

Antibacterial effect of mango (*Mangifera indica* Linn.) leaf extract against antibiotic sensitive and multi-drug resistant *Salmonella typhi*

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Abstract: Alternative herbal medicine has been used to treat various infections from centuries. Natural plants contain phytoconstituents having similar chemical properties as of synthetic antibiotics. Typhoid fever is a serious infection and failure of its treatment emerged multi-drug resistant (MDR) bugs of *Salmonella typhi*. Due to multiple and repeated issues with antibiotics efficacy, it became essential to evaluate biological properties of plants from different geographical origins. Mango leaves have been reported for various medicinal effects like antioxidant, antimicrobial, antihelminthic, antidiabetic and antiallergic etc. Objective of present study was to investigate anti-typhoid properties of acetone mango leaf extract (AMLE) against antibiotic sensitive and MDR *S. typhi* isolates. A total of 50 isolates of *S. typhi* including MDR (n=30) and antibiotic sensitive (n=20) were investigated. *Staphylococcus aureus* (ATCC 25923) and *Salmonella typhimurium* (ATCC14028) were used as quality control strains. AMLE was prepared and its antibacterial activity was evaluated by agar well diffusion screening method and minimum inhibitory concentration (MIC), by agar dilution technique. Zone of inhibition (mm) of AMLE against MDR and antibiotic sensitive isolates was 18±1.5mm (Mean±S.D). Zone of *S. aureus* (ATCC 25923) and *S. typhimurium* (ATCC14028) was 20±1.5mm (Mean±S.D). MIC of AMLE was reported in range from 10-50 mg/ml. The present study described the inhibitory effects of mango leaves against *S. typhi*

Keywords: Antibacterial, Mango, Extract, Multi-drug resistant, *S. typhi*.

INTRODUCTION

Natural plants as herbal remedies are being employed to prevent and cure several illnesses vary in different communities (Kubmarawa *et al.*, 2007). These herbal plants are largely raw source for the production of modern antibiotics. For many years, medicine had been explored exclusively from leaves, flowers and barks of plants; only recently the synthetic drugs used to treat different infections have same chemical constituents as identified in plants. According to WHO, a medicinal plant could be any plant that contains substances which can be obtained from its different parts and can be applied for beneficial purposes or can be predecessor for the production of useful drugs (Junaid *et al.*, 2006).

Continuous spread of infectious diseases is a major apprehension for health institutions, pharmaceutical companies and government think tanks all over the world. Failure of treatment, particularly with the current escalating trends of multi-drug resistance (MDR) to the available modern drugs or antibiotics among emerging and re-emerging bacterial pathogens leads to serious risks (Franklin and Snow, 1989; Prescott *et al.*, 2002). Typhoid fever is a serious health problem due to poor personal hygiene, improper sewage disposal, contaminated food and unsafe drinking water in Pakistan (Khan and Kamal, 2004). According to WHO in 2000, the estimated global incidence of typhoid fever was about 21.6 million and

mortality rate was up to 216,000 per year (Ochiai *et al.*, 2008). In 2008, it was reported that the incidence of typhoid fever is 451/100,000 in Pakistan (Kothari *et al.*, 2008). Multi drug resistant (MDR) *Salmonella typhi* showed resistance to all three first line drugs {Ampicillin (AMP), Chloramphenicol (C), and Trimethoprim-sulfamethoxazole (SXT)} (Ackers *et al.*, 2000). Recently extended spectrum beta lactamase (ESBL) production has been reported in *S. typhi* isolated from the 54 year old Dutch man returned from Philippines (Naiemi *et al.*, 2008).

The multiple and repeated difficulties with antibiotics has prompted research to explore phototherapeutic agents, used as folk medicine. Therefore it became very necessary to search for newer antibiotic sources; be a continued process. Plants are easily accessible, safer and cost effective sources for alternative medicines (Pretorius and Watt, 2001; Sharif and Banik, 2006; Doughari *et al.*, 2007).

Mangifera indica L, commonly called as mango, is a plant belonging to the family *Anacardiaceae* which consists of about sixty genera and six hundred species (Akinpelu and Onakoya, 2006). It is one of the most popular tropical fruit bearing trees in the world (Kabuki *et al.*, 2000).

Conventionally, the mango plant has several biological properties as like to treat mouth infections in children, diarrhea, dysentery, gastrointestinal tract disorders,

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typhoid fever, sore throat and scurvy (Fowler, 2006; Campbell *et al.*, 2002). It is also a source of vitamin A when eaten in combinations with salt and honey for the treatment of blood disorders. Ground seeds and leaves have been used as remedy for diabetes, as colic and to treat irritations, scorpion and bee stings (Doughari and Manzara, 2008). Stem bark of mango has been found to possess anti-helminthic and anti allergic properties. Different parts of mango have been investigated for their antibacterial, antifungal and antioxidant activities. In one of study, mango seed kernel extract showed its inhibitory effect against coliform and *E. coli* isolates (Abdalla *et al.*, 2007). A mango kernel triturate or mango kernel extract is being used in food products or cosmetics due to its bacteriostatic and antibacterial properties (Nakajima *et al.*, 1998).

The leaves of *M. indica* have been analyzed chemically and reported to contain glucoside and mangiferin which is potent antimicrobial agent. Mangiferin has been demonstrated to possess antiviral activity against herpes simplex type 2 viruses, hypoglycemic, anti-hyperlipidemic and antiatherogenic activities (Zakaria *et al.*, 2006).

Pakistan has a special place for the cultivation of various species of mangoes. Unfortunately, no scientific data is available about antibacterial properties of mango trees. In view of the importance of *M. indica* in ethno-botany as healthy remedy, we focused to investigate Chounsa specie of mango against drug sensitive and resistant isolates *Salmonella typhi*. The objective of the present study was to screen acetone mango leaf extract (AMLE) for antibacterial activity and also to determine minimal inhibitory concentration (MIC) against *S. typhi*.

MATERIAL AND METHODS

Prior to the start of the study, approval was obtained from the Ethical Committee, University of Health Sciences, Lahore, Pakistan.

Bacterial isolates

Fifty clinical isolates of *S. typhi* were obtained by the Department of Microbiology, Armed Force Institute of Pathology (AFIP), Rawalpindi, and Microbiology Department of Sheikh Zayed Medical Complex, Lahore, Pakistan. Out of total 50 isolates, 30 were multi-drug resistant (MDR) while other 20 were antibiotic sensitive isolates. The isolates were preserved in 16% (v/v) glycerol in brain heart infusion (Oxoid Ltd, UK) and were stored at - 70°C until use.

Purification of bacterial isolates

The bacterial isolates were identified by the standard morphological, cultural and biochemical characteristics including Gram staining, purify cultures, biochemical assays, API 20-E and serological confirmations.

Quality control strains

Two ATCC reference strains, *S. aureus* (ATCC 25923) and *S. typhimurium* (ATCC14028) were included to monitor the quality control of the procedures.

Preparations of acetone mango leaf extract (AMLE)

Fresh mango leaves of Chounsa specie were obtained from D.G.Khan; a city of Southern Punjab, Pakistan. These leaves were properly washed with distilled water to remove dirt and air-dried for a week. Then dried leaves were blended using a household electrical blender. The leaf powder (500 g) was soaked in 1 liter of acetone in a sterile conical flask and allowed to stay at ambient temperature for 72 h. The bioactive components were extracted using the methods of Akerele *et al.* (2008) with slight modification. The extracts were then filtered using What man no. 1 filter paper and the filtrate was concentrated in vacuo at 40°C using a rotary evaporator (Heidolph apparatus) to evaporate acetone at Pakistan Council of Scientific and Industrial Research (PCSIR) laboratories, Lahore by a standard procedure.

Assay for antibacterial activity/Agar well diffusion Assay

Acetone mango leaf extract (AMLE) was screened for its antibacterial activity against 30 MDR and 20 antibiotic sensitive isolates of *S. typhi* by agar well diffusion assay. *S. aureus* ATCC (25923) and *S. typhimurium*, ATCC (ATCC14028) were used to monitor quality control. The isolates were sub-cultured on blood agar and incubated at 37°C for 24 hours. After 24 hours incubation, 3-4 morphologically identical colonies were picked up and mixed in saline. Then the bacterial suspension was adjusted to 0.5 McFarland turbidity standard (10^8 cells equivalent). A series of dilutions of AMLE were prepared by dissolving in 10%DMSO at different concentrations of 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml and 250 mg/ml to determine its antibacterial property. Phenol (6%) was used as positive control. From 0.5 McFarland suspension micro-organisms were loaned on Muller Hinton agar plates. Seven wells were cut into the MH plates with 6 mm cork borer and removed with needle of disposable syringe. The wells were labeled at the backside of plates. Five wells were filled with 170 µl of AMLE serial dilutions. Remaining of two wells was filled with positive (6% phenol) and negative (10%DMSO) controls. MH plates were kept to settle down on working bench for 5 minutes; allowing extract to diffuse. Then plates were incubated at 35-37°C for overnight in upside down position. The diameter of the clear zones was measured in mm with digital calipers (Sylvac, Fowler, Ultra-Call11). The mean of inhibition zones of AMLE against 50 *S. typhi* isolates were calculated at various concentrations.

Determination of minimal inhibitory concentration (MIC)

Minimum inhibitory concentration of acetone extract was determined against fifty *S. typhi* isolates, comprising of

thirty MDR and twenty antibiotic sensitive by agar dilution technique. The procedure was performed in triplicates at varying concentrations of extract of 10, 20, 30, 40 and 50 mg/ml. Then the bacterial suspension of each test isolate was adjusted to 0.5 McFarland standard. Mixing of AMLE with autoclaved MHA plates were performed at 50°C and dispensed into Petri dishes (20 mm). The poured plates were allowed to dry at 45°C for about 10 to 15 minutes. Three control plates were also set up in parallel, one of MH agar without extract inoculated with all strains to confirm the viability of the cultures (positive control). Second control plate contained medium only (sterility control) and third medium with AMLE as to check extract sterility. Inoculation of MH plates was performed by multi-inoculator. A 500 µl of each bacterial suspension (equivalent to 10^8 cells) was filled in the grid circles of multi-inoculator. Grid was adjusted and extract incorporated MH plates were placed on multi-inoculator. Plates were inoculated with 3 µl of bacterial suspension. Simultaneously 35 isolates were inoculated at single plate. Control strains were inoculated on each plate. The plates were allowed to stand for 5 minutes and incubated at 35-37°C for 24 hours. MIC was reported as the lowest concentration of AMLE at which all bacterial isolates/strains were inhibited was MIC. The data was analyzed by using computer software, Statistical Package for Social Sciences (SPSS) 17.0 version.

RESULTS

Results of antibacterial properties of AMLE against *S. typhi* were measured as zone of inhibition in mm. It is demonstrated that zone size was directly proportional to the increase in concentration of AMLE. *S. typhimurium* (ATCC14028) and *S. aureus* (ATCC 25923) showed the largest zone of inhibition (20 ± 1.5 mm) at the lowest concentration of extract (50mg/ml) followed by MDR and antibiotic sensitive *S. typhi* (18 ± 1.5 mm). *S. typhi* isolates showed zone of inhibition >12 mm at all concentrations of AMLE (table 1). The positive control (phenol) showed the zone of 26 ± 1 mm against MDR.

The maximum numbers of MDR and antibiotic sensitive isolates of *S. typhi* were inhibited at concentration of 30 mg/ml (table 2). The percentage inhibition of organism was directly proportional to increasing concentration of AMLE (fig.1 & fig.2). Out of 50 *S. typhi* isolates, 50% isolates were inhibited at less than 30mg/ml, 90% at less than 40mg/ml and 100% at 50mg/ml of AMLE concentrations (table 3). The same results were obtained for 50% and 100% isolates inhibition in case of both MDR and antibiotic isolates. But in case of MDR isolates (n=30) 90% isolates were inhibited at less than 40 mg/ml while 50% antibiotic sensitive *S. typhi* (n=20) were inhibited at 40 mg/ml. MIC range for both MDR and antibiotic sensitive isolates was reported same as 10-50mg/ml. *S. typhimurium* (ATCC 14028) and *S. aureus* (ATCC 25923) were inhibited at 10 mg/ml.

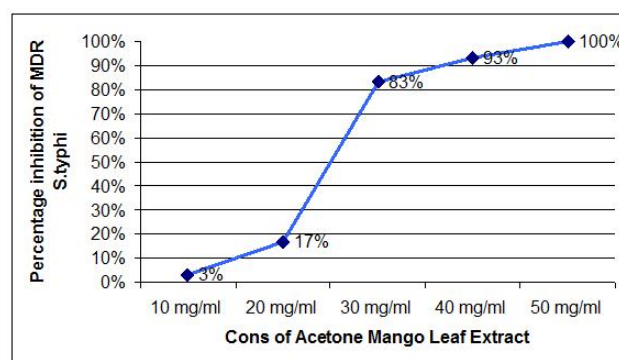


Fig. 1: Cumulative Percentage of Multi-drug resistant *Salmonella typhi* (n=30) inhibited at different concentrations of AMLE.

Fig. illustrates the cumulative percentage inhibition of MDR *S. typhi*. It shows that at 10 mg/ml, 20mg/ml, 30mg/ml, 40mg/ml and 50mg/ml, percentage of isolates inhibited were 3%, 17%, 83%, 93%, and 100% respectively.

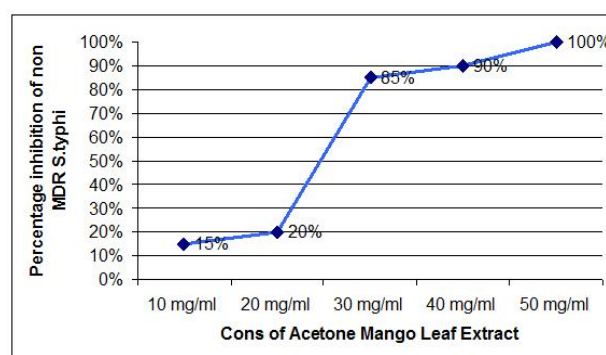


Fig. 2: Cumulative Percentage of antibiotic sensitive (non-MDR) *Salmonella typhi* (n=20) inhibited at different concentrations of AMLE.

Fig. illustrates the cumulative percentage inhibition of antibiotic sensitive isolates of *S. typhi*. It represents that at 10 mg/ml, 20 mg/ml, 30mg/ml, 40mg/ml and 50mg/ml, percentage of isolates inhibited were 15%, 20%, 85%, 90%, and 100% respectively.

DISCUSSION

In this study AMLE showed antibacterial activity against MDR *S. typhi* as it was inhibited in all dilutions tested. Zone of inhibition of AMLE against both MDR and antibiotic sensitive *S. typhi* were 15.5 ± 3.5 , 17.25 ± 3.75 , 18.0 ± 4.0 , and 18.5 ± 3.5 and 19.75 ± 3.75 mm (Mean $\pm$ SD) at 50, 100, 150, 200 and 250 mg/ml respectively. The present study can be compared with a Nigerian study that showed zones of 4.0, 7.0, 9.0, 12.0, 14.0 mm at concentrations of 50, 100, 150, 200 and 250 mg/ml respectively (Doughari and Manzara, 2008). It represents that mango extract of Pakistani origin showed more activity and exhibited wide zones (mm) at the same concentrations as in previous study. The likely explanation might be due to the difference in specie of mango and methodology employed. That Nigerian study also demonstrated that acetone extract of mango leaf

Table 1: Zone of inhibition (mm Mean $\pm$ SD) of mango leaf extract against *Salmonella typhi* and ATCC strains

Bacterial isolates	Acetone Mango leaves Extract (zone in mm)				
	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	250 mg/ml
<i>S. typhi</i> (n=50)	15.5 $\pm$ 3.5	17.25 $\pm$ 3.75	18.0 $\pm$ 4.0	18.5 $\pm$ 3.5	19.75 $\pm$ 3.75
MDR <i>S. typhi</i> (n=30)	15.25 $\pm$ 3.75	17 $\pm$ 3.5	17.5 $\pm$ 3.5	18.25 $\pm$ 3.25	19.75 $\pm$ 3.75
Antibiotic sensitive <i>S. typhi</i> (n=20)	15.5 $\pm$ 3.5	17.5 $\pm$ 3.5	18.5 $\pm$ 3.5	18.5 $\pm$ 3.5	19.75 $\pm$ 3.75
ATCC <i>S. aureus</i> (ATCC 25923)	20.0 $\pm$ 1.5	22.0 $\pm$ 1.5	24.0 $\pm$ 1.5	26.0 $\pm$ 1.5	26.0 $\pm$ 1.5
ATCC <i>S. typhimurium</i> (ATCC14028)	20.0 $\pm$ 1.5	20.0 $\pm$ 1.5	22.0 $\pm$ 1.5	22.0 $\pm$ 1.5	24 $\pm$ 1.5

Table 2: Inhibition of *Salmonella typhi* with acetone mango leaf extract

Bacterial isolates (number)	Concentrations of AMLE (mg/ml)				
	10 mg/ml	20mg/ml	30mg/ml	40 mg/ml	50 mg/ml
<i>S. typhi</i> (n=50)	04	05	33	04	04
MDR <i>S. typhi</i> (n=30)	01	04	20	03	02
Antibiotic sensitive <i>S. typhi</i> (n=20)	03	01	13	01	02

Table 3: MIC of AMLE against *Salmonella typhi* (n=50)

No. of bacterial isolates	Minimum Inhibitory Concentration (mg/ml)			
	MIC RANGE (mg/ml)	MIC ₅₀	MIC ₉₀	MIC ₁₀₀
<i>S. typhi</i> (n=50)	10-50	<30	<40	50
MDR <i>S. typhi</i> (n=30)	10-50	<30	<40	50
Antibiotic sensitive <i>S. typhi</i> (n=20)	10-50	<30	40	50

exerted highest activity against *S. typhi* compared to aqueous and methanolic extracts of mango leaves.

In one of another study conducted in USA, ethanolic and methanolic extract of mango leaf showed relatively high zones of inhibition against *S. typhi*. Both ethanolic and methanolic extract of mango leaf showed inhibition zones of >13.0 mm and <16.0 mm at 50 and 100 mg/ml (Zakaria *et al.*, 2006). The differences in the observed activities of various extracts may be due to varying degrees of solubility of the active constituents in the solvent used. It has been documented that different solvents have different solubility capacities for different phytoconstituents (Doughari and Manzara, 2008). In this study, AMLE demonstrated high inhibition zones against *S. aureus* (ATCC 25923) followed by *S. typhimurium* (ATCC 14028). Previous studies have revealed that mango leaf extract is more affective against Gram positive bacteria than Gram negative (Zakaria *et al.*, 2006; Doughari and Manzara, 2008). This may be due to presence of lipopolysaccharides (LPS) in the Gram negative bacteria (Zakaria *et al.*, 2006).

Regarding MIC, in present study MIC range for fifty *S. typhi* isolates came out to be 10-50 mg/ml by agar dilution method for both MDR and non MDR *S. typhi*. Whereas in previous study MIC range of acetone mango leaf extract against *S. typhi* was 12.5-100 mg/ml by tube method (Doughari and Manzara, 2008). The most obvious reason for this variation may be due to the fact that mango leaf composition depends upon many factors like origin, age,

storage conditions, type of processing and consumption methods and these may in turn its activity.

In previous studies, screening or MIC of mango leaf extract against MDR *S. typhi* was not being reported. The interesting observation of this study is that both MDR *S. typhi* (n=30) and antibiotic sensitive (n=20) isolates were inhibited with same range that is 10-50mg/ml. This showed that mango leaf is equally effective against MDR and antibiotic sensitive isolates. It might be due to the difference in mechanism of action of mango leaf; because the other drugs have single mode of action and it is easier to develop resistance against certain antibiotics drugs but mango leaf has been reported to possess strong bioactive agent mangiferin that shows multiple mode of action (Navarro *et al.*, 2003).

The basic ground for selecting AMLE in this study was because of high concentration of mangiferin (antibacterial component) that has been reported to possess antimicrobial activity. In a study conducted in Vietnam, antimicrobial effect of polyphenol mangiferin obtained from mango leaves was screened against *Salmonella* species (Navarro *et al.*, 2003). The pure bioactive agent showed remarkable zones of inhibition at 15, 20, 25 mg/ml of mangiferin that were 22 $\pm$.64, 26 $\pm$ 0.30, 29 $\pm$ 0.70 mm (Mean $\pm$ SD) respectively. This demonstrates that there is a need to analyze the chemical composition of mango leaves and to explore the most effective agents, used for antibacterial properties. It is also a need of hour to investigate extracts of mango species of different

geographical locations for the most active ingredients responsible for their antibacterial activity.

CONCLUSION

Demonstration of antibacterial activity of AMLE against MDR *S. typhi* is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the development of newer antibacterial agents. The results achieved by present study indicate that leaves of mango tree in our country have the potential to be used as antibacterial agent. The research must go on beyond what is done so far, so that we may utilize our natural sources to treat any ailment associated with *S. typhi*.

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