PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL STUDIES OF AQUEOUS AND ALCOHOLIC EXTRACTS OF *MANGIFERA INDICA (ANACARDIACEACE)* STEM BARK

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Summary

The flora of Indian medicinal plants is potent source of bioactive principal. Extracts of stem barks of *Mangifera indica* were subjected to preliminary phytochemical screening for the presence of plant secondary metabolites and *invitro* antibacterial and antifungal studies. The result of the preliminary investigation revealed the presence of alkaloids, Glycosides, Tannins, flavonoids and Triterpenes. The alcoholic and aqueous extract of *Mangifera indica* barks were investigated for *invitro* antimicrobial activity using the agar disc diffusion technique. Eight strains of human pathogenic microorganisms comprising 3 gram positive *i.e., Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus*, 3 gram negative bacteria *i.e., Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and 2 fungi *i.e., Aspergillus niger* and *Candida albicans* were utilized for the studies, the antibacterial activies were compared with standard drug Ciprofloxacin at the concentration of 50 μg/disc. Similarly the antifungal activities were compared with standard drug Ketoconazole at the concentration of 50 μg/disc. Both the extracts displayed overwhelming concentration dependent antimicrobial properties. The aqueous extract showed good activity against gram (+) ve bacteria compared to gram (-) ve bacteria. Similarly, the alcoholic extract showed good activity against gram (-) ve bacteria than gram (+) ve bacteria. In the anti fungal assay, the growth of *Aspergillus niger* and *Candida albicans* used, were inhibited in the same manner comparable to ketoconazole the reference drug included in the study. So, at conclusion both the extracts of stem bark of *Mangifera indica* seems to justify their ethnomedical uses.

KEY-WORDS: *Mangifera indica;* Antibacterial activity; Antifungal Activity; agar disc diffusion.

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Introduction

The different systems of medicine practiced in India like Ayurveda, Siddha, Unani, Amchi and local health traditions, utilize a large number of plants for the treatment of human diseases. But the efficacy of many of the plants yet to be verified (1, 2).

An estimate suggests that about 13,000 plant species world wide are known to have used as drugs. The trend of using natural products has increased and the active plant extracts are frequently screened for new drugs discoveries and for the presence of antimicrobials (3). Moreover India is a country with a vast reserve of natural resources and rich history of traditional medicine (4).

Presently, the medical fraternity and the patients have increasingly started using plants to overcome various illness and sufferings mainly to obviate the profound side effects encountered in usage of modern drugs (5, 6). The Present article deals with the screening of *Mangifera indica* for their antimicrobial activity against various micro organisms. The plant *Mangifera indica* belongs to family *Anacardiaceace*. It has been used in the indigenous system of Medicine for the treatment of various ailments. Several therapeutic uses as Anti Parasitic, Antiseptic, Anti asthmatic, Expectorant, cardiotonic and parasiticide have been described in literatures.

Phytochemical review showed the presence of ascorbic acid, beta – carotene, carotenoids, tannic acid, geraniol, limonene, mangiferine, mangiferolic acid, myristic acid, quercetin and xanthophylls etc.

Recently, pharmacological function of mangiferin, an active phytochemical and natural polyphenotic antioxidant and glusosyl xanthone derivative present in different parts such as in bark and leaves of *Mangifera indica* Linn (7) in altering the oxidative mechanisms have received much attention (8), Mangiferin has cardiotonic (9, 10), diuretic properties and strong antioxidant activity in the biological peroxidation system.

Materials and Methods

Collection of plant materials

The stem bark of *Mangifera indica* was collected from mature trees and its botanical identification was confirmed by Prof.P.Jayaraman, Ph.D., Director, Plant Anatomy Research Centre (PARC), Chennai. A voucher specimen was deposited in the herbarium of PARC, Chennai.

Plant extraction

The collected plant material was cut into small pieces and shade dried for several days until a constant weight was obtained and powdered with the help of an electric grinder. A wide range of solvents of increasing polarity were used for extraction. Among the different solvent extracts, the aqueous and alcoholic extracts possessing higher concentration of active compounds were selected and prepared for bulk extraction by process of continuous extraction (soxhlation).

Total Alcoholic extract

500g of dried and coarse powdered bark of *M. indica* was extracted with 95% ethanol for a period of 24hrs. The filtrate was taken and concentrated on a water bath using petridish. The temperature was maintained at 55˚C. The concentrate obtained was weighted 9.0% w/w (11).

Total Aqueous extract

500g of dried and coarse powdered bark of *M. indica* was extracted with double distilled water for 24 hrs. The filtrate was taken and concentrated on a water bath using petridish. The temperature was maintained at 60˚C. The concentrate obtained was weighted 16.0% w/w (11). Both dried extracts was stored in a desiccator and used for the further study.

Preliminary Phytochemical Screening

Standard methods (12, 13) were used for preliminary phytochemical screeing of alcoholic and aqueous extracts to know the nature of phyto constituents present in it.

Screening of Antimicrobial Activity

Test Organisms used

Bacterial Test organisms used were *Escherichia coli, Klebsiella Pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtills* and *staphylococcus aureus*. Fungal test organisms used were *Candida albicans* and *Aspergillus niger*. The bacterial culture were maintained in nutrient broth (Himedia), fungal cultures were maintained in Sabouraud dextrose agar (Himedia) at 4˚ C.

Antimicrobial Assay

The Crude Plant extract were tested for their antimicrobial activity using the diffusion technique on solid media (14, 15). Sterile discs of 6mm in diameter (made from whatmann filter paper previously sterilized in UV lamp) were impregnated with 100µg of alcoholic and aqueous extracts separately and placed on nutrient agar (in case of bacteria) or sabouraud dextrose agar (in case of fungi). Seeded with the micro organisms (10^6 CFU /m1).

The plates were incubated for 24hrs at 37˚C for bacteria and 48hrs at 24˚C for fungi. Control discs were soaked with the same extract solvents and treated as sample discs. The experiments were carried out as duplicate three times and corrected for the control discs. Additionally, Ciprofloxacin 50µg/disc and Ketoconazole 50 µg/disc were tested as positive standards. The diameters of the inhibition zones were measured.

Results and Discussion

The alcoholic and aqueous extracts of *M. indica* were prepared and its yield in percentage was calculated. The highest yield was found to be in aqueous extracts.

The results of the Preliminary phytochemical screening of alcoholic and aqueous extracts were given in (Table 1). It reveals the presence of alkaloids, Glycosides, Tanning, Flavonoids and Triterpenes. Compared to alcoholic extract, Aqueous extract found to contain these phyto constituents in high concentration.

Table 1: Preliminary Phytochemical screening of aqueous and alcoholic extracts of stem bark of *Mangifera indica*.

Phyto Constituents	Extracts	
	Alcoholic	Aqueous
Alkaloids	++	-
Carbohydrates & Glycosides	-	+++
Phytosterol	-	-
Fixed Oil &Fats.	-	-
Saponins	-	-
Phenolic Compounds & Tannins	-	++
Proteins & Amino acids.	-	+
Gums& Mucilage	-	+
Flavonoids	+++	++
Lignins	-	-
Triterpenes.	+	++

(-): Absent, (+): Slightly Present, (+) (+): Fairly Present, (+) (+) (+): Abundant

Alcoholic & aqueous extracts were screened for antibacterial and antifungal activities. The results of antimicrobial activity of aqueous and alcoholic extract of *M. indica* were given in (Table 2). These data revealed that the aqueous extract exhibits potent antibacterial activity against gram (+) ve than gram (-) ve bacteria. However, it exhibits only a moderate activity against gram (-) ve bacteria and fungal organism.

On the other hand, the alcoholic extract showed good activity against gram (-) ve bacteria, *Candida albicans* and *Aspergillus niger* which was comparable with standard drug.

Table 2: Antimicrobial activity of Aqueous and Alcoholic extracts.

		Zone of Inhibition in (mm)					
		Micro organisms					
Extract	**Concentration** µg / disc	*S. aureus*	*B. subtills*	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
Alcoholic	100 µg / disc	25.67±0.58	23.67±0.29	23.5±0.50	25.67±0.29	22.33±0.76	25.5±0.50
Aqueous	100 µg / disc	27.83±0.29	23.67±0.29	25.83±0.28	23.00±0.00	20.67±0.58	24.5±0.50
Ciprofloxacin (Control)	50 µg / disc	31.83±0.29	32.83±0.29	33.5±0.50	35.0±0.50	32.5±0.50	33.5±0.50

		Aspergillus niger	***Candida albicans***
Alcoholic	100 µg / disc	25.67±0.58	23.33±0.577
Aqueous	100 µg / disc	18.5±0.50	17.33±0.58
Ketoconazole (control)	50 µg / disc	33.83±0.29	30.67±0.29

Conclusion

Generally all plant extracts showed good and moderate antimicrobial activity. On the other hand aqueous extract of *M. indica* had impressive antibacterial and antifungal properties and could lead to the discovery of new antibiotics. Thus becomes more relevant as the current antibiotics in use are fast losing effectiveness due to emergence of resistant microorganisms. The antimicrobial activity may be due the presence of phytoconstituents like triterpenes, flavonoids and tannins. The literature showed that the plant contains triterpenoids which are well known antifungal agents. Thus, the antifungal activity of the extracts may be due to the presence of triterpenes in the extract.

The results of this study support the use of these plants for human and animal disease therapy and reinforce the importance of the ethnobotanical approach as potential sources of bio active substances. Future work will be emphasizing upon isolating and identifying the potential principle (s) responsible for antimicrobial activities.

References

1. Tyler V. Herbal medicine, from the past to future. J Pub Health Nutrition 2000;3:447-452.
2. Eisenberg D, Davis R, Ettner S., Appel S, Wilkey S, Van Rompay A, Kessleer R. Trends in alternative medicine use in the united states. J Am med Assoc 1998;280:1569 – 1575.
3. Robbers JE, Tyler VE. Tyler's Herbs of Choice: the therapeutic use of phyto medicinals. Vol I. New Delhi: CBS Publishers and Distributors; 2002. p. 23-29.
4. Grover JK, Vats V. Shifting paradigm from conventional to alternate medicine. An introduction on Traditional Indian Medicine. Asia Pacific Biotech News 2001;5:28-32.
5. BhavaPriya V, Kalpana S, Govindasamy S, Apparanantham T. Biochemical studies on hypoglycaemic effect of Aavirai Kudineer – an herbal formulation in alloxan diabetic rats. Ind J Exp Biol 2001;39(9):925 - 28.
6. Dharmananda S. Congestive heart failure and other cardio vascular disorders of aging. Treatment of cardio vascular diseases with Chinese herbs. PhD thesis, Insititue of Tradional medicine, Oregon, Portland, (2002).
7. Aritomi M, Kawasaki T. A new Xanthone C-glucoside, Position isomer of mangiferin from Anemarrhena asphodeloids Bunge. Tetra Lett 1969;12(6):941-44.
8. Sanchez GM, Re L, Giuliani A, Nunez – Selles AJ, Davison GP, Ferandez OSL. Protective effect of Mangifera indica L. extract, Mangiferin and selected antioxidants against TPA induced biomolecules oxidation and peritoneal macrophage activation in mice. Pharmacol Res 2000;42(6):565-573.
9. Ansari MA, Rajadurai S, Nayudamna Y. Studies on the polyphenols of *Mangifera indica* bark. Leath Sci 1967;14(1):247-51.
10. Srinivasan KK, Subramanian SS. Isolation of Mangiferin from *Urena Labata.* Arog J Health Sci 1981;7(1):140-41.
11. Natrajan B, Dhananjayan R. Pharmacological effects of *Trigonella foenum graecum* Linn. Seeds on various isolated perfused smooth muscle preparations. Phcog Mag 2007;3(10):77-82.
12. Trease GE, Evans WC. Pharmacognosy. 13th Ed. New Delhi: ELBS Publication; 1989. p. 171.
13. Harborne JB. Phytochemical methods; A guide to modern techniques of plant analysis. 2nd ed. New York: Chapman and Hall; 1984. p. 85.
14. Janssen AM, Scheffer JC, Suendsen AB. Antimicrobial activity of essential oils: A.1976 – 1986 literature Review. Aspects of the test methods. Planta Med 1987;53:395 – 398.
15. Kirby, Bauer, Sherries, Turck. Am J Clin Path 1996;45:498.