ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. III:111- 116; 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.in

PHARMACOLOGICAL EFFICACY OF SOME MEDICINAL PLANTS USED FOR TREATMENT OF GASTROINTESTINAL DISEASES

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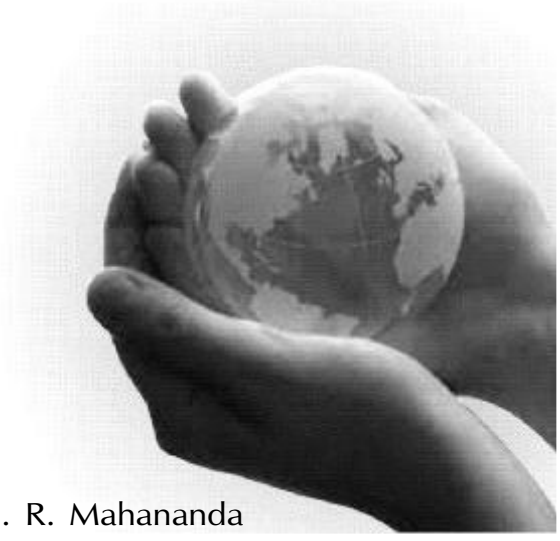
KEYWORDS

Gastrointestinal
Phytochemical
Antimicrobial
Extracts



Prof. P. C. Mishra Felicitation Volume

Paper presented in
National Seminar on Ecology, Environment & Development
25 - 27 January, 2013
organised by
Deptt. of Environmental Sciences,
Sambalpur University, Sambalpur
Guest Editors: S. K. Sahu, S. K. Pattanayak and M. R. Mahananda

The Ecoscan
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ABSTRACT

Success of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains. Methanol extracts of five plant species traditionally used in Indian folklore medicine for the treatment of various gastrointestinal bacterial infections were investigated for *in vitro* antimicrobial activity against pathogens namely *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholera and Salmonella typhi* by agar diffusion method. Methanolic and aqueous extracts of *Mangifera indica, Moringa oleifera, Psidium guajava, Murraya koneigii, Ficus infectoria* showed the toxicity against all the bacteria. Minimum inhibitory concentration (MIC) assay were determined for these extracts against bacteria and revealed antimicrobial activity. The phytochemical analysis carried out revealed the presence of flavaonoids, glycosides, tannins, saponins, alkaloids and steroids and many other metabolites. The results provide justification for the use of the plants in folk medicine to treat various gastrointestinal diseases.

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INTRODUCTION

One of the major concerns of human life throughout the globe is health care and there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures, cheap, easily available with novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use (Parekh and Chanda, 2008). In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens (Martins *et al.,* 2001; Clardy and Walsh, 2004; Verpoorte *et al.,* 2006). The pharmacological actions and the medicinal uses of aqueous extracts of leaves in folk medicine include the treatment of various type of gastrointestinal disturbances such as vomiting, diarrhoea, inhibition of the peristaltic reflex, gastroenteritis, spasmolytic activity, dysentery, abdominal distention, flatulence and gastric pain Lutterodt, 1992; Mittal *et al.,* 2011). The importance of plant secondary metabolites in medicine, agriculture and industry has led to numerous studies on the synthesis, biosynthesis and biological activity of these substances. It has been estimated that over 40% of medicines have their origins in these active natural products (De Fatima *et al.,* 2006, 2008; Vohra and Gupta, 2011).

The important active constituents are essential oils, flavonoids, carotenoids, polyphenolic compounds, pentacyclic triterpenoids, esters, and aldehydes etc. These natural products are found in stem, berry, bark, leaf, flower, and root tissue (Borchardt, 2008) as well as essential oils (Afolayan and Ashafa, 2009; Hassan *et al.,* 2009) and include such compounds as alkaloids, anthocyanins, anthraquinoes, flavonoids, glycosides, phenols, saponins, tannins, and terpenoids (Cowan, 1999; Hemaiswarya *et al.,* 2008; Afolayan and Ashafa, 2009; Banso, 2009; Ekpo and Etim, 2009; Hassan *et al.,* 2009; Ladan *et al.,* 2009). These compounds which are soluble in a variety of solvents including ethanol and water, and are safe for human exposure, have been shown to be highly effective in the preparation of biologically active extracts (Rasool *et al.,* 2008; Afolayan and Ashafa, 2009; Banso, 2009; Ekpo and Etim, 2009; Hassan *et al.,* 2009; Gillitzer *et al.,* 2012).

In the present study five medicinal plants (*Mangifera indica, Moringa oleifera, Psidium guajava, Murraya koenigii, Ficus infectoria)* have been screened for their anti-bacterial efficacy against multi-drug resistant bacteria including *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella*

typhi, Vibrio cholerae.

MATERIALS AND METHODS

Collection of plant material

Five folk medicinal plants leaves viz. *Mangifera indica, Moringa oleifera, Psidium guajava, Murraya koenigii, Ficus infectoria* were screened. The leaves were shade dried and coarsely powdered and were used for further studies.

Extract preparation

50g of the powder samples were extracted against ~350mL of methanol and water separately for 24h. The extracts obtained were dried at room temperature and used for the further study.

Phytochemical analysis

Freshly prepared extracts were subjected to standard phytochemical analyses to find the presence of the following phyto- constituents such as flavanoids, alkaloids, carbohydrates, glycosides, tannins, saponins and steroids, proteins, lipids, oils as listed below.

Molisch's test for carbohydrates: Few drops of Molisch's reagent were added to the test solutions, this was then followed by addition of 1mL of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test (Sofowora, 2008).

Test for polysaccharides: To 1mL of test solutions, 2 drops of iodine solution was added. Blue coloured solution observed confirmed positive test for polysaccharides (Sofowora, 2008).

Test for glycosides: To 1mL of test solutions, 3mL of anthrone reagent was added and mixed well. Formation of green coloured complex was a positive test (Sofowara, 2008).

Test for proteins (free amino acids): To 1mL of test solutions, 5drops of ninhydrin was added and boiled for 2mins. Formation of purple coloured solution indicates the presence of free amino acids (Sofowara, 2008).

Bradford's test for proteins: To 0.5mL of the test sample 2mL of Bradford's reagent was added occurrence of blue colour indicates the presence of proteins (Sofowara, 2008).

Test for alkaloids: Few quantity of the each portion was stirred with 5mL of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1mL was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second 1mL, Mayer's reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids (Sofowora, 2008).

Liebermann-Burchard test for steroids: To 0.2g of each portion, 2mL of acetic acid was added, the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring (Sofowora, 2008).

Salkwoski test for steroids: About 2mL of sample was taken and 2mL of conc. H_2SO_4 was added and mixed vigorously. Steroid and H_2SO_4 layers gets separated and sample layer forms cherry red colour and acid layer forms green layer which confirms the presence of steroids (Sofowara, 2008).

Test for triterpenes: To the methanolic extracts chloroform and conc. H_2SO_4 was added, formation of red colour confirms the prescence of triterpenes (Trease and Evans, 2002).

Test for tannins: About 0.5g of the extracts were stirred with about 10mL of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2mL of the filtrate occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins (Trease and Evans, 2002).

Shinoda's test for flavonoids: About 0.5 of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids (Trease and Evans, 2002).

Sodium hydroxide test for flavonoids: Few quantity of the each portion was dissolved in water and filtered; to this 2mL of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease and Evans, 2002).

Test for saponins: One gram of each portion was boiled with 5mL of distilled water, filtered. To the filtrate, about 3 mL of distilled water was further added and shaken vigorously for about 5min. Frothing which persisted on warming was taken as an evidence for the presence of saponins (Sofowora, 2008).

Test for oils: 1 drop of sample was placed on the filter paper and allowed to dry, clear greasy spot indicates presence of oils.

Test for lipids: To 2mL of test sample, iodine solution was added dropwise. Original colour of iodine disappears, which confirms the presence of lipids (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 potencial causative pathogen for different diseases as listed below (Todar, 2008).

Microorganisms	Diseases Caused
E. coli	Diarrhoea, colic dysentery, inflammation of the small intestine, bloody diarrhea, fatigue, nausea and abdominal cramps
S.aureus	Gastroentritis (nausea and vomiting, abdominal pain and diarrhoea)
S.typhi	Thyphoid, 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
P. aeruginosa	Pediatric diarrhea, typical gastroenterities and necrotizing enterocolitis
V. cholerae	Cholera (stomach aches, very watery and continuous diarrhoea and vomiting)

Concentrations screened: 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0mg for agar diffusion method

Agar diffusion method

Media Used: Peptone-10g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000mL of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18h. 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, 10^4 cfu) and spread evenly on the plate. After 20min, 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 24h and the diameter of inhibition zones were noted.

RESULTS AND DISCUSSION

The methanol extracts of the medicinal plants were tested against the pathogenic microbes which causes gastroenteritis and other serious gastrointestinal ailments such as diarrhoea and dysentery due to the consumption of contaminated food and water (Table 1).

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defence mechanism against prediction by many microorganisms, insects and other herbivores (Bonjar *et al.,* 2004). The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical constituents of the selected plants investigated are summarized in Table 2. Analysis of plant extracts revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroids and tannins in most of the selected plants which could be responsible for the observed antimicrobial property. These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Tannins bind to proline rich proteins and interfere with the protein synthesis (Shimada, 2006) and it was observed that tannins were absent in. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie, 1999). Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz *et al.,* 1996). Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes (Raquel, 2007). But in our study steroids were absent in *F.infectoria* plant extracts.

All the plants tested for antimicrobial activity showed antibacterial activity by inhibiting one or more

Table 1: Medicinal plants used for treatment of various gastrointestinal diseases

Botanical name	Common name	Ailments treated	Reported relevant pharmacological and anti bacterial activities
Moringa oleifera	Sahjan, Munga	Acidity, indigestion, constipation, lack of appetite	Mollick *et al.,* 2010
Mangifera indica	Aam	Diarrhoea, dysentery, chronic dysentery(frequeht thurst, frequent watery stool,stomach pain, fever) Stomach ache,gastrointestinsal disorders	Anti-bacterial effects on *E.coli, S.aureus, P.aeruginosa* (Akinpelu and Onokoya, 2006; Engels, 2009, 2010), Islam *et al.,* 2011
Murraya koenigii	Curry patta	Acidity, indigestion, constipation, lack of appetite, vomiiting	Anti-bacterial activity of alcoholic extracts against *S.aureus, E.coli, V.cholerae, S.typhi*(Mylarappa *et al.,* 2009; Khuntia and Panda, 2010; Muthumani *et al.,* 2010; Vohra and Gupta, 2011), Mollick *et al.,* 2010
Psidium guajava	Amrud	Diarrhoea, gastroenteritis, dysentery, abdominal distention, flatulence, gastric pain,	Anti-bacterial activity of leaf extract demonstrated against multi drug resistant *S.aureus* (Anas, 2008); anti bacterial activity of methanol extracts of leaves against diarrhoea causing enteric bacteria, *S.aureus E.coli*, and *Salmonella* spp. (Caceres, 1990,1993;Goncalves, 2008; Chandekar and Madgugiri, 2011), Antimicrobial activity against bacterias causing diarrhoea and dysentery (Abdelrahim *et al.,* 2002; Ojewole *et al.,* 2008) , Lutterodt, 1992; Hernandez *et al.,* 2003; Kagyung *et al.,* 2010; Mittal *et al.,* 2010
F.infectoria	Phutkal	Colic dysentery	Sinha *et al.,* 2007

Table 2: Phytochemical screening of the plant extracts

Plants name	Carbohydrates	Glycosides	Lipids	Oils	Alkaloids	Steroids	Triterpenoids	Tanins	Flavonoids	Saponins
M.oleifera	+	+	+	+	+	+	-	-	+	-
M.indica	+	+	+	+	+	+	+	+	+	-
M.koneigii	+	+	+	+	+	+	-	-	-	-
P.guajava	+	+	+	+	+	+	+	-	-	-
F.infectoria	+	+	+	+	+	-	-	-	+	-

+ = present, - = absent

microorganisms. The results of the antimicrobial activity of plant extracts tested against microorganisms by disc diffusion method are shown in Table 3.

Table 3: Antimicrobial activity of *Mangifera indica, Moringa oleifera, Psidium guajava, Murraya koenigii, Ficus infectoria* tested against microorganisms by disc diffusion method

Name of plant	Mean diameter of growth inhibition zone in mm Conc. (mg/mL)	*E.coli*	*S.aureus*	*P.aeruginosa*	*V.cholerae*	*S.typhi*
M. oleifera	0.125	-	-	-	-	-
	0.25	-	-	-	-	-
	0.5	-	-	-	-	-
	1.0	-	-	-	-	-
	2.0	-	-	-	-	-
	4.0	-	-	3	-	9
M.indica	0.125	-	-	-	-	-
	0.25	-	-	-	-	-
	0.5	-	-	-	-	-
	1.0	-	-	-	-	-
	2.0	-	-	-	-	3
	4.0	-	3	9	-	6
M. koenigii	0.125	-	-	-	-	-
	0.25	-	-	-	-	-
	0.5	-	-	-	-	-
	1.0	-	-	-	-	-
	2.0	-	-	-	-	-
	4.0	5	-	-	2	7
P. guajaya	0.125	-	-	-	-	-
	0.25	-	-	-	-	-
	0.5	-	-	-	-	-
	1.0	-	-	-	-	-
	2.0	-	-	-	-	5
	4.0	2	-	3	-	8
F. infectoria	0.125	-	-	-	-	-
	0.25	-	-	-	-	-
	0.5	-	-	-	-	-
	1.0	-	-	-	-	-
	2.0	-	-	-	-	-
	4.0	3	-	-	2	6

The agar disc diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zone (in mm) against the test microorganisms and the range observed was from 2mm-9mm at 2mg/ml-4mg/mL. *M.oleifera* exhibited the prominent antibacterial activity in the range 3-9mm against *P.aeruginosa* and *S.typhi* whereas *M.indica* was more susceptible against *S. aureus, S.typhi* as well as *P.aeruginosa* in the range 3-9mm). *M. koenigii* showed antibacterial activity against *E.coli, S.typhi* and *V.cholerae* in the range 2-7mm. Similarly *P.guajava* exhibited antimicrobial activity against *E.coli, P.aeruginosa* and *S.typhi* in the range 2-8mm whereas *F.infectoria* showed antibacterial activity against *E.coli, S.typhi* and *V.cholerae* in the range 2mm-6mm. The same organisms were also inoculated with standard antibiotic gentamycin and minimum inhibitory concentration was in the range 1-18mm at 25-100μ/mL.

In the present study, *S.typhi* was found to be the most sensitive to all the extracts exhibiting the minimum zone of inhibition (ZOI) of 3mm at 2mg/mL *(Psidium gvajava)* while pure antibiotics gentamycin exhibited 2mm at 25μg/mL. The present study revealed that the extracts of *Psidium gvajava* is very effective in inhibiting *S.typhi* hence we suggest use of *Psidium gvajava* extracts in treating bloody and watery diarrhoea and dysentary.

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