

FIGURE 2. GC-MS analysis of hexanic extract of marigold leaves.Microscopical Characters

Microscopical examination of the dry leaves of marigold plant showed the presence of pitting vessels, spherical shape small size starch, stone cell, and fiber as shown in Fig. 3 and 4, respectively. Anomocytic stomata and glandular multicellular multiseriate stalk trichomes of fresh leaves are depicted in Fig. **5**.

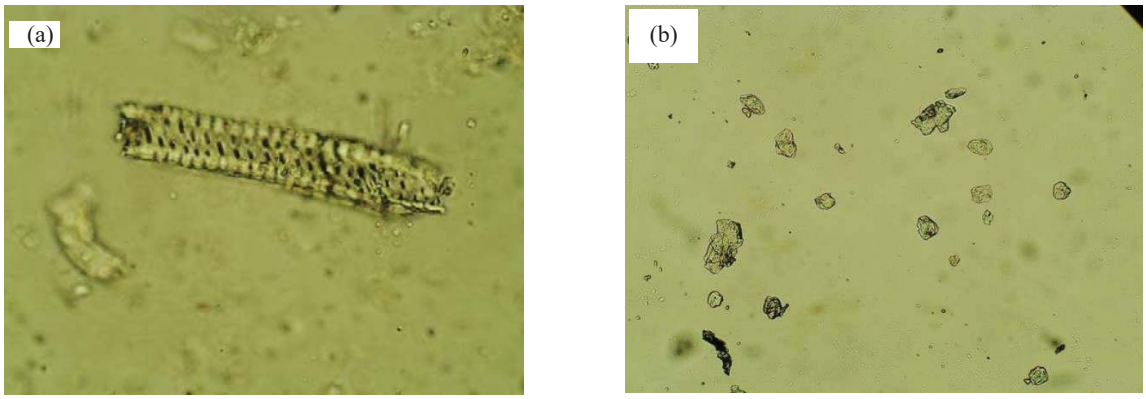

FIGURE 3. (a) Pitting vessels and (b) starch of dry marigold leaves.

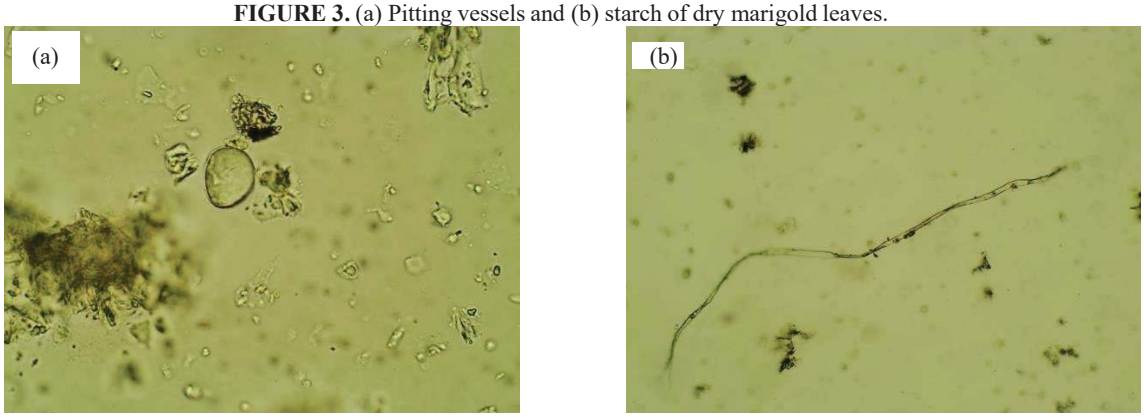

FIGURE 4. (a) Stone cell and (b) fiber of dry marigold leaves.

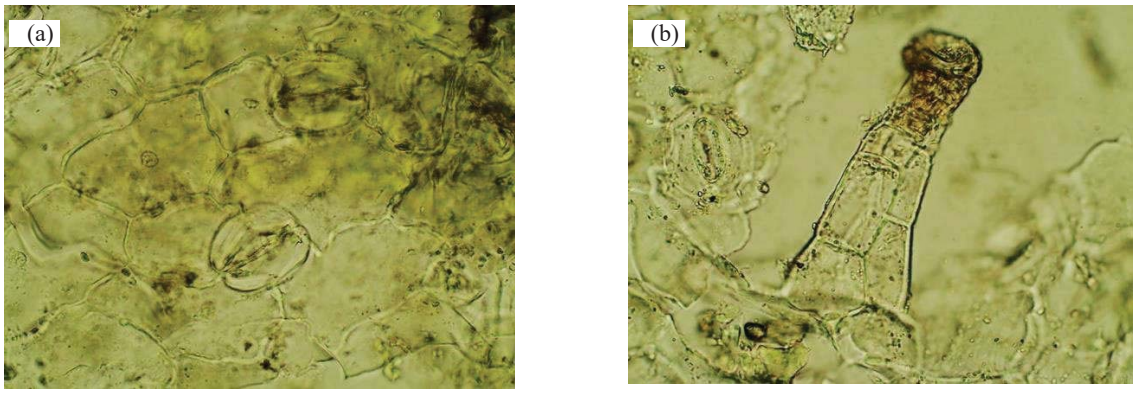

FIGURE 5. (a) Anomocytic stomata and (b) glandular multicellular multiseriate stalk trichomes (10x) of fresh marigold leaves.

CONCLUSIONS

Twenty seven different compounds were identified in different *C. officinalis* extracts as characterized by GC-MS. Phytochemical study revealed that the methanolic crude extract has saponins, tannins, and flavonoids, while *n*-hexanic extract contains terpenoids. Regarding microscopical analysis, dry leaves showed the presence of pitting vessels, starch, stone cell, and fiber, while anomocytic stomata and glandular multicellular multicerate stalk trichomes were presented in fresh leaves microscopical analysis. All these findings could be due to the Iraqi environmental factors that may affect the plant growth and its active compounds. Therefore, the Iraqi cultivated Marigold needs further separation and purification for its pure components and study their biological applications.

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