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RESEARCH ARTICLE

ANALYSIS OF PHYTOCHEMICAL PROFILE OF CARDANTHERA DIFFORMIS DRUCE WHOLE PLANT EXTRACT WITH ANTIBACTERIAL PROPERTIES

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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 phytochemical screening of ***Cardanthera difformis*** whole plant extract. For phytochemical screening, some common and available standard tests were done. Antibacterial bioassay was done through agar well diffusion method. Phytochemical screening showed the active compounds present in high concentration, such as flavonoids, phenolic compounds, terpenoids and cardiac glycosides. The greater performance for antibacterial activity was found against Gram negative bacteria. The whole plant extract revealed the presence of bio-active constituents which are known to exhibit medicinal activity.

INTRODUCTION

Phytochemical are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them [1] . They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components [2].The quantity and quality of phytochemicals present in plant parts may differ from one part to another. In fact, there is lack of information on the distribution of the biological activity in different plant parts essentially related to the difference in distribution of active compounds (or active principles) which are more frequent in some plant parts than in others [3].

Phytochemicals have been recognized as the basis for the tradition herbal medicine practiced in the past and currently envogue in parts of the world [4]. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region [5].WHO has projected that the global herbal market for the medicinal plant has been estimated to be worth around US $120 billion which is growing at 7to10 percent. Every year and it is likely to increase to more than US $5 trillion by 2050 [6-8]. The plant *Cardanthera difformis* which is chosen, is a weed. It is a tropical aquarium plan under the family *Acanthacae* and common known as water wisteria, used as environmental ornaments, found in marshy habitats on the Indian subcontinent including Bangladesh, Bhutan, and Nepal [9]. Detection of phytochemicals is the main aim of this work from whole plant extracts in various solvents. The existence of antimicrobial compounds in various plants have been investigated [10-14]. Central Nervous System depressant activities and anthelmentic activity of ethanol extract of aerial parts of *Hygrophila difformis* in mice have been experimentally observed [15,16]. The antibacterial and phytochemical analysis of *Cardanthera difformis* has been done to a limited extend. In the present investigation attempts have been made to find out the antibacterial and phytochemical properties of *C. difformis*.

MATERIALS AND METHOD

Selection of plant

Cardanthera difformis Druce has been selected for experiment tools. It is collected in the month of march , 2014 form Paschim Medinipur district , West Bengal , India and it is available in any season of year.

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Plant material extraction process

After collection of plant material, they are cuted in small pieces and they are dried under shade for 12 days. After drying in finally grinded in powder by grinder machine. Then the powdered material was extracted with acetone, ethanol, methanol, chloroform, and 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 chloroform respectively at 60^0C for 12 hours. Thereafter, it was filtered with the help of Whatman No.1 filter paper and use for various phytochemical and antimicrobial tests.

Bacterial strain and culture conditions

Two Gram negative and two Gram positive indicator bacteria used for antimicrobial assay respectively, *Escherichia coli (E. coli* MTCC 443), *Klebsiella pneumonia (K. pneumonia* MTCC 109), *Staphylococcus aureus (S. aureus* MTCC 3160) and *Salmonela typhi (S. typhi* MTCC 890) were provided by microbiological laboratory and clinical detection center Paschim Medinipur ,India. They were cultured in tryptone soy broth or agar (TSB or TSA) in aerobic condition at 37 °C.

Antibacterial activity

The antimicrobial activity was determined in the methanolic *C. difformis* extract using agar well diffusion method. The antibacterial activities of *C. difformis* extract (concentration of compound 50%, 100 %) were tested against two Gram-positive *S. aureus, S. typhi* and two Gram negative *E. coli and K. pneumoniae*, human pathogenic bacteria. Zone of inhibition of *C. difformis* bark extract were compared with standards like chloramphenicol for antibacterial activity. The results showed that the remarkable inhibition of the bacterial growth was against the tested organisms [17].

Phytochemical Screening

Phytochemical analysis of the test sample was carried out according to standard methods[18-20].

Test for Alkaloids

A fraction of extract was treated with 3-5 drops of Wagner's reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml of water] and observed for the formation of reddish brown precipitate (or coloration).

Tests for Carbohydrates

Few drops of Molisch's reagent were added to 2ml portion of the various extracts. This was followed by addition of 2ml of concentrated H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet color at the interphone of the two layers was a positive test.

Test for Cardiac glycosides

5 ml of each extract was treated with 2 ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

Test for Flavonoids

2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Phenols

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color.

Test for Phlobatannins

Deposition of a red precipitate when 2 ml of extract was boiled with 1ml of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Amino acids and Proteins

2 ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple color.

Test for Saponins

To 2 ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Tannins

2mls of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish color solution.

Test for Terpenoids

1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Test for Quinones

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or coloration).

RESULT

Preliminary phytochemical screening

The preliminary phytochemical analysis in *C. differmis* different extract showed the active compounds presence in high concentration, such as flavonoids, phenolic compounds, glycosides, terpenoids. Also, the active compounds are absent or in low concentration, such as saponins, phlobatannins, alkaloids, carbohydrates and amino acids and quinones are shown in Table 1.

Table1 Result of phytochemical screening of *Cardanthera differmis*

Phytochemicals	Methanol Extract	Ethanol Extract	Chloroform Extract	Acetone Extract	Aqueous Extract
Alkaloids	-ve	-ve	+ve	-ve	-ve
Carbohydrate	+ve	+ve	-ve	-ve	-ve
Cardiac glycosides	+ve	+ve	-ve	+ve	+ve
Flavonoids	+ve	+ve	+ve	+ve	+ve
Phenol	+ve	+ve	+ve	+ve	-ve
Phlobatannins	-ve	-ve	-ve	-ve	-ve
Amino acid	-ve	-ve	-ve	-ve	-ve
Saponins	-ve	-ve	+ve	-ve	-ve
Tannins	-ve	-ve	-ve	-ve	-ve
Terpenoids	+ve	-ve	+ve	-ve	+ve
Quinones	-ve	-ve	-ve	-ve	-ve

+ve = Present; -ve = Absent

Fig. 2 Inhibition zone against *E. coli* and *S.typhi*

Antimicrobial activity

Antimicrobial activity of *C. differmis* whole plant methnolic extract showed greater result against gram negative bacteria than Gram positive bacteria (Figure 2, 3 and Table 2).

DISCUSSION

Medicinal plants were of great importance to the health of individuals and communities [21]. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [22]. Analysis of the plant extracts revealed the presence of phytochemicals, such as proteins, charbohydrates, phenols, tannins, flavonoids, saponins, glycosides, phlobatannins, terpenoids and alkaloids. In this present study, preliminary phytochemical analysis revealed a large amount of flavonoids, phenolic compounds, terpenoids and cardiac glycosides present in different extract of *C. differmis* whole plant.

Table 2 Antimicrobial activities of *C. differmis* methanolic extract and zone of inhibition

	Diameter of zone of inhibition (mm)* against			
Extract	***K.pneumoniae***	***E. coli***	***S. aureus***	***S.typhi***
Methanolic	10	28	12	21
Chloramphenical (std)	26	39	19	30

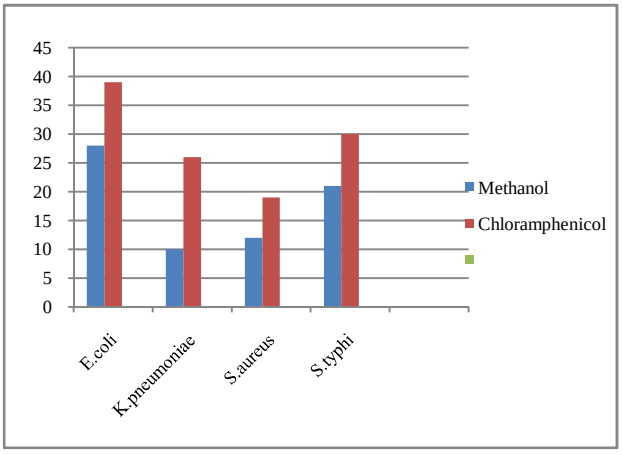

Fig 3 Antibacterial activity of *C.differmis* methanolic extract against four types of pathogenic bacteria.

Be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro.

Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [23]. Activity of methanolic extract of *C. differmis* was comparable to that of reference standard drug chloramphenicol. *C. differmis* whole plant extract exhibited good antimicrobial activity.

The maximum inhibition zone of methanolic extract shows against gram negative bacteria. Thus, the methanolic extract of *C. differmis* has great antimicrobial activities. It has been shown that *C. differmis* whole plant extract consists of many useful compounds, such as flavonoids, phenols, terpenoids and cardiac glycosides.

CONCLUSION

Phytochemicals found present in whole plant extracts of *Cardanthera difformis* indicates their potential as a source of principles that may supply novel medicines. Further studies are therefore suggested to ascertain their anti-microbial, antispasmodic and anti-helminthic activities. Furthermore, isolation purification and characterization of the phytochemicals found present will make interesting studies.

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