

In vitro Antioxidant Activity and Phytochemical Screening of *Achyranthes aspera*

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Abstract: *In the past few decades, the therapeutic value of plants has attained a significant dimension by exploring a diverse array of secondary metabolites with antioxidant potential. Several synthetic antioxidant agents are commercially available but are being restricted due to their carcinogenicity. Hence, the current study was intended to evaluate the antioxidant potential of phytoconstituents of the herb, Achyranthes aspera. The investigation of phytochemical compounds of the plant material extracted in aqueous and ethanol, revealed the presence of coumarins, flavonoids, alkaloids, tannins, phenolic compounds, saponins, sterols, quinone and so on. Antioxidant activity of the plant extracts was also assessed by DPPH assay, reducing power assay and superoxide radical scavenging assay. They showed commendable antioxidant effect as that of the ascorbic acid. The antioxidant properties were due to the presence of promising phytoconstituents in the plant.*

Keywords: Antioxidant, Phytoconstituents.

1. Introduction

Reactive oxygen species such as superoxide anions, hydroxyl radicals, hydrogen peroxide and singlet oxygen are formed as a result of normal metabolic activity and due to exogenous sources. The oxidative stress created by these radicals, leads to a range of biological and physiological lesions culminating in metabolic impairment, cell death, and degenerative diseases such as cancer, diabetes, obesity and neural disorder [1].

Numerous synthetic antioxidant agents including butylated hydroxyanisole and butylated hydroxytoluene (BHT) are commonly in use, yet, are reported to be deadly to animals and humans. Herbal plants are the treasury of antioxidant drugs that are potent free-radical scavenging molecules. This has encouraged the interest of many investigators to search natural antioxidants from herbal origin such as plant polyphenols and flavanoids which scavenge and inhibit free radicals. Many medicinal plants play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. [2]

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Achyranthes aspera of the family Amaranthaceae is an annual herb that grows throughout India. In traditional medicinal system, *A. aspera* is known for diuretic and hepatoprotective properties and used to cure several diseases viz., malarial fever, dysentery, asthma, hypertension and diabetes. Also it is widely studied for its medicinal properties and reported to have immunostimulatory, wound healing activity [6], antioxidant activity, hemolytic activity [13] and anti-inflammatory properties [8]. The present investigation was undertaken to examine the extracts of *A. aspera* for antioxidant potential by using *in vitro* assays such as DPPH radical scavenging, hydroxyl radical scavenging and reducing power assays.

2. Materials and Methods

2.1 Collection and processing of plant material

Whole plant of *Achyranthes aspera* was collected from the local market of Srirangam and various places in and around Tiruchirapalli. The plant was identified and authenticated by herbal division of Srimad Andavan Arts and Science College. The plant was dried in shade and powdered by making use of mechanical blender. The powder was stored in an airtight container and was used for solvent extraction.

2.2 Evaluation of organoleptic and physicochemical parameters

Organoleptic characters and physicochemical parameters were analyzed by the methods given in the ayurvedic pharmacopoeia of India.

2.3 Preparation of Extracts

The powdered plant material (150g) was extracted with water and ethanol separately using cold extraction method. Extracts were collected and filtered with a muslin cloth and then subjected to evaporation. Oven temperature was maintained at 45°C. The extracts were stored in labeled sterile screw capped bottles at -15°C and used for further studies.

2.4 Qualitative phytochemical analysis

Achyranthes aspera extracts were subjected to the analysis of macromolecules and secondary metabolites such as alkaloids, flavonoids, sterols, coumarins, lignin, gum, starch, terpenes, saponins, tannins, proteins, quinine and phenols by using standard methods [5].

2.5 Quantitative phytochemical analysis

The total tannin content in the plant extract was determined by using Folin-Denis reagent [12]. Aluminum chloride

colorimetric method [4] was used for flavonoids determination. Total phenols were determined by using Folin Ciocalteu reagent [9].

In-vitro antioxidant Assay

2.6 DPPH Radical Scavenging Activity

The free radical scavenging capacity of the aqueous and ethanolic extracts of *Achyranthes aspera* was determined using DPPH [3]. DPPH solution (0.004% w/v) was prepared in 95% methanol. The extracts were mixed with methanol to prepare the stock solution (10mg/100ml). The concentration of extract solution was 100µg/mL. From stock solution, 2mL, 4mL, 6mL, 8mL and 10mL of the solution were taken in five test tubes and serially diluted, this was made up to final volume of each test tubes to 10mL whose concentration was then 20µg/mL, 40µg/mL, 60µg/mL, 80µg/mL and 100µg/mL respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing extracts and after 10minutes, the absorbance was taken at 517nm using a spectrophotometer. Ascorbic acid was used as standard.

2.6.1 DPPH assay by TLC

An aliquot (3µL) of each extract and standard (Quercetin and Ascorbic acid) were carefully loaded onto a silica gel plate and allowed to dry for 3 minutes. After 5 minutes, the TLC plate was sprayed with 0.2% DPPH in methanol. Discoloration of DPPH indicates scavenging potential of the compound tested [15].

2.7 Reducing power assay

1ml of plant extract solution was mixed with 2.5ml phosphate buffer (0.2M, pH 6.6) and 2.5ml potassium ferricyanide (10g/L) was added. It was centrifuged at 3000 rpm for 10 minutes. Finally, 2.5ml of the supernatant solution was mixed with 2.5ml of distilled water and 0.5ml FeCl₃ (1g/L) and the absorbance was measured at 700nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer as blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power [17].

2.8 Superoxide radical scavenging activity

Phenazine methosulfate - nicotinamide adenine dinucleotide (PMS-NADH) system was used for the generation of superoxide anion. It was assayed by the reduction of nitroblue tetrazolium (NBT). About 1 ml of nitro blue tetrazolium, 1ml NADH in 100mM phosphate buffer at pH 7.8 and 0.1 ml of sample solutions of different concentrations were mixed. The reaction was started by adding 100µl PMS and the reaction mixture was incubated at 25°C for 5 minutes. The absorbance of the mixture was measured at 560nm [11].

2.9 Assessment of % inhibition and IC₅₀

Radical scavenging activity of extracts and standard were expressed in terms of % inhibition. It is calculated by using the formula $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$. Where A_{control} is the absorbance of control, and A_{sample} is the absorbance in

the presence of the sample of aqueous and ethanolic extracts. The IC₅₀ value is defined as the concentration (in µg/ml) of extracts that produced 50% antioxidant effect.

$$IC_{50} = \text{Concentration of extract} / \% \text{ inhibition} \times 50.$$

3. Results and Discussion

Achyranthes aspera powder was light green colored with pungent odour and bitter taste (Table 1). Physicochemical data of the plant powder also provide specific nature of the plant.

Results of physicochemical parameter in Table 2 reveal that the plant powder did not have any of the foreign matter. Ash content of the powdered material was directly proportional to the quantity of foreign matter present in the sample. *Achyranthes aspera* exhibited 7.4% total ash, 2.4% water-soluble ash and 3.2% acid soluble ash. Powder showed higher percentage of water soluble extractive (11.5%) followed by alcohol soluble extractive (18.45%). Higher extractive values indicated that the plant showed higher polar compounds like alkaloid, flavonoids, phenolic compounds etc., 0.18-0.34µm size particle were used for assaying biological activity.

Table 1: Organoleptic characters of Plant *A. aspera*

Character	Observation
Colour	Light green
Odour	Pungent
Taste	Slightly sweet, sometimes bitter
Height	0.5 to 1 meter

Table 2: Physicochemical parameters of *A. aspera*

Parameter	Results
Foreign matter	Nil
Dry Powder Particle size	0.18-0.34µm
Wet powder Particle size	0.23-0.42µm
Acid insoluble ash value	3.2%
Water soluble ash value	2.4%
Total ash	7.4%
Water soluble extractive Value	11.5%
Alcohol soluble extractive value	18.45%

The phytochemical screening of the aqueous extract of plant depicted in Table 3 show the presence of sterols, quinone, alkaloids, flavonoids, saponins, phenolic compounds, tannins, and protein where as the ethanolic extract of *Achyranthes aspera* showed the presence of coumarins, sterols, quinone, alkaloids, flavonoids, phenolic compounds, tannins and proteins. This test could elicit the primary nature of phytochemicals available in the extracts.

Table 3: Qualitative Phytochemical screening

Phytoconstituent	<i>A. aspera</i> aqueous extract	<i>A. aspera</i> ethanolic extract
Coumarin	-	+
Sterol	+	+
Quinone	+	+
Lignin	-	-
Saponin	+	-
Flavonoid	+	+
Gum	-	-

Triterpene	-	-
Alkaloid	+	+
Tannin	+	+
Starch	-	-
Protein	+	+
Phenolic compound	+	+

In order to quantify important phenolic compounds available in the extracts, standard spectrophotometric methods were used and the results indicated that ethanolic extract of *Achyranthes aspera* yield 2.3% flavonoids, 1.7% tannins and 7.2% phenols and the aqueous extract showed lower concentrations of these phytochemicals with 1.1% flavonoids, 0.7% tannins and 4.62% phenols (Table 4).

Table 4: Quantitative Phytochemical screening

Phytoconstituents	% Yield (Ethanol extract)	% Yield (Aqueous extract)
Flavonoids	2.3	1.1
Tannins	1.7	0.7
Phenols	7.2	4.62

In DPPH assay, the DPPH solution is decolorized when the odd electron becomes paired off in the presence of free radical scavenger. The color becomes light yellow from deep violet. DPPH TLC assay clearly has indicated the radical scavenging nature of the extracts (Figure: 1).

Corresponding increase in absorbance is noted in extracts as well as standard when the concentrations of extracts and standard were increased. The percentage of DPPH radical scavenging activity of aqueous and ethanolic extracts of *Achyranthes aspera* (at 100µg/ml) and standard (at 50µg/ml) were 34.37%, 72.06% and 52.67% respectively.

DPPH TLC Spray Assay

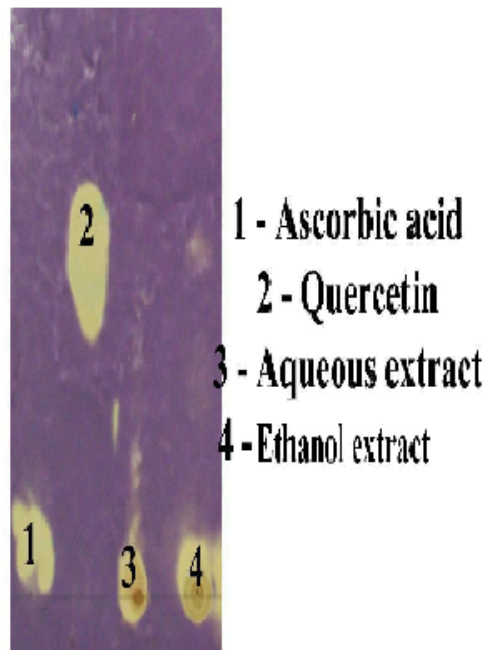
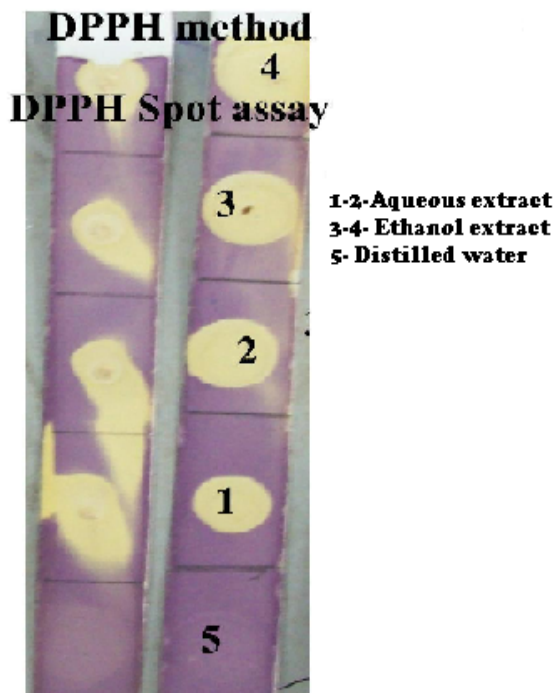


Figure 1: DPPH assay by TLC

Aqueous and ethanol extracts of the plant showed potent antioxidant power by reducing power ability. Aqueous extract of *A. aspera* exhibited better antioxidant power (61.32%) than ethanolic extract (40.78%) at 100µg/ml concentrations. Results of reducing power assay were significantly different among various concentrations tested. Likewise, aqueous extract of *A. aspera* showed significant free radical scavenging activity against superoxide ions. The percentage of scavenging was found to be 66.53% which is a slightly higher than ethanolic extract (63.18%). Ascorbic acid exhibited 50.70% superoxide radical scavenging power at 50µg/ml concentration.



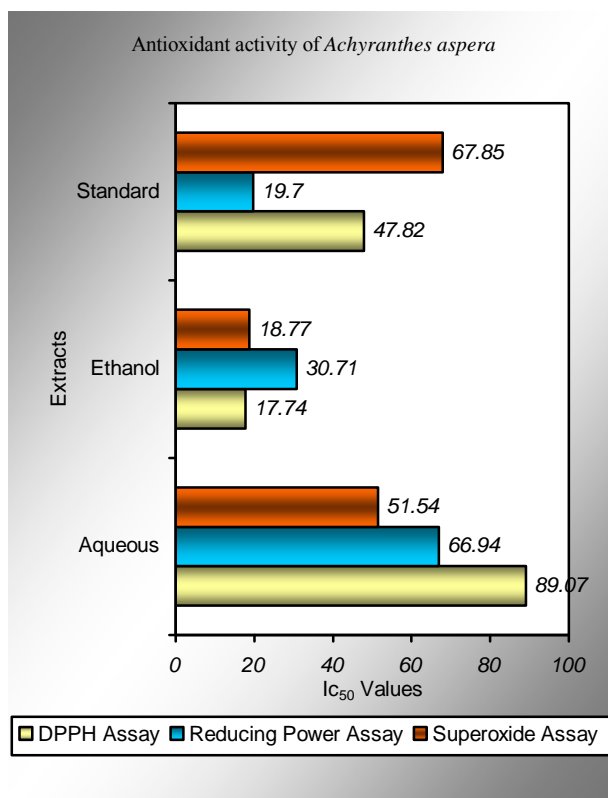


Figure 2: Antioxidant activity of *A. aspera*

The concentration of extracts that showed that produced 50% antioxidant effect i.e IC_{50} values were represented in Figure: 2.

Many researchers [7, 10, 14 and 16] have reported the effectiveness of antioxidant property of the plant. This research also suggests that *A. aspera* can be a proficient alternative medicine for infectious diseases encountered today which could raise the optimism of scientists about the future of phytomedicine.

4. Conclusion

Antioxidant is one of the most essential ingredient of today's therapy because the anti oxidative system protects us against reactive oxygen species (H_2O_2 , superoxide, OH, singlet oxygen & nitrogen species) induced oxidative damage.

Aqueous & ethanolic extracts of the plant have been studied for their antioxidant properties using different *in vitro* antioxidant methods. Flavonoids, phenolics, tannins, steroids are found in these two extracts of plant. These extracts showed good antioxidant effect, which could be due to the available phytoconstituents. In this respect, poly phenolic compounds commonly found in plants have been reported to have multiple biological effects like anticancer, antiproliferative, antimicrobial, wound healing, and antibacterial activities including antioxidant activity.

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6. Future Scope

Herbs have been used traditionally for various purposes, of which, their medicinal values are of great significance. The potentiality of *Achyranthes aspera* has been studied *in vitro* and proved to be an outstanding antioxidant. In future, this study can be further extended *in vivo* in animal cell lines and tissue cultures in the aim of discovering a new drug to combat human diseases.

Author Profile

Ms. S. Varalakshmi received M.Sc., and M. Phil. degrees in Microbiology from Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. She was awarded with Gold Medal for securing the University First Rank in Post Graduation from the same University. Currently she is working as a faculty in Srimad Andavan Arts and Science College, T. V. Koil, Tiruchirappalli.

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