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Anti-enteric bacterial activity and phytochemical analysis of the seed kernel extract of *Mangifera indica* Linnaeus against *Shigella dysenteriae* (Shiga, corrig.) Castellani and Chalmers

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ABSTRACT

Objective: To evaluate the phytochemical and anti-bacterial efficacy of the seed kernel extract of *Mangifera indica (M. indica)* against the enteropathogen, *Shigella dysenteriae (S. dysenteriae)*, isolated from the diarrhoeal stool specimens. **Methods:** The preliminary phytochemical screening was performed by the standard methods as described by Harborne. Cold extraction method was employed to extract the bioactive compounds from mango seed kernel. Disc diffusion method was adopted to screen antibacterial activity. Minimum inhibitory concentration (MIC) was evaluated by agar dilution method. The crude extracts were partially purified by thin layer chromatography (TLC) and the fractions were analyzed by high performance thin layer chromatography (HPTLC) to identify the bioactive compounds. **Results:** Phytochemical scrutiny of *M. indica* indicated the presence of phytochemical constituents such as alkaloids, gums, flavanoids, phenols, saponins, steroids, tannins and xanthoproteins. Antibacterial activity was observed in two crude extracts and various fractions viz. hexane, benzene, chloroform, methanol and water. MIC of methanol fraction was found to be (95±11.8) μg/mL. MIC of other fractions ranged from 130–380 μg/mL. **Conclusions:** The present study confirmed that each crude extracts and fractions of *M. indica* have significant antimicrobial activity against the isolated pathogen *S. dysenteriae*. The antibacterial activity may be due to the phytochemical constituents of the mango seed kernel. The phytochemical tannin could be the reason for its antibacterial activity.

1. Introduction

Diarrhoea is a most prevalent cause of childhood death in developing countries. *Shigella dysenteriae (S. dysenteriae)* is one of the most important etiology of inflammatory diarrhoea and dysentery. It is estimated that 165 million cases of *Shigella* associated diarrhoea occur annually and 69% of episodes occur in children under five years of age[1]. The prevalence of Shigellosis among diarrhoeal patients in India is 14.05%[2]. Antimicrobial resistance pattern of *Shigella* sp. varies in different parts of the world. This microorganism is resistant to most of the antibiotics when used as a drug for diarrheal therapy. For most of the infectious diseases antibiotics are used as cure. But they have many side effects. The common side effects of antibiotic treatment include kidney problems, abnormal blood clotting, and increased sensitivity to the sun, blood disorders and defenses. Because of these serious threats of synthetic drugs people are turning towards the use of medicinal plants as cure for infectious disease particularly diarrhoea. *Mangifera indica (M. indica)* is one such medicinal plant used as a cure for diarrhea among the traditional and indigenous people of India since ages.

M. indica Linn. is a large evergreen tree, belongs to the family Anacardiaccae. It is commonly known as 'Ma Maram' in Tamil, 'Mango' in English, 'Aam' in Hindi and 'Aamra' in Sanskrit. Different varieties of mango have been cultivated throughout the world and also in India[3]. Seed kernel of mango has been used as a remedy for diarrhoea and dysentery since ancient past[4]. Recent literatures from India also revealed the same[5-7]. Reports from other

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countries like Fiji and Mexico[8,9] also revealed the same. Extracts and fractions of mango seed kernel showed anti-diarrhoeal[10,11], antibacterial[12,13], anti-helminthic[14], anti-ascardial[15] and anti-inflammatory activity[12]. In India not only in ancient past but also today *M. indica* seed kernel powder is used as a remedy for the treatment of gastrointestinal disorders in villages. It is taken along with honey to expel worms[16].

Phytochemical analysis of plants showed the presence of highly effective bioactive compounds. Plant chemicals reported to have antibacterial[17-37], anti-fungal[38], anti-inflammatory[39-42], anti-diabetic[43] antioxidant activity[44-48], *etc*. Secondary metabolites of plants are responsible for odours, pigments, flavour and medicinal properties[49,50]. They are responsible for their defense against many invaders. Phenols and phenolic compounds have gastroprotective activity. Having known these facts, *i.e*., diarrhoea as the major reason for morbidity and mortality of children around the world, particularly in developing countries like India, and the role of *S. dysenteriae* in its etiology and the importance of herbal medicine in the treatment of diarrhoea, this study was undertaken to look for the antibacterial activity of *M. indica* against the enteropathogenic *S. dysenteriae* isolated from the stool samples of diarrhoeal cases.

2. Materials and methods

2.1. Collection and identification of mango seeds

The mango seeds were collected from different mango gardens of Puthalam village of Kanaykumari district, Tamil Nadu, India. The collected plant material was identified and authenticated by using the herbarium specimens available in the department of Botany, Nesamony Memorial Christian College, Marthandam, Kanyakumari district, Tamilnadu, India.

2.2. Preparation of seed kernel extracts

The mango seeds were washed with running tap water to remove sand and other adhering soil particles. Then the seeds were dried in sunlight for 15 d. The seed coats were removed and the kernels are powdered using mortar and pestle, made by solid Thai granite stone. The powdered kernels were stored in an air tight container for further use. Known quantity of the coarse powder was subjected for cold extraction with water and ethyl alcohol (100%) separately. The aqueous and ethyl alcohol extracts were collected and concentrated in vacuum desicator. The concentrated extracts were stored in DMSO and kept under refrigeration at −20 ℃ for further studies. In addition to this, the seed kernel coarse powder was successively extracted with various solvents like hexane, benzene, chloroform, ethylacetate, methanol and water. Different fractions were collected, filtered and evaporated in vacuum desicator and the extractive values were calculated (Table 1).

Table 1
Extractive values of the extracts and fractions of *M. indica* seed kernel.

S. No	Extract / fraction	Extractive value (%) (w/w)
1	Aqueous extract	24.60
2	Crude alcohol extract	15.30
3	Hexane fraction	5.61
4	Benzene fraction	3.25
5	Chloroform fraction	1.34
6	Ethyl acetate fraction	1.40
7	Methanol fraction	16.62
8	Water fraction	19.16

2.3. Phytochemical screening

All the extracts were subjected to preliminary phytochemical screening as per the standard methods[51].

2.4. Bacterial strains used

S. dysenteriae was isolated from the stool samples of diarrhoeal patients, and used as test organism and stored on nutrient agar slants under refrigeration at −4 ℃. The isolated test organism was identified by the procedure described by Bergey's manual of determinative bacteriology[52]. Standard referral *S. dysenteriae* strain − MTCC 1842 obtained from Microbial Type Culture Collection Centre, Chandigarh was also used as reference culture.

2.5. Preparation of inoculum

Clinical isolates and *S. dysenteriae* MTCC 1842 were inoculated in nutrient broth individually and incubated at 37 ℃ for 4 h in a shaker (Orbitech, Scigenics, India) and the seed culture was assay and minimal inhibition concentration (MIC) studies.

2.6. Preparation of disc

Known quantity of extracts and fractions of *M. indica* seed kernel were dissolved in DMSO: Methanol in a ratio of 1:1. This intern was diluted with equal volume of phosphate buffered saline (PBS pH−7). It was then filtered and sterilized by making use of Sortorious syringe filter of pore size 0.22 μ m. Sterile discs of 6 mm diameter (Hi-Media) were loaded with various concentrations of extracts & fractions and were dried. Dried discs were stored in sterile containers. Solvent loaded discs were also prepared and were used as negative control. Oxytetracycline loaded Hi-Media discs were used as positive control.

2.7. Determination of antibacterial activity and MIC

Disc diffusion method was performed to study the antibacterial activity of various extracts and fractions of *M. indica*. The assay was performed in triplicates. The zone of inhibition was measured by making use of Antibiotic Zone Scale (Hi-Media, Mumbai). Agar dilution method was used to find out the MIC[53]. MIC was recorded based on the growth of the test organism.

2.8. Partial purification of bioactive compounds by TLC

TLC was performed by making use of ready made silica coated aluminium plate supplied by M/s Qualigens chemicals Pvt Ltd, Mumbai. The thickness of the TLC plate is about 0.25 mm. TLC plates were dried at 110 ℃ in hot air oven to activate the column allowed to cool. Kernel extract was spotted on the baseline by making use of capillary tube with 15mm space in between the samples. TLC chamber was saturated with solvent mixture (chloroform: ethyl acetate: formic acid in the ratio of 5:4:1). The plates were placed in the solvent system. The chromatogram was developed at room temperature by allowing the solvent to ascent the specified distance. TLC plate was removed from the chamber and marked the solvent front. The plates were sprayed with 50% H_2SO_4 and dried at 60 ℃ for 10 min in hot air oven. The coloured spot developed was observed and recorded the R_f value.

2.9. HPTLC analysis

HPTLC was performed by making use of a Camag – HPTLC unit of M/s Asthagiri Herbal foundation, Chennai. Plant extracts and fractions were dissolved in respective solvents. Sample was applied on 0.22 mm thick silica gel plate by making use of Camag automatic TLC sampler. Each sample was applied as band and as not spot. Chromatograms were developed with active stationary, mobile and vapour phases. Stationary phase plate was put into the mobile phase containing organic solvents like hexane: ethyl acetate: methanol in the ratio of 1:2:1. Stepwise automatic procedure was followed at room temperature to run the column. Automatic developing chamber was used to develop the chromatogram. Chemical compounds are quantitated and evaluated through spectral scanner. Scanning was controlled by Camag software© 1998 available in the instrument. Computerized scanning HPTLC report provided the informations like R_f value, λ_{max} and percentage of chemical constituents present in the sample. The results were recorded and interpreted.

2.10. Statistical analysis

Statistical analysis was done by Student's *t* test and one-way ANOVA using Origin 6.0 software for epidemiological studies. Antimicrobial activity was assessed by calculating mean ± SD.

3. Results

The cold and solvent extractive values of *M. indica* seed kernel was ranged from 1.4% to 19.16% (Table 1). Aqueous extract showed maximum extractive value (24.6%). Chloroform and ethyl acetate fraction had less extractive values (1.34% and 1.4%).

Antibacterial activity of *M. indica* seed kernel extracts and fractions were summarized in Table 2. It was observed that aqueous extracts and five different fractions such as

Table 2
Antibacterial activity of *M. indica* seed kernel extract against *S. dysenteriae* (mean±SD) (*n*=10).

S. No	Extract / fraction	Zone of inhibition (mm)					
		Positive control (30 μg)	Negative control (20 μL)	100 μg	200 μg	400 μg	800 μg
1	Aqueous extract	20.00±1.40	Nil	Nil	Nil	10.30±1.50	12.60±0.57
2	Crude alcohol extract	20.80±1.30	Nil	Nil	12.00±0.00	13.30±1.15	16.00±1.00
3	Hexane fraction	21.60±1.51	Nil	Nil	12.60±0.57	15.00±1.00	17.60±0.57
4	Benzene fraction	21.20±0.83	Nil	Nil	17.60±1.52	19.60±1.52	20.00±1.00
5	Chloroform fraction	19.80±2.40	Nil	Nil	12.00±1.00	14.00±2.00	15.00±1.00
6	Ethyl acetate fraction	19.00±1.00	Nil	Nil	Nil	Nil	Nil
7	Methanol fraction	19.60±0.54	Nil	Nil	9.60±2.08	17.00±1.00	18.60±1.52
8	Water fraction	20.20±0.44	Nil	Nil	Nil	11.30±1.15	11.60±1.50

Positive control – Oxytetracycline; Negative control – Solvent used (PBS, PBS: MeOH, PBS: MeOH: DMSO).

Table 3
Minimum inhibitory concentration of *M. indica* against *S. dysenteriae* (mean±SD) (*n* = 10).

S. No	Extract / fraction	MIC of clinical isolate (μg/mL)
1	Aqueous extract	380.00±20.90
2	Crude alcohol extract	190.00±54.70
3	Hexane fraction	260.00±62.70
4	Benzene fraction	135.00±22.30
5	Chloroform fraction	95.00±11.18
6	Ethyl acetate fraction	450.00±11.20
7	Methanol fraction	130.00±27.30
8	Water fraction	215.00±22.30

Positive control – Oxytetracycline; Negative control – Solvent used (PBS, PBS: MeOH, PBS: MeOH: DMSO).

hexane, benzene, chloroform, ethyl acetate, methanol and water showed significant antibacterial activity. Benzene fraction showed maximum antibacterial activity [(20.00±1.00) mm], followed by methanol fraction (18.60±1.52), hexane fraction [(17.60±0.57) mm], crude alcohol extract [(16.00±1.00) mm], chloroform fraction [(15.00±1.00) mm] and water extract (aqueous) [(12.60±0.57) mm]. Water fractions showed minimum zone of inhibition [(11.60±1.50) mm]. Surprised to our knowledge ethylacetate fraction had no antibacterial activity.

MIC of chloroform fraction was found to be (95.00±11.18) g/mL followed by methanol fraction [(130.00±27.30) μg/mL], benzene fraction [(135.00±22.30) μg/mL], crude alcohol extract [(190.00±54.70) μg/mL]. Hexane fraction and water fraction showed MIC at (215.00±22.30) μg/mL concentrations (Table 3).

M. indica seed kernel extracts had various bioactive compound such as alkaloids, phenolic compounds, tannins, gum and saponins. Methanol fractions have steroids. Tannin and saponin were observed in all the extracts and fractions. Terpenoids and quinines were not observed in each fractions and extracts. Ethanolic extracts and fractions had flavonoides. Phenolic compounds were observed in aqueous and crude alcohol extracts (Table 4).

To confirm the presence of bioactive compounds assessed by qualitative analysis, TLC was performed. Based on the developed chromatogram and calculated R_f value, it was found that *M. indica* had quantifiable level of alkaloids, tannins and other phenolic compounds (Table 5).

Crude water extract of *M. indica* seed kernel had eight compounds and tannin was found to be present in all the extracts. Ethanolic extract had seven bioactive compounds. Fractions like hexane, benzene, chloroform, methanol and water also showed the presence of tannin (Table 6 and Figure 1).

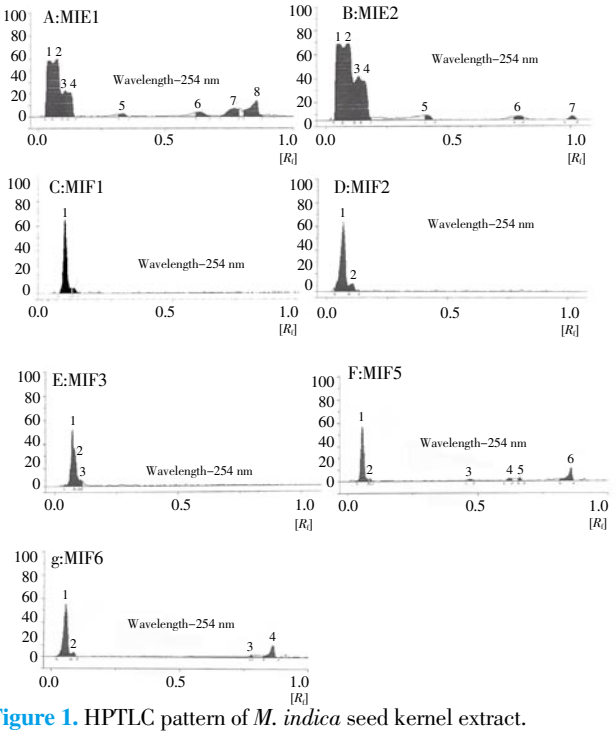

Figure 1. HPTLC pattern of *M. indica* seed kernel extract.

Table 4

Qualitative analysis of bioactive compounds – *M. indica* seed kernel extracts and fractions.

S. No	Extract / fraction	Steroids	Triterpenoids	Alkaloids	Phenolic compounds	Saponins	Quinone	Tannins	Flavonoids	Gum	Xanthoprotein
1	Aqueous extract	–	–	–	+	+	–	+	–	+	+
2	Crude alcohol extract	–	–	+	+	+	–	+	+	+	+
3	Hexane fraction	–	–	+	–	+	–	+	–	+	+
4	Benzene fraction	–	–	+	–	+	–	+	–	+	–
5	Chloroform fraction	–	–	+	–	+	–	+	–	+	–
6	Ethyl acetate fraction	–	–	+	–	+	–	+	–	+	–
7	Methanol fraction	+	–	+	–	+	–	+	–	+	+
8	Water fraction	–	–	+	–	+	–	+	–	+	+

Table 5

Thin layer chromatographic pattern of *M. indica* fruit rind extracts andfractions.

S. No	Chemical and extract / fraction	R_f value				
		Spot 1	Spot 2	Spot 3	Spot 4	Spot 5
1	Gallic acid	0.05	0.70	0.50	0.40	–
2	Aqueous extract	0.05	0.30	0.56	0.80	–
3	Crude alcohol extract	0.05	0.40	0.60	0.80	–
4	Hexanefraction	0.05	0.40	0.60	0.70	–
5	Benzenefraction	0.05	0.40	0.60	0.70	–
6	Chloroform fraction	0.05	0.40	0.60	0.70	–
7	Ethyl acetatefraction	0.05	0.40	0.50	0.70	–
8	Methanolfraction	0.05	0.40	0.50	0.70	0.80
9	Waterfraction	0.05	0.40	0.50	0.80	–

Table 6
HPTLC pattern of various extracts andfractions of *M. indica* seed kernel.

S. No	Extract / fraction	Spot	R_f value	λ_{max}	%
1	Hexanefraction	1	0.06	285	93.83
2	Hexanefraction	2	0.10	290	6.17
3	Benzenefraction	1	0.06	283	89.53
4	Benzenefraction	2	0.10	290	10.47
5	Chloroformfraction	1	0.06	285	63.27
6	Chloroformfraction	2	0.17	353	29.74
7	Methanolfraction	1	0.05	282	64.74
8	Methanolfraction	2	0.08	297	1.75
9	Methanolfraction	3	0.46	251	3.66
10	Methanolfraction	4	0.62	200	4.84
11	Methanolfraction	5	0.66	200	3.68
12	Methanolfraction	6	0.86	200	21.32
13	Waterfraction	1	0.05	283	73.79
14	Waterfraction	2	0.08	311	5.06
15	Waterfraction	3	0.78	200	1.90
16	Waterfraction	4	0.86	200	19.25
17	Aqueous extract	1	0.04	286	24.66
18	Aqueous extract	2	0.07	289	32.30
19	Aqueous extract	3	0.12	289	8.76
20	Aqueous extract	4	0.33	331	1.67
21	Aqueous extract	5	0.63	302	3.29
22	Aqueous extract	6	0.77	200	7.38
23	Aqueous extract	7	0.86	200	11.63
24	Crude alcohol extract	1	0.04	293	29.08
25	Crude alcohol extract	2	0.08	295	38.56
26	Crude alcohol extract	3	0.12	277	13.88
27	Crude alcohol extract	4	0.41	299	13.69

M1EI – Aqueous extract; M1E2 – Crude alcohol extract; M1F1 – Hexane fraction; M1F2 – Benzene fraction; M1F3 – Chloroform fraction; M1F5 – Methanol fraction; M1F6 – Water fraction.

4. Discussion

In the present study, it is proved that, extracts and fractions of mango seeds are effective against the pathogen responsible for dysentery and diarrhoel diseases. The extractable value was maximum in aqueous extracts and fractions followed by alcoholic extract and methanol fraction. This indicates that among the different solvents tested, aqueous media was the suitable solvent for the maximum extraction of bioactive compounds. The active secondary metabolite in aqueous extract could be tannins. Similar results was reported by Gen-ichiro[54] in isolation and structure elucidation of tannins.

Methanolic fraction of seed kernel showed highest antibacterial activity. Similar results were observed by Sairam *et al*[13] and Gabino *et al*[55]. Ethanolic extract and other fractions showed good antibacterial activity. Past studies also showed the similar results in mango seed kernel[10,55]. In this study effective MIC was observed in chloroform fraction *i.e.* (95.00 ± 11.18) μg/mL. Whereas Das *et al*[12] showed variable results, who showed that MIC of 3 mg for *E. coli*, *Proteus* sp., *Bacillus* sp., 1.5 mg for *Agrobacterium* sp., *Sarcina* sp. (2mg) and 4 mg for *Pseudomanas* sp. Shigella is one of the dangerous pathogen which is responsible for bacillary dysentery, if untreated it may leads to haemolytic uremic syndrome[56]. This infection is more severe in children. Similar kind of work was reported by Kaur *et al*[11] using *E. coli*, *Staphylococcus aureus* and *Vibrio vulnificus*. These organisms may not directly involved in adult human dysentery but *Shigella* sp. used in this study directly involved in human dysentery. Most of the scientists are looking for some new therapies for the treatment of bacillary dysentery. In this context, mango seed kernel extracts and fractions showed good antimicrobial activity against *Shigella* sp. isolated from dysentery stool. Phenolic compounds, tannins, flavonoids were the major compound isolated from mango seed kernel extracts. These chemicals are found to be responsible for antimicrobial property. This may be due to iron binding capacity of tannic acid[57]. They also prevent microorganisms by inhibiting extracellular microbial enzymes, by depriving the substrates required for the microbial growth and by avoiding oxidative phosphorylation. Flavonoids may precipitate proteins of microorganisms and there by growth is completely arrested[58].

Mango seed kernel is a refrigerant and is used to kill

abdominal worms and also given as a cure for vomiting, diarrhoea and hyperacidity[59]. Seed kernel extracts showed the presence of alkaloids, phenolic compounds, tannin, gum and saponins. Methonal fraction had steroids. In addition to flavanoids, xanthoprotein were also found in crude extract and fractions. Similar results were shown by the past workers[60-64] who reported the presence of tannins, ellagitannins, phenolic compounds etc., in the seed kernel of mango.

TLC and HPTLC results of this study showed the presence of major secondary metabolites of this plant particularly tannin and R_f value of which ranges between 0.05 - 0.95. Tannic acid produced four major spots corresponding to gallic acid, digallic acid and trigallic acid. Miroslov and Guy[65] described that the R_f value of tannin ranges from 0.05, 0.06, 0.07 and up to 0.36. From the above findings it is proved that all the extracts and fractions of *M. indica* seed kernels had significant antimicrobial activity against the isolated pathogen *S. dysenteriae*. It may be due the presence of tannin in the mango seed kernel. Further work is in progress on the isolation, purification and identification of the active bioactive components in the seed kernel extract of *M. indica*.

Conflict of interest statement

We declare that we have no conflict of interest.

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