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

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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 hydrodistillation method were realized by gas 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, Klebsiella 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 (8.25%) and 8-Cadinol (8.29%). 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 Klebsiella 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

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 antispicrotechal 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 material

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 (RI) 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
- *Klebsiella 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 24 h 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%).

![Mass spectrum of C. morifolium L essential oil](https://example.com/mass-spectrum.png)
The results obtained in the study of Chang and Kim (2013) [9] 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 chrysanthenyl acetate (21.6%), octadecanoic acid (19.5%) and borneol (15.5%) [2]; 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 Aoualas). 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)-1,6-dioxaspiro[4.4]non-3-ene (21.41%) [12]; another study [9] reported that major components in C. morifolium essential oils from Geonggi (Korea) were Chrysanthanlyl 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
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 Lenede oxide-(I) [15]; C. indicum: trans-Sabinol and C. boreale: β-Thujone[16], C. arcticum: Chrysanthene (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.

![Antibacterial activity](image)

**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 100% | Concentration 50% | Gentamicin |
|------------------------------------------|--------------------|-------------------|------------|
| 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    |
| Klebsiella 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 another 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.
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 chrysantheneone, 4-Epi-cubedol and δ-Cadinol as dominant components. This chemical composition 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 have an important role to identify this vegetal species.

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