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Extraction and Characterization of Mango seed kernel oil and its antimicrobial activity

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***Abstract*— The waste mango seed generated from Mango pulp industry in India is a major difficulty in handling the waste and hence conversion of mango seed kernel into kernel oil was studied using extraction process. Mango seeds were collected and processed for oil extraction. The study was carried out for proximate composition and characteristics such as unsaponifiable matter and fatty acid composition. The result showed 1.354% moisture, 6.36% crude proteins, 3.4% Crude fiber, 14.468% carbohydrate (by difference), and 1.432% ash. The fatty acid composition includes 45.22% of Oleic acid (C18:1), 39.57% of Stearic acid (C18:0), 7.89% of Palmitic acid (C16:0) and 7.32% of Linolenic acid (C18:2). Higher saponification value of 187 mg of KOH/g is an indication for the use of oil in soap making industries. Antimicrobial studies were carried using the extracted oil. Studies to find out the Minimum Inhibitory Concentration (MIC) were carried out for bacterial species such as *Proteus vulgaris and Klebsiella pneumoniae* at different concentrations- 10 µl, 15 µl and 20 µl, inhibition zone shown were 1.5 cm, 1.75 cm and 2.0cm respectively. The corresponding inhibition zones for Klebsiella pneumonia were 1 cm, 1.5 cm and 2.0 cm. For fungal species such as *Cladosporium and Penicillium chrysogenum* respective zones of inhibition were 1.5 cm, 1.8 cm and 1.6 cm. It can be concluded that Mango seed kernel extract and oil can be used as natural antioxidant due to the rich content of different phenol compounds with rich fatty acid and mono-unsaturated oleic acid.**

Keywords: **Mango kernel; oil; seed oil; phenolic compound; antimicrobial.**

I. INTRODUCTION

Mango seed is an abundant residue discarded by mango juice manufacturing industries of mango juice and its amount is increasing due to the expansion of fruit production. An alternative for the use of this residue is necessary. India is the world's largest producer of mangoes. With world's total production of 37.12 million tons India produced 40.48% of total world Mango production. As given by APEDA (Agricultural and Processed Food Products Export Development Authority, India) India reported an export of 63.4 lakh metric tons in the form of processed mangoes in the year 2011-12. So complete exploitation of these mango seed kernels not only eliminates the disposal problem but also results in valuable products production. Oils and fats are important components of foods and their derivatives and products play an important role in non-food applications. The main components of food lipids are triacylglycerols, but minor components are also important for quality characteristics, stability and application areas.

With dramatic increase in number of food borne diseases adding microbial agents will prevent spoilage of foods. Here we report the anti microbial activity of Mango seed Kernel extract with anti-microbial properties against most prevalent food-borne pathogenic microorganisms.

II. RELATED WORK

Mango is known as the 'King of fruit' throughout the world and cultivated as a fruit tree in frost-free tropical and warmer subtropical climates like that of the India which accounts for 23 lakh hectare cultivation with more than half of the total world's total area under mango cultivation[1]. Mango processing yields about 40-50% of by-products which can be used to feed livestock [2].

After consumption or industrial processing of the fruits, considerable amounts of mango seeds are discarded as waste. The seed represents from 10% to 25 % of the whole fruit weight [3]. The kernel inside the seed represents from 45% to 75% of the seed and about 20% of the whole fruit [4].

Amina [14] proposed the possibility of utilization mango kernel as antimicrobial agent against food borne pathogenic bacteria after the extracting with ethanol and also studied the possibility of utilization mango kernel powder (MKP) as antioxidant agent to determine their content of phenolics to identify the polyphenol which protect the ghee from oxidation.

Soong et al [6] indicated that mango seed kernel has potent antioxidant activity with relatively high phenolic contents. Recent developments in the area of oils and fats has led to the production of specialty lipids from novel sources such as fruit seeds, nuts, and other minor plant sources[5]. Conventional extraction methods from plant materials include hydro distillation, steam distillation and solvent extraction. Supercritical fluid extraction, a novel and environmentally benign separation technology represents a green alternative to the conventional extraction methods for the production of natural extracts [5, 6].

Extraction of oil from Jatropha curcas L. seed kernels was done by use of ultrasonication as a pretreatment before aqueous oil extraction and aqueous enzymatic oil extraction, the maximum yield of 74% was obtained by ultrasonication process [7].Extraction of oil with conventional organic solvents produces low quality that requires extensive refining [12].

Many oils were extracted from its flour by a multistage process using n-hexane It appeared that what may be desirable is a paradigm shift in the extraction strategy. Hence three phase partitioning method generally used for protein separation for oil extraction because of its simplicity and short processing time may be worth exploring. [7].

Ahmed et al [10] carried his studies on Egyptian mango seed kernels to clarify their proximate composition, amino acids, phenolic compounds and the characteristics of the extracted oil including unsaponifiable matter constituents, lipid classes and fatty acid composition.

Phenolic compounds could be a major determinant of antioxidant potentials of foods and could therefore be a natural source of antioxidants [9]. The aqueous-methanolic extracts of pulp, peel and seed kernels was analyzed for antioxidant activity by free radical-scavenging and reducing power and the results showed that Uba pulp presented higher antioxidant activity than standard [8].

Nagendran Balasundram et al [11] worked on antimicrobial activity of crude water extract and methanolic extract of Raphanus sativus was investigated in vitro using Agar well diffusion method. The extracts displayed highest antibacterial activity against *Hafnia alvei, Enterobacter agglomerans, Lactobacillus* and *Bacillus thuringiensis* while fungal species viz. *Penicillium lilacinum, Paecilomyces variotii, Spadicoides Stoveri, Penicillium funiculosum* showed variable degrees of inhibition at lower concentration.

Winkelhausen et al [13] proposed the antifungal activity of strains *Alternaria solani, Botrytis cinerea and Fusarium culmorum* was tested with the phenolic compounds extracted from Olive pomace, a byproduct of olive oil production. Phenolic compounds resulted in reduction of mycelia growth by promising as a natural fungicide against most prevalent pathogens.

III. MATERIALS AND METHODS

A. Preparation of Mango Seed Kernel Powder

About 50 kg of mango seeds as by-products (waste) was collected from the local juice manufacturing industry GALLA FOODS (P) Ltd. The seeds were washed and air dried to remove the hard seed coat manually from the processed waste. These dried seeds were finely powdered using domestic blender into powdery form and sieved using a sieve of size 425 microns and that sieved particles was used in all further experiments. This powdery form is kept in a closed dark glass bottle and stored at 4°C until utilization.

B. Proximate analysis

The proximate composition of the samples was determined using the methods of the AOAC (1990).Moisture content, ash content , crude fiber, and crude protein were determined for MSK according to AOAC (1990), while total lipids were extracted and determined according to FolchLe and Stanley (1957).

C. Procedure for Oil Extraction by three phase partitioning Technique

5g of Mango Seed Kernel powder was dispersed in 20ml distilled water and gently stirred on a magnetic stirrer to make the slurry. Ammonium sulphate (50%, w/v) was added to the slurry and vortexed gently followed by the addition of 20 ml t-butanol (organic solvent).The mixture was kept at 37˚C for 1 h. Formation of three distinct phases, upper organic phase, lower aqueous phase and interfacial precipitate layer was observed. These were separated by centrifugation at 2000 rpm for 10 min. The upper organic layer was collected and evaporated to obtain oil extracted in this phase. Each extraction was run in duplicate.

D. Characterization of mango seed kernel oil

Physical properties included specific gravity, odor, melting point and colour was determined according to AOAC (Association of Official Analytical Chemists-1989). Chemical characteristics including free fatty acids (FFA), peroxide value (PV), iodine value (IV), Ester Value, Unsaponifiable matter and saponification value (SV) were determined according to AOAC (1989).

E. Preparation of MSK Ethanol extract

Mango seed kernels are obtained by washing the processed mango waste, drying it and removing the shells which was obtained from local juice manufacturing industry. The obtained kernels were ground using domestic mixie. The ethanol (99.5%) was added to the mango seed kernel triturate to 2:1 (v/w) and kept overnight in a dark place with gentle shaking. After removing insoluble materials the solvent was evaporated with a rotary evaporator. The residue was used as MKE for the following experiments.

F. Determination of total phenolic content

Total phenols were extracted from mango seed kernels powder samples according to the methods of Rodriguez, et al., 1994 using methanol alcohol 95% under cooling. The total phenol content of methanol extract was assayed calorimetrically using the Folin-ciocalteu method where an aliquot (1 ml) of the extract was mixed with diluted Folin-ciocalteu (0.5 ml) and 2% ethanol amine (1 ml) at room temperature. After 5 min, the absorbance was measured at 750 nm using a spectrophotometer with a blank sample (water plus reagent) in the reference cell. The phenolic compounds of ethanolic extracts from mango seed kernels were identified and determined using high performance liquid chromatography (HPLC).

G. Antimicrobial activity

The antibacterial and antifungal evaluated with ethanolic extract of Mango (Mangifera indica) kernels by Well diffusion method against most prevalent food borne pathogens such as *Proteus vulgaris, Klebsiella pneumonia,Pencillium chrysogenum, and Cladosporium.*1.3 g of HIMEDIA instant nutrient broth supplemented with nutrients was dissolved in 100 ml of distilled water. The prepared broth was sterilized and stored 4°C until required. Bacterial and fungal strains were sub-cultured on to their respective growth medium and then incubated for 48 hrs at 25-30°C. From these plates, several colonies were transferred to 5 ml of sterile distilled water. The suspensions were mixed for 15 s to ensure homogeneity and then sterilized and supplemented to working

suspension.5g of nutrient agar (Muller Hinton Agar) medium available instantly was dissolved in 200ml of distilled water and autoclaved along with petri plates. Then the medium was taken from autoclave and kept in laminar air flow chamber and poured into sterilized plates for solidification. When the medium solidify, 4 wells of 8mm were made by well cutter in which one well was taken as control. Then the bacterial strains were swabbed in Muller Hinton agar plates with the help of sterile cotton. Ethanol extracts of mango seed kernel of 10µl, 15µl and 20µl were serially pipetted into the wells. The Petri plates were incubated at 37˚C for 7 days to find the zone of inhibition for fungal strains were as for bacterial strains zone of inhibition was observed within 24 hours. The zone of inhibition produced by the ethanol extract was compared with control and measured with a scale.

IV. Results and Discussion

A. Proximate analysis of Mango seed kernel

Results obtained showed that the seeds contained 1.354% moisture,6.36% crude proteins, 3.4% Crude fiber,14.468% carbohydrate (by difference), and 1.432% ash (Table 1).The results indicate that the highest among these is the carbohydrate value which is an important constituent of nutrients

Table.1. Proximate analysis of Mango Seed Kernel Powder

Characteristic	Obtained Value (%)
Moisture Content	1.354
Ash content	1.43
Crude fiber	1.43
Crude protein	6.36
Crude Fat	2.395
Total Carbohydrate Value	14.468

B. Characteristics of mango seed kernel oil

Oils and fats are oxidized during processing, circulation and preservation. This causes deterioration in taste, flavor, odor, color, texture, and appearance and a decrease in the nutritional value of the foods. So therefore from quality and safety perspective it is important to test the physical and chemical characteristics.

Physical properties includes specific gravity, melting point, odor and color were as chemical characteristics includes free fatty acid (Acid Value), peroxide value, Saponification value, Ester Value and unsaponifiable matter. The low acidity of MSKO indicated that the mango seed was almost free from hydrolytic rancidity brought almost by lipases and enables the direct use of such oil in industries without further neutralization. On the other hand, MSKO had a high quality due to the low level of peroxide value.

The fatty acid composition includes 45.22% of Oleic acid (C18:1), 39.57% of Stearic acid (C18:0), 7.89% of Palmitic acid (C16:0) and 7.32% of Linolenic acid (C18:2). Higher saponification value of 187 mg of KOH/g is an indication for use of oil in soap making industries. Table 2 shows the characteristics of Mango Seed Kernel Oil.

Table 2.Characteristics of Mango Seed Kernel Oil

Physical Characteristics	
Color	Light Yellow
Odor	Fatty Odor
Specific Gravity	0.93
Melting Point	24.5°C
Chemical Characteristics	
Peroxide Value(meqO2/kg oil)	0.90
Saponification Value(mg of KOH/g)	187
Acid Value(FFA)	0.37
Ester Value	186.63
Unsaponifiable matter (% of total lipid)	2.113

C. Total Phenolic Content

Phenolic compounds sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. Phenolics have unique properties and have higher acidities due to the aromatic ring's tight coupling with the oxygen and a relatively loose bond between the oxygen and hydrogen. Some phenolics are germicidal and are used in formulating disinfectants others possess estrogenic or endocrine disrupting activity. The contents of total phenolics are expressed as Gallic acid equivalents (GAE), either as mg GAE/g or mg GAE 100/g. Phenolic compounds react with Folin-Ciocalteau reagent only under basic conditions (pH- 10) due to a much higher reactivity of the phenolate anion with the molybdenum-based compounds present in the reagent. The value was obtained from the absorbance of the extracts treated with Folin-Ciocalteau reagent. The total phenolic contents for Mango seed kernels 79.825 mg/kg of dry matter. This indicates Mango Seed Kernels as a promising source for polyphenolics.

D. Antibacterial activity

Minimum Inhibitory Concentration (MIC) was carried out for *Proteus vulgaris and Klebsiella pneumoniae* at different concentrations such as 10 µl, 15 µl and 20 µl. Antibacterial assay revealed that the mango seed kernel extract showed significant activity against pathogenic bacteria.Table.3. shows the details of zone of inhibition of mango seed kernel ethanol extract against different bacterial strains. The antimicrobial spectrum of MKE extract was effective against Gram negative bacteria. The structures of cell envelope including cytoplasmic membrane and cell wall component are different between gram-positive and negative bacteria. As antimicrobial agents generally make contact with the cell envelope first the structural difference plays a key role in the susceptibility. The zone of inhibition for Proteus *vulgaris*(Gram negative) and *K. pneumonia*(Gram negative) was depicted in Fig.1 and Fig.2.

Table3.Antibacterial activity of Mango Kernel Extract

Name of the organism	Zone of Inhibition		
	10 µl	**15** µl	**20** µl
P. vulgaris	1.5	1.75	2.0
K. pneumonia	1.0	1.5	2.0

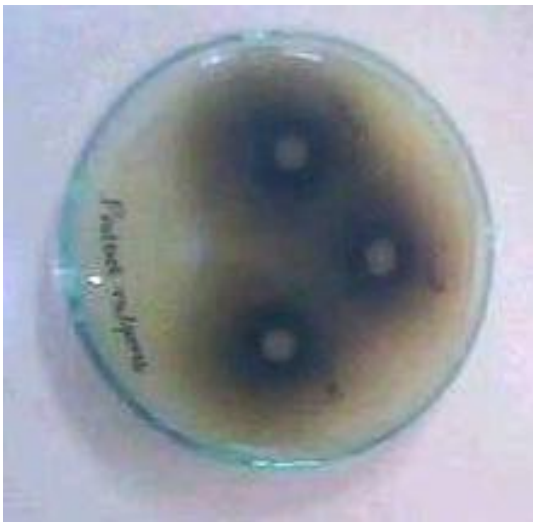

Figure.1.Zone of Inhibition for *P. vulgaris*

Figure.2. Zone of Inhibition for *K.pneumoniae*

E. ***Antifungal activity***

Antifungal activity with different concentrations was done against two different pathogenic fungi namely *Cladosporium* species *and Penicillium chrysogenum*. The zone of inhibition is shown in Fig.3 and Fig.4 for *Cladosporium* sps *and Penicillium chrysogenum* having 1.5cm,1.8cm,1.6cm for 10 µl,15 µl & 20µl concentrations and for *P. chrysogenum* the zones of inhibition at 10 µl,15 µl & 20µl concentrations was shown as 0.9cm,1.4cm & 1.7 cm.

Table4.Antifungal activity of Mango Kernel Extract

Name of the organism	Zone of Inhibition		
	10 µl	**15** µl	**20** µl
Cladosporium sps	1.5	1.8	1.6
P. chrysogenum	0.9	1.4	1.7

Figure.3 Zone of Inhibition for *Cladosporium sps*

Figure.4 Zone of Inhibition for *P. chrysogenum*

V. CONCLUSION

Proximate analysis of mango seed kernel powder was analyzed by standard methods and result shown the highest composition of potassium in the sample is a promising source in soap making industries. The percentage of oil extraction was nearly 65%, which can be recommended for oil extraction at industrial level. The extracted Mango Seed Kernel Oil (MSKO) was analyzed for physical and chemical characteristics. MSKO has a low content of protein. Stearic and oleic acids were the principal fatty acids and the proportion of unsaturated fatty acids was greater than the saturated fatty acids. High unsaponifiable matters content guarantees the use the oils in cosmetics industry. Lower the

peroxide value best is the quality of the oil. The antimicrobial spectrum of the mango kernel extract is more effective against gram- negative bacteria and some pathogenic fungi. Mango seed kernels contain considerable amounts of total phenolic compounds and total lipids with high levels of unsaponifiable matter. It can be concluded that mango seed kernel extract and oil can be use as natural antioxidant in 54 different kinds of foods due to high content of different phenolic compounds their fatty acid pattern rich with saturated fatty acids and with mono-unsaturated oleic acid.

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