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Antioxidant Activity of Wood and Leaf Extracts of *Achyranthes aspera* Using Radical Scavenging Method

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Abstract

***Achyranthes aspera* Linn. is an indigenous herb. It is traditionally used as an abortifacient. The aqueous extract is given as a medicine against pneumonia. The aqueous extracts of this plant were screened for their antioxidant activity using DPPH radical scavenging method. The standard used is an ascorbic acid and the phytochemicals exhibited better activity than the standard compound. This extract is comprised of alkaloids and terpenoids as its major constituent and the biological activity can be attributed to the presence these compounds.**

Keywords:* *Achyranthes aspera; Aqueous extract; Phytochemicals; Antioxidant activity

Introduction

The countries like India and China are well known for the various applications of medicinal plants for the treatment of diseases. The polyherbal and herbal therapies are employed combine for effective medication with minimum or no side effect [1]. In the treatment of cancer and diabetics, some of these methods were found to be more useful than the conventional methods. Many medicinal plants show the antioxidant and antidiabetic properties [2]. Most of these plants contain glycosides, alkaloids, terpenoids and flavanoids [3]. Many countries are using different plant species for the prevention and control of diabetics. Naturally derived drugs are having low side effects as compared to synthetic drugs. The root extracts of *Achyranthes aspera* is known to possess the diuretic property [4]. The dried plant is used for the treatment of gonorrhoea, fever, dysentery, asthama, hypertension and diabetics. The flowering spikes are used for external application in case of reptile and snake bite treatment [5]. The plant is useful in liver complaints, skin diseases [6]. Ash of the plant is used for the treatment of ulcer and warts [7]. Considering the wide range of applications of this plant, we have designed our study to

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screen the biological activity of the same. For that purpose, we have prepared the aqueous extracts of the plant and stored them in the refrigerator and used whenever required.

Materials and Methods

Collection of plants

Achyranthes aspera was collected from Pune in Maharashtra state, India. Fresh plants were used for extraction and isolation of their phytochemicals. Phytochemicals and extracts were stored in the refrigerator and used whenever needed.

Extraction

Aqueous extraction: The 25 g of dried and crushed plant material was soaked in 25 mL, 50 mL and 100 mL of distilled water respectively for 24 h. The extracts were filtered through the muslin cloth. The final volumes were corrected to viz. 25 mL, 50 mL and 100 mL by washing residue with distilled water and used for all activity.

Procedure for isolation of phytochemicals: The phytochemicals were isolated using known method [8]. Whole fresh plant material weighing 35 g *Achyranthes aspera* was dried, powdered and homogenized by methanol: water mixture (4:1). Then it was filtered and the filtrate was evaporated. $2MH_2SO_4$ was added and the organic compounds were extracted with chloroform to furnish terpenoids. Aqueous layer was basified with NH_4OH and then extracted with chloroform-methanol (3:1). This extract afforded most of the alkaloids whereas remaining aqueous basic layer was evaporated and extracted with methanol yielded quaternary alkaloids. During purification of quaternary alkaloids, tannins were separated.

Phytochemicals: The phytochemicals were analyzed and identified using existing characterization techniques.

Dilution and concentration: Dilution and concentrations were prepared using literature procedure [9].

Dilution of water extracts: 25 mL, 50 mL and 100 mL of these dilutions were prepared by washing residue with distilled water and these dilutions were used for evaluation of all the activity.

Concentration of isolated phytochemicals: 50 µg/mL, 100 µg/mL, 200 µg/mL and 500 µg/mL concentration were prepared using respective solvent for DPPH radical scavenging activity [10,11].

DPPH radical scavenging activity: Free radical scavenging activity of different extracts of leaves and flowers of *Achyranthes aspera* Linn. Plant was measured by 1, 1-diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 mL) was added to 3 mL. of different extracts in Methanol at different concentration (50, 100, 200, 500 ppm). Here, only those extracts are used which are soluble in methanol and their various concentrations were prepared by dilution. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min; the absorbance was measured at 517 nm by using spectrophotometer.

Reference standard compound used was ascorbic acid and experiment was done in triplicate. The value of the sample, which is the concentration of samples required to inhibit 50% of the DPPH free radical, was calculated using long dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity (TABLES 1 to 3). The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or % inhibition=A0-A1/A0 × 100.

Where A0 was the absorbance of control reaction and A1 was the absorbance in presence of test or standard sample. Control absorbance=0.2492.

Results and Discussion

TABLE 1. **Absorbance of different isolated phytochemicals with standard ascorbic acid at 517 nm.**

Isolated phytochemicals of plant	Absorbance			
	50 ppm	100 ppm	200 ppm	500 ppm
Terpenoids	0.2003	0.1972	0.1771	0.1436
Alkaloids	0.2369	0.2261	0.2161	0.2132
Quaternary alkaloids	0.2442	0.1741	0.1349	0.1212
Tannins	0.2476	0.2400	0.2292	0.1247
Ascorbic acid	0.0530	0.0425	0.0190	0.0054

TABLE 2. **% Inhibition of different isolated phytochemicals with standard ascorbic acid.**

Isolated phytochemicals of plant	% of inhibition			
	50 ppm	100 ppm	200 ppm	500 ppm
Terpenoids	19.62	20.86	28.93	42.37
Alkaloids	4.93	9.26	13.28	14.44
Quaternary alkaloids	2.00	30.13	45.86	51.36
Tannins	0.64	3.69	7.78	49.95
Ascorbic acid	78.73	82.94	92.37	97.83

TABLE 3. **Absorbance of different aqueous extracts at 517 nm.**

Extraction of plant	Concentration in mL	Absorbance
Aqueous extract	E-1	0.2243
	E-2	0.2395
	E-3	0.2447
E-1: 25 g crushed plant material in 25 mL distilled water E-1: 25 g crushed plant material in 50 mL distilled water E-1: 25 g crushed plant material in 100 mL distilled water		

This study determined that isolated phytochemicals and aqueous extract of *Achyranthes aspera* species showed better antioxidant potential by DPPH radical scavenging method compare to standard ascorbic acid (TABLE 4). The absorbance at 517 nm by UV visible spectrophotometer were found to be as compared to ascorbic acid more % of inhibition showed at 25% means it is nothing but 100% aqueous extract. In isolated phytochemicals 50 ppm terpenoid showed high percentage of inhibition as compared to other phytochemicals. In 100 ppm, 200 ppm and 500 ppm quaternary alkaloid showed high % of inhibition as compared another isolated phytochemical.

TABLE 4. **% Inhibition of different aqueous extract.**

Extraction of plant	Concentration in mL	% Inhibition
Aqueous extract	E-1	9.11
	E-2	2.95
	E-3	0.85
E-1: 25 g crushed plant material in 25 mL distilled water E-1: 25 g crushed plant material in 50 mL distilled water E-1: 25 g crushed plant material in 100 mL distilled water		

Conclusion

By DPPH radical scavenging activity aqueous and isolated phytochemicals are exhibited better activity or better % of inhibition with standard ascorbic acid. The method used for activity is simple, fast and economical.

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