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BACTERICIDAL EFFECT OF TENDER AND MATURED SEED KERNEL OF MANGIFERA INDICA – A COMPARITIVE STUDY

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Abstract: **Traditional Indians uses tender as well as matured fruit rind of *Mangifera indica* for the treatment of diarrhoea, dysentery and other intestinal troubles. Microbial infections are mainly responsible for gastrointestinal disorders. Hence in this study antibacterial activity is performed with the extracts of *Mangifera indica* against diarrhoeal causative agents. The aim of the present study is to assess the antibacterial activity of tender and mature fruit rind of *Mangifera indica*. The following techniques were used to assess antibacterial effect which includes disc diffusion assay, MIC, MBC, % inhibition and IC_{50} were used to assess antibacterial efficiency of *Mangifera indica* seed kernel aqueous and phenolic extracts (MIMSKAE, MIMSKPE, MITSKAE AND MITSKPE). All the extracts showed good antibacterial activity against all the clinical pathogens tested with better percentage of inhibition and IC_{50}.**

Keywords: **Bacterial pathogens, MIC, MBC, Antibacterial activity, IC_{50}, Percentage inhibition, *Mangifera indica*, Seedkernel.**

1. INTRODUCTION

Traditional Indian System of Medicine (ISM) like siddha utilizes herbs and herbominerals as a remedy for the treatment of infectious disease. Village healers of India harvest medicinal plants from the wild and used for healing. *Mangifera indica* is one among the medicinal plant belonging to the family anacardiaceae, different parts of the plant at different stages used for healing. *Mangifera indica* tender seed kernel (MITSK) along with immature fruit (Aavakai) is used to prepare pickle in India. Masuad Parvez [1] and Rajan et al.,[2] indicated the uses of MITSK in diarrhoea, dysentery, haemorrhages, haemorrhoids, diabetes, heat burn etc.,. *Mangifera indica* seed kernel (MISK) is one of the most important ingredients in siddha and ayurvedha preparations. Allopathic therapy reduces burden of diseases but microorganisms developed resistant and become vulnerable pathogen. Despite a various advancements in modern medicine, the prevalence of infectious disease and development of multidrug resistance among human pathogens, haphazard use of synthetic drugs and side effects created by the modern medicine the need to screen medicinal plants for novel bioactive compounds as they are biodegradable, safe and have fewer side effects (Prusti *et al.*, 2008). Seed kernel of this plant also treats chronic diarrhoea. It has the ability to expel tapeworms (Antihelminthic) and other worms in ulcers (Prabhu and Rajan, 2015). It also possesses antioxidant, and antimicrobial properties. The kernel powder is used as astringent in bleeding piles. MITFSK is reported in traditional medicine as a cure for vomiting, dysentery and burning (Prabhu and Rajan, 2014). *Mangifera indica* seed kernel both matured and tendered were selected in this study to screen antibacterial potentials of these products on MDR clinical isolates.

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2. MATERIALS AND METHODS

Preparation of plant material: Seed kernel of young (tender) mango and matured fruit of *Mangifera indica* were collected as wild from Perambalur, Tamilnadu. The collected seed kernels were sundried, ground into powder and then sieved using a sieve. Five hundred grams of powdered plant were transferred into airtight containers and stored at room temperature.

Extraction of plant powder: Active components of the MITSK and MIMSK plant were extracted using the cold extraction method (Fransworth, 1988). Water was added to 50g of the plant powder in sterile conical flasks and allowed to soak at room temperature for 48 hours. A shaker set at 120 rpm was used to improve extraction of phyto-chemicals. The filtrate was obtained by means of a vacuum filter pump. Filtering was repeated for three times with same plant material until the solution was clear. The filtrate was evaporated in a weighed flask, with a water bath set at 40°C. Extracts were reconstituted by re-dissolving in DMSO. The final filtrate was filter-sterilized by using syringe filter with a pore size of 0.45µm. Sterile extracts obtained were stored separately in labelled, sterile capped bottles in a refrigerator at 4°C. Aqueous extract of the tender and matured seed kernel was coded as MITSKAE and MIMSKAE respectively.

Preparation of Phenolic extract: Phenolic extract was collected by making use of soxhlet extraction. It was performed by placing 50gm plant material with 1:1 ethanol and methanol. Extraction was performed at 90˚C for 12 hours. The extracts were filtered under the vacuum through Whatman filter paper (No. 1) under gravity. Extract was dried under vaccum evaporator for removing the solvent. The remaining residues were stored in refrigerator till further use (Shi *et al.*, 2005). MITSKPE and MIMSKPE were the codes used for the phenolic extracts of tender and matured fruit seed kernel.

Determination of antibacterial activity: Antimicrobial activity was performed by standard methods like the disc diffusion method on Mueller Hinton agar (Bauer *et al.*, 1966) and MIC, MBC were calculated using modified drug dilution methods (Kowser and Fatena, 2009). Cells used for antibacterial assays were harvested at log phase while they are most active.

Assessment of MIC, MBC and IC_{50}: It was performed by making use of the method of Kowser and Fatena, 2009 with few modifications.

Determination of % inhibition: It is a calculation of inhibitory effect of extracts at 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 Vitro*. It is obtained from the %inhibition and the concentration of extract used. IC_{50} was calculated by using the formula.

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

3. RESULTS

Phenolic extract of *Mangifera indica* seed kernel showed good antimicrobial activity at 400µg/ disc concentrations which was better than aqueous extract. MITSKPE produced 23.0±2.00mm zone of inhibition against *Streptococcus pyogenes*. This is followed by *E.coli*, *Pseudomonas*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Shigella sp.*, and *Salmonella typhi*. Good antimicrobial activity was also exhibited by the phenolic extracts of MIMSKPE (Table 1). *Mangifera indica* tender and matured extracts showed more or less similar effects as like commercial antibiotics, which confirmed the effect of MISK.

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Table 1: Antibacterial activity of extract of *Mangifera indica* tender and matured seed kernel at 400µg/ml

S. No	Clinical isolates	Zone of Inhibition in mm					
		+ve control	-ve control	MIMSKPE	MIMSKAE	MITSKPE	MIMSKAE
1	*Escherichia coli*	21.6±0.56	-	19.7±2.08	20.3±1.15	20.8±1.05	24.6±0.57
2	*Salmonella typhi*	18.5±1.57	-	17.6±2.30	17.7±1.53	17.4±1.40	24.3±1.52
3	*Staphylococcus aureus*	21.3±2.67	-	18.3±1.52	18.6±1.16	18.2±0.28	23.0±1.00
4	*Pseudomonas aeruginosa*	22.5±0.30	-	20.7±3.05	22.0±2.64	20.7±1.20	22.3±1.15
5	*Streptococcus pyogenes*	20.2±1.52	-	19.6±2.51	20.3±1.52	23.0±2.00	23.3±3.78
6	*Shigella sp.*	15.8±1.15	-	15.3±2.51	16.3±1.52	17.7±0.58	18.7±1.52
7	*Klebsiella pneumoniae*	23.4±1.53	-	18.0±2.00	20.0±2.64	20.3±1.53	22.0±1.73

Drug dilution method was followed to assess minimal inhibitory concentration of aqueous and phenolic extracts. Growth inhibition study was assessed specifically by the way of expressing MIC. MIMSK phenolic extract showed effective bacterial inhibition at lower doses when compared to aqueous extract (Table 2). MITSKPE inhibited *Salmonella typhi* at 200.0±50.0 µg/ml concentration whereas MIMSKPE inhibited *Salmonella* at 300.0±50.0 µg/ml. MIMSKPE inhibited best at 283.3±28.8 µg/ml against *E. coli*. Aqueous extract of both products of *Mangifera indica* also showed good MIC against all the test isolates, MIC ranges from 216.7±28.9 µg/ml to 583.3±28.8.

Table 2: Minimal inhibitory concentration of *Mangifera indica* aqueous and phenolic extract

S. No	Test Organism	MIMSKAE	MIMSKPE	MITSKAE	MITSKPE
1	*E. coli*	400.0±86.6	283.3±28.8	266.7±76.4	250.0±50.0
2	*Salmonella typhi*	333.3±28.7	300.0±50.0	216.7±28.9	200.0±50.0
3	*Staphylococcus aureus*	450.0±50.0	383.3±76.4	400.0±50.0	250.0±50.0
4	*Pseudomonas aeruginosa*	416.7±28.9	416.7±28.8	433.3±57.7	266.7±28.9
5	*Streptococcus pyogenes*	433.3±28.9	383.3±57.7	450.0±50.0	283.3±104.1
6	*Shigella dysenteriae*	583.3±28.8	516.7±76.4	566.7±76.4	316.7±28.9
7	*Klebsiella pneumoniae*	400.0±50.0	300.0±50.0	433.3±57.7	300.0±50.0

MBC effects of *Mangifera indica* seed kernel aqueous and phenolic extracts were ranges from 400.0±050.0 to 633.3±028.7 µg/ml for phenolic extracts and 433.3±104.1 to 666.7±115.5 µg/ml for aqueous extract. Among the clinical isolates *Pseudomonas aeruginosa* and *Streptococcus pyogenes* were inhibited effectively by MITSKPE.

Table 3: Minimal Bactericidal concentration of *Mangifera indica* aqueous and phenolic extract

S. No	Test Organism	MIMSKAE	MIMSKPE	MITSKAE	MITSKPE
1	*E. coli*	516.7±115.4	550.0±050.0	516.7±076.4	466.7±057.7
2	*Salmonella typhi*	433.3±104.1	516.7±076.4	466.7±057.7	483.3±125.8
3	*Staphylococcus aureus*	550.0±132.3	466.7±028.7	533.3±057.7	450.0±100.0
4	*Pseudomonas aeruginosa*	516.7±160.7	516.7±104.1	550.0±050.0	400.0±100.0
5	*Streptococcus pyogenes*	633.3±104.1	416.7±076.4	550.0±086.6	400.0±050.0
6	*Shigella dysenteriae*	666.7±115.5	633.3±028.7	583.3±104.1	533.3±076.4
7	*Klebsiella pneumoniae*	450.0±050.0	500.0±100.0	450.0±132.3	416.7±104.3

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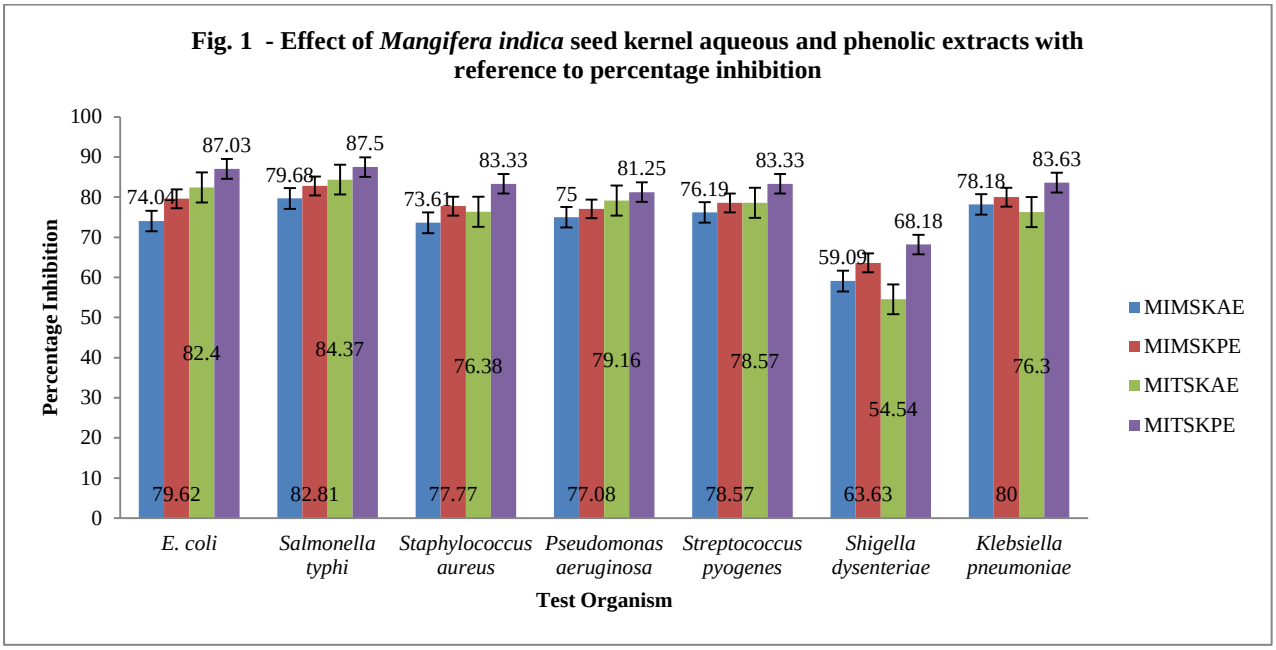
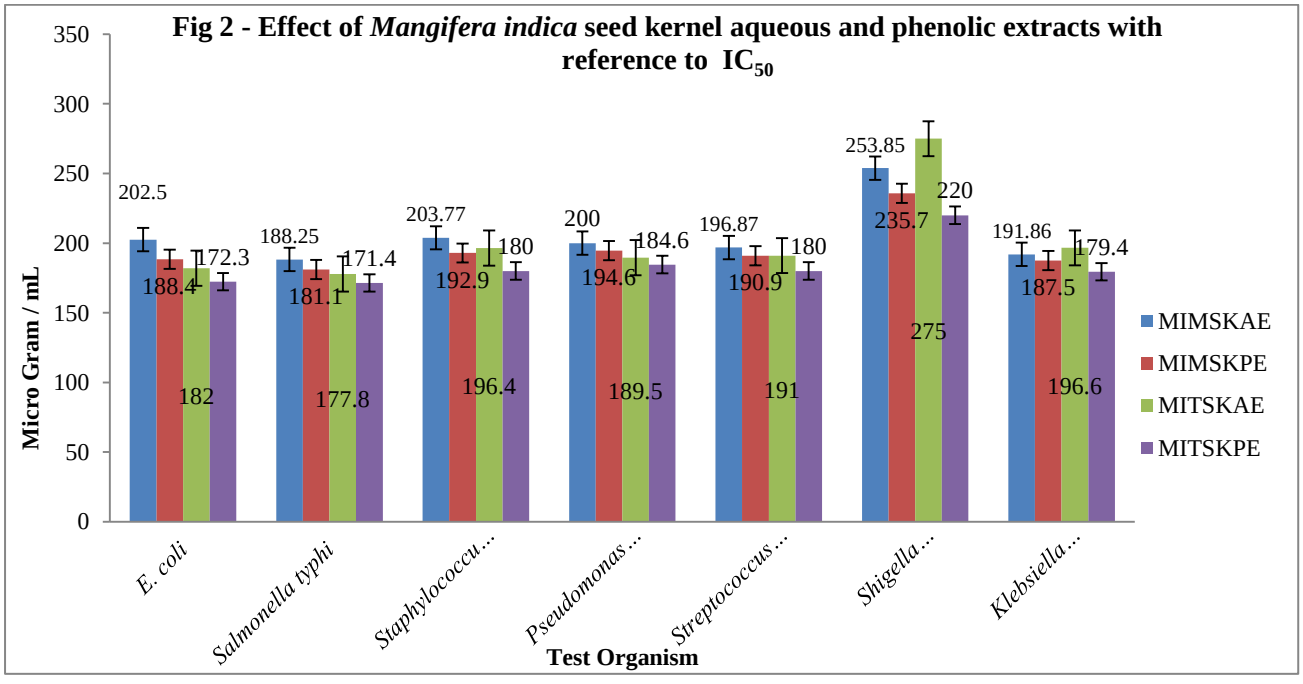


MIMSKAE, MIMSKPE, MITSKAE and MITSKPE showed variable percentage of inhibition against different clinical isolates. MITSKPE inhibited *Salmonella typhi* upto 87.5% at 200µg/ml concentration. About 54.5% of *Shigella sp*. was inhibited by MITSKAE at 200µg/ml concentrations. *Shigella* is the bacterial agent which was inhibited least by the extracts of both tender and matured seed kernel (Fig.1). *Salmonella typhi* was best inhibited by all the extracts of seed kernel.

Effect of herbal drugs were also measured with to IC_{50}, The half maximal inhibitory concentration. Among all extracts studied MITSKPE showed effective IC_{50} against all the clinical isolates. Best IC_{50} was noted against Salmonella typhi (171.4 µg/ml), followed by *E. coli* (172.3 µg/ml), *Klebsiella pneumonia* (179.4 µg/ml), *Staphylococcus aureus*, *Streptococcus pyogenes* (180.0 µg/ml each), *Pseudomoans aeruginosa* (184.4 µg/ml) and *Shigella* (220 µg/ml). Concentration required for killing 50% of *E. coli* by MITSKAE was at 182 µg/ml concentration, MIMSKPE produced best activity against *Salmonella typhi* at 181.1 µg/ml concentration. Tender seed kernel extracts showed better IC_{50} than matured seed kernel (Figure 2).



4. DISCUSSION

In the present study, *Mangifera indica Seed kernel* extracts exhibited good antimicrobial activity. Phytoconstituents like tannins, phenolic compounds could be responsible for these activities. In this study we noticed that phenolic extracts were best when compared to aqueous extracts. One of the previous studies indicated the presence of steroids, terpenoids, flavonoids, phenolic compounds in both extracts of matured seed kernel [2]. Bharathi and Rajan [8] also reported the presence of these compounds in tender sed kernel of this plant. This is also evidenced by Rajeshwari & Ramachandramurty [9]. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and are thought to be responsible for coagulating the wall proteins of pathogenic organisms. Thus, *MISK* extract containing this compound may serve as a potential source of bioactive compounds in the treatment of infectious diseases. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane bound enzymes such as the ATPase and phospholipase [10]. They also serve as health promoting compounds as a result of their anion radicals [11].

There are many reports available that plants have been evaluated *In Vitro* for their antibacterial potency against some important human pathogenic bacteria [12], [13], [14], [15]. Patni *et al.*, [16], Karou *et al.*, [17], Masika and Afolayan, [18] reported that gram positive bacteria are more susceptible than gram negative bacteria because of its nature of cell wall. Our report doesnot showed huge variability in terms of MISK susceptibility. Antimicrobial property of a plant depends on its biologically active phytoconstituents. A wide range of antiinfective actions have been assigned to tannins [19]. Some authors have found that more highly oxidized phenols are inhibitorier [20], [21]. Flavonoids are synthesized by plants in response to microbial infection [22]. Terpenoids are active against bacteria [23], fungi [24], viruses [25] and protozoa [26].

5. CONCLUSION

Hence, the plant which was subjected to this investigation reveals the presence of active phytochemicals, which exhibits many beneficial properties. *MISK* extracts inhibited the growth of all types of microbial population. Tannins and phenolic compounds found in MISK, which precipitates cell wall proteins of microorganisms and also suppress prokaryotic DNA replication.

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