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Research Article

Essential oils and morphological study of *Mentha aquatica*

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ABSTRACT: As part of the development of plant resources, we are interested in the chemical study of essential oils of *Mentha aquatica* that is widely used in Algerian traditional medicine. Essential oil extracted from this plant was obtained by hydrodistillation with a Clevenger type apparatus for a period of three hours. The extraction produced yellowish essential oils with a very strong odor. Chemical analysis of the essential oils was achieved by gas chromatography coupled with mass spectrometry (GC/MS). The major compound present in the essential oil of *M. aquatica* was found to be linalyl acetate (26.1 %), other constituents included α-Pinene (22.7 %), Linalol (13.8 %). The morphological study of *M. aquatica* shows two different types of glandular trichomes on both leaves and stems but lacunar parenchyma is only present in stem section.

KEYWORDS: Essential oils, Chemical analysis, *M. aquatica,* (water mint), Morphological study.

INTRODUCTION

Most of mentha species are perennial herbs growing wildly in damp and wet places throughout temperate regions of Eurasia, North Africa, Australia and South Africa.[1] The plants belong to Mentha genus is considered as an industrial group as they are a source of essential oils. They are rich in certain monoterpenes, which are widely used in food as flavour, cosmetic and pharmaceutical industries. Several mentha species are used across the globe for their medicinal and culinary properties. They usually taken after a meal for their ability to reduce indigestion and colonic spasms.[2] In addition to countering colds, coughs, constipation, stomach-ache or inflammation of the appendix, stomach trouble and heart complaints'.[3]

Mentha aquatica (Water Mint) is a perennial plant of genus Mentha. It is herbaceous rhizomatous plant growing to 90 cm tall. The stems are square in cross sections, green or purple, and variably hairy to almost hairless. The rhizomes are widely spread fleshy and bear fibrous roots. The leaves are ovate to ovate-lanceolate, 2–6 cm long and 1–4 cm wide, green (sometimes purplish), opposite, toothed and vary from hairy to nearly hairless. Flowers are tiny, densely crowded, purple, tubular, and pinkish to lilac in color. Flowering period starts in mid to late summer. The plant is pollinated by insects and also spreads by underground rhizomes, as with other species of mint. All parts of the plant have a distinctly minty smell.[4] The aim of this study is to identify the chemical composition of essential oil and the anatomical study (glands trichomes) of *M. aquatic*a that is widely used in Algerian traditional medicine, *M. aquatic*a is used as tea to treat cough, digestive diseases and cold.

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MATERIALS AND METHODS

Plant material

The aerial parts of *Mentha aquatica* at flowering stage were collected from north east of Algeria. These were washed with tap water, to eliminate soil and other surface contaminants and then air-dried at shade in ambient temperature. The plant material was subsequently cut into small pieces for extraction.

Extraction of the essential oil

The aerial parts of the plant were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil

obtained was collected and dried over anhydrous sodium sulfate and stored at 4°C in sealed brown vials until analysis. The oil analysis was carried out using GC-FID and GC/MS.

Gas chromatography (GC-FID) analysis

The gas chromatography analysis of the two volatile oils was performed using a HP 6890 equipped with: Flame ionization detectors (FID), HP Innowax (DB5; polyethylenglycol) 30 m 9 x 0.25 mm ID, 0.25 µm film thickness fused capillary column. The carrier gas was Hydrogen: (1ml min -1). The oven temperature program was 5 min isothermal at 50 °C, then 50–300 °C (BP- 20) at rate of 5 °C/min and held isothermally (300 °C) for 5 min. The injection port temperature was 280 °C, detector 300 °C. Volume injected: 1 µl of 1: 60 (v: v in hexane) solution. Percentages of the constituents were calculated by electronic integration of FID peak areas.

Gas chromatography-mass spectrometry (GC/MS) analysis

The analyses of the volatile constituents were run on a Hewlett-Packard GC-MS system (GC: 7890; MSD-HP 5975-C.). The fused-silica DB5 capillary column (30 m 9 x 0.25 mm ID, film thickness of 0.25 µm) was directly coupled to the MS. The carrier gas was helium, with a flow rate of 1.0 ml min-1. Oven temperature was programmed (50 °C for 5 min, then 50-300 °C at 5 °C/min) and subsequently, held isothermally (300 °C) for 5 min. Injector port: 250 °C, detector: 280 °C, split ratio 1:100. Volume injected: 1 µl of 1 % solution (diluted in hexane): HP 5975 recording at 70 eV; scan time 1.5 s; mass range 40–300 amu. The identification of the oil components was based on comparison of their mass spectra with those of NIST mass spectral library,[5,6] and those described by,[7] as well as on comparison of their retention indices either with those of authentic compounds or with literature values.[7].

Preparation of Sections for Anatomical Study

The anatomical study was realized on young fresh parts of stems and leaves and sections were taken manually using sambucus wood, sharp riser blade and double coloration.[8]

RESULTS AND DISCUSSION

Chemical composition

The analysis of the essential oils of *M. aquatica* by GC and GC/MS identified 43 components corresponding to 97.8 % of the total oil (Table 1), the gas chromatogram of the oil on a HP-5 MS capillary column is shown in (Figure 1). The major compound in essential oil of *M. aquatica* is linalyl acetate (26.1 %), followed by α-pinene (22.7 %), linalol (13.755%), α-terpeneol (3.42%) and geranyl butyrate (3.39%). In the

Table 1: chemical composition for *Mentha aquatica* essential oil.

Pic	RT	%	Compounds
1.	9.981	0.1376	α-Thujene
2.	10.263	22.7087	α- Pinene
3.	10.775	0.1151	Fenchene
4.	11.671	0.0843	Camphene
5.	11.730	1.0965	Sabinene
6.	11.830	0.6362	β- Pinene
7.	12.277	2.2353	Myrcene
8.	12.919	0.4105	Delta-3-Carene
9.	13.219	0.1115	α-Terpinene
10.	13.492	0.1093	Para-cymene
11.	13.641	0.6738	Limonene
12.	13.758	2.8512	Eucalyptol
13.	13.884	1.5932	Z- β- Ocimene
14.	14.234	1.2305	E- β- Ocimene
15.	14.620	0.6246	Gamma Terpinene
16.	15.504	1.5917	Terpinolene
17.	16.025	13.7556	Linalol
18.	16.092	0.4534	α-Terpinolene
19.	16.958	0.1886	1-Terpineol
20.	18.488	0.2961	Terpinene-4-ol
21.	18.928	3.4295	α-Terpeneol
22.	19.744	0.5596	Trans-geraniol
23.	20.166	1.8357	Pulegone
24.	20.473	26.1093	Acetate de linalyle
25.	21.366	0.9707	Acetate de lavandulyle
26.	23.354	1.6343	Geranyl octanoate
27.	23.862	3.3909	Geranyl butyrate
28.	24.056	0.6262	Mentha-1,4,8-triene
29.	24.190	0.1702	Elemene<BETA->
30.	24.973	1.2637	β- Caryophyllene
31.	25.705	0.2549	Trans-b -Farnesene
32.	25.846	0.1328	α- Humulene
33.	26.455	0.984	Germacrene-D
34.	27.295	0.1776	β- Cadinene
35.	28.004	3.0035	Elemol
36.	28.664	0.1191	Gamma-cadinene
37.	29.084	0.3682	Epi-globulolemophilene
38.	29.891	0.2237	α-Selinene
39.	30.412	0.6995	Eudesmol
40.	38.289	0.1549	Abietatriene
41.	40.598	0.2597	Sandra copimarinal
42.	41.068	0.3461	Zierene
43.	42.867	0.1469	Ferruginol
	Total	97.7647	

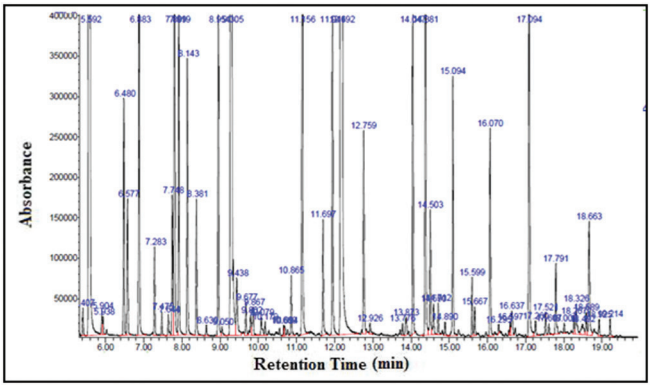

Figure 1: Chromatogram of *M. aquatica* essential oil obtained by GC

stem essential oil of *M. aquatica* from Iran, β-caryophyllene (22.4%), viridiflorol (11.3%) and 1, 8-cineole (10.9%) were recognized as the main component whereas the major compounds of the leaf oil of the this plant were piperitenone oxide (25.7%), β-caryophyllene (12.0%) and 1.8-cineole (10.3%).[9] The composition of essential oil of in the present study is different from those reported in recent studies.[2, 10]

The identical Botanical plant in fact can give more or less important different extracts, biochemical specificity and proprieties of some plants are varied in different regions.[11] In present study, results are compared with other previous studies and examined the similarities and the differences of this plant in different regions and with other species that belong to the same genus.

Znini M. et al, 2011[12] found in their study that in *M. spicata* essential oils from Morocco carvone (29 %) and trans-carveol (14 %) were the main components. Another study by Derwich et al, 2012[13] showed that piperitone (35.6 %) and menthol (40.5 %) were the major constituents of *Mentha pulegium* and *Mentha rotundifolia* essential oils respectively. Gulluce et al.[1] reported *cis*-piperitone epoxide (18.4 %), followed by pulegone (15.5 %) and piperitenone oxide (14.7 %) as the main compounds of essential oil from *M. longifolia*, growing in Turkey. A further study[14] showed that piperitone (38 %) and piperitenone (33 %) were the main compounds of *Mentha pulegium* essential oil; in addition, results published by Vian et al.[15] present that pulegone (83.70 %) was the main compound of *M. pulegium* essential oil.

Similar result was found by Zaks et al.[16] whereas the main compounds of *M. aquatica* var, *citrata* volatiles are the monoterpenes (alcohol linalool and its ester linalyl), as well as the results of Gulluce et al.[1] The essential oils of mint genus mainly consisted of oxygenated monoterpenes such as carvone, menthone were the principal oxygenated monoterpenes in the essential oils of *M. spicata, M pulegium.*

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,[17] the period of collection and the part of plant used.[11] Tuker et al., 2007[18] said that the genetics of the essential oil pathways and the enzymes involved are well known in mentha. Typically, the predominant essential oil pattern consists of acyclic monoterpenes like linalool, 2-oxygenated monoterpenes like carvone, and 3-oxygentated monoterpenes for example menthol.

Morphology of the Plant

Morphological variations were observed among the plants growing in the natural habitat. Plant height varies from 45 to 80 cm and grows in moist damp streams. The plant is a perennial and aromatic with squared stems and green opposite short-stalked leaves. Flowering period is between August and October.

Anatomical Study

The anatomical study which was performed on young fresh stems showed the presence of pith, vascular stacks, cortex and epidermis whereas collenchyma tissue (supporting tissue) is existed in the cortex in four corners of the stem (Figure 3); The cortex is characterized by lacunous parenchyma tissue. However, in leaves, vascular bundles are surrounded by lacunous parenchyma tissue (Figure 6). Observations by light microscope show the epidermis layer have stoma joined with subsidiary cells to form stomatal complex. Both stems and leaves of this plant contain glandular and covering trichomes (Figures 3–7**).**

Two types of trichomes at different developmental stages: simple hairs (covering trichomes) and secretive glands with short stalks are present **(**Figure 7,8**)**. The first is unicellular and very small with globule head (capitate, glandular trichomes); the second is big and multi-cellular (peltate glandular trichomes). Peltate glandular trichomes

Figure 2: M. *aquatic* general view.

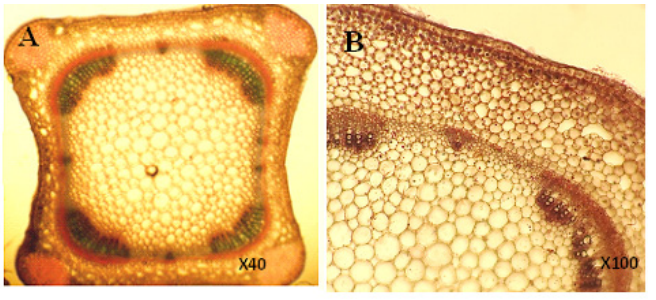

Figure 3: Cross sections of stem of *M. aquatica* (A) general view, (B) lacunous parenchyma tissue in cortex.

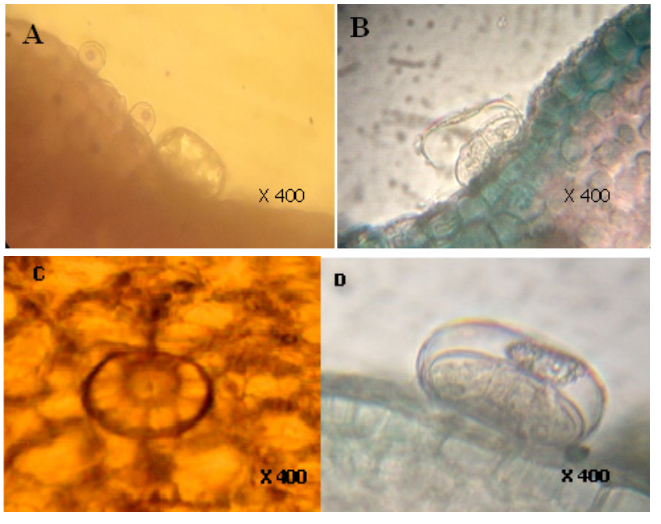

Figure 4: Cross sections of stem of *M. aquatica* (A), capitates and peltate glandular trichomes, (B), (C) and (D) peltate glandular trichomes.

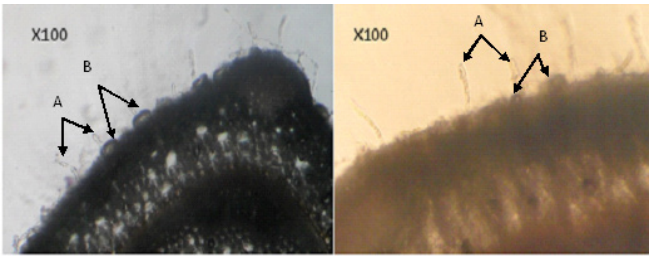

Figure 5: Cross sections of *M. aquatica* stems (glandular and covering trichomes), A: covering trichomes, B: glandular trichomes.

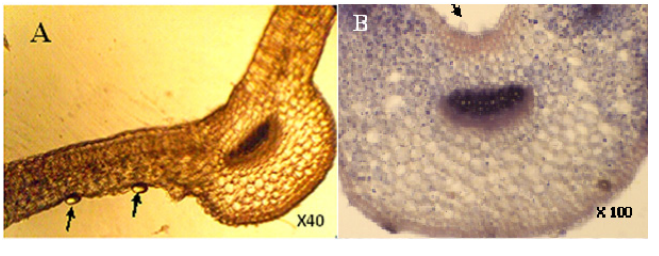

Figure 6: Cross sections of *M. aquatica* leaves, (A) peltate glandular trichomes, (B) capitates glandular trichomes.

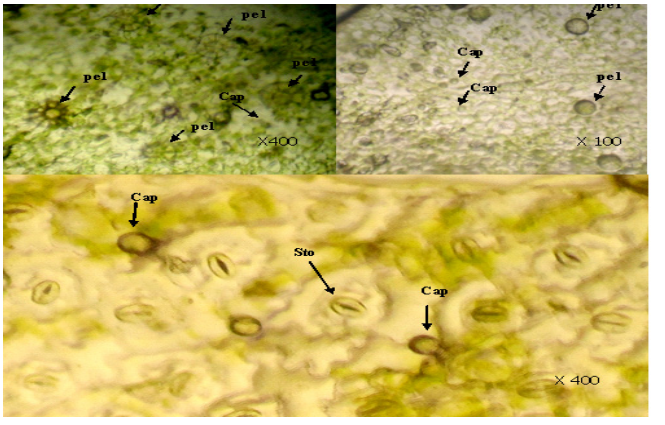

Figure 7: glandular trichomes on leaves of *M. aquatica* (pel) peltate glandular trichome, (cap) capitate glandular trichome, (Sto) stomata.

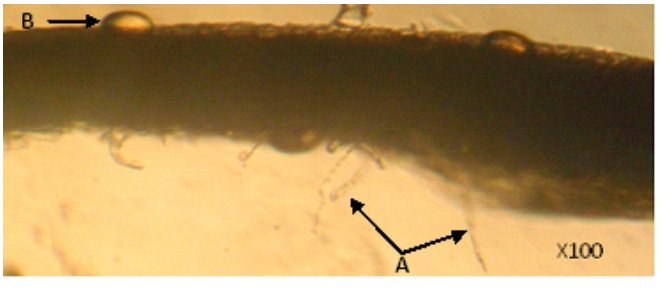

Figure 8: glandular and covering trichomes on young leaves of *M. aquatic* A: covering trichomes, B: glandular trichomes.

are bigger than capitate trichomes. Covering trichomes are simple and multi-cellular. Simple hairs are composed of elongated cells with a large base cell**.**

At mature stage, the peltate glandular trichome is composed of a disc of eleven cells surmounted by a large sub cuticular storage space **(**Figure 3B,D**).** peltates glandular trichomes were more abundant on abaxial surface of the leaf while capitates glandular trichomes were more dense on adaxial side in young and mature leaves.

In aged leaves and stems, the density of the three types of glandular trichomes were lower compared to young shots and varies with the side of the leaves.

Hatamneia et al.[19] reported that stomata on both epidermises of intercostal regions and leaves are amphistomatic (adaxial and abaxial surface), except for *Ballota nigra* subsp *curdica, Mentha aquatica* and *Mentha longifolia,* however, they are absent at the veins of the leaves. In stem, collenchyma tissue is located under the epidermis.

In another study,[20] leaves and stems of M. pulegium were shown to contain simple covering hairs of three cells and two types of glandular trichomes. The first type of glandular

trichomes is capitate with two cells (stalk and head) and the second type is peltate (gland oval of ten cells covered with large cuticle). In addition, the number of glandular cells diver from one species to another according to stages of growth.

Different results were found by Zaks *et al* [16] in which they mentioned that Lemon mint (*M. aquatica* var. *citrata*) contains peltate glandular trichomes that is formed by a stalk cell and eight secretory cells encompassed by an elevated cuticle.

The medicinal plant *(Mentha aquatica)* which was chosen in the present study is one of the most renowned medicinal plants in Algeria. It is known by the ease of hybridization (Mentha genus) and by a high polymorphism in morphology. Its composition of essential oil is diverse according to different regions of the world. Therefore research is carried out in all regions at all times.

CONCLUSIONS

The composition of essential oil of *M. aquatica* is diverse according to different regions of the world. However, our analysis of this plant essential oil by GC and GC/MS identified 43 components and the main compounds are the monoterpenes. The anatomical studies carried out on young fresh stems and leaves of *mentha aquatica* show the presence of two types of trichomes at different developmental stages. The first is the simple hairs (covering trichomes) and the second is the secretive glands. Under light microscope these secretive glands were: big glandular peltate trichomes "multicellular oval head" and small glandular capitate «unicellular globule head". It would be more beneficial if research is carried out in different region and comparisons are made to gain better understanding of this plant.

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