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Anti-salmonella activities of *Mangifera indica* seed kernel aqueous extract (MISKAE)

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ABSTRACT

To assess antibacterial activities of MISKAE on Salmonella sp., isolated from Acute Gastroenteritis (AGE). Salmonella causes major AGE outbreaks among children. It also causes typhoid and intestinal invasive infections. Antibiotics are used for the treatment of these infections. Now a day, microorganism's develope resistance. Alternative treatment strategy is needed to curtail the effect of Salmonella. Traditional System of Medicine (TSM) is most useful for the treatment of multidrug resistant (MDR) pathogens. MISK is selected and extracted using water (MISKAE). It was subjected for antimicrobial assay by disc diffusion method. MIC, MBC, % inhibition, IC_{50} along with qualitative phytochemical analysis of this plant extracts were done using standard methods. results revealed that MISKAE showed good antisalmonella activity and produce 7.3±0.6 to 15.0±1.0mm zone of inhibition a t 200µg/disc concentration. MISKAE showed good MIC and MBC with 98.8% inhibition at 200µg/ml concentration for Salmonella typhi 14. IC_{50} required for killing Salmonella ranges from 101.3 to 800 µg/ml concentration. MISKAE could be considered as a effective medicine for the treatment of Salmonella infection. Flavonoid, Tannins and Polyphenols were considered as a chemical prevents or inhibits the growth of Salmonella.

Key words: salmonella, antisalmonella, miskae, antibacterial activity, MIC, MBC, ic_{50}.

INTRODUCTION

Salmonella is one of the most important causative agents of AGE as well as typhoid. Non typhoidal Salmonella is a major reason for gastroenteritis and play a major role in outbreaks [1]. They also cause major outbreaks among children [2]. *Salmonella* are also considered as a major causative agent of food borne illness. This bacterium also causes life threatening invasive infection like septic arthritis [3]. Though gastroenteritis is a self limited infection may cause invasive infection in children and immmunocompromized individuals and needs antibiotic treatment. Quinolones were used for the treatment of *Salmonella* causing infections. Now a day, antibiotics are not effective due to the development of drug resistance, which is evidenced through various scientific findings from India and abroad [4, 5, 6, 7]. In this situation, use of antibiotics leads to various side effects and need the development of an alternative strategy for better treatment. One such strategy is the development of medicine from the plants. *Mangifera indica* is commonly called as mango in English and Manga in Tamil and belongs to the family Anacadiaceae. *Mangifera indica* seed kernel is one of the most powerful plant part traditionally used for the treatment of diarrhoea, dysentery etc., [8, 9, 10, 11, 12, 13, 14]. Few studies are also reported in antimicrobial activity of this plant using different microbial species. This study also describes antimicrobial activity of seed kernel in a holistic manner and taken this differently with the aim of screening antisalmonella activities of *Mangifera indica* seed kernel aqueous extract (MISKAE).

MATERIALS AND METHODS

Preparation of plant material

Mangifera indica seed kernel was collected from the local market of Madurai, Tamilnadu, India. Seed kernel was dried properly and was ground into powder and then sieved using a sieve. Two hundred grams of powdered plant were transferred into airtight containers and stored at room temperature.

Extraction of the plant material

Plant active components were extracted using the cold extraction method [15]. Water was used for the extraction. The filtrate was obtained by means of a vacuum filter pump. The final filtrates were filter-sterilized with syringe filter (pore size of 0.45µm). Sterile extracts obtained were stored separately in labelled, sterile capped bottles, in a refrigerator at 4°C.

Determination of antibacterial activity

Antimicrobial activity was performed by disc diffusion method [16].

Assessment of MIC, MBC and IC50

It was performed by making use of the method of Kowser and Fatena [17] with a few modifications. It is performed as mentioned in Table 1

Determination of % inhibition

It is a calculation of inhibitory effect of extracts at a particular concentration by making use of total viable count value of GC tube and dilution tubes. It was calculated by making use of the following formula.

$$\frac{\text{Number of colonies in tube GC} - \text{Number of colonies in dilution tube}}{\text{Number of colonies in tube GC}} \text{X } 100$$

Determination of IC_{50}

According to the FDA, IC_{50} represents the concentration of a drug that is required for 50% inhibition in *in-vitro*. It is obtained from the %inhibition and the concentration of extract used. IC_{50} was calculated by using the formula.

$$\frac{\text{Concentration of Extract}}{\text{\% inhibition}} \text{X } 50$$

Table – 1 Assessment of MIC, MBC and IC_{50}

Tube No.	AC	1	2	3	4	5	6	7	8	9	10	11	GC	Blank
Volume of Mueller Hinton broth in µl	1900	1800	1810	1820	1830	1840	1850	1860	1870	1880	1890	1895	1900	2000
Volume of Extract / antibiotics in µl	100	100	90	80	70	60	50	40	30	20	10	5	0	0
Initial Total Extract concentration in µg	100	2000	1800	1600	1400	1200	1000	800	600	400	200	100	0	0
Bacterial Suspension in µl	0	100	100	100	100	100	100	100	100	100	100	100	100	0
Final Volume in µl	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000
Final extract conc. µg/ml	50	1000	900	800	700	600	500	400	300	200	100	50	0	0
A.C = Antibiotic Control, G.C = Growth Control														
Incubate for 24 hours at 37°C														
Read OD at 620nm, it will provide MIC value														
Inoculate each growth contents (0.1ml) on Mueller Hinton Agar by spread plate method (After Proper Dilution)														
Incubate for 24 hours at 37°C														
Count number of colonies														
Calculate % growth inhibition and MBC														
Finally calculate IC_{50}														

Qualitative Phytochemical Screening

Freshly prepared MISKAE were tested for the presence of phytochemical constituents using standard methods [18].

Statistical analysis

All the results were expressed as mean ±SD. The data were statistically analyzed by one way ANOVA and P values <0.05 were considered significant

RESULTS AND DISCUSSION

MISKAE is one of the most important phytomedicine, traditionally claimed for its antidiarrhoeal activity [19]. It is a refrigerant, employed to kill abdominal worms, cure for vomiting, diarrhoea and hyperacidity [20]. Recent scientific reports also support the use of this plant as an antidiarrhoeal agent [12, 13]. MISK is considered as an effective medicine for AGE caused by microbial agents. *Salmonella* is one of the invasive bacterium [21, 22] caused various life threatening infection. These organisms were resistant to multiple numbers of antibiotics [7]. Availability of surface factors and enzymes are responsible for antimicrobial resistance [23, 24, 25].

MISKAE was subjected for antimicrobial assay against the strains of *Salmonella enteritidis*, *Salmonella paratyphi A, Salmonella typhi* and *Salmonella typhemurium*. All these organisms were responsible for causing AGE. These organisms were isolated from the stool samples of infected patients admitted in inpatients ward of Meenachi medical Mission, Madurai, Tamilnadu, India. Concentrations like 50, 100, 150 and 200 µg/disc of MISKAE were used for antisalmonella assay. Out of ten strains of Salmonella tested, only *Salmonella enteritidis* 135 was inhibited at 50 µg/disc concentration (Table 2). Similarly *Salmonella enteritidis* 101 inhibited at 100 µg/disc with 7.7±0.6 zone of inhibition. Other strains were inhibited at 150 µg/disc concentration with a zone of inhibition ranges from 7.3±0.6 to 10.0±0.6mm and 9.7±0.6 to 15.0±1.0 at 200 µg/disc concentration. MISKAE produced the best zone of inhibition against *Salmonella typhi* 14 and *Salmonella typhimurium* 7 (15.0±1.0 and 15.0±1.2 respectively).

Table 2: Antisalmonella activities of MISKAE (n=3)

S. No.	Test Strain	Zone of Inhibition in mm (mean±SD)			
		50µg/disc	100µg/disc	150µg/disc	200µg/disc
1	*Salmonella enteritidis 51*	-	-	07.3±0.6*	11.0±2.0*
2	*Salmonella enteritidis 93*	-	-	08.0±1.0*	12.0±0.6*
3	*Salmonella enteritidis 101*	-	7.7±0.6	08.3±0.6*	11.0±1.2*
4	*Salmonella enteritidis 135*	7.3±0.6	8.3±0.6	10.0±0.6*	13.0±1.0*
5	*Salmonella paratyphi A 2*	-	-	09.3±1.2*	12.0±1.2*
6	*Salmonella paratyphi A 3*	-	-	09.7±1.2*	12.0±0.6*
7	*Salmonella paratyphi A 9*	-	-	09.0±2.6*	14.0±0.6*
8	*Salmonella paratyphi A 10*	-	-	07.7±0.6*	09.7±0.6*
9	*Salmonella typhi 14*	-	-	10.0±1.7*	15.0±1.0*
10	*Salmonella typhimurium 7*	-	-	08.3±2.3*	15.0±1.2*

** one way ANOVA and P values <0.05 were considered significant*

MIC and MBC assessment along with Percentage inhibition and IC_{50} is essential to validate the efficiency of the drug. Hence a new modified procedure was adopted to assess these parameters. The MIC value of MISKAE against *Salmonella* sp., were varied from 053.6±14.4 µg/mL and 144.7±28.8 µg/mL. Effective MIC of MISKAE was noted for *Salmonella enteritidis* 135 (053.6±14.4 µg/mL) followed by *Salmonella enteritidis* 101(066.1±14.4 µg/mL). Least MIC of MISKAE was noted against *Salmonella enteritidis* 51(144.7±28.8 µg/mL). Bacteriostatic nature of the extracts was revealed in MIC assay whereas bactericidal concentration of the extracts was assessed by MBC assessment. MISKAE showed best MBC against *Salmonella enteritidis* 101 (116.6±28.8 µg/mL). 366.6±28.9 µg/mL concentration of MISKAE was needed for killing of *Salmonella enteritidis* 51(Table 3).

Table 3: MIC and MBC activities of MISKAE against Salmonella strains

S. No.	Test Strain	MIC (µg/mL)	MBC (µg/mL)
1	*Salmonella enteritidis 51*	144.7±28.8*	366.6±28.9*
2	*Salmonella enteritidis 93*	137.5±25.0*	258.3±38.1*
3	*Salmonella enteritidis 101*	066.1±14.4*	116.6±28.8*
4	*Salmonella enteritidis 135*	053.6±14.4*	141.6±14.4*
5	*Salmonella paratyphi A 2*	144.7±28.8*	350.0±50.0*
6	*Salmonella paratyphi A 3*	132.2±28.8*	316.6±28.8*
7	*Salmonella paratyphi A 9*	107.2±28.8*	233.3±50.0*
8	*Salmonella paratyphi A 10*	100.0±25.0*	300.0±28.8*
9	*Salmonella typhi 14*	072.3±14.4*	216.6±28.8*
10	*Salmonella typhimurium 7*	085.8±43.3*	183.3±28.8*

** One way ANOVA and P values <0.05 were considered significant*

Number of bacterial cells in a test Vs control explains the nature of inhibition by MISKAE on *Salmonella*. MISKAE showed effective control of *Salmonella* strains which is expressed in terms of % inhibition. Percentage inhibition of MISKAE at 200 µg/mL ranges from 12.5 to 98.8%. *Salmonella typhi* 14 culture was inhibited upto 98.8% at 200 µg/mL concentration. Least % inhibition was noted against *Salmonella paratyphi A* 10 (Figure 1). Concentration required to kill 50% of the population completely were assessed in IC_{50} assay. IC_{50} concentration of MISKAE ranges from 101.3 µg/mL concentration to 800 µg/mL concentration. This indicated that all the test organisms were inhibited at any one of the concentration (Figure 2). *Salmonella typhi* and *Salmonella typhimurium* were best inhibited at 101.3 and 104.3 µg/mL concentration respectively.

Figure 1
Effect of MISKAE with reference to % inhibition at 200µg/ml

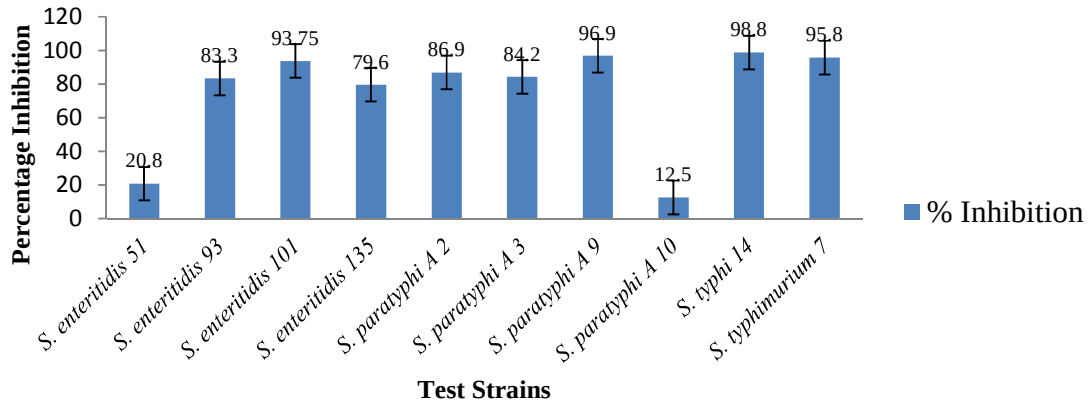
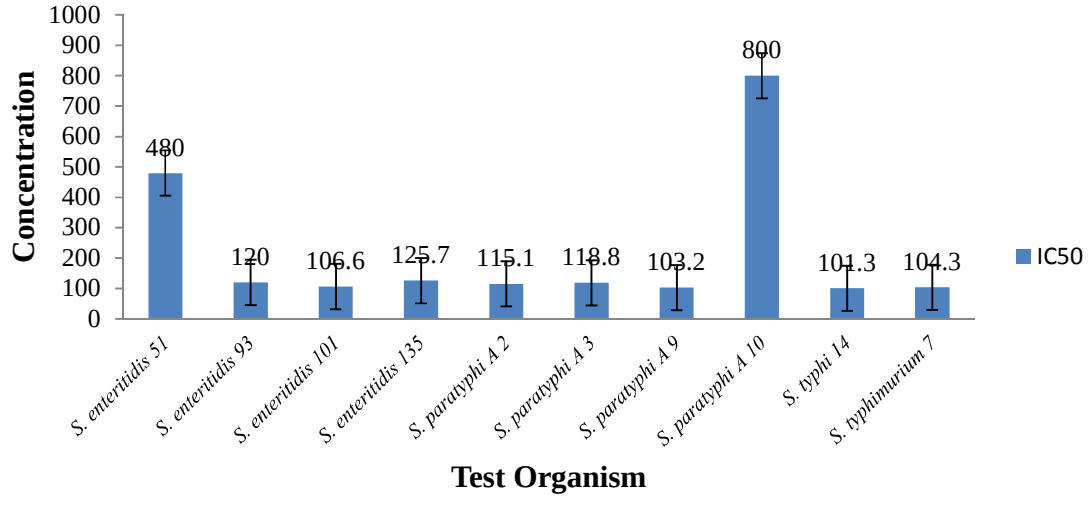


Figure 2
Effect of MISKAE with reference to IC_{50}



Secondary metabolites of the plants are considered as a defense mechanism exists in plant body. These secondary metabolites are also used as a defense system of human also. To understand the phytochemials available in MISKAE, this study was conducted and detected the availability of flavonoids, tannins, phenolic compounds, triterpenoids, saponins and steroids (Table 4).

Table 4: Phytochemical features of MISKAE

S. No	Phytochemicals	Result
1	Flavonoids	+
2	Anthraquinones	-
3	Triterpenes	+
4	Tannins	+
5	Saponin	+
6	Alkaloids	-
7	Steroids	+

Sometimes microorganisms do not allow the entry of phtochemicals by producing biofilm. Biofilms are major factor responsible for antimicrobial resistance. Hence in this study, MDR pathogens with multiple virulent factors were selected and subjected for antsalmonella screening. In the present study, ten different strains of *Salmonella* were selected and subjected for screening antisalmonella activity of MISKAE. MISKAE are helpful in controlling the growth of *Salmonella*, which is evidenced in different mm of zone of inhibition at 50 to 200 µg/disc concentrations. Peoples from different parts of the world use various parts of this plant for screening antimicrobial activities. They used different pathogenic or nonpathogenic organisms but none of them used *Salmonella* isolated from Madurai district of Tamilnadu, India [14, 26, 27). MISKAE produced a maximum of 15mm zone of inhibition at 200 µg/disc concentrations. Some strains were inhibited at 50 µg/disc concentrations also. MIC and MBC results were also expressed the effectiveness of this plant part as an antimicrobial agent. It could show bacteriostatic as well as bactericidal action on Salmonella strains.

Salmonella enteritidis 135 *and Salmonella enteritidis* 51 are belong to same category of species but variability was noted in the inhibitory pattern. 101 strain was inhibited at 50 µg/disc concentration whereas 51 inhibited only at 150 µg/disc concentration. When comparing *Salmonella typhi* and *Salmonella typhimurium*, *Salmonella enteritidis* were less inhibitory by MISKAE. Difference in inhibitory pattern could be due to the availability of variable surface and virulence factors. Due to the availability of these factors pathogenic bacteria thrive in any kind of stressful environment. Surface factors mediate flow of food materials from outside environment to inside. Extracts may have efficient phytochemicals but it may not enter inside the host cell. This could be a reason for difference in sensitivity pattern. One of our unpublished data revealed the presence of multiple virulence factors in strains 31 and 101. Rajan *et al.*, [11] showed that *Mangifiera indica* seed kernel contains phenolic compounds, tannins, flavonoids, which is closely related to this study report and confirms the availability of these phytochemicals. Iron binding capacity of tannic acid prevents the growth of microorganisms by preventing the action of extracellular enzymes. This deprives the entry of growth factors required for microbial growth. It also prevents oxidative phosphorylation. Tannins also precipitates extracellular proteins thereby growth is prevented. Phytochemicals available in the MISKAE directly or indirectly interferes with microbial metabolism and prevents microbial growth [28, 29, 30, 31, 32].

This study showed MISKAE inhibited the growth of MDR virulent strains of *Salmonella*. It may due to prevention of biofilm and action on pathogenic islands of the pathogen. One of our unpublished data revealed that expression of virulence genes like *stn, pef* and *sef* were stopped by the action of MISKAE [33].

CONCLUSION

MISKAE could be considered as an effective phytomedicine for the treatment of typhoidal as well as non typhoidal strains of *Salmonella*. Further studies on fractional and molecular characterization of the phytochemicals on virulent pathogens confirm the uses of this plant material.

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