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Chemical composition, antibacterial activity of essential oil and anatomical study of *Chrysanthemum morifolium*

Boukhebti H.[1], Demirtas I.[2], Omar L.[3], Chaker A.N.[1]

1 Laboratory of Natural Resource Valorization. Faculty of Nature Life Sciences. Ferhat Abbas University Setif-1. 19000, Setif. Algeria
2 Department of Chemistry. Faculty of Natural Sciences. Cankiri Karatekin University. 18200. Cankiri. Turkey
3 Department of Ecology and Vegetal Biology . Faculty of Nature and Life Sciences. Ferhat Abbas University Setif-1.,19000 Setif. Algeria

ABSTRACT

The aim of this study is to identify the chemical composition and to evaluate the antimicrobial activity of *Chrysanthemum morifolium*. The analysis and identification of essential oil which obtained by hydrodistilation method were realized by gaz chromatography and mass spectroscopy. The antibacterial activity was tested by using the agar diffusion test and the Gram positive and negative pathogenic bacteria: *Staphylococcus aureus* ATCC 25923. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Citrobacter freundii* ATCC 8090, *Kleibseilla pneumoniae* ATCC 700603 and *Shigella sonnei* were used to evaluate this activity. This analysis led to the identification of 26 compounds representing 88.40 % of the total essential oil mass. The major compound was Verbenone (17.33 %). Other components present in appreciable contents were: Chrysanthenone (9.71%). 4-epi-cubedol (07.25%) and δ-Cadinol (05.29 %). Essential oil of *Chrysanthemum morifolium* exhibited an antibacterial effect against pathogenic bacteria, like those observed against *Staphylococcus aureus* ATCC 25923 (35±1.2mm) and *Citrobacter freundii* ATCC 8090 (21±0.87mm), however *Pseudomonas aeruginosa* ATCC 27853 and *Kleibseilla pneumoniae* ATCC 700603 were resistant. The anatomical study showed the presence of several types of trichomes including the glands secreted for essential oils and protector trichomes.

Keywords: essential oil, antibacterial activity, *Chrysanthemum morifolium*, anatomical study, chemical composition

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***Address for Correspondence:**

Boukhebti H., Laboratory of Natural Resource Valorization. Faculty of Nature Life Sciences. Ferhat Abbas University Setif-1. 19000, Setif. Algeria

INTRODUCTION

The genus *Chrysanthemum* belongs to the Asteraceae family and consists of 300 species **[01].** The *Chrysanthemum* is one of the most popular traditional used species and one of the most popular cut flowers in the world **[02]**.

Chrysanthemum morifolium is well known not only as an ornamental plant, but also as an important medicinal plant and a major source of natural products (flavonoids, sesquiterpene lactones, essential oils, triterpene diols and triols) used as pharmaceutical ingredients because this plant possess antibacterial, antifungal, antiviral and antispirochetal as has been reported in many studies **[03]**, several studies show that the extracts of *C. morifolium* have antioxidant, cardiovascular protective, anti-inflammatory functions and potent neuroprotective activity and therefore, might be a potential candidate in neurodegenerative diseases such as Parkinson's disease. It therefore occupies a very important position in the world flower industry. The flowers of *C. morifolium* have been used in Vietnam and other Asian countries for the treatment of eye diseases, headaches, insomnia and hyperglycemia **[02]**. In Algeria the genus includes 20 species with 8 endemic **[04].** *Chrysanthemum* has been investigated for its biological activities and chemical compositions.

Fig. 01: *Chrysanthemum morifolium*

MATERIALS AND METHODS

Plant materiel

Aerial parts of *Chrysanthemum morifolium* were harvested during June 2015 from the North of wilaya of Sétif (north east Algeria), then plant parts were washed with tap water to eliminate soil and other surface contaminants, after the dryness at laboratory temperature and obscurity. The plant material was cut to small pieces with universal knife.

Extraction of the essential oil

The air-dried aerial parts of *C. morifolium* were subjected to hydrodistillation for 3 h with distilled water using a Clevenger-type apparatus **[05]**. The oil obtained was collected and dried over anhydrous sodium sulfate and stored in screw capped glass vials in a refrigerator at 4– 5°C prior to analysis. Yield based on dried weight of the samples was calculated.

GC and GC-MS analysis

GC analyses were carried out on a Perkin-Elmer Clarus 500 Series. in split mode. 50:1. Equipped with a flame ionization detector (FID) and a mass spectrometer-equipped BPX-5 a polar capillary column (30 m×0.25 mm. 0.25 m i.d.). The injection temperature was fixed and FID was executed at 250 ºC. The carrier gas was helium at a rate of 1.0 mL/min. The initial column oven temperature was

50 ºC and was raised to 220 ºC at a rate of 8 ºC/minute. In the mass spectrometer transfer line temperature was at 250 ºC, ionization energy was 70 eV. Analytical standards were used for the identification of components and Kovats retention indices (RIs) were determined for all the sample components using the Van den Dool and Kratz equation according to the retention times of homologous series of n-alkane **[06]**.

Antibacterial activity

Two Gram positive and four Gram negative bacterial species were used in present study:

- *Staphylococcus aureus* ATCC25923
- *Pseudomonas aeruginosa* ATCC27853
- *Escherichia coli* ATCC 25922
- *Citrobacter freundii* ATCC 8090
- *Kleibseilla pneumoniae* ATCC 700603
- *Shigella sonnei*

The antibacterial activity of oil samples were evaluated by disc diffusion assay **[07]**. The bacterial inoculums were prepared (OD: 0.08-01 at 625 nm). Muller-Hinton agar (MH agar) was poured in Petri dishes solidified and surface dried before inoculation. Sterile discs (6 mm Φ) were placed on inoculated agars, by test bacteria filled with 10 µl of mother solution and diluted essential oil (1:2. 1:5. and 1:10 v:v of DMSO). DMSO was used as negative control while Gentamicin (GM) was used as positive control. Petri dishes were incubated at 37°C during 18 to 24h aerobically; after incubation. inhibition zone diameters were measured and documented.

Preparation of sections for anatomical study

Young sections of the plant containing stems and leaves were selected to make cross sections by hand with sharp blade and then coloring them using double coloration method **[08]**. Light microscope was used to check up transverse sections.

RESULTS AND DISCUSSION

Chemical composition

The hydrodistillation of the essential oil of *C. morifolium* gave a viscous liquid with a greenish color. The yield of the sample essential oil is 0.09%. The essential oil tested in this study was analyzed using GC-MS to identify its major components. The retention time and chemical composition of essential oils of *C. morifolium* are presented in Table 01. The mass spectrum of *C. morifolium* L. essential oil is shown in Fig. 02.

Chemical analysis led to the identification of 26 compounds representing 88.40 % of the total essential oil mass. Most essential oil compounds were terpenic compounds (Fig. 03); the major compound was verbenone (2-Pinene-4-one) (17.33 %). other components present in appreciable contents were: Chrysanthenone (9.71%). 4-Epi-cubedol (07.25%) and δ-Cadinol (05.29%).

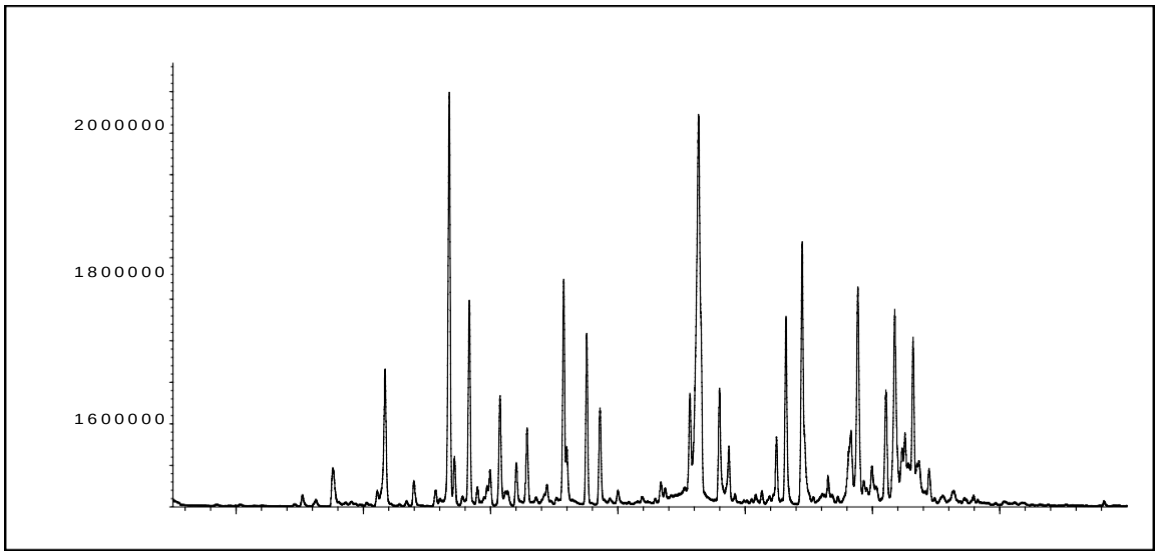

Fig. 02: Mass spectrum of *C. morifolium* L. essential oil

Table 01: Chemical composition of the essential oil extracted from *C. morifolium*

	RT (min)	IR	%	Compounds
1	13.808	974	1.26	Morillo
2	15.854	1035	3.78	Eucalyptol
3	16.989	1068	0.57	Safranal
4	18.376	1106	**9. 71**	Chrysanthenone
5	18.577	1113	1.06	1.3-Cyclopentadiene. 5.5-dimethyl-2-ethyl-
6	19.977	1154	1.19	Camphor
7	20.371	1165	2.28	Verbenol (2-pine-4-ol)
8	21.014	1183	0.95	4-terpineol
9	21.432	1194	1.59	Cyclohexene. 3-(3-methyl-1-butenyl)
10	22.872	1237	5.8	Bicyclo[3.1.1]hept-2-en-4-ol. 2.6.6-trimethyl-. acetate
11	24.297	1279	2.24	p-Mentha-1.8-dien-3-one (limonene)
12	27.833	1386	3.3	alfa-Copaene
13	28.171	1396	**17.33**	verbenone (2-Pinene-4-one)
14	29.361	1435	1.38	Caryophyllene
15	31.23	1494	1.6	β-Cubebene
16	31.603	1507	4.27	Cubedol
17	32.239	1529	7.25	4-Epi-cubedol
18	34.153	1594	2.86	Nerolidol
19	34.425	1603	5.4	Caryophylleneoxide
20	34.98	1623	1.07	Globulol
21	35.53	1643	2.61	β-Guaiene
22	35.878	1656	5.29	δ-Cadinol
23	36.168	1666	0.85	9-Isopropyl-1-methyl-2-methylene-5oxatricyclo[5.4.0.0(3.8)]undecane
24	36.282	1670	1.43	α-Cadinol
25	36.597	1681	2.7	Junipercamphor
26	37.225	1703	0.63	Murolan-3.9(11)-diene-10-peroxy
		Total	**88.4**	

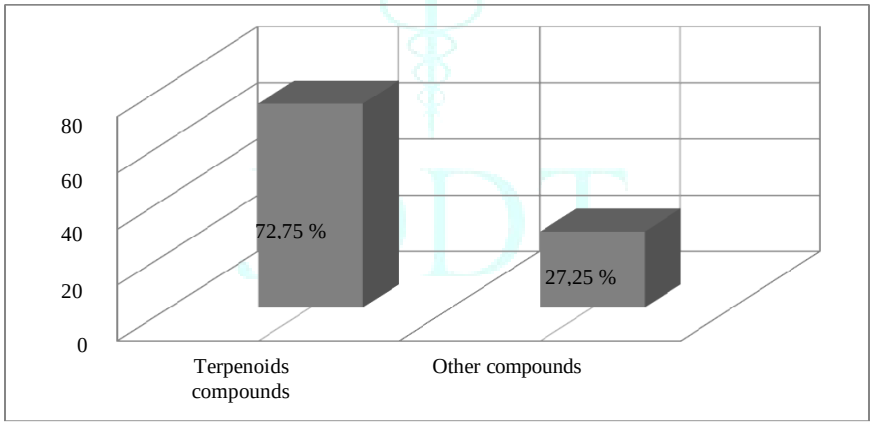

Fig. 03: the major chemical compounds of the *C. morifolium* essential oil

The results obtained in the study of Chang and Kim (2013) [09] and those obtained in the present study are similar where the yield of their sample essential oil is 0.1%; whereas the yields of essential oil were 0.1% for *C. fontanesii* and 0.07% for *C. coronarium*[10], however the yield of essential oil of *C. trifurcatum* was 0.055%[11].

The results of present study are different from those reported in previous studies. The chemical constituents of the essential oil of *Chrysanthemum morifolium* Ramat from Nigeria are different, for example the compounds Verbenone. 4-epi-cubedol and δ-Cadinol were absent while the main constituents were *cis* chrysantheny acetate (21.6%), octadecanoic acid (19.5%) and borneol (15.5%)**[02]**; also results of **Lograda et *al.* (2013) [10]** indicate different major components in essential oil of *C. fontanesii* and *C. coronarium* from two localities in eastern Algeria (of the region Aouakas). These compounds are triene (22.3%) and 1.1-Difluror-tetramethylcyclopropane (11.52%) respectively; while the main components in essential oil of *C. indicum* flowers from China were 2.6.6-trimethyl- bicyclo[3.1.1]hept-2-en-4-ol (21.67%) and 2-(2.4-hexa diynylidene)-1.6dioxaspiro[4.4]non-3-ene (21.41%) **[12]**; another study **[09]** reported that major components in *C. morifolium* essential oils from Geonggi (Korea) were Chrysanthenyl acetate (43.74%) and verbenol (27.85%); whereas in another study **[13]** the components are Germacrene D (10.6%). 1.8-Cineole (10.4%) and Camphor (10.12%) were the major constituents in *C. indicum* essential oil from the area of Mt. Mireuk in

Korea. Previous studies confirmed that the major constituents for essential oil of *Chrysanthemum* species are monoterpenoids **[01]**.

The variability of the composition of essential oils of *Chrysanthemum* species has been reported in several other studies, by comparing present results with it for example we observed that the components α-Curcumene **[14],** Hulene-β followed by Ledene oxide-(I) **[15];** *C indicum* :trans- Sabinol and *C. boreale:* β-Thujone**[16],** *C. arcticum*: Chrysanthenone (13.98%), *C. parthenium*: Camphor 38.51% **[17],** trans-Verbenyl acetate **[03]** were the dominant compounds.

The reason for these variations in essential oils composition may be attributed to factors related to ecotype, the environment including temperature, relative humidity, irradiance, photoperiod, the period of collection and the part of plant used **[18] [19].**

Antibacterial activity

Antibacterial activity of the essential oil was tested by the disc diffusion assay. Table 02 shows diameter of inhibition of the different concentrations of the essential oil in DMSO (v/v) against either Gram positive and negative pathogenic bacteria.

The essential oil of *C. morifolium* exhibits significant inhibitory effects toward most tested bacteria (Fig. 04); nevertheless. *Pseudomonas aeruginosa* ATCC 27853 and *Kleibseilla pneumoniae* ATCC 700603 were resistant to the essential oil. In general tested Gram negative bacteria appear more resistant than Gram positive ones. *Staphylococcus aureus* ATCC 25923 and *Citrobacter freundi* ATCC8090 were more susceptible to essential oil. The activity of *C. morifolium* essential oil against *S.aureus* ATCC 25923 strain is significant and expressed in varying diameters of concentration 35±1.2mm in 100% concentration and 14±0.98mm in 50% dilution, while the inhibition zone of Gentamicin (GM) was 26±0.23mm; the activity of this essential oil against *C. freundii* ATCC 8090 strain is significant and expressed in varying diameters of concentration, 21±0.87mm in 100% concentration and 12±1.03 mm in 50% dilution, however the inhibition zone of Gentamicin (GM) was 20±0.12 mm. Essential oil tested present moderate activity against *Escherichia coli* ATCC 25922 and *Shigella sonnei*. this activity expressed by inhibition diameters of 14±0.88mm and 13±1.99mm respectively in 100% dilution and 11±1.01mm and 09±0.17mm respectively in 50% dilution; whereas the inhibition zones of Gentamicin (GM) were 23±0.25mm and 25±0.09mm respectively.

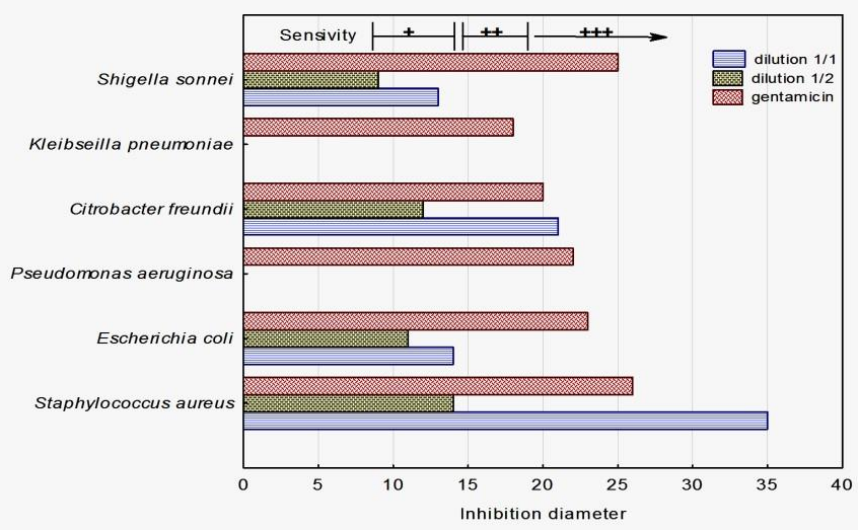

Fig 04. Antibacterial activity of *C. morifolium* essential oil

Table 02: Antibacterial activity of *C. morifolium* measured as diameter of inhibition (mm)

Bacteria strains	Concentration		Gentamicin
	100%	50%	
Staphylococcus aureus ATCC 25923	35±1.2	14±0.98	26±0.23
Escherichia coli ATCC 25922	14±0.88	11±1.01	23±0.25
Pseudomonas aeruginosa ATCC27853	00	00	22±0.66
Citrobacter freundii ATCC8090	21±0.87	12±1.03	20±0.12
Kleibseilla pneumoniae ATCC 700603	00	00	18±0.33
Shigella sonnei	13±1.99	09±0.17	25±0.09

It's noticed that Gram positive bacteria were more susceptible to essential oils than Gram negative bacteria; these results are similar to those in previews studies. This phenomenon was ascribed to possession of these bacteria to a hydrophilic polysaccharide chains as a barrier to hydrophobic essential oils **[20] [21]**. In previous studies the different extracts of *Chrysanthemum* species especially essential oils showed a significant activity against microbial activity and exhibited significant activity against both Gram positive and Gram negative bacteria **[01]**.

The *Chrysanthemum parthenium* essential oil from Iran showed inhibitory effects on *Escherichia coli* and *Salmonella typhi*, but were not active against *Staphylococcus aureus* **[22]**, while the essential oils of *C.coronarium* from Italy have no activity against *Escherichia coli*. *Staphylococcus aureus* and *Pseudomonas aeruginosa* **[23],** however the activity of *C. coronarium*

essential oils from Houfa (Irbid governorate. Jordan) against five bacterial strains was moderate with gram-positive strains and weak with gram-negative strains **[24].**

In other study, the antibacterial activities of essential oil of *C. coronarium* and *C. fontanesii* showed that the oils of both species have a very low activity**) [10]**, in contrast the essential oil of

C. viscidehirtum exhibited significant activity against *Salmonella typhi* and *Proteus mirabilis*, also the essential oil of *C. boreale* exhibited the activity against six Gram negative bacteria and eight Gram positive bacterial strains **[01].** *Chrysanthemum* species have pharmacological effects such as antiviral, antihypertensive, anti-bacterial and anti-inflammatory effects **[25].**

Anatomical study

The anatomical study that is carried out on young fresh stem of *C. morifolium* showed that Pith, xylem, phloem, cortex and epidermis are the most important tissue found (**Fig 05. a. b. c**) while the epidermis, mesophyll and conductive vessel are the constituent tissues of the leaf (**Fig 06. a. b. c**).

Observations by light microscope showed the epidermis layer of stems and leaves contained two types of hairs glandular and covering trichomes (**Fig 05. d. e. f. g. h**) and (**Fig 06 d. e. f. g.**). The protector trihomes are pluricellular (3-4 cells) having the form of the letter T, while glandular trichomes consists of 03 cells. a base cell, cervical cell and the glandular cell in the stems (**Fig 05 d.e. f. h**) and without cervical cell in the leaves (**Fig 06 a. b. c. d. e**).

The protector and glandular trichomes were spread on the lower side of the leaves more than the upper side (Fig. 5d. f. g).

The results of present study are similar (protector trihomes pluricellular having the form of the letter T) with those obtained by **[26] [27]** and **[28]**. Several studies confirm that climatic and environmental conditions play an important role in the growth and shape of plants.

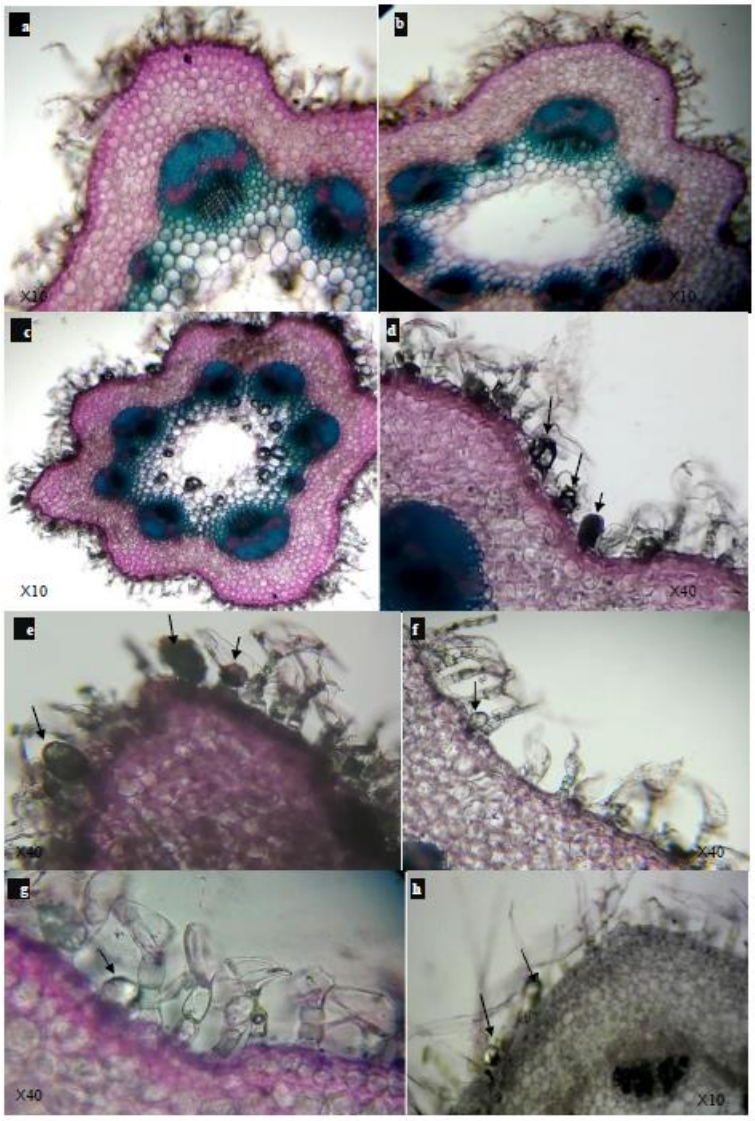

Fig 05: Cross section of *C. morifolium* (stem)

(a.b.c) Cross section in stem showing différent tissue. (d.e.f.g.h) Cross section in stem showing covering and glandular trichomes

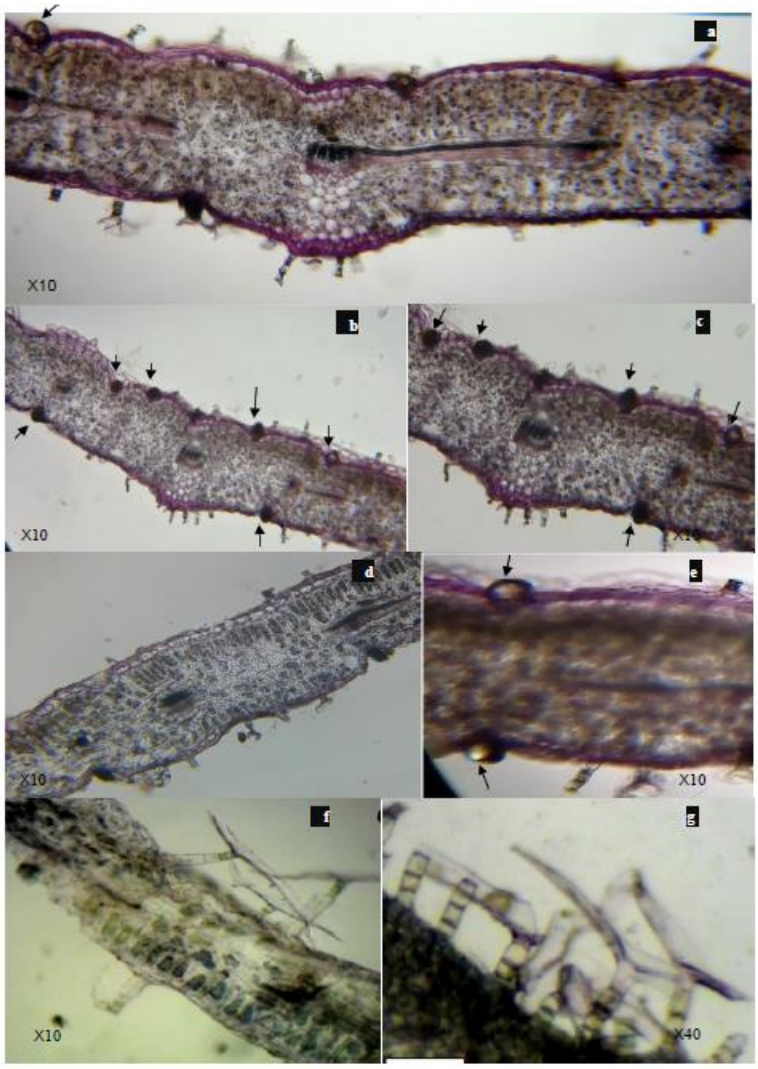

Fig 06: Cross section of *C. morifolium* (leaf)

(a) Cross section in leaf showing différent tissue, (b. c. d) Cross section in leaf showing larger vascular bundle, (e) glandular trichomes, (f. g) protector trichomes

CONCLUSION

The chemical composition of the essential oil of *Chrysanthemum morifolium* aerial parts is characterized by the presence of verbenone (2-Pinene-4-one, followed by Chrysanthenone, 4-Epi- cubedol and δ-Cadinol as dominant components. this chemical composition is differs comparatively from the oil composition given in previous studies.

Essential oil of *C. morifolium* present significant antibacterial activity, also it is noted that the effect of this essential oil on positive bacteria is greater than negative bacteria. The antibacterial activity of *C. morifolium* essential oil on positive bacteria strains was exceeded those obtained with antibiotic.

The anatomical study which was performed on young fresh stems showed the presence of two types of trichomes: protector trichomes (pluricellular (3-4 cells) having the form of the letter T) and secretor trichomes (small and simple). *C. morifolium* is rich in secretor glands and therefore rich in essential oils; these trichomes has an important role to identified this vegetal species

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