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Antimicrobial and Phytochemical Screening of *Mangifera indica* against Skin Ailments

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The most implicated organism involved in skin ailments is *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* and *Corynebacterium species*. Some of these isolates were found to be resistant to various antibiotics like tetracycline, erythromycin, penicillin, Clindamycin and ciprofloxacin, commonly prescribed for bacterial skin infections. Cold and hot solvent extracts of mango leaves and rind were tested against bacterial pathogens isolated from various types of skin ailments including acne, cellulitis, folliculitis, furuncles, carbuncles, boils, impetigo etc. The cold ethyl acetate + ethanolic extracts showed remarkable antimicrobial activity followed by cold ethyl acetate, cold ethanolic and cold methanolic extracts.

Key words: *Mangifera indica;* Plant extracts; Antibiotic Resistance; Antimicrobial agent; Phytoactive constituents; Formulations.

Skin infections are commonly seen in both primary care and dermatological practice. Yet as multi-drug resistant strains grow to be more common and cross-resistance to antibiotics becomes widespread, treatment of these infections can be increasingly challenging[1, 2, 3].

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Skin ailments are dealt with chemotherapeutic agents. Though effective, these drugs have their own side effects. Another problem being faced in treatment of these diseases is development of antibiotic resistance by the implicated organisms. Excessive use of antibiotics resulted in the emergence of bacterial resistance[4]. The more often a drug is used, the more likely bacteria are to develop resistance to it[5]. In the wake of these facts the mainstream medicine is becoming increasingly receptive to the use of antimicrobial and other drugs derived from plants. Plant extracts have been used for a wide variety of purposes for thousands of years[6]. The antimicrobial activity of plant oils and extracts has formed the basis of many medical applications, including pharmaceuticals, alternative medicine and natural therapies [7, 8].

Research indicates that mango has notable antibacterial activity. Mango (*Mangifera indica*, family- Anacardiaceae) is a large evergreen tree, with a heavy, dome-shaped crown. The mango is the most popular fruit in India. Over

500 named varieties have evolved and have been described in India [9]. Microbiological assays testing methanolic extracts of Mango leaves for antibacterial activity against *Salmonella typhi*, *Salmonella paratyphi*, and *Salmonella typhimurium* were carried out by Nkuo *et al.,*[10]. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were measured and it was found that *Mangifera indica* leaves inhibited bacterial growth at very low concentrations[10].The plant has been shown to possess anti-inflammatory properties[11,12]. Barks, leaves and seeds of *Mangifera indica* contain tanins[13-15] and this may also explain its antibacterial activity[16].

The Negritos of the Philippines utilise the gum resin of the tree mixed with coconut oil to apply directly to scabies and other parasitic diseases of the skin. The gum resin and the fruit are also used for healing sores caused by herpes and venereal disease like syphilis. The ashes of the leaves are a popular remedy for burns and scalds. The resin is also used for curing aphthae[17]. The Indians use mango leaves to relieve the pain of scorpion stings and the unripe fruit to help heal a wide variety of skin eruptions, ranging from leprosy and sores to boils and cysts[18]. The pulp of the ripe fruit is used as a poultice for tender breasts and sore nipples[19].

The present work has been undertaken with a view to study the antimicrobial efficacy of some plant products, which are considered as waste materials and are not very popular as a therapeutic agent. These common products included leaves and rind of mango. The antimicrobial property of these plant parts was studied using Cold and Hot Ethanolic extracts (CEE & HEE), Cold and Hot Methanolic extracts (CME& HME), Cold and Hot Ethyl acetate extracts (CEaE & HEaE) and Cold and Hot Ethyl acetate + Ethanolic extract (CEa+EE & HEa+EE). These plant extracts were tested for their efficiency to inhibit the causative agents of various skin diseases using Agar ditch and Agar dilution method.

MATERIAL AND METHODS

Collection and Identification of Plant Material

Fresh plant parts were collected randomly from the Botanical Garden in the college premises. The plants and the parts screened, together with their families and vernacular names, are given in Table 1. The taxonomic identities of these plants were confirmed by Dr.Aruna Rai, Department of Botany, Smt. C.H.M. College, and the voucher specimen numbers of the plants were preserved. The leaves were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles. The rind was peeled from the fruit, air dried and then ground to fine powder.

Preliminary Phytochemical Analysis

Qualitative phytochemical analysis of the crude powder of the plant parts was determined as follows: Tannins (200 mg plant material was mixed with 10 ml distilled water and filtered); 2 ml filtrate + 2 ml $FeCl_3$ gave blue-black precipitate indicating the presence of Tannins.

Alkaloids (200 mg plant material was dissolved in 10 ml methanol and filtered); a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayor's reagents/Wagner's reagent/ Dragendroff reagent, a creamish precipitate/ brownish-red precipitate/orange precipitate indicated the presence of alkaloids in the sample. Saponins (0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated presence of saponins.

Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + conc. H_2SO_4); green-blue color showed the presence of cardiac glycosides.

Steroids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO_4. Blue-green ring indicated the presence of terpenoids.

Flavonoids (200 mg plant material in 10 ml ethanol, filtered); 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red color showed the presence of flavonoids[20].

Thin Layer Chromatography

The presence of the phytochemical compounds detected in the Preliminary Phytochemical Analysis was confirmed using TLC[21]. Silica gel 60 F254 TLC aluminium sheets (5×7.5 cm) (Merck) were used to perform the analysis.

Preparation of the extracts

The cold and hot ethanolic, methanolic, ethyl acetate and ethyl acetate + ethanolic extracts[22, 23] of the plant parts (mango leaves and mango rind) were prepared and tested for their antimicrobial efficacy.

Microorganisms

The samples, for the present study were collected from patients suffering from different types of skin infections. Various hospitals and clinics were approached for the same. Human volunteers were also the sources for the samples.

The identification of the isolates was done on the basis of their Gram nature, cultural characteristics observed on the selective media, pigments, haemolysis and their biochemical properties. The reference used for the identification was Bergey's Manual of Determinative Bacteriology, 8th Edition.

Antibiotic sensitivity test

The antibiotic sensitivity of the organism was tested using the "Agar disc diffusion" method[24].

Antimicrobial screening of the plant extracts

This was carried out using Agar dilution method[25]. A series of the dilution ranging from 1.0mg/ml to 20mg/ml was prepared by adding different volumes of stock solution i.e. 50mg/ml to molten Nutrient agar (for bacterial isolates) and Sabouraud's agar (for fungal isolate)cooled to 45°C. These were then poured into sterile petriplates. Each plate was then spot inoculated with the test organisms. After incubation for 24 hours at 37º C, the plates were observed for growth and the minimum inhibitory concentration for different extracts was determined.

Formulations

Creams/ Ointments/ Gels are semisolid preparation, usually containing medicament, used for application to the skin. Two formulations were prepared using 1) water based gel (*Aloe vera*-Unprocessed)[26] and 2) Oil in water emulsion base (Hydrophilic ointment)[27], by the following procedure.

The required amount (2 gms) of the active extract was weighed and mixed with a weighed quantity (100 gms) of sterile base to give a homogenous product.

***In vitro* testing of the formulations**

Antimicrobial Activity[28, 29]

The basic principle underlying the test is to determine the antimicrobial activity of the formulations against the isolates by measuring the zone size of inhibition. The method used for this test is agar well diffusion method where a cup in the agar was punched using a 10 mm cork borer. The activity of the test compound is measured by correlating with the inhibition zone around it.

RESULTS AND DISCUSSION

23 samples were collected and each case yielded identifiable bacteria, out of which 10 were found to be dominated by single organisms. The causative agents of the remaining 12 were found to be associated along with Micrococcus. This high incidence of *Micrococcus* can be explained by the fact that *Micrococcus* forms a part of the normal flora of the skin.

The organism mainly associated with skin infections like impetigo, carbuncle, furuncle etc is *Staphylococcus aureus*[30,31]. *Pseudomonas* is found to be the main causative agent of acute cellulitis, Hot tub folliculitis etc[32, 33]. The present study confirms the presence of these respective organisms in different types of skin ailments.

The six most resistant isolates were selected for further studies, after studying the antibiotic profile of the organisms.

These isolates include *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Aeromonas* spp, *Corynebacterium* spp and *Candida albicans.*

The qualitative phytochemical profile of the plant extracts was carried out. The results of the same are presented in Table 2. The presence of the same was confirmed using Thin Layer Chromatography technique specific for the various groups of phytochemical components.

The MIC was performed using a range of 1.0mg/ml to 20mg/ml. From the results presented in Table 3 and 4 it can be concluded that the Cold Ethyl acetate+ Ethanolic extract of mango leaves is most efficient in inhibiting the growth of the micro organisms at a low concentration.

A medium control (without plant extract) was maintained where complete growth of organisms was observed.

Appropriate solvent controls were maintained to eliminate the possibility of the

Table 1.

Plant Species	Family	Common name	Parts used	Therapeutic use
Mangifera indica	Anacardiaceae	Aam, Amra	Leaves, roots, bark, fruit, seed kernel	Antiinflammatory, antiseptic, antioxidant, antitumour, antimicrobial

Table 2. Phytochemical analysis

Plant part	Tannins	Volatile oil	Flavonoids	Steroids	Saponins	Cardiac Gycosides	Alkaloids
Mango Leaf	+	+	+	+	+	+	+
Mango Rind	+	+	+	+	+	+	+

+; Presnent, - Absent

Table 3. Minimum inhibitory concentration (MIC) of the mango leaf extracts

Mango leaves extract (mg/ml)	Sa	Ps	Kp	Ca	Aero. spp	Cory. spp
CME	12.5	15	17.5	12.5	12.5	17.5
HME	15	15	17.5	15	12.5	17.5
CEE	10	12.5	15	12.5	10	10
HEE	12.5	15	17.5	12.5	12.5	12.5
CEaE	10	12.5	15	10	10	10
HEaE	12.5	12.5	15	12.5	12.5	12.5
CEa+EE	7.5	10	12.5	7.5	7.5	7.5
HEa+EE	7.5	12.5	12.5	10	7.5	7.5

*The values are the mean of three readings
CME: Cold Methanolic Extract,HME: Hot Methanolic Extract,CEE: Cold Ethanolic Extract,HEE: Hot Ethanolic Extract,CEaE: Cold Ethyl acetate extract,HEaE: Hot Ethyl aceteate extract,CEa+EE: Cold Ethyl acetate + Ethanolic Extract,HEa+EE: Hot Ethyl acetate + Ethanolic Extract

Table 4. Minimum inhibitory concentration (MIC) of the mango rind extracts

Mango leaves extract (mg/ml)	Sa	Ps	Kp	Ca	Aero. spp	Cory. spp
CME	15	15	17.5	12.5	12.5	17.5
HME	15	17.5	17.5	15	17.5	17.5
CEE	10	12.5	17.5	12.5	10	10
HEE	12.5	15	17.5	17.5	12.5	15
CEaE	10	12.5	15	10	10	10
HEaE	12.5	12.5	15	12.5	12.5	12.5
CEa+EE	7.5	12.5	12.5	7.5	7.5	7.5
HEa+EE	10	12.5	12.5	10	10	12.5

*The values are the mean of three readings
CME: Cold Methanolic Extract,HME: Hot Methanolic Extract,CEE: Cold Ethanolic Extract,HEE: Hot Ethanolic Extract,CEaE: Cold Ethyl acetate extract,HEaE: Hot Ethyl aceteate extract,CEa+EE: Cold Ethyl acetate + Ethanolic Extract,HEa+EE: Hot Ethyl acetate + Ethanolic Extract

Table 5. *In-vitro* analysis of Formulations

Organisms	BASES		Zone of Inhibition (mm)					
	AV	O/W	MR+AV	ML+AV	MR+O/W	ML+O/W	MR+ML+AV	MR+ML+O/W
Sa	-	-	18	20	17	18	22	20
Ps	-	-	16	19	15	17	21	19
Kp	-	-	14	16	13	15	19	18
Ca	-	-	18	20	16	18	22	21
Aero. spp	-	-	16	18	15	18	22	21
Cory. spp	-	-	17	18	15	16	21	20

AV-Aloe vera base, O/W-Oil in water emulsion base, MR-Mango rind, ML-Mango leaves -:- no inhibition

inhibition of the organisms by the solvent used. All the organisms showed complete growth even at 2% concentration of the solvent control (40% alcohol).

The formulations were tested for their in vitro efficacy using the agar cup method. On the basis of the zone size (Table 5) it was inferred that the formulation prepared using the combination of mango rind and mango leaves extract in aloe vera base showed maximum inhibition of all the organisms.

The bases used in the preparation of the formulation were tested for their antimicrobial activity and were found to be non-inhibitory.

CONCLUSION

In conclusion, *Mangifera indica* extracts (leaf and rind) possess a broad spectrum of activity against a panel of microorganisms responsible for the most common skin diseases. These promissory results open up new avenues and the possibility of finding new antimicrobial compounds in mango to prove its clinical efficacy.

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Abbreviations:
CME: Cold Methanolic Extract, HME: Hot Methanolic Extract, CEE: Cold Ethanolic Extract, HEE: Hot Ethanolic Extract, CEaE: Cold Ethyl acetate extract, HEaE: Hot Ethyl aceteate extract, CEa+EE: Cold Ethyl acetate + Ethanolic Extract, HEa+EE: Hot Ethyl acetate + Ethanolic Extract, MIC: Minimum Inhibitory Concentrations , MBC:Minimum Bactericidal Concentrations , AV:Aloe vera base , O/W:Oil in water emulsion base , MR:Mango rind, M:-Mango leaves