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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF MURRAYA KOENIGII

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ABSTRACT

The worldwide increase of multidrug resistance in both community and health-care associated bacterial infections has impaired the current antimicrobial therapy, warranting the search for other alternatives. We aimed to find the *in vitro* antibacterial activity of ethanolic extracts of *Murraya koenigii* traditionally used medicinal plants and the leaves have tremendous medicinal values**.** The present investigation deals with qualitative screening of secondary metabolite and antimicrobial activity of *M. koenigii* leaves extract belongs to family of Rutaceae. Plant metabolites screenings were performed by using various solvents systems of varying polarity of acetone, ethanol and aqueous extracts. In this examination the crude extracts showed the presence of flavonoids, carbohydrates, saponins, phlobatannins, and volatile oil while Phenol, steroids and terpenoids were absent in all the solvents. Alkaloids present in acetone and ethanolic extracts. On the other hand tannins were absent only in acetone extracted. The ethanolic leaf extract was tested against Gram positive and Gram negative bacterial pathogens. Plant extract showed antibacterial activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis* while no inhibitory activity against *Klepseilla pneumoniae.* The phytochemical property of *M. koenigii* may be attributed to the presence of flavonoids and phenolic compounds with rich antioxidant potential. Thus, curry leaves could be effective for prevention of bacterial infections and may be considered as an alternative to antibiotic regimens.

KEYWORDS: Antibacterial activity, *Murraya koenigii*, phytochemical analysis.

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Now a day, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. In fact plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Boominathan and Ramamurthy, 2009).

The use of crude extracts of plants parts and phytochemicals of known antimicrobial properties can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning. The screening of plant products for antimicrobial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants. Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4,00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities (Selvamohan et al., 2012).

Curry leaves (*Murraya koenigii*) are a popular leaf-spice used in very small quantities for their distinct aroma due to the presence of volatile oil and their ability to improve digestion. These leaves are widely used in Asian cuisines for flavoring foods. The leaves have a slightly pungent, bitter and feebly acidic taste, and they retain their flavor and other qualities even after drying. Curry leaf is also used in many traditional cultures namely Indian Ayurvedic and Unani prescriptions (Suman Singh et al, 2014). The leaves of *Murraya koenigii* contain proteins, carbohydrate, fiber, minerals, carotene, nicotinic acid, Vitamin C, Vitamin A, calcium and oxalic acid. It also contains crystalline glycosides, carbazole alkaloids, koenigin, girinimbin, iso-mahanimbin, koenine, koenidine and koenimbine. Triterpenoid alkaloids cyclomahanimbine, tetrahydromahanimbine are also present in the leaves. Murrayastine, murrayaline, pyrayafoline carbazole alkaloids and many other chemicals have been isolated from *Murraya koenigii* leaves (Bhandari, 2012).

A broad range of phytochemicals present in plants are known to inhibit bacterial pathogens (Medina et al., 2005). The determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds. To maximize up take the recovery of plant antimicrobials for human consumption, establishing optimal and specific extraction condition using various solvent system. Hence, the objective of this study was to determine qualitative investigation was carried out to evaluate the presence of phytochemicals. Furthermore, the ethanolic leaf extract as a good source for the determination of the antimicrobial activity against various human pathogens.

MATERIALS AND METHODS

Collection and Identification

Murraya koenigii was collected from Thanjavur District. The plant was authenticated by Director, Plant Anatomy & Research Center, Chennai and the voucher specimen is deposited in our laboratory.

Preparation of ethanolic extract of *Murraya koenigii*

The whole plant was shade dried and pulverized. 100gm of the powder was soaked in 150ml of ethanol (w/v) for 3-5 days with intermediate shaking. This was filtered through a fine cheese cloth and the filtrate was pooled after 3 days of repeated extractions. The filtrate

obtained was evaporated to dryness using rotary evaporator. The concentrate was lyophilized and used for the study.

Phytochemical Analysis

The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered (50 gm) were extracted in a Soxhlet apparatus with ethanol successively. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described (Sofowora, 1993; Trease and Evans, 1983; Harborne, 1973). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Microorganisms

Bacterial strains were obtained from Department of Microbiology, Pathology section, Thanjavur Tamil Nadu and were used for assay of antibacterial activity; Microorganisms were maintained at 4°C on nutrient agar slants. The studied bacterial strains comprised: *Bacillus subtilis, Staphylococcus aureus, enterococcus faecalis, Escherichia coli, Klebsiella pneumonia.*

Antibacterial Assay

The antibacterial assay was performed by agar well diffusion method (Perez et al., 1990) for solvent extract. The Muller Hinton Agar media was inoculated with the 100 µl of the inoculum ($1x10^8$ Cfu) and poured in to petriplates. In this method a well was prepared in the plate using a cork-borer (0.85) 50,100µg of test sample was introduced in to the well. The plates were incubated overnight at 37^0C and microbial growth was determined by measuring the diameter of zone of inhibition. The controls were maintained where pure solvent was used instead of the extract for each strain.

RESULTS AND DISCUSSION

The result of phytochemical screening of the alcoholic extracts of *Murraya koenigii* revealed that the presence of alkaloids, flavanoids, phytosterols, tannins and phenols (Table 1). The plant extract of *M. koenigii* used for the present work was choosing on the basis of their medicinal values. Previous study in the naturally the ethanolic extracts of *Avicennia* spp.

were subjected for phytochemical analysis. Phytochemical screening of the crude extract revealed that the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids and steriods, but reducing sugars, carbonyl (aldehyde) and Phlobatanin show negative results (Makinde et al., 2007).

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by Sofowora (1993). In the present study, it was clearly understood that the alcohol extracted maximum amount of the different type of metabolites present in the *M. koenigii*. Boominathan and Ramamurthy (2009) reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

The presence of some of these secondary metabolites suggests that the plant might be of medicinal significance and supports the origin for some of the ethno-uses. For instance, the presence of flavonoids suggest that the plant have been reported to exert multiple biological effects including, anti-allergic, anti-inflammatory, anti- microbial antioxidant, anti- cancer activity (Kunle and Egharevba, 2009). It also suggests that the plant might have diuretic properties (Jayyir et al., 2002). The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing and burns (Haslem, 1989). Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions (Oloyode, 2005). Some researchers have also investigated that some saponins have anti-cancer and immune modulatory properties (Evans, 2002). Volatile oils are used in the industries for various purposes, both as a pharmaceutical/cosmetic raw material for production of emollients and active ingredient for the respiratory tract infections. They are also used as flavouring agents, in aromatherapy, perfumery etc. egs are eucalyptus oil, lemon oil and peppermint.

In the present study plant extract of *M. koenigii* showed higher antibacterial activity against *Escherichia coli* than *Bacillus subtilis, Staphylococcus aureus Enterococcus faecalis* while no inhibitory activity against *Klebsiella pneumonia*. This study also shows the presence of different phytochemicals with biological activity that can be valuable therapeutic index. From

the result, it is concluded that *M. koenigii* have great potential use as phytomedicine and have pharmacological activities. Development of phytomedicine is inexpensive and less time consuming and suitable to our economic conditions. In overall conclusion the medicinal plants have the great therapeutic and economic values in all over the world. The present results offer a scientific basis for traditional use of *M. koenigii* against various ailments.

Curry leaf oil extracts have demonstrated (Rajendran et al., 2014) the strongest inhibition zone against *Proteus mirabilis, Staphylococcus aureus, Corynebacterium pseudotuberculosis, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterobacter aerogenes* and a moderate level zone of inhibition was observed with *Salmonella enterica* (11mm) and *Streptococcus pyrogens* (10 mm) respectively. Also, in this study *Murraya koenigii* displayed antibacterial activity against the *M. koenigii* showed higher antibacterial activity against *E. coli* than *B. subtilis, S. aureus E. faecalis* while no inhibitory activity against *K. pneumonia*. Another study by Das and Biswas (2012) indicated that ethyl acetate and dichloromethane soluble partition of methanolic leaf extract of *M. koenigii* exhibited mild activity against *E. coli* forming zone of inhibition of 9 mm to 11 mm. Mathur et al. (2010) have demonstrated that the methanolic extract of curry leaves inhibited *S. aureus, S. epidermidis, S. uberis, P. aeruginosa, E. coli, C. gravis* and *B. cereus*. In comparison the present study revealed that the ethanolic extracts of *Murraya koenigii* screened for their antibacterial activity against bacterial organism. The antibacterial effect on the later bacterial species could have been limited by contamination in the laboratory procedures.

Murraya koenigii (Curry leaves) extracts have demonstrated antibacterial effects particularly on bacteria as compared to commercial antibiotics such as Gentamycin and Amikacin in our study. Therefore, curry leaves could be effectively used as a natural remedy in everyday meal, for the prevention of bacterial infections. Indeed this phenomenal plant may serve as a useful resource in the food industry and clinical medicine. This research could be further extended to test the bioactive properties of curry leaves for therapeutic use.

Table 1: Qualitative Phytochemical screening on extracts of *Avicennia germinans.*

S. No	Name of Test	Test applied / Reagent used	Leaves extract
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D]) Dragndorff's test	+ + + +
2	Flavanoids	HCl and magnesium turnings	+
3	Carbohydrate	Molisch's test	+
4	Tannins & Phenols	A] 10% Lead acetate B] $Fecl_3$	+ +
5	Test for Steroids	A] Salkowski's Test B] Libermann-Burchard's Test	+ +
6	Gums & Mucilages	Alcoholic Precipitation	-
7	Fixed oil & Fats	Spot test	+
8	Saponins	Foam test	+
9	Phytosterols	LB test	+
10	Volatile oils	Hydro distillation method	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C] Xanthoprotein test	+ + +

-, absents; +, present;

Table: 2 Antibacterial activity of leaf extract of *Avicennia germinans.*

Microorganisms	100 µg/mL	200 µg/mL
	Diameter of inhibition zone(mm)	
Gram positive bacteria		
Bacillus subtilis	5	9
Staphylococcus aureus	7	13
Enterococcus faecalis	8	14
Gram negative bacteria		
E.coli	12	20
Klebsiella pneumoniae	10	12

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