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Research Article

PHYTOCHEMICAL SCREENING AND EVALUATION OF (*INVITRO)* ANTIOXIDANT ACTIVITY OF *ACHYRANTHES ASPERA LINN* ROOT EXTRACT

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ABSTRACT

Objective: The aim of the present study was to investigate the different Phytoconstituents and *invitro* antioxidant potential of the Bethe part of the plant (*Achyranthes aspera Linn*).

Methods: Preliminary Phytochemical Screening analysis was determined using J.B. Harborne standard protocol methods. The antioxidant activity of the crude plant extracts of *Achyranthes aspera Linn* were determined using spectroscopic method against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Hydroxyl Radical Scavenging method.

Results: Qualitative phytochemical screening analysis reveals presence of alkaloids, tannins, saponins, steroids, terpenoids, flavanoids, phenols, tannins, phytosterols, fixed oil, fats and cardiac glycosides. The crude plant root extracts showed potent antioxidant activities against the tested methods.

Conclusion: It signifies that the Plant-derived phenolics and flavonoids represent good sources of natural antioxidants. From the above results it seen that this plant exhibits significant antioxidant activity.

Keywords: *Achyranthes aspera Linn*, Phytoconstituents, Antioxidant activity, DPPH and Hydroxyl radical scavenging activities.

INTRODUCTION

Many medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, (or) decomposing peroxides. Many of these phytochemical possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases [1]. Various human diseases are caused through oxidative damage that results from an imbalance between the development and neutralization of pro-oxidants [2]. Oxidative damage is caused by free radicals, such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxynitrite [3-5]. Antioxidants are known to inhibit oxidation stress [6]. Reactive Oxygen species (ROS), including free radicals such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxynitrite radicals are active oxygen species that are often generated by biological oxidation reaction by exogenous factors [7,8]. These oxidative mediators can lead to the damage of biological structures such as nucleic acids, proteins and lipids [9]. Many free radicals have been implicated in the causation of several diseases such as liver chirrhosis, atherosclerosis, cancer, diabetes, ageing and Alzheimer's disease [10-15].

Achyranthes Aspera L. belongs to the family *Amarantheceae*. It is an erect, annual herb, distributed in the hilly districts of India [16]. The plant is used in indigenous system of medicine such as anti-bacterial [17], anti-viral [18], anti-cancer [19], anti-oxidant [20], anti-inflammatory and anti-arthritic activity [21],

Anti-fertility [22], anti-plasmodic [23] and anti-tumor activities [24, 25]. It is also used in the treatment of dropsy, rheumatism, stomach problems, cholera, skin diseases and rabies [26, 27]. The juice extracted from the root of this plant, mixed along with the root extracts of *Urena lobata* and the bark of *Psidium guajava*, are used in the treatment of diarrhoea and dysentery [28]. Extensive literature survey has been done on this particular plant material showed that, only few phytoconstituents were isolated and identified in this plant material [29].

With this view in mind, the present attempt has been made to study the qualitative phytochemical screening analysis and antioxidant activities of the root part of the plant material - *Achyranthes Aspera L.*

MATERIALS AND METHODS

Plant material

The root part of the plant material *Achyranthes Aspera L.* was collected from ABS Botanical Gardens, Salem District, during the month of December 2010. The plant material was authenticated by Dr. A. Balasubramanian - Executive Director, ABS Botanical Conservation, Research and Training centre, Salem District, Tamil Nadu, and India. The roots were cleaned and dried. The leaves were dried in hot air woven at 55°C for 3 days and at 40°C for the next 4 days.

Extraction

The dried roots were crudely powdered and extracted with solvents like n-hexane, petroleum ether, ethyl acetate, ethanol, chloroform and water using Soxhlet apparatus (J.B.Harbrone 1973) at 50°C. The solvent was completely removed and the dried crude extracts were used for investigation. Further the crude plant extracts were subjected to phytochemical study as well as antioxidant screening.

Phytochemical Screening analysis

Chemical tests were carried out on the different crude plant extracts using standard protocol to identify the different Phytoconstituents [30,31].

Antioxidant activity - DPPH method

The antioxidant properties were determined by DPPH radical scavenging method [32]. The different extracts were measured in terms of hydrogen donating (or) radical scavenging ability using a stable radical DPPH. Briefly, 1mL of 0.1mM methanolic solution of DPPH was added to 3mL of the crude plant extracts at different concentrations in methanol (10, 20, 50, 75, 100 ppm). The samples were kept in the dark for 30minutes after which the absorbance was measured at 517 nm in UV spectrophotometer (Systronics 2202). In the radical form, DPPH absorbs light radiations at 517 nm, but upon reduction by an antioxidant (or) a radical species its absorption

decreases. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Butylated Hydroxy Toluene (BHT), which is a good antioxidant, is taken as a standard in this study. The capability to scavenge the DPPH radical was calculated using the following equation:

$(A_0 - A_{test})/ (A_0 - A_{ref}) \times 100$

Whereas A_0 is the initial absorbance (DPPH + sample absorbance) and A_{ref} and A_{test} are absorbance after 5 min with BHT solution and sample solution.

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was determined using standard protocol [33]. Various concentrations (10, 20, 50, 75, 100 ppm) of the crude plant extracts in ethanol were taken in different test tubes and evaporated to dryness. One milliliter of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5mL of EDTA (0.018%), and 1mL of DMSO (0.85% v/v in 0.1M phosphate buffer, pH-7.4) were added to these tubes, and the reaction was initiated by adding 0.5mL of 0.22% ascorbic acid. The test tubes were capped tightly and heated on a water bath at 80-90°C for about 15mins. The reaction was completed by the addition of 1mL of ice-cold TCA solution (17.5% w/v). Three milliliters of Nash reagent (18.75g of ammonium acetate, 0.75mL of glacial acetic acid and 0.5mL of acetyl acetone were mixed and raised to 250mL with distilled water) was added to all of the tubes and left at room temperature for about 15mins for color development.

The intensity of the yellow color formed was measured spectrophotometrically at 412nm against reagent blank. The percentage hydroxyl radical scavenging is calculated by the following formula:

% hydroxyl radical scavenging activity) = 1-(difference in absorbance of sample/difference in absorbance) x 100

RESULTS AND DISCUSSION

The crude root extracts of *Achyranthes aspera L.* were subjected to their qualitative investigation for the presence of Phytoconstituents or secondary metabolites like alkaloids, carbohydrates & glycosides, saponins, phytosterols, terpenoids, phenols, tannins, and flavanoids.

Preliminary phytochemical analysis results were mentioned in the Table-1.

Table 1: Phytochemical Screening analysis of the crude plant extracts – *Achyranthes aspera L.*

Plant Constituent	n-hexane	Pet. ether	$CHCl_3$	Ethyl acetate	Ethanol	Water
Alkaloids	----	----	**++++**	----	**++++**	**++++**
Carbohydrates & glycosides	----	----	----	----	**++++**	**++++**
Phytosterols	----	**++++**	----	----	**++++**	----
Phenols & Tannins	----	----	----	----	**++**	**++++**
Terpenoids	----	**++++**	**++++**	----	----	----
Saponins	----	----	----	----	**++++**	++++
Flavanoids	----	----	----	----	**----**	**++++**
Fixed oil and fats	**++++**	----	----	----	----	----
Steroids	**----**	**----**	**----**	**++++**	**----**	**----**

++++ / ++ = Presence of Phytoconstituents; ---- = Absence of Phytoconstituents.

The qualitative phytochemical investigation tests were performed in all six crude plant extracts (n-hexane, Pet. ether, Chloroform, Ethyl acetate, Ethanol and water) revealed the presence of alkaloids, carbohydrates & glycosides, saponins, phytosterols, terpenoids, phenols & tannins, fixed oil & fats and flavanoids. Ethanolic extract of the drug showed the existence of carbohydrates and glycosides, saponins in high concentration followed by alkaloids and phytosterols, while the phenols and tannins were present in lower concentration. But in the case of ethyl acetate and water extracts, phenols and tannins were present in higher concentration. In other hand, terpenoids were present in high concentration in chloroform and petroleum ether extracts. The existence of flavanoids in aqueous crude plant extracts in a lower concentration with high percentage yield. The occurrence of phytoconstituents is considered to be responsible for the therapeutic action.

Antioxidant activity of the crude plant extracts were studied using two different methods - DPPH and hydroxyl radical scavenging. The results were illustrated in Table -2&3. The samples were prepared at different concentrations in alcoholic solutions (10, 20, 50, 75, 100 µg/mL). Butylated hydroxytoluene and ascorbic acid were used as a standard in this study. Petroleum ether, ethyl acetate and water extracts of *Achyranthes aspera L.* showed potent antioxidant activity, where n-hexane, chloroform and ethanolic extracts showed modest antioxidant activity.

The IC_{50} [DPPH = 0.13, 0.96 and 0.76 µg/ml; (OH^{-}) = 0.13, 0.94 and 0.73µg/ml] values of petroleum ether, ethyl acetate and water extracts of *Cleome gynandra* L. showed potent antioxidant activities, whereas IC_{50} [DPPH = 0.11, 0.04 and 0.26µg/ml; (OH^{-}) = 0.86, 0.04 and 0.25µg/ml] values of n-hexane, chloroform and ethanolic extracts were attributed less antioxidant activity. Significant antioxidant activity of the petroleum ether, ethyl acetate and water extracts were there due to the presence of secondary metabolites like phenols, flavanoids and terpenoids. The plant (*Achyranthes aspera L.*) studied here can be seen as a potential source of free radical scavenging drugs. Further studies are going on the plant (*Achyranthes aspera L.*) in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

Table 2: Antioxidant activity of the crude plant extracts - *Achyranthes aspera Linn* – DPPH Method

Crude Plant Extracts	% Inhibition Concentration (µg/ml)						
	10	**20**	**50**	**75**	**100**	***IC50***	***r²***
n-hexane	11.63	28.96	41.45	63.62	78.97	0.11	0.977
Pet. Ether	9.07	19.25	57.24	74.92	83.71	0.13	0.956
Ethyl acetate	22.32	85.77	91.92	95.83	97.84	0.96	0.511
Ethanol	7.81	15.62	29.36	52.33	67.35	0.04	0.988
Chloroform	16.71	24.91	33.34	49.15	59.42	0.26	0.985
Water	36.71	56.21	67.75	81.82	96.92	0.76	0.957
BHT	44.75	62.44	81.32	93.21	96.56	0.97	0.908

Table 3: Antioxidant activity of crude plant extracts – *Achyranthes aspera Linn - Hydroxyl Radical Scavenging Activity*

Crude Plant Extracts	% Inhibition Concentration (µg/ml)						
	10	**20**	**50**	**75**	**100**	***IC50***	***r²***
n-hexane	8.93	20.67	41.12	63.67	78.75	0.86	0.994
Pet. Ether	9.14	19.48	57.52	75.12	84.02	0.13	0.955
Ethyl acetate	22.07	84.82	91.76	95.55	97.27	0.94	0.518
Ethanol	7.77	15.42	29.06	51.97	67.13	0.04	0.988
Chloroform	16.35	24.36	32.65	48.25	58.45	0.25	0.985
Water	35.75	55.85	67.33	81.42	96.76	0.73	0.954
BHT	44.56	62.34	81.22	93.12	96.36	0.95	0.906

CONCLUSION

The results of the preliminary phytochemical screening analysis clearly confirmed the presence of several phytoconstituents in the root part of the plant - *Achyranthes aspera Linn*. In both antioxidant methods, the petroleum ether, ethyl acetate and water extracts (IC_{50} range -0.13-0.96µg/mL) showed an interesting antioxidant property of *Achyranthes aspera Linn* which have an inhibiting capacity against the reactive oxygenated species and anticancerigenos. Based on the antioxidant activity results, the plant material can be used as a drug for radical scavenging diseases in future.

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