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Research Article

PHYTOCHEMICAL INVESTIGATION OF VARIOUS EXTRACTS OF LEAVES AND STEMS OF *ACHYRANTHES ASPERA* LINN.

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ABSTRACT

Objectives: Plants and plant based medications are the basis of many of the modern pharmaceuticals we use today for various ailments. The main objective of this study was to appraise antioxidant activity of different sequential extracts of leaves and stems of *Achyranthes aspera* by phytochemical analysis.

Methods: The plant material was dried in shade, crushed and subjected to prepare different sequential and non-sequential extracts using soxhlet apparatus.

Results: Our findings revealed that both stems and leaves possess the phytochemicals like alkaloids, cardiac-glycosides, terpenoids, flavonoids, saponins, steroids, proteins and reducing sugars in different amounts.

Conclusion: The results exhibited the presence of different phytochemicals. All these phytochemicals have potential therapeutic or physiological actions on human system, for that the leaves and stems of *A. aspera* can stand as a potential source of some vital drugs.

Keywords: *Achyranthes aspera*, Antioxidants, Oxidative stress, Phytochemical.

INTRODUCTION

In biological systems, reactive oxygen species (ROS) are conventionally viewed as toxic by-product of cellular metabolism [1]. Reactive oxygen species include all highly reactive, oxygen-containing molecules, including free radicals as hydroxyl radical (OH^-), singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), nitric oxide radical (NO_2^-), hypochlorite radical ($HOCl^-$), and various lipid peroxides which causes oxidative damage to genetic material, proteins, and other macromolecules has been implicated in the pathogenesis of a wide variety of diseases [2].

Recently, there have been great efforts to find safe, cost-effective and potent phytochemicals which act as natural antioxidants from various plant sources. Phytochemicals are non-nutritive plant chemicals and recent research demonstrates that many phytochemicals can protect humans against diseases [3]. This resurgence of interest in plant derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the synthetic drugs, many of which are costly and have adverse side effects [4]. Medicinal plants contain phenols, flavonoids, saponins and many other phytochemicals which exert a multiple biological effects including antioxidants, free radical scavenging activities, anti-inflammatory, anti-carcinogenic and others. *Achyranthes aspera* belongs to the family Amaranthaceae, is a perennial stiff erect herb, and grows up to 1000 m height found as weed in India, Baluchistan and other tropical Asian countries.

A. aspera is used in Indian traditional medicine for the treatment of ophthalmic and other eye infections. Various studies have been reported for its hypoglycemic effect, anti-cancer, anti-fungal and potential to increase the thyroid hormone levels [5]. Leaf extracts were also reported to possess thyroid stimulating and anti-per oxidative properties [6].

Keeping above beneficial properties of *A. aspera*, the current investigation was planned to evaluate the phytochemical screening of different sequential extracts of stems and leaves of *A. aspera.*

MATERIALS AND METHODS

Plant material

Achyranthes aspera was selected for the current work. The stems and leaves of the plant were collected from the roadsides of University campus, Banasthali University, Banasthali, Banasthali, Tonk District, Rajasthan, India. Botanical identification was performed by the Botanist of Krishi Vigyan Kendra of this University. The collected stems and leaves were washed, shade dried and milled into coarse powder using electric grinder and stored in an air-tight container at 25^0 C.

Preparation of extracts

Dried powdered materials were placed in the soxhlet thimble to obtain sequential extracts of different solvents ranging from non-polar to polar - petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water by placing them in 250 ml round bottom flask. The materials were refluxed with each solvent for 12- 14 hours at 40-60^0 C. Extracts were collected and cooled at room temperature and poured in glass Petri dishes & then evaporated at 40^0C using hot air oven. Dried extracts were kept in desiccators for two days and stored at 5^0C in air tight containers [7].

Chemicals and Reagents

Ferric chloride, folin-ciocalteu's reagent, gelatin, HCl, dragendorff's reagent, methanol, gallic acid, H_2SO_4, Na_2CO_3, vanillin, tannic acid, acetic anhydride, fehling solutions were all purchased from Merck, USA. All other unlabelled chemicals and reagents were of analytical grade and of high purity.

Qualitative Phytochemical Screening

The Phytochemical screening of all six extracts was performed by the standard methods [8,9,10].

Test for alkaloids

a) Mayer's Test- Test solution (1 ml) was taken in test tube and few drops of Mayer's reagent (Potassium mercuric iodide solution) were added into it and cream color precipitate was observed.

b) Dragendroff's Test- Test solution (1 ml) was taken in test tube and few drops of Dragendroff's reagent (Potassium bismuth iodide solution) were added into it and observed for reddish brown precipitate.

c) Tannic acid Test- Test solution (1 ml) was taken in test tube and few drops of 10 % tannic acid solution was added to it and observed for buff coloration.

Test for tannins

a) $FeCl_3$ Test- About 0.5 mg of dried powdered samples were boiled in 20 ml water in test tubes and filtered. A few drops of

0.1 % ferric chloride solution was added and observed for brownish green or blue-black coloration.

b) Gelatin Test- About 1ml test solution was taken in a clean dried test tube and 1 % gelatin solution was added followed by 10 % sodium chloride solution and observed for white precipitate to form.

c) Vanillin hydrochloride Test- Test solution was treated with few drops of vanillin hydrochloride reagent and observed for purplish- red color.

Test for cardiac glycosides

a) Keller killiani Test- Test solution (1 ml) was taken in a test tube and 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added to it. Carefully added 0.5 ml of concentrated sulphuric acid by the side of the test tube and observed for blue color to appear in the acetic acid layer.

b) Salkowski Test- Test solution (1 ml) was taken in a clean and dried test tube and 2 ml chloroform and few drops of sulphuric acid were added into it. Shaken well and allowed to stand for some time and observed for reddish brown color at interface.

c) Baljet Test – Test solution (1 ml) was placed in test tube and 2-3 drops of baljet solution was added to it and observed for orange to deep red color to appear.

Test for Steroids

a) Liebermann Buchard test- Test solution of 1 ml was treated with few drops of acetic anhydride, boiled and cooled, concentrated sulphuric acid was added from the sides of the test tube and observed for a brown ring at the junction of the two layers and green layer in upper layer.

Test for Flavonoids

a) Alkaline reagent test- About 1 ml test solution was treated with few drops of sodium hydroxide solution and observed for intense yellow coloration which disappeared on the addition of dilute HCl.

b) Lead acetate Test- Test solution (1 ml) was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow colored precipitate.

Test for Terpenoids

a) Salkowski Test- Test solution (1 ml) was taken in a clean and dried test tube and 2 ml chloroform and few drops of sulphuric acid were added into it. Shaken well and allowed to stand for some time and observed for reddish brown color at interface.

Test for Proteins

a) Ninhydrin Test- Test solutions were boiled with 0.2 % solution of ninhydrin and observed for violet color to appear.

Test for Reducing sugars

a) Fehling Test- Test sample of 1 ml was taken into a clean and dried test tube and 0.5 ml of Fehling A and Fehling B solutions were added to it, boiled and observed for brick red coloration.

Test for Saponins

a) Froth test- Test solution (1 ml) was placed in a test tube containing water and shaken well and noted for a stable froth that persists for at least 2 min.

RESULTS

Yield of different extracts of *Achyranthes aspera*

Extraction of botanical compounds from the plant materials is mainly dependent on the type of solvent used in the extraction procedure. Table-1 represents the extraction values with % yield of dried stems and leaves in different solvents (non-polar to polar sequentially). The values were high in petroleum ether extract for leaves (7210 mg) and aqueous extract of stems (7177 mg). Among other solvents used for study, ethyl acetate showed minimum extraction value as 230 mg for stem and 730 mg for leaves. After the petroleum ether extract, benzene extract has shown high value as 5840 mg in leaves and after aqueous extract, 479 mg in ethanolic extract of stems. Chloroform when used as extract solvent than the yield was 350 mg for stems and 800 mg for leaves.

Table 1: Extraction yield of different extracts of *Achyranthes aspera* Linn stems and leaves.

Solvents	Yield of extract of (mg/50 g) leaves		Yield of extract of (mg/50 g) stems	
	Yield (M±SD)	Yield%	Yield (M±SD)	Yield%
Pet Ether	7210±0.02	14.42	340±0.06	6.8
Benzene	5840±0.13	11.68	116±1.20	2.32
Chloroform	800±0.05	1.6	350±0.03	7.00
Ethyl Acetate	730±0.78	1.46	230±1.54	4.6
Ethanol	4118±0.03	8.2	479±0.98	9.58
Aqueous	4240±0.07	8.48	7177±1.41	14.35

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

Phytochemical screening of leaves

Preliminary phytochemical screening of the various extracts of *Achyranthes aspera* leaves revealed the presence of various bioactive components which include cardiac glycosides, reducing sugars, alkaloids, and tannins in different amount.

Phytochemical screening of stems

The phytochemical screening of the all the six sequential extracts of stems of *A.aspera* showed the presence of terpenoids, reducing sugars, cardiac glycosides in prominent amount.

DISCUSSION

The traditional healers or practitioners make use of water primarily as a solvent, but according to this study showed that ethyl acetate extract of these plant parts were certainly much better than a aqueous extract. This may be due to the better solubility of the active compound in organic solvents [11].

Preliminary phytochemical screening of the various extracts of *Achyranthes aspera* leaves revealed the presence of cardiac glycosides, reducing sugars, alkaloids, and tannins were the most prominent. Terpenoids, reducing sugars, cardiac glycosides were prominently found in sequential extracts of stems. These compounds may be responsible for antioxidant activity and antimicrobial activity and may serve as a substitute for synthetic drugs [12]. For leaves, flavonoids and terpenoids were found to be present in moderate amounts. Flavonoids were present in ethanolic and aqueous sequential extracts of leaves and terpenoids were found to be present in petroleum ether, chloroform, benzene and aqueous extracts. Steroids were also present in petroleum ether and chloroform extract. The results of phytochemical screening of leaves are tabularized in table 2.

Table 2: Qualitative phytochemical screening of various extracts of *Achyranthes aspera* leaves.

Phytochemicals		PEAA	BEAA	CEAA	EAEAA	EEAA	AEAA
Alkaloids	**Dragendroff's test**	-	+	++	+++	+++	+++
	Tannic acid t-est	+	+	-	-	-	++
Tannins	**Ferric chloride test**	-	+	++	-	++	++
	Gelatin test	-	-	-	-	++	++
	Vanillin-HCl test	+	++	++	++	++	+
Cardiac glycosides	**Keller-Killiani test**	+++	++	+++	++	-	-
	Salkowski test	+	++	+	-	-	+
	Baljet test	++	-	+	-	+	+++
Steroids	**Liebermann-Buchard test**	++	-	++	-	-	-
Flavonoids	**Alkaline Reagent test**	-	-	-	-	+	++
	Lead Acetate test	-	-	+	-	-	+
Terpenoids	**Salkowski test**	+	++	+	-	-	+
Proteins	**Ninhydrin test**	-	-	-	-	-	-
Reducing sugars	**Fehling's test**	+++	++	++	++	++	+++
Saponins	**Froth test**	-	+	+	+	+	+

(+++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent. 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*.

For various extracts of stems, alkaloids were found in appreciable amounts in ethanolic and aqueous extracts of stems. Tannins were found to be present in appreciable amounts in almost every extract. Vanillin- HCl test suggested that tannins were present in moderate amounts in benzene and chloroform extracts of stems. Cardiac-glycosides were found to be present in appreciable amounts in chloroform extract while in other extracts they were present in moderate amounts. Steroids were present in appreciable amounts in all the extracts except benzene extract. Flavonoids were present in ethanolic and aqueous extract in moderate amounts. Terpenoids were present in appreciable amounts in chloroform, ethanolic and aqueous extracts and absent in benzene extract. Aqueous extracts were containing appreciable amounts of reducing sugars. Froth test for saponins screened their presence in chloroform, ethyl acetate, ethanolic and aqueous extracts of stems. The results of phytochemical screening for stems are tabularized in table 3.

Table 3: Qualitative phytochemical screening of various extracts of *Achyranthes aspera* stems.

Phytochemicals		PEAA	BEAA	CEAA	EAEAA	EEAA	AEAA
Alkaloids	**Dragendroff's test**	-	-	+	++	++	+++
	Tannic acid t-est	+	-	+	-	++	+++
Tannins	**Ferric chloride test**	-	-	-	-	++	++
	Gelatin test	-	-	-	-	-	+
	Vanillin-HCl test	++	+++	+++	+	+	+
Cardiac glycosides	**Keller-Killiani test**	++	+++	+++	++	-	-
	Salkowski test	++	-	+++	+++	++	++
	Baljet test	+	+	++	-	++	++
Steroids	**Liebermann-Buchard test**	+++	-	+++	+++	+++	+++
Flavonoids	**Alkaline Reagent test**	-	-	+	++	++	+++
	Lead Acetate test	-	-	-	-	+	++
Terpenoids	**Salkowski test**	++	-	+++	+++	++	++
Proteins	**Ninhydrin test**	-	-	-	-	-	-
Reducing sugars	**Fehling's test**	+	+	+	++	+	+++
Saponins	**Froth test**	-	-	++	+	+	+

(+++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent. 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*.

All these phytochemicals are reported to possess various pharmacological actions and anti-oxidant properties. A water soluble alkaloid, Achyranthine was isolated from the plant which is reported [13] to possess cardiovascular activities and broncho-protective activities. The presence of flavonoids in the extract was observed for the free radical scavenging effect observed. Plant phenolics like flavonoids and tannins act as primary antioxidants or free radical scavengers.

CONCLUSIONS

The phytochemicals content is high in different sequential extracts of stems than sequential extracts of leaves. Ethyl acetate extract of stems possesses higher phytochemical contents than other extracts of *A. aspera* stems, while chloroform extract of leaves contains higher amounts of such phytochemicals. Both parts (leaves and stems) of *A. aspera* contain alkaloids, tannins, cardiac glycosides, steroids, flavonoids, terpenoids, reducing sugar and saponin in appreciable, moderate and trace amount.

It can also be concluded that the leaves and stems of *A. aspera* are the good sources of antioxidants and surely helpful in treating the disease associated with oxidative stress. Due to rich source of phytochemicals, this plant may be used for herbal medicine and useful for food and drugs.

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