



ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. 1: 419 - 423: 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.in

ANTIBACTERIAL EFFICACY AND PHYTOCHEMISTRY OF METHANOLIC LEAF EXTRACTS OF MANGIFERA INDICA LINN

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KEYWORDS

Mangifera indica
Extracts
Gastrointestinal
Pathogens

Proceedings of International Conference on
Anthropogenic Impact on Environment & Conservation Strategy
(ICAIECS - 2012)
November 02 - 04, 2012, Ranchi,
organized by
Department of Zoology, Ranchi University, Ranchi
&
Departments of Zoology and Botany,
St. Xavier's College, Ranchi
in association with
NATIONAL ENVIRONMENTALISTS ASSOCIATION, INDIA
www.neaindia.org



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ABSTRACT

Mangifera indica is a well known traditional medicinal plant used in various indigenous systems of medicine. It is widely distributed throughout India. The present study provides phytochemical and antimicrobial details of the methanolic leaf extract of *Mangifera indica* against clinically important gastrointestinal pathogens viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholera*. The phytochemical analysis carried out revealed the presence of flavanoids, glycosides, alkaloids, tannins and steroids and many other metabolites and absence of saponins. Minimum inhibitory concentration (MIC) assay was determined for the extract. The methanolic extract showed toxicity against all the bacteria, *S.typhi* being highly susceptible with a zone of inhibition of 2mm at 4mg/mL.

INTRODUCTION

For decades, diarrhoeal diseases continue to be a major cause of morbidity and mortality throughout the world not only in the developing world, but also in the developed too. The implications are however more evident in the former (Farthing, 2000). The immediate source of concern in diarrhoea is dehydration and the electrolyte imbalance. The type of dehydration: hypertonic, isotonic or hypotonic is independent of the pathogen (Koletzko and Stephanie, 2009). With repeated episodes, protein energy malnutrition (PEM) results, followed by complications such as shock and renal failure, and subsequently death, if the diarrhoea is not restricted, and therefore this has renewed interest in the discovery of novel compounds that can be used to fight these diseases. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years.

Medicinal properties of plants are due to the active chemical constituents present in different parts of the plant (Palombo, 2006). Numerous studies have validated the traditional use of anti-diarrhoeal medicinal plants by investigating the biological activity of extracts of such plants, which have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water adsorption or reduce electrolyte secretion. Of the numerous phytochemicals (such as alkaloids, tannins, flavonoids and terpenes) present in active extracts, tannins and flavonoids are thought to be responsible for anti-diarrhoeal activity by increasing colonic water and electrolyte reabsorption. Others act by inhibiting intestinal motility (Alkizim, 2012). As some of the active ingredients are potentially toxic, there is a need to evaluate the safety of plant preparations. A few clinical trials have evaluated the safety and tolerability of traditional and herbal medicine preparations used to treat diarrhoea and generally indicate that minimal side effects are observed.

Mango fruit (*Mangifera indica* L., Anacardiaceae), often called the "King of fruits" originated in India, Myanmar, and the Andaman Islands (Wauthoz, 2007). It is now cultivated throughout the tropical and subtropical world. The chemical composition of this plant has been studied extensively over the past years and has been shown to have numerous therapeutic uses (Shah et al., 2010). Extracts of diverse parts of *Mangifera indica* L., viz. stem bark, leaf, seed, kernel and peel, have been reported to possess several biological and pharmacological properties (Masibo and He, 2008; Barreto et al., 2008). Chemical constituents of *Mangifera indica* are always of an interest, especially the polyphenolics, flavonoids, triterpenoids, mangiferin a xanthone glycoside major bio-active constituent, isomangiferin, tannins and gallic acid derivatives (Scartezzini and Speroni, 2000; Shah et al., 2010). Anti-diarrhoeal activity of the extracts may also be due to the presence of tannins and tannic acids, which probably precipitates pathogenic proteins (e.g. toxins) forming protein tannates (irreversible complexes with proline rich proteins resulting in the inhibition of the cell protein synthesis), hence detaching them from the mucosa and reducing their effect (Sairam et al., 2003).

The present study is justified as an attempt to establish credibility, awareness, and scientific data to support the therapeutic use of methanolic extracts of *Mangifera indica* leaves as an alternative to conventionally used pharmaceutical drugs for the

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treatment of diarrhoea and other gastrointestinal ailments against a few clinically important multi-drug resistant gastrointestinal pathogens including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*.

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.

Extract preparation: 50g of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using ~350mL methanol and distilled water separately. The extract obtained was filtered, concentrated after dryness in rotary flash evaporator maintained at 45°C, 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 37°C for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18h old cultures (100µL, 10⁴ 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 37°C for 24 h and the diameter of inhibition zones were noted.

Broth dilution method: Media Used: Peptone-10g, 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 37°C for 18h. 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 (100µL, 10⁴ 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 37°C on a shaker with 140 rpm for 24h and the growth was measured at 660 nm. The % of inhibition was calculated by using the formula below.

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

RESULTS AND DISCUSSION

Anti-dysentric 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). The results of the evaluation of phytochemical screening of methanolic extracts of *Mangifera indica* revealed the presence of glycosides, steroids, alkaloids, triterpenoids, tanins and flavanoids and absence of saponins. These constituents may mediate the anti-diarrhoeal property of *Mangifera indica* 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.

Table 1: Zone of inhibition (in mm) of methanolic leaf extract of *Mangifera indica*

Concentration (mg/mL)	<i>E.coli</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>	<i>V.cholerae</i>	<i>S.typhi</i>
0.125	-	-	-	-	-
0.25	-	-	-	-	-
0.50	-	-	-	-	-
1.0	-	-	-	-	-
2.0	-	-	-	-	3
4.0	-	3	4	-	6
MIC(mg/mL)	NF*	4	4	NF*	2

*NF-not found

Table 2: MIC of Gentamycin against the test organisms

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

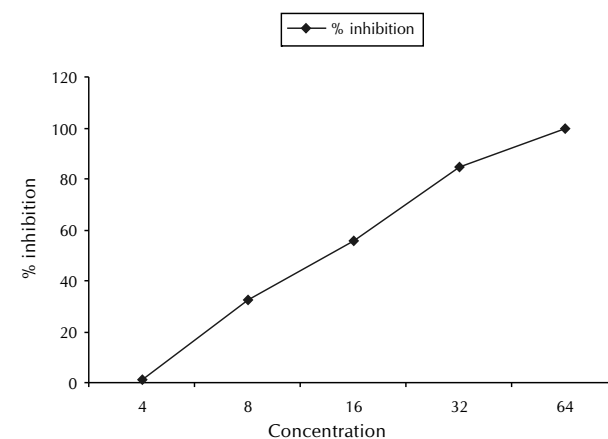


Figure 1: Inhibition (%) of *E.coli* by methanolic extract of *M.indica* in broth medium

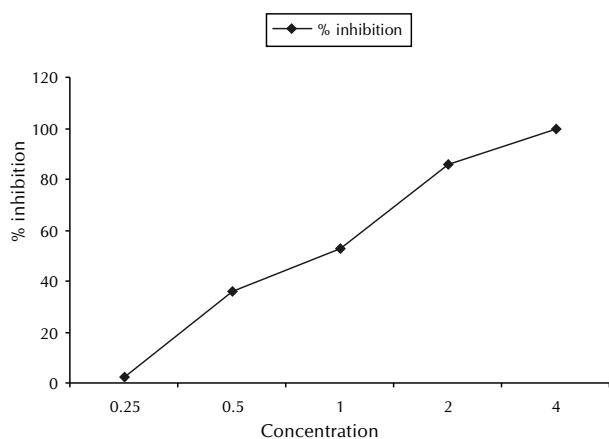


Figure 2: Inhibition (%) of *P.aeruginosa* by methanolic extract of *M.indica* in broth medium

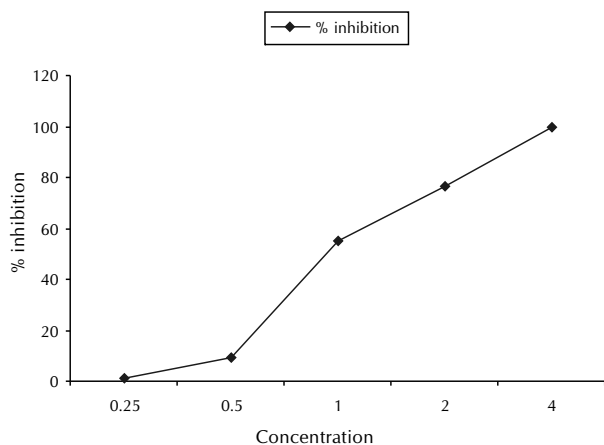


Figure 3: Inhibition (%) of *S.aureus* by methanolic extract of *M.indica* in broth medium

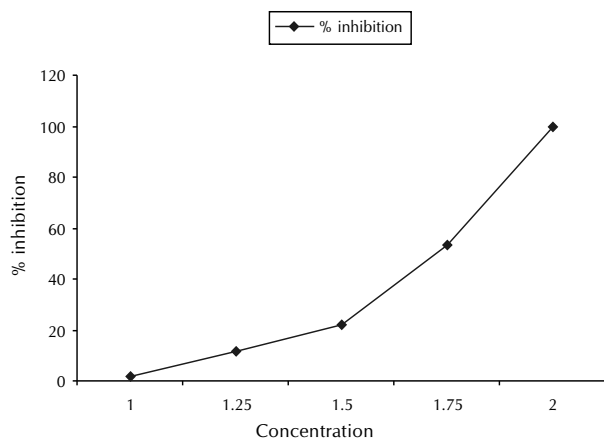


Figure 4: Inhibition (%) of *S.typhi* by methanolic extract of *M.indica* in broth medium

The antimicrobial efficacy of the extracts of *M.indica* leaves was quantitatively assessed on the basis of inhibition zone (in mm) and the results are shown in 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

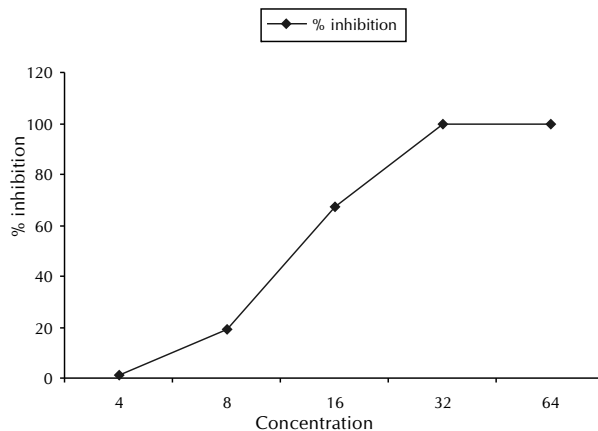


Figure 5: Inhibition (%) of *V.cholerae* by methanolic extract of *M.indica* in broth medium

efficacy of leaf extract for their antimicrobial properties and the results are shown in 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 2-4mm at 2-4mg/mL concentration of the extract. *S.typhi* was found to be highly susceptible as it showed an inhibition zone of 3mm at 2mg/mL concentration whereas *S.aureus* and *P.aeruginosa* were comparatively less sensitive by showing 4mm and 3mm ZOI at 4mg/mL concentration. *E.coli* and *V.cholerae* did not show any zone of inhibition reflecting their insensitiveness towards the methanolic extract of the leaf.

The broth dilution method showed more pronounced antimicrobial activity through 100% inhibition for all the pathogens in the range of 2-64mg/mL concentration. The MIC for *S.typhi* was 2mg/mL (Fig. 1), for *P.aeruginosa* and *S.aureus* 4mg/mL (Fig. 2, 3), for *V.cholerae* 32mg/ml (Fig. 4) and for *E.coli* 64mg/mL (Fig. 5).

All the pathogens screened in the present study are potent causative agents of typhoid, watery diarrhoea (influx of water and ions to the intestinal lumen increase in intestinal motility and watery stools), diarrhoea (usually non-bloody), nausea, vomiting, abdominal pain, pediatric diarrhoea, typical gastroenteritis, and necrotizing enterocolitis (Todar, 2008). Earlier studies have reported that various medicinal plant extracts exhibit antimicrobial activity against the screened gastrointestinal pathogens (Ogbulie *et al.*, 2004; Bajracharya *et al.*, 2008; Borokini and Omotayo, 2012, Choudhury *et al.*, 2012). Alkizim *et al.*, (2012) have reported inhibitory effects of *M.indica* extracts on intestinal motility therefore it can be conclusively stated the *M.indica* leaf extract is potential antibacterial agent for the bacteria causing gastrointestinal problem and can be used for such ailments.

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