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Antioxidant Potential and Phytochemical Screening of *Aristolochiabraceelata*: An *in vitro* Approach

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Abstract: The present study was designed to investigate the antioxidant activity of *Aristolochiabraceelata* whole plant and fruit. Phytochemical study was piloted to detect the bioactive compounds, which have been responsible for the biological activities. The antioxidant activities were conducted via DPPH radical scavenging assay. Potential antioxidant activity was presented by ethanol crude extract was motivated to evaluate the fractions of n-hexane chloroform, ethyl acetate, n-butanol and water, the radical scavenging activities of whole plant were found to be 84 ± 0.02, 82.5 ± 0.04, 78.2 ± 0.04, 92.9 ± 0.03, 88.9 ± 0.02, 77.3± 0.05 respectively, while the fruit extracts displayed a higher potentials in crude and lower with fractions. The results of phytochemical screening showed the presence ofFlavonoids, Saponins, Alkaloid, Tannins, Phenols, Triterpene, Phytosterol, Anthraquinones and Carbohydrates. This study give rise to antioxidant property of studied plant, and showed interesting correlation with the phytochemical constituents and biological activities.

Keywords: *Aristolochiabraceelata*, Antioxidant, Phytochemical Screening, Radical Scavenging

1. Introduction

Oxidation is a chemical reaction that transfers electrons from asubstance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chainreactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducingagents such as thiols, ascorbic acid or polyphenols (Sies, 1997). Oxidative stress refers to the physiological condition at which the capacity of the endogenous antioxidant system fails to cope with the damaging effects of free radicals. Strong experimental evidences have been established about the oxidative stress theory of Alzheimer's disease pathogenesis where oxidative damage plays a major role in neurological degeneration (Mariani et al., 2005).

All plants produce chemical constituents, part of their normal metabolic activities(Tyler et al., 1981, Rosenthal et al., 1979). These, can be divided into primary metabolites, such as sugars, amino acids, nucleotides and fats, found in all plants, and secondary metabolites which have no obvious function in a plant'sprimary metabolism as well as in growth, photosynthesis, or other ''primary'' functions of theplant cell. They may possess an ecological role, as pollinator attractants, represent chemical adaptations to environmental stresses, or to be responsible for the chemical defence of the plant against microorganisms, insects and higher predators (Harborne et al., 1982, Evans et al., 1989). Many plant compounds have an outstanding role in medicine as drugs or as chemical model for the design and synthesis (or semisynthesis) of new drug molecules such as the opiates (from morphine and codeine models), aspirin from the naturally occurring salicylic acid (from willow-Salix spp.), or etoposide (semisynthetic

antineoplastic agent derived from the mayapple-Podophyllumpeltatum). Their pharmacological and economical value has lost nothing of its importance until today (Balandrin et al., 1985, Mann et al., 1994, Cassady et al., 1980). Phytotherapyhas been found to show potential in the treatment of ailments. Furthermore, the advantage of using phytotherapy resides in the concoction of chemicals found within plants, which potentially provide protection against a wide range of aetiological factors (Prerna and Vikas, 2010).The chemical diversity of the compounds found in nature makes plant and marine materials important potential sources of new drugs (Gareth Thomas, 2007). Almost all the medicinal plants available in the world have great potential sources for discovery as well as protection of new drugs of benefit to mankind. Presently, there is lot of approaches available to reach for new biologically active ingredients in the medicinal plants for the preparation of safe drugs. In ethnomedicine it is very common for a single plant species to be used to treat various ailments due to the multiplicity of bioactive molecules they contain. In this respect, medicinal plants provide a rich source of biologically active constituents with multiple activities. Check and need to check or systematic screening of these available traditional herbs may result in the discovery of novel effective bioactive compounds for the formulation of drugs. Phytochemical screening of the active morphological samples is extremely valuable in giving us information about the nature of constituents found in each plant sample (Tiwari et al., 2011).

*Aristolochiabraceolata*is commonly called “worm killer” in English due to supposed anthelminticactivity andtrypanocidal effect. It is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery, and snakebites. Aristolochiaceaehasbeenused bySudanese people as analgesic, antiscorpion, andantisnake. It is also used in the treatment of tumors and malaria and for fevers.

2. Materials and Methods

2.1. Plant Material

The *Aristolochiabraceelata*were purchased from the local market in Omdurman. The plant was identified in the Botany department, Faculty of science and technology, Omdurman Islamic University, by comparison with herbarium of the department. The plant was spread and dried in the shade for ten days and then pulverized with mechanical grinder.

2.2. Extraction

Extraction was carried out according to the method described by (Harbone., 1984). The fresh samples were dried in shades for 7 days, powdered then used for extraction. The shade-dried samples were socked in 80% ethanol in the ratio of (1:10) at room temperature for 3 days then filtered and they were left to dry at room temperature this process was repeated till the solvent at the last time returned to colourless. The weight of the solid residues were recorded and taken as yield of crude extracts. The yield a percentage was calculated as follows:

Percentage = (weigh of extract/weigh of sample) X 100.

2.3. Fractionation

The crude extracts were fractionated using liquid- liquid extraction methodology, which were carried by dissolving the samples in dist. H_2O then they were partitioned between n-hexane chloroform, ethyl acetate, and n-butanol using separation funnel apparatus.

2.4. DPPH Free Radical Scavenging Activity

The DPPH radical scavenging was determined according to the method of (Shimada et.al. 1992), with some modification. The test samples were allowed to react with 2.2 di(4-tretoctylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37^{o}C in 96-wells plate. The concentration of DPPH was kept at (300µM). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation decrease in absorbance was measured at 517nm using multiplat reader spectrophotometer. Percentage of radical scavenging activity of the sample was determined in comparison with a DMSO treated control. All tests were conducted triplicate.

2.5. Qualitative Phytochemical Evaluation

Phytochemical screening was conducted to determine the presence of natural products in the extract an obtained from the *Aristolochiabraceelata*fruit and whole plant.

The extracts obtained were subjected to following chemical tests for identification of various phytoconstituents using standard methods of (Trease and Evans, 1989; Odebiyi and Sofowora, 1978).

2.5.1. Test for Carbohydrates

Molisch’s test.

To the extract 1ml of the Molisch’s reagent was added then along the walls of the test tube carefully conc H_2SO_4 was added. Formation of a brown ring at the junction of the two liquids was observed.

Benedict’s Test.

Filtrates were treated with Benedict’s reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Barfoed’s test.

To the extract in a test tube 1ml of Barfoed reagent was added and boiled on the water bath. The solution was observed for the colour change reaction.

2.5.2. Detection of Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.5.3. Test for Tannins (Ferric Chloride)

0.5ml of the extract was boiled with 10ml of Distilled

water in a test tube and then, few drops of 0.1% Ferric Chloride solution was added and the reaction mixture was observed for blue, greenish black colour change.

2.5.4. Test for Saponins (Frothing Test)

0.5ml of the extract was added to 5ml of Distilled water in a test tube. The solution was shaken vigorously and observed for the stable persistent froth.

2.5.5. Test for Flavonoids

ThreeDifferent Tests Were Used for the Flavonoid Identification.

Alkaline reagent test.

To 0.5ml of extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Lead acetate Test.

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

KOH test.

To 0.5ml of extract was treated with few drops ofalcoholicpotassium hydroxide solution. Formation of intense yellow colour.

2.5.6. Test for Terpenoid and Steroids

Salkowski test was used to identification steroid and terpenoid.

To 0.5ml of each of the extract 2ml of chloroform was added and then 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids and steroids.

2.5.7. Test for Alkaloids

Two different tests were used for the identification of alkaloids

Dragendroff's Test.

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Wagner's Test.

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

2.5.8. Test for Anthraquinones (Borntragor's Test)

To 0.5ml of the extract 5 - 10ml of dilute HCL was added and boiled on water bath for 10 minutes and filtered. Then the filtrate was extracted with carbon tetra chloride and the equal amount of ammonia was added. Aftershaking the reaction mixture was observed for the formation of pink – red colour in the ammonia layer.

3. Results and Dissection

*Aristolochiabraceelata*ethanolic extracts of fruit and whole plant were able to inhibit the DPPH activity with 86 ± 0.05 and 84 ± 0.02 respectively theirs fractions was showed varied potentials shown in table (1). Ethyl acetate was mainly the most active maybe due the polarity of the active constituents.

Table 1. *Antioxidant activityAristolochiabraceelata extracts.*

sample	Antioxidant activity percentage					
	crude	hexane	$CHCl_3$	EtOAc	BuOH	H_2O
fruit	86 ± 0.05	41.4 ± 0.09	51.4 ± 0.06	87.2 ± 0.01	87.9 ± 0.01	77.3 ± 0.01
Whole plant	84 ± 0.02	82.5 ± 0.04	78.2 ± 0.04	92.9 ± 0.03	88.9 ± 0.02	77.3 ± 0.05

Table (2 and 3) indicate the presence of pharmacologically useful classes of compounds (Flavonoids, Saponins, Alkaloid, Tannins, Phenols, Triterpene, Phytosterol, Anthraquinones) tested for. These secondary metabolites have been shown to have therapeutic activities in plants and function in a synergistic or antagonistic fashion for the treatment of diseases (Trease and Evans, 1996).

Table2. *Phytochemical screening ofAristolochiabraceelata fruit.*

Family of compounds	Type of test	Inference				
		Hexane	$CHcl_3$	EtoAc	BuOH	H_2O
Carbohydrates	Molish's	+v	+v	+v	+v	+v
	Bendect test	+v	+v	+v	+v	+v
	Barafoids	+v	+v	+v	+v	+v
Flavonoids	Lead acetate	+v	+v	+v	+v	+v
	KOH	+v	+v	+v	+v	+v
	Alkiline test	+v	+v	+v	+v	+v
Saponins	Forth	-v	+v	-v	-v	+v
Alkaloid	Dragendorff's	+v	+v	+v	-v	-v
Tannins	$FeCl_3$	+v	+v	+v	+v	+v
Phenols	$FeCl_3$	+v	-v	+v	+v	+v
Triterpene	Salkowski	+v	+v	+v	+v	+v
Phytosterol	Salkowski	+v	+v	+v	+v	+v
Anthraquinones	Borntragor's	+v	+v	+v	+v	+v

+ve = positive result -ve = negative result.

Table3. *Phytochemical screeningofAristolochiabraceelatawhole plant.*

Family of compounds	Type of test	Interference				
		hexane	$CHCl_3$	EtOAc	BuOH	H_2O
Carbohydrates	Molish's	+v	+v	+v	+v	+v
	Bendect	+v	+v	+v	+v	+v
	Barafoids	+v	+v	+v	+v	+v
Flavonoids	Lead acetate	+v	+v	+v	+v	+v
	KOH	+v	+v	+v	+v	+v
	Alkiline test	+v	+v	+v	+v	+v
Saponins	Forth	+v	+v	+v	-v	+v
Alkaloid	Dragendorff's	+v	+v	+v	-v	-v
Tannins	$FeCl_3$	+v	+v	+v	+v	+v
Phenols	$FeCl_3$	-v	-v	+v	+v	+v
Triterpene	Salkowski	+v	+v	+v	+v	+v
Phytosterol	Salkowski	+v	+v	+v	+v	+v
Anthraquinones	Borntragor's	+v	+v	+v	-v	-v

+ve = positive result -ve = negative result.

The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. Plant phenolic compounds especially flavonoids are currently of growing interest owning to their supposed properties in promoting health (anti-oxidants) (Rauha et al., 2000); Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages (Mark Percival, 1998). Tannins are reported to possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitatingmicrobial proteins and making nutritional proteins unavailable for them; they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent anti-oxidants (Trease and Evans, 1983; Tyler et al., 1988; Awosika, 1991; Ogunleye and Ibitoye, 2003). They act as binders and for treatment of diarrhea and dysentery (Dharmananda, 2003). Tannin also reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannin are able to inhibit HIV replicationselectivity and is also used as diuretic (Heslem, 1989). Plant tannin has been recognized for their pharmacological properties and is known to make trees and shrubs a difficult meal for many caterpillars (Aiyelaagbe and Osamudiamen, 2009). Plant steroids are known to be important for their cardiotonic, insecticidal and anti-microbial properties. They are also used innutrition, herbal medicine, cosmetics and they are routinely used in medicine because of their profound biological activities (Denwick, 2002). Saponins have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotonicin nature and are reported to have anti-diabetic and anti-fungal properties (Finar, 1989; Trease and Evans, 1989; Kamel, 1991).

4. Conclusion

All extracts of *Aristolochiabraceelata*fruit and W. P showed potent antioxidant activity. This might be attributed to the presence of phenols, flavonoids and tannins compounds in this plant. The effect of this plant bioactivities, and toxicological investigation and Further purification, need to be carried out also further phytochemical investigation of active constituents might be present is required.

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