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Phytochemical Analysis and Assessment of Antimicrobial Activity of *Mangifera indica*

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Abstract

Nowadays, the use of medicinal plants and bioactive phytocompounds has seen a more growing interest. The present study investigated the qualitative screening of phytochemicals and evaluation of the antimicrobial activity of leaf extract of *Mangifera indica*. Aqueous, chloroform and butanol extracts of dried and ground plant materials were prepared using Soxhlet apparatus. Qualitative phytochemical screening of the extracts showed positive for carbohydrates, chloride, tannins, alkaloids, flavanoids, saponins phenolic compounds and steroids. Antimicrobial activities of the extracts were evaluated by agar well diffusion method. The extracts exhibited significant antimicrobial effects against all the tested bacterial and fungal pathogens. The aqueous extracts of leaf showed highest activity on the Gram positive bacteria *S.aureus* and fungus *A.fumigatus*. The analysis of bioactive compounds present in the plant extracts involving the applications of common phytochemical screening assay as well as FT-IR are discussed. The research findings concluded that the presence of phytochemical components and antimicrobial activity.

Keywords: Medicinal plants, phytochemicals, *Mangifera indica* and antimicrobial activity.

Introduction

In India, almost 95% of the prescriptions are plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha. The study of plants continues principally for the discovery

of novel secondary metabolites. The leaves have been reported to contain saponins, glycosides, unsaturated sterols, polyphenols, euxanthin acid, mangiferine and tannins etc. The leaf extracts are used as antiseptics in the treatment of various infections in humans and animals has been reported. Phytochemical screening is very important in identifying a new sources of therapeutically and industrially important compounds like alkaloids, flavonoids phenolic compounds, saponins, steroids, tannins, terpenoids etc. The medicinal plants are used as herbal remedies to prevent and cure several diseases which differs from community to community (Kubmarawa *et al*., 2007). Mango (*Mangifera indica L*.) is one amoung the most important tropical plants in the world (Severi *et al.*, 2009). It mostly found in the tropical and subtropical areas and its parts are mostly used in folk medicine for a wide variety of remedies (Garrido *et al*., 2004). The presence of phytoconstituents in the leaf extracts may be responsible for the antibacterial activity of the plant. It has been documented that different solvents have diverse solubility capacities for different phytoconstituents (Manjorie *et al.*, 1999). It has recently been reported that extract of Mangifera indica leaf inhibited lipid peroxidation (Badmus *et al*., 2011), exerted antifungal activity (Kanwal *et al*., 2010), and exhibited antiulcerogenic action (Severi et al.,2009). In our present study, we assessed the phytochemical components and anti-microbial effect of the leaf extract.

Materials and Methods

Plant sample

The *Magnifera indica* leaves were collected from Marthandam, Kanyakumari District, Tamil Nadu, India. The leaf of the plant used for this study was rinsed severally with clean tap water and shade dried in a dark place at room temperature for few days. The dried plant parts were cut into small pieces ground in electric chopper to get fine powder for further use.

Preparation of extracts

The powdered leaves were subjected to soxhlet extraction using aqueous, acetone, dimethyl sulfoxide, chloroform and ethanol. Each 5 g of powder of plant material was filled separately in the thimble and extracted successively with 60 ml of solvents using a soxhlet extractor for 3 h. After solvent evaporation, the solvent extract was weighed and stored at room temperature for further use.

Qualitative analysis of phytochemical constituents

All the extracts were subjected to systematic phytochemical screening for testing the presence of various phytochemical constituents by the method followed by standard protocols (Harborne, 1973). Phytochemical test includes carbohydrates, amino acids, proteins, vitamin C, chloride, tannin, alkaloids, flavonoids, phlobatannins, steroids, phenols and saponins.

Antimicrobial activity of plant extracts

Antimicrobial activities of the plant extracts were determined by well diffusion method (Anushia, 2010). Four bacterial pathogens such as *E. coli, K. Pneumonia, Bacillus subtilis,* and *Staphyloccocus aureus* and three fungal pathogens such as *Aspergilus niger, Aspergillus fumigatus* and *Penicillium* sp. were used for this investigation. The bacterial strains were inoculated into Nutrient broth and incubated at 37°C for 24 h. After the incubation period, the culture were compared with the standard. Fungal inoculums were prepared by suspending the spores of fungus (as previously cultured) in saline water mixed thoroughly, made turbidity standard and used.

Fresh bacterial culture of 0.1 ml was spread on Muller Hinton agar plates using sterile cotton swabs. The fungal strains were spread on Potato dextrose agar. Wells of 6 mm diameter were punched off with sterile cork borer and loaded with 50 µl of plant extracts using micro pipette under aseptic condition. The plates were kept in refrigerator to allow diffusion of extract for 30 min and then incubated at 37°C for 24 hr. The antimicrobial activity was evaluated by measuring the zone of inhibition.

Thin Layer Chromatography

The slurry of silica gel G prepared with glass distilled water in the ratio 1:2 (w/v) was poured on glass slide with a layer of silica gel in 500 µm thickness. The coated plates were activated at 80^{o}C for 3 hours. In this study, n-Butanol, Acetic acid and methanol (2:2:1) was used as solvent. The concentrated plant extract of 0.5 mg was loaded on the TLC plates just above 2 cm from the bottom using a capillary tube. The plates were reserved in a developing jar containing the solvent mixture. The plates were removed and allowed at room temperature for 30 minutes and results were observed by UV radiation.

Analysis of plant extracts by Infrared Spectrophotometer (FT-IR)

ATR model FT-IR Spectrophotometer (Bruker Co., Germany) was used for the analysis of the of leaves extract of *Magnifera indica.* The spectrum (400-4000nm) was recorded using Attenuated Total Reflectance (ATR) technique beach measurement.

Results

Qualitative analysis phytochemical constituents

The leaves extract of *Magnifera indica* has been analyzed for their phytochemical constituents. In the leaf material, aqueous extract showed positive results for amino acid, chloride, tannins, alkaloids, flavonoids, Phlobatannins, steroids, phenolic compounds and saponins; Butanol extract showed positive results only for chloride, tannins and phenolic compounds; chloroform extract showed positive results for carbohydrate, tannins, phenolic compounds and saponins (Table 1).

Table 1: Qualitative analysis of phytochemical constituents

Sl. No.	Chemical constituents	Leaf extracts		
		Aqueous	Chloroform	Butanol
1	Carbohydrates	+	+	-
2	Protein	-	-	-
3	Amino acid	+	-	-
4	Vitamin C	+	-	-
5	Chloride	-	-	+
6	Tannins	+	+	+
7	Alkaloids	+	-	-
8	Flavonoids	+		-
9	Phlobatannins	+	-	-
10	Steroids	+	-	-
11	Phenolic compounds	+	+	+
12	Saponins	+	+	-

'+' presence of compound; '-'absence of compound

Antimicrobial activity of leaf extracts

Antimicrobial activity of the leaf extracts were determined by well diffusion method against various bacterial and fungal pathogens. The leaf of butanol extract showed inhibition activity only on *E.coli* (11mm), *K. pneumonia* (10 mm), *S. aureus* (16 mm), *B.cereus* (11 mm), and *A. fumigatus* (10 mm), *A.niger* (18 mm) and *Penicillium sp* (14 mm); chloroform extract showed activity on *K. pneumonia* (15 mm), *S. aureus* (11 mm), *B.cereus* (10 mm), *A. fumigatus* (14 mm), *A.niger* (10 mm) and *Penicillium sp* (11 mm); and aqueous extract showed activity on *K. pneumonia* (11 mm), *S. aureus* (10 mm), *B.cereus* (15 mm) and *A.niger* (22 mm) (Table 2).

Table 2: Antimicrobial activity of *Magnifera indica* leaf extracts

Sl. No.	Test organisms	Leaf		
		Aqueous	**Chloroform**	**Butanol**
1.	*E.coli*	-	-	11mm
2.	*K. pneumoniae*	11mm	15mm	10mm
3.	*S. aureus*	10mm	11mm	16mm
4.	*B. cereus*	15mm	10mm	11mm
5.	*A. fumigatus*	-	14mm	10mm
6.	*A. niger*	22mm	10mm	18mm
7.	*P. chrysogenum*	-	11mm	14mm

Zone of inhibition in 'mm'

Thin Layer Chromatography

Thin layer chromatography was performed for leaf material. In this study, two spots were observed in each leaf and stem sample, the Rf value was calculated using the following formula: Rf = Distance travelled by solute / Distance travelled by solvent. The leaf sample showed green and pale green with the Rf value of 0.93 and 0.72 respectively. (Table 3).

Table. 3: TLC analysis results of plant material

Sample name	Color of band	Rf value
Caladium leaf	1. Green	0.93
	2. Pale green	0.72

Identification and characterization

FT-IR spectroscopy is one of the reliable and sensitive methods for detection of biomolecular fractions of various plant extract (Joshi *et al*., 2012). In this study, different functional groups such as alcohols, phenols, alkanes, aldehydes, primary amines, and alkynes were identified using FT-IR (Figure1).

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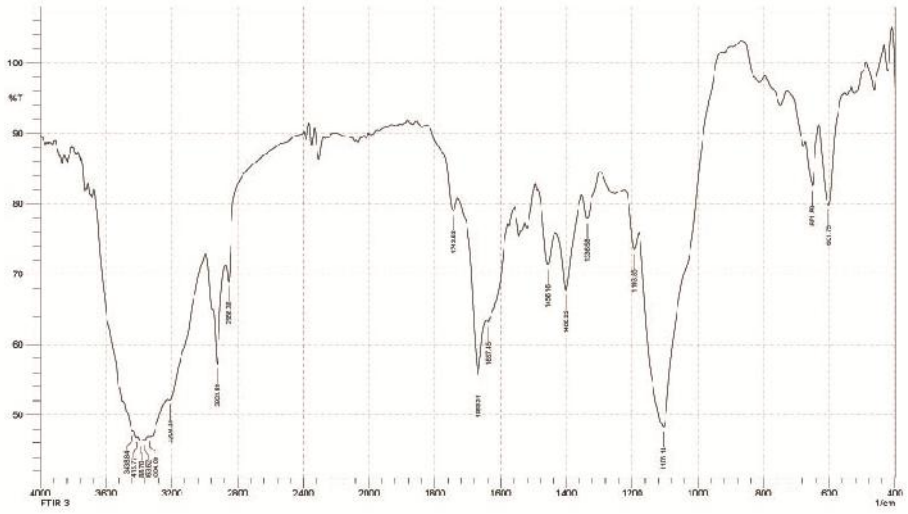

Figure 1: Fourier Transform Infrared analysis of *Magnifera indica* leaves extract

Discussion

The present study confirmed the presence of carbohydrates, amino acids, chloride, tannins, alkaloids, flavonoids, phenolic compounds and steroids. In the antimicrobial activity test, the leaf extract exhibited inhibition activity against all the tested organisms includes *E. coli, K. pneumoniae, S. aureus, B. cereus, A. fumigatus, A. niger* and *P. chrysogenum*. The zone of inhibition was varied with different solvents used. Further, a total of six phytochemical components were identified from the leaf extract by FTIR analysis. The *Magnifera indica* extract showing phytochemical constituents also have pharmacological properties such as antioxidant and antimicrobial properties that can be attributed by the phytochemicals such as flavones, iso flavones, flavonoids, anthocyanin, coumarin, lignans, catechins, and isocatechins (Avelesco *et al*., 2014). The results are in line with the previous findings reported by (Shah *et al*.,2010). In

this study, different extracts of *Magnifera indica* leaves have potent antimicrobial activity against Gram positive and Gram negative bacteria, and fungal pathogens were equally affected by the leaf extract of *Magnifera indica* indicating the presence of broad spectrum of antibacterial substance in the plant.

Conclusion

The phytochemicals of *Mangifera indica* was demonstrated by simple phytochemical screening and by FT-IR spectroscopy. In this study *Mangifera indica* was chosen because they are easily available, economical and have high medicinal values. The study revealed that the leaf extract of *Mangifera indica* was more effective against the pathogens. There is a possibility for extracting bioactive substances from the effective medicinal plants. This deserves mole research.

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