academicJournals

Vol. 7(35), pp. 2538-2544, 17 September, 2013
DOI: 10.5897/JMPR2013.5069
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Essential oil composition and pharmacological activities of *Micromeria biflora* (Buch.- Ham. Ex D. Don) Benth. collected from Uttarakhand region of India

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Accepted 5 August, 2013

By a combination of gas chromatography (GC) and gas chromatography/mas spectrometry (GC/MS) sixteen compounds contributing to 91.9% of the total essential oil of *Micromeria biflora* (Buch.-Ham. ex D. Don) Benth. (*Lamiaceae*) were identified. The oil was dominated by oxygenated sesquiterpenoids; caryophyllene oxide (42.5%), epi-α-cadinol (5.7%), β-eudesmol (9.3%), oplapanone (4.5%) and guaial (4.6%). α-Terpeneol (1.1%) was the only identified oxygenated monoterpene. The major mono and sequiterpene hydrocarbons identified in the essential oil were þ-cymene (4.7%), γ-cadinene (2.6%), δ-cadinene (3.4%), and α-cadinene (2.8%) besides other minor constituents. Both the oil and the extract were also tested for anti-inflammatory, analgesic and antipyretic activity on Swiss albino mice which exhibited good to moderate pharmacological activity in a dose dependent manner in comparison with the standard drug, namely; ibuprofen, paracetamol and indomethacin.

Key words: Lamiaceae, *Micromeria biflora*, caryophyllene oxide, essential oil, phenolic assay antioxidant, analgesic and antipyretic activities, anti-inflammatory.

INTRODUCTION

The genus *Micromeria* (Lamiaceae family) is distributed from the Macaronesian-Mediterranean region to Southern Africa, India and China. Various species of *Micromeria* are effective against heart disorders, headache, wounds, skin infections in cattle and treating cold (Bentham, 1994; Pedro et al., 1995; Baytop, 1984; Ali-Shtayeh et al., 1998; Mallavarapu et al., 1997). The leaf powder mixed with oil is used in ulcer and fungal infection (Uniyal and Shiva, 2005). About 100 species of this genus are widely distributed throughout the globe. Only three species have been reported from Indian Himalayan region, namely *Micromeria biflora* (Buch.-Ham ex D. Don) Benth., *Micromeria capitellata* Banth. and *Micromeria biflora* var. hispida Kitamura ex Murata. (Gaur, 1999; Babu, 1977). It has been reported that several essential oils posses anti-inflammatory activity (Salud et al., 2011; Al-Reza et al., 2010; Silva et al., 2003). No report exists on the essential oil composition, and pharmacological activity of *M. biflora* (Buch.- Ham ex D.Don) Benth. which grows wildly in the Uttarakhand region of the Indian Himalayas.

This study reports on the essential oil composition and pharmacological activity of *M. biflora* (Buch.-Ham ex D. Don) Benth., because of its various therapeutic uses in the present study.

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MATERIALS AND METHODS

Plant

Fresh aerial parts of the plant were collected from Jiyolikot (Nainital) during August to September, 2011 and were identified by Dr. D. S. Rawat (Assistant Professor and Taxonomist), Department of Biological Science, G.B. Pant University of Agriculture and Technology, Pantnagar. The herbarium specimen has been preserved and stored for future reference.

Isolation of essential oil (MIbEO)

The plants were subjected to hydrodistillation for 8 h using Clevenger' type apparatus. The extraction of the distillate with diethyl ether was followed by drying using anhydrous Na_2SO_4. Removal of solvent using rotatory evaporation under vacuum yielded the essential oil (0.15% v/w).

Preparation of hydro-alcoholic extracts (MIbE)

Hydro-alcoholic extract was prepared by soaking (for one week) the fresh plant material (250 g) with 98 ml of 10% ethyl alcohol in distilled water at room temperature (25°C). The liquid was then percolated; the extraction process was twice repeated. The extracts were combined and evaporated to dryness under vacuum (30°C). The residue (600 mg) was dissolved in water before use.

Gas chromatography (GC) analysis

The GC analysis of the essential oil was done with a Nucon-GC 5765 system equipped with flame ionization detector (FID) and 25 M capillary column DB-5 (30 m×0.32 mm id). The initial 60°C column temperature was maintained for 5 min and then programmed to increase in temperature at the rate of 3°C min^{-1} to 210°C, which was then maintained for 10 min. The detector temperature was 210°C; N_2 was used as the carrier gas (flow rate 50 ml/min). The percentage composition of the oil was determined from the computer read out of the FID data and rounded to the first decimal place.

GC/Mass spectrometry (MS) analysis

The GC/MS analysis was performed with a Thermo Quest-Trace 2000 GC coupled with a Finnegan Mat-Polaris Q ion trap Mass Spectrometer, Detector-MS using DB-5 capillary column (30 m×0.25 mm id) with an initial temperature of 60°C and then temperature programmed, to increase in temperature, at the rate of 3°C min^{-1}, to 210°C. Helium was used as the carrier gas (flow rate 1 ml/min). The ion source temperature was 210°C. The MS was recorded by ionizing the molecules under electron impact (EI) conditions (70 eV) with a split ratio of 40:1. The compounds were identified and compared by matching their retention indices and mass spectra to those recorded in NIST/Wiley Library and those published in the literature (Adams, 1995).

Evaluation of *in vivo* pharmacological activities

The experiments were carried with the permission of the institutional ethical committee (Registration No. 330/CPCSEA). Swiss albino mice were procured from the Laboratory Animal Division of the Central Drug Research Institute, Lucknow, India. They were randomly divided into seven groups, with six mice in each group, and were maintained under standard laboratory conditions (temperature, 25±2°C; humidity, 40±5%). Two concentrations of the dried extract (50 and 100 mg/kg body weight) and three concentrations of essential oil (5, 10 and 20%) were administered orally at 10 ml/kg body weight. All concentrations of the extract and the oil were prepared separately by the addition of a small amount (0.3 to 0.5 ml) of Tween-20 and saline water with constant triturating to make a final volume of 10 ml. Ibuprofen, indomethacin and paracetamol were used as standard drugs and saline water was used as the control.

Anti-inflammatory activity

Both acute and sub-acute anti-inflammatory activities of the essential oil and the hydro-alcoholic extract were investigated.

Carrageenan-induced paw oedema

The method of Henriques et al. (1987) was used to determine the anti-inflammatory activity of the essential oil and the extract. Oedema was induced by the injection of carrageenan (0.1 ml, 1% w/v in saline) into the sub plantar tissue of the right hind paw. Three groups of mice were given essential oil (5, 10 and 20% at 10 ml/kg body weight), two groups were given hydro-alcoholic extract (50 and 100 mg/kg body weight), one group was given Ibuprofen (40 mg/kg body weight), and one group distilled water (10 ml/kg body weight). The paw volume was measured using a plethysmometer (UGO Basile, Italy) at 1, 3 and 24 h after the carrageenan injection. Oedema formation in the paw results from a synergy between various inflammatory mediators that increase vascular permeability, and mediators that increase the blood flow (Italenti et al., 1995). The reduction in the volume displacement of hind foot in comparison to control and between 0, 4 and 24 h was taken as an anti-inflammatory effect.

Sub-acute anti-inflammatory activity

The sub-acute anti-inflammatory activity of the hydro-alcoholic extract and essential oil on formaldehyde induced arthritis was determined using the method of Selye (1949). A 1% solution of formaldehyde (0.1 ml) was injected into the right hind paw of the mice on the first day of the experiment (Selye, 1949). The extract and oil as the same dose rate mentioned earlier were administered orally every morning for 10 days. For the standard drug group, Ibuprofen suspension (40 mg/kg body weight) was administered in the same way (orally) for the same period of time. The control group received only saline water. Paw volume was determined using a plethysmometer every evening for 10 days.

Analgesic activity

Hot plate test

The analgesic activity was determined using the hot plate method (Langerman et al., 1995). A hot plate was maintained at 55.0±0.5°C and the mice were placed into a perspex cylinder on the heated surface. The discomfort reaction time (licking paws or jumping) was recorded prior to and after the administration of the extract (50 and 100 mg/kg body weight) and essential oil (5, 10 and 20% at 10 ml/kg body weight) at 30, 60, 120, and 150 min so as to determine response latency. For the standard drug group, indomethacin was used at 5 mg/kg body weight and for control saline, water (0.2 ml) was used. The reaction time was recorded after 60 min of oral administration of the extract and essential oil.

Table 1. Chemical composition of essential oil from aerial part of *M. biflora*.

S/N	Compound	KI	FID response (%)
1	α- Pinene	936	0.1
2	Sabinene	975	0.1
3	α- Phellendrene	1008	2.7
4	þ-Cymene	1029	4.7
5	α- Terpineol	1193	1.1
6	β- Caryophyllene	1406	4.9
7	Aromadendrane	1439	1.6
8	β- Selinene	1485	1.3
9	γ-Cadinene	1513	2.6
10	δ-Cadinene	1513	3.4
11	α- Cadinene	1530	2.8
12	Caryophyllene oxide	1580	42.5
13	Guaial	1602	4.6
14	β- Eudesmol	1649	9.3
15	Epi- α-Cadinol	1656	5.7
16	Oplopanone	1740	4.5
Total	-	-	91.9

FID: Flame ionization detector.

Acetic acid-induced abdominal writhing test

Glacial acetic acid was administered intraperitoneally to create pain sensation (Collier et al., 1968). After 1 h, 0.2 ml of essential oil (5, 10 and 20% at 10 ml/kg body weight), extracts (50 and 100 mg/kg body weight), the standard drug Ibuprofren at 40 mg/kg body weight and saline water were administered orally to their respective groups. The number of writhings was counted for 30 min for each mouse. The inhibition of writhing in mice by the standard analgesic Ibuprofen was compared with the groups receiving extract/essential oil and the percentage of pain protection was calculated using the following formula:

Writhing (%) = (T/C) × 100
Inhibition (%) = (C-T/C) × 100

where T= treatments (groups III to VII) and C= control saline group I.

Antipyretic activity

Pyrexia was induced by subcutaneous injection of 10 mg/kg body weight of 20% suspension of Brewer's yeast *(Sacchromyces cerevisiae)* (Rao et al., 1997). At the nineteenth hour post administration, their body temperature was taken rectally and recorded. Following which the extract at a dose level of 50 and 100 mg/kg body weight, essential oil at of 5, 10, and 20% (at 10 ml/kg body weight) and standard drug paracetamol (33 mg/kg body weight) were administered orally. The control group received 0.2 ml normal saline. The temperature was recorded at hourly intervals in all the mice for up to 3 h and the percentage reduction in rectal temperature was calculated by using the following relationship, and considering the fall in temperature to normal level as 100%.

Reduction (%) = {(B – C / B- A) × 100}

where A = normal temperature, B= Pyrexia temperature, and C = temperature at hourly intervals.

Assessment of toxicity

Lethality was determined by oral administration of 400, 600 and 800 mg/kg of body weight of the extract and 40, 60 and 80% of the essential oil. Clinically animals were examined for 24 h and the number of deaths, if any, was recorded up to 48 h.

Statistical analysis

The data were expressed as mean±standard error (SE) and the results were analysed using one way analysis of variance (ANOVA) and P≥0.05 which were considered to be statistically significant.

RESULTS AND DISCUSSION

By a combination of GC and GC/MS, the presence of over 30 compounds was detected of which 16 compounds contributing to 91.9% of the oil were identified. The oil was rich in oxygenated sesquiterepenes. Caryophellene oxide (42.6%) was the major compound. The other identified oxygenated sesquiterpens included β-eudesmol (9.3%), epi-α-cadinol (5.7%), oplapanone (4.5%) and guaioll (4.6%). The identified sesquiterpenoide hydrocarbons were β-caryophyllene (4.9%), aromadendrane (1.6%), β-selinene (1.3%), γ-cadinene (2.6%), α-cadinene (2.8%) and δ-cadinene (3.6%). The only identified oxygenated monoterpenoid was α-terpineol (1.1%). þ-Cymene (4.7%) and α-phellendrene (2.7%) were the major monoterpene hydrocarbons besides minor amount of α-pinene and sabinene (Table 1).

It has been reported (Mallavarapu et al., 1997) that the essential oil of *M. biflora* collected from South India

Table 2. Effect of essential oil (MIbEO) and extract (MIbE) of *M. biflora* on carrageenan-induced paw oedema (Mean ± SE, n=6).

Group	Treatment	Dose (mg/kg body weight extract) and percent (at 10 ml/kg body weight oil)	Change in paw volume (mm^3) after different times		
			0 h	4 h	24 h
I	Control	0.2 ml	2.40±0.02	2.33±0.03	2.32±0.01
II	Ibuprofen	40 mg/kg	2.20±0.01[b]	1.73±0.02[a] (25.75%)	1.18±0.02[a] (49.14%)
III	MIbE	50 mg/kg	2.55±0.05[ab]	2.27±0.03[ab] (3.57)	2.13±0.02[ab] (8.19%)
IV	MIbE	100 mg/kg	2.38±0.03[b]	2.17±0.04[ab] (6.87%)	1.95±0.04[ab] (15.95%)
V	MIbEO	5% (0.2 ml)	2.34±0.01[ab]	2.31±0.05[b] (0.86%)	2.23±0.01[ab] (3.87%)
VI	MIbEO	10% (0.2 ml)	2.50±0.01[ab]	2.26±0.03[ab] (3.1%)	2.20±0.01[ab] (5.17%)
VII	MIbEO	20% (0.2 ml)	2.61±0.02[ab]	2.21±0.02[ab] (5.15%)	2.05±0.01[ab] (11.64%)

There is a statistically significant difference (P<0.05). One way ANOVA followed by Dunnett's multiple comparison test. [a]Significant (P<0.05) as compared to control, [b]Significant (P<0.05) as compared to Ibuprofen. % = Perent reduction in paw volume at different times.

mostly contains monoterpene hydrocarbons and oxygenated monoterpenes neral (25.3 and 32.2%) and geranial (26.7 and 41.3%). Pulegone (66.55 and 64.10 %), cis-p-menthone (21.71 and 25.31%), trans-p-menthone (9.59 and 5.59%), nerol (0.35 and 2.49%) and 3-octanol (2.81 and 0.25%) were reported in the essential oil of *M. cilicica* from Turkey (Duru et al., 2004). Trans-caryophyllene (43.7%) and caryophyllene oxide (18.0%) were reported as the major compounds besides spathulenol (8.5%) and α-humulene (3.1%) in the essential oil of *M. biflora* species *arabica* K.Walth collected from Pakistan (Al-Rehaily, 2006). The composition of the oil analysed in the present study did not match that of the essential oils from the species of *M. biflora* reported earlier which might be possibly due to different climatic conditions. The analysis thus reveals that it is different chemo variant.

Anti inflammatory activity

Carrageenan-induced mice paw oedema

In the Ibuprofen treated group, a significant reduction in oedema was observed (Table 2). MIbEO reduce only 0.86% in paw volume at the dose level of 5% (10 ml/kg body weight) concentration in comparison to the control after 4 h. The peak inhibitory effect of MIbEO and MIbE showed maximum at the dose levels of 20% (10 ml/kg body weight) and 100 mg/kg body weight, respectively after 24 h. Their values were 11.64 and 15.95%, respectively while 40 mg/kg body weight of Ibuprofen produced 49.06% reduction in the same time period.

Sub-acute anti-inflammatory activity

Perusal of Table 3 revealed that both MIbEO and MIbE were found to be comparatively ineffective at lower dose levels (5 to 10% in MIbEO and 50 mg/kg body weight in MIbE). At higher concentrations, MIbEO showed only moderate activity by reducing the paw volume from 2.13 (at 0 day) to 2.37 (at day one) and 2.23 mm^3 (at day 10) (at dose level of 20%, 10 ml/kg body weight). MIbE also showed moderate activity (on day 10) (at dose level of 100 mg/kg body weight) in comparison to the standard (Ibuprofen) (10 mg/kg body weight).

Analgesic activity

Hot plate method

Both MIbEO and MIbE exhibited a dose dependent increase in latency time when compared with the standard drug, indomethacin. The hot plate reaction time for indomethacin treated mice reached a maximum (4.96 min) at 60 min. MIbEO and MIbE treated mice significantly attenuated the acetic acid induced writhing and exhibited an increase in the paw licking and jumping time on hot plate in a dose dependent manner maximum hot plate reaction time (3.93 min) were observed in MIbEO at the dose level of 20% (10 ml/kg body weight) and MIbE (3.91 min) at the dose level of 100 mg/kg body weight followed by other concentrations of extract and oil (Table 4). The hot plate test is useful for the evaluation of centrally acting analgesics which are known to elevate the pain threshold of mice towards heat (Hiruma-Lima et al., 2000). It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally (Ibironke and Ajiboye, 2007).

Acetic acid-induced abdominal writhing test

Both MIbEO and MIbE treated mice showed a significant reduction in writhing induced by acetic acid after oral administration in a dose dependant manner. The standard drug Ibuprofen was found to be more potent, then both MIbE and MIbEO at all the dose levels. MIbE showed significant analgesic activity with 36.77% inhibition at 100 mg/kg body weight, while 31.67% inhibition was observed with MIbEO at 20% (10 ml/kg

Table 3. Effect of essential oil (MIbEO) and extract (MIbE) of *M. biflora* on formaldehyde-induced mice paw oedema (Mean ± SE, n=6).

Treatment	Dose (mg/kg body weight extract) and percent (at 10 ml/kg body weight oil)	Volume of inflammation (mm^3)										
		0 Day	1 Day	2 Day	3 Day	4 Day	5 Day	6 Day	7 Day	8 Day	9 Day	10 Day
Control	0.2 ml	2.13±0.02	2.33±0.04	2.36±0.03	2.52±0.04	2.38±0.03	2.37±0.02	2.40±0.02	2.38±0.01	2.27±0.01	2.27±0.01	2.26±0.01
Ibuprofen	10 mg/kg	2.17±0.02	2.13±0.02[a]	2.19±0.01[a]	2.27±0.01[a]	2.21±0.02[a]	2.19±0.01[a]	2.15±0.01[a]	2.26±0.01[a]	2.17±0.01[a]	2.19±0.01[a]	2.15±0.01[a]
MIbE	50 mg/kg	2.19±0.01	2.27±0.02[ab]	2.39±0.02[b]	2.48±0.02[b]	2.38±0.04[b]	2.35±0.01[b]	2.34±0.01[ab]	2.31±0.01[ab]	2.32±0.01[b]	2.33±0.04[ab]	2.30±0.01[b]
MIbE	100 mg/kg	2.17±0.02	2.38±0.01[ab]	2.51±0.02[ab]	2.52±0.02[b]	2.33±0.04[b]	2.32±0.01[b]	2.28±0.03[ab]	2.36±0.01[b]	2.30±0.01[b]	2.28±0.03[b]	2.26±0.01[b]
MIbEO	5% (0.2 ml)	2.12±0.02	2.35±0.02[b]	2.57±0.03[ab]	2.52±0.04[b]	2.49±0.03[ab]	2.42±0.01[b]	2.47±0.01[ab]	2.51±0.02[ab]	2.33±0.03[ab]	2.31±0.01[b]	2.28±0.03[b]
MIbEO	10% (0.2 ml)	2.14±0.03	2.38±0.03[ab]	2.48±0.02[ab]	2.48±0.03[b]	2.44±0.01[ab]	2.39±0.02[b]	2.37±0.01[b]	2.41±0.01[b]	2.35±0.01[ab]	2.34±0.01[ab]	2.25±0.01[b]
MIbEO	20% (0.2 ml)	2.13±0.03	2.37±0.01[ab]	2.49±0.03[ab]	2.47±0.01[b]	2.28±0.03[ab]	2.28±0.02[ab]	2.32±0.01[ab]	2.36±0.01[b]	2.31±0.01[b]	2.26±0.01[b]	2.23±0.01[b]

There is a statistically significant difference (P = <0.05). One way ANOVA followed by Dunnett's multiple comparison test. [a]Significant (P<0.05) as compared to control. [b]Significant (P<0.05) as compared to Ibuprofen.

Table 4. Effect of essential oils (MIbEO) and extracts (MIbE) of *M. biflora* on hot plate induced pain in mice. (Mean ± SE, n=6).

Group	Treatment	Dose (mg/kg body weight extract) and percent (at 10 ml/kg body weight oil)	Hot plate reaction time (min) after administration of drug/sample					
			0 min	30 min	60 min	90 min	120 min	150 min
I	Control	0.2 ml	3.00±0.04	2.99±0.03	2.97±0.04	2.89±0.03	2.88±0.06	2.87±0.02
II	Indomethacin	5 mg/kg	3.46±0.04	3.84±0.04[a]	4.96±0.05[a]	4.21±0.02[a]	4.06±0.05[a]	3.84±0.04[a]
III	MIbE	50 mg/kg	3.46±0.02[ab]	3.52±0.05[ab]	3.64±0.02[ab]	3.58±0.05[ab]	2.69±0.10[ab]	2.29±0.15[ab]
IV	MIbE	100 mg/kg	3.58±0.03[b]	3.62±0.03[ab]	3.91±0.06[ab]	3.77±0.06[ab]	3.00±0.07[ab]	2.50±0.01[ab]
V	MIbEO	5% (0.2 ml)	3.44±0.01	3.00±0.03[b]	3.08±0.09[ab]	2.98±0.03[b]	2.22±0.10[ab]	1.89±0.04[ab]
VI	MIbEO	10% (0.2 ml)	3.40±0.04	3.42±0.03[ab]	3.70±0.04[ab]	3.44±0.10[ab]	2.61±0.08[ab]	2.17±0.10[ab]
VII	MIbEO	20% (0.2 ml)	3.69 ±0.05	3.77±0.04[a]	3.93±0.05[ab]	3.81±0.03[ab]	2.89±0.06[b]	2.28±0.09[ab]

There is a statistically significant difference (P<0.050). One way ANOVA followed by Dunnett's multiple comparison test. [a]Significant (P<0.05) as compared to control. [b]Significant (P<0.05) as compared to Indomethacin.

body weight).Ibuprofen caused 43.53% inhibition (at 40 mg/kg body weight) (Table 5). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria et al., 2008). Any substance that has got analgesic activity reduce the number of writhing of animals within a given time and with respect to the control group will help analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Collier et al., 1968; Duarte et al., 1988; Ferdous et al., 2008). The significant pain reduction of both the oil and extract might be due to the presence of analgesic principles acting with the prostaglandin pathways. It was found that the observed analgesia.

Antipyretic assay

MIbEO and MIbE both produced significant antipyretic effect in a dose dependent manner. The yeast significantly ($p<0.05$) increased body temperature (1.05 to 1.28°C) 18 h after yeast injection. Before administration (-18 to 0 h) of sample (essential oil, extract and standard), body

Table 5. Effect of essential oil (MIbEO) and extract (MIbE) of *M. biflora* on acetic acid induced writhing reflex in mice (Mean ± SE, n=6).

Group	Treatment	Dose (mg/kg body weight extract) and percent (at 10 ml/kg body weight oil)	No. of writhings	Writhing (%)	Inhibition (%)
I	Control	0.2 ml	140.50±1.88	100	-
II	Ibuprofen	40 mg/kg	79.33±0.435	56.47	43.53[a]
III	MIbE	50 mg/kg	109.17±0.223	77.70	22.30[ab]
IV	MIbE	100 mg/kg	88.83±0.367	63.23	36.77[ab]
V	MIbEO	5% (0.2 ml)	127.17±0.0498	90.51	9.49[ab]
VI	MIbEO	10% (0.2 ml)	115.17±0.180	81.97	18.03[ab]
VII	MIbEO	20% (0.2 ml)	96.00±0.316	68.33	31.67[ab]

There is a statistically significant difference (P<0.050). One way ANOVA followed by Dunnett's multiple comparison test. [a]Significant (P<0.05) as compared to control. [b]Significant (P<0.05) as compared to Ibuprofen.

Table 6. Effect of essential oils (MIbEO) and extracts (MIbE) of *M. biflora* on yeast induced pyrexia in mice (Mean±SE, n=6).

Group	Treatment	Dose (mg/kg body weight extract) and percent (at 10 ml/kg body weight oil)	Body temperature before administration of drug (°C)		Body temperature after administration of drug (°C)		
			-18 h	0 h	1 h	2 h	3 h
I	Control	0.2 ml	37.31	38.43	38.64±0.05	38.46±0.12	38.61±0.06
II	Paracetamol	33 mg/kg	37.26±.01[a]	38.42±0.05	37.42±0.08[a] (86.31%)	37.39±0.02[a] (88.76%)	37.40±0.02[a] (87.90%)
III	MIbE	50 mg/kg	37.51±0.04	38.68±0.06[ab]	38.31±0.06[ab] (31.62%)	38.11±0.04[ab] (48.71%)	37.95±0.05[ab] (62.39%)
IV	MIbE	100 mg/kg	37.51±0.05	38.79±0.03[ab]	38.23±0.03[ab] (43.75%)	37.79±0.02[ab] (78.12%)	37.77±0.05[ab] (79.68%)
V	MIbEO	5% (0.2 ml)	37.52±0.04	38.57±0.02[ab]	38.32±0.03[ab] (23.80%)	38.16±0.04[ab] (39.04%)	38.03±0.03[ab] (51.42%)
VI	MIbEO	10% (0.2 ml)	37.44±0.05	38.70±0.04[ab]	38.31±0.05[ab] (30.95%)	38.03±0.04[ab] (53.17%)	37.87±0.05[ab] (65.87%)
VII	MIbEO	20% (0.2 ml)	37.44±0.03	38.72±0.03[ab]	38.20±0.05[ab] (40.62%)	37.89±0.02[ab] (64.84%)	37.77±0.05[ab] (74.21%)

There is a statistically significant difference (P<0.050). One way ANOVA followed by Dunnett's multiple comparison test. [a]Significant (P<0.05) as compared to control. [b]Significant (P<0.05) as compared to paracetamol.

temperature increases between 1.05 and 1.28°C in different groups. The maximum reduction in body temperature was observed in standard drug (paracetamol) group, about 1.03°C (88.76%) at 2h after administration of standard drug and the antipyretic activity was evident at all dose level of MIbEO and MIbE with body temperature falling over the 3 h. The initial and final rectal temperatures in the groups treated with essential oil at 20% (10 ml/kg body weight), extract (100 mg/kg body weight) and paracetamol (33 mg/kg body weight) were 38.72°C±0.03 to 37.77°C±0.05, 38.79°C±0.03 to 37.77°C±0.05 and 38.42°C±0.05 to 37.40°C±0.02, respectively. Paracetamol, extract and essential oil showed 87.90, 79.68 and 74.21% reduction in body temperature, respectively, with significant (P<0.05) antipyretic activity throughout the test period of 3 h (Table 6). Previous studies suggest that the increase in body temperature intensified the lipid peroxidation process, which indicates that pyrexia is associated

with increased antioxidant supplementation decreased the lipid peroxidation processes (Brazezinska-Slebodzinska, 2001).

Acute toxicity

Both MIbEO and MIbE did not cause any behavioral changes and no deaths were observed. Thus they were considered to be practically non-toxic substances.

Conclusion

It can be concluded from results that the plant *M. biflora* can be a source of caryophyllene oxide and β-eudesmol and can be used as anti-inflammatory analgesic and antipyretic agent besides its other traditional and indigenous applications. To best of our knowledge, this is the first study to provide data on the evaluation of essential oil composition and pharmacological activities of *M. biflora.*

ACKNOWLEDGEMENT

Authors are thankful to Uttarakhand Counccil for Science and Technology, Dehradun for financial assistance and the GC/MS Laboratory, Kumaun University (D.S.B. Campus) Nainital is thankfully acknowledged for the GC and the GC/MS data acquisition.

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