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Antibacterial activity and phytochemical analysis of *Cardanthera difformis* druce leaf extracts from West Bengal, India.

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Abstract

In rural and backward areas of west Bengal of India several plants are commonly used as herbal medicine for the treatment of infectious diseases without any phytochemical and biological information in detail. The current study was to investigate the antibacterial activity and phytochemical analysis of the leaf extracts of *Cardanthera difformis*. Methanol, ethanol, chloroform, acetone and petroleum ether extracts of shed dried plant leaves of *Cardanthera difformis* were tested for antibacterial activity and phytochemical screening.The antibacterial behaviour of different extracts (methanol, acetone, ethanol, chloroform and petroleum ether) of leaves of Cardanthera difformis using the standard well diffusion assay were investigated against the seven strains of bacterial species, viz., Enterobactor faecalis, Eschirichia coli, Klebsiella pneumonia, Pseudomonous aerusinosa, Salmonella typhi, Shiegella dysentriae and Staphylococcus aureus.. Among the various solvent extract the acetone leaf extract exhibit the best antibacterial property followed by chloroform, ethanol, methanol and petroleum ether. The ethanol extract showed significant activity against Staphylococcus aureus, Pseudomonous aeruginosa, Enterobactor faecalis and Shiegella dysentriae. The acetone extract showed the highest magnitude of inhibition against Salmonella typhi among the extracts under study. Phytochemical screening of the leaf extracts using standard qualitative methods revealed the presence of saponins, flavonoids, terpenoids, tannins, quinone, carbohydrates and amino acids. Both the petroleum ether and methanol extracts showed the maximum efficiency for biochemical constituents. The present study experimentally proved the justification of traditional use of *C.difformis* for the treatment of various diseases and reported for the first time in the world to focus the antibacterial activity of leaf extracts of *C.difformis* and may lead to the development of a new generation of drugs having both chemotherapeutic and chemo preventive properties in future.

Keywords: Agar well diffusion method; Antibacterial activity; *Cardanthera difformis*; Phytochemicals.

Introduction

Man has been using plants to cure different diseases associated with pathogenic bacteria since antiquity. According to the study conducted by W.H.O. based on publications on pharmacopoeia's and medicinal plant in 91 countries, the number of medicinal plants is nearly 21000. Nearly 6-7 thousand species of medicinal plants out of about 17-18 thousand flowering plants are known to be use in folk and officially recognized system of medicine in India, i.e., Ayurveda, Sidha, Unani and homeopathy. The wide range of medicinal plants existing in India owing to a vast range of agro climatic variability is accountable for increased demand of Indian medicinal plants in the international market in recent years. WHO has projected that the global herbal market for medicinal plants has been estimated to be worth around US $ 120 billion which is growing at 7% - 10% every year and it is likely to increase to more than US $ 5 trillion by 2050[1-3]. Plants grow in this region are not systematically tested for their biological activities in general and antimicrobial activity in particular. Exceptional ways to available antibiotics for disease management have been increasingly felt due to the increase in the resistance of bacterial isolates. This has urgently demanded the requirement second and third line drug and plants are considered potent candidates to overcome such inevitable problems associated with the complications of antimicrobial resistant bacteria[4-13].

Medicinal plants are an important source for the therapeutic remedies of various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century[14]. Natural antimicrobials have been often derived from plants, microorganisms or animal tissues[15]. India is known for its rich diversity of medicinal world[16]. Nearly 70 percent of the world population is dependent on the traditional medicines for primary health care.

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as fungi, bacteria or protozoan's as well as destroying viruses[17]. Antimicrobial drugs either kill microbes or

prevent the growth of plants with a new eye for their antimicrobial usefulness and as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agents with general as well as specific activity[18].

There are many reports on the presence of antibacterial compounds in various plants[19,20]a very limited superficial work have done on *C.*difformis[21] but there is no reports on antibacterial potential on *Cardanthera difformis* up till now. Therefore, present study is an attempt in this direction to find out a new concept from *C.difformis.*

Materials and Methods

Selection of medicinal plant as experimental tool

Cardanthera difformis Druce has been selected as experimental tool there is no report up till now on antibacterial activities of this plant throughout the globe. It is the tropical aquarium plant under the family Acanthaceae and commonly known as water wisteria, used as environmental ornaments, 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, glandular hairy. Leaves membranous, glandular hairy on both surfaces, highly variable, lower ones usually pinnatifid in water, large and pectinate; upper ones ovate or rounded, decurrent at base in a short petiole, rounded at apex, closely and sharply crenate-serrate, 1.5-3 1.2-2.5 cm. Flowers 1-3 in auxiliary whorls forming terminal spikes by gradual reduction of leaves; bracteoles oblong, 5-6 mm long sepals linear-subulate, glandular hairy, acute, one larger than others. Corolla 0.8-1.2 cm long, Pubescent outside, purple; upper lip erect inflated below shortly 2- lobbed limb; lower lip broad, sparsely hairy within, with darker veins. Capsule 6-8 mm long, pubescent, 30-60 seeded. Retinacula minute, conical,soft, straight.

Plant material extraction

Fresh plant parts are collected from the different agricultural field of Eastern India. The taxonomic identities of this plant are determined by the expertise of the department of botany of our college. The leaves were washed thoroughly using tap water and dried under shed for 11 days, then finely grinded to a powder. Then the powdered material was extracted with acetone, ethanol, methanol, chloroform, petroleum ether, distilled water using soxhlet apparatus. About 10 grams of powder was loaded in soxhlet extraction unit and exhaustively extracted using 100ml of solvents such as acetone, ethanol, methanol, chloroform, distilled water and petroleum ether respectively at 60 c for 12 hours. Thereafter, it was filtered with the help of Whatman No.1 filter paper. Then the extracts were used for antibacterial and phytochemical analysis.

Test Microorganism

The leaf extracts of *Cardanthera difformis* were screened against seven pathogenic bacteria collected from NICED (National Institute of Cholera and Enteric Disease) Research Centre Beleghata, Kolkata. The test organisms were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobactor faecalis, Shigella dysentria* and *Salmonella typhi*. 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.

Antibacterial activity

Antibacterial assay was carried out by Agar well diffusion method. Fresh microbial culture of 100µl (10^6 cells/ml) was spread on Muller Hilton Agar plate with cotton swab. A well of 6mm diameter was punched off into agar medium with sterile cork borer and filled with 100µl of each (100µg/ml) ethanol, methanol, acetone, petroleum ether, distilled water and chloroform extracts by using micropipette in each well in aseptic condition. The petriplates were then kept in a refrigerator to allow pre diffusion of extracts for 30 minutes and further incubated in a incubator at 37 c for 30 minutes for 24 hours extroverted position. The antibacterial screening was evaluated by measuring the zone of inhibition. An inhibition zone of 6mm or greater (incubating diameter of well) was considered antibacterial activity. The experiment was done in triplicate and the mean diameter of inhibition zone was calculated.

Phytochemical analysis

The ethanol, acetone, methanol and chloroform extracts of *Cardanthera difformis* were screened for the presence of secondary metabolites using the procedure Harborne and Kokate et al.[22,23]

Test for carbohydrates (Molisch's test)

5 ml of solution of each extract was mixed with few drops of Molisch's reagent and 2ml con.H_2SO_4 was added from side wall of test tube. Formation of purple colour ring at the junction indicated the presence of carbohydrates[24].

Test for proteins and amino acids (ninhydrin test)

Each solution extract was heated with ninhydrin reagent. Characteristic deep blue or pale yellow colour developed which indicates the presence of amino acids and proteins[24].

Test for tannins

The substance mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of tannins.

Test for saponine (foam test)

The substance shaken with water, foamy latner indicates presence of saponine.

Test for flavonoids (Ammonia test)

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each extract followed by addition of con. H_2SO_4. A yellow colour observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing[24].

Test for terpinoids (Salkowski's)

5ml of each extract was mixed in 2ml of chloroform. After that 3ml of concentrated $H_2SO_4$was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive test for presence of terpenoids.

Test for quinone

To test the substance sodium hydroxide was added. Blue, green or red colour indicates the presence of quinone.

Statistical analysis

Agar well diffusion activity was performed in three replicates under strict aseptic conditions to ensure consistency of all conclusions. Data of all experiments were statistically analysed and expressed as mean.

Results and discussion

Recent reports on ethno medicine have brought to the light that our rich floristic heritage is one of the reliable sources which can be traced pharmacologically for their possible antibacterial potential. The plant bio-constituents have been a good source of antibacterial agents but still today many of the plant species remained unexplored or under explored[25]. 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 have been implicated in the conferment of antibacterial activities[26,27]. The presence of some such secondary metabolites in a significant amount in the investigated part of *C. difformis* have confirmed the antibacterial activity. In this regard, higher concentration of these phytochemicals may have been responsible for higher degree of inhibition on the bacterial strains.

The results of the present study showed that the five types of extracts (methanol, ethanol, acetone, petroleum ether and chloroform) from the leaves of *C. difformis* revealed antibacterial properties against all the seven human pathogens collected from NICED. All the extracts exhibit broad spectrum of antibacterial activity (Table 1 and Figure 1).

Tables 1: Antibacterial activity of leaf extracts of *Cardanthera difformis* against pathogenic bacteria.

Leaf extract	Zone of inhibition(mm) including 6 mm diameter of well						
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Enterobactor faecalis*	*Shiegella dysentriae*	*Salmonella typhi*
Acetone	-	10	11	-	9	11	21
Methanol	-	-	8	10	-	13	12
Ethanol	-	12	10	-	14	13	-
Chloroform	7	-	-	-	-	-	20
Petroleum ether	14	7	7	-	-	-	12
Tetracycline(50µg/ml)	17	20	10	30	15	10	30

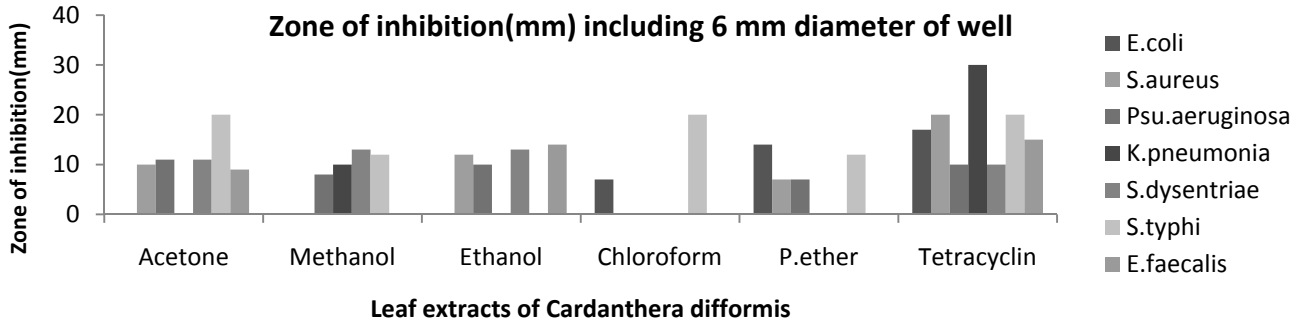

Figure 1. Antibacterial activity of Cardanthera difformis against pathogenic bacteria.

Acetone extract showed the highest potentiality in comparison to others. The diameters of inhibition zones ranging from 9 – 21 mm and the highest zone of inhibition observed against *S. typhi* (21 mm), followed by *S. dysentriae* (11 mm), *P. aerusinosa* (11 mm), *S. aureus* (10 mm) and least inhibition zone was against *E. faecalis* (9 mm). It is noteworthy the chloroform and the acetone extract have as good as similar potency with standard antibiotic Tetracycline in most of the cases. Acetone extract showed no zone of inhibition in case of *E. coli* and *K.pneumonia* and meant acetone extraction did not have any role in extracting antibacterial phytochemicals for killing *E. coli* and *K. pneumonia.* Again ignoring the type of extracts *C. difformis* exhibited maximum inhibition to *P. aeruginosa* and *S. typhi* but tremendously significant result have been found against *S. typhi*. *C. difformis* also highly efficient than the tetracycline to kill the *P. aeruginosa* and *S. dysentriae.* Chloroform extract have saponins and terpenoids and inhibit only to *E. coli* and *S. typhi*.

The major phytochemical constituents such as saponins, flavonoids, tannins, terpenoids, carbohydrates, proteins, amino acids and quinones are found to be present in the leaf extracts of *C. difformis* (Table 2).

Table 2: Qualitative analysis of the phytochemicals in the leaf extracts of *Cardanthera difformis.*

Phytochemicals	Methanol extract	Ethanol extract	Chloroform extract	Acetone extract	Petroleum extract
Saponins	+	+	+	+	_
Flavonoids	+	+	_	+	+
Tannins	+	_	_	_	+
Terpenoids	_	_	+	_	+
Carbohydrates	+	+	_	_	+
Proteins and Amino acids	_	_	_	_	+
Quinone	+	+	_	+	_

(+) – Positive (-) – Negative

Presence of these phytochemicals suggest that the plant might have the medicinal importance and would be the significant tool in pharmaceutical industry. Acetone extract showed saponins, flavonoids and quinone compound in the extraction and these three compounds showed inhibition except *E. coli* and *K. pneumonia* but chloroform extract showed saponins and terpenoids compounds which inhibit *E. coli* and *S. typhi* only. From this account it may be suggested that the terpenoids are responsible for inhibition to *E. coli.* Saponins present in both the acetone and chloroform extract and showed the high degree of inhibition (20 mm) to *S. typhi*. These finding gives credence to the traditional medicine application of *C. difformis* as remedies for internal and external wounds and infections. Several phytoconstituents like flavonoids[28] tannins[29] and saponins[30] are effective antimicrobial substances against a wide range of microorganisms. Flavonoids show anti-allergic, anti-inflammatory and anticancer activity. Saponins possess hypocholesterolemic and antidiabetic properties[31] and also responsible for central nervous system activities[32] and terpenoids show analgesic properties[33]. This result suggest that the bioactive compounds present in the leaf extracts of *C. difformis* have good potential for development of novel antibacterial herbal products.

Conclusion

Experimental findings reveal *C. difformis* is the best herbal to control specially *S. typhi*, *P. aeruginosa* and *S. dysentriae.* The phytochemical constituents which are responsible for many pharmacological activities, may be useful for the evolution of pharmaceutical and for the therapy of ailments. This is the first ever experimental findings of antibacterial activity as well as demonstration of any biological properties in the globe but deserve further investigation to develop new antibiotics that may help in combating several bacterial diseases in tropical countries. To some extend further studies are also going on this plant in order to isolate, identify, characterized and elucidate the structure of the bioactive principles present within it to develop new antibacterial medication.

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References

[1]. Kala CP, Dhyani PP, Sajwan BS. Developing the medicinal plants sector in Northern India: challenges and opportunities. J Ethanobiol Ethnomed. 2006;2:32.

[2]. Pei SJ. Ethnobotanical approaches of traditional medicinal studies: some experiences from Asia. Pharm Biol.2001;39:74-79.

[3]. Das DC, Sinha NK, Chattopadhyay JC, Das M, Samanta P. The use of Medicinal Plants for the treatment of Gonorrhoea and Syphilis in South West Bengal of India. Int J Phytomed. 2013;5(1).

[4]. Goossens H, Ferench M, Vander SR, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet. 2005;365:579-587.

[5]. Kinghorn AD, Chai HB, Sung CK, Keller WJ. The classical drug discovery approach to define bioactive constituents of botanicals. Fitoterapia.2011;82(1):71-79.

[6]. Cos P, Vlietinck AJ, BergheDV, Maes L. Anti-infective potential of natural products;how to develop a stronger in vitro 'proof-of concept'. J ethnopharmacol.2006;106:290-302

[7]. Houghton PJ. Old yet new-pharmaceuticals from plants. J Chem Edu . 2001;78:175

[8]. Nahrstedt A, Butterweck V. Lessons learned from herbal medicinal products:the example of St John's Wort. J Nat Prod. 2010;73:1015-1021

[9]. Gerald B. Biologically active compounds from marine organism. Phytother Res. 2001;15:89-94.

[10]. Newman DJ, Cragg GM.Natural products as sources of new drugs over the last 25 years. J Nat Prod. 2007;70:461-477.

[11]. Dossey A. Insects and there chemicals weaponry:new potential for drug discovery. Nat Prod Rep. 2010;27:1737-1757.

[12]. Dorman HJ, Deans SG. Antimicrobial agents from plants : activity of plant volatile oils. J Appl Microbiol. 2000;88:308-316.

[13]. Asghari J, Ondruschka B, Mazaheritehrani M. Extraction of bioactive chemical compounds from the medicinal Asian plants by microwave irradiation. J Med Plant Res. 2011;5:495-506.

[14]. Zaika LL. Species and herbs their antimicrobial activity and its determination. J.Food Safty. 1975;9:97-118.

[15]. Gordon MC, David J. Natural Product drug discovery in the next millenium. Pharm Bio. 2001;139: 8-17.

[16]. Vedavaty S, Mrudal V, Sudhakar A. Tribal medicine in Chitton district, Andhra Pradesh, India. Vedamse Books P Ltd. 1997.

[17]. Chan-Bacab MJ, Pena-Rodriguez LM. Plant natural products with leishmanicidal activity. Nat Prod Rep. 2001; 18 : 674 – 688.

[18]. Evans WC. Trease and Evan's Pharmacognosy. 14th Edition. W B Saunders Co. 1996; 290.

[19]. Perez C, Paul M , Bazerque P. Antibiotic assay by agar by agar – well diffusion method. Acta Biol Med Exp. 1990 15 :113 -115.

[20]. Olurinola PF. A Laboratory Manual of Pharmaceutical Microbiology. National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. 1996; 69-105.

[21]. Samanta K, Hossain E, Pal DK. Anthelmentic activity of *Hygrophila difformis* Blume. J Buffalo science. 2012; 1:35-38.

[22]. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 14 edition. Nirali Prakasanan, Pune, India. 2008; 1 – 635.

[23]. Harborne JB, Harborne JB. Phytochemical methods ; A Guide to Modern Techniques of plant analysis. Champman and Hall, New York. 1998 ; 1- 320.

[24]. Harborne JB. Phytochemical methods : A Guide to Modern Techniques of plant of analysis. 3rd Edition. Chapman and Hall , New York. 1998; 1 – 302.

[25]. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol. 2005;100:80-84.

[26]. Cao Y, Wei X, Xu H, Tang W. Antifungal properties of methanol extracts and its active compounds from *Brickellia rosmarinifolia* .Vent Fitoterapia. 2010;81(8):1176-1179.

[27]. Sule A, Ahmed QU, Samah OA, Omar MN, Hassan NM, Kamal LZM. Bioassay guided isolation of antibacterial compounds from *Andrographis paniculata*. Am J Appl Sci. 2011;8:525-534.

[28]. Tsuchiya H, Sato M, Miyazaki T, Fujuwara S, Tanigaki S, Ohayama M, Tanaka T, Linuma M. Comparative study on the antibacterial activities of phytochemical flavonones against methicillin resistant *Staphylococcus aureus*. J Ethnopharmacol. 1996;50:27-34.

[29]. Ya C, Gaffney SH, Lilley TH, Haslam E. Carbohydrate-Polyphenol Complexation.In: Chemistry and Significance of condensed tannins. Hemingway RW, Karchesy JJ, ed. In Plenum Press, New York.1988;553.

[30]. Nabila B, Fawzia BA, Tatjana PK. Antioxidant and Antimicrobial activities of *Pistacia lentiscus* and *Pistacia atlantica* extracts. Afr J Pharm Pharmacol.2008;2 (2):22-28.

[31]. Rupasinghe HP, Jackson CJ, Poysa V, Berardo CD, Bewley JD, Jenkinson J. Soyasapogenol A and B distribution in soyabean (*Glycine max* L. Merr) in relation to seed physiology, genetic

variability and growing location. J Agr Food Chem.2003;51:5888-5894.

[32]. Argal A, Pathak AK. CNS activity of *Calotropis gigantea* roots. J Ethnopharmacol. 2006;106:142-145.

[33]. Malairajan P, Gopalakrishnan G, Narasimhan S, Veni KJK. Analgesic activity of some Indian medicinal plants. J Ethnopharmacol. 2006;106:425-8.