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Evaluation of antioxidant and antimicrobial potential of mango seed kernel

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Abstract
The present study evaluated the antioxidant and antimicrobial activity of mango seed kernel extracts prepared in different extraction solvents. Three different solvents *viz*. acetone, methanol, and water were used to extract the bioactive compounds of mango seed kernel and their antioxidant potential was estimated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis 3 –ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging assays. The results revealed that methanolic extract of mango seed kernel showed higher antioxidant activities than acetonic and water extracts. Based on the higher antioxidant potential, methanolic extract of mango seed kernel was further assessed for antimicrobial activity against selected bacterial species namely *Xanthomonas campestris* and *Escherichia coli* using well diffusion method. This research shows that mango seed kernel is an abundant and cost-effective source of potential natural antioxidant and this would probably become an alternative source of antimicrobial agents.

Keywords: Mango seed kernel, DPPH, ABTS and antimicrobial activity

Introduction
Mango (*Mangifera indica* L.) is known as one of the most prominent tropical and sub-tropical fruits in the world. It is famous for deliciousness, flavor, taste, nutrition and also called as "King of fruits" and "National fruit of India". India is the largest producer of mangoes (40% of the world total) with21.25 million tonnes production during 2018. The main mango producing states in India were Uttar Pradesh, Andhra Pradesh, Karnataka, Telangana, Bihar, Maharashtra, Gujarat, and Tamil Nadu with the production of 23.06%, 16.07%, 9.29%, 8.54%, 7.52%, 2.63%, 6.38% and 5.88%, respectively (MoA&FW, 2018) [6]. Mango is consumed in its fresh form as well as in processed form such as nectar, jam, juices, dehydrated products, frozen mango and canned slices all over world (Masibo and He, 2009) [4]. Large amount of by-products are produced during industrial processing of fruits. One of the by-products of the food processing industry is the mango seed kernel, which is not commercially used, but is discarded as waste. Depending on the variety of mango, its seed accounts for 20% to 60% of the total fruit weight, and the kernel present constitutes 45% to 75% of the total seed (Ashoush and Gadallah, 2011) [1]. It is estimated that around 35-60% of the mango by-products in form of peels and stones are discarded as waste after processing (O'Shea *et al.* 2012) [8]. After processing of mangoes million tons of mango seed are discarded and its disposal may cause environmental pollution (Leanpolchareanchai *et al.* 2014) [2]. Mango seed kernel (MSK) possessesa good amount of carbohydrates (58-80%), protein (6-13%), kernel oil (6-16%) and an excellent amount of essential amino acids (Siaka, 2014). Besides this, it is Alsoa rich source of phenolic compounds, antioxidants and bioactive compounds that improve human health (Abdalla *et al.* 2007; Jahurul *et al.* 2015). But still it is underutilized. Considering the above facts in view, the present study was conducted to assess the antioxidant and antimicrobial potential of mango seed kernels.

Materials and Methods
Source of materials and sample preparation
The mango stones were collected from the processing industries and dried in tray dryer at 50 °C to remove the outer moisture present in fibrous seed coat.

After complete drying the MSK was manually separated from the stone and cut into small pieces. After that these small pieces were oven dried to remove their initial moisture and ground in hammer mill to obtain fine powder of particle size of 0.5 mm and stored in air tight containers under refrigerated conditions. All the chemicals used in the present study were of analytical grade.

Antioxidant properties of mango seed kernel extracts

The MSK extracts were prepared using different extraction solvents such as acetone, methanol, and water. For this purpose, 100 mg of dried MSK powder was taken in 50 ml conical flask and 10 ml of solvent was added to it. The content were refluxed for 1 h and centrifuged at 6,000 rpm for 15 min.

The supernatant was further diluted 10 times with methanol and used for the estimation of antioxidant activity. The supernatant was collected and served as extract to evaluate antioxidant potential.

DPPH activity

Antioxidant activity was measured using stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as per the method described by Shimada *et al.* (1992).

Twenty-five mg DPPH dye was dissolved in 10 ml methanol with vigorous shaking. One ml of this solution was diluted to 100 ml. Three ml of dye was mixed with 0.5 ml diluted sample and incubated in dark for 20 min. The absorbance was read at 517 nm on spectrophotometer. Dye mixed with 0.5 ml methanol was used as blank. Antioxidant activity was expressed as per cent scavenging of DPPH and it was calculated by using formula:

$$\% \text{ scavenging capacity of DPPH} = \frac{A0-A1}{A0} X100$$

Where

A_0 = Absorbance of blank, A_1 = Absorbance of sample

ABTS activity

Free radical scavenging activity of MSK sample was determined by ABTS radical cation decolorization assay (Re *et al.* 1999). ABTS+ cation radical was produced by reaction between 7mM ABTS in water and 2.45mM potassium per-sulphate (1:1), stored in the dark at room temperature for 12-16 h before use. ABTS+ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 0.1ml of extract to 3.995 ml of diluted ABTS+ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay.

All the measurements were taken in triplets. Calculations were done on the basis of following formula:

$$\% \text{ Scavenging Activity with ABTS} = \frac{A0-A1}{A0} X100$$

Where,

A_0= Absorbance of blank, A_1 = Absorbance of sample

Antimicrobial properties of mango seed kernel extracts

One g of MSK sample was taken and mixed it with 10 ml of methanol and refluxed the content. After 24 hthe supernatant was taken out and filtered. The supernatant was collected and extracts of different concentrations between 50-200 mg/ml were prepared for evaluating the antimicrobial potential.

The antimicrobial activity was evaluated with methanolic extracts of MS Kagainst gram negative bacterial pathogens such as *Escherichia coli* (*E. coli*) and *Xanthomonas campestris (X. campestris).*These bacterial strains were grown in 500 mL nutrient broth at 37°C and maintained in nutrient agar slants at 4°C. The bacterial cultures were diluted with distilled water and collected in sterile centrifuge tubes and standardized by obtaining absorbance to 0.5493 Abs at 610 nm spectrophotometrically. The agar well diffusion technique was used for antimicrobial test (Mirgani *et al.* 2009) [5]. Nutrient agar plates were prepared and overnight suspension culture of 100µl of bacterial culture (*E. coli* and *X. campestris)* was spread on them gently. With the help of sterile well puncture, wells were drawn on plates and 100 µl of methanolic extract of MSK of different concentrations was filled in it. The commercially available antibiotic streptomycin (1 mg/ml) was used as positive control and methanol was used as negative control to determine the antimicrobial activity of MSK against these bacterial strains. The inoculated plates were incubated at 37°C for 24 h. The antimicrobial activity was evaluated by measuring diameter of the inhibition zone formed around the well using ruler in centimeters.

Statistical analysis: All data were reported as mean (± standard error) of three replicates. Analyses of variance (ANOVA) were performed using open access R software. Differences at $p< 0.05$ were considered significant.

Results and Discussion

Antioxidant potential of mango seed kernel extracts

The antioxidant activity of food is a result of the action of each of its antioxidants, which can interact and produce synergistic or inhibitory effects (Lee *et al.* 2003) [3]. The antioxidant activities of mango seed kernel extract using different extraction solvents are presented in Fig 1. DPPH radical scavenging method is broadly used to evaluate antioxidant activity. This method is based on hydrogen donating ability or radical scavenging ability of any extract in alcoholic medium that results in color change from purple to yellow. While the ABTS method is based on electron transfer ability of the sample with long life and reactive radical anion 2,2-azinobis (3 -ethylbenzothiazoline-6-sulfonic acid). In this study, the MSK extract prepared in methanol showed highest per cent scavenging of DPPH and ABTS radical with 87.70±0.70% and 89.43±0.87%, respectively than the extracts obtained by acetone and water extraction. Mutua *et al.* (2016) [7] reported that methanolic extract of mango seed kernel extract at the concentration of 20 mg/ml showed 92.22% DPPH scavenging activity. Similar to this, Ashoush and Gadallah (2011) [1] have reported 95.08% ± 0.10 DPPH scavenging ability of mango seed kernel extract.

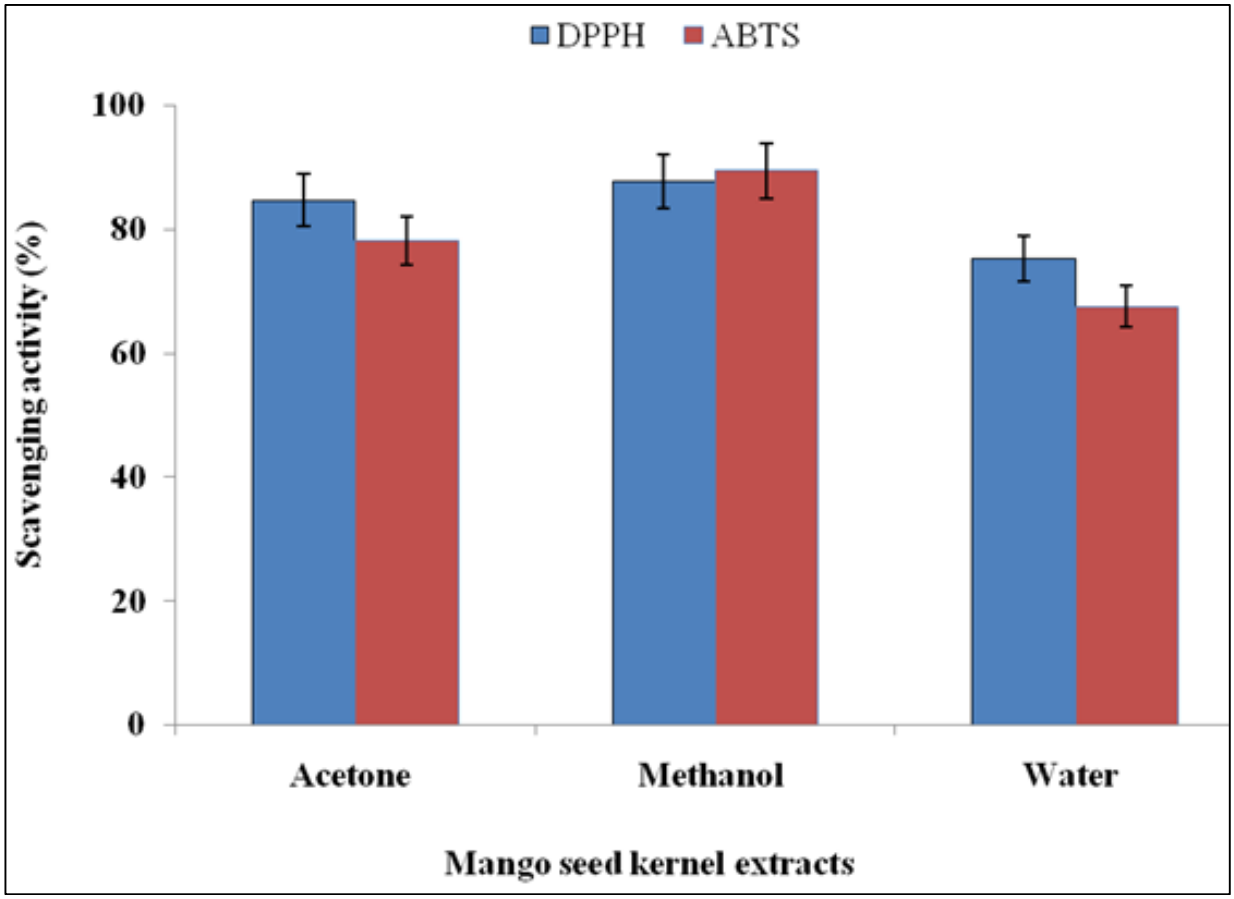

Fig 1: Antioxidant activity of mango seed kernel extracts using different extraction solvents

Antimicrobial activity of mango seed kernel extracts against bacteria

Extracts were prepared and to check the efficiency of extracts against air borne microbes, slight exposer of air was given to extracts and then incubated at 28 ± 2 °C. After 48 hrs, growth of different fungi was seen in water extract. It means water extract of MSK doesn't show any anti-mycotic and when tried with disc method same results were observed. Extracts were prepared and to check the efficiency of extracts against air borne microbes, slight exposer of air was given to extracts and then incubated at 28 ± 2 °C. After 48 hrs, growth of different fungi was seen in water extract.

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The results of antimicrobial activity studies showed that methanolic extract of MSK exhibited higher antimicrobial activity against *E. coli* followed by *X. campestris*. The antimicrobial activity of methanolic extracts of mango seed kernel was higher compared to the commercial antibiotic streptomycin which showed 0.5±0.12 cm and 0.31±0.07 cm zone of inhibition against *E. coli* and *X. campestris*, respectively. Different zones of inhibition against *E. coli* and *X. campestris* with different concentrations of methanolic extracts of MSK are shown in Table 2. It was found that the antimicrobial activity of mango seed kernel extract with concentration of 200 mg/ml showed maximum zone of inhibition against both gram negative bacteria *E. coli and X. campestris.* Mutua *et al.* (2016) [7] also reported the similar findings against *E. coli* bacteria. The strong antimicrobial activity exhibited by the mango kernel extracts could be due to their high amount of phytochemical composition such as flavonoids, terpenes, tannins, and coumarins (Orijajogun *et al.* 2014) [9]. The zone of inhibition for *E. coli and X. campestris* was depicted in Fig. 2 and Fig. 3. The results revealed that as the concentration of extracts increased the size of inhibition zone also increased.

Table 2: Inhibition zones of *Escherichia coli* and *Xanthomonas campestris* with different concentration of methanolic extracts. The values are represented as mean of five readings ± Standard deviation.

Sr. no.	Extract concentration (mg/ml)	Zone of Inhibition (cm)			
		Escherichia coli		*Xanthomonas campestris*	
		24 h	48 h	24 h	48 h
1	Methanol (negative control)	No Zone	No Zone	No Zone	No Zone
2	50	0.54±0.20	0.65±0.28	0.24±0.11	0.24±0.11
3	100	0.64±0.25	0.7±0.30	0.33±0.097	0.33±0.081
4	150	0.7±0.31	0.8±0.23	0.39±0.022	0.44±0.15
5	200	1.0±0.23	1.0±0.26	0.47±0.31	0.48±0.12
6	Streptomycin (positive control)	0.5±0.12	0.5±0.12	0.31±0.07	0.31±0.07

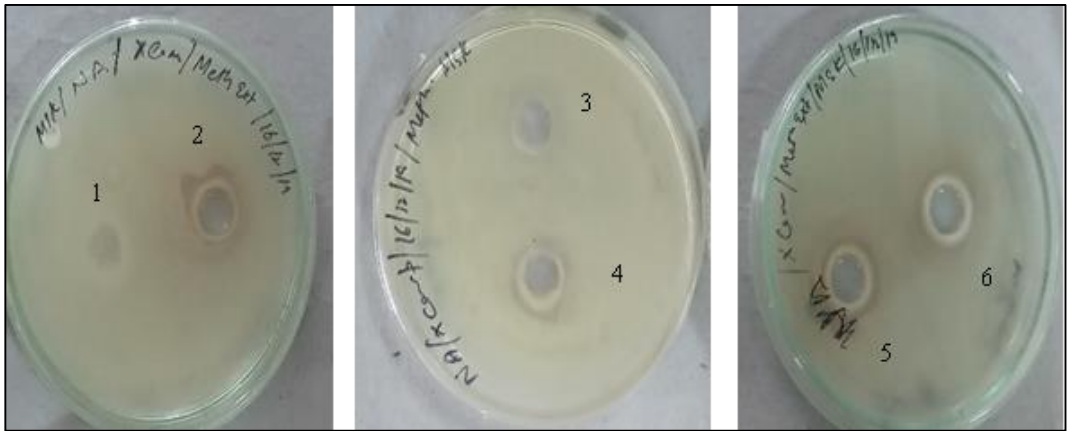

Fig 2: Comparison of antimicrobial activity ofdifferent concentrations of methanolic extract of mango seed kernel with streptomycin by well diffusion method against *Xanthomonas campestris*. 1 Methanol (negative control), 2. 50mg/ml MSK extract, 3. 100mg/ml MSK extract, 4. 150mg/ml MSK extract, 5. 200mg/ml MSK extract, and 6. Streptomycin (positive control).

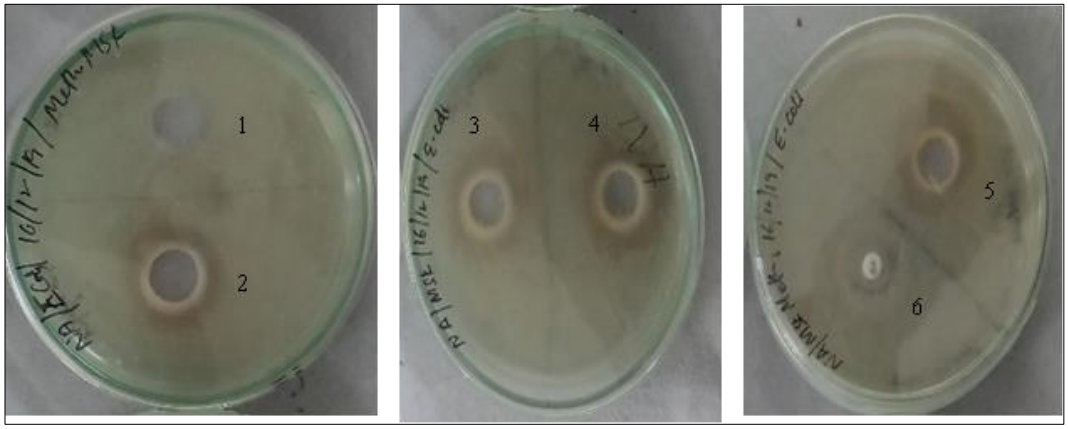

Fig 3: Comparison of antimicrobial activity of different concentrations of methanolic extract of mango seed kernel with streptomycin by well diffusion method against *Escherichia coli*.1. Methanol (negative control), 2. 50mg/ml MSK extract, 3. 100mg/ml MSK extract, 4. 150mg/ml MSK extract, 5. 200mg/ml MSK extract and 6.Streptomycin (positive control).

Conclusions

In the search for a variety of biomolecules from various parts such as stem bark, leaves and pulp, mango plants have been the subject of thorough research. However, medicinal properties of the mango seeds kernel are less explored. The results of this study revealed that among the three studied solvents, the methanolic extract of mango seed kernel showed good antioxidant and antimicrobial activity. Therefore, methanolic extract could find applications in food industry as natural antioxidant and antibiotic source to address the challenge of infections caused by pathogenic microbes. The mango seed kernel is discarded as waste, the availability and cost factor will be negligible in generating cheap and effective plant-based natural antioxidants. It will also play a major role in utilization of mango seed waste generated worldwide.

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