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CHEMICAL AND BIOLOGICAL STUDY OF *MENTHA SPICATA* L. ESSENTIAL OIL FROM IRAN

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ABSTRACT

The essential oil from spearmint (*Mentha spicata* L., Labiatae) was extracted by hydrodistillation, and analysed by GC and GC/MS. Twenty eight components were identified representing 90.14% of total oil composition. The major constituents were carvone (22.40%), linalool (11.25%) and limonene (10.80%). The essential oil showed good activity against larvae of *Anophel stephensi* and *Artemia salina* (LD_{50}= 9±5 µg /ml and 9.2 µg/ml respectively).

Key words: *Mentha spicata*, Labiatae, Essential oil, Carvone, Linalool, Limonene, *Anophel stephensi*, *Artemia salina*

INTRODUCTION

The family of Labiatae, which are rich in essential oil have commercial and medical values are widespread throughout the world as well as Iran (1-5). Several studies have been reported on the phytochemistry of the *Mentha* species in literatures (6-7). Spearmint has normally about 0.7% volatile oil consisting 50 to 70% carvone with lesser amounts of dihydrocarvone ,phellandrone and limonene (8). In a study *Mentha spicata* collected from Qom (Iran), 5 components consisting 96.02% of total oil were identified. Among them, Carvone (87.6%) was the major constituent (9). Spearmint oil obtained from *Mentha spicata* is an important raw material that has been used as carminative, antispasmodic, diuretic, and as a flavouring agent for confectionary, chewing gums, cosmetics, toothpastes and many other products (10). The quality of spearmint that is determined by its chemical composition and its flavour is very important. The objective of this study was to characterise the volatile components of cultivated *Mentha spicata* L. and to determine its biological activity against fourth instar of *Anopheles stephensi* larvae an important factor for malaria transmission in Iran.

MATERIALS AND METHODS

Plant materials: The aerial parts of fully flowered *M. spicata* cultivated in Karadj (Iran) which were collected during summer (August 1998) identified by a local taxonomist and confirmed by Dr. Mozaffarian (Research Institute of Forests and Rangelands). Voucher specimens were deposited at the Department of Pharmacognosy, Tehran University of Medical Sciences, Tehran, Iran.

Isolation of the essential oil: The collected material was air-dried at room temperature and then was grossly pulverized and submitted to hydrodistillation for 3 h using a clevenger type apparatus. The oil was decanted, dried over anhydrous sodium sulphate, to yield 1.1% v/w on the basis of the dried plant material.

GC Analysis: The analysis was carried out by gas chromatography (Shimadzu 9A, Data processor, ChromatoPac c-R3A, Column: DB-1, 60 m, 0.25 mm, I.D., 0.25 micron film thickness) with helium as the carrier gas.

GC/MS Analysis: GC/MS analysis was carried out in Finnigan-Mat, Model Incos-50. The mass spectra corresponding to GC peaks were scanned at 70 eV. The GC and GC/MS column temperature were

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Table 1: Chemical composition of the essential oil of *Mentha spicata* L.

Compound	Relative Retention Indices	Percent
α-Pinene	935	0.46
Camphene	950	0.05
β-Pinene	947	0.88
Octanol	979	0.96
Limonene	1025	10.8
Linalool	1087	11.25
α-Thujone	1091	0.73
Menthofuran	1154	1.64
trans-Dihydrocarvone	1178	4.04
cis-Dihydrocarvone	1182	7.08
Carvone	1218	22.4
Piperitone	1232	1.83
Isopulegyl acetate	1259	8.43
Mentha-1,3-dien-7-al	1261	0.34
Menthyl acetate	1280	0.42
Carvacrol	1284	0.64
trans-Carvyl acetae	1319	2.71
cis-Carvyl acetae	1349	0.94
Piperitone epoxide	1360	1.58
Bourbonene	1386	1.24
β-Caryophylene	1427	5.84
α-Humulene	1453	0.78
Germacrene-D	1480	0.87
Calamenene	1498	0.48
Caryophylene epoxide	1537	2.16
Globulol	1582	0.44
Tau-Cadinol	1630	0.67
α-Cadinol	1642	0.47
Total identified	------	90.14

ranked from 50°C to 280°C at 4°C/min. Injection and ion source temperature were 280°C and 270°C respectively. The oil components were identified by comparing their retention indices and mass spectra data with those of authentic samples and published data (11).

Biological Study: The biological experiment was conducted according to WHO recommendation (12). Different concentrations of spearmint oil in dimethylsulfoxide were prepared (5, 10, 15, 20, 30 µg/ml) and 25 batches of *Anapheles* larvae were exposed to these concentrations at different replicates. The efficacy of *M. spicata* essential oil against larvae of *Artemia salina* was determined at four concentrations (2, 5, 7, 10 µg/ml). Mortality was scored after 24 hr. of exposure time. From the regression line plotted by computer, the LC_{50} was determined (13).

RESULTS AND DISCUSSION

As shown in Table 1, twenty-eight components consisting 90.14% of the total oil including the monoterpene hydrocarbons (76.22%), sesquiterpene hydrocarbons (12.95%) and oxygenated terpenoids (63.07%) were identified.

The major components were carvone (22.4%), linalool (11.25%), limonene (10.8%), *cis* and *trans* dihydrocarvone (7.08 and 4.04% respectively). The essential oil of *M. spicata* was rich in carvone derivatives (*cis* and *trans* dihydrocarvone and *cis* and *trans* carvyl acetate) which constitute 37.17% of the total oil.This oxygynated derivative of the essential oil was a major constituent of *M. spicata* (14-15). LC_{50} for *M. spicata* essential oil against *Anopheles* and shrimp larvaes were found 9±5 and 9.2 µg/ml respectively.

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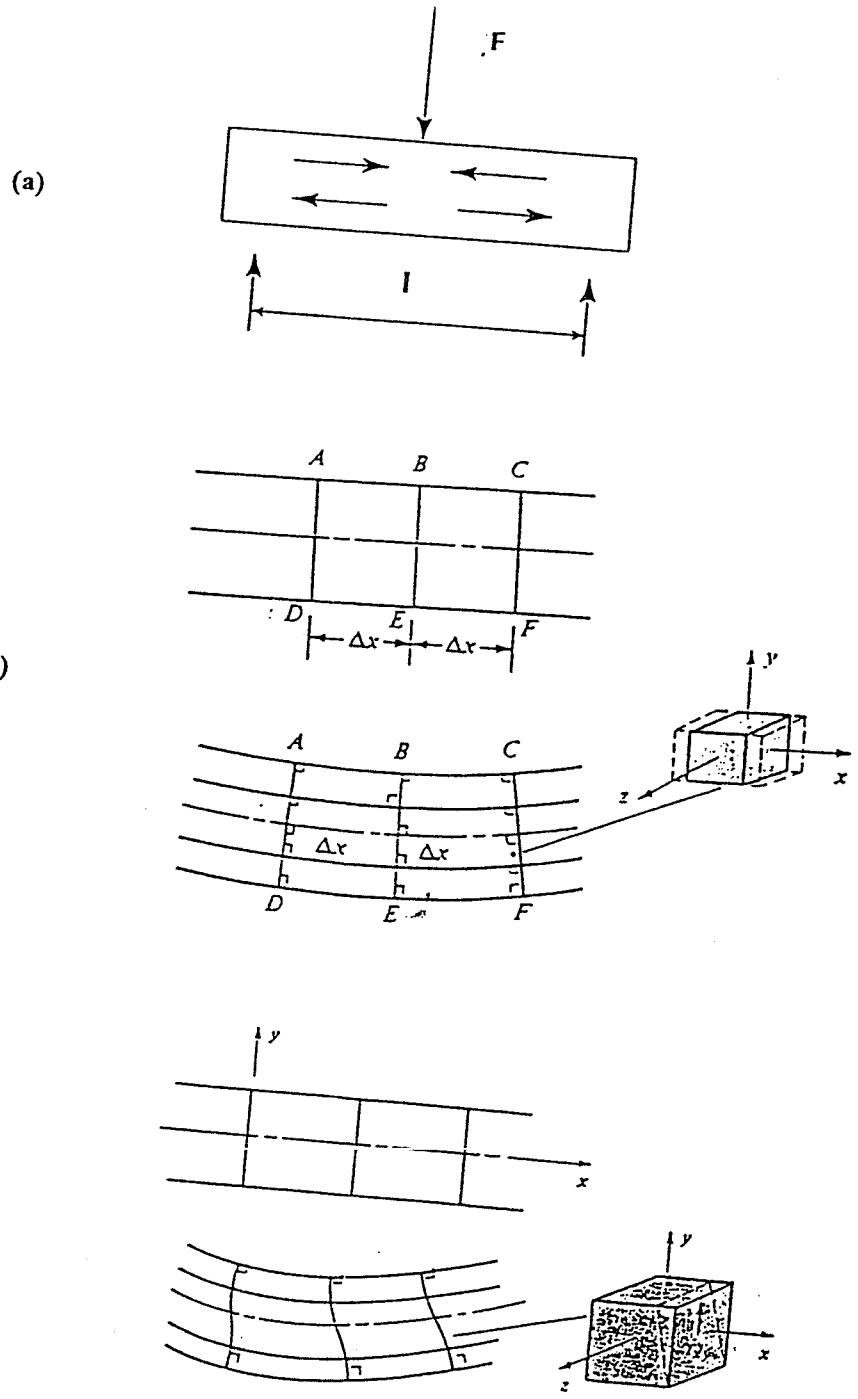

Fig. 1. a: beam loading to produce bending
b: section subjected to pure bending
c : section subjected to transverse shear and bending
F is fracture load, and I is the distance between two supports.

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