# REPORT

# Constituents and biological activity of the essential oil and the aqueous extract of *Micromeria fruticosa* (L.) Druce subsp. *serpyllifolia*

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**Abstract**: *Micromeria fruticosa* L Druce subsp. *serpyllifolia* is a medicinal herb that is widely used as folk medicine in the treatment of abdominal pains, diarrhea, eye infections, heart disorders, elevated blood pressure, colds and wounds. This study aims to investigation the constituents and biological activity of the essential oil and aqueous extract of the plant that had been collected from Nablus. The oil was prepared by hydro-distillation method and analyzed by GC/MS. The oxygenated constituents were prevalent (87.4%) with the pulegone (58.5%) was the major constituents.
Antitumor and analgesic activities of the isolated oil and the aqueous extract of *M. fruticosa* were investigated. Both the oil and the aqueous extract exhibited marked antitumor activities against Human Colon Tumor cells (HCT) and Mammary Carcinoma F7 (MCF7). The oil showed less $IC_{50s}$ against both cell lines (10, 12.7 µg/ml respectively). Also the extract significantly inhibited acetic acid-induced writhing response ($p<0.05$) and increased hot-plate pain threshold of mice at doses of 100 and 200 mg/kg while the oil did not show any analgesic activity on both models. Therefore, we concluded that the aqueous extract of *M. fruticosa* has a remarkable inhibitory activity in non-inflammatory reactions as well as inflammatory pain.

**Keywords**: *Micromeria fruticosa*; antitumor; analgesic; essential oil.

## INTRODUCTION

*Micromeria* species (family Lamiacea) is widely used as herbal infusion in many Mediterranean countries. The aerial parts of the plants are used as sedative, anaesthetic, antiseptic, antirheumatic, CNS-stimulant and in the treatment of heart disorders and colds (Davis 1985; Tabanca *et al*., 2001;Vera and Chane-Ming, 1999). White micromeria [*Micromeria fruticosa* (L.) Druce subsp. *serpyllifolia*; Lamiaceae], is a medicinal herb, widely distributed in northern and central Israel, Lebanon, Syria, Jordan and Turkey. The aerial parts of *M. fruticosa* are widely used as medicinal teas and infusions and to treat many diseases including abdominal pains, diarrhea, eye infections, heart disorders, elevated blood pressure, colds and wounds (Dafni *et al*., 1984; Yaniv *et al*., 1982).

Previous studies on the plant reported that the composition of the essential oil of *Micromeria* species may differ due to variation in cultivation, origin, vegetative stage and seasons (Dudai *et al*., 2001; Telci and Ceylan, 2007). There have been a few efforts to study the chemical composition of *M. fruticosa* ssp *serpyllifolia* growing in different areas of Turkey and Israel (Dudai *et al*., 2001; Medine *et al*., 2004; Telci and Ceylan, 2007). Literature review showed that no documented studies are available concerning the volatile oil constituents as well as the analgesic and anti-tumor activities of *M. fruticosa* plants collected from Nablus. Thus the objectives of this study were to evaluate the analgesic and antitumor activities of both aqueous extract and the oil of *M. fruticosa* collected from Nablus region, and to analyze and compare the chemical composition of the hydro-distilled oil by the composition of the previous isolated oil from both Turkey and Israel.

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## MATERIALS AND METHODS

### *Phytochemical study*

*Equipments*

GC/MS analysis of the volatile oil obtained from *M. fruticosa* plant cultivated in Nablus was comparatively performed on a fused silica capillary column HP-5MS (5% phenyl polysiloxane, 30m x 0.25mm i.d. and 0.25 µm film thickness), varian Chrompack CP-3800 GC/MS - 2000 (Darmstadt, Germany) adopting the following operating conditions: The injector port temperature was set at 220°C for 5 min with a 1:10 split ratio. Helium was used as carrier gas at flow rate of 1.00 ml/min. One µl volume of l000 ppm oil solution in n-hexane was injected. The linear temperature program was as following: initially set at 50°C for 2 min, ramped at a rate of 10°C/min to 150°C at which it was maintained isothermal for 5 min; a second ramp (20°C/min) up to 220°C and maintained isothermal for 10 min. The temperatures of the transfer line and ion source were maintained at 230

and 180°C, respectively. The mass detector (El: 70 eV) was programmed to scan ions between 40-400 m/z using full-scan fixed mode electron impact. The individual components of the volatile was identified by comparing their retention times and mass spectra with those stored in the Saturn and NIST library databases and by referring to published data (Adams 1989; Adams 1995).

*Plant material*

The aerial parts of *Micromeria fruticosa* were collected before the flowering stage (March, 2010) from, Nablus, is a Palestine city in the northern west bank. The taxonomic identification of plant materials was confirmed by Dr. Hassnaa Ahmed Hosny, Professor of Plant Taxonomy, Department of Botany, Faculty of Science, Cairo University, Egypt. Collected plant materials were dried in the shade and grounded to course powder.

A voucher specimen has been kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

*Drugs, chemicals and solvents*

Indomethacin and morphine were purchased from E. Merck, glacial acetic acid from Fisher Scientific, USA and Carboxy Methyl Cellulose (CMC) from Sigma.

*Extraction and isolation*

The aqueous extract of the air-dried powdered plant under the investigation was prepared by infusion method. One liter of boiled water was added to the plant (300 grams) then left in the refrigerator for 3 days, filter and the filtrate was lyophilized. The residue (11.25 grams) obtained was stored in the refrigerator for biological activity.

For hydro-distillation of the volatile, sample of dried plants (300 gram) was directly processed after collection (1984). The oil obtained was dried over anhydrous sodium sulphate and kept in a refrigerator till further analysis.

***Biological study***

*Animals*

Albino mice of both sexes weighing 20±2 g, obtained from the Dubai Pharmacy College, were used. They were kept in cages at 22±2 ◦C with free access to pellet food and water and on a 12-h light/dark cycle. The animals were kept without any food during 24 h before the experiments and allowing water ad libitum. Animal welfare and experimental procedures were carried out according to the guide for the care and use of laboratory animals (ASTM F719 - 81 2007).

*Drug administration*

The aqueous extract, oil and indomethacin were given orally to the animals after suspension in carboxymethyl cellulose (CMC) 1% except the oil suspended in 5% Tween solution. Morphine was given intraperitoneal (ip). The animals of control groups received orally CMC 1% or Tween 5%

*Acute toxicity*

$LD_{50}$ was calculated using 50% death within 72 h following oral administration of the extract and the oil at different doses (250, 500, 1000, 2500 and 5000 mg/kg for the extract and 20, 40, 80 and 160 mg/kg for the oil). Male albino mice weighing 22±2 g (10 per group) were used in this experiment. The number of animals, which died during the observation period, was expressed as a percentile, and the $LD_{50}$ estimated by probit test using a death percent versus doses' log (Lorke 1983).

*Analgesic activity*

Acetic acid -induced writhing response

Fifty six mice have been selected and divided into 7 groups consisting of 8 animals each. Five groups were treated orally with the aqueous extract and the isolated oil of the plant under investigation at different doses (50, 100 and 200 mg $kg^{-1}$ for the extract and 4 and 10 mg $ml^{-1}$ for the oil) one group received indomethacin at dose of 10 mg/kg as standard and the last one received 10 ml/kg of 1% solution of Tween 20 as control. All the groups received the extract, oil and idomethacin one hour before intraperitoneal injection of 1% v/v acetic acid in a volume of 10 ml/kg. The test was performed according to that described by and Anderson (1959). Animals were then placed individually into glass beakers and 5 min were allowed to elapse before they were observed for 10 min. The number of writhes during 10 min was recorded for each animal. Significant reduction in the number of writhes compared to the control was considered analgesic response

Hot plate method

The test was carried out according to Adzu method using female mice (Adzu *et al*., 2003). The hot-plate was set at 55±0.5◦C. animals were placed on the hot-plate and the time elapsed until either a hind paw licking or a jump off the surface was recorded as latency. Mice with baseline latencies of less than 5 s or more than 30 s were eliminated from the study. After the determination of baseline response, latencies were determined at 30, 60, 90, 120, 150, 180 and 210 min after oral administration the plant extract (50, 100 and 200 mg $kg^{-1}$) as well as the isolated oil (4 and 10 mg $ml^{-1}$) or morphine (10 mg $kg^{-1}$) as positive reference.

*Anti-tumor activity*

Mammary carcinoma F7cells (MCF7) and Human colon tumor cells (HCT) were purchased from National Cancer Institute, Cairo University, Egypt. Potential cytotoxicities of the aqueous extract and the hydro-distillate oil were tested using the method of Skehan *et al*. (1990). Intensity of the color was measured at λ570 nm in an ELISA

reader. The percentage of surviving fraction of each tumor cell line was recorded for each concentration of the different extracts tested.

## STATISTICAL ANALYSIS

The data's were expressed as mean ± S.D., statistical analysis was performed by one way ANOVA followed by Student-Newman-Keuls test, *p* values <0.05 were considered as significant.

## RESULTS

### *Phytochemical study*

The yield of hydro-distilled volatile oil of *M. fruticosa* was 2.2 % v/w (calculated on dried weight basis). The volatile oil was obtained as viscous liquid, colorless and with aromatic odor. The identified components belong to different groups as displayed in tables 1 and 2.

**Table 1**: Chemical compositions of hydro-distilled oil

| No. | Identified Constituents | R.T. (min) | KI | % content |
|---|---|---|---|---|
| 1 | alpha-pinene | 5.788 | 934 | 0.3 |
| 2 | beta-pinene | 5.788 | 980 | 0.8 |
| 3 | Myrcene | 5.788 | 989 | 0.2 |
| 4 | 3-Octanol | 5.788 | 999 | 0.4 |
| 5 | para-Cymene | 8.923 | 1026 | 0.5 |
| 6 | Limonene | 8.923 | 1031 | 1.2 |
| 7 | Z-beta-Ocimene | 8.923 | 1035 | 0.2 |
| 8 | gamma-Terpinene | 8.923 | 1059 | 0.2 |
| 9 | para-Mentha-3,8-diene | 8.923 | 1073 | 3.7 |
| 10 | Terpinolene | 8.923 | 1088 | 0.4 |
| 11 | para-Menth-3-en-8-ol | 12.88 | 1148 | 2.0 |
| 12 | Menthone | 12.88 | 1152 | 0.5 |
| 13 | iso-Isopulegol | 12.88 | 1159 | 0.1 |
| 14 | iso-Menthone | 12.88 | 1162 | 3.9 |
| 15 | neo-Menthol | 12.88 | 1166 | 1.5 |
| 16 | Isopulegone | 12.88 | 1175 | 3.2 |
| 17 | neoiso-Isopulegol | 12.88 | 1172 | 0.9 |
| 18 | iso-Menthol | 12.88 | 1184 | 3.3 |
| 19 | neoiso-Menthol | 12.88 | 1188 | 8.7 |
| 20 | alpha-Terpineol | 12.88 | 1190 | 0.1 |
| 21 | Pulegone | 17.21 | 1238 | 58.5 |
| 22 | Piperitone | 17.21 | 1253 | 0.2 |
| 23 | Thymol | 17.21 | 1293 | 1.6 |
| 24 | Carvacrol | 21.59 | 1302 | 0.4 |
| 25 | Piperitenone | 21.59 | 1345 | 0.3 |
| 26 | Nepetalactone | 21.59 | 1354 | 0.3 |
| 27 | Piperitenone oxide | 21.59 | 1366 | 0.2 |
| 28 | alpha-Copaene | 21.59 | 1378 | 0.2 |
| 29 | beta-Bourbonene | 21.59 | 1386 | 0.1 |
| 30 | E-Caryophyllene | 25.86 | 1422 | 3.9 |
| 31 | alpha-Humulene | 25.86 | 1457 | 0.2 |
| 32 | Germacrene D | 25.86 | 1484 | 0.5 |
| 33 | delta-Cadinene | 29.96 | 1521 | 0.2 |
| 34 | Spathulenol | 29.96 | 1581 | 0.2 |
| 35 | Caryophyllene oxide | 29.96 | 1586 | 0.9 |

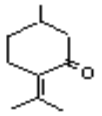

**Fig. 1**: Pulegone chemical structure

**Table 2**: Relative percentages of the volatile constituents identified from *M. fruticosa*

| Constituents | Relative Percentage |
|---|---|
| *Oxygenated constituents* | |
| Aliphatic | 0.4 |
| Aromatic | 2.0 |
| Monoterpenoid | 83.7 |
| Sesquiterpenoid | 1.3 |
| *Total oxygenated constituents* | 87.4 |
| Alcohols | 17.2 |
| Ketones | 67.1 |
| Phenols | 2.0 |
| Oxides | 1.1 |
| *Non-oxygenated constituents (hydrocarbons)* | |
| Monoterpenoid | 7.5 |
| Sesquiterpenoid | 5.1 |
| Total non-oxygenated constituents | 12.6 |

### *Biological study*

*Acute toxicity test*

Oral administration of *M. fruticosa* aqueous extract in doses up to 5 g kg-1 body weight produced no mortality and no morbidity signs or behavioral changes in any of the treated animals during observation period. However the oil showed 50% mortality at dose of 52 mg $kg^{-1}$ which considered as highly toxic.

*Analgesic activity*

In hot-plate test, the extract in dose dependant manner significantly increased latency of mice at doses of 100 and 200 mg/kg ($p< 0.05$ and $p<0.01$ respectively) with a peak at 1.5 h after drug administration. The positive drug, morphine (10 mg/kg, i.p.), also markedly increased latency of mice while the oil did not show any considerable analgesic effect even at higher dose ($p> 0.05$) as shown in table 3.

**Table 3**: Effects of the extract and the oil on acetic acid-induced writhing response and hot-plate in mice

| Groups | Dose (mg/kg) | No. Of writhing | Inhibitory ratio (%) | Hot plat latency (s) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0h | 1h | 1.5h | 2h |
| Control | - | 22.3 ± 7.68 | - | 18.45 ± 2.36 | 19.59 ± 3.23 | 18.82 ± 3.68 | 18.65 ± 3.59 |
| Indomethacin | 10 | 5.9 ± 4.77** | 73.5 | - | - | - | - |
| plant extract | 50 | 19.2 ± 4.42 | 13.9 | 19.14 ± 3.02 | 22.14 ± 5.24 | 25.57 ± 3.47 | 21.69 ± 6.37 |
| plant extract | 100 | 12.6 ± 5.53* | 43.5 | 18.52 ± 4.98 | 30.56 ± 3.41* | 34.68 ± 3.15* | 29.89 ± 3.56* |
| plant extract | 200 | 6.7 ± 4.32** | 70 | 18.43 ± 3.25 | 36.15 ± 3.32** | 38.26 ± 4.64** | 32.13 ± 3.74** |
| Oil | 4 | 21.4 ± 5.32 | 4 | 16.45 ± 3.45 | 18.59 ± 2.14 | 17.82 ± 4.56 | 17.34 ± 3.79 |
| Oil | 10 | 19.8 ± 6.53 | 11.2 | 17.74 ± 2.98 | 18.27 ± 4.58 | 18.48 ± 3.32 | 17.83 ± 4.19 |
| Morphine | 10 | - | - | 19.35 ± 2.63 | 36.54 ± 3.48** | 39.17 ± 3.89** | 32.37 ± 4.68 |

In acetic acid induced writhing response, as shown in table 3, the extract but not the oil inhibited the writhing response at the two doses of 100 and 200mg/kg with inhibition percentage of 43.5% and 70%, respectively. The positive drug, indomethacin (10 mg/kg), also inhibited the writhing response significantly (73.5%)

*Anti-tumor activity*

As shown in table 4, both oil and extract exhibited marked antitumor activities against both Mammary Carcinoma F7 (MCF7) and Human Colon Tumor Cells (HCT).

**Table 4**: Anti-tumor activities of the aqueous extract and the oil against MCF-7 and HCT

| Conc. µg/ml | Percentage of Survival | | | |
|---|---|---|---|---|
| | Aqueous extract | | Isolated Oil | |
| | MCF-7 | HCT | MCF-7 | HCT |
| 0.0 | 100 | 100 | 100 | 100 |
| 5.0 | 72 | 64 | 79 | 67 |
| 12.5 | 56 | 46 | 49 | 43 |
| 25 | 45 | 25 | 19 | 17 |
| 50 | 25 | 21 | 10 | 14 |

## DISCUSSION

***Phytochemical study***

The yield of the Nablus oil, which collected before flowering stage, (2.2% v/w) was being higher than that of the Turkey sample which collected in the flower stage, 1.85% (Medine *et al*., 2004).

GC/MS analysis of the volatile oil under the aforementioned conditions led to the identification of 35 components (table 1). The chromatographic profile of the volatile was dominated by oxygenated constituents (amounting to 87.4%) among which pulegone, fig. 1, was prevalent (58.5%), (comparing to North Israel and Turkey samples in which pulegone content was 65-78% and 29.19 respectively) (Dudai *et al*., 2001; Medine *et al*., 2004), followed by neoiso-menthol (8.7%) which did not identify in the Turkey sample (Medine *et al*., 2004).

The isolated oil was mainly rich in ketonic and alcoholic compounds (67.1 % and 17.2% respectively). However, an obvious variability was noticed among the minor components of the oxygenated compounds between the sample under investigation and which collected from Turkey and North Israel (Dudai *et al*., 2001; Medine *et al*., 2004).

The relative percentage of non-oxygenated constituents reached 12.6% among which E- Caryophyllene was the major compounds (3.9%).

The environmental conditions of growth (including climatic and geographical factors) obviously affected the yield and composition of the volatile oil of the plant under investigation comparing to Turkey sample (Medine *et al*., 2004).

***Biological study***

*Acute toxicity test*

The high toxicity of the oil could be in part due to its high pulegone content. Hepatotoxicity and death have been reported in mice after ingestion of Pennyroyal oil which its primary constituent was pulegone (Sztajnkrycer *et al*., 2003).

*Analgesic activity*

The analgesic activity was assessed by two animal models. The hot-plate test was chosen to investigate the central analgesic activity since the involvement of endogenous substances like prostaglandins is minimized in this model (Woolfe and Mc Donald 1995).

In acetic acid induced writhing response model is usually used for peripheral analgesics screening (Deraedt *et al*., 1980).

Pain in this model is generated by the release of various endogenous mediators such as prostaglandins E2 and F2, thus causing inflammatory pain by increasing capillary permeability (Amico-Roxas *et al*., 1984).

Many researches have showed that flavonoid and other polyphenolic compounds and triterpenoids own analgesic properties (Li *et al*., 2003; Taesotikul *et al*., 2003).

Therefore, we believed that the aqueous extract of *M. fruticosa* has a remarkable inhibitory activity in non-inflammatory reactions and inflammatory pain, and this action may be related to suppression of synthesis and/or release of some endogenous proinflammatory substances like prostaglandins.

However, a further detailed study on *M. fruticosa* is required to reveal the major active constituents and the mechanism involved in analgesic action.

*Anti-tumor activity*

The oil showed more activity against both cell lines with least $IC_{50s}$ (HCT and MCF-7 10 and 12.7 µg/ml respectively) while the IC50s against HCT and MCF-7 for the aqueous extract were 11 and 19.6 µg/ml respectively. The antitumor activities may be attributed to the oxygenated compounds in the oil (87.4%) (Khamsan *et al*., 2011) and the flavonoidal glycosides content in the aqueous extract (Kandaswami *et al*., 2005).

## CONCLUSION

The results of this study showed that the oil and aqueous extract of *M. fruticosa* possesses marked antitumor activities against Human Colon Tumor cells and Mammary Carcinoma F7. On the other hand, the aqueous extract has significant analgesic activities in non-inflammatory reactions as well as inflammatory pain.

Meanwhile, activity-guided fractionation of the total extracts of *M. fruticosa* remained to be performed in the further studies.

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