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, IC₅₀ 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 at 200µg/disc concentration. MISKAE showed good MIC and MBC with 98.8% inhibition at 200µg/ml concentration for Salmonella typhi 14. IC₅₀ 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. Flavonoids, Tannins and Polyphenols were considered as a chemical prevents or inhibits the growth of Salmonella.

Key words: salmonella, antisalmonella, miskae, antibacterial activity, MIC, MBC, IC₅₀.

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 immunocompromized 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 Anacardiaceae. 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:

\[
\frac{\text{Number of colonies in tube GC} - \text{Number of colonies in dilution tube}}{\text{Number of colonies in tube GC}} \times 100
\]

**Determination of IC50**
According to the FDA, IC50 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. IC50 was calculated by using the formula.

\[
\text{Concentration of Extract} \times 50
\]

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**Table 1**

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>AC</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>GC</th>
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<tbody>
<tr>
<td>Volume of Mueller Hinton broth in µl</td>
<td>1900</td>
<td>1800</td>
<td>1810</td>
<td>1820</td>
<td>1830</td>
<td>1840</td>
<td>1850</td>
<td>1860</td>
<td>1870</td>
<td>1880</td>
<td>1890</td>
<td>1895</td>
<td>1900</td>
<td>2000</td>
</tr>
<tr>
<td>Volume of Extract / antibiotics in µl</td>
<td>100</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Initial Total Extract concentration in µg</td>
<td>100</td>
<td>2000</td>
<td>1800</td>
<td>1600</td>
<td>1400</td>
<td>1200</td>
<td>1000</td>
<td>800</td>
<td>600</td>
<td>400</td>
<td>200</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bacterial Suspension in µl</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Final extract conc. µg/ml</td>
<td>50</td>
<td>1000</td>
<td>900</td>
<td>800</td>
<td>700</td>
<td>600</td>
<td>500</td>
<td>400</td>
<td>300</td>
<td>200</td>
<td>100</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

A.C = Antibiotic Control, G.C = Growth Control

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**Qualitative Phytochemical Screening**
Freshly prepared MISKAIE 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 typhimurium. 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)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test Strain</th>
<th>Zone of Inhibition in mm (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50µg/disc</td>
</tr>
<tr>
<td>1</td>
<td>Salmonella enteritidis 51</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Salmonella enteritidis 93</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Salmonella enteritidis 101</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Salmonella enteritidis 135</td>
<td>7.3±0.6</td>
</tr>
<tr>
<td>5</td>
<td>Salmonella paratyphi A 2</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Salmonella paratyphi A 3</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Salmonella paratyphi A 9</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Salmonella paratyphi A 10</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Salmonella typhi 14</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Salmonella typhimurium 7</td>
<td>-</td>
</tr>
</tbody>
</table>

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

MIC and MBC assessment along with Percentage inhibition and IC₅₀ 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

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test Strain</th>
<th>MIC (µg/mL)</th>
<th>MBC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Salmonella enteritidis 51</td>
<td>144.7±28.8*</td>
<td>366.6±28.9*</td>
</tr>
<tr>
<td>2</td>
<td>Salmonella enteritidis 93</td>
<td>137.5±25.0*</td>
<td>258.3±38.1*</td>
</tr>
<tr>
<td>3</td>
<td>Salmonella enteritidis 101</td>
<td>066.1±14.4*</td>
<td>116.6±28.8*</td>
</tr>
<tr>
<td>4</td>
<td>Salmonella enteritidis 135</td>
<td>053.6±14.4*</td>
<td>144.7±28.8*</td>
</tr>
<tr>
<td>5</td>
<td>Salmonella paratyphi A 2</td>
<td>144.7±28.8*</td>
<td>350.0±50.0*</td>
</tr>
<tr>
<td>6</td>
<td>Salmonella paratyphi A 3</td>
<td>132.4±28.8*</td>
<td>316.6±28.8*</td>
</tr>
<tr>
<td>7</td>
<td>Salmonella paratyphi A 9</td>
<td>107.2±28.8*</td>
<td>233.3±50.0*</td>
</tr>
<tr>
<td>8</td>
<td>Salmonella paratyphi A 10</td>
<td>100.0±25.0*</td>
<td>300.0±28.8*</td>
</tr>
<tr>
<td>9</td>
<td>Salmonella typhi 14</td>
<td>072.3±14.4*</td>
<td>216.6±28.8*</td>
</tr>
<tr>
<td>10</td>
<td>Salmonella typhimurium 7</td>
<td>085.8±43.3*</td>
<td>183.3±28.8*</td>
</tr>
</tbody>
</table>

* 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<sub>50</sub> assay. IC<sub>50</sub> 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.

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).
Sometimes microorganisms do not allow the entry of phytochemicals 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 anti-salmonella screening. In the present study, ten different strains of Salmonella were selected and subjected for screening anti-salmonella 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 Mangifera 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.

**Acknowledgement**

We are quite thankful to the Management of Meenachi Mission Hospital, Madurai and Management of M. R. Government Arts College, Mannargudi, Tamilnadu, India for providing all facilities for the completion of the study.

**REFERENCES**