PHYTOCHEMICAL AND ANTIMICROBIAL STANDARDIZATION OF THE METHANOLIC LEAF EXTRACTS OF *MURRAYA KOENIGII* LINN.

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ABSTRACT

Antibacterial activity of methanolic leaf extract of *Murraya koenigii* was tested against five strains of both Gram positive and Gram negative bacteria viz. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholera and Salmonella typhi* by agar disc diffusion method and broth dilution. The susceptibility of the microorganisms to the extracts was compared with each other and with pure antibiotics Gentamycin. The result showed that, the methanolic extracts exhibited high activity against the common gastrointestinal pathogens. The minimum inhibitory concentration (MIC) of the methanolic extract was in the range 4-64 mg/mL With *S.typhi* being the most susceptible showing a zone of inhibition (ZOI) of 7mm at 4mg/mL. The phytochemical analysis carried out revealed the presence of carbohyrdates, proteins, oils, lipids, glycosides, alkaloids and steroids and absence of tannins, flavanoids and saponins.

1. INTRODUCTION

Medicinal plant-based drugs owe the advantage of being simple, effective and exhibit broad spectrum activity and also have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Indigenous systems of medicine that use plant-based drugs could provide both concepts of therapy as well as therapeutic agents to complement modern medicine in management of diseases and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index**.** Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs in the pharmaceutical industry (Baker et al., 1995). It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents (Vlietinck et al., 1995).

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased (Nascimento et al., 2000). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with

reduced susceptibility to antibiotics raises the spectre of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradzki et al., 1999). Diarrhoeal diseases continue to be a major cause of morbidity and mortality throughout the world. Thus, their treatment by using medicinal plant is an important public health issue. Medicinal properties of plants are due to the active chemical constituents present in different parts of the plant (Mitscher et al., 1980, Bajracharya et al., 2008).

Murraya koenigii Linn. Spreng belonging to family Rutaceae, commonly known as "Curry patta", is an aromatic plant that has been widely used in India as Ayurvedic herbal medicine. Since antiquity, it is used to treat a wide array of unrelated ailments that include dysentery, diarrhoea, microbial growth and stomach ache. Of the fourteen global species belonging to the genus *Murraya*, only two are available in India, viz. *Murraya koenigii* Spreng and *Murraya paniculata* Linn. Jack, (syn. with *M. exotica* Linn). The former is more popular due to its large spectrum of medicinal properties and also because of the use of its leaves for centuries as a natural flavouring agent in various curries and food items (Nayak et al., 2010). Triterpenoid alkaloids cyclomahanimbine, tetrahydromahanmbine, murrayastine, murrayaline, pypayafolinecarbazole alkaloids and many other chemical compounds have been reported in the leaves of *Murraya koenigii* (Chakraborty and Das, 1968; Kureel et al., 1969).

Thus the present study aims to evaluate the antimicrobial potential of *Murraya koenigii* leaf extracts to treat gastrointestinal infection against pathogenic bacteria such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Vibrio cholerae* as most of the pathogens develop drug resistance against commonly used antibiotics.

2. MATERIALS AND METHODS

Collection of Plant material: The fresh and tender leaves were collected, dried in a shade under room temperature for six to seven days and then crushed into coarse powdery substance by using electric grinder. The coarse powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until required.

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***Extract Preparation*:** 50 g of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using ~350 mL methanol. The extract obtained was filtered, concentrated after dryness in rotary flash evaporator maintained at 45ºc, percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies.

***Phytochemical Analysis*:** Freshly prepared extracts of the powdered leaves were subjected to phytochemical analyses to find the presence of the following phyto constituents such as flavanoids, alkaloids, carbohydrates, glycosides, tannins, saponins, steroids, proteins, lipids, oils by standard methods (Trease and Evans, 2002; Sofowara, 2008).

Anti-bacterial analysis

Test Microorganisms: The organisms namely *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Vibrio cholerae* used during the present experiment were procured from Hi-media which are potential causative pathogen for different diseases.

***Concentrations screened*:** 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 mg for agar diffusion method and for broth dilution method up to 64 mg/mL concentrations were used according to the sensitivity of samples.

***Agar diffusion method**:* Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µL, 10^4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with Gentamycin along with solvent. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zones were noted.

***Broth dilution method*:** Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g, in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The tubes containing above media were prepared, autoclaved and respective concentrations of the samples were added. Each tube was inoculated with 18 h old cultures (100 µL, 10^4 cfu). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37ºC on a shaker with 140 rpm for 24 h and the growth was measured at 660 nm. The % of inhibition was calculated by using the formula below.

$$\% \text{ Inhibition} = 100 - \left[\frac{\text{OD of culture with sample (Test)}}{\text{OD of culture without sample (Control)}} \times 100 \right]$$

3. RESULTS AND DISCUSSION

Anti-dysenteric and anti-diarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars (Vimala et al., 1997; Pampattiwar and Advani, 2011, Choudhury et al., 2012a, b) and there are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (De Boer et al, 2005). The results of the evaluation of phytochemical screening of methanolic extracts of *Murraya koenigii* revealed the presence of carbohydrates, glycosides, proteins, oils, steroids, alkaloids and absence of triterpenoids, tannins, flavanoids and saponins. Alkaloids possess anti-oxidizing effects, thus reduces the nitrate generation which is useful for protein synthesis, suppresses the transfer of sucrose from stomach to small intestine and steroids enhance intestinal absorption of sodium ions and water. Isaac and Chinwe (2001) reported that alkaloids are responsible for the antibacterial activity. Triterpenoid alkaloids such as cyclomahanimbine, tetrahydromahanmbine, murrayastine, murrayaline, pypayafoline carbazole alkaloids and many other chemical compounds have been reported in the leaves of *Murraya koenigii* (Chakraborty and Das, 1968; Kureel et al., 1969). The carbazole alkaloids (Koenimbine and Kurryam) were isolated from *Murraya koenigii* seed and oral administration of 50 mg/kg of Koenimbine and Kurryam exhibited significant ant-diarrhoeal effects in castor oil-induced diarrhoea

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rats (Mandal et al., 2010). The ethanol and petroleum ether extract at a dose of 300 and 500mg/kg of leaves of the plant were subjected to Swiss albino mice to determine their effect on gastrointestinal motility. The study resulted in acceleration the intestinal transit dose dependently. Verapamil was used for assessing the impact of calcium channel on acceleratory intestinal transit by extracts. This finding indicates that plant may act by increasing the intracellular calcium concentration through calcium channel (Tembhurne and Sakarkar, 2009). The result is also supported with reported ionotropic effect of plant through voltage gated calcium channel (Shah et al., 2006). The constituents listed above may mediate the anti-diarrhoeal property of *Murraya koenigii* which could make the plant useful for treating different ailments and having a potential of providing useful and safe drugs and drug leads for human use.

The antimicrobial efficacy of the extracts of *M.koenigii* leaves was quantitatively assessed on the basis of inhibition zone in mm (Table.1) following the agar disc diffusion method and minimum inhibitory concentration by broth dilution method. The test organisms were also inoculated with pure antibiotics- Gentamycin to compare the efficacy of leaf extract for their antimicrobial properties (Table.2)

In the present investigation the extract was found to be effective against all the pathogens. When the above pathogens were screened by agar disc diffusion method the zone of inhibition (ZOI) observed for the methanolic extract was in the range 2-7mm at 2-4mg/ml concentration of the extract. *S.typhi* was found to be highly susceptible as it showed an inhibition zone of 7mm at 4mg/ml concentration whereas *E.coli* and *V.cholerae* were comparatively less sensitive by showing 5mm and 2mm ZOI at 4mg/ml concentration. *S.aureus* and *P.aeruginosa* did not show any zone of inhibition reflecting their insensitiveness towards the methanolic extract of the leaf. In a similar study by Vohra and Gupta (2011) the antibacterial activity of petroleum ether and alcoholic extracts of *Murraya koenigii*, alcoholic extract at a dose of 10mg/mL produced potent antibacterial activity as it showed inhibitory zone as compared to other individual concentrations of petroleum ether. The activities are comparable with the reference drug tetracycline at a dose of 10µg/mL (Khuntia and Panda, 2011). The

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present findings indicate that pathogenic organisms such as *S.typhi, E.coli and V.cholerae* are inhibited at a dose of 4mg/mL of the extract showing its higher efficacy.

The broth dilution method showed more pronounced antimicrobial activity through 100% inhibition for all the pathogens in the range of 0.25-64mg/mL concentration. The MIC for *S.typhi, E.coli and V.cholerae* was 4mg/ml. For *P.aeruginosa* it was 32mg/ml (Fig.4) and for *S.aureus* it was 64mg/ml (Fig.5).

The results of the present study also support the medicinal usage of the methanolic leaf extracts of *Murraya koneigii* and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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Fig.1: Inhibition (%) of *E.coli* by methanolic extract of *M.koenigii* in broth medium.

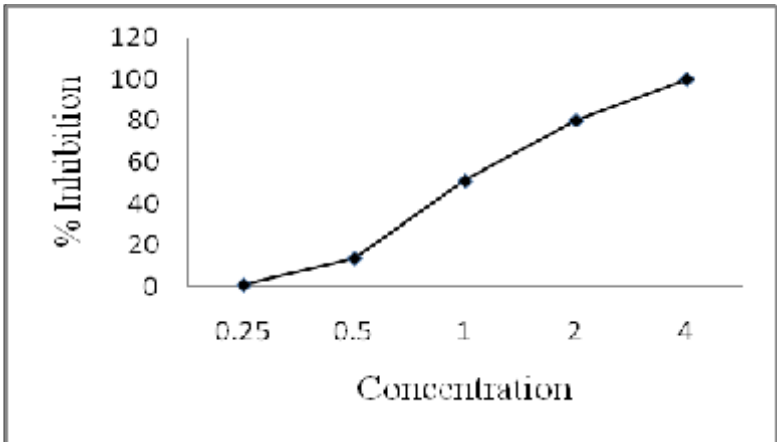

Fig.2: Inhibition (%) of *P.aeruginosa* by methanolic extract of *M.koenigii* in broth medium.

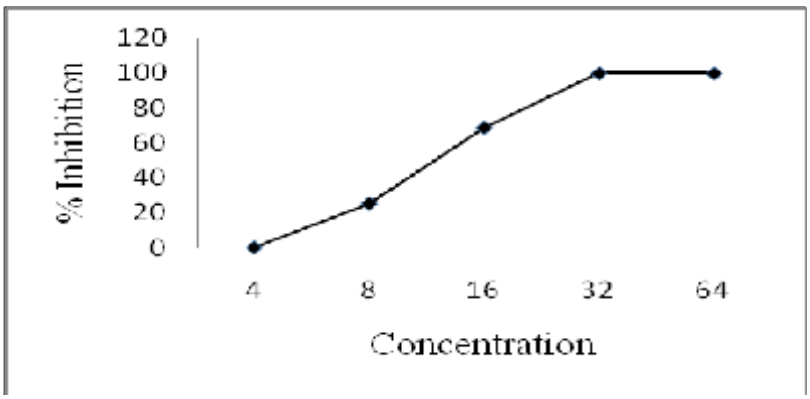

Fig.3: Inhibition (%) of *S.aureus* by methanolic extract of *M.koneigii* in broth medium.

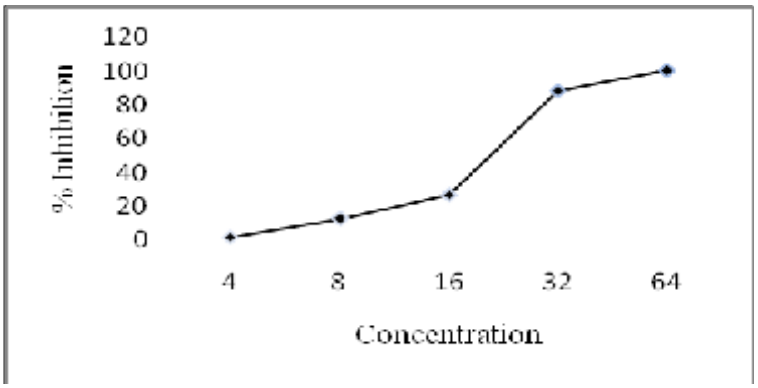

Fig.4: Inhibition (%) of *S.typhi* by methanolic extract of *M.koenigii* in broth medium.

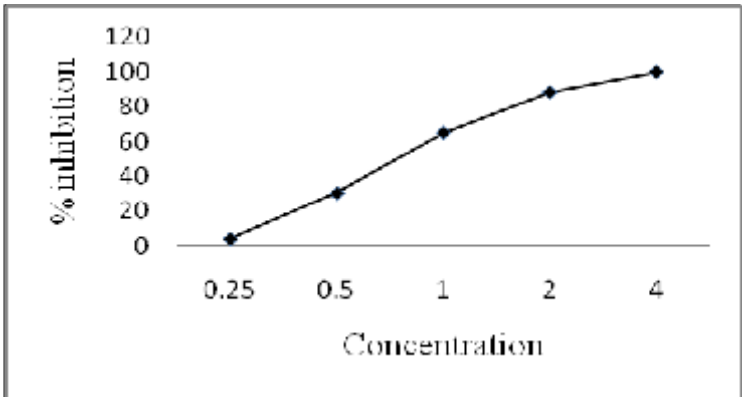

Fig.5: Inhibition (%) of *V.cholerae* by methanolic extract of *M.koenigii* in broth medium.

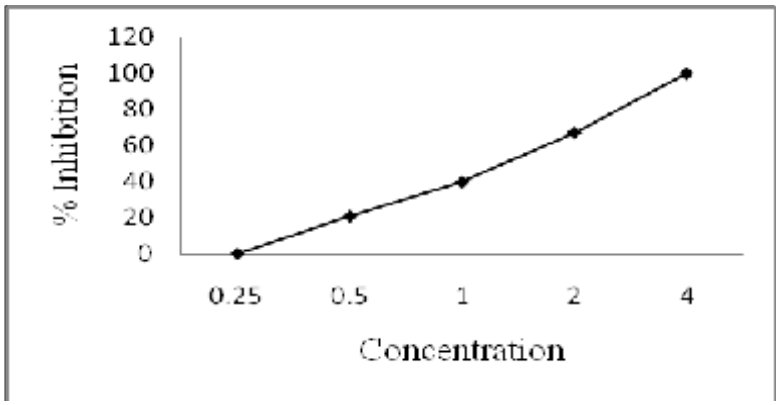

Table.1: Zone of inhibition (in mm) of methanolic leaf extract of *M. koenigii*

Concentration(mg/ml)	*E.coli*	*S.aureus*	*P.aeruginosa*	*V.cholerae*	*S.typhi*
0.125	-	-	-	-	-
0.25	-	-	-	-	-
0.50	-	-	-	-	-
1.0	-	-	-	-	-
2.0	-	-	-	-	-
4.0	5	-	-	2	7
MIC(mg/ml)	4	NF*	NF*	4	4

***NF-not found**

Table.2: MIC of Gentamycin against the test organisms

Microorganisms	MIC(µg/ml)	ZOI(mm)
E.coli	25	18
P.aeruginosa	100	1
S.aureus	25	13
S.typhi	25	2
V.cholerae	25	13