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GC-MS analysis, Antibacterial, Antioxidant and Brine Shrimp Lethality Assay of *Murraya koenigii* Spreng

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Abstract
Extraction of essential oil of leaves of *Murraya koenigii* Spreng. was done by hydro distillation in Clevenger apparatus. Also, it was subjected to extraction with methanol. The composition of essential oil so collected were determined by GC-MS system and showed the presence of 23 different compounds. Caryophyllene (27.89%), *α*-Pinene (14.22%), Caryophyllene oxide (7.74%), *α*-Caryophyllene (7.25%), *β*-Pinene (6.01%), *α*-Selinene (5.36%) and D-Limonene (5.04%) were the most abundant. Antibacterial, antioxidant activity and brine shrimp lethality assay were studied. Oil exhibited moderate antibacterial activity. Extract showed efficient DPPH antioxidant activity with IC_{50} 93.2 ± 7.5µg/mL. From Brine shrimp lethality assay, the LD_{50} of the sample of plant was found to be 125µg/mL.

Keywords: *Murraya koenigii* Spreng. Essential oil, GC-MS, Activity

Introduction
Murraya koenigii Spreng. belongs to the family Rutaceae, and commonly known as curry leaves, is mostly cultivated for the aromatic and appetizing nature of its leaves.[1] *Murraya koenigii* Spreng. is called as Karipatta in Nepali. It is distributed in tropical and subtropical region of Himalaya of Nepal, India, Srilanka, Myanmar, China. These plants are trees with pinnate, leaflets, alternate, entire, aromatic, glandular and glaborous leaves. The inflorescence is corymb [2, 3, 4].
Curry leaf was found to contain eight carbazole alkaloids; from bark: mahanimbine, girinimbine, murrayacine, murrayazoline and murrayanine; from leaves: mahanimbine and mahanine; and from roots: girinimbinemurrayanine, 3-methylcarbazole and murrayafoline-A [1]. *β*-caryophyllene, bicyclogermacrene, *α*-cadinol, caryophyllene epoxide, *α*-pinene, *β*-pinene, limonene, terpinen-4-ol, *γ*-terpinene, *β*-Selinene, bornyl acetate and humulene are the major constituents [4]. Phytochemical investigation revealed the presence of amino acids in the leaves. The amino acids namely Proline and its analog N-Methyl Proline were reported to have antioxidant and a number of other pharmacological activities [5].
Root is purgative. Leaves and root are externally used to cure bites of poisonous animals. Plant is useful in hyperdipsia, burning sensation, leprosy, skin diseases, anorexia, helminthiasis, colic, dysentery, vomiting, inflamations [3]. Constituents of Karipatta shows anticancer, hepatoprotective, antipyretic, antioxidant, anti-obesity, antimutagenic, antimicrobial, antifungal, insecticidal, antibacterial effect [4].

Experimental
Collection of Plant Materials
The plant material (leaves) was collected from Kathmandu, Nepal. The plants were identified by Department of Botany, Amrit Campus, Lainchour, Kathmandu.

Preparation of Plant Extracts
The clean and dried leaves were grinded to powder and further proceeded via cold percolation process for 7 days for three times with 2.5 liters methanol. The methanol extract was concentrated by evaporation on rotavapour. Plants extracts were stored at 4°C.

Extraction of Essential Oil
The mature leaves of *Murraya koenigii* were crushed for hydro distillation and subjected to a Clevenger apparatus for three hours. By this process about 3.2 ml of yellow coloured essential oils were collected and stored in a sealed glass vials at low temperature (0-4ºC) prior to analysis.

GC-MS Analysis

The essential oils sample of *Murraya koenigii* was subjected to GC-MS analysis. GC-MS analysis was performed on a gas chromatography mass spectrometer GCMS-QP2010 under the following condition: injection volume 1µL with split ratio 1:50; Helium as a carrier gas with a Rtx-5MS column of dimension 30m×0.25mm×0.25µm, temperature programmed at 40, 200 and 280°C with a hold time of 2.0, 3.0 and 4.0 min identification was accompanied by comparison of MS with those reported in NIST 05 and FFNSCI.3 libraries. It was performed in Department of Food Technology and Quality Control, Nepal Government, Babarmahal, Kathmandu, Nepal.

Antioxidant Assay (DPPH method)

First of all, 1mg of sample to be tested was dissolved in 1ml methanol to get stock solution of concentration 1mg/ml. 100µl of these solutions were added to 100µl of 0.1mM DPPH (prepared in methanol) and was left for 30 minutes in dark room. After 30 minutes, their absorbance was taken at 517 nm against DPPH and DMSO as a blank. Quercetin was prepared as standard. The extracts or essential oil, which do not show antioxidant property was discarded and for the sample with the yellow colour (more than 50% inhibition then control) was taken for further testing as they were expected to be the potential antioxidants.

Different concentration of the extracts were prepared by two-fold dilution method to find the IC_{50} value.

Antibacterial activity assays

Antimicrobial assay of extracts of plants was performed by agar well diffusion method in Muller Hilton Agar (MHA) and the minimum bactericidal concentration of those extract was determined by micro dilution method. All the strains of bacteria was cultured in Nutrient broth (NB) and incubated at 37^0C for 18 hours. After incubation each stain were diluted with sterile distilled water. The turbidity of dilution was compared with 0.5 McFarland standards (approximately 10^8 CFU/ml). The suspensions were then diluted (1:100) in Muller Hilton Broth (MHB) to obtain 10^6 CFU/ml. Prepared inoculums were incubated for 30 minutes at 37 ^{0}C prior to use.

Plant extracts (30 µl) were loaded into the respective wells with the help of micropipette. The solvent (50% DMSO) was tested for its activity as a control at the same time in the separate well. The Neomycin 20 µg/ml was used as a positive control. The plates were then left for half an hour with the lid closed so that extracts diffused to the media. The plates were incubated overnight at 37 °C. After proper incubation (18-24 hours) the plates were observed for the zone of inhibition around well which is suggested by clean zone without growth. The ZOI were measured with the help of the ruler and mean was recorded for the estimation of potency of antibacterial substance.

Determination of Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) was determined by micro dilution method. The methanol extracts were diluted by two fold to get series of concentrations from 0.048 to 25 mg/ml in freshly prepared sterile nutrient broth. 20µl of the microorganism suspension (correspond to 10^6 CFU/ml) was added to each of the sample dilutions. These were incubated for 18 hours at 37°C and each tube content was subculture in fresh nutrient agar separately and minimum bactericidal concentration was determined that showed no growth at all.

Determination of the Minimum Inhibitory Concentration

The smallest amount of compounds required to kill or inhibit the growth of micro-organism *in vitro* can be determined by the dilution method. This amount is referred as minimum inhibitory concentration (MIC). It is a measure of potency which is expressed in terms of either µg or mg/ml. A stock solution of 25 mg/ml was prepared. This was serially diluted to obtain various ranges of concentrations between 25 mg/ml to 0.048 mg/ml.

Brine Shrimp Lethality Assay

Ten nauplii were exposed to each of different concentrations of the plant extract and number of survivors were calculated the percentage of death after 24 hours.

Result and Discussion

GC-MS Analysis

GC-MS analysis of essential oils of *Murraya koenigii* shows the presence of 23 different compounds. The chemical compound identified in essential oils of the leaves of the *M. koenigii* plant are presented below:

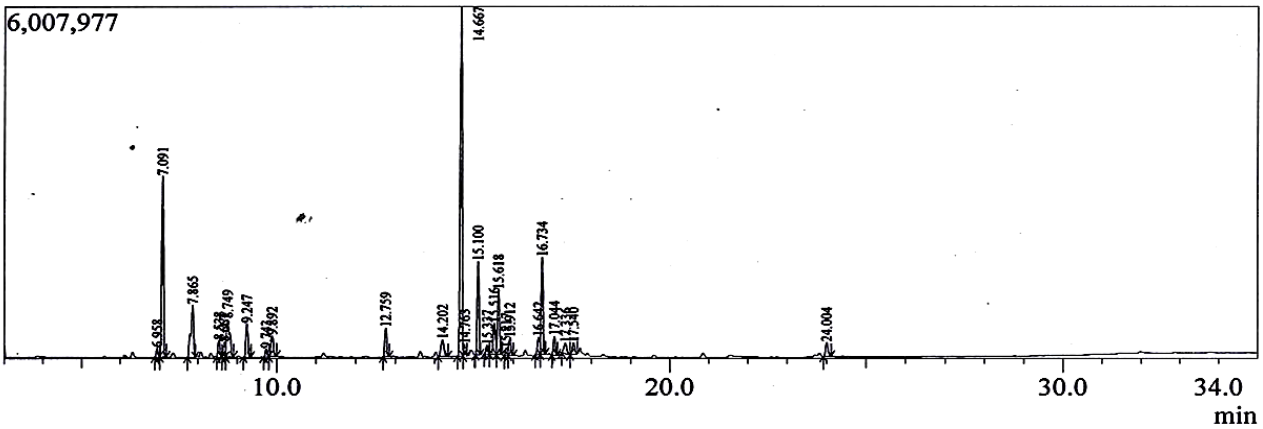

Fig 1: Chromatogram of essential oils of *M. koenigii.*

The major constituents present in the essential oils of *M. koenigii* were Caryophyllene (27.89%), *α*-Pinene (14.22%), Caryophyllene oxide (7.74%), *α*-Caryophyllene (7.25%), *β*-Pinene (6.01%), *α*-Selinene (5.36%) and D-Limonene (5.04%). Constituents of essential oils of *Murraya koenigii* (L.) are tabulated as follows.

Table 1: List of compounds in essential oils of *M. koenigii*

S.N.	Name of the compounds	Molecular Formula	Molecular Weight	Retention Time	Area %	Height %
1.	α-Phellandrene	$C_{10}H_{16}$	136	6.958	0.70	0.77
2.	α-Pinene	$C_{10}H_{16}$	136	7.091	14.22	15.33
3.	β-Pinene	$C_{10}H_{16}$	136	7.865	6.01	4.48
4.	2-Carene	$C_{10}H_{16}$	136	8.538	1.32	1.42
5.	o-Cymene	$C_{10}H_{14}$	134	8.667	1.16	1.27
6.	D-Limonene	$C_{10}H_{16}$	136	8.749	5.04	3.26
7.	γ-Terpinene	$C_{10}H_{16}$	136	9.247	2.66	2.86
8.	Terpinolene	$C_{10}H_{16}$	136	9.743	0.63	0.68
9.	β-Linalool	$C_{10}H_{18}O$	154	9.892	1.79	1.79
10.	Bornyl acetate	$C_{12}H_{20}O_2$	196	12.759	2.32	2.49
11.	β-Bourbonene	$C_{15}H_{24}$	204	14.202	1.99	1.43
12.	Caryophyllene	$C_{15}H_{24}$	204	14.667	27.89	29.63
13.	Germacrene	$C_{15}H_{24}$	204	14.763	0.87	0.99
14.	α-Caryophyllene	$C_{15}H_{24}$	204	15.100	7.25	7.96
15.	γ-Muurolene	$C_{15}H_{24}$	204	15.337	0.82	0.76
16.	β-Selinene	$C_{15}H_{24}$	204	15.516	2.79	2.68
17.	α-Selinene	$C_{15}H_{24}$	204	15.618	5.36	5.52
18.	γ-Cadinene	$C_{15}H_{24}$	204	15.817	0.69	0.62
19.	δ-Amorphene	$C_{15}H_{24}$	204	15.912	1.52	1.50
20.	Spathulenol	$C_{15}H_{24}O$	220	16.642	1.73	1.49
21.	Caryophyllene oxide	$C_{15}H_{24}O$	220	16.734	7.74	8.26
22.	Caryophyllene oxide	$C_{15}H_{24}O$	220	17.044	1.57	1.63
23.	Viridiflorol	$C_{15}H_{26}O$	222	17.336	1.23	0.93
24.	Viridiflorol	$C_{15}H_{26}O$	222	17.540	1.32	1.01
25.	Phytol	$C_{20}H_{40}O$	296	24.004	1.38	1.22
					100.00	100.00

Antibacterial activity

Table 2: Antibacterial activity of the extract of *M. koenigii*.

Sample	MIC values		MBC Values	
	MRSA	KP	MRSA	KP
Neomycin* (µg/ml)	0.156	0.0195	5	0.156
Extracts (mg/ml)	1.56	0.048	6.25	6.25

*Control Antibiotics
MRSA = Methicillin resistance *Staphalococcus aureus* (MRSA)
KP = *Klebsiella pneumoniea* (MDR)

Antioxidant Activity

The antioxidant potential is in an inverse relation with IC_{50} value, which can be calculated from linear regression of the % inhibition verses antioxidant activity. Lower the IC_{50} value indicates high antioxidant activity.

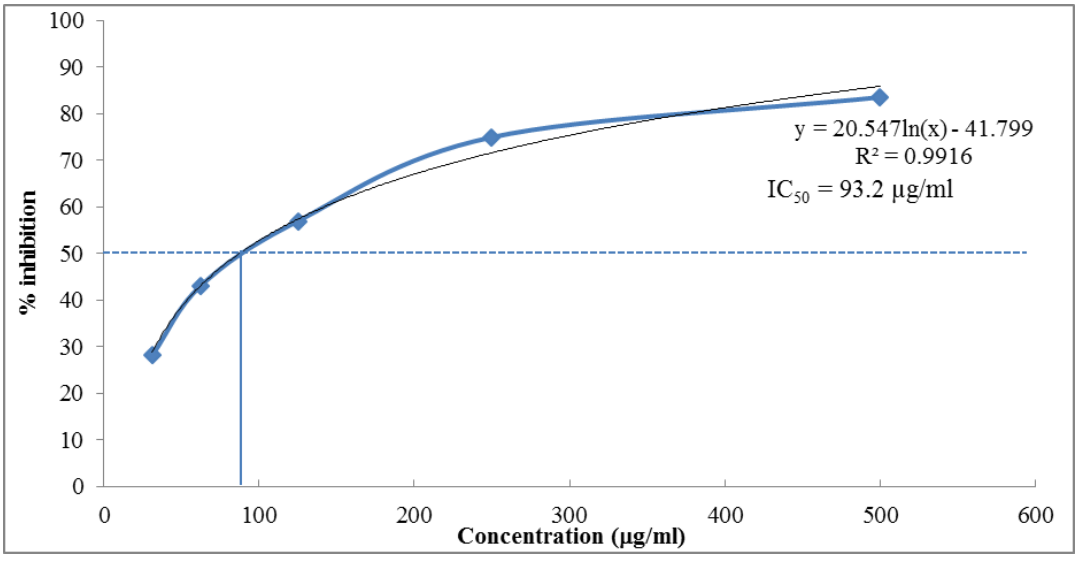

Fig 2: Graphical representation of DPPH assay of the methanolic extract of *M. koenigii*.

The IC_{50}±SEM of the extract was found to be 93.2 ± 7.5µg/mL and the standard, quercetin was 2.28±0.1µg/mL.

Brine Shrimp Lethality Assay

The LD_{50} of the sample extract was found to be 125 µg/mL in Brine shrimp lethality assay.

Conclusion

Caryophyllene (27.89%), *α*-Pinene (14.22%), Caryophyllene oxide (7.74%), *α*-caryophyllene (7.25%), *β*-Pinene (6.01%), *α*-Selinene (5.36%) and D-Limonene (5.04%) were the major constituents present in the essential oils of *M. koenigii*. Plant extract was effective against Methicillin resistance *Staphalococcus aureus* (MRSA) and *Klebsiella pneumoniae* (MDR). The IC_{50}±SEM of the extract was found to be 93.2 ± 7.5µg/mL. In Brine shrimp lethality assay, the LD_{50} of the sample was found to be 125 µg/mL.

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