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Chemical Composition of Leaf Essential Oil of Wild and Domestic Genotypes of *Murraya paniculata* L.

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Abstract: The plant *Murraya paniculata* L. (locally known as Kamini) collected from two different locations of Uttarakhand were subjected to hydrodistillation for the isolation of essential oils which yielded about 0.6 % oil in both the collections. The essential oils of both the collections were studied for their chemical composition and antifungal activity against two phytopathogenic fungi such as *Rhizoctonia solani* and *Sclerotium rolfsii*. The GC-FID analysis revealed the presence of over 10 compounds in each essential oil of which over 11-14 constituents have been identified which contribute 86.99 % and 76.25 % of the total oil respectively. Both the collections were dominated by sesquiterpenoids. Essential oil of *Murraya paniculata* (wild variety) was rich in oxygenated sesquiterpenoids mainly dominated by *trans*-verbenol (26.25 %) while essential oil of *Murraya paniculata* (domestic variety) was rich in sesquiterpene hydrocarbons being dominated by germacrene B (41.91 %) besides other minor constituents. The essential oil of *Murraya paniculata* (wild variety) showed significant antifungal activity, hence can be used as antifungal agent against the tested fungi after its proper clinical trials while domestic verity has non-significant antifungal activity. This was may be due to the presence of oxygenated sesquiterpenoids.

Key words: *Murraya paniculata,* sesquiterpenoids, chemical composition, antifungal activity.

Introduction

Medicinal plants throughout the world have been used in traditional medicines. Since ancient times, plants have been exemplary source of medicine. Ayurveda and other oriental systems of medicine mention the use of plant in treatment of various human ailments. India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties [3]. *Murraya paniculata* (Orange jasmine) commonly known as "kamini" in India belongs to the family Rutaceae, which represents more than 150 genera and 1600 species [4], is a highly valuable plant due to its characteristic aroma and medicinal values. It possess higher amount of phytochemicals like alkaloids, flavonoids, tannins and phenolic compounds which are responsible for their antinociceptive [5], antioxidant [6,13], anti-diabetic [7], antimicrobial [8] and analgesic activities [9].

Marraya paniculata is geographically the most wide-spread species of section Murraya occurring in either the tropics or subtropics of Asia and Oceania. It is distributed over the greater part of India and the Andaman Islands to an altitude of 1500 m. It is an evergreen shrub or occasionally a small tree, usually 2 to 3 m in height but reaching 7.5 m and 13 cm in stem diameter [1]. Leaves are stimulant and astringent and are used in the treatment of diarrhea, dysentery and diseases of

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teeth and gum and also useful against rheumatism, coughs and hysteria [2].

Essential oils, secondary metabolites of plants, are derived from terpenes and their oxygenated compounds, show various pharmacological activity. They are also used in aromatherapy, food preservation and in fragrance industry. In the earlier report the main constituents of essential oil of leaves of *Murraya paniculata* were β-cyclocitral (22.9 %), methylsalicylate (22.4 %), *trans*-nerolidol (11.7 %), α-cubebene (7.9 %), (-)-cubenol (6.8 %), β-cubebene (5.8 %) and isogermacrene (5.7 %) and the most prominent compounds were β-caryophyllene (24.1 %), with lesser amounts of germacrene D (11.9 %) and bicyclogermacrene (11.8 %).

In another study 60 compounds were identified from volatile and essential oil from the leaves. The major compounds were γ-elemene (31.7 %), perolidol (10 %), t-caryophyllene (11.6 %), caryophyllene oxide (16.6 %), β-caryophyllene (11.8 %), spathulenol (10.2 %), β-elemene (8.9 %), germacrene D (6.9 %) and cyclooctene, 4-methylene-6-(1-propenylidene) (6.4 %) [12]. The major compound (E)-caryo-phyllene was found to posses cytotoxic against MDA-MB-231 and Hs 578T human tumor cells [11]. On the other hand, *M. paniculata* contained abundance of caryophyllene oxide which has antifungal activities [10]. In view of pharmacological activity and industrial potentiality to use the oil of *Murraya paniculata*, it is essential to check the chemical variation in the essential oils of *M. paniculata* grown in different climatic habitats and compare their antimicrobial activity.

Material and methods

Plant material

Fresh leaves of *M. paniculata* were collected from different locations of Kumaun region of Uttarakhand in the month of September. Wild verity was collected from Dogaon district Nanital and domestic verity from Pantnagar district Udham Singh Nagar. Both the plant samples were identified by Dr. D.S. Rawat, Assistant Professor, plant taxonomy, Department of Biological Sciences, G.B. Pant University of Agricultural and Technology, Pantnagar.

Extraction of essential oils

Fresh aerial parts of each collection of *M. paniculata* were subjected to Clevenger's type apparatus for 8 hours separately. Extraction of distillate by diethylether or dichloromethane followed by drying over anhydrous Na_2SO_4 and removal of solvent yielded essential oils. The oil yields were 0.68 % for samples collected from Pantnagar (altitude 300 feet) and 0.54 % for samples collected from Dogaon (altitude 1000 feet). The oil samples were stored at 0°C in air-tight containers for gas chromatography mass spectrometry (GC-MS) analysis.

GC-MS analysis

The essential oil from both the leaves sample was analyzed by GC-MS electron impact ionization (EI) method on GC-Thermo Quest, Trace 2000 coupled with finnigan Mat Polaris Q MS; DB-5 Non-polar silica capillary column (30 m x 0.32 mm i.d.); column temperature 60°C to 210°C at the rate of 3°C/ min; carrier gas, helium at constant pressure of 90 KPa.

The compounds were identified by matching their mass spectra and GC retention indices with those in NIST-MS Wiley library, comparing with literature reported and published data **(Adams, 1995).**

Antimicrobial activity

The essential oil of both sample leaves was screened for its antifungal activity. The two phytopathogenic fungi such as *Rhizoctonia solani* and *Sclerotium rolfsii* were maintained and grown on potato dextrose agar medium. Pathogenicity test was carried out in glasshouse using autoclaved soil filled in 2 kg capacity plastic pots. The soil was artificially infested by inoculums (2 kg) of *R. solani* in top 5 cm soil layer. After seven days, 10 seeds of soybean variety "Bragg" were sown after surface sterilization with mercuric chloride (0.1 %) and washed with distilled water. Pots were watered as and when required to maintain proper moisture level. Observations of diseased seedling were recorded. The symptoms expressed were matched and re-isolated of the causal organism was done PDA slants and was compared with previously isolated fungus as well a symptoms

produced by it.

Sterilized petri plates of 90 mm diameter were used for pouring medium. In each petri plate about 20 ml, sterilized melted medium was aseptically poured near burner flame in a sterilized laminar floor chamber. The medium in the plates were centrally inoculated by placing a 5 mm mycelia disc which was cut from the margin of 5 days old culture of the test fungus. Sterilized three filter paper discs was placed in sterilized petri plates and 5 µl each essential oil was added with the help of sterilized micropipette on each filter paper disc. The plate was sealed with parafilm immediately. Inoculated petri plates were incubated at 28±1°C in a BOD incubator. The growth of the fungus was measured in mm at an interval of 24 hours and percent inhibition of growth was calculated by using the following formula:

$$I = (C-T / C) \times 100$$

I= Inhibition percentage, C= Colony radius in check (mm), T = colony radius in treatments (mm)

Results and discussion

In the present study the essential oils of the two collections of *Murraya paniculata* collected from different locations (Pantnagar and Dogaon) were analyzed by combination of GC and GC-MS (Table 1). The GC-FID analysis revealed the presence of over 10 compounds in each essential oil of

Table 1. Comparative essential oil composition of *Murraya paniculata* from different regions of Uttarakhand

No.	Compounds	RI	*M. Paniculata* (%) (domestic variety)	*M. Paniculata* (%) (wild variety)
1	β-Caryophyllene	1408	3.25	-
2	α-Humulene	1452	4.44	-
3	Germacrene D	1484	21.67	-
4	ar-Curcumene	1479	3.54	-
5	β-Selinene	1489	2.44	-
6	δ-Cadinene	1522	1.62	-
7	α-Cadinene	1537	1.12	-
8	Germacrene B	1559	41.91	2.18
9	β-Bisabolol	1674	1.10	-
10	α-Bisabolol	1684	1.50	-
11	*trans*-Verbenol	1683	-	26.25
12	α-Copaene	1374	-	1.4
13	*E*-Caryophyllene	1417	-	1.79
14	*epi*-Cubebol	1493	-	4.74
15	Germacrene D-4-ol	1574	-	13.74
16	Caryophyllene oxide	1582	-	12.34
17	β-Cuzcumin	1553	-	2.05
18	γ-Eudesmol	1630	-	1.37
19	Cubenol	1645	-	1.20
20	α-Muurolol	1644	-	2.25
21	β-Eudesmol	1649	-	1.55
22	*E,E*-Farnesol	1724	-	2.03
	Total		84.39	76.25
	Monoterpene Hydro carbons (MTHC)		-	-
	Oxygenated Monoterpenes (MTO)		-	-
	Sesquiterpenes Hydro carbons (STHC)		84.39	7.42
	Oxygenated Sesquiterpenes (STO)		2.60	68.83

which over 11-14 constituents have been identified which contribute 86.99 % and 76.25 % of the total oil respectively. Both the collections were dominated by sesquiterpenoids. Sample collected from Dogaon was rich in oxygenated sesquiterpenoids mainly dominated by *trans*-verbenol (26.25 %), germacren-D-4-ol (13.74 %), caryophyllene oxide (12.34 %), *Epi*-cubebol (4.74 %), α-muurolol (2.25 %), *E,E*-farnesol (2.03 %), β-eudesmol (1.55 %), γ-eudesmol (1.37 %) and cubenol (1.20 %) while *Murraya paniculata* (Pantnagar) contained only α-bisabolole (1.50 %) and β-bisabolole (1.10 %) as the major oxygenated sesquiter-penoids but was rich in sesquiterpene hydrocarbons namely germacrene B (41.91 %), germa-crene D (21.67 %), α-hummulune (4.44 %), ar-curcumene (3.54%), β-caryophyllene (3.25 %), β-selinene (2.44 %), δ-cadinene (1.62 %) and α-cadinene (1.12 %). *Murraya paniculata* (Dogaon) contained a few sesquihydrocarbons i.e. germacrene B (2.18 %), β-cuzcumin (2.05 %), E-caryophyllene (1.79 %) and α-copaene (1.4 %). Oxygenated monoterpenoid could not be detected in any collection. The detailed comparative composition of the oils has been presented in Table 1.

The essential oils of these two different collections were also studied for their antifungal activity against *Rhizoctonia solani* and *Sclerotium rolfsii* fungus. The oil of Pantnagar collection did not exhibit antifungal activity against *Rhizoctonia solani* as well as *Sclerotium rolfsii*. In case of *R. solani*, the lowest mean radius for Pantnagar collection was observed at the concentration of 100 % (5.33 mm) while at a concentration of 40 and 80 % (5 mm) in Dogaon collection. However, in case of *Sclerotium rolfsii*, the lowest mean radius of Pantnagar collection was observed at the concentration of 100 % (0 mm) after 24 hrs of incubation whereas Dogaon collection showed no growth (0 mm) at the concentrations i.e. 10, 20, 40, 80 and 100 %. The observations are presented in Table 2-5, indicating the variation of mean radii in mm, % growth of fungal mycelia at different concentrations including controls (C-1 & C-2) and critical differences (cd-1 & cd-5) with coefficient of variations (cv) as obtained by applying statistical analysis"ANOVA".

In our study, a wide variation in the essential oil yield and composition was observed in between the domestic (Pantnagar collection) and wild variety (Daogaon collection) of *M. paniculata*. In domestic variety, the more amounts of sesquiterpene hydrocarbons were reported whereas in wild variety oxygenated sesquiterpenoids was found. It was also reported that the wild variety of *M. paniculata* collected from Dogaon showed better antifungal activity in comparision to domestic variety collected from Pantnagar. This is may be due to the presence of more amounts of oxygen-

Table 2. Antifungal activity of different samples of *Murraya paniculata* against *R. solani* at different concentrations

Sample name	% Growth (mm) of *R. solani* at different concentration during different time interval									
	Time interval	10 %	20 %	40 %	80 %	100 %	C-1	C-2	cd-1	cd-5	cv
MP	24 hr	9.00	8.00	7.33	6.66	5.33	10	10.00	-	-	-
Pant	48 hr	27.66	24.66	21.33	18.33	14.33	27	31.66	-	-	-
	72 hr	40.00	40.00	40.00	40.00	40.00	40	40.00	0	0	0
MP	24 hr	7.8.00	6.00	5.00	5.00	5.50	10	10.00	-	-	-
Dogaon	48 hr	21.00	20.00	18.50	16.50	14.00	27	31.66	-	-	-
	72 hr	30.00	28.50	31.00	21.50	21.00	40	40.00	1.452	1.046	1.973

C-1= Control (acetone); C-2= Blank (without aceton)
cd-1= Critical difference at 1%; cd-5= Critical difference at 5%
cv= Coefficient of variation

ated sesquiterpenoids found in wild variety. Finally, we concluded that the essential oil of *M. paniculata* (Dogaon) sample under study has significant antifungal activity, hence can be used as antifungal agent against the tested organism/fungi after its proper clinical trials while *M. paniculata* (Pantnagar) has non-significant antifungal activity and cannot be used as antifungal agent.

Table 3. Antifungal activity of different samples of *Murraya paniculata* against *Sclerotium rolfsii* at different concentrations

Sample name	Time interval	% Growth (mm) of *Sclerotium rolfsii* at different concentration during different time interval									
		10 %	20 %	40 %	80 %	100 %	C-1	C-2	cd-1	cd-5	cv
MP	24 hr	8.33	6.33	5.33	4.00	-	4.00	4.30	-	-	-
Pant	48 hr	21.00	16.33	13.66	10.33	8.00	17.30	17.00	-	-	-
	72 hr	35.66	27.00	24.00	21.66	18.33	25.00	25.00	-	-	-
	96 hr	40.00	40.00	40.00	40.00	40.00	40.00	40.00	0	0	0
MP	24 hr	-	-	-	-	-	4.00	4.3.00	-	-	-
Dogaon	48 hr	-	-	-	-	-	17.30	17.00	-	-	-
	72 hr	36.00	29.00	18.00	13.00	8.00	25.00	25.00	-	-	-
	96 hr	40.00	34.50	28.50	18.00	13.50	40.00	40.00	1.215	0.876	1.632

C-1= Control (acetone); C-2= Blank (without acetone)
cd-1= Critical difference at 1%; cd-5= Critical difference at 5%
cv= Coefficient of variation

Table 4. Effect of essential oils from different samples of *Murraya paniculata* on growth (%) of *R. solani* at different concentrations after 72 hrs

No.	Sample name	After 72 hrs									
		10 %		20 %		40 %		80 %		100 %	
		M.R (mm)	I (%)	M.R (mm)	I (%)	M.R (mm)	I (%)	M.R (mm)	I (%)	M.R (mm)	I (%)
1	MP Pant	40	0	40.0	0	40	0	40.0	0	40	0
2	MP DG	30	25	28.5	28.75	31	22.5	21.5	46.25	21	47.5

M.R: Mean radius of fungal growth; I (%): Growth of inhibition of fungus

Table 5. Effect of essential oils from different samples of *Murraya paniculata* on growth (%) of *S. rolfsii* at different concentrations after 96 hrs

No.	Sample name	After 96 hrs									
		10 %		20 %		40 %		80 %		100 %	
		M.R (mm)	I (%)	M.R (mm)	I (%)	M.R (mm)	I (%)	M.R (mm)	I (%)	M.R (mm)	I (%)
1	MPPant	40	0	40.0	0	40.0	0	40	0	40.0	0
2	MPDG	40	0	34.5	13.75	28.5	28.75	18	55	13.5	66.25

M.R: Mean radius of fungal growth; I (%): Growth of inhibition of fungus

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