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Preliminary Phytochemical Screening and Antimicrobial Activity of Cotyledon of Two Variety of *Mangifera indica L.*

U. Arul Pamila[1], S. Karpagam[2]

[1]Ph.D Research Scholar, Department of Botany, Queen Mary's College (A), Chennai-600 004, India

[2]Associate Professor, Head Department of Botany, Queen Mary's College (A), Chennai-600 004, India

Abstract: ***Mangifera indica, (Mango) commonly used plant in Ayurvedic medicine. Mangoes belong to genus Mangifera which consists of about 30 species of tropical fruiting trees in the family Anacardiaceae. Mango peels and seed kernels are the major by-products of mango juice industry, they are rich sources of natural bioactive compounds which play an important role in prevention of diseases. Preliminary phytochemical analysis was performed with five different solvent extracts such as ethanol, acetone, petroleum ether, chloroform and aqueous extracts of Mangifera indica seed kernel extracts that showed alkaloid, carbohydrates, saponins, phenol, diterpenes, terpenoids, protein, xanthoprotein, tannins, quinone, steroids and coumarin. The ethanol and aqueous extract showed increased amount of phytoconstituents when compared to other extracts. This study emphasized specifically on the potential of the mango seed kernel by discovering the prospective usage of mango seed kernels as a source of phytoconstituents and antimicrobial constituents against medically important pathogen Staphylococcus aureus and Enterococcus faecalis (Gram positive); Escherichia coli and Klebsiella pneumonia (Gram negative); and fungi such as Aspergillus niger, Epidermophyton floccosum and Candida albicans. The present study endeavor to screen two varieties of mango kernels; Banganapalli and Rumani extracted using five extraction solvents (acetone, aqueous, chloroform, ethanol and petroleum ether) examine the potential of mango kernel as natural antimicrobial against four bacterial strains and three fungal strains. Well diffusion method was employed to determine the antimicrobial activity. The extracts were compared with standards like Amoxicillin and Ketoconazole for antibacterial and antifungal activity respectively. It was found that Banganapalli had the best antimicrobial activity in the ethanol and aqueous extracts when compared to Rumani. This finding would probably become an alternative source of new and natural antibacterial and antifungal agents.***

Keywords: Mangifera Indica seed kernel, phytoconstituents and antimicrobial activity

1. Introduction

Mangifera indica L., Anacardiaceae family, is a large evergreen tree of tropical and subtropical regions of the world. It is commonly used in folk medicine for a wide variety of remedies (Coe and Anderson, 1996). *Mangifera indica L.* is one of the choicest fruit, especially in Asia. Its population and importance can easily be realized by the fact that it is often referred as "King of Fruits in the Tropical World". Mango is popular due to its excellent flavour, delicious taste, delicate fragrance, attractive colour and nutritive value which make rank among the best fruits of world (Singh Hemango, 1960). All parts of plants are rich in tannins and flavonoids (Nunez-Selles 2005). Different parts of mango have a broad range of medicinal properties, such as antimicrobial (Keita *et al.*, 2004), antiviral, antifungal (Cojocaru 1986), anti-inflammatory (Garrido *et al.*,1986), anti-diarrhoeal (Sairam *et al*., 2003), antioxidant (Scartezzini *et al.*, 2002), as well as immunomodulatory effect (Makare *et al.*, 2001). Of all the by-products of mango tree, its kernel is the cheapest and most readily available. Huge amounts of unutilized mango kernels, can be used as feed additives to enhance non-specific immunity in fish (Sahu *et al.*, 2007). Mango seed kernel is one of the by-products of food processing industry and is not commercially exploited, but are discarded as waste.

Mango seed represents from 20% to 60% of the whole fruit weight, depending on the mango variety and the kernel inside represents from 45% to 75% of the whole seed (Maisuthisakul and Gordon 2009). Thus, the present study was evaluated for the phytoconstituents, antibacterial and antifungal potential of the Banganapalli and Rumani mango seed kernel.

2. Materials and Methods

Collection of Mango Seeds and Processing of Mango Seeds

The two variety of *Mangifera indica* seed kernels were collected separately from the local market of Chennai, Tamilnadu, India. Banganapalli and Rumani seeds were washed repeatedly under running tap water. The kernels were air-dried at room temperature and then made into powder using electric blender. The powder was preserved in an airtight container.

Preparation of Extract

Each sample of 10g was taken and soaked for 24h in 50ml of ethanol, petroleum ether, acetone, chloroform and aqueous separately. The extracts were filtered using Whatman filter paper No. 1, evaporated to dryness and re-dissolved in their respective solvents. The extracts were preserved in airtight container and kept at 4-5°C for further use.

Phytochemical Screening

Phytochemical screening was carried out by using the standard protocol as described by Harborne (1973). The alkaloids are determined by Wagner's Test (Tiwari *et al.*, 2011); carbohydrates by Benedict's Test; saponin by Foam Test, phenol by Ferric Chloride Test; flavonoids by Lead Acetate Test; diterpenes by Copper Acetate Test; Tannins by

Ferric Chloride Test; terpenoids by Salkowski's Test, and proteins by Biuret Test (Khanam *et al*., 2014). Further detection of steroids was carried out by Harborne (1973); detection of coumarin was done by Mace method (Mace, 1963) and quinone by conc. H_2SO_4; xanthoproteins by conc. HNO_3 and NH_3 Test, and glycosides by Modified Borntrager's Test (Kokate *et al*., 2006).

Antimicrobial Assay

Antibacterial Activity: The antimicrobial activity of five different extracts of the *Mangifera indica* was evaluated by well diffusion method on Mueller Hinton broth (Samy and Ignacimuthu, 2000). The selected microorganisms of bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* (Gram positive), *Klebsiella pneumoniae* and *Escherichia coli* (Gram negative) were inoculated as lawn culture using sterile swab. Wells were made in the agar plate using sterile cork borer (6 mm diameter)**.** The extracts were applied to different wells in serially increasing volumes 30µl, 40µL and 50µL. Respective solvents served as negative control and Amoxicillin (10µg) was used as the reference. The plates were labelled, covered and incubated at 37°C for 24h. The activity was assessed by the diameter of the zone of inhibition and results were recorded.

Antifungal Activity: Fungal cultures were *Candida albicans*, *Epidermophyton floccosum* and *Aspergillus niger* were maintained on Potato Dextrose Agar (PDA). *In vitro* activity was carried out by well diffusion method. PDA was poured into sterile Petri plates and allowed to solidify. Wells with diameter of 6mm were made on the plates and the extracts were applied to different wells in serially increasing volumes of 30µl, 40µL and 50µL by using micropipette. Respective solvents served as negative control whereas Ketoconazole (10µg) was used as the reference. The plates were labelled, covered and incubated at 28°C for 72 hr. The activity of the extracts was determined by measuring the diameter of zone of inhibition.

3. Results and Discussion

Table 1: Phytochemical content of two variety of Mangifera indica L. (Banganapalli and Rumani)

Name of the Test	Ethanol		Chloroform		Acetone		Petroleum ether		Aqueous	
	B	R	B	R	B	R	B	R	B	R
Alkaloids	-	-	-	-	-	+	-	-	+	+
Carbohydrates	+	-	-	-	-	-	+	-	+	+
Saponins	+	+	+	-	-	-	+	+	++	+
Phenols	++	+	-	++	+	+	-	+	++	-
Flavonoids	+	+	-	-	+	-	-	-	+	-
Diterpenes	-	-	+	+	+	+	+	-	+	+
Tannins	+	+	+		++	-	-	+	++	+
Terpenoides	++	+	-	-	-	-	+	+	+	+
Protein	-	-	-	-	+	+	-	+	-	+
Steroid	++	+	+		-	-	+	+	+	-
Glycosides	+	+	-	+	-	-	-	+	-	-
Xanthoprotein	+	+	-	-	-	-	-	-	-	-
Coumarin	+	-	-	-	+	-	-	-	-	+
Quinones	-	+	-	-	+	-	-	+	+	-

Note: "++" Abundantly presence;"+" indicates presence; "-" indicates absence; B: Banganapalli and R: Rumani.

Table 2: Antimicrobial activity of the Acetone extract of Mangifera indica L

Microorganisms	Diameter of inhibition zone in mm						
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 *µg*		40 *µg*		50 *µg*	
		B	R	B	R	B	R
Staphylococcus aureus	25	10	9	11	10	12	11
Klebsiella pneumoniae	23	10	9	10	11	11	12
Escherichia coli	21	9	9	10	10	12	11
Aspergillus niger	23	-	-	9	9	11	11
Candida albicans	21	9	8	11	10	12	11
Epidermophyton floccosum	20	-	-	10	9	11	10

Table 3: Antimicrobial activity of the Aqueous extract of Mangifera indica L.

Microorganisms	Diameter of inhibition zone in mm						
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 *µg*		40 *µg*		50 *µg*	
		B	R	B	R	B	R
Staphylococcus aureus	25	11	11	12	11	15	13
Klebsiella pneumoniae	23	11	10	12	11	12	12
Escherichia coli	21	9	9	10	10	11	14
Aspergillus niger	23	9	10	10	11	13	12
Candida albicans	21	10	9	12	11	11	13
Epidermophyton floccosum	20	10	9	11	10	10	11

Table 4: Antimicrobial activity of the chloroform extract of Mangifera indica L

Microorganisms	Diameter of inhibition zone in mm						
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 *µg*		40 *µg*		50 *µg*	
		B	R	B	R	B	R
Staphylococcus aureus	25	-	-	9	9	11	11
Klebsiella pneumoniae	23	-	-	7	7	8	8
Escherichia coli	21	-	-	10	8	9	9
Aspergillus niger	23	-	-	-	-	8	-
Candida albicans	21	-	-	7	6	8	7
Epidermophyton floccosum	20	-	-	-	-	-	-

Table 5: Antimicrobial activity of the Ethanol extract of Mangifera indica L

Microorganisms	Diameter of inhibition zone in mm						
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	50 *µg*		40 *µg*		50 *µg*	
		B	R	B	R	B	R
Staphylococcus aureus	25	16	15	17	16	19	18
Klebsiella pneumoniae	23	12	11	13	12	15	14
Escherichia coli	21	13	11	15	13	17	15
Aspergillus niger	23	10	9	11	11	13	12
Candida albicans	21	11	10	13	11	15	13
Epidermophyton floccosum	20	10	9	11	10	13	11

Table 6: Antimicrobial activity of the Petroleum ether extract of Mangifera indica L

Microorganisms	Diameter of inhibition zone in mm						
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µg		40 µg		50 µg	
		B	R	B	R	B	R
Staphylococcus aureus	25	--	-	11	10	13	11
Klebsiella pneumoniae	23	-	-	7	-	9	7
Escherichia coli	21	-		9	8	11	10
Aspergillus niger	23	-	-	-		9	8
Candida albicans	21	-	-	7	8	9	9
Epidermophyton floccosum	20	-	-	-	-	-	-

Note: B: Banganapalli and R-Rumani.

The phytochemical analysis of the two variety of *Mangifera indica* (Banganapalli and Rumani) seed kernel extracts revealed the presence of important bioactive compounds i.e., alkaloid, carbohydrates, saponin, flavonoids, diterpenes, tannins, terpenoids, protein, steroid, glycosides, xanthoprotein, coumarin and quinine (Table 1). The ethanol and aqueous extracts of Banganappalli seed kernel showed higher phytoconstituents when compared to Rumani. The acetone, aqueous, chloroform, ethanol, and petroleum ether extracts of two variety of *Mangifera indica* (Banganapalli and Rumani) were tested for growth inhibiting activity against four bacterial microorganisms and three fungal microorganisms in three varying concentrations. The results show (Tables 2, 3, 4, 5 and 6) that Banganappalli variety of mango seed kernel possessed good antibacterial and antifungal activity when compared to Rumani.

Ethanol extracts from both Banganappalli and Rumani were more effective against *Staphylococcus aureus* and *Escherichia Coli.* The aqueous and ethanol extracts of mango seed kernel showed good antimicrobial activity against the tested strains. The acetone extract showed moderate activity against the tested microorganisms. Chloroform and petroleum ether extracts of mango seed did not show any activity against the *Epidermophyton floccosum*. Comparatively, the chloroform extract was found to be less active than other extracts.

There was clear indication that the solvent system plays a significant role in the solubility of the bioactive components and influences the antibacterial activity (El-Mahmood *et al*., 2008). Phytochemicals generally exert their antimicrobial activities through different mechanisms to that of synthetic drugs (Scalbert 1991). Mangiferin is the bioactive compound that has strong antioxidant, antidiabetic, and wound healing activities (Shah *et al*., 2010). Sowmiya *et al*. (2009) mentioned the antibacterial activity for mango seeds ethanolic extract, the extract showed good activity against pathogenic bacteria such as: *E.coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebisella pneumoniae* and *Streptococcus pyogenes*. Phenolic, tannins and flavonoids compounds in mango seed are found to be responsible for antimicrobial property. They act on microorganisms by inhibiting extracellular microbial growth and by avoiding oxidative phosphorylation. Also mango seed kernel oil used to kill abdominal worm and so given as a cure for vomiting, diarrhea and hyperacidity (Schiber *et al.*, 2003). Mango seeds are used in treatment of disorders of female reproductive organs namely vaginal leucorrhoea, vaginitis while mango bark juice is used to control menorrhagia (Sarmah and Hazarika 2012). Ethanol and aqueous extract of *Mangifera indica* was found to be more active than the other extracts.

4. Conclusion

From the present study, it could be concluded that the mango (*Mangifera indica L.*) seed kernel (Banganapalli and Rumani) is more potent antimicrobial agent than Rumani mango. It is recommended that there is need for further investigations in terms of toxicological studies and purification of active constituents with the view to using the mango seed in novel drug development.

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Author Profile

Arul Pamila graduated from Queen Mary's College (A) Chennai-600 004 Affiliated to University of Madras. Currently she is doing PhD. She secured First Rank in M.Sc. Botany from Queen Mary's College and scored 1st Class and Distinction in B.Ed. Biological Science from Apollo College of Education affiliated to Tamil Nadu Teachers Education University. She published six papers in International Journals and presented nine papers in various National and International Conferences from different colleges. She won Cash Prize for paper presentation in National Seminar from Meenakshi College for Women, Chennai 60 0024.