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SCREENING FOR ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF VARIOUS LEAF EXTRACT OF *MURRAYA KOENIGII*

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ABSTRACT
To investigate the antimicrobial activity and phytochemical screening Ethanol, methanol, Ethyl acetate, aceton,chloroform, Petroleum ether, hexane, hot water, and extracts of *Murraya koenigii.* The aim of the present study was to evaluate the qualitative analysis of phytochemicals and antimicrobial activity of various solvent extracts of *Murraya koenigii*. The antimicrobial activity of different solvent extracts of *Murraya koenigii* were tested against the Gram-positive and Gram-negative bacterial strains and fungus by observing the zone of inhibition. The Gram-positive bacteria used in the test were *Staphylococcus aureus, Bacillus cereus and Micrococcus luteus*, and the Gram-negative bacteria were *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, fungus like *Aspergillus niger, Candida albicans, Candida tropicalis, Cryptococcus neoformans* and *Candida kefyr*. It was observed that ethanol, methanol, ethyl acetate, aceton, chloroform, petroleum ether, hexane and aquas extracts showed activity against bacteria and fungus. The Hot water extract of *Murraya koenigii* showed more activity against *Staphylococcus aureus*, zone of diameter 28.17±0.29mm and Ethanol extract of *Murraya koenigii* showed more activity against *Aspergillus niger* and *Candida tropicalis*, zone of diameter 12.17±0.15mm compared to other solvent extracts. In this study Hot water Extract in bacteria and Ethanol Extract in fungus showed a varying degree of inhibition to the growth of tested organism, than methanol, Ethyl acetate, aceton, chloroform, Petroleum ether, hexane, and acetone extracts, The results confirmed the presence of antibacterial activity of *Murraya koenigii* extract against various human pathogenic bacteria. Presences of phytochemical and antimicrobial activity are confirmed.
Keywords: phytochemicals, crude extract, Antimicrobial activity, *Murraya koenigii.*

INTRODUCTION

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to an alarming increase in the incidence of new and reemerging infectious diseases and development of resistance to the antibiotics in current clinical use[1]. The screening of plant extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases[2]. Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments[3-5].

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds[6]. Many plant leaves have antimicrobial principles such as tannins, essential oils and other aromatic compounds[7,8]. In addition, many biological activities and antibacterial effects have been reported for plant tannins and flavanoids[9-11]. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives[12]. These compounds protect the plant from microbial infection and deterioration[13]. Some of these phytochemicals can significantly reduce the risk of cancer due to polyphenol antioxidant and anti-inflammatory effects. Some preclinical studies suggest that phytochemicals can prevent colorectal cancer and other cancers[14-16].

The Indian plant *Murraya koenigii* belong to family Rutaceae, commonly called curry leaf in English and locally known as Karivepu[17]. The species is native of India and found everywhere in India[18]. It commonly occurs in foothills of Himalaya, Assam, Skim, Kereala, Tamil Naidu, Andra Pradesh, Maharastra[18]. The leaves are pinnate, with 11-21cm broad, and flower are small white and fragrant[23]. On phytochemical investigation researcher claimed that leave of *Murraya koenigii* found to contain alkaloids, volatile oil, Gycozoline, Xanthotoxine and sesquiterpione[19]. The leaf has been found to show antioxidant activity[20], hypoglycemic activity[21], antibacterial activity[17], anti- dysentery[22] and also act as a hepatoprotective[23].

MATERIALS AND METHODS

Plant materials

The *Murraya koenigii* leaf collected during June-July of 2010 in and around Arakkonam, Tamilnadu were authenticated by Department of Botany. The voucher specimens were kept in the Department of Botany in C. Abdul Hakeem College, Melvisharam, Vellore, Tamilnadu, India.

Extraction procedure

All the laboratory works are done in Microlabs, Institute of Research and Technology, Arcot, Tamil Nadu, India. The plants washed with fresh water and dried under shade at room temperature, cut into small pieces and powdered in a mixer grinder. The roots were powdered and stored in sterile containers for further use. Then this powdered samples (100g/100ml) in hot water, ethanol, methanol, chloroform, Ethyl acetate, Petroleum ether, hexane and acetone extracts for Overnight at room temperature. Soxhelt apparatus are used for this extraction[24,25]. The extract from three consecutive soaking are pooled and evaporated under pressure.

The crude samples were subjected to phytochemical screening for the presence of amino acids, proteins, saponins, triterpenoids, flavonoids, carbohydrates, alkaloids, phytosterols, glycosidal sugars, protein, tannins, and phenols.

Phytochemical test

The extracted samples were stirred with dil HCl and filtered. This filtrated is tested carefully and used for compound analysis. In this Alkaloids (Mayer's test), Carbohydrates and Glycosides (molish test), Saponins (Chloroform and H2SO4 test), Protein and aminoacid (Millon's Test), Phytosterols (Libermann- Burchard's Test), Phenolic compound and Tannin (Ferric chloride test and Lead acetate test) Adopting the Procedures Described by Stephen (1970)[28] and Parekh and Chanda (2007)[29] are analyzed.

Test organisms

The bacterial spp. used for the test were *Staphylococcus aureus (S. aureus*), *Bacillus cereus (B. cereus), Micrococcus luteus (M.luteus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumonia (K.pneumonia)* The fungus spp. used for the test were *Aspergillus niger (A.niger), Candida albicans (C.albicans), Candida tropicalis (C. tropicalis), Candida kefyr* and

Cryptococcus neoformans. All the stock cultures were obtained from Microlab, Institute of Research and Technology, Vellore, Tamilnadu, India.

Culture media and inoculums preparation

Nutrient agar /broth (Himedia, India.) were used as the media for the culturing of bacterial strains. Loops full of all the bacterial cultures were inoculated in the nutrient broth and incubated at 37° C for 72 hrs and Potato dextrose agar /and potato dextrose broth (Himedia, India)were used as the media for the culturing of fungal strains. Loops full of all the fungus cultures were inoculated in the potato dextrose broth (PDA) and incubated at room temperature for 72 hrs.

Preliminary phytochemical screening

All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites.

Antibacterial activity

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic Ciprofloxacin (100 µg/mL) in-vitro by well diffusion method[26,27]. Lawn culture was prepared using the test organism on Muller Hinton Agar (MHA). The inoculated plates were kept aside for a few minutes. Using well cutter, four wells were made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol. Using sterilized micropipettes 30µl of different solvents with selected *Murraya koenigii* leaf extract was added in to the well. The plates were incubated at 37°C for overnight. The activity of the leaf extract was determined by measuring the diameters of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents without extracts were used.

Antifungal activity

The extracts were also screened for their antifungal activity in comparison with standard antibiotic ketoconazole (10 µg/mL) *in-vitro* by well diffusion method [26,27]. Lawn culture was prepared using the test organism on Sabouraud's Dextrose agar (SDA). The inoculated plates were kept aside for a few minutes. Using well cutter, four wells were made in those plates at required distance. Using sterilized micropipettes 30µl of different solvents with selected *Murraya koenigii* leaf extract was added in to the well. The plates with yeast like fungi were incubated at 37°C for overnight. The plates with mold were incubated at room temperature for 48 hrs. The activity of the root extract was determined by measuring the diameters of zone of inhibition. For each fungal strain, controls were maintained where pure solvents were used instead of (*Murraya koenigii)* extracts.

RESULTS

The results of antibacterial activity are given in the Table 1, which clearly show that all the extracts have shown antibacterial activity equivalent to that of standard against the entire tested organisms. Ethanol, methanol, Ethyl acetate, acetone, chloroform, Petroleum ether hexane and hotwater extracts have shown better activity against all the six microorganisms. Ethanol extract was more effective against *M. luteus* and *S. aureus* . Methanol extract was more effective against *B. cereus* and *S. aureus*. Ethyl acetate extract was more effective against *M. luteus, E. coli* and *S. aureus*. Acetone extract was more effective against *M.luteus, B. cereus* and *E. coli*. Chloroform extract was more effective against *S. aureus* and *B. cereus*. Petroleum ether extract was more effective against *B. cereus* and *S. aureus*. Hexane extract was more effective against *K. pneumonia* and *B. cereus*. Hot water extract was more effective against *S. aureus* and *K. pneumonia* .

The results of antifungal activity are given in the Table 2, which clearly show that all the extracts have shown antifungal activity against the entire tested organisms. Ethanol, methanol, Ethyl acetate, acetone, chloroform, Petroleum ether, hexane, and hot water extracts have shown better activity against all the five microorganisms. Ethanol extract was more effective *against A.niger, C. tropicalis* and *C. albicans*. Methanol extract was more effective against *C. albicans* and *A. niger*. Ethyl acetate extract was more effective against *C.albicans, A.niger* and *C. kefyr*. Acetone extract was more effective against *C. albicans, C. tropicalis* and *C. kefyr*. Chloroform extract was more effective against *A. niger* and *C. albicans*. Petroleum ether extract was more effective against *C.tropicalis* and *C.kefyr*. Hexane extract was more effective against *C. albicans, C. kefyr, A .niger* and *C .tropicalis*. Hot water extract was more effective against *A.niger* and *C. tropicalis* .The presence of various phytochemicals was shown in Table 3.

DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay[30]. The Therapeutic value of medicinal plants lies in the various chemical constituents in it. The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane[31]. Flavonoids are a major group of phenolic compounds reported for their antiviral[32], antimicrobial[33] and spasmolytic[34] properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties[35]. Extract of the seeds of vitex agnus-castus was reported to possess antimicrobial activity which was associated with its alkaloids, saponins, tannins, flavnoids and glycosides contents[36]. The antibacterial activity of the leaf extract of *Murraya koenigii* as recorded in present study may therefore be attributed to the presence of above phytochemicals *i.e* Alkaloids, Carbohydrates, Saponins, Glycosides, Proteins & aminoacids, Phytosterol, Phenol, Flavinoids, Terpinoids, in Ethanol extracts and Alkaloids, Saponins, Glycosides, Proteins & aminoacids, Phytosterol, Phenol, Flavinoids, Terpinoids, in Methanol extracts and Alkaloids, Saponins, Glycosides, Proteins & aminoacids, Phytosterol, Flavinoids, Terpinoids, Tannins in Ethyl acetate extracts and Alkaloids, Carbohydrates, Saponins, Proteins & aminoacids, Phytosterol, Phenol, Flavinoids, Terpinoids in Acetone extracts and Alkaloids, Saponins, Proteins & aminoacids, Phytosterol, Flavinoids, Tannins in Cloroform extracts and Alkaloids, Proteins& aminoacids, Phytosterol, Phenolic, Flavinoids, Terpinoids, in Petroleum ether extracts and Glycosides, Phytosterol, Phenolic, Flavinoids, Terpinoids, Tannins in Hexane extracts and Saponins, Proteins & aminoacids, Phytosterol, Flavinoids in Aquas extracts.

It is concluded that the plant extract possess microbial activity against tested organisms. The zone of inhibition varied suggesting the varying degree of efficacy and different phytoconstituents of herb on the target organism. The antimicrobial activity of the plants may be due to the presence of various active principles in their leaf. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs.

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Table 1: Inhibition zone diameter of Extracts against Bacteria

Antibacterial activity of different extracts of *Murraya koenigii (L)* against Differents organisms (Mean±SEM) (mm)

Organism	Ethanol	Methanol	Ethyl Acetate	Acetone	Chloroform	Petroleum either	Hexane	Hot water	ciproflaxacin
Escherichia coli	12.27±0.25	10.07±0.12	15.17±0.29	16.20±0.20	10.10±0.10	6.0±0.0	10.07±0.12	12.20±0.20	20.20±0.20
Micrococcus luteus	25.90±0.10	10.03±0.25	16.83±0.29	20.17±0.15	8.03±0.06	8.0±0.0	-	10.03±0.06	21.17±0.29
Pseudomonas aeruginosa	10.20±0.20	8.10±0.10	10.77±0.25	10.13±0.11	8.03±0.06	-	-	10.07±0.12	22.17±0.29
Bacillus cereus	13.20±0.20	16.13±0.23	11.17±0.15	16.17±0.15	11.17±0.15	14.20±0.20	11.10±0.10	-	23.07±0.12
Klebsiella pneumoniae	12.17±0.15	10.0±0.20	12.17±0.15	11.17±0.15	10.03±0.06	-	11.17±0.15	20.10±0.10	15.03±0.06
Staphylococcus aureus	22.10±0.17	15.07±0.16	15.0±0.20	12.13±0.23	12.13±0.23	12.07±0.12	10.10±0.10	28.17±0.29	20.20±0.20

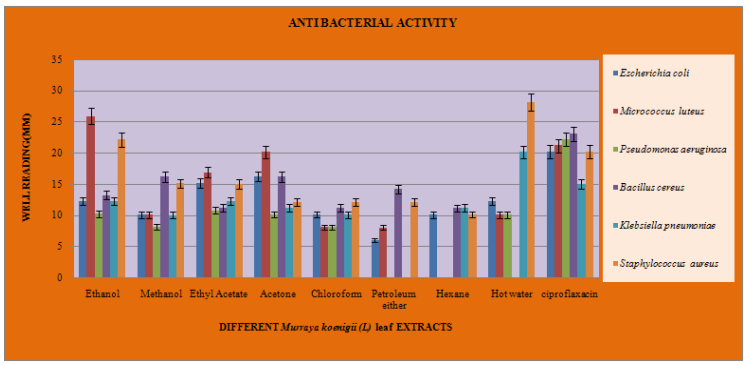

Table 2: Inhibition Zone Diameter of Extracts against Fungus

Antifungus activity of different extracts of *Murraya koenigii (L)* of against Differents organisms (Mean±SEM) (mm).

Organism	Ethanol	Methanol	Ethyl Acetate	Acetone	Chloroform	Petroleum either	Hexane	Hot water	ketoconozole
Aspergillus niger	12.17±0.15	11.17±0.15	9.07±0.12	8.07±0.12	9.07±0.12	8.07±0.12	9.10±0.17	10.17±0.15	14.30±0.26
Candida albicans	11.07±0.12	12.13±0.23	10.07±0.12	10.07±0.12	9.07±0.12	7.0±0.00	11.20±0.20	9.07±0.11	15.20±0.20
Candida tropicalis	12.17±0.15	10.07±0.12	8.07±0.12	10.07±0.12	8.07±0.12	10.07±0.12	9.07±0.12	10.10±0.17	15.30±0.26
Cryptococcus neoformans	10.07±0.12	8.07±0.12	8.23±0.06	9.00±0.00	8.07±0.12	8.0±0.00	7.07±0.12	6.00±0.00	14.10±0.17
Candida kefyr	10.0±0.00	8.07±0.12	9.07±0.12	10.07±0.12	8.07±0.12	9.17±0.15	9.20±0.20	8.03±0.06	17.30±0.26

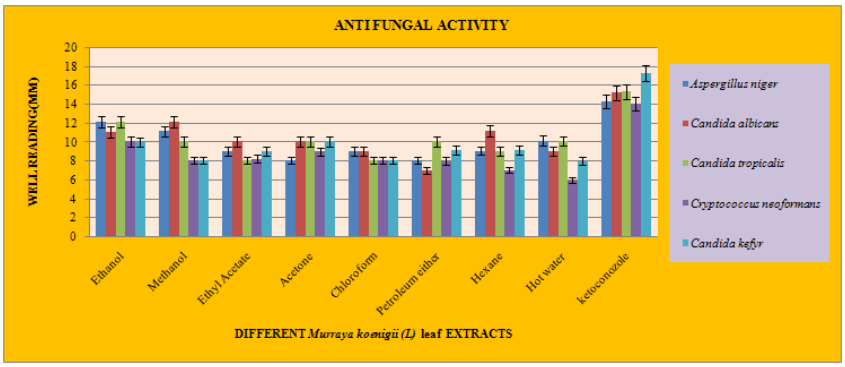

Table 3: Preliminary phytochemical analysis of *Murraya koenigii (L)*.

S.NO	Phytochemicals	Test performed	Ethanol extracts	Methanol extract	Ethyl acetate	Acetone extracts	Cloroform	Petroleum ether	Hexane	Aquas extract
1	Alkaloids	Dragendorff's test	+	+	+	+	+	+	-	-
2	Carbohydrates	molish test	+	-	-	+	-	-	-	-
3	Saponins	Chloroform and H2SO4 test	+	+	+	+	+	-	-	+
4	Glycosides	molish test	+	+	+	-	-	-	+	-
5	Proteins& aminoacids	Millon's Test	+	+	+	+	+	+	-	+
6	Phytosterol	Libermann-Burchard's Test	+	+	+	+	+	+	+	+
7	Phenolic compounds	Ferric chloride test and Lead acetate test	+	+	-	+	-	+	+	-
8	Flavinoids	Shinoda test	+	+	+	+	+	+	+	+
9	Terpinoids	Noller's test	+	+	+	+	-	+	+	-
10	Tannins	Neutral $FeCl_3$	-	-	+	-	+	-	+	-

(+) Positive (-) Negative

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