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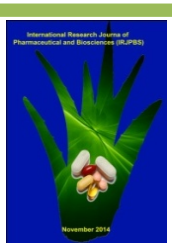

Phytochemical evaluation and Antioxidant potential of ethanolic leaf extract of *Achyranthes aspera*

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Abstract

The essential for antioxidant agents, which can inhibit oxidative stress, has become a major priority. *Achyranthes aspera* could constitute a reservoir of effective biomolecules useful as antioxidants. *Achyranthes aspera* is a species of plant in the Amaranthaceae family. It is distributed throughout the tropical world. It can be found in many places growing as an introduced species and a common weed. As it is known to be a source of chemical compounds which are characterized by antioxidant properties, this medicinal shrub is used in traditional Pharmacopoeia. The originality of the present study consists in scrutinizing the phytochemical composition and evaluating the antioxidant activity of ethanolic extract of *Achyranthes aspera* in conditions corresponding to its traditional use. Phytochemical screening was performed to assess the qualitative chemical composition of *Achyranthes aspera* using precipitation and coloration reactions. In addition, the total alkaloid contents were determined by using standard method. Finally, *Achyranthes aspera* was assayed to determine its antioxidant activities using Phosphomolybdenum assay. The results reveal the presence of several biomolecules in *Achyranthes aspera* such as alkaloids, steroids, saponins, cardiac glycosides, and fixed oils. Besides, the quantitative analyses show a considerable total alkaloid contents. In sum, it was found that *Achyranthes aspera* possess a high antioxidant activity against many problems, which justifies its use by traditional healers. Consequently, *Achyranthes aspera* is a good source of antioxidants.

Key words: *Achyranthes aspera*, phytochemicals, antioxidants, alkaloid, phosphomolybdenum method.

INTRODUCTION

Achyranthes aspera L. (Amaranthaceae) is distributed as weed throughout India, tropical Asia and other parts of the world. Ayurvedic, Yunani practitioners and Kabirajes use different parts of the plant to treat leprosy, asthma, fistula, piles, arthritis, wound, insect and snake bite, renal and cardiac dropsy, kidney stone, diabetes, dermatological disorders, gynecological disorders, gonorrhea, malaria, pneumonia, fever, cough, pyorrhea, dysentery, rabies, hysteria, toothache etc. The plant is a popular folk remedy in traditional system of medicine throughout the tropical Asian and African countries. The plant is reported to be used as antimicrobial, larvicidal, antifertility, immunostimulant, hypoglycemic, hypolipidemic, anti-inflammatory, antioxidant, diuretic, cardiac stimulant, antihypertensive, anti-anasacra, analgesic, antipyretic, antinoiceptive, prothyrodic, antispasmodic and hepatoprotective. Phytochemical investigations revealed the presence of sterols, alkaloids, saponins, steroid, cardiac glycosides etc from ethanolic extract of *Achyranthes aspera* [1].

Oxidative stress refers to an imbalance between the production of free radicals and the antioxidant system. Free radicals are widely believed to be involved in the etiology of many diseases such as ageing, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammation as indicated by the signs of oxidative stress. This brings the need for antioxidant agents which can prevent oxidative stress by retarding or inhibiting the oxidation possibly by free radicals in biological system. However, synthetic antoxidants such as butylated hydroxyanisole and butylated hydroxytoluene are considered to be responsible for liver damage and carcinogenesis. Therefore, it is essential to develop natural non-toxic antioxidants to protect human body from free radicals and retard the progress of many chronic diseases. Several plants are known to be a source of chemical compounds which are characterized by antioxidant properties. These antioxidants compounds could possess the ability to protect the cellular organelles from damage caused by free radicals induced oxidative stress. *Achyranthes aspera* are known by their resistance to several stress factors. Under extreme climatic conditions, Achyranthes aspera could constitute a reservoir of new natural, safe and effective biomolecules potentially useful as Antioxidants [2].

The aims of the present study was to evaluate phytochemical screening and also measure the alkaloid content using harborne method and identify an antioxidant activity of ethanol extract of *Achyranthes aspera* using phosphomolybdenum assay.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in the present study were purchased from reliable firms like Merck, Southern scientific and were of analytical grade.

Experimental plant

Achyranthes aspera was selected for the present study. Leaves of *Achyranthes aspera* were collected seasonally from campus of Adhiparasakthi Agricultural College, G.B.Nagar, Kalavai. Vellore district. Tamilnadu.

Extraction of phytochemicals using soxhlet apparatus by ethanol as a solvent

The leaf powder (*Achyranthes aspera*) of 25 g was weighed using weighing balance. Then the leaf powder was covered by muslin cloth and kept in soxhlet extractor. In the Round bottom flask, 170 ml of solvent namely ethanol was added. The set up was switched on. After completion of seven cycles, the round bottom flask containing ethanolic extract (*Achyranthes aspera*) was set to maintain at room temperature.

Concentration by Rotary vacuum evaporator (ROTEVA)

The ethanolic extract was concentrated by rotary vacuum evaporator at the respective boiling point. Here the solvent and the compound were collected separately. The concentrated ethanolic extract was transferred to the preweighed beaker. Also the weight of the extract in the beaker was recorded.

Extractive yield (%)

Further the extract yield % was calculated.

Extract yield % = ((Extract + Beaker weight) - (Empty beaker weight))/Sample weight *100

These ethanolic extract were further used for the following experiments.

Preliminary phytochemical screening

Qualitative phytochemical analysis of etanolic extract of *Achyranthes aspera* was carried out as follows using standard procedures.

Carbohydrate

Benedict's test: To 0.5 ml of filtrate 0.5ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 minutes. A reddish brown precipitate indicates the presence of reducing sugar [3] .

Protein

Millon's Test: To 2 ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins [4].

Alkaloid

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids [3].

Tannin

1 g of each powdered sample was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot.1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2- 3) of 10 % ferric chloride were observed for any formation of precipitates and any colour change. A bluish-black or brownish-green precipitate indicated the presence of tannins [3].

Phenol

Nitric acid test: The extract was treated with dilute nitric acid separately. Formation of reddish to yellowish colour indicates the presence of phenol.

Flavonoids

Shinoda Test: To the extract, few fragments of Magnesium ribbon and concentrated hydrochloric acid was added. After a few minutes, appearance of red to pink colour indicates the presence of flavonoids [4].

Terpenoids/Triterpenoids

Salkowski Test: To the extract few drops of concentrated H_2SO_4 and 2ml chloroform and shaken then allow standing, appearance of golden yellow colour indicates the presence of triterpenes (4).

Steroid

Bubble test: To the 1ml extract, add 5 ml of distilled water shake vigorously. Formation of foam indicates presence of steroids [7].

Saponin

Foam Test: Small amount of extract add little quantity of water. If foam produced on shaking persists for 10 minutes, indicates presence of Saponins [3].

Glycosides

To the extract, 2 ml of glacial acetic acid and 2 drops of ferric chloride was added. Finally 2 ml of conc. H_2SO_4 was added along the sides of the test tube. A brown ring indicates the presence of glycosides (7).

Volatile Oil

To the extract, 1ml of 0.1M NaOH solution and 1% aqueous HCl was added to form white precipitate for the presence of volatile oil (6).

Fixed Oil

To the extract, 1 ml of 1% copper sulphate and few drops of (10%)sodium hydroxide solution was added. The formation of clear blue colour confirms the presence of fixed oil (8).

Balsams

To the extract, add 10ml of 90% ethanol and filter. Add two drops of 10% alcoholic ferric chloride solution to form dark green coloured solution for the presence of balsams (6).

Resins

Dissolve the extract in acetone and pour the solution in distilled water. Turbidity indicates presence of resins [6].

Estimation of Alkaloid

Alkaloid determination using Harborne (1973) method: 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [9].

$$\% \text{ of Alkaloid} = \frac{W2 - W1}{W} * 100$$

Where W = weight of sample; W1 = Weight of empty filter paper;

W2 = Weight of paper + precipitate.

Evaluation of total antioxidant activity by phosphomolybdenum method

To a series of fresh tubes respective volumes of ascorbic acid standard and sample was added. The volume was made up to 1ml with distilled water. Reagent solution of 3ml was added to the entire test tubes and vortexed well. The test tubes were incubated at 90° C for 90 minutes. After cooling the tubes; the absorbance was read at 695nm. A standard graph equivalent of ascorbic acid was determined to the antioxidant concentration of sample [10].

RESULT AND DISCUSSION

Biologically active compounds usually occur in low concentration in plants. An extraction technique is that which is able to obtain extracts with high yield and with minimal changes to the functional properties of the extract required [11]. Soxhlet apparatus and rotary vacuum evaporator is used for extraction process is shown in figure 3 and 4. Ethanolic extract yields percentage is indicated in table 1 and in figure 5. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [12]. Analysis of the plant extracts revealed the presence of phytochemicals such as saponins, glycosides, steroids and alkaloids.

Figure 1: *Achyranthes aspera*

Figure 2:Sample preparation

Figure 4: Rotary Vacuum Evaporator

Figure 3: Soxhlet extraction

Figure 5: Concentrated ethanolic extract

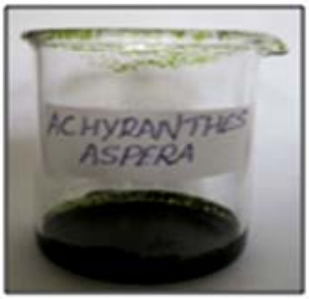

Figure 6: Ethanolic extract of *Achranthes aspera* (After drying)

Table 1: Extract yield (%) for the ethanolic extract of *Achyranthes aspera.*

Extract yield (%)					
Name of the extract	**Empty beaker wt (g)**	**Beaker + Sample wt (g)**	**Sample wt (g)**	**Extract yield % (25g)**	**Extract yield % (100g)**
Ethanol	30.6136	32.2015	25	1.5879	6.3516

The phytochemical characteristics were summarized in the (Table 2 and in figure 7). Identification of plant chemical constituents is desirable because such information will be value for synthesis of complex chemical substances. The plant products over synthetic compound in the treatment of diseases are needed, because it does not have a deleterious effect in higher plants and animals including man. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plant proved to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Based upon the preliminary phytochemical test, Quantitative determination of alkaloid phytoconstituents were carried out for the powdered plant material by standard methods and found that alkaloid 0.626%w/w, present in this plant is noted in Table no 3 and in figure 8.

Table 2: Phytochemical analysis of ethanolic extract of *Achyranthes aspera.*

S.NO	PHYTOCHEMICALS	TEST	ETHANOLIC EXTRACT OF *ACHYRANTHES ASPERA*
1	CARBOHYDRATE	BENEDICT'S TEST	-
2	PROTEIN	MILLON'S TEST	-
3	ALKALOID	WAGNER'S TEST	+
4	TANNIN	FERRIC CHLORIDE TEST	-
5	PHENOL	NITRIC ACID TEST	-
6	FLAVONOID	SHINODA TEST	+
7	STEROID	BUBBLE TEST	+
8	SAPONIN	FOAM TEST	+
9	GLYCOSIDES	KELLER-KILANI TEST	+
10	FIXED OIL		+
11	VOLATILE OIL		-
12	RESIN		-
13	BALSAM		+
14	TERPENOID	SALKOWSKI TEST	-

+ - Present; - -Absent

Antioxidant activity of the antioxidants is concerning with those compounds capable of protecting the organism system against the potential harmful effect of oxidative stress [13].

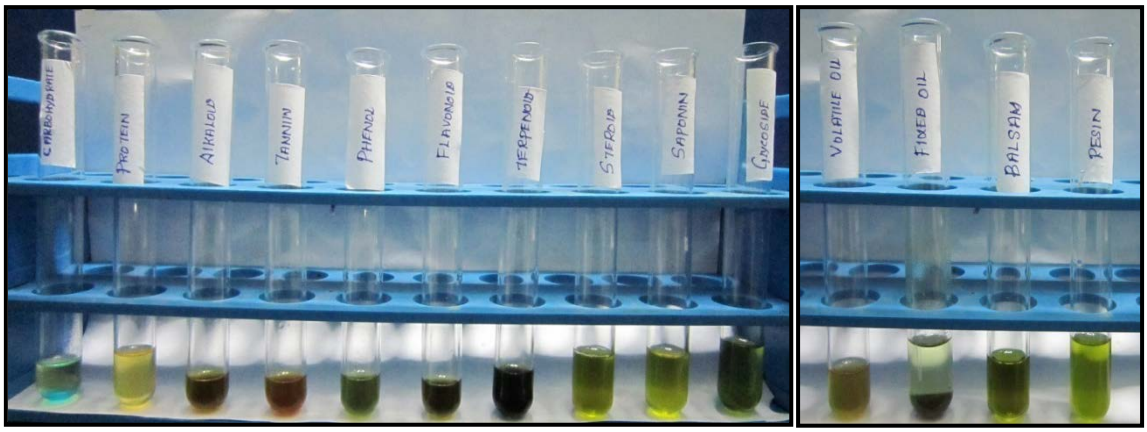

Figure 7: Phytochemical screening (qualitative method) for the ethanolic extract of *Achyranthes aspera*

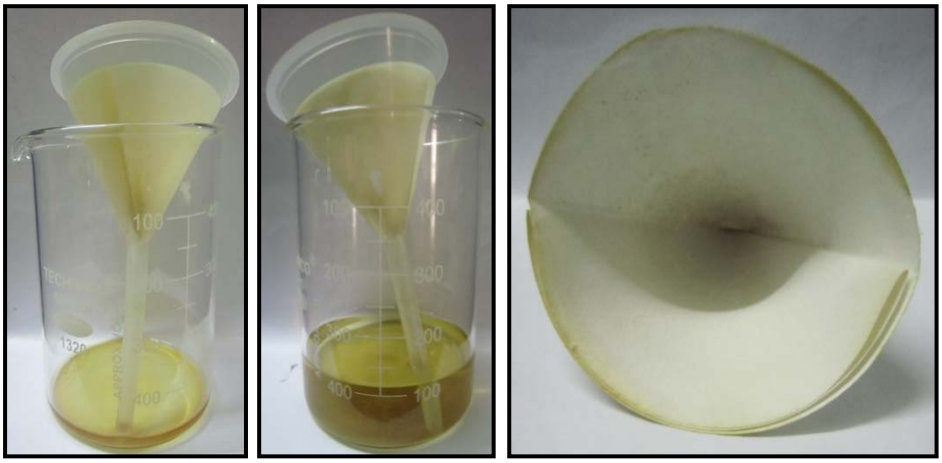

Figure 8: Quantitative method (Alkaloid)

Table 3: Alkaloid estimation of *Achyranthes aspera* ethanolic extract

Estimation of alkaloid				
Sample name	**Sample weight (g)**	**Empty filter paper weight (g)**	**Precipitate weight (g)**	**Alkaloid (%)**
Achyranthes aspera	5	1.0812	1.1125	0.626

Table 4: *Achyranthes aspera* ethanolic extract antioxidant assay by PMA

Description	B	S1	S2	S3	S4	S5	A1	A2	A3	A4	A5
Standard /Sample (ml)	0	0.1	0.15	0.2	0.25	0.3	0.1	0.15	0.2	0.25	0.3
Distilled water	Make up the volume to 1ml										
Reagent solution	3ml										
Incubation	95 °C for 90 minutes										
Absorbance	695nm										

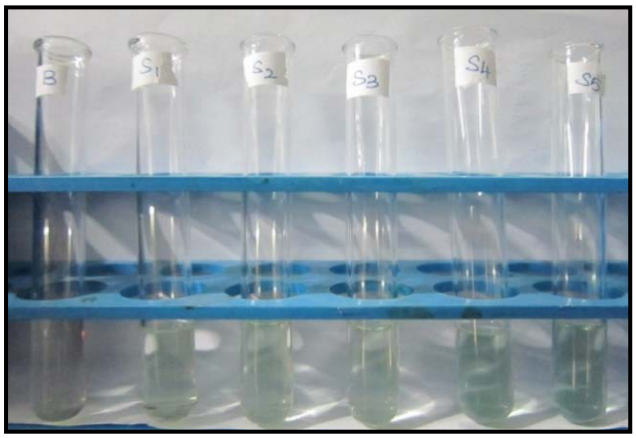

Figure 9: Standard Ascorbic acid – PMA

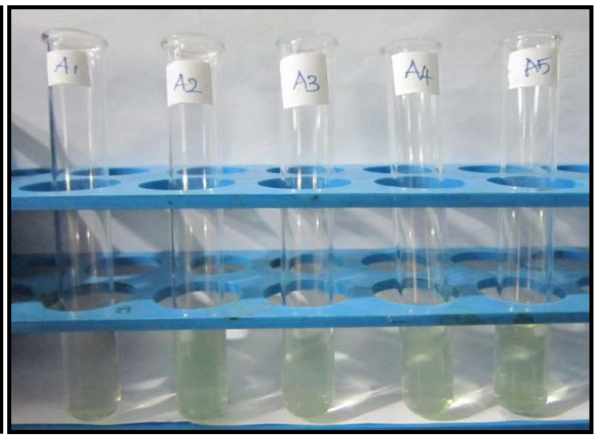

Figure 10: Ethanolic extract of *Achyranthes Aspera* - PMA

Table 5: Standard Ascorbic acid – PMA

Standard name	Standard volume (µl)	Standard concentration (µg)	Standard Abs (nm)
S1	100	10	0.038
S2	150	15	0.075
S3	200	20	0.09
S4	250	25	0.152
S5	300	30	0.199
		IC_{50}(µg/ml)	**11.73**

Table 6: Ethanolic extract of *Achyranthes Aspera* – PMA

Sample name	Sample volume (µl)	Sample concentration (µg)	Sample Abs (nm)
A1	100	10.276	0.028
A2	150	12.135	0.047
A3	200	14.19	0.071
A4	250	15.432	0.086
A5	300	17.21	0.105
		IC_{50}(µg/ml)	**7.49**

The phosphomolybdinum method has been used routinely to evaluate the total antioxidant capacity of plant extract [14]. In the ethanolic extract, Mo (VI) is reduced to Mo (V) and forms a green colored phosphomolybdenum V complex, which shows a maximum absorbance at 695 nm. Figure 9, 10, Table 4, 5 and 6 shows that the antioxidant capacity of the ethanolic extracts. IC_{50} of ethanolic extract of *Achyranthes aspera* compared to standard ascorbic acid. The result shows, ethanolic extract of *Achyranthes aspera* having good antioxidant activity.

CONCLUSION

The present study propose that qualitative phytochemical analysis of crude extracts of *Achyranthes aspera* confirm the occurrence of bioactive compounds such as alkaloids, Saponins, steroids, glycosides, fixed oils in the medicinal plant and thus responsible for the antioxidant activities. Etanolic extract of *Achyranthes aspera* possesses persuasive antioxidant activity is determined by phosphomolybdenum assay. Therefore, it could be a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing down the progress of ageing and age associated oxidative stress related degenerative diseases. Further research is recommended for better characterization of important bioactive constituents responsible for antioxidant activity. The revealed antioxidant property of extracts may provide potential therapeutic intervention against oxidative threats and degenerative disorders.

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