



Original Research Article

INVESTIGATION OF THE ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *CARDANTHERA DIFFORMIS* DRUCE WHOLE PLANT EXTRACTS AGAINST SOME CLINICAL PATHOGENSSomnath De¹, Dulal Chandra Das^{2*}, Tanushree Mondal³ and Monalisa Das⁴¹Department of Biotechnology, Panskura Banamali College, West-Bengal, India.²Department of Botany, Raja Narendra Lal Khan Women's College, West-Bengal, India.³Department of Biotechnology, Oriental Institute Science and Technology, West-Bengal, India.⁴Department of Nutrition and Dietetics, Vidyasagar Institute of Health, Midnapore, West-Bengal, India.

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Abstract: In rural and backward areas of West Bengal in India several plants were commonly used as herbal medicine for the treatment of many diseases without studying any phytochemical and biological information in detail. The current study was to investigate the antibacterial and antifungal analysis of the whole plant extracts of *Cardanthera difformis*. Methanol and aqueous extracts of shed dried whole plant of *Cardanthera difformis* were tested for antibacterial and antifungal activity. The antibacterial behavior of methanol and aqueous extracts of whole plant of *Cardanthera difformis* using the standard well diffusion assay were investigated against the eight strains of bacterial species (*Bacillus subtilis*, *Enterobacter faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*) and antifungal activity were also studied against two fungal species (*Aspergillus niger* and *Candida albicans*). Among two types of solvent extracts the methanol extract comparatively exhibited the best results. The experimental findings proved the justification of traditional use of *C. difformis* for the treatment of various diseases of human beings. The phytochemical analysis may lead to the development of a new generation of drugs which may have both chemotherapeutic and chemo preventive properties in future.

Key Words: *Cardanthera difformis*, Antibacterial activity, Antifungal activity, Agar well diffusion.

INTRODUCTION

In developing countries and particularly in India marginal people such as farmers, people of small isolated villages and native communities were used folk medicine for the treatment of common infections[1] but were not tested systematically for the biological activities in general and antimicrobial activities in particular since past. Plants are the potent source of antimicrobial agents and have started investigation throughout the globe [2-11]. Plant extracts have numerous health related effects such as antibacterial, anti-mutagenic, anti-carcinogenic, anti-thrombotic and vasodilator activities [12]. Medicinal plants are an important source for the therapeutic remedies of various ailments. Scientific experiments on the antimicrobial properties of plants components were first documented in the last 19th century [13]. Natural antimicrobials have been often derived from plants, microorganisms or animal tissues [14]. India is well known for its richness in diversity of medicinal plants [15]. Nearly 70% of the world population is dependent on the traditional medicines for primary health care.

The existence of antimicrobial compounds in various plants have been investigated [16-20]. Central Nervous System depressant activities and anthelmintic activity of ethanol extract of aerial parts of *Hygrophila difformis* in mice have been experimentally observed [21,22]. The antibacterial and phytochemical analysis of *Cardanthera difformis* has been done to a limited extend [23]. In the present investigation attempts have been made to find out the antibacterial and antifungal

potentialities of *C. difformis* against some selected clinical pathogens.

MATERIAL AND METHODS

Cardanthera difformis Druce was selected as experimental tool as this plant was used in traditional healthcare by the common and marginal people since past for the treatment of some disease. It is tropical aquarium plant under the Acanthaceae family, commonly known as water wisteria used as environmental ornaments and found in marshy habitats on the Indian subcontinent including Bangladesh, Bhutan and Nepal. It is decumbent, annual herbs, stem 20-40 cm long, rooting at base and with glandular hair.

Collection of sample

Cardanthera difformis was collected along with flowers and fruits from the wet places of different districts of West Bengal.

Sterilization

The disease free and fresh plants were selected. About 25 gm. fresh and healthy whole plant were taken for each solvent extraction and were washed with distilled water for three times. Then surface sterilization was done with 0.1% mercuric chloride for 20 seconds. Finally plants were washed with distilled water again at least three times.

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Extraction

The fresh and washed plants were dried under shed for 11 days and then finely grinded to a powder. The powder was stored in an air tight container [24].

Preparation of aqueous extract

For aqueous extraction the powdered materials were taken in soxhlet apparatus with distilled water. About each 25 grams of powdered material was packed in soxhlet extraction unit and exhaustively extracted using 250 ml of distilled water at 60°C for 12 hours. Thereafter, it was filtered with the help of Whatman No.1 filter paper; the filtrates were used for antibacterial and antifungal activity against the tested microorganisms.

Preparation of methanol extract

For methanol extraction the powdered materials were taken in soxhlet apparatus with methanol as organic solvent. About each 25 grams of powdered material was packed in soxhlet extraction unit and exhaustively extracted using 250 ml of methanol at 60°C for 12 hours. Thereafter, it was filtered with the help of Whatman No.1 filter paper; the filtrates were used for antibacterial and antifungal activity against the tested microorganisms.

Antimicrobial screening

Screening for antimicrobial activity was done by the agar well diffusion method.

Pathogens tested for antimicrobial activity

Tested microorganisms: The eight pathogenic bacteria were isolated from NICED (National Institute of Cholera and Enteric Disease Research Centre) Belehata, Kolkata. The test organisms include *Bacillus subtilis*, *Escherichia coli*, *Enterobacter faecalis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella dysentria*, *Salmonella typhi*. The clinical fungal test organisms used for study are *Aspergillus niger* and *Candida albicans* were collected from the Department of Microbiology, Vidyasagar University, Paschim Medinipur, west Bengal, India.

Media for test organisms: The bacterial cultures were revived in nutrient broth medium and incubated at 37°C for 48 hours. Each bacterial culture was further maintained at 37°C on nutrient agar slants and nutrient broth after every 48 hours of transferring. The fungal cultures were revived in PDA broth medium and incubated at 23°C for 48 hours. Each fungal culture was further maintained at 23°C on Potato dextrose agar medium plates and PDA broth after every 48 hours of transferring.

Antibacterial activity: Antibacterial activity of two types of extracts from *C. difformis* were

investigated against *Bacillus subtilis*, *Escherichia coli*, *Enterobacter faecalis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella dysentriae* and *Salmonella typhi*.

Antibacterial assay was carried out by Agar Well diffusion method [25-26]. Fresh microbial culture of 0.1ml having 10⁸ CFU was spread on nutrient agar plate with glass spreader. A well of 6 mm diameter was punched off into agar medium with sterile cork borer and filled 50µg/ml of aqueous and methanol extracts using micropipette in each well under aseptic condition. The Petri plates were kept in a refrigerator to allow pre- diffusion of extract for 30 minutes and further incubated in incubator at 37°C for 24 hours. The antibacterial screening was evaluated by measuring the zone of inhibition. The experiment was done in triplicate and the mean diameter of the inhibition zone was calculated. Antibiotic Ciprofloxacin at a concentration of 25µg/ml was used as positive control.

Antifungal activity: The method of Bauer et al., [27-28] was adopted for the study. Antifungal activities of whole plant extracts of *C. difformis* were proved in radical growth inhibition activity. A fungal plug was placed in the centre of the Potato Dextrose Agar plate. Extracts of 50µg/ml concentration was pipette into the wells. The Petri plates were incubated in the dark at 23°C for 48 hours. Antifungal properties were observed as a crescent shaped zone of inhibition at the mycelia form. The effect of fungal growth was expressed quantitatively. Comparison of various extracts was done with standard antifungal Fluconazole at a concentration (1mg/1 ml) 25 µg as a positive control. The diameters of zone of inhibition surrounding each of the well were recorded.

RESULTS AND DISCUSSION

At the beginning of the 21st century the herbal medicines achieved the reliability in the mind set of global people as because it have no side effects relatively less expensive and better patient tolerance. Recent reports on ethno medicine have brought to the light that our rich floristic heritage in one of the reliable sources which can be traced pharmacologically for their possible antimicrobial and antifungal potential. The plants bio- constituents have been a good source of antimicrobial agents but still many of the plant species remained unexplored or under explored [29]. Plants are important sources of potentially useful substances for the development of new chemotherapeutic agents. Various phytochemical compounds which are naturally present in plants as secondary metabolites may have been implicated in the conferment of antibacterial and antifungal activities [30 and 27]

The presence of some such secondary metabolites in a significant amount in the investigated part of *C. difformis* may have confirmed the antibacterial activity on the whole plant extracts. In this regard, higher concentration of these phytochemicals may have been responsible for a higher degree of inhibition on the bacterial and fungal strains.

The results of the methanol extract of *C. difformis* revealed antibacterial properties against the eight human pathogens collected from NICED. In methanol extract the diameter of inhibition zones ranging from 11 to 28 mm of which the highest degree of inhibition was found against *S. typhi* (28mm) (Table 1 & Fig.1). followed by *K. pneumoniae* (18 mm), *B. subtilis* (17 mm), *E. coli* (17mm), *S. dysenteriae* (16 mm) *E. faecalis* (13mm), *Pseudomonas aeruginosa* (13mm) and *Staphylococcus aureus* (11mm). The most significant result is that the degree of inhibition of methanol extract (28mm) exceed the degree of inhibition of standard drug ciprofloxacin (20mm) to control *S. typhi* and showed more or less similar activity to control *K. pneumoniae*.

Table 1: Antibacterial activity of whole plant extract *C. difformis* against pathogenic bacteria.

Name of microorganisms	Zone of inhibition(mm)		
	Ciprofloxacin (25 µg/ml)	Methanol extract (50 µg/ml)	Aqueous extract (50 µg/ml)
<i>Escherichia coli</i>	39mm	17mm	11mm
<i>Staphylococcus aureus</i>	29mm	11mm	**
<i>Pseudomonas aeruginosa</i>	31mm	13mm	**
<i>Klebsiella pneumoniae</i>	21mm	18mm	8mm
<i>Enterobacter faecalis</i>	26mm	13mm	12mm
<i>Shiegella dysenteriae</i>	32mm	16mm	11mm
<i>Salmonella typhi</i>	20mm	28mm	**
<i>Bacillus subtilis</i>	31mm	17mm	**

Data given are mean of three replicates I standard error. ** - No inhibition

The aquous extracts of *C. difformis* reveal the inhibition against the four pathogens only (Table 1 & figure 1) and the degree of inhibition ranging from 8-12 mm. The highest degree of inhibition observed in *E. faecalis* (12mm) and lowest degree found in *K. pneumonia* (8mm). The surprising result of aqueous extract is that it cannot inhibits *S. typhi* whereas methanol extract showed the extreme activity even super succeeding the standard drug ciprofloxacin activity against *S. typhi*. Again methanol and aqueous extract have similar response to control *E. faecalis* (12-13mm).

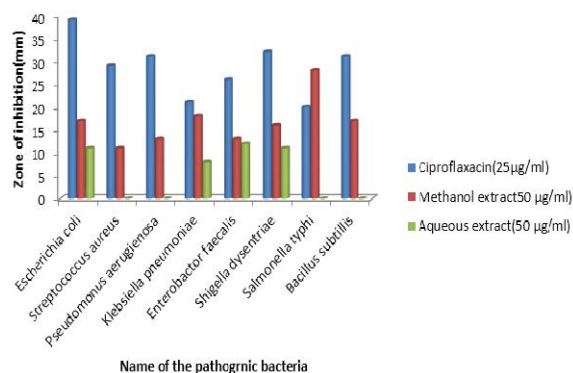


Figure 1: Antibacterial activity of whole plant extract *C. difformis* against pathogenic bacteria.

The antifungal activity was also determined by measuring the diameter of zone of inhibition. The methanol extracts of *C. difformis* showed antifungal activity against *A. niger* (16mm) but no activity against *C. albicans*. The results are illustrated in Table 2 as figure 2. Again it is also very much interesting is that the degree of antifungal activity is more or less same of both the methanol extract and standard antibiotic the fluconazole. The aqueous extract is completely unable to inhibit both the fungal pathogens.

Table 2: Antifungal activity of whole plant extract of *C. difformis* against pathogenic fungi.

Name of the microorganisms	Zone of inhibition(mm)		
	Fluconazole (25 µg/ml)	Methanol extract (50 µg/ml)	Aqueous extract (50 µg/ml)
<i>Aspergillus niger</i>	18mm	16mm	**
<i>Candida albicans</i>	**	**	**

Data given as mean of three replicates I standard error. ** - No inhibition

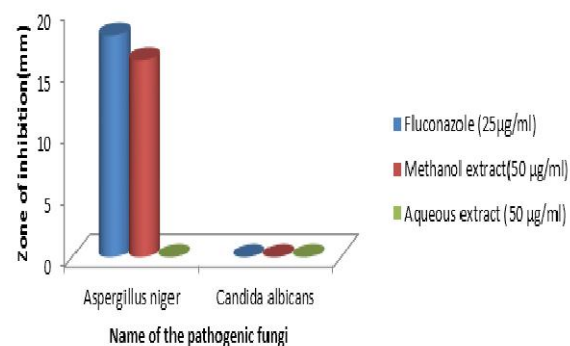


Figure 2 Antifungal activity of whole plant extract of *C. difformis* against pathogenic fungi.

CONCLUSION

Experimental findings revealed that methanol extract is better than aqueous extract to control both the bacteria and fungi. From the findings it may also be concluded that the methanol solvent is unable to release some bioactive compounds from *C. difformis* to

inhibit the *C. albicans*. Similarly aqueous solvent may be unable to release the biomolecules from *C. difformis* to control *C. albicans*, otherwise it can be explained no such bio molecules are present in *C. difformis* to control the *C. albicans*. Future detail research work will find out the actual answer and will open a new avenue to formulate and develop antibacterial and antifungal medication.

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