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Effects of aqueous young leaves extract of *Mangifera indica* on gm (-) microorganisms causing gastro-intestinal disorders

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ABSTRACT

Plants have, for generations, been a source of various kinds of remedies and been used for medicinal purpose to cure different types of ailments and will continue to provide remedies for these ailments, especially in rural areas in developing countries. Consumption of medicinal herbs is tremendously increasing over a past decade as an alternative approaches to improve the quality of life and to maintain a good health. Gastro-intestinal disorders (mainly diarrhea) is one of the major health threats to populations in tropical and subtropical poor countries, responsible for about 5 million deaths annually, of which 2.5 million are children of less than 5 years. Mangifera indica (anacardiaceae family) grows in the tropical & subtropical region & its parts are commonly used in folk medicine for a wide variety of remedies. One of the folk uses is to take the young leaves juice in gastrointestinal disorder. Hence an attempt is taken here to evaluate the effects of aqueous young leaves extract against different gm (-) organisms causing gastro-intestinal disorders. Qualitative phytochemical analysis of the aqueous extract revealed the presence of tannins, flavonoid, steroid, cardiac glycoside, alkaloids & carbohydrate. The presence of this secondary metabolite may be responsible for the antidiarrhoeal properties of the crude extract. Aqueous extract of young leaves of Mangifera indica were tested against five gm (-) organisms like E,coliTG1; S.typhi NCTC 74; S. typhi 62; Vibrio cholera 1023 and S. sonnei NK4010 at concentrations of 300, 200, 100, 50 mg/ml. The growths of all the tested organisms are inhibited and the growth inhibition is dose dependent. Hence it could be concluded that the aqueous young leaves extract of Mangifera indica could be utilized in the management of gastro-intestinal disorders and its effect could be potentiating as required.

Key words: *Mangifera indica*, Mangiferin, leaves, Antidiarrheal, Gm (-) Microorganism

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INTRODUCTION

Infectious diseases are the world's leading cause of premature death, killing about 50,000 people every day. Morbidity & mortality due to diarrhea (one of the major intestinal disorder) continuous to be a major problem in many developing countries, especially amongst the children. Infection due to a various bacterial etiologic agents, such as pathogenic *Escherichia coli, Vibrio cholera, Shigella spp, Salmonella spp, Staphylococcus aureus* are most common. In recent years, drug resistant to human pathogenic bacteria has been reported commonly all over the world. With continuous uses of antibiotics microorganism have become resistant. Therefore, there is a need to develop alternative antimicrobial drug\s for the treatment of infectious diseases; one approach is to search local medicinal plants for possible antimicrobial properties.

Diarrhea is the passage of loose stool, by an individual, at least three times a day, or more frequent than normal. It is most commonly caused by intestinal infection and is transmitted via faeco- oral route [1]. For decades, diarrhea has been described as one of the leading cause of mortality not only in the developing country, but also in the developed too. The implications are however more evident in the former [2]**.** In 1979, WHO reports [3] expressed concern on the matter & more than 2 decades later the same agency reported diarrhea as the second leading cause of infant mortality worldwide, causing up to 1.5 million deaths annually [4].This accounts 16% of deaths in this age group, a toll greater than that of AIDS, malaria, measles combined [4]

Mangifera indica is the species of mango in the anacardiaceae family. *Mangifera indica* (anacardiaceae) grows in the tropical & subtropical region & its parts are commonly used in folk medicine for a wide variety of remedies. Various parts of plant are used as a dentrifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative and diuretic and to treat diarrhea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and piles [5]. Mangiferin, being a polyphenolic antioxidant and a glucosyl xanthone, it has strong antioxidant, anti lipid peroxidation, immunomodulation, cardiotonic, hypotensive, wound healing, antidegenerative and antidiabetic activities. Mangiferin is extracted from mango at high concentration from young leaves (172 gm/kg) & from old leaves (94 gm/kg). Hence the objective of the present study is to evaluate the activity of young leaf extract on the organisms (gm-) causing gastro-intestinal disorders including diarrhea.

MATERIALS AND METHODS

Plant Material

Mangifera indica young leaves were collected from different area of Ashoknagar, North-24 parganas, West Bengal. The leaves were identified by Botanical Survey of India (BSI), Howrah, and West Bengal. The leaves were cleaned; shade dried and was crushed to moderately coarse powder. The powder was stored in an air tight container for subsequent use.

Chemicals

Ciprofloxacin injection (RANBAXY-), Sodium Chloride crystal (MERCK-Mumbai), Beef Extract (MERCK-Mumbai), Agar powder purified (MERCK- Mumbai), Peptone (MERCK- Mumbai) were procured from local supplier.

Preparation of plant extract

500 ml of boiled distilled water was added to 50 g of the powdered sample in a 1 liter of round bottom flask, stirred with a glass rod, covered, shaken continuously for 6 h using a mechanical shaker. Then it is allowed to stand for another 18 h at room temperature. The extract was then filtered using Whatmann filter paper No. 91 (18.5 cm). The filtrate was concentrated on a water bath at a controlled temperature. Then the concentrated extract of *M. indica* was completely evaporated in a lyophilizer (MAC) to get in the dry powder form [6]

Phytochemical screening

Phytochemical screening was carried out on young leaves extract of *M. indica* using standard methods [7] for detecting the presence of secondary metabolites; alkaloids, carbohydrates, tannins, saponins, glycosides, steroids, protein, fat & fixed oil and flavonoids. [8]

Determination of antidiarrheal activity by disc diffusion method [9]

The antidiarrheal activity of crude aqueous extract of young leaves of *M. indica* were tested against five different gm(-)microorganisms causing diarrhea like *Escherichia coli TG1, Vibrio cholera 1023 and Shigella sonnei NK4010, Salmonella typhi 62* and *Salmonella typhi NTCC 74* using disc diffusion method. The disc diffusion technique is a widely accepted in vitro investigation for primary screening of agents which may possess any antibacterial activity. It is a qualitative or quantitative test indicating the sensitivity or resistance of the microorganism to the test materials.

Preparation of solid-agar (Nutrient agar) media

For the Preparation of solid agar media, first sodium chloride was taken in a clean conical flask, then take agar powder, then peptone and at last take beef extract and make the volume up to specified amount with distilled water

with proper stirring and make the pH alkaline, then plug it with cotton and kept it in autoclave at 121ºc for 15 psi for 15 minute.

Preparation of liquid-agar media

For the preparation of liquid agar media first take Sodium Chloride in a conical flask then take peptone and make the volume up to specified amount with distilled water with proper stirring and make the pH alkaline with the help of sodium hydroxide solution, then plug it with cotton and kept it in autoclave at 121^0c for 15 psi for 15 minute.

Inoculation of micro-organisms

The inocula of five test isolates (*Escherichia coli TG1, Vibrio cholera 1023, Salmonella typhi 62, Salmonella typhi NTCC 74* and *Shigella sonnei NK4010)* were prepared directly from subculture in test tube. Under asepsis, each isolate was transferred into liquid agar media in Biju Bottle & incubate over night in 35-37ºC until the density of the inoculums become turbid. The density of the bacterial suspension was standardized by standard McFarland method [10].

Preparation of standard dilution of aqueous extract for antimicrobial assay

The aqueous extract of young leaves of M. indica was lyophilized and stored in desiccators as dry powder. The powder was then dissolved in distilled water which was previously sterilized to make solutions having different concentrations like 300mg/ml, 200mg/ml, 100mg/ml and 50mg/ml. Finally the solutions were sterilized by filtration using 0.45 µm Millipore filters.

The sterile discs (6 mm in diameter) were impregnated with 10 µl of above extract solutions to achieve desired concentration of 3000 µg/disc; 2000 µg/disc; 1000 µg/disc and 500 µg/disc and placed in inoculated agar. Ciprofloxacin (CF) (20 µg/disc) was used as standards.

The solid agar medium flooded with the inoculums prepared in liquid medium and kept inverted in the incubator until the plates become dry. On the inoculated solid medium the test and standard discs are placed in proper position and are incubated at 37°C for 24 h.

RESULTS AND DISCUSSION

Percentage yield calculation:

The yields of the young leaves aqueous extract is calculated on the basis of weight of crude drug taken for extraction and the amount of solid residue obtained after lyophilization. We had taken 50 gm of crude drug for maceration in boiled distilled water. From there we obtained 4.19 gm of dry residue. Hence the percentage yield is 8.38%w/w.

Phytochemical composition:

Results of photochemical screening of aqueous extract of *M. indica* young leaves is shown in Table 1.

Table 1. Results of photochemical screening of the young leaves aqueous extract of *Mangifera indica*

Secondary metabolites	Water extract
Carbohydrate	+
Tannins	+++
Alkaloids	+
Saponins	_
Steroid	+++
Cardiac glycosides	+++
Flavonoids	+++
Protein	_
Fat & fixed oil	_

+ Slightly present; ++ present; +++ highly present; - Absent.

Qualitative phytochemical analysis of the young leaves water extract of *Mangifera indica* revealed the presence of tannins, alkaloids, steroid, carbohydrate, glycoside and flavonoid. The presence of this secondary metabolite may be responsible for the antidiarrhoeal properties of the crude extract. This result is consistent with previous studies [6, 11, 12]. These authors opined that the antidiarrhoeal and anti-dysenteric properties of medicinal plants were due to tannins, alkaloids, saponins, reducing sugars, sterols and triterpenes.

Antimicrobial effects of young leaves aquous extract of *Mangifera indica* on microorganisms implicated in intestinal disorders:

Aqueous extract of young leaves of *Mangifera indica* were tested against five pathogenic organisms at concentrations of 300, 200, 100, 50 mg/ml. As because the filter paper disc can absorb 0.01ml of solution, hence the drug containing per disc is calculated accordingly and shown in Table 2, they showed varying degrees and specificity of activity against these bacteria.

Table2: Zones (mm) of growth inhibition by young leaves aqueous extract of *Mangifera indica* & Ciprofloxacin injection as standard

Name of Organisms	Ciprofloxacin(20 µg/disc)	500 µg/disc	1000 µg/disc	2000 µg/disc	3000 µg/disc
E. coli TG1	33.2	9.3	10	11.3	13.7
S.typhi NTCC 74	31.2	8.3	10.2	10.3	11.7
S. typhi 62	28.3	9.7	11	11.3	12
Vibrio cholera 1023,	25.7	9	9.8	10.1	10.5
S. sonnei NK4010	26.8	7.15	7.8	9.3	9.8

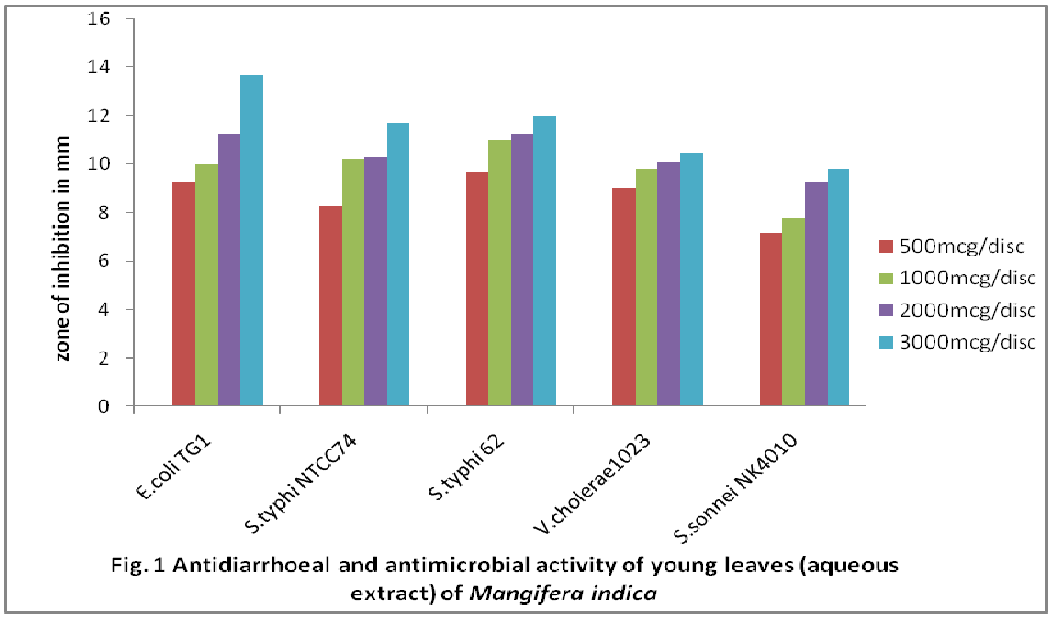

Fig. 1 Antidiarrhoeal and antimicrobial activity of young leaves (aqueous extract) of *Mangifera indica*

Fig.2 Petridish showing growth inhibition of E.coli by aqueous maceration Of *M. indica* young leaves

It has been found from the above results that the aqueous extract of young leaves of *Mangifera indica* has sufficient antibacterial and antidiarrhoeal activity. For all the above five gm (-) organisms it inhibited the growth and the growth inhibition is dose dependent. As the dose is increased the zone of diameter of inhibition is also increased. The highest growth inhibition (13.7mm) was found in case of *E.coli TG1* at the concentration 300mg/ml. Fig. 2 shows that the growth of E.coli TG1 is inhibited by aqueous maceration (AM1=3000µg/disc and AM2= 2000µg/disc) and from that figure it can be easily noted that in higher dose the diameter of zone inhibition is more (AM1).

Determination of minimum inhibitory concentration:

Aqueous extract, maceration of young leaves of *Mangifera indica* were tested for Minimum Inhibitory Concentration against five pathogenic organisms at concentrations of 5, 10, 15, 20, 30, 40 mg/ml.

Table 3: Minimum Inhibitory Concentration of young leaves aqueous extract (maceration) of *M. indica*

S. No.	Name of Organisms	5 mg/ml	10 mg/ml	15mg/ml	20 mg/ml	30 mg/ml	40 mg/ml
1	*E. coli TG1*	+	+	-	-	-	-
2	*S.typhi NCTC 74*	+	+	-	-	-	-
3	*S. typhi 62*	+	+	-	-	-	-
4	*Vibrio cholera 1023*	+	-	-	-	-	-
5	*S. sonnei NK4010*	+	-	-	-	-	-

+ means growth, - means no growth

Above table no.3 shows the MIC of (gm-) organisms by aqueous maceration of *M.indica* young leaves using gradually increasing strengths. The result shows that the Minimum Inhibitory Concentration for *E. coli TG; S.typhi NTCC 74* and *S. typhi 62* is 15 mg/ml, whereas for *V. cholera 1023* and *S. sonnei NK4010* is 10 mg/ml.

CONCLUSION

The aqueous extract of *Mangifera indica* leaves contain carbohydrate, tannins, alkaloids, saponins, steroid, cardiac glycosides, flavonoids, and protein. From the antibacterial and antidiarrhoeal study against five gm (-) organisms causing intestinal disorders revealed that the growth of all organisms is inhibited and the growth inhibition is dose dependent.

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