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Investigation of Antioxidant Properties of *Cardanthera Difformis* Druce Whole Plant Extract

KEYWORDS: 'Cardanthera difformis', antioxidant activity and whole plant materials.

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ABSTRACT *The current study was to investigate the thin layer chromatographic separation of flavonoid components, antioxidant activity and total flavonoid compound of Cardanthera difformis Druce whole plant extract. Detection of antioxidant activity and flavonoid coumpounds were done through thin layer chromatography. Total antioxidant activity was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in colorimetric method. Aluminum chloride colorimetric method was used for total flavonoid determination. This methanolic extract showed a promising antioxidant DPPH ridicules decreased in DPPH free radical scavenging assay. Flavonoids components having antioxidant property present in the methanol extract at a level of 178 mg quercetin equivalent/g of dried methanol extract in colorimetric method. The C.difformis whole plant extract revealed the presence of bio-active constituents which are known to exhibit medicinal as well as physiological activity. 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 antioxidant activity of whole plant extract of C.difformis and may lead to the development of a new generation of drugs having both chemotherapeutic and chemopreventive properties in future.*

INTRODUCTION:-

During the last few decades, there has been an increasing interest in the study of traditional plants and their medicinal value in different parts of the world[1]. Antioxidants may offer resistance against oxidative stress by scavenging free radicals, lipid peroxidation and many other mechanisms thus prevent disease[2,3]. The use of traditional medicine is widespread and plants provide a large source of natural antioxidants that might serve as leads for the development of novel drugs. Therefore, investigations of natural antioxidants and bioactive compounds for preservation of traditional medicines and use in treating certain human diseases have received much attention[4]. Plants are the natural sources for their property of having antioxidants, including polyphenolic compounds, tocopherols, vitamin C and carotenoids, and are attracting the food industry. These compounds are the replacements for synthetic ones, whose usage is being restricted due to their harmful effects on human health. The natural antioxidants from the plant source protect the body from free radicals. BHA (Butylated hydroxyl anisole) and BHT (Butylated hydroxyl toluene) are the very effective synthetic antioxidants but they are toxic to human health and need to be replaced by natural antioxidants. So, there is a necessity for identifying alternative natural and safe source of antioxidants and the search for natural antioxidants, especially of plant origin has been increased in recent years[5].

Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs[6]. Flavonoids and other plant phenolics, such as phenolic acids, stilbenes, tannins, lignans, and lignin, are especially common in leaves, flowering tissues, and woody parts such as stems and barks[7]. The search of new antioxidants and phenolics from herbal source has taken very large attention in last decade. Antioxidant and antimicrobial properties are responsible for well being of human body hence, they are very much important for further characterization of plant material. Secondary metabolites from plants, mainly phenolics having antioxidants, antimicrobial, antitumour, antiviral, enzyme inhibiting and radical scavenging properties[8,11]. The plant Cardanthera difformis which is chosen is a weed. It is a tropical aquarium plan under the family *Acanthaccae* and common known as water wisteria, used as environmental ornaments , found in marshy habitats on the Indian subcontinent including Bangladesh, Bhutan ,and Nepal[12]. In the present investigation attempts have been made to find out the antioxidant properties of C. difformis.

MATERIAL AND METHOD

Selection of plant material

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.

Preparation of methanolic extract of C.difformis whole plant

The collected C.difformis plant were cut into small pieces. The plant parts were dried in an incubator for 7 day at 40 °C,crushed in an electrical grinder and then the powder was separated. A total of 100 g of powder of said plant material was washed in 400 mL of petroleum ether for 24 h to remove the greasy pigmented non polar materials. Then the petroleum ether was discarded and residue was dissolved in 500 mL diethyl ether for 2 h in a soxhlet apparatus. The extract was filtered through Whatman No. 1 filter paper and the resulting filtrate was dried in the air. The ether solid extract was dissolved in 300 mL acetone for 1 h in a soxhlet apparatus. Then the extract was filtered through Whatman No. 1 filter paper and the resulting filtrate was dried under reduced pressure at 40°C on a rotary evaporator. The acetone solid extract was dissolved in 200 mL methanol and was dried in the air. The methanol

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extract was stored in refrigerator for antioxidant activity study.

Thin layer chromatography (TLC) analysis for antioxidant constituents

About 2 µg of extracts of C.difformis was loaded on TLC plates (Merck, 20 cm ×20 cm). The plates were developed in methanol: chloroform: hexane (7:2:1, v/v/v) to separate various constituents of the extracts. The developed plates were air dried. Then the antioxidant constituents were analyzed by DPPH technique[13,14]. For this 0.05% of DPPH solution in methanol was sprayed on the surface of developed TLC plates and incubated for 10 min at room temperature. The active antioxidant constituents of the C.difformis extract was detected as yellow spots produced via reduction of DPPH by resolved bands against purple back ground on the TLC plates. Ascorbic acid was used as standard antioxidant[15].

TLC analysis for flavonoid constituents

About 2 µg of extracts of C. difformis was loaded on TLC plates (Merck, 20 cm ×20 cm). The plates were developed in ethyl acetate: glacial acetic acid: water (10:2:2.5, v/v/v) to separate flavonoid compounds of the extracts. The developed plate was air dried. The present flavonoid compound of this extracts was detected as yellow spot on developed TLC plate. The Rf value of the bands were also determined.

Determination of total flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination[16]. About 1 mL of the plant extracts/ standard of different concentration solution was mixed with 3 mL of methanol, 0.2 mL of aluminum chloride, 0.2 mL of 1 mol/L potassium acetate and 5.6 mL of distilled water. It remained at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. Methanol served as blank. The total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated by the following equation:

$$C=(c\times V)/m$$

Where C is total content of flavonoid compounds, mg/g plant extract, in quercetin equivalent; c is the concentration of quercetin established from the calibration curve in mg/mL, V is the volume of extract in mL, and m is the weight of crude plant extract in g.

Antioxidant activity determination by DPPH free radical scavenging assay

DPPH radical scavenging activity of the extract was measured by the method described by Barros et al[17]. For this, different concentrations of extract and ascorbic acid (standard) were prepared with methanol (Sigma- Aldrich) as the test solutions. About 1 mL of each prepared concentrations were placed into test tubes and 0.5 mL of 1 mmol/L DPPH solution in methanol was added. The test tubes were incubated for 15 min and the absorbance was read at 517 nm. A blank solution consisted of DPPH dissolved in same amount of methanol. The DPPH radical scavenging activity percentage was calculated by using the following formula:

DPPH raadcitcivailt ysc avenging (%)

$$\frac{Acontrol-Aextract}{Acontrol} \times 100$$

Result

TLC analysis for antioxidant constituents

The plates TLC were developed in methanol: chloroform: hexane (7:2:1, v/v/v) and sprayed with 0.05% DPPH reagent. Purple colour of DPPH reagent was bleached by yellow spots which was the indication of positive antioxidant activity. The whole plant extract of C.difformis in terms of DPPH free radical scavenging activity showed one resolved TLC band with strong antioxidant activity and another spot with antioxidant activity as compared to standard antioxidant Quercetin (Figure 1). standard

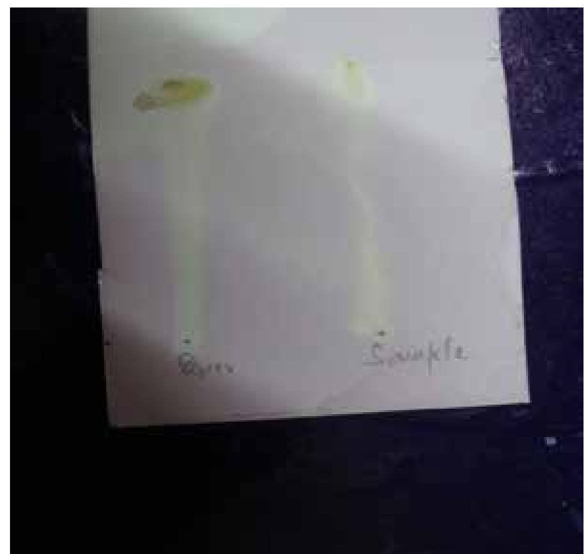

Figure 1. TLC antioxidant activity analysis of C.difformis constituents. Standard: Quercetin; sample

Determination of total flavonoid content

The total flavonoid content of the C.difformis whole plant was estimated by using aluminum chloride colorimetric technique and found to be 178 mg quercetin equivalent/g of dried methanol extract.

TLC analysis for flavonoid constituents

The plates were developed in ethyl acetate: glacial acetic acid: water (10:2:2.5, v/v/v) to separate flavonoid compounds of the extracts. The developed plate was air dried. The present flavonoid compound of this extracts was detected as yellow spot on developed TLC plate. The Rf value of the bands were also determined. The eluted compounds showed blue color corresponding with flavonoid behavior (Figure 2 and Table 2).

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Table3 :-Detection of flavonoids through TLC.

Extract	Solvent system	Rf value
Standard: Quercetin	ethyl acetate: glacial acetic acid: water (10:2:2.5, v/v/v)	0.97
Sample	ethyl acetate: glacial acetic acid: water (10:2:2.5, v/v/v)	0.97

Figure 4. TLC analysis for flavonoid compounds in C.difformis whole plant methanolic extract.

Antioxidant activity determination by DPPH free radical scavenging assay

DPPH is a free radical and it gives strong absorption band at 517 nm in the visible region of electromagnetic radiation. As antioxidant compounds donate protons to these radicals, the absorption decreased. The decrease in absorption was activity is largely due to flavonoids. The antioxidant and anti-microbial properties of C.difformis are responsible on presence of large amount of flavonoid components. So the results further supported the view that the bark of C.difformis is promising source of natural useful therapeutic agents. The traditional medicine practice is recommended strongly for this plant as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the bioactivity study.

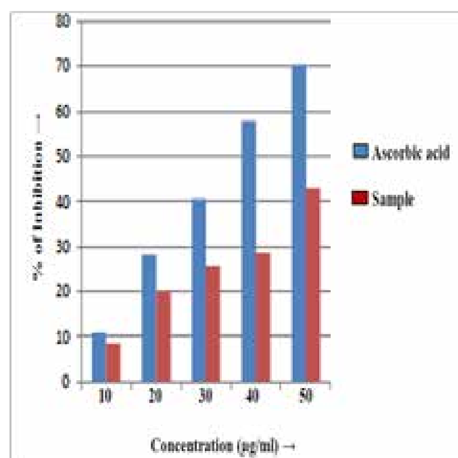

Figure: DPPH radicals scavenging activity of methanol whole plant extract of *C. difformis* and ascorbic acid as a standard.

Concentration (in µg/ml)	% of Inhibition of Ascorbic acid	% of Inhibition of Sample
10	10.9375	8.571429
20	28.125	20
30	40.625	25.714286
40	57.8125	28.571429
50	70.3125	42.857143

DISCUSSION

Medicinal plants were of great importance to the health of individuals and communities[18]. Several studies have described the antioxidant properties of different parts of various medicinal plants which are rich in phenolic compounds[19,20]. In this present study, preliminary phytochemical analysis revealed a large amount of flavonoids of C.difformis whole plant. Natural antioxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherols etc[21].The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation[22]. This methanolic extract has great free radical scavenging property and also contains liberal amount of flavonoid components. Flavonoids are hydroxylated phenolic substances known to 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]. More than 2000 flavonoids have been reported among woody and non-woody plants[24]. Its antioxidant versatile medicinal plants having a wide spectrum of biological activity. In this present study, authors have demonstrated antioxidant, along with total flavonoid compound measure respectively.

CONCLUSION

The isolated flavonoid compound from sample and the standard flavonoid Quercetin are the same Rf value. The antioxidant activity of C.difformis was found to be comparable to standard Ascorbic acid. For future work on the profile and nature of chemical constituents of C.difformis whole plant will provide more information on the active principles responsible for their pharmacological properties. This may also lead to the development of a new generation of drugs that possess both chemotherapeutic and chemopreventative properties which can results in ways of combating the serious problems of dieases.

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