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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *ACHYRANTHES ASPERA*

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ABSTRACT

Nowadays, production of plant-based-drugs increases for the treatment of many diseases. The present study investigates the presences of antimicrobial activity and phytochemical compounds of *Achyranthes aspera*. *Achyranthes aspera* (Amaranthaceae) is an important medicinal herb found as a weed and its parts are used in traditional systems of medicine. The physicochemical, phytochemical and fluorescence analysis was carried out. Those activities were due to the presence of secondary metabolites. However, further detailed studies are required to determine the active components responsible for these effects and mechanism pathway.

KEYWORDS: *Achyranthes aspera*, physicochemical, phytochemical and fluorescence analysis.

INTRODUCTION

Plants have been used as medicines for thousands of years. People depend on plants for several purposes like for wood, timber, non-timber forest products, food, medicine etc. (Jain *et al.*, 2005). They have always been used as a rich source of biologically active drugs and have numerous traditional uses to serve mankind for many thousand years (Kirtikar *et al.*, 1999). Now a day, they are used widely because of growing awareness of people towards unwanted side effects and high cost of the allopathic medicines which makes them beyond the reach of common people.

The therapeutic properties of medicinal plants are mainly due to the secondary metabolites present in it. The most significant of these bioactive constituents of plants are alkaloids,

tannins, proteins, phenolic compounds and flavonoids. These phytochemicals are known to possess antioxidant (Wong *et al.*, 2009)[17], antibacterial (Nair *et al*., 2005)[18], antifungal (Khan and Wassilew, 1987)[19], antidiabetic (Singh and Gupta, 2007), anti-inflammatory (Kumar *et al.*, 2008)[20], hypolipidemic activity (Durkar *et al.* 2014) etc and due to these properties they are largely used for medicinal purpose. Therefore, qualitative phytochemical screening of these selected plants is necessary and the present study is designed to evaluate the bioactive chemical constituents of Achyranthes aspera commonly used as medicine in India.

Flavonoids are water soluble phytochemical showing the antioxidant, anticancer and anti-inflammatory activities. These prevent cells from oxidative damage and carcinogenesis. Flavonoids are also used to cure some heart related diseases (Hussain *et al*., 2011).

Alkaloids and their derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal activities (Harisaranraj *et al*., 2009).

Saponins are widely distributed in nature, occurring primarily in the plant kingdom. These are generally known as nonvolatile surface active compounds having sweet or bitter, foaming emulsifying, pharmacological, medicinal and haemolytic properties, as well as antimicrobial, insecticidal, and molluscicidal activities (Vincken *et al*., 2007).

Phenols and phenolic compounds prevent the platelest from clumping and have the ability to block specific enzyme that cause inflammation; these are antioxidant, immune enhancer and hormone modulators and are also used for curing skin infection and other wounds (Hussain *et al*., 2011).

Tannins have stringent propreties, hasten the healing of wounds and inflamed mucous membranes. Tannins present in the cells of plants are inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens (Dash, 2008).

The crude fiber content is commonly used as a measure of the nutritive value of poultry and livestock feeds. It is analyzed in various foods and food products to assess adulteration, quality and quantity (Stary, 1998).

Pectin or pectic substances are complex polysaccharides universally present in the cell walls of plants, especially in the spaces between the cell walls together. These are reported to have

antihypercholesterolemic property and helps in excreting heavy metal and lowering fat absorption properties (Leung and Foster, 1996).

Achyranthes aspera L. (Latjeera) is an erect or procumbent, annual or perennial herb of about 1-2 meter in height, often with a woody base (Rawat *et al.,* 2008). Stems angular, ribbed, simple or branched from the base, often with tinged purple colour, branches terete or absolutely quadrangular, striate, pubescent, leaves thick, 3.8 - 6.3 × 22.5 - 4.5 cm, ovate – elliptic or obovate rounded, finely and softly pubescent on both sides, entire, petiolate, petiole 6 – 20 mm long, flowers greenish white, numerous in axillary or terminal spikes up to 75 cm long, seeds subcylindric, truncate at the apex, rounded at the base, reddish brown (Jain *et al.,* 2006).

MATERIALS AND METHODS

Collection, Identification and Authentication of plant materials

The plant species namely *Achyanthes aspera* L. plant was collected by in and around Koothanallur, Thiruvarur District, Tamil Nadu, India. The plant was identified with the help of the Flora of Presidency of Madras and authenticated by Dr. S. John Britto, RAPINAT Herbarium and Centre for Molecular Systematics, St. Joseph's college, Tiruchirappalli (Voucher number of the specimen, AMTA 001) (Gamble, 1997). The plant was air dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powder matter was used for further analysis.

Preparation of the aqueous extract

The plant material (Whole plant) was shade dried and coarsely powdered with electrical blender. 200g of *Achyranthes aspera* was mixed with 1200ml of water. Then it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to preclinical screening.

Preparation of the Ethanol extract

Ethanolic extracts was prepared according to the methodology of |Indian pharmacopoeia (Anonymous, 1996). The coarse powder material was subjected to Soxhlet extraction separately and successively with 210ml ethanol and 90ml distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40^0C – 50^0C). The paste form of the extracts was put in an air tight container

stored in refrigerator.

Preliminary phytochemical analysis

The preliminary phytochemical investigation of the whole plant of *Achyranthes aspera* was carried out with the standard protocol. The extracts are subjected to preliminary phytochemical analysis (Kokate *et al.*, 1995). The physicochemical characters such as moisture content, total ash, acid insoluble ash, water insoluble ash, and alcohol soluble extract value, water soluble extract value. The qualitative and quantitative analysis such alkaloids, flavonoids, glycosides, phytosterols, saponins, phenols, tannins, crude fiber and pectin substances. Powder of *Achyranthes aspera* treated with acetone, water, nitrate, nitrite, picric acid, sulphuric acid, chloroform, sodium hydroxide, sodium chloride, sodium nitrite, iodide, 1N HCl, 1N KOH, conc. nitric acid, ammonia.

RESULTS AND DISCUSSION

In recent years, although technology and medicine have developed extensively, some countries have made it obligatory to use natural products for many different purposes due to decrease in natural richness and draw-backs. Like in many other countries, the plants known by people with health benefits are picked up and used for the treatment of various purposes.

Physico-chemical analysis of *Achyranthes aspera*

Physico-chemical characteristic such as moisture content, ash content, acid insoluble ash content, water soluble ash content, alcohol soluble extractive and water soluble extractive were represented in Table 1.

Table 1: Physico-chemical characteristics of *Achyranthes aspera*

S.No.	Parameters tested	Percentage Yield (%)
1.	Moisture content	2.97
	Ash value	
2.	Total ash	40
3.	Acid insoluble ash	25
4.	Water insoluble ash	26.66
	Extractive value	
5.	Alcohol soluble extractive	6.14
6.	Water soluble extractive	3.68

The moisture content of the *Achyranthes aspera* was found to be 2.97%. The ash content of *Achyranthes aspera* was found to be 40%. Since the accepted range was 50%, which implies that the plant has normal complexes of inorganic and organic components (British

Pharmacopoeia, 1980). The high ash content is reflection or the mineral contents preserved in the food material. The result suggested a high deposit of mineral elements in the leaves (Anonymous, 1955). The acid insoluble ash value was found to be 25% and water insoluble ash value was found to be 26.66%. The normal acid insoluble ash has a portion of the ash contents, which was acid soluble and hence may be physiologically important as salts in the body when consumed. It is also indicative of high digestibility of the plant when eaten (Rajurkar *et al.*, 2009). Alcohol soluble and water soluble extractive were found to be 6.14% and 3.68% respectively.

PHYTOCHEMICAL ANALYSIS

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis of aqueous and ethanolic extract of *Achyranthes aspera* was represented in Table 2.

The medicinal properties of plants are due to the presence of different complex chemical substances as secondary metabolites, which are exclusively accumulated in

Table 2: Phytochemical screening of *Achyranthes aspera*

S.No.	Name of the test	Name of the extract	
		Aqueous extract	Ethanolic extract
1.	Alkaloids	+	+
2.	Carbohydrates	-	-
3.	Glycosides	+	+
4.	Saponins	-	-
5.	Phytosterols	+	+
6.	Fixed oils and fats	+	+
7.	Resins	-	-
8.	Phenols	-	-
9.	Tannins	+	+
10.	Flavonoids	+	+
11.	Proteins and amino acids	+	+
12.	Diterpenes	+	+
13.	Terpenoids	+	+
14.	Gums and Mucilage	+	+
15.	Coumarins	-	-
16.	Chlorogenic acid	-	-
17.	Steroids	-	-

(+) Indicates Presence; (-) Indicates Absence

Different parts of the plants and produce marked healing action on human body (Bashir *et al.*, 2012). The most important of these agents are alkaloids, flavanoids and tannins

(Edeoga *et al.*, 2005). These compounds have been associated with antimicrobial effects in various studies using plant extracts (Nwaogu *et al.*, 2007).

In the present study, identification of phytochemical screening of ethanol extract of flowers in *Achyranthes aspera*. The result revealed that the ethanolic extract of *Achyranthes aspera* recorded the presence of alkaloid, flavonoid, tannins, fixed oils and fats, proteins and amino acids, diterpenes, terpenoids, gums and mucilage, phytosterol and glycosides followed by other extract. Phytochemical constitutes such as tannins, flavonoids and several aromatic compounds or secondary metabolites of plants serves as defense mechanism against predation by many microorganisms. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloid, flavonoid, tannins, phenolic compounds, saponins and phytosterols (Britto and Sebastian, 2012). The presence of alkaloids, saponin, flavonoids, phenolic compounds, tannins, phytosterol and terpenoids are used in analgesic and antiplasmodic and bacteriocidal activities (Stary, 1998). Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Quantitative Phytochemical analysis

Quantitative phytochemical analysis of aqueous and ethanolic extract of *Achyranthes aspera* was represented in Table 3.

Table 3: Quantitative Phytochemical analysis of *Achyranthes aspera*

S.No.	Phytochemical	Concentration in Percentage (%)
1.	Flavonoids	12.2
2.	Alkaloids	5.8
3.	Saponins	22.5
4.	Phenols	10.6
5.	Tannins	40.4
6.	Crude fiber	6.5
7.	Ash content	10.8
8.	Pectic substances	35.8

The results revealed the presence of following important classes of natural constituents; flavonoids, alkaloids, saponins, phenols and tannins. The crude fiber and ash content were also analyzed and quantified whereas; pectic substances were calculated as (35.8%). Very high flavonoids content (12.2%) of *Achyranthes aspera* makes it a potential candidate bearing antioxidant and anticancer properties. Tannins and phenols although found in low concentrations, (40.4% and 10.6% respectively) can synergize the antioxidant and anticancer

potential of flavonoids. Phenols are reported to prevent the platelets from clumping and have the ability to block specific enzymes that cause inflammation. These also act as immune enhancers, anti-clotting and hormone modulators. Tannins in the plant cell inhibit hydrolytic enzymes like proteolytic macerating enzymes used by plant pathogens. The presence of alkaloids indicates the pharmacological importance of plant because alkaloids and their derivatives are used as basic medicinal agents due to their analgesic, antispasmodic and bactericidal activities.

The plant is an enriched source of alkaloids (5.8%). Saponins in the plant are responsible for antimicrobial, molluscidal and insecticidal activities and current analysis showed that leaves contain 22.5% saponins. High fiber content indicates the nutritive value of the plants. Younger leaves are palatable, although their intake is low. *Achyranthes aspera* can also be used as low cost, highly nutritive and digestible For age after processing with molasses or other feeds (Heuze *et al*., 2011). Ash content roughly represents minerals and 10.8% of ash content adds to the nutritive value of plant as fodder. Pectic substances are complex polysaccharides, present in the plant cell wall and act as binder. Pharmacologically pectins help in heavy metal excretion and act as anti-hypercholestrolemic.

Fluorescence analysis of *Achyranthes aspera*

Table 4 showed the behavior of *Achyranthes aspera* Linn plant powder on treatment with different reagents.

Powder of *Achyranthes aspera* appeared to be green in colour. Powder of *Achyranthes aspera* is added to water gives green colour, dark green colour at 24 hours and balck colour at 48 hours. On treatment with concentrated H_2SO_4 it gives black colour and brown colour at 24 hours and dark brown colour at 48 hours. When treated with acetone it gives sandal colour and pale green colour at 24 hours and green colour at 48 hours. When powders of *Achyranthes aspera* is treated acetic acid, NaOH and 1N HCl gives green colour, pale green colour at 24 hours and dark green colour at 48 hours. Treated with 1N KOH gives dark grey colour, green colour at 24 hours and dark green colour at 48 hours.

Table 4: Fluroscence analysis of *Achyranthes aspera*

S.No.	Particulars of the Treatment	Under ordinary light		
		0 Hours	24 Hours	48 Hours
1.	Powder as such	Green	Green	Green
2.	Powder + water	Green	Dark Green	Black

3.	Powder + conc.H_2So_4	Black	Brown	Dark brown
4.	Powder + Acetone	Sandal	Pale green	Green
5.	Powder + Acetic acid	Green	Pale green	Dark green
6.	Powder + $Fecl_3$	Dark green	Pale green	No change
7.	Powder + NaOH	Green	Pale green	No change
8.	Powder + $CHCl_3$	Sandal	Pale sandal	Sandal
9.	Powder + Sodium nitrite	Pale green	Brown	No change
10.	Powder + NaCl	Pale grey	Green	Pale green
11.	Powder + NH_3OH	Brown	Dark brown	Pale brown
12.	Powder + picric acid	Yellow	Green	No change
13.	Powder + Iodide	Grey	Pale green	No change
14.	Powder + conc.HCl	Pale brown	Dark brown	Black
15.	Powder + 1N HCl	Green	Pale green	Dark green
16.	Powder + 1N KOH	Dark grey	Green	Dark green

When powders of *Achyranthes aspera* is treated with NaCl gives pale grey colour, green colour at 24 hours and pale green colour at 48 hours. On treatment with ferric chloride gives dark green colour, pale green colour at 24 hours and there is no change at 48 hours. When treated with NH_3OH gives brown in colour, dark brown colour at 24 hours and pale brownish colour at 48 hours. On treatment with $CHCl_3$ gives sandal colour, pale sandal colour at 24 hours and sandal colour at 48 hours. When powders of *Achyranthes aspera* is treated with sodium nitrite gives pale green colour, brown colour at 24 hours and there is no change at 48 hours. When powders of *Achyranthes aspera* is treated with picric acid gives yellow colour, green colour at 24 hours and there is no change at 48 hours.

On treatment with concentrated HCl gives pale brownish colour, dark brown colour at 24 hours and balck colour at 48 hours. When powders of *Achyranthes aspera* is treated with iodide gives grey colour, pale green colour at 24 hours and there is no change at 48 hours.

CONCLUSION

These phytochemical compounds are the key candidates in the medicinal value of the plant. The presence of alkaloids in plants extract may be participating in plant metabolism sequences and the presences of terpinoids may be show cytotoxic activity against a wide range of organisms, ranging from bacteria and fungi. Saponins are the glycoside of triterpenes or steroids and therefore saponins may be used in traditional medicine as anti-infecting agents. The presence of flavonoids and tannins in the plants is probable to be responsible for the free radical scavenging effects. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary

antioxidants and free radical scavengers. All these phytochemicals possesses good antioxidant activities and can be served as a substitute for synthetic drugs.

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