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In-vitro investigation of antioxidant activity of *Cissus adnata* in different fractions

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Abstract
This study was conducted to evaluate the antioxidant property of *Cissus adnata.* The plant belongs to the vitaceae family and locally used for the treatment of tumors, boils and buboes. This study provides a scientific basis for the use of *Cissus adnata* in traditional medicine. The whole plant was extracted using methanol and different fractions were obtained using different organic solvents, e.g., dichloromethane, petroleum ether, chloroform. All these fractions were subjected to antioxidant activity determination. Among all the fractions, aqueous soluble fraction showed highest level of total phenolic content of 34.65 mg of GAE / gm of extractives comparing to the others. Whereas, for the DPPH free radical scavenging assay, petroleum ether fractions provided the highest IC_{50} value of 546.29 □g / ml.

Keywords: antioxidant, *Cissus adnata*, DPPH, phenolic content

Introduction
The folkloric concepts of a wide range of medicinal plants have been proven scientifically and had led to the development of drugs to fight various infectious diseases [1]. A brief exploration has been performed on plant extracts for their therapeutic activities against most microbial infections. It has been established that about 80% of the world's population relies on plant derived medicines for their healthcare needs and 3.5 billion people in the world depend on the exploitation medicinal plants and herbal products around them for their health needs [2]. Free radicals are atoms, groups of atoms or molecules that have one or more unpaired electrons in the outermost orbital, so they are chemically highly reactive and tend to seek electron pairs to be able to bind to achieve stability. Free radical atoms are constantly attacking body's cells including normal cells for obtaining electron pairs [3, 4]. Free radicals react with biological compounds in body continuously and if they do not stop, they can damage body cells and provides harmful effects to body health. This free radical reaction will also cause various diseases such as cancer, heart, cataract, premature aging, and other degenerative diseases [5]. Secondary metabolite compounds derived from plants, especially phenolic compounds has potential to be natural antioxidants. Many studies suggest that phenolic group compounds such as flavonoids, phenolic acids, lignins, cinnamic acids, coumarins, tocopherols and tannins have their activities as natural antioxidants [6]. Antioxidants derived from plants are highly recommended in this case, they are safer for body and can block oxidative damage through reduction with free radicals, forming chelates with catalytic metal compounds, and capturing oxygen [7]. *Cissus adnata* (Roxb.) has been first time reported in our study for evaluation of several pharmacological activities. The other species of *Cissus* genus has been proven to possess certain activities. *Cissus hamaderohensis* possesses anti-viral activity [8]. *Cissus verticillata* can be used as an anti-cholesterol and anti-diabetic agent [9]. *Cissus rubiginosa* provides anti-diarrheal activity [10]. Considering the several activities the other species of Cissus genus plants possesses, *Cissus adnata* has been chosen for the determination of antioxidant property by performing the total phenolic content test and 2,2-diphenyl-1-picrilhidrazine (DPPH) free radical scavenging assay.

Materials and Methods
Plant collection and identification
The plant was collected from Bikrampur in Munshiganj district beside Dhaka city in February, 2017. After the collection of the plant, it was identified by Bangladesh National Herbarium providing the Accession ID 45961.

Chemicals
1, 1-diphenyl-2-picrylhydrazyl (DPPH), Dichloromethane, Petroleum ether, Chloroform, Methanol, Folin-Ciocalteu reagent, Na_2CO_3 were used.

Preparation of plant extract

After proper washing the whole plants were sun dried for a couple days. The dried plant were then ground to a coarse powder and extracted by soaking it in 2.5 liter of methanol. The mixture was kept for 15 days and occasional stirring was maintained. The whole mix was then filtered through a perfect cotton plug ultimately with a Whatman No.1 filter paper. The volume of the filtrate was then diminished using a Rotational evaporator at low temperature and weight. The heaviness of the grungy focus was 40gm.

***In vitro* antioxidant potential measuring assays**

Determination of DPPH radical scavenging activity

The free radical searching exercises (cell reinforcement limit) of the plant separates on the steady radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were assessed by the strategy [13]. For 2.0 ml of a methanol arrangement of the concentrate at various fixations were blended with 3.0 ml of a DPPH methanol arrangement (20 µg/ml). The cell reinforcement potential was examined from the blanching of purple shaded methanol arrangement of DPPH radical by the plant extricate when contrasted with that of tert-butyl-1-hydroxytoluene (BHT) and ascorbic corrosive (ASA) by UV spectrophotometer. DPPH was used to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plants [11, 12]. 2.0 ml of a methanol arrangement of the specimen (extractives/control) at various focus (500 µg/ml to 0.977 µg/ml) were blended with 3.0 ml of a DPPH methanol arrangement (20 µg/ml). After 30 min response period at room temperature in dim place the absorbance was measured at 517 nm against methanol as clear by UV spectrophotometer.

Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$(I\%) = (1 - A_{sample}/A_{blank}) \text{ X } 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).Extract concentration providing 50% inhibition *(IC_{50})* was calculated from the graph plotted inhibition percentage against extract concentration.

Determination of total phenolic content

The phenolic mixes apply their cancer prevention agent properties by redox response, which can assume an imperative part in retaining and killing free radicals, extinguishing singlet and triplet oxygen, or breaking down peroxides (Osawa, 1994). The antioxidative impact is primarily because of phenolic segments, for example, flavonoids, phenolic acids, and phenolic diterpenes [14 15]. Numerous phytochemicals have huge cell reinforcement limits that might be related with lower occurrence and lower death rates of malignancy in a few human populaces [16]. In the alkaline situation phenols ionize totally. At the point when Folin-Ciocalteu reagent is utilized as a part of this ionized phenolic arrangement the reagent will promptly oxidize the phenols. Common color of Folin-Ciocalteu reagent is yellow and after the oxidation procedure the color change into plain blue. The rate of changing in color is measured in a spectrophotometer at 760 nm. The absorbance will estimate the aggregate phenolic substance of the compound [17].

Result and Discussion

Table 1: % of inhibition in different concentration

Conc.(µg/ml)	ASA	MEF	PESF	DCMSF	CSF	ASF
500	98.59	98.59	44.23	44.51	98.59	93.24
250	98.31	98.31	27.04	72.96	97.18	69.29
125	95.77	95.77	17.46	42.54	77.75	43.38
62.5	93.24	93.24	12.96	33.8	57.18	29.86
31.25	80.85	80.85	12.39	20.85	34.93	17.18
15.625	72.39	72.39	6.19	15.49	23.19	9.58
7.813	60.85	60.85	6	6.19	9.01	9.29
3.906	47.61	47.61	3.09	4.89	8.73	8.73
1.953	50.7	50.7	1.97	3.66	8.16	7.32
0.977	45.63	45.63	0.36	0.85	7.64	6

ASA= Ascorbic acid, MESF= Methanol soluble fraction, PESF =Petroleum ether soluble fraction,
DCMSF= dichloromethane soluble fraction, CSF=Chloroform soluble fraction, ASF=Aqueous soluble fraction.

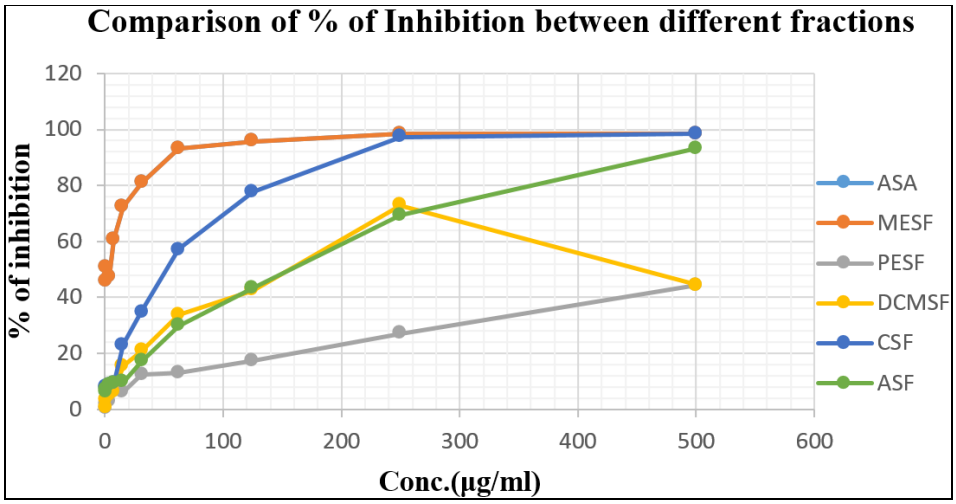

Fig 1: Result for DPPH radical scavenging assay of *Cissus adnata.*

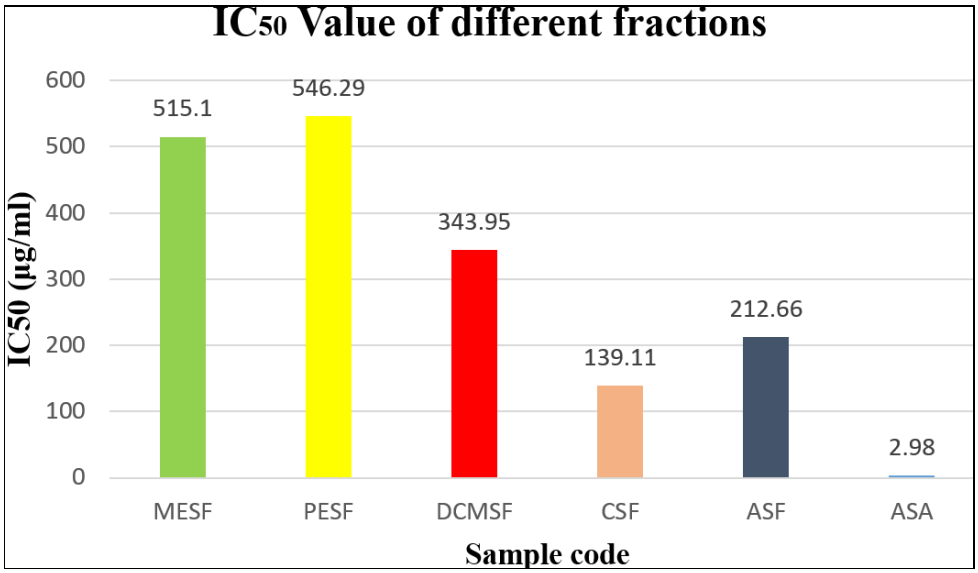

Fig 2: Comparison of IC_{50} values for different fraction

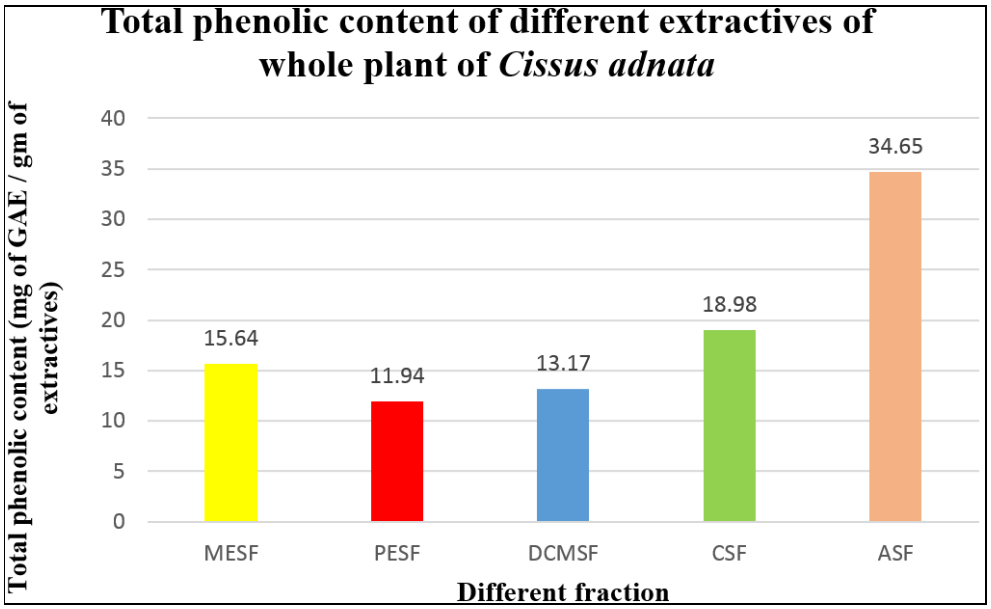

Fig 3: Graphical representation of total phenolic content (mg of GAE/ gm of extractives) of different extractives of whole plant *Cissus adnata*

DPPH free radicals are decolorize through the reaction of antioxidant compound where they further reacts with DPPH radical that produces purple color and it is converted in to 1-1-diphenyl-2-picryl hydrazine which have colorless property and mainly measured at 517 nm. This is used in vitro antioxidant activity to determine the DPPH radical scavenging activity of crude plant extracts. The whole plant *Cissus adnata* has shown potent scavenging activity at 500 µg/ml in methanol, petroleum ether, dichloromethane, chloroform and aqueous soluble fraction which is 98.59 µg/ml, 44.23 µg/ml, 44.51 µg/ml, 98.59 µg/ml, 93.24 µg/ml as compared to the positive control ascorbic acid. The activities were to some extent less than ascorbic acid fraction. The ascorbic acid fractions showed 98.59 µg/ml DPPH scavenging activity. The results are extrapolated in FG 1. DPPH radical scavenging activities are in dose dependent manner when the concentration increase extract inhibition of DPPH radical is also increased. Antioxidant plays a powerful role in food as well as biological system that evaluation the antioxidant activity.

Phenols and flavonoids are potent antioxidants. The total phenolic content of the whole plant *Cissus adnata* denotes that the aqueous soluble fraction has the highest level of phenol content comparing to the other fractions. So the phenolic content in methanol, petroleum ether, dichloromethane, chloroform and aqueous soluble fraction is 15.64 (mg of GAE/ gm of extractives), 11.94 (mg of GAE/ gm of extractives), 13.17 (mg of GAE/ gm of extractives), 18.98 (mg of GAE/ gm of extractives) and 34.65 (mg of GAE/ gm of extractives).

Conclusion

In this present study it can be seen that the extract of the whole plant of *Cissus adnata* in several solvent extractions were found to be effective DPPH scavenging assay. The methanolic extract is expected to be the ideal choice for oxidation reduction. The plant extract also denoted presence of phenol component which are to be potent antioxidants. So present study supports interesting inhibitory antioxidant activity against the reactive oxygen species. Further investigations will ensure much better consideration.

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