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ANTIBACTERIAL ACTIVITY OF LEAVES OF *MURRYA KOENIGII* (LINN.) SPRENG WETTST

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ABSTRACT
Antibacterial activity of methanol, ethanol, petroleum ether and acetone extracts of leaves of *Murrya koenigii* Linn. Spreng Wettst (*M. koenigii* L.), a medicinal plants of North Eastern Himalaya, was evaluated against four Gram - positive bacteria viz. *Bacilus megaterium, Staphylococcus aureus, Bacilliu subtilis* and *Streptococcus pyogenes* as well as four Gram- negative bacteria like *Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae* and *Salmonella typhi.* Disc diffusion technique was used for *in vitro* antibacterial screening. Result showed that all extracts showed anti bacterial activity but the acetone extract of leaves of *M. koenigii* L., had larger zone of inhibition in disc diffusion against the said bacteria. Antibacterial activity was more in Gram - positive bacteria than in Gram - negative bacteria. Highest activity was noted against *Bacilus megaterium* and lowest activity was found for *Pseudomonas aeruginosa*. The MIC (minimum inhibitory concentration) values of extract of leaves of *M. koenigii* L. against the bacteria ranged from 8 – 64 microgram/mL. Results, therefore, suggests that acetone extract of *M. koenigii* L had good anti bacterial activity against the tested bacteria.

KEYWORDS: Antibacterial activity, *Murrya koenigii* Linn., Disc diffusion technique, Zone of inhibition, Minimum inhibitory concentration.

1. INTRODUCTION
Infectious diseases are the world's leading cause of premature deaths. It is estimated that infectious diseases kill almost 50,000 people every day. A large number of antibacterial agents have been discovered but pathogenic bacteria are constantly developing resistance to these agents. Due to this, life threatening bacterial infection has been increased worldwide and is becoming an important cause of morbidity and mortality.[1] Under the circumstances, search for antibacterial agent is going on and is extended even in the field of medicinal plants to develop safer antibacterial drugs.[2]

M. koenigii L (family, Rutaceae) is a medicinal plant widely distributed at foothills of Himalayas from Kumaon to Sikkim, Bengal, Assam, middle and lower hill forests up to the height of 5000 ft. It is a small tree with dark green bark, often cultivated. February to May is the flowering time of the plant. The plant has several names. In Nepali it is called 'meehi saag', in Hindi 'bursunga' and in English it is known as 'curry leaf tree'. Leaves of the plant are often used in curries for flavouring due to their typical flavour.

In traditional medicine *M. koenigii* L. has several uses. Leaves and roots are used to treat kidney pain, piles, leukoderma and blood disorders. Burk is used to cure eruptions, poisonous animal bites etc. The plant is also used for its stomachic and tonic properties.[3, 4] Several bio active compounds have been isolated and characterized from this plant. These bioactive compounds possess antidiarrheal, hepatoprotective, antimicrobial, anthelmintic, anti ulcer, antioxidant, analgesic, cytotoxic, antitumor and anti inflammatory properties.[5] We also reported anti gastric ulcer activity[6,7] and anti oxidative activity[8] of *M. koenigii* L. leaves in albino rats.

The crude extract of *M. koenigii* roots[9] and chloroform soluble fraction of *M. koenigii* leaves[10] showed strong antibacterial activity. The present work was aimed to see the antibacterial activity of leaves of *M. koenigii* L. of this region against a number of gram positive and gram negative bacteria.

2. METHODOLOGY
2.1 Collection of plant materials
Leaves of *M. koenigii* L. were collected in morning hours (9 – 10 AM) from the medicinal plants garden of the

University of North Bengal, Dist. Darjeeling, west Bengal, India randomly. Leaves were authenticated by the taxonomist of the department of Botany of the said University. Voucher specimens of the leaves were deposited in the department for future references.



***Murrya koenigii* Linn. Spreng Wettst**

2.2 Preparation of leaves for Anti bacterial screening

Leaves of *M. koenigii* L. were washed thoroughly, shed dried and powered. 10 grams of this powder was extracted separately with 100 ml of methanol, ethanol, petroleum ether and acetone in soxhlet at 37^0C for 15 minutes. The whole extract was filtered and the solvent was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50 ^{0}C. A brownish mass was obtained. 500 micro gram of the mass was extracted with 1 ml water and the solution obtained was used to evaluate the anti bacterial activity against the tested bacteria.

2.3 Bacteria

Four Gram - positive bacteria *viz. Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus* and *Streptococcus pyogenes* and four Gram-negative bacteria *viz. Shigella dysenteriae, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* were employed to determine antibacterial activity and minimum inhibitory concentration. All these bacteria were collected from the department of Microbiology, North Bengal Medical College Hospital.

2.4 Media

Nutrient agar media (Difco laboratories) pH 7.2 and nutrient broth media (Difco laboratories) pH 6.8 were used for antibacterial screening and minimum inhibitory concentration determination.

2.5 Antibacterial screening

In vitro antibacterial screening was carried out by disc diffusion method.[11] According to this method, 20 ml quantities of nutrient agar were placed in a petri dish with 0.1 ml of 10^{-2} dilution of bacterial culture of 20 hours old. Filter paper discs (6 mm diameter) impregnated with 60 µg per disc and 120 µg per disc concentration of the solution prepared from *M. koenigii* L. were placed on test bacteria-seeded plates. Blank disc impregnated with water was used as negative control. Zone of inhibition was recorded after 18 hours of incubation. Diameters of zone of inhibition produced by the solution prepared from *M. koenigii* L. were compared with that of standard antibiotic kanamycin 40 µg per disc, Each sample was used for five times for the determination of anti bacterial activity.

2.6 Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration is defined as the lowest concentration of antibiotic completely inhibiting visible growth of bacteria after 18 – 24 hours of incubation at 37 ^{0}C. This was done by the method of Mosaddik and Haque.[12] According to this method, extract of *M. koenigii* L. (1.0 mg) was dissolved in 2 ml nutrient broth media to obtain a stock solution of concentration 500 µg/ml. 3 drops of Tween 80 was added in nutrient broth to facilitate dissolution. Serial dilution technique was followed to obtain 250 µg/ml concentration of the compound. One drop (0.02ml) of prepared suspensions of organism (10^6 organism/ml) was added to each broth dilution. These dilutions were then incubated for 20 hours at 37^0C. Growth of bacteria was examined by noting turbidity of the solution. The nutrient broth media with 3 drops of Tween 80 was used as negative control while kanamycin was used as positive control.

2.7 Statistical analysis

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20^{th} versions. Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

3. RESULTS

In vitro antibacterial activity of methanol extract of *M. koenigii* L. is given in Table – 1. Results were compared with standard antibiotic kanamycin. Results showed that methanol extract of *M. koenigii* L. exerted anti bacterial activity at 40 µg per disc concentrations for all the tested bacteria but the activity was much lower than that of reference drug kanamycin at same concentration. Zone of inhibition in disc diffusion was noted. Antibacterial activity was more in Gram - positive bacteria than Gram - negative bacteria. Highest activity was noted against Gram.

Table 1: *In vitro* antibacterial activity of the methanol extract of *M. koenigii* L. and kanamycin [Zone of inhibition (diameter in mm)].

Bacteria	Strain	*M. koenigii* L. (40 µg per disc)	Kanamycin (40 µg per disc)
Gram – positive			
Bacillus subtilis	ATCC 19659	16 ± 0.6	36 ± 1.0
Bacillus megaterium	NBMC 1122	18 ± 1.0	35 ± 1.0
Staphylococcus aureus	ATCC 25923	15 ± 1.0	38 ± 1.1
Streptococcus pyogenes	NBMC 1321	12 ± 0.6	30 ± 0.8
Gram – negative			
Escherichia coli	ATCC 25922	11 ± 1.0	27 ± 1.0
Shigella dysenteriae	NBMC 1127	12 ± 1.0	30 ± 1.0
Pseudomonas aeruginosa	NBMC 1243	10 ± 1.1	31 ± 1.1
Salmonella typhi	MTCC 733	13 ± 0.6	32 ± 1.1

Data was in mean SEM (n = 5). Control was made with water. It had no zone of inhibition. So data has not been shown. positive *Bacillus megatorium* and lowest activity was for Gram negative *Pseudomonas aeruginosa.*

Table -2 showed *In vitro* antibacterial activity of ethanol extract of *M. koenigii* L. Kanamycin was used as standard antibiotic. It was found out that ethanol extract of *M. koenigii* L. exerted anti bacterial activity at 40 µg per disc concentrations for all the tested Gram positive and Gram negative bacteria. The activity, however, was lower than that of reference drug kanamycin at same dose, 40 µg per disc concentration. In disc diffusion technique zone of inhibition was

Table 2: *In vitro* antibacterial activity of the ethanol extract of *M. koenigii* L. and kanamycin [Zone of inhibition (diameter in mm)].

Bacteria	Strain	*M. koenigii* L. (40 µg per disc)	Kanamycin (40 µg per disc)
Gram – positive			
Bacillus subtilis	ATCC 19659	17 ± 0.7	37 ± 1.1
Bacillus megaterium	NBMC 1122	20 ± 1.0	35 ± 0.9
Staphylococcus aureus	ATCC 25923	16 ± 0.8	39 ± 1.0
Streptococcus pyogenes	NBMC 1321	17 ± 0.7	32 ± 0.9
Gram – negative			
Escherichia coli	ATCC 25922	14 ± 0.9	26 ± 0.9
Shigella dysenteriae	NBMC 1127	13 ± 1.0	29 ± 1.0
Pseudomonas aeruginosa	NBMC 1243	12 ± 1.0	30 ± 1.2
Salmonella typhi	MTCC 733	13 ± 0.7	31 ± 0.8

Data was in mean SEM (n = 5). Control was made with water. It had no zone of inhibition. So
data has not been shown.

developed. Antibacterial activity was more in Gram - positive bacteria than Gram - negative
bacteria. Highest activity was noted against *Bacillus megatorium* followed by *Bacillus subtilis, Staphylococcus aureus* etc. Among Gram negative bacteria lowest activity was found for *Pseudomonas aeruginosa.*

Table 3: *In vitro* antibacterial activity of the petroleum ether extract of *M. koenigii* L. and kanamycin [Zone of inhibition (diameter in mm)].

Bacteria	Strain	*M. koenigii* L. (40 µg per disc)	Kanamycin (40 µg per disc)
Gram – positive			
Bacillus subtilis	ATCC 19659	17 ± 0.6	36 ± 0.9
Bacillus megaterium	NBMC 1122	22 ± 0.7	34 ± 0.8
Staphylococcus aureus	ATCC 25923	15 ± 0.8	38 ± 1.1
Streptococcus pyogenes	NBMC 1321	19 ± 0.6	34 ± 0.8
Gram – negative			
Escherichia coli	ATCC 25922	16 ± 0.7	26 ± 0.8
Shigella dysenteriae	NBMC 1127	15 ± 0.8	29 ± 1.1
Pseudomonas aeruginosa	NBMC 1243	14 ± 0.9	30 ± 1.0
Salmonella typhi	MTCC 733	15 ± 0.6	31 ± 0.7

Data was in mean SEM (n = 5). Control was made with water. It had no zone of inhibition. So data has not been shown.

Kanamycin was used as standard antibiotic. Results showed that petroleum ether extract of *M. koenigii* L. exerted anti bacterial activity at 40 µg per disc concentrations for all the tested bacteria but the activity was lower than that of reference drug kanamycin in same dose. Zone of inhibition in disc diffusion technique suggested that in Gram - positive bacteria antibacterial activity of the plant leave was maximum against *Bacillus megatorium* and for Gram negative bacteria it was against *Escherichia coli.* Lowest activity was, however, found for *Pseudomonas aeruginosa* among Gram negative bacteria and *Staphylococcus aureus* among Gram positive bacteria.

Table 4: *In vitro* antibacterial activity of the acetone extract of *M. koenigii* L. and kanamycin [Zone of inhibition (diameter in mm)].

Bacteria	Strain	*M. koenigii* L. (40 µg per disc)	Kanamycin (40 µg per disc)
Gram – positive			
Bacillus subtilis	ATCC 19659	26 ± 0.8	32 ± 1.0
Bacillus megaterium	NBMC 1122	33 ± 1.1	35 ± 1.0
Staphylococcus aureus	ATCC 25923	25 ± 1.0	30 ± 1.1
Streptococcus pyogenes	NBMC 1321	22 ± 0.7	29 ± 0.8
Gram – negative			
Escherichia coli	ATCC 25922	25 ± 1.0	26 ± 1.0
Shigella dysenteriae	NBMC 1127	26 ± 1.1	29 ± 1.0
Pseudomonas aeruginosa	NBMC 1243	18 ± 1.0	28 ± 1.1
Salmonella typhi	MTCC 733	27 ± 0.7	30 ± 1.1

Data was in mean SEM (n = 5). Control was made with water. It had no zone of inhibition. So data has not been shown.

Table-4 showed *In vitro* antibacterial activity of acetone extract of *M. koenigii* L. leaves and kanamycin. Acetone extract of *M. koenigii* L. exerted anti bacterial activity at 40 µg per disc concentrations for all the Gram positive and Gram negative bacteria. Activity was comparable to that of reference drug kanamycin in 40 µg per disc concentration. In disc diffusion method zone of inhibition indicated that antibacterial activity of acetone extract of *M. koenigii* L. leaves was more in Gram - positive bacteria than Gram - negative bacteria. Highest activity was noted against *Bacillus megatorium* and lowest was found for *Pseudomonas aeruginosa.*

Table – 5 indicates results of minimum inhibitory concentration of acetone extract of *M. koenigii* L. leaves and kanamycin. The MIC (Minimum Inhibitory Concentration) of acetone extract of *M. koenigii* L. leaves ranged from 8 to 32 for Gram positive bacteria and 16 to 64 microgram/mL for Gram negative bacteria respectively. For kanamycin MIC value for Gram positive bacteria was found to vary between 2 and 8 and for Gram positive bacteria it came between 4 and 16

Table 5: Minimum inhibitory concentration of *M. koenigii* L. and kanamycin.

Bacteria	MIC values of *M. koenigii* L. (microgram/mL)	MIC values of kanamycin (microgram/mL)
Gram – positive		
Bacillus subtilis	16	2
Bacillus megaterium	8	4
Staphylococcus aureus	16	8
Streptococcus pyogenes	32	8
Gram – negative		
Escherichia coli	16	8
Shigella dysenteriae	32	4
Pseudomonas aeruginosa	64	16
Salmonella typhi	16	8

Negative control containing water had no MIC value. Thus, it has not been shown

4. DISCUSSION

Several medicinal plants showed antimicrobial property.[13-15] Akerel and Ayinde showed antibacterial activity of the volatile oil and aqueous extract of *Murraya koenigii* leaves. Essential oil and aqueous extract of leaf were found active against *Staphylococcus epidermidis, S. aureus* and streptococcus species[16] Murrayanol, mahanimbine, and mahanine isolated from fresh leaves of *M. koenigii* L. showed anti microbial activity.[17]

In the present study antibacterial activity of *M. koenigii* L. leaves was observed against four Gram - positive bacteria viz. *Bacilus megaterium, Staphylococcus aureus, Bacilliu subtilis* and *Streptococcus pyogenes* as well as four Gram- negative bacteria like *Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae* and *Salmonella typhi.* Maximum antibacterial activity was found by the acetone extract of *M. koenigii* L. leaves when compared with methanol, ethanol and petroleum ether extract of the plant leaves (Figures – 1& 2). Argal et al.[18] and Harbi et al.[19] also observed anti bacterial activity of methanol and ethanol extract of *M. koenigii* L. leaves but they have not studied anti bacterial activity of acetone extract of the leaves.

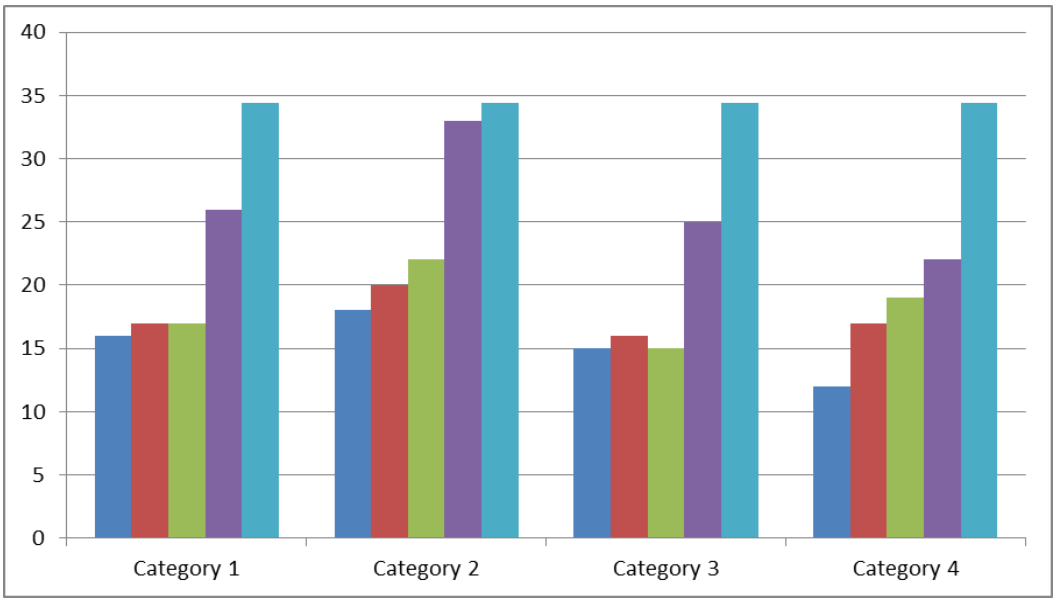


Figure 1: Showing anti bacterial effect [Zone of inhibition (diameter in mm)] of different solvent extract of *M. koenigii* L. leaves on Gram positive bacteria.

Category 1: *Bacillus subtilis*, Category 2: *Bacillus megaterium*, Category 3: *Staphylococcus aureus*, Category 4: *Streptococcus pyogenes*

■ Methanol ■ Ethanol ■ Petroleum ether ■ Acetone ■ Kanamycin (Mean value)

Present study shows that acetone extract of *M. koenigii* L. has maximum anti bacterial activity which is comparable to that of standard antibiotic kanamycin. This indicates that the anti bacterial factor present in *M. koenigii* L. leaves may act as antibiotic in future. Therefore, isolation of this anti bacterial factor from *M. koenigii* L. leaves is important specially in view of bacterial resistant to antibiotics globally.

It is known that season can change biological activity of plants.[20-22] Studies in relation to effect of season on anti

bacterial activity of *M. koenigii* L. leaves are now in progress in our laboratory.

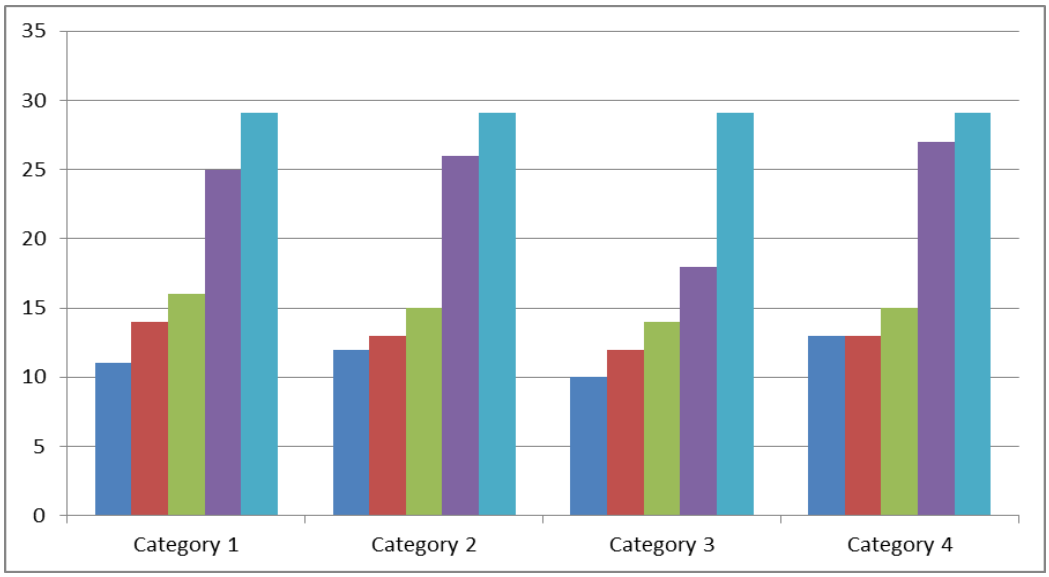


Figure 2: Showing anti bacterial effect [Zone of inhibition (diameter in mm)] of different solvent extract of *M. koenigii* L. leaves on Gram negative bacteria.

Category 1: *Excherichia coli*, Category 2: *Shigella dysenteriae*, Category 3: *Pseudomonas aeruginosa*, Category 4: *Salmonella typhi*
■ Methanol ■ Ethanol ■ Petroleum ether ■ Acetone ■ Kanamycin (Mean value)

5. CONCLUSION

Present study showed that acetone extract of *M. koenigii* L. leaves possess anti bacterial effect against number of gram positive and gram negative bacteria which is comparable to that of standard antibiotic kanamycin. Effort should, therefore, be given to isolate the antibacterial compound present in *M. koenigii* L. leaves which may be included in the list of antibiotic in future.

Conflict of interest: Nil.

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