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Achyranthes Aspera: Phytochemical Estimation

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ABSTRACT

Phytochemicals are dependable sources for the treatment of different health problems. In this study, antioxidant activities of different sequential extracts (petroleum ether, benzene, chloroform, ethyl acetate, ethanol and aqueous) of root and inflorescences of *Achyranthes aspera* Linn. (Family: Amaranthaceae) was done by different standard methods of phytochemical screening. Our finding reveals that all the extracts of *A. aspera* root and inflorescences contain alkaloids, tannins, cardiac glycosides, steroids, flavonoids, terpenoids, reducing sugar and saponin in appreciable, moderate and trace amount. Observed result showed that the phytochemical contents are high in different sequential extracts of root than sequential extracts of inflorescences. Due to rich source of phytochemicals, this plant is may be used for herbal medicine.

Keywords: *Achyranthes aspera*, Antioxidants, Flavonoid.

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INTRODUCTION

Reactive oxygen species (ROS) encompasses all highly reactive oxygen molecules including the hydroxyl radicals (OH^-), superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), nitric oxide radicals (NO^-), hypochlorite radicals and various lipid peroxides radicals (LOO^-)[1].

Reactive oxygen species (ROS) on accumulation in the body subsequently induces oxidative stress, directly or indirectly damage cellular DNA and protein. Accumulation of ROS like hydroxyl radicals, superoxide radicals and hydrogen peroxides causes aberrant gene expression at low concentrations and lesions of lipids, proteins and DNA in higher concentrations which eventually lead to cellular death[2].

Many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been used to retard the oxidation process; however, the use of synthetic antioxidants must be under strict regulation due to potential health hazards like some side effects, low therapeutic index, inability to penetrate cellular membrane and non specificity. The search for natural antioxidants as alternatives is therefore of great interest among researchers. They are easily available and non toxic[3-4].

Medicinal plants are used for treatment of various ailments due to their potent pharmacological activities, low toxicity and economic viability then the synthetic drugs[1] and are the only affordable and accessible source of primary health care, especially in the absence of access to modern medical facilities. They mainly contain biologically active ingredients and are used primarily for treating mild or chronic ailments. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. They are also some times added to foods meant for pregnant and nursing mothers for medicinal purposes[5].

Achyranthes aspera Linn. (Amaranthaceae) is an important medicinal plant, commonly known as Chirchita in Hindi, an annual, pubescent stiff erect herb, found as weed throughout India, tropical Asia and other parts of the world [6-7]. The inflorescences are pale to bright purple, loosely arranged below and compact above in long spikes. The fruits are oblong cylindrical, yellowish brown, smooth and glabrous. Root extract exhibit pronounced insect molting hormonal activity. These are also used as astringents to wounds, in abdominal tumor and stomach pain[8]. This plant is popularly supposed to act as a safeguard against scorpions and snakes[9].

Keeping in view the above mentioned medicinal properties of the *A. aspera,* the present study was carried out to test the phytochemical constituents of different sequential extracts (petroleum

ether, benzene, chloroform, ethyl acetate, ethanol and aqueous) of *A. aspera* root and inflorescences.

MATERIALS AND METHODS

Chemicals & reagents

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

Experimental plant

A. aspera was selected for the present study. Roots and inflorescence of *Achyranthes aspera* were collected seasonally from campus of Banasthali University, Tonk, Rajasthan, India. The plant was identified with the help of available literature and authenticated by Botanist of Krishi Vigyan Kendra of this University.

Preparation of different fractions of *A. aspera*

The plant parts (root and inflorescences) were cleaned, dried and powdered with the help of mixer grinder separately. Various extracts were prepared using sequential solvents from non polar to polar (petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water). These extracts were concentrated using rotary evaporator and stored at 4 ºC in air tight containers for further experimental studies.

Preliminary phytochemical screening

Qualitative phytochemical analysis of *A. aspera* extracts (petroleum ether, benzene, chloroform, ethyl acetate, ethanol, aqueous) were carried out as follows using standard procedures.

TEST FOR ALKALOIDS

Mayer's Test-

Test solution (1ml) was taken in test tube and few drops of Mayer's reagent (Potassium mercuric iodine solution) were added into it and then cream color precipitate was observed[10].

Dragendorff's test-

To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added by the side of the test tube. A prominent red precipitate indicates test as positive[10].

Tannic acid test- Alkaloids give buff color precipitate with 10 % tannic acid[10].

TEST FOR TANNINS[10,11]

Ferric Chloride test-

To test solution added 10 ml distilled water, then filtered, in the filterate 2 ml $FeCl_3$ (10%) was added, blue-black or green precipitate formed, indicate the presence of tannins.

Gelatin test-

To the test solution added 1 ml of 1 % gelatin solution and 1 ml of 10 % NaCl, white precipitate of gelatin indicate the presence of tannins.

Vanillin hydrochloride test-

To the test solution added 1ml of vanillin hydrochloride solution; purple red color indicates the presence of tannins.

TEST FOR CARDIAC GLYCOSIDES

Keller-Killiani test-

To an extract, added 4 ml of glacial acetic acid, few drops of ferric chloride and concentrated sulfuric acid (2 ml) was added. Brown ring obtained at interface, indicate the presence of cardiac glycosides[11].

Salkowski test-

To the test solution added 2 ml of chloroform and 1 ml H_2SO_4, reddish brown color at interface, indicate the presence of cardiac glycosides[10].

Baljet test-

Solution-1: picric acid (1 g) in 100 ml of ethanol. Solution-2: NaOH (10 g) in 100 ml of water. Mixed both solution. To the test solution added 2 to 3 drops of combined solution, orange to deep red color, indicate the presence of cardiac glycosides[12].

TEST FOR STEROIDS

Libermann test:

To the test solution added 10 ml of chloroform then filtered. To the 2 ml filterate added 2 ml of acetic anhydride and con. H_2So_4. Blue green ring indicate the presence of steroids in the sample[13].

TEST FOR FLAVONOIDS

Alkaline reagent test-

To the test solution added few drops of NaOH solution, formation of intense yellow color, which turns to colorless on the addition of few drops of diluted acid, indicates presence of flavonoids[13].

Lead acetate test-

The extracts were treated with few drops of 10 % lead acetate solution. The formation of precipitate confirmed the presence of flavonoids[13].

TEST FOR TERPINOIDS

Salkowski test-

To the test solution added 2 ml of chloroform and 1 ml H_2So_4, reddish brown color at interface,

indicate the presence of terpinoids[15].

TEST FOR PROTEINS

Ninhydrin test-

To the test solution added 1 ml of 0.2 % ninhydrin solution, violet color indicate the presence of protein in sample[14].

TEST FOR REDUCING SUGAR

Fehling's test-

Filtrate (1 ml) was boiled on water bath with 1ml each of Fehling solution A & Fehling solution B; a colored product, indicates the presence of sugar[11].

TEST FOR SAPONINS

Foam test-

To the 0.5 ml of test solution added 2 ml distilled water and shake the all tubes, if foam produced persist for 10 min, indicate the presence of saponins[10].

RESULTS AND DISCUSSION

Yield of different extracts of *A. aspera*

Extraction of botanical compounds from the plant materials is mainly dependent on the type of solvent used in the extraction procedure. Quantitative estimation of the extraction values and percentage (%) yield of the six extracts (sequential: i.e. from non-polar to polar) from two parts of *A. aspera* (roots and inflorescences) were studied and summarized in table 1. The extraction values were high in aqueous extract for both root (854.5 mg) and inflorescences (4020 mg), whereas, ethyl acetate extract had shown minimum extractive value as 40 mg for root and 120 mg for inflorescences. After the aqueous extract, ethanolic extract had shown high value as 540 mg in root and 2040 mg in inflorescences. Petroleum ether when used as extract solvent than yield was only 210 mg for root and 1010 mg for inflorescences.

Table 1: Extraction value of *A. aspera* Linn. root and inflorescences.

S. No.	Solvents	Yield of extract (mg/50 g) of root		Yield of extract (mg/50 g) of inflorescence	
		Yield (M±SD)	Yield %	Yield (M±SD)	Yield %
1.	Petroleum ether	210±1.02	0.42	1010±0.45	2.02
2.	Benzene	170±0.99	0.34	360±0.50	0.72
3.	Chloroform	180±1.12	0.36	330±0.75	0.66
4.	Ethyl acetate	40±1.25	0.08	120±1.12	0.24
5.	Ethanol	540±2.21	1.08	2040±2.55	4.08
6.	Aqueous	854.5±2.24	1.71	4020±3.65	8.04

The values are the average of three determinations and are expressed as mean±SD.

Aqueous extract showed the highest % yield of 1.71 % (in root) and 8.04 % (in inflorescences) while ethyl acetate showed the lowest % yield of 0.08 % (root) and 0.24 % (inflorescences). The traditional healers or practitioners make use of water primarily as a solvent. According to this study, water and ethanol extract of these plant parts were found to be certainly much better. This may be due to the better solubility of the active compound in these solvents[16].

Qualitative phytochemical screening

In the present study, preliminary phytochemical screening of the different extracts of root and inflorescences of *A. aspera* revealed the presence of various bioactive components of which alkaloids, saponins, reducing sugars, tannins, cardiac glycosides, steroids, flavonoids, terpenoids and proteins were identified. All these phytochemicals possesses good antioxidant activities and has been reported to exhibit multiple biological effects including antimicrobial, anticancer and anti-inflammatory activities and served as a substitute for synthetic drugs. Flavonoids and steroids are responsible for antioxidant and antibacterial activity[17].

Table 2: Qualitative analysis of Phytochemical of various extracts of *A. aspera* root.

Phytochemicals	Tests	PEAA	BEAA	CEAA	EAEAA	EEAA	AEAA
Alkaloids	Dragendroff's Test	_	+	++	++	++	++
	Tannic acid Test	_	_	++	_	++	++
	Mayer´s Test	_	+	++	++	+	+
Tannins	Ferric Chloride Test	_	_	_	_	++	++
	Gelatin Test	_	_	_	_	+	+
	Vanillin HCl Test	+	++	+	+	+	+
Cardiac Glycosides	Keller- Killiani Test	+++	+++	+++	++	++	+
	Salkowski Test	_	_	_	++	+	+++
	Baljet Test	++	++	++	_	++	++
Steroids	Libermann-Buchard Test	++	++	+	_	+++	+++
Flavonoids	Alkaline Reagent Test	++	_	+++	_	+	_
	Lead Acetate Test	++	_	+++	_	+	++
Terpenoids	Salkowski Test	_	_	_	++	+	+++
Proteins	Ninhydrin Test	_	_	_	_	_	_
Reducing Sugar	Fehling's Test	++	++	++	++	++	++
Saponins	Froth Test	+	-	++	+	+	+

(+++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent. Abbreviations: PEAA- Petroleum ether extract of *Achyranthes aspera*; BEAA- Benzene extract of *Achyranthes aspera;* CEAA- Chloroform extract of *Achyranthes aspera;* EAEAA- Ethyl acetate extract of *Achyranthes aspera*; EEAA- Ethanolic extract of *Achyranthes aspera*; AEAA- Aqueous extract of *Achyranthes aspera*

The phytochemical characteristics of the sequential extracts of *A. aspera* root were investigated and are summarized in table 2. The results reveal the presence of medicinally active phyto-

constituents studied in the six extracts. From table 2, tannin, cardiac glycosides and carbohydrates (reducing sugar) were present in all extracts in appreciable, moderate or in trace amount while proteins were absent in all extracts. Different extracts of root of *A. aspera* were tested and showed that alkaloids were absent in only petroleum ether extract, while steroids were absent in only ethyl acetate extract, but both were present in appreciable, moderate or in trace amount in remaining extracts. Flavonoids were absent in both benzene and ethyl acetate extracts while present in appreciable, moderate or in trace amount in remaining extracts. Observed results showed that the terpenoids were absent in petroleum ether, benzene and chloroform extracts and present in appreciable, moderate or in trace amount in remaining extracts. A new aliphatic acid n-hexacos-14-enoicacic were isolated and identified in the ethanolic extract of root by Sharma *et al*. (2009)[18], oleanolic acid was also isolated from glycosidic fraction of the roots by Khastgir *et al*. (1958)[19] and Srivastav *et al*. (2011)[9].

Table 3: Qualitative analysis of phytochemical of various extracts of *A. aspera* inflorescences.

Phytochemicals	Tests	PEAA	BEAA	CEAA	EAEAA	EEAA	AEAA
Alkaloids	Dragendroff's Test	+	+	++	+++	+++	+++
	Tannic acid Test	_	_	_	_	_	_
	Mayer´s Test	+	+	++	+++	++	++
Tannins	Ferric Chloride Test	_	_	_	_	_	_
	Gelatin Test	+	+	+	++	+++	+++
	Vanillin HCl Test	+++	++++	+	+	+	++
Cardiac Glycosides	Keller- Killiani Test	++	_	++	++	+	+
	Salkowski Test	++	_	+++	+++	++	+
	Baljet Test	++	++++	++	++	++	+++
Steroids	Libermann-Buchard Test	++	+++	-	-	-	+
Flavonoids	Alkaline Reagent Test	_	_	+	++	+++	+++
	Lead Acetate Test	++	_	+++	_	+	++
Terpenoids	Salkowski Test	++	_	++++	+++	++	+
Proteins	Ninhydrin Test	_	_	_	_	_	_
Reducing Sugar	Fehling's Test	+++	+++	+++	+++	+++	+++
Saponins	Froth Test	++	+	+	++	+++	++

(+++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent. Abbreviations: PEAA- Petroleum ether extract of *A. aspera*; BEAA- Benzene extract of *A. aspera;* CEAA- Chloroform extract of *A. aspera;* EAEAA- Ethyl acetate extract of *A. aspera*; EEAA- Ethanolic extract of *A. aspera;* AEAA- Aqueous extract of *A. aspera*.

The phytochemical characteristics of the sequential extracts of *A. aspera* inflorescences were investigated and are summarized in table 3. Different extracts of inflorescences of *A. aspera* showed the presence of tannins, alkaloids, cardiac glycosides, reducing sugars and saponins in

appreciable, moderate and in trace amount. It was seen that alkaloids, tannins, cardiac glycosides, reducing sugar and saponins were present in all extracts while proteins was absent in respectively all extracts. This indicates that the presence of secondary metabolites may have suppressed the activity of protein. In addition to this, the solvents might have also denatured the protein. Steroids were present in all extracts except chloroform, ethanol and ethyl acetate extract, whereas flavonoids and terpenoids were present in all extracts except benzene extract.

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities[10].The presence of plant phenolics like flavonoids and tannins present in the plant extracts may act as primary antioxidants or free radical scavengers[1]. All these phytochemicals are reported to possesses various pharmacological actions and anti-oxidant properties. A water soluble alkaloid achyranthine isolated from the plant possess cardiovascular activities and broncho-protective activities[20]. The presence of glycosides was detected in both parts (root and inflorescences) of *A. aspera*. Glycosides have been known to regulate blood pressure, although some workers have attributed the cardiac action of these oils due to the presence of alkaloids[21].

CONCLUSION

Both parts (root and inflorescences) of *A. aspera* contain alkaloids, tannins, cardiac glycosides, steroids, flavonoids, terpenoids, reducing sugar and saponin in appreciable, moderate and trace amount. Observed result concluded that the phytochemicals content is high in different sequential extracts of root than sequential extracts of inflorescences. Due to rich source of phytochemicals this plant is used for herbal medicine. Also due to beneficial properties, *A. aspera* is used as antimicrobial, larvicidal, anti-fertility, immunostimulant, hypoglycemic, hypolipidemic, anti-inflammatory, antioxidant, diuretic and for many purposes.

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