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RESEARCH ARTICLE

PHYTOCHEMICAL INVESTIGATION AND ANTI MICROBIAL ACTIVITY OF *Corchorus aestuans* (TILIACEAE)

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ABSTRACT

To investigate the antimicrobial activity and phytochemical screening ethanol, methanol, ethyl acetate, acetone, chloroform, petroleum ether, hexane, hot water, and extracts of *Corchorus aestuans* (Family: Tiliaceae). The aim of the present study was to evaluate the qualitative analysis of phytochemicals and antimicrobial activity of various solvent extracts of *Corchorus aestuans* against the clinical isolates of 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, Candida kefyr and Cryptococcus neoformans*. It was observed that ethanol, methanol, ethyl acetate, acetone, chloroform, petroleum ether, hexane and aqueous extracts showed activity against bacteria and fungus. The Ethyl acetate extract of *Corchorus aestuans* showed more activity against *Micrococcus luteus* ,zone of diameter 13±0.15mm and *Escherichia coli*, zone of diameter 13.07±0.12mm and hot water extract of *Corchorus aestuans* showed more activity against *Candida kefyr*, zone of diameter 12.20±0.20mm and *Cryptococcus neoformans,* zone of diameter 11.17±0.29mm when compared to other solvent extracts. In this study ethyl acetate extract in bacteria and hot water Extract in fungus showed a varying degree of inhibition to the growth of tested organism, than ethanol, methanol and acetone extracts. The results confirmed the presence of antibacterial activity of *Corchorus aestuans* extract against various human pathogenic bacteria. Presence of phytochemical and antimicrobial activity is confirmed.

INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. Scientific interest in phytomedicine has burgeoned due to increased efficacy of new plant-derived drugs, emerging interest in natural products and increasing concerns about the side effects of conventional medicine [1]. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary health care systems [2]. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [3]. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of infectious disease and cancer [4]. Recent trends, however, show that the discovery rate of active novel chemical entities is declining [5]. *Corchorus* is a genus of about 40-100 species of flowering plants in the family *Tiliaceae*, native to tropical and subtropical regions throughout the world [6]. Different common names are used in different contexts, with jute applying to the fiber produced from the plant, and mallow-leaves for the leaves used as a vegetable. The English common name 'mallow' (also applied to other members of *Tiliaceae* [7]. The fibers from *Corchorus* (known as jute) are the most widely cultivated vegetable fiber after cotton [8]. The leaves of *Corchorus* are rich in betacarotene, iron, calcium, and Vitamin C. The plant has an antioxidant activity with a significant α-tocopherol equivalent Vitamin E. In North Africa and the Middle East, the young leaves of *Corchorus* species are known in Arabic as *malukhiyah* and are used as green leafy vegetables . *Malukhiyah* is eaten widely in Egypt and some consider it the Egyptian national dish. It is featured in cuisines from Lebanon, Palestine, Syria, Jordan and Tunisia. In Turkey and Cyprus, the plant is known as *molohiya* and is usually cooked into a kind of chicken stew [9].

MATERIALS AND METHODS

Plant materials

The *Corchorus aestuans* whole plant 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, Vellore & 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. Soxhlet apparatus are used for this extraction [10,11]. 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 diluted 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 H_2SO_4 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) [21][12] [13], 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 Microlabs, Institute of Research and Technology, Vellore, Tamilnadu, India.

Culture media and inoculum preparation

Nutrient agar /broth (Himedia, India.) were used as the media for culturing of bacterial strains. Loopful of all the bacterial cultures were inoculated in the nutrient broth (NB) at 37°C for 24 hrs, and Sabouraud's dextrose agar(SDA) /and broth (Himedia, India) were used as the media for the culturing of fungal strains. Loopful of all the fungus cultures were inoculated in the potato dextrose broth (SDB) 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 (10 µg/mL) in-vitro by well diffusion method [14, 15]. Muller Hinton Agar (MHA) with lawn culture using desire test organism was prepared in a conical flask using required quantity of powdered media. The inoculated plates were kept aside for 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 *Corchorus aestuans* extract was added in to well. The plates were incubated at 37°C for overnight. The microbial growth was determined by measuring the diameters of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of *C.aestuans* extracts.

Antifungal activity

The Antifungal activity was determined by well diffusion method [14, 15]. Sabouraud's dextrose agar (SDA) with lawn culture using desire test organism was prepared in a conical flask using required quantity of powdered media. The medium was sterilized by autoclaving and was allowed to cool at room temperature. The medium was poured into the sterile Petri plate. The inoculated plates were kept aside for few minutes, using well cutter. Two wells are made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol. Using sterilized micropipettes 30 µl of different solvents with selected *Corchorus aestuans* extract was added in to one well and in another well the same volume of corresponding controls. The plate was incubated at room temperature 72 h. The microbial growth was determined by measuring the diameters of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of (*Corchorus aestuans*) extracts.

RESULTS

The results of antibacterial activity are given in the Table I and Fig1, which clearly show that all the extracts have shown antibacterial activity. Ethanol, methanol, ethyl acetate, acetone, chloroform, petroleum ether and hexane extracts have shown better activity against all the six microorganisms. Ethanol extract was more effective against *Escherichia coli* and *Staphylococcus aureus*. Methanol extract was more effective against *Staphylococcus aureus* and *Bacillus cereus*. Ethyl acetate extract was more effective against *Micrococcus luteus* and *Escherichia coli.* Acetone extract was more effective against *Staphylococcus aureus* and *Klebsiella pneumoniae.* Chloroform extract was more effective against *Micrococcus luteus* and *Staphylococcus aureus.* Petroleum ether extract was more effective against *Bacillus cereus* and *Klebsiella pneumonia.* Hexane extract was more effective against *Micrococcus luteus* and *Klebsiella pneumonia* and *Staphylococcus aureus.* Hot water extract was no effective against the six microorganisms. The results of antibacterial activity are given in the Table I and Fig1, which clearly show that all the extracts have shown antibacterial activity. Ethanol, methanol, ethyl acetate, acetone, chloroform, petroleum ether

Table I. Inhibition zone diameter of extracts against bacteria

Antibacterial activity of different extracts of *Corchorus aestuans (*Tiliaceae*)* against Different organisms (Mean±SEM) (mm).

Organism BACTERIA	Ethanol	Methanol	Ethyl acetate	Acetone	Chloroform	Petroleum either	Hexane	Hot water	Ciproflaxacin
Escherichia coli	13.17±0.15	10.07±0.12	13.07±0.12	10.07±0.11	6.0±0.00	8.07±0.12	8.07±0.12	Nil	22.17±0.29
Micrococcus luteus	10.07±0.11	11.07±0.11	13.17±0.15	9.03±0.06	13.17±0.15	9.07±0.12	9.17±0.15	Nil	20.30±0.26
Pseudomonas aeruginosa	8.0±0.00	10.10±0.17	11.17±0.15	8.07±0.12	10.07±0.12	Nil	Nil	Nil	25.37±0.32
Bacillus cereus	10.07±0.12	11.20±0.20	11.17±0.15	10.07±0.11	Nil	10.17±0.15	8.00±0.00	Nil	25.40±0.36
Klebsiella pneumoniae	9.07±0.12	8.07±0.12	12.13±0.12	11.07±0.12	11.17±0.15	10.07±0.12	8.13±0.12	Nil	20.00±1.00
Staphylococcus aureus	10.93±0.11	14.20±0.20	12.07±0.12	12.0±0.20	13.07±0.12	9.07±0.12	8.13±0.11	Nil	35.50±0.50

Table II. INHIBITION ZONE DIAMETER OF EXTRACTS AGAINST FUNGUS

Antifungal activity of different extracts of *Corchorus aestuans (*Tiliaceae*)* of against Different organisms (Mean±SEM) (mm)

Organism FUNGUS	Ethanol	Methanol	Ethyl Acetate	Acetone	Chloroform	Petroleum either	Hexane	Hot water	Ketocona-zole
Aspergillus niger	7.07±0.12	6.07±0.11	6.0±0.00	7.0±0.00	5.0±0.00	Nil	Nil	10.17±0.15	15.30±0.26
Candida albicans	10.17±0.15	8.13±0.12	8.07±0.13	12.20±0.20	8.07±0.16	8.07±0.12	5.0±0.00	9.07±0.12	14.20±0.20
Candida tropicalis	9.07±0.12	Nil	8.07±0.11	Nil	5.0±0.00	5.03±0.06	5.07±0.12	9.07±0.11	13.17±0.29
Cryptococcus neoformans	11.17±0.15	Nil	11.17±0.15	Nil	8.07±0.11	Nil	Nil	11.17±0.29	14.37±0.32
Candida kefyr	Nil	Nil	9.09±0.17	Nil	8.03±0.06	Nil	Nil	12.20±0.20	15.23±0.25

TABLE III: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *CORCHORUS AESTUANS (*TILIACEAE*)*

S.NO	Phytochemicals	Test performed	Ethanol extracts	Methanol extract	Chloroform	Ethyl acetate	Petroleum ether	Hexane	Aqueous extract	Acetone extracts
1	Alkaloids	Dragendorff's test	+	+	-	+	-	-	+	+
2	Carbohydrates	Molish test	+	+	-	+	-	-	+	+
3	Saponins	Chloroform and H_2SO_4 test	-	-	+	+	+	+	-	-
4	Glycosides	Molish test	+	+	-	+	-	-	+	+
5	Proteins& amino acids	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 $FeCl_3$	+	+	-	+	-	-	+	+

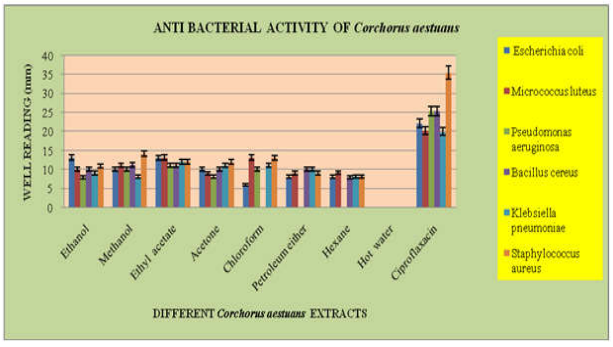

Fig.1. Antibacterial activity of different extracts of *Corchorus aestuans (*TILIACEAE*) against* Different organisms

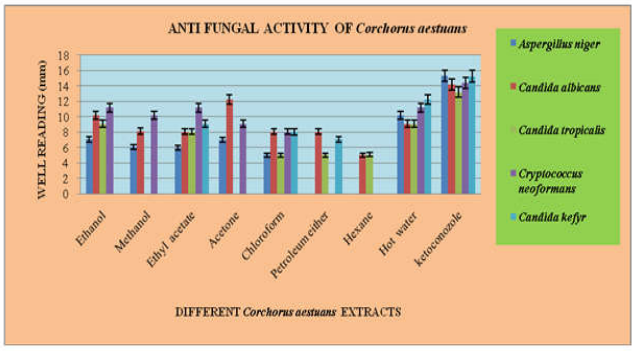

Fig 2. Antifungal activity of different extracts of *Corchorus aestuans (*Tiliaceae*) of* against Different organisms

and hexane extracts have shown better activity against all the six microorganisms. Ethanol extract was more effective against *E.coli* and *S.aureus*. Methanol extract was more effective against *S.aureus* and *B.cereus*. Ethyl acetate extract was more effective against *M.luteus* and *E.coli.* Acetone extract was more effective against *S.aureus* and *B.cereus*.

Ethyl acetate extract was more effective against *M.luteus* and *E.coli.* Acetone extract was more effective against *S.aureus* and *K.pneumoniae.* Chloroform extract was more effective against *M.luteus* and *S.aureus.* Petroleum ether extract was more effective against *B.cereus* and *K.pneumonia.* Hexane extract was more effective against *M.luteus* and *K.pneumonia*

and *S.aureus*. Hot water extract was no effective against the six microorganisms. The results of antifungal activity are given in the Table II and Fig 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 *C.neoformans* and *C.albicans*. Methanol extract was more effective against *C.neoformans* and *C.albicans*. Ethyl acetate extract was more effective against *C.neoformans* and *C.kefyr*. Acetone extract was more effective against *C.albicans and C.neoformans*. Chloroform extract was more effective against *C.albicans, C.kefyr and C.neoformans*. Petroleum ether extract was more effective against *C. albicans* and *C.kefyr*. Hexane extract was more effective against *C.tropicalis* and *C.albicans*. Hot water extract was more effective against *C.kefyr, C.neoformans and A.niger*. The presence of various phytochemicals was shown in Table III.

DISCUSSION

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 [16]. Flavonoids are a major group of phenolic compounds reported for their antiviral [17], antimicrobial[18] and spasmolytic [19] properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties [20]. The antibacterial activity of the *Corchorus aestuans* extract as recorded in present study may therefore be attributed to the presence of above phytochemicals *i.e* alkaloids, carbohydrates, glycosides, proteins& amino acids, phenolic, flavonoids, terpenoids, and tannins in ethanol extracts and alkaloids, carbohydrates, glycosides, proteins, phenolic, flavonoids, terpenoids, tannins in methanol extract and saponins, phytosterol in chloroform extract and alkaloids, carbohydrates, saponins, glycosides, phenolic, flavonoids, tannins in ethyl acetate extract and saponins, phytosterol in petroleum ether extracts and saponins and saponins, phytosterol in hexane extracts and alkaloids, carbohydrates, glycosides, proteins& amino acids, phenolic compounds, flavonoids, terpenoids, tannins in aqueous extracts and alkaloids, carbohydrates, glycosides, proteins& amino acids, phenolic compounds, flavonoids, terpenoids, tannins in acetone extracts. Based on the results of the present study it is concluded that the *Corchorus aestuans* extract plants have potent antimicrobial activity against various. Bacteria and fungi which might be due to the phytochemicals present in the plants. Also, there is further scope to study the identification and purification of active compound(s) involved in this antimicrobial activity of *Corchorus aestuans* extract.

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