

Contents lists available at [ScienceDirect](http://ScienceDirect)

## Asian Pacific Journal of Tropical Medicine

journal homepage: [www.elsevier.com/locate/apjtm](http://www.elsevier.com/locate/apjtm)

Document heading doi:

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

Rajan S<sup>1</sup>, Thirunalasundari T<sup>2</sup>, Jeeva S<sup>3\*</sup><sup>1</sup>Department of Microbiology, Srimad Andavan Arts and Science College, Tiruchirapalli – 620 005, Tamilnadu, India<sup>2</sup>Department of Biotechnology, Bharathidasan University, Tiruchirapalli – 620 024, Tamilnadu, India<sup>3</sup>Centre for Biodiversity and Biotechnology, Department of Botany, N.M. Christian College, Marthandam, Kanyakumari – 629 165, Tamilnadu, India

## ARTICLE INFO

## Article history:

Received 07 January 2011

Received in revised form 1 February 2011

Accepted 15 March 2011

Available online 20 April 2011

## Keywords:

Antibacterial activity

*Mangifera indica* (seed kernel)

Minimum inhibitory concentration

Phytochemistry

*Shigella dysenteriae*

## 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  $(95 \pm 11.8) \mu\text{g/mL}$ . MIC of other fractions ranged from 130–380  $\mu\text{g/mL}$ . **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<sup>[1]</sup>. The prevalence of Shigellosis among diarrhoeal patients in India is 14.05%<sup>[2]</sup>. 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 diarrhoeal 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 Anacardiaceae. 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<sup>[3]</sup>. Seed kernel of mango has been used as a remedy for diarrhoea and dysentery since ancient past<sup>[4]</sup>. Recent literatures from India also revealed the same<sup>[5–7]</sup>. Reports from other

\*Corresponding author: Dr. Jeeva S, Assistant Professor, Department of Botany, NM Christian College, Marthandam, Kanyakumari – 629 165, Tamil Nadu, India.

Tel: +91 9952202112

E-mail: [solomonjeeva@gmail.com](mailto:solomonjeeva@gmail.com)

countries like Fiji and Mexico<sup>[8,9]</sup> also revealed the same. Extracts and fractions of mango seed kernel showed anti-diarrhoeal<sup>[10,11]</sup>, antibacterial<sup>[12,13]</sup>, anti-helminthic<sup>[14]</sup>, anti-ascarial<sup>[15]</sup> and anti-inflammatory activity<sup>[12]</sup>. 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<sup>[16]</sup>.

Phytochemical analysis of plants showed the presence of highly effective bioactive compounds. Plant chemicals reported to have antibacterial<sup>[17–37]</sup>, anti-fungal<sup>[38]</sup>, anti-inflammatory<sup>[39–42]</sup>, anti-diabetic<sup>[43]</sup> antioxidant activity<sup>[44–48]</sup>, etc. Secondary metabolites of plants are responsible for odours, pigments, flavour and medicinal properties<sup>[49,50]</sup>. 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 desiccator. The concentrated extracts were stored in DMSO and kept under refrigeration at  $-20\text{ }^{\circ}\text{C}$  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 desiccator 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<sup>[51]</sup>.

### 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  $-4\text{ }^{\circ}\text{C}$ . The isolated test organism was identified by the procedure described by Bergey's manual of determinative bacteriology<sup>[52]</sup>. 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  $37\text{ }^{\circ}\text{C}$  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 Sortorius syringe filter of pore size  $0.22\text{ }\mu\text{m}$ . 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<sup>[53]</sup>. 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 °C 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<sub>2</sub>SO<sub>4</sub> and dried at 60 °C for 10 min in hot air oven. The coloured spot developed was observed and recorded the R<sub>f</sub> 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<sub>f</sub> value, λ<sub>max</sub> 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).

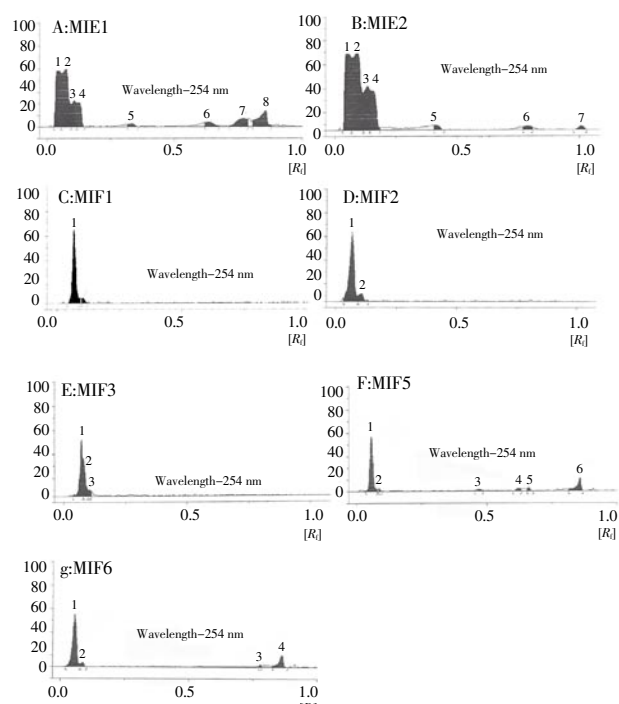


Figure 1. HPTLC pattern of *M. indica* seed kernel extract.

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 and fractions.

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	Hexane fraction	0.05	0.40	0.60	0.70	–
5	Benzene fraction	0.05	0.40	0.60	0.70	–
6	Chloroform fraction	0.05	0.40	0.60	0.70	–
7	Ethyl acetate fraction	0.05	0.40	0.50	0.70	–
8	Methanol fraction	0.05	0.40	0.50	0.70	0.80
9	Water fraction	0.05	0.40	0.50	0.80	–

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

S. No	Extract / fraction	Spot	R <sub>f</sub> value	λ <sub>max</sub>	%
1	Hexane fraction	1	0.06	285	93.83
2	Hexane fraction	2	0.10	290	6.17
3	Benzene fraction	1	0.06	283	89.53
4	Benzene fraction	2	0.10	290	10.47
5	Chloroform fraction	1	0.06	285	63.27
6	Chloroform fraction	2	0.17	353	29.74
7	Methanol fraction	1	0.05	282	64.74
8	Methanol fraction	2	0.08	297	1.75
9	Methanol fraction	3	0.46	251	3.66
10	Methanol fraction	4	0.62	200	4.84
11	Methanol fraction	5	0.66	200	3.68
12	Methanol fraction	6	0.86	200	21.32
13	Water fraction	1	0.05	283	73.79
14	Water fraction	2	0.08	311	5.06
15	Water fraction	3	0.78	200	1.90
16	Water fraction	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

M1E1 – 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 diarrhoeal 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 *Pseudomonas 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<sup>[59]</sup>. Seed kernel extracts showed the presence of alkaloids, phenolic compounds, tannin, gum and saponins. Methanol fraction had steroids. In addition to flavanoids, xanthoprotein were also found in crude extract and fractions. Similar results were shown by the past workers<sup>[60–64]</sup> 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<sup>[65]</sup> 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.

### Acknowledgements

The authors thank the patients who have given samples for isolation of enteric pathogens. The authors also thank the management of Srimad Andavan Arts and Science College, Tiruchirappalli for providing required facility to carry out this work and MTCC, Chandigarh, India for providing referral strain.

### References

- [1] John C, Karen K, Bradford K. *Generic protocol to estimate the burden of Shigella diarrhoea and dysenteric mortality*. Geneva: World Health Organization; 1999.
- [2] WHO. *World health report shaping the future*. Geneva: World Health Organization; 2003.
- [3] Jeeva S. Horticultural potential of wild edible fruits used by the Khasi tribes of Meghalaya. *J Hortic For* 2009; 1(9): 182–192.
- [4] Sharma PV. Treatment of diarrhoea, In: *Charak samhitha–critical notes*. 7th edition. Varanasi: Chaukhambha Orientalia Publishers; 2003.
- [5] Kiruba S, Jeeva S, Das SSM. Enumeration of ethnoveterinary plants of Cape Comorin, Tamil Nadu. *Indian J Tradition Know* 2006; 5(4): 576–578.
- [6] Jasmine TS, Jeeva S, Febreena GL, Mishra BP, Laloo RC. Wild edible plants of Meghalaya, North–east India. *Nat Prod Rad* 2007; 6(5): 410–426.
- [7] Jeeva S, Kingston C, Kiruba S, Kannan D. Sacred forests – treasure trove of medicinal plants: a case study from south Travancore. In: Trivedi PC. *Indigenous medicinal plants*. Jaipur: Pointer Publishers; 2007, p. 262–74.

- [8] Singh YN. Traditional medicine in Fiji – some herbal folk cures used by Fiji Indians. *J Ethnopharmacol* 1986; 15: 57–88.
- [9] Ponce MM, Navarro AI, Martinez GMN, Alvarez CR. *In vitro* anti-giardiac activity of plant extracts, *LaRevi Stade Invest Clinica* 1994; 46(5): 343–347.
- [10] Kabuki T, Kakajima H, Arai M, Veda S, Kuwabara Y, Dosaiko S. Characterization of novel antimicrobial compounds from mango (*Mangifera indica*) seed kernel. *Food Chem* 2000; 71: 61–66.
- [11] Kaur J, Rathinam X, Kasi M, Leng KM, Ayyalu R, Kathiresan S, et al. Preliminary investigation on the antibacterial activity of mango (*Mangifera indica* L: Anacardiaceae) seed kernel. *Asian Pac J Trop Med* 2010; 3(9): 707–710.
- [12] Das PC, Das A, Mandal S, Islam CN, Dutta MK, Patra BB, et al. Anti-inflammatory and antimicrobial activities of the seed Kernel of *M. indica*. *Fitoterapia* 1989; 60: 235–240.
- [13] Sairam K, Hemalatha S, Kumar A, Srinivasan T, Jaiganesh S, Shankar M, et al. Evaluation of anti-diarrhoeal activity in seed extracts of *Mangifera indica*. *J Ethnopharmacol* 2003; 84: 11–15.
- [14] Sharma LD, Bhage HS, Srivastava PS. *In vitro* antihelminthic screening of indigenous medicinal plants. *Indian J Anim Res* 1971; 5: 33–38.
- [15] Feroz H, Khare AK, Srivastava MC. Review of scientific studies on antihelminths from plants. *J Sci Res Appl Med* 1982; 3: 6–12.
- [16] Kurian JC. *Plants that heal*. 3rd edition. Pune: Orient watchman Publishing house; 1998.
- [17] Anpin Raja RD, Prakash JW, Jeeva S. Antibacterial activity of some medicinal plants used by Kani tribe, southern Western Ghats, Tamilnadu, India. In: Trivedi PC. *Ethnic tribes and medicinal plants*. Jaipur: Pointer Publishers; 2010, p. 28–45.
- [18] Bhattacharjee I, Chatterjee SK, Chandra G. Isolation and identification of antibacterial components in seed extracts of *Argemone mexicana* L. (Papaveraceae). *Asian Pac J Trop Med* 2010; 3(7): 547–551.
- [19] Bhimba BV, Meenupriya J, Joel EL, Naveena DE, Kumar S, Thangaraj M. Antibacterial activity and characterization of secondary metabolites isolated from mangrove plant *Avicennia officinalis*. *Asian Pac J Trop Med* 2010; 3(7): 544–546.
- [20] Darabpour E, Motamedi H, Nejad SMS. Antimicrobial properties of *Teucrium polium* against some clinical pathogens. *Asian Pac J Trop Med* 2010; 3(2): 124–127.
- [21] Irudayaraj V, Janaky M, Johnson M, Selvan N. Preliminary phytochemical and antimicrobial studies on a spike–moss *Selaginella inaequalifolia* (hook. & grev.) Spring. *Asian Pac J Trop Med* 2010; 3(12): 412–420.
- [22] Jarrar N, Abu–Hijleh A, Adwan K. Antibacterial activity of *Rosmarinus officinalis* L. alone and in combination with cefuroxime against methicillin – resistant *Staphylococcus aureus*. *Asian Pac J Trop Med* 2010; 3(2): 121–123.
- [23] Jeeshna MV, Manorama S, Paulsamy S. Antimicrobial property of the medicinal shrub, *Glycosmis pentaphylla*. *J Basic Applied Bio* 2009; 3(1&2): 25–27.
- [24] Johnson M, Wesely EG, Zahir Hussain MI, Selvan N. *In vivo* and *in vitro* phytochemical and antibacterial efficacy of *Baliospermum montanum*(Willd.) Muell. Arg. *Asian Pac J Trop Med* 2010; 3(11): 894–897.
- [25] Kannan RRR, Arumugam R, Anantharaman P. Antibacterial potential of three seagrasses against human pathogens. *Asian Pac J Trop Med* 2010; 3(11): 890–893.
- [26] Kingston C. Medicinal plants used in the endemic art of Travancore. *J Basic Appl Biol* 2007; 1(1): 38–39.
- [27] Koochak H, Nejad SMS, Motamedi H. Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran).

- Asian Pac J Trop Med* 2010; **3**(3): 180–184.
- [28]Laila Banu NR, Sreeja S, Pinky VR, Prakash JW, Jeenath Jasmine A. Medicinal plants used by the rural people of Kattathurai, Kanyakumari district, Tamilnadu. *J Basic Appl Biol* 2007; **1**(1): 18–22.
- [29]Mansour A, Enayat K, Neda MS, Behzad A. Antibacterial effect and physicochemical properties of essential oil of *Zataria multiflora* Boiss. *Asian Pac J Trop Med* 2010; **3**(6): 439–442.
- [30]Moghadam MS, Maleki S, Darabpour E, Motamedi H, Nejad SMS. Antibacterial activity of eight Iranian plant extracts against methicillin and cefixime resistant *Staphylococcus aureus* strains. *Asian Pac J Trop Med* 2010; **3**(4): 262–265.
- [31]Naik MI, Fomda BA, Jaykumar E, Bhat JA. Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some selected pathogenic bacterias. *Asian Pac J Trop Med* 2010; **3**(7): 535–538.
- [32]Nejad SMS, Koochak H, Darabpour E, Motamedi H. A survey on *Hibiscus rosa-sinensis*, *Alcea rosea* L. and *Malva neglecta* Wallr as antibacterial agents. *Asian Pac J Trop Med* 2010; **3**(5): 351–355.
- [33]Okoye TC, Akah PA, Okoli CO, Ezike AC, Mbaoji FN. Antimicrobial and antispasmodic activity of leaf extract and fractions of *Stachytarpheta cayennensis*. *Asian Pac J Trop Med* 2010; **3**(3): 189–192.
- [34]Pugazharasi G, Meenakshi SA, Ramesh Kannan N, Bastin Churchill M, Natarajan E. Screening of antimicrobial activity of *Phyllanthus maderaspatensis* L. *J Basic Appl Biol* 2009; **3**(3&4): 43–49.
- [35]Rajan S, Jeevagangai TJ. Studies on the antibacterial activity of *Aegle marmelos* – fruit pulp and its preliminary phytochemistry. *J Basic Appl Biol* 2009; **3**(1&2): 76–81.
- [36]Sadheeshna Kumari S, Huxley AJ, Sasikala. *In vitro* propagation of medicinally important plant *Mimosa invisa*. *J Basic Applied Biol* 2009; **3**(3&4): 27–32.
- [37]Suresh SN, Nagarajan N. Preliminary phytochemical and antimicrobial activity analysis of *Begonia malabarica* Lam. *J Basic Applied Biol* 2009; **3**(1&2): 59–61.
- [38]Suresh Kumar P. Anti-fungal activity of *Leptadenia reticulata* in rat animal model *in vivo*. *J Basic Appl Biol* 2008; **2**(1): 9–13.
- [39]Emamuzo ED, Miniakiri SI, Tedwin EJO, Ufouma O, Lucky M. Analgesic and anti-inflammatory activities of the ethanol extract of the leaves of *Helianthus annuus* in Wistar rats. *Asian Pac J Trop Med* 2010; **3**(5): 341–347.
- [40]Georgewill OA, Georgewill UO, Nwankwoala RNP. Anti-inflammatory effects of *Moringa oleifera* lam extract in rats. *Asian Pac J Trop Med* 2010; **3**(2): 133–135.
- [41]Saha S, Goswami G. Study of anti ulcer activity of *Ficus religiosa* L. on experimentally induced gastric ulcers in rats. *Asian Pac J Trop Med* 2010; **3**(10): 791–793.
- [42]Shenoy S, Shwetha K, Prabhu K, Maradi R, Bairy KL, Shanbhag T. Evaluation of antiinflammatory activity of *Tephrosia purpurea* in rats. *Asian Pac J Trop Med* 2010; **3**(3): 193–195.
- [43]Osadebe PO, Omeje EO, Uzor PF, David EK, Obiorah DC. Seasonal variation for the antidiabetic activity of *Loranthus micranthus* methanol extract. *Asian Pac J Trop Med* 2010; **3**(3): 196–199.
- [44]Kumar BSA, Lakshman K, Jayaveera KN, Shekar DS, Kumar AA, Manoj B. Antioxidant and antipyretic properties of methanolic extract of *Amaranthus spinosus* leaves. *Asian Pac J Trop Med* 2010; **3**(9): 702–706.
- [45]Melinda KP, Rathinam X, Marimuthu K, Diwakar A, Ramanathan S, Kathiresan S, Subramaniam S. A comparative study on the antioxidant activity of methanolic leaf extracts of *Ficus religiosa* L, *Chromolaena odorata* (L.) King & Robinson, *Cynodon dactylon* (L.) Pers. and *Tridax procumbens* L. *Asian Pac J Trop Med* 2010; **3**(5): 348–350.
- [46]Kannan RRR, Arumugam R, Anantharaman P. *In vitro* antioxidant activities of ethanolic extract from *Enhalus acoroides* (L.F.) Royle. *Asian Pac J Trop Med* 2010; **3**(11): 898–901.
- [47]Haripriya D, Selvan N, Jeyakumar N, Periasamy RS, Johnson M, Irudayaraj V. The effect of extracts of *Selaginella involvens* and *Selaginella inaequalifolia* leaves on poultry pathogens. *Asian Pac J Trop Med* 2010; **3**(9): 678–681.
- [48]Selvamaleeswaran P, Wesely EG, Johnson M, Velusamy S, Jeyakumar N. The effect of leaves extracts of *Clitoria ternatea* Linn against the fish pathogens. *Asian Pac J Trop Med* 2010; **3**(9): 723–726.
- [49]Ponni V, Thenmozhi S, Rajan S. Screening of bioactive potentials and phytochemical nature of *Solanum trilobatum* extracts. *J Basic Appl Biol* 2009; **3**(3&4): 134–139.
- [50]Jeeva S, Jasmine T Sawian, Febreena G Lyndem, Laloo RC, Venugopal N. Medicinal plants in Northeast India: past, present and future scenario. National Seminar on Past, Present and Future Scenario in Medicinal Plants and Phytochemistry. Thiruchirappalli Tamil Nadu: Department of Plant Science, Bharathidasan University; 2007.
- [51]Harboune JH. *Phytochemical methods*. 2nd edition. New York: London Compant Hall; 1984.
- [52]Garrity GM. *Bergey's manual of systematic bacteriology*. 2nd ed. New York: Springer-Verlag; 2001.
- [53]National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disk susceptibility test – third edition – Approved standards*. Villanova: National Committee for Clinical Laboratory Standards; 1993.
- [54]Gen-ichiro N. Isolation and structure elucidation of tannins. *Pure Appl Chem* 1989; **61**: 357–60.
- [55]Gabino G, Deyarina G, Yeny L, Dagmar G, Lizt L, Gypsy Q, et al. *In vivo* and *in vitro* anti-inflammatory activity of *Mangifera indica* L. extract. *Pharmacol Res* 2004; **50**: 143–149.
- [56]Rajan S. *Medical microbiology in dysentery*. Chennai: MJP Publishers; 2007, p. 167–188.
- [57]Chung KT, Luz CMW. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. *Food Chem Toxicol* 1998; **36**: 1053–1060.
- [58]Scalbert A. Antimicrobial properties of tannins. *Phytochem* 1991; **30**: 3875–3883.
- [59]Vatsayan R. Medicinal Plants. In: Hari Jaisingh. *Mango, the fruit medicine*, Chandigarh: The Tribune House Publication; 2002.
- [60]Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H, et al. Antibacterial activity of hydrolysable tannins derived from plants against *Helicobacter pylori*. *Microbiol Immunol* 2004; **48**(4): 251–261.
- [61]Paul RK, Irudayaraj V, Johnson M, Patric RD. Phytochemical and anti-bacterial activity of epidermal glands extract of *Christella parasitica* (L.) H. Lev. *Asian Pac J Trop Biomed* 2011; **1**(1): 8–11.
- [62]Habbal O, Hasson SS, El-Hag AH, Al-Mahrooqi Z, Al-Hashmi N, Al-Bimani Z, et al. Antibacterial activity of *Lawsonia inermis* Linn (Henna) against *Pseudomonas aeruginosa*. *Asian Pac J Trop Biomed* 2011; **1**(3): 173–176.
- [63]Yean YS, Philip JB. Antioxidant activity and phenolic content of selected fruit seeds. *Food Chem* 2004; **88**: 411–417.
- [64]Okuda T. Systematic and health effects of chemically distinct tannin in medicinal plants. *Phytochem* 2005; **66**: 2012–2031.
- [65]Miroslav D, Guy B. *Determination of tannic acid in Beer by thin layer chromatography*. Toronto: American Society of Brewing chemist, Inc.; 1978.