

A promising Natural remedy for Drug Resistant Bacteria Associated Diarrhea from *Mangifera indica. L*

Sudha A¹, Sathiyathan S² & Amutha R^{2*}

¹ *Department of Microbiology, Vivekanandha College of Arts and Sciences for Women,
Ellayampalayam, Tiruchengode- 636725, Tamil Nadu, India*

² *Department of Biotechnology, Periyar University PG Extension centre Dharmapuri. Tamil
Nadu, India*

*Corresponding author

Email: draramutha@rocketmail.com

Abstract

We examine the antibacterial activity of *Mangifera indica.L* against the drug resistant bacterial pathogens. The Indian traditional mango fruit exposed promise in the prevention of diarrhea. However, there is less information existing regarding activity against drug resistance bacteria causing diarrhea. In this study, we performed isolation and identification of bacterial pathogens from diarrheal stool samples and analyzed their antibiotic resistance pattern with three generation antibiotics. Antibiotic activity of mango kernel exhibited remarkable activity from $15.2\pm0.5\text{mm}$ to $19.0\pm0.3\text{mm}$ zone of inhibition. MIC of kernel extract was noted as $10\text{ }\mu\text{g /ml}$. Current study provide the potent antibacterial activity of mango kernel against the drug resistant bacteria. In conclusion, the traditional Indian fruit become the natural remedy for the treatment of diarrhea.

Keywords: Antibiotic sensitivity, Diarrhea, Drug resistant, MIC, Natural remedy.

Introduction

Diarrhoea is a very essential health issue, chiefly in developing countries where it is predictable to be the second major cause of death in children less than five years, killing more children than AIDS, Malaria and Measles combined (Lescano et al.2016). Various bacteria can cause the diarrhea. Among the bacterial pathogens, *enteropathogenic Escherichia coli*, *Shigella sp* and *Salmonella sp* are causing moderate to severe diarrhea in children (Kirkby D Tickell et al.2020).

The increased levels of resistance have reduced the efficiency of before preferred first-line antibiotics (David R. Tribble 2017). Random antibiotic treatment is critical for children and weak individuals with severe infections of bacteria. Besides, emerging of multi-drug resistance (MDR) strains are increased in Enterobacteriaceae family in recent years, which leads to the treatment impasse and challenges in diarrheal illness (Shun-Xian Zhang *et al.*, 2018).

Worldwide, diverse documents are reported by various institutes and agencies about this severe global problem in public health. There are several proposed recommendations and resolutions from various researches, in addition to that several reports are documented about this drug resistance, but still made a small advancement. Unfortunately, the increase in antibiotic resistance is a persistent issue (Roca *et al.*, 2015). There are many mechanisms are reported about antibiotic resistance in bacteria. However, abuse of antibiotics or misuse in treating diarrhea which increases antibiotic resistance in bacteria (Reza Ranjbar & Abbas Farahani 2019).

Now a day's treatment failure is common due to antibiotic resistance and multi-drug resistance; Alternative treatment procedures are under consideration to fight against bacterial infection (Sojib Bin Zaman *et al.*, 2017). In India, the mango fruit has a great cultural, socio-economic and religious worth since early times (Gumte *et al.*, 2018). Traditionally *M. indica* seed kernel (MISK) has been used for its anti-diarrheal activity in Indian medicine (Subburaj, 2017).Current study focused on the antibacterial activity against the drug resistant bacterial strains which are associated with diarrhea. Antibiotic sensitivity analysis provided the resistance pattern of pathogens which is useful in the treatment and management of infectious diseases.

Materials and methods

Diarrheal sample collection and Identification of bacterial pathogens

Diarrheal stool samples were collected from Erode Micro lab in Tamil Nadu. Samples were carefully transferred to the Microbiological lab and processed for the isolation of bacterial pathogens. Samples were directly streaked on the selective media plates. Identification of bacteria done by standard microbiological methods using Bergey's manual of systematic bacteriology through Microscopy(Gram staining) , biochemical analysis(IMViC) and further identification by using 16 s rRNA partial sequencing and submitted to GENBANK.

Antibiotic Sensitive Profile of Isolated Bacterial pathogens

Antibiotic susceptibility test was carried out to check the resistance pattern of isolated bacterial pathogens using disc diffusion method described by Mamuye Y (2015). Totally 10 different antibiotics were used for the analysis. Ampicillin (10 mcg), Tetracycline (15mcg), Chloramphenicol (30 mcg), Amikacin (30 mcg), Gentamicin(15mcg) and Trimethoprim (5 mcg) are used for first generation antibiotics. Ciprofloxacin, (5mcg), Norfloxacin (10 mcg) and Azithromycin (15mcg) are used for second generation antibiotics. Ceftriaxone (30 mcg) was used for third generation antibiotics. Based on the inhibition of zone around the disc was calculated and the results were interpreted as per the CLSI guidelines.

Plant sample collection and Extraction

Mangifera indica fruits were collected from the various places of Erode in Tamil Nadu. Seeds were removed from the fruits and thoroughly washed with tap water and sun dried. After drying kernel were carefully removed from the seed coat and made a powder for extraction. Extraction was carried out using cold percolation method described by Talaat et al. 2016. Briefly, known amount of kernel powder was extracted overnight with 70% ethanol in 1:3 ratio. After centrifugation the filtrate extract were concentrated and stored at -20°C for further use.

Antibacterial activity of Mango kernel extract against isolated pathogens

Mango kernel ethanol extract antibacterial activity was assessed by using agar disc diffusion method with various concentrations. (Divya et al. 2020). For positive control,

Penicillin was used and Ethanol was used as a negative control to calculate the sensitivity of identified bacterial pathogens.

Minimum inhibitory concentration (MIC) of Mango kernel ethanol extract

The minimum inhibitory concentration (MIC) of Mango kernel ethanol extract was determined by 96-well micro-titre plate technique with Resazurin as an indicator (Divya et al. 2020). Briefly, various concentrations of extract (0–100 $\mu\text{g ml}^{-1}$) in 100 μL of nutrient broth were analyzed with 10 μL of bacterial suspension (5×10^6 CFU ml^{-1}) and 10 μL of indicator solution. The MIC was noted as the lowest concentration and there is no growth of bacteria.

Results and Discussion

Totally 13 diarrheal stool samples were collected and processed from isolation of bacterial pathogens. Based on the growth on selective media and microbiological methods, three bacterial pathogens were isolated and identified through 16 s rRNA partial sequencing. Kirkby D Tickell et al.2020 reported *enteropathogenic Escherichia coli*, *Shigella sp* and *Salmonella sp* are causing moderate to severe diarrhea in children. Hence the current study focused to isolate the important pathogens such as *Escherichia coli*, *Salmonella enterica* and *Shigella flexneri*. (Fig: 1) The sequence was submitted to GENBANK (Accession number: MW829499.1) as *Escherichia coli* strain AS1. GENBANK (Accession number: MW829564.1) as *Salmonella enterica* strain ASSH. GENBANK (Accession number: MW830164.1) as *Shigella flexneri* strain ASSA.

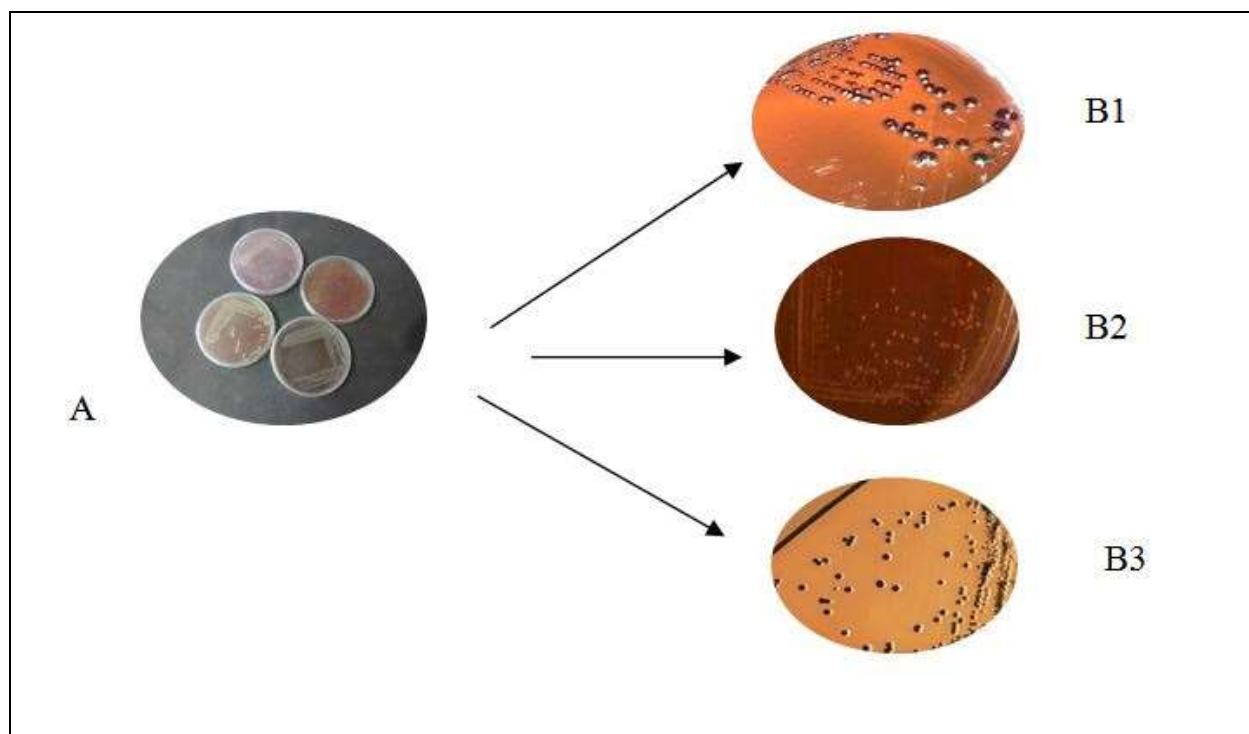


Fig: 1 A) Diarrheal stool sample were inoculated on Selective media plates. B1) *E.coli* colonies on EMB plate. B2) *Shigella* sp, colonies on SS agar plate. B3) *Salmonella* sp, colonies on SS agar plate

Shigellosis is a foremost cause of diarrheal disease worldwide especially in Low and Middle Income Communities. After rotavirus *Shigella* causing the moderate to severe diarrhoea among children under the age of five in developing countries (M.S. Riddle et al.2018). Khalil et al. 2018 reported that shigellosis disease burden of is more than that of proceeding estimates, especially in the children and also elderly, while the burden is lower in *Enterotoxigenic E.coli*. *Enterotoxigenic Escherichia coli*, *Shigella* spp, *Salmonella* spp, revealed to be more frequent among under the age of five year children with Acute Malnutrition. Acute malnutrition is too a risk factor for mortality associated with diarrhoea (Kirkby D Tickell et al. 2020). Hence the current study focused on the important three bacterial pathogens associated with diarrhea.

Emerging of bacterial antibiotics resistance is an important global problem nowadays (Elaheh Sadat Nazoori & Ashraf Kariminik, 2018). In modern medicine antibiotic treatment is tremendously essential to treat infections; in addition it plays a crucial role in reducing prevalence and rate of mortality in infectious disease (Reza Ranjbar & Abbas Farahani 2019). Present study demonstrated the resistance pattern of identified bacterial pathogens such as

E.coli, *Salmonella enterica* and *Shigella flexneri*. The identified strain *E.coli* showed resistance to conventional antibiotics tetracycline, chloramphenicol and amikacin (Fig: 2). Similarly resistant *E.coli* strains were reported by Seung-Hak Cho et al. 2011 but chloramphenicol and gentamicin sensitive strains were reported by Elton Carvalho Costa et al.2016.

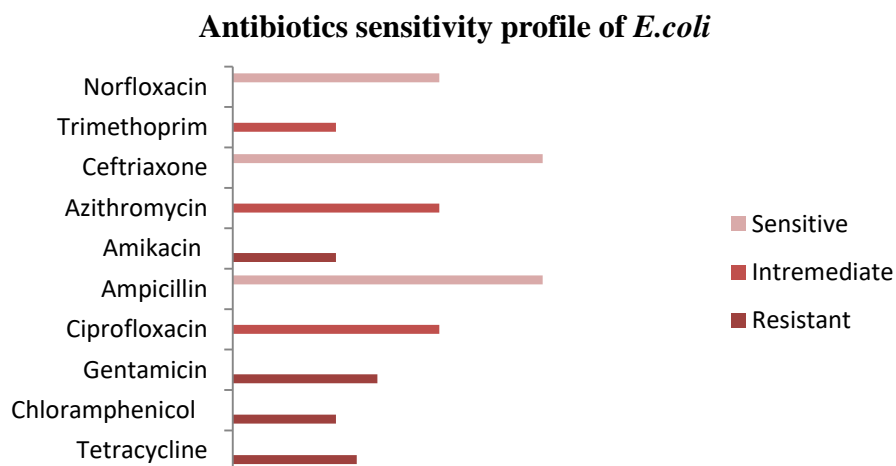


Fig: 2 Antibiotic sensitivity profile of *E.coli* isolated from diarrheal stool samples

Salmonella is one of the important invasive bacterium which causing various infectious diseases including diarrhea. Due to the development of multi drug resistance *Salmonella* strains, the antibiotics are not effective to treat infections (Azhagesan et al. 2015). Current study showed the antibiotic resistance of identified strain *Salmonella enterica* (Fig: 3). Present study exhibited resistance against the second generation antibiotics. Similarly, Mohammad Sharif Uddin et al. 2017 reported the resistant strains of *Salmonella* sp in Bangladesh.

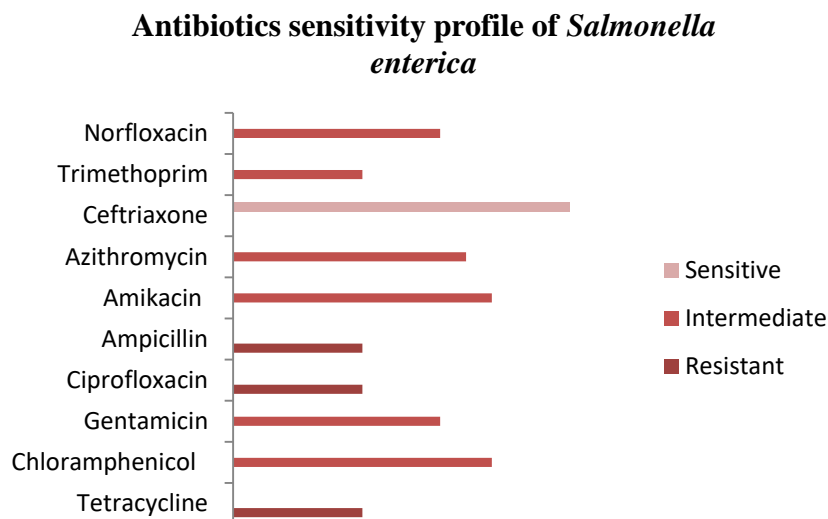


Fig: 3 Antibiotic sensitivity profile of *Salmonella enterica* isolated from diarrheal stool samples

In worldwide, Shigellosis is a universal human health problem and moreover *Shigella* is a leading cause of childhood diarrhoea (Minakshi Puzari et al., 2018). In the present study the isolated strain *Shigella flexneri* showed resistance against third generation antibiotic Ceftriaxone(Fig:4). Similarly previous studies also reported the resistant strains of *Shigella* sp, by Madhavan et al. 2018 and Addisu Assefa and Mengistu Girma 2019.

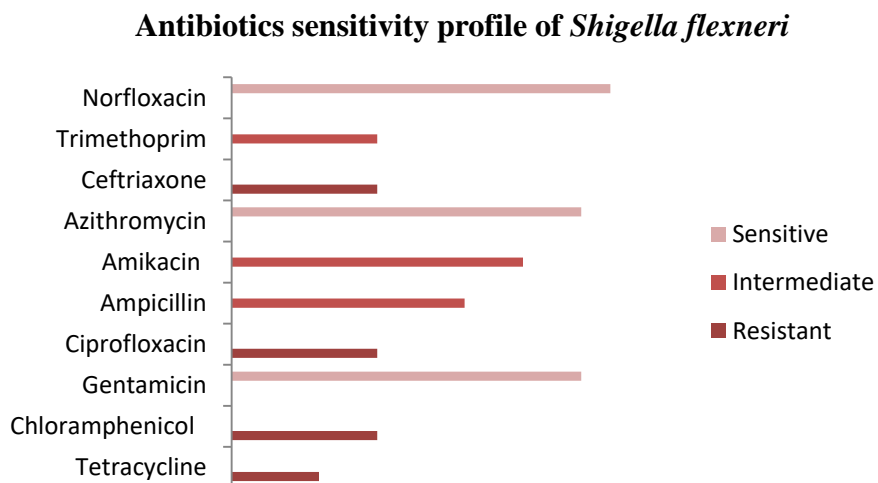


Fig: 4 Antibiotic sensitivity profile of *Shigella flexneri* isolated from diarrheal stool samples

In recent researchers focused on the medicinal plants and their phytochemicals to find novel solutions to the growing demand for antibacterial agents for the treatment of infectious diseases like pediatrics diarrhea and dysentery. Among the tropical fruits, mango (*Mangifera indica* L.) is an important traditional and also seasonal fruit which possess huge nutritional value (Torres-Leo *et al.*, 2016). Mango fruits were collected from different places in Erode. Mango kernels were used for the extraction process. By using cold percolation method extraction was done with 70% ethanol. 9.36% of extract was obtained from the process using 1:3 ratio (Fig:4).



Fig: 4 A) Collected Mango fruits B) mango kernel powder C) Cold percolation method using 70% ethanol solvent

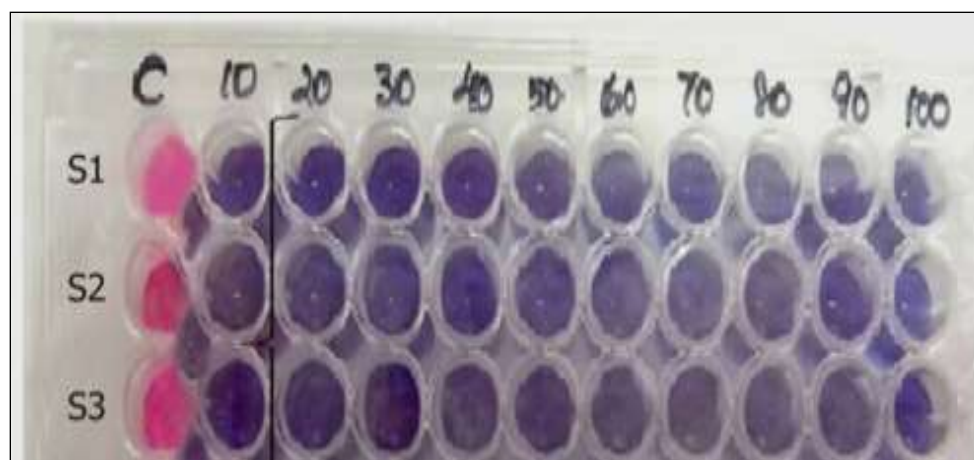
Numerous studies reported the mango kernel antibacterial activity (Sairam *et al.*, 2003, Rajan *et al.*, 2011 and Yakubu and Salimon 2015). Previous studies provided valuable findings but still less information about conventional antibiotic resistant strains associated with diarrhea. Current study evaluated the antibacterial activity of mango kernel extract against the drug resistant bacterial pathogens isolated from diarrheal stool samples. Antibacterial activity of mango kernel extract exhibited highest zone of inhibition 19.2 ± 0.2 mm against *E. coli* at 250 μ g concentration. Antibacterial activity showed the remarkable activity when compared to positive control. Similarly, many previous studies reported the same findings of activity against the gram negative bacteria (Jasminder Kaur *et al.*, 2010 Amit Sarker *et al.*, 2017 and Venkata Raju *et al.*, 2019). In the present study extract at 50 μ g concentration exhibited the 15.2 ± 0.5 mm zone and 19.0 ± 0.3 mm zone was observed at 250 μ g. The test were done in triplicate and the results were noted and recorded in the table:1.

Table: 1 Antibacterial activity of mango kernel extract against the three identified strains

Identified strains	Concentrations of mango seed kernel ethanol extract			Tetracycline 30 µg	70% Ethanol
	50 µg	100 µg	250 µg		
<i>E.coli</i>	15.2±0.5mm	16.3±0.5mm	18.2±0.2mm	18.3±0.2mm	NZ
<i>Salmonella enterica</i>	16.3±0.3mm	17.0±0.3mm	19.0±0.3mm	19.3±0.4mm	NZ
<i>Shigella flexneri</i>	17.2±0.2mm	17.8±0.2mm	18.1±0.1mm	18.6±0.3mm	NZ

NZ- no zone

Mangifera indica seed kernel ethanol extract was found to be the most potent activity in inhibiting the growth of resistant isolated strains of *Shigella flexneri*, *Salmonella enterica* and *E.coli* with the efficient MIC value of 10 µg /ml. (Fig:5). Similarly, Omara et al ., 2017 reported MIC of mango kernel was found to be 16µg/mL.

**Fig: 5 MIC of mango kernel extract against isolated strains. S1 S2 & S3 –Strains no**

Conclusion

Based on the findings *Mangifera indica* exhibited the significant antibacterial activity against the conventional drug resistant bacterial pathogens which are isolated from diarrheal stool samples. *E.coli*, *Salmonella enterica* and *Shigella flexneri* are the prominent etiological agent for moderate to severe diarrhea especially in children who are living in Low and Middle income communities. After further need analysis mango kernel become a prominent natural remedy for bacteria associated diarrhea from the traditional Indian fruit background.

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