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Research Article

PHYTOCHEMICAL SCREENING & EVALUATION OF ANTIMICROBIAL ACTIVITY OF *CHONEMORPHA FRAGRANS* AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT

The present study aimed at evaluating the antimicrobial efficiency of ethanolic leaf extracts of Chonemorpha fragrans, a medicinal plant against four human pathogenic bacteria like Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa using agar well diffusion method and minimum inhibitory concentration and minimum bacterial concentration. Chonemorpha fragrans showed significant activity against all pathogens with its ethanolic extract which showed maximum zone of inhibition and minimum inhibitory concentration against all the experimental strains.The phytochemical analysis carried out revealed the presence of phytochemicals such as flavonoids, glycosides, alkaloids, gums, mucilage, phytosterols and saponins. The results provide justification for the use of the plant in folk medicine to treat various infectious diseases.Hence the use of plant extract with known antimicrobial properties can be of great significance in therapeutic treatments.

KEYWORDS: *Antimicrobial activity, Chonemorpha fragrans, Agar well diffusion method, MIC, MBC, Zone of inhibition, Human pathogenic bacteria.*

INTRODUCTION

A wide range of medicinal plants extracts are used to treat several infections because they have potential antimicrobial activity. These herbal products are today the symbol of safety in contrast to the synthetic drugs that are regarded as unsafe to human beings and environment. *Chonemorpha fragrans* (moon), Alston belongs to family Apocynaceace. It has been included in the list of endangered medicinal plants [1]. Many plants species have pharmacological properties as there are known to possess various secondary metabolites like alkaloids, flavonoids, carbohydrates, glycosides, saponins, proteins and phytosterols therefore should be utilized to combat the disease causing pathogens [2-4]. The entire plant, leaves are used for fever and stomach disorders. *Chonemorpha fragrans* is traditionally been used as anthelminthic, diuretic, astringent and stomachic. The roots are sweet, bitter, astringent, laxative, thermogenic, depurative, carminative, anthelmintic, digestive, antiscorbutic, anodyne, expectorant and febrifuge. They are useful in vitiated conditions of vata and kapha, skin diseases, leprosy, scabies, dyspepsia, constipation, hyperacidity, cardiac debility, diabetes, jaundice, bronchitis cough and intermittent fevers. It is also used in diseases like anemia (pandu), fever (jwara), diabetes (prameha), stomach disorders (udararoga), typhoid (visamajwara), urinary infections (asmari) and cough (ksaya). It is also used in the treatment of diarrhoea, polyuria, boils, leprosy, eye diseases, vomiting and poisoning [5]. The literature study revealed that *Chonemorpha fragrans* medicinal importance as an antimicrobial agent have not been studied so far since there are no reports on antimicrobial activity and there are scanty reports on secondary metabolite production in vivo and in vitro for this plant. Hence the present research was set up to evaluate the antimicrobial activity of leaves of *Chonemorpha fragrans* on selected human pathogenic bacterial strains.

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MATERIALS AND METHODS

Collection of plant material:

Fresh leaf samples of *Chonemorpha fragrans* of family Apocynaceae were obtained from botanical gardens of Sri Venkateshwara University; Tirupathi. The species were taxonomically identified and authenticated by Dr. Madhav Chetty, Assistant professor of Botany, Department of Pharmacognosy, Sri Venkateshwara University, Tirupathi.

Preparation of ethanolic extract:

The leaves of *C.fragrans* were washed with tap water in order to remove the dust and shade dried in room temperature for about 10 days and then powdered which were later extracted by cold maceration technique using ethanol as solvent for 7 days with intermittent shaking. The mixture is then filtered through a Whatman no.4 filter paper and the solvent

was removed using rotary vaccum evaporator until it reaches one fourth of its volume. Then the extract was stored at 4ºC in air tight bottles for further studies.

Test microorganisms:

Bacterial strains of Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa were obtained from Department of Microbiology, CMR College of Pharmacy, Kandlakoya Medchal.

Chemicals:

Muller Hinton agar media (MHA) and Ampicillin (10µg/disc) were purchased from Hi- Media lab Ltd, Mumbai, India. All other chemicals and media used were of analytical grade.

Phytochemical screening:

Preliminary phytochemical screening was performed as per the standard methods [6]. The ethanolic leaf extracts of *C.fragrans* were screened for the presence of various phytochemicals by employing standard conventional protocols [7-9].

Microbiological screening:

Antimicrobial activity of ethanolic leaf extract of *Chonemorpha fragrans* was evaluated by the agar well diffusion method and minimum inhibitory concentration (MIC) [10].

Agar well diffusion method:

It is widely used to evaluate the antimicrobial activity of plants or microbial extracts.In this method, the agar plate surface is inoculated by spreading a volume of the microbial inoculums over the entire agar surface. Then a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20-100µl) of the antimicrobial agent or extract solution at desired concentration is introduced into the cell.Then agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

Minimum inhibitory concentration (MIC):

The antibacterial activity of the plant extract was determined using sterile 2ml 96-well plates. The 12 wells of each row were filled with 0.5 ml sterilized Mueller Hinton agar. Sequentially, wells 2–11 received an additional 0.5 ml of a mixture of culture medium and plant extract serially diluted to create a concentration sequence from 0.512 ml to 0.008 ml. Well 1 served as growth control, well 12 as antibiotic control. Ampicillin (0.1µg/disc) was used as controls for the *S. aureus* and *E. coli* assays respectively. This antibiotic was chosen because it is often employed as first line antibiotic in the respective bacterial infections. The MIC of Ampicillin (for *E. coli* assays) was 8µg/ml. The deep-wells were incubated for 24h at 37°C. At least three repetitions were run for each assay. Strong activity was defined as MIC < 5 mg/ml.

Minimum bacterial concentration (MBC):

A pure culture of a specified microorganism grown overnight, then diluted in growth-supporting broth (typically Mueller Hinton Broth) to a concentration between 1 x 10^5 and 1 x 10^6cfu/ml. A stock dilution of the antimicrobial test substance is made at approximately 100 times the expected MIC (if known).Further 1:1 dilutions are made in test tubes or 96 well microtiter plates. All dilutions of the test product(s) are inoculated with equal volumes of the specified microorganism.

A positive and negative control tube or well is included for every test microorganism to demonstrate adequate microbial growth over the course of the incubation period and media sterility respectively. Turbidity indicates growth of the microorganism and MIC is the lowest concentration where no growth is visually observed. To determine the MBC, the dilution representing the MIC and at least two of the more concentrated test product dilutions are plated and enumerated to determine viable CFU/ml.The MBC is the lowest concentration that demonstrates a predetermined reduction (such as 99.9%) in CFU/ml when compared to the MIC dilution.

Preparation of inoculum:

Test for antibacterial activity:

The antibacterial assay was carried out by microdilution method in order to determine the antibacterial activity of compounds tested against the pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 X 10^7 CFU/ml. The inocula were prepared and stored at 4ºC until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum.All experiments were performed in duplicate and repeated three times

Determination of MIC:

The minimum inhibitory concentrations (MIC), minimum bacterial concentration (MBC) were performed by a serial dilution technique using 96-well microtiter plates. The ethanolic plant extract was taken (1 mg/ml) and serial dilution of the extract with luria broth for bacterial culture with inoculum was used. The microplates were incubated for 72 hours at 28ºC respectively. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

Determination of MBC:

The MBC was determined by serial sub-cultivation of 2 µl into microtiter plates containing 100 µl of broth per well and further incubation for 72 hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate reader (Perlong, ENM8602) and compared with the standards Ampicillin for Bacteria as the positive control. All experiments were performed in duplicate and repeated three times.

RESULTS AND DISCUSSION

In the present investigation, the inhibitory effect of ethanolic leaf extract from *Chonemorpha fragrans* was evaluated against four different bacterial strains. The antimicrobial activity was determined using agar well diffusion method and micro dilution method. The activity was quantitatively assessed on the basis of inhibition zone with minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) was also calculated.

Measurement of Anti microbial activity using agar well diffusion method:

The antimicrobial potential of the experimental plant was evaluated according to their zone of inhibition against various pathogenic bacterial strains and the results (zone of

inhibition) were compared with the activity of the standards, viz., Ampicillin (10 µg/disc).The results showed that the ethanolic leaf extract of *Chonemorpha fragrans is* a potent antimicrobial against all the microorganisms studied. For all the tested microorganisms in ethanolic extract the maximum zone of inhibition diameter was obtained in gram positive Bacillus subtilis and in gram negative Pseudomonas aeruginosa with diameter of 22.4 mm and 18.95 mm respectively (Table 2).

Determination of MIC, MBC values:

Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit growth of organisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of microorganism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. The MBC was determined by sub culturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 h. The concentration of plant extract that completely killed the bacteria was taken as MBC. Ethanolic extract of *Chonemorpha fragrans is* showed least MIC value of 25.4 µg/ml against gram positive Bacillus subtilis and 42.4µg/ml against gram negative Pseudomonas aeruginosa and least MBC value of 40.6µg/ml against gram positive Bacillus subtilis and 72.5µg/ml against gram negative Escherichia coli respectively (Table 3).

Table No. 1: Phytochemical screening of ethanolic leaf extract of *Chonemorpha fragrans*

S.NO	TEST	OBSERVATION	INFERENCE
1	Alkaloids	+ve	Alkaloids are present
2	Carbohydrates	+ve	Carbohydrates are present
3	Glycosides	+ve	Glycosides are present
4	Saponins	+ve	Saponins are present
5	Phenols	-ve	Phenols are absent
6	Tannins	-ve	Tannins are absent
7	Gums and Mucilage	+ve	Gums and mucilage are present
8	Flavonoids	+ve	Flavonoids are present
9	Fixed oils & fats	-ve	Fixed oils & fats are absent
10	Phytosterols	+ve	Phytosterols are present
11	Proteins	+ve	Proteins are present

Table No. 2: Anti microbial activity Zone of inhibition (mm) of *Chonemorpha fragrans* ethanolic leaf extract against four human pathogenic bacterial strains

Test organism	Zone of inhibition (mm) & (Concentration in µg/ml)				Ampicillin (10µg/disc)
	50(µg/ml)	100(µg/ml)	200(µg/ml)	400(µg/ml)	
Bacillus subtilis	12.82	16.3	20.6	22.4	25.64
Staphylococcus aureus	10.3	15.5	17.3	20.51	22.82
Escherichia coli	11.24	13.4	15.2	16.32	18.52
Pseudomonas aeruginosa	9.4	12.6	14.4	18.95	21.36

Table 3: Minimum inhibitory concentration MIC (µg/ml) and minimum bactericidal concentration (MBC) (µg/ml) of *Chonemorpha fragrans* ethanolic leaf extract against four human pathogenic bacterial strains

Microorganisms	Type	Ethanolic Extract (MIC) (µg/ml)	Ethanolic Extract (MBC) (µg/ml)
Bacillus subtilis	Gram +ve	25.4	40.6
Staphylococcus aureus	Gram +ve	36.5	70.8
Escherichia coli	Gram –ve	45.3	72.5
Pseudomonas aeruginosa	Gram –ve	42.4	76.4

CONCLUSION

Medicinal plants continue to play a central role in the healthcare systems of large proportions of the world's population, particularly in developing countries, where herbal medicine has a long and uninterrupted history of use [11]. Medicinal plants provide accessible and culturally relevant sources of primary health care. The remedies based on these plants often have minimal side effect [12].

In the present investigation it can be concluded that ethanolic leaf extract fraction of medicinal plant *Chonemorpha fragrans* showed potent antimicrobial activity against the tested bacterial strains. The antimicrobial activity may be due to strong occurrence of active compounds i.e. flavonoids, saponins, alkaloids, glycosides, steroids and proteins. The metabolites have been shown to be responsible for various therapeutic activities of medicinal plants [13]. Flavonoids especially are known to be effective antimicrobial agent against a wide array of microorganisms. The activity is attributed to their ability to complex with extra cellular and soluble proteins and with bacterial cell wall [14]. There are several reports published on antibacterial activity of different herbal extracts [15-22]. However, this medicinal plant species may be subjected to detailed phytochemical and pharmacological studies in order to find out new drugs against pathogenic bacterial strains. While *Chonemorpha fragrans* has been used successfully in Ayurvedic

medicine for centuries, an extensive research and development work should be undertaken on *Chonemorpha fragrans* and its products for their better economic and therapeutic utilization.

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