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Tamanna Talreja
CVAS, Rajasthan University of Veterinary and Animal Sciences, Bikaner (Rajasthan) India.

Asha Goswami
Retd. Principal, M.L.B. Govt. College, Nokha, Bikaner (Rajasthan) India

Tribhuwan Sharma
CVAS, Rajasthan University of Veterinary and Animal Sciences, Bikaner (Rajasthan) India

Correspondence
Tamanna Talreja
CVAS, Rajasthan University of Veterinary and Animal Sciences, Bikaner (Rajasthan) India

Preliminary phytochemical analysis of *Achyranthes aspera* and *Cissus quadrangularis*

Tamanna Talreja, Asha Goswami and Tribhuwan Sharma

Abstract
Phytochemicals are major sources for the treatment of various health problems. The present study was aimed to investigate the preliminary phytochemical screening of different sequential extracts (methanol, ethyl acetate, benzene, aqueous) of seeds of *Achyranthes aspera* Linn. (Family: Amaranthaceae) and stem of *Cissus quadrangularis* Linn. (Family: Vitaceae) by different standard methods of phytochemical screening. Our results reveals that the extracts of *A. aspera* seeds and *C. quadrangularis* stem contain alkaloids, phenolic compounds and tannins, steroids and terpenoids,, flavonoids, reducing sugar and saponin in appreciable, moderate and trace amount**.** Due to rich source of phytochemicals, these plant is may be used for herbal medicine.

Keywords: *Achyranthes aspera, Cissus quadrangularis,* phytochemical screening

Introduction
Plants have been used in traditional medicine for several thousand years. In India, it is reported that 2500 plant species serve as a regular source of medicine. Today, according to W.H.O. (World Health Organization), as many as 80% of the world's people depend on traditional medicine for their primary health care needs. India has its long tradition and history of health care through herbal drugs and even today more than 76% of total population depends for their health care needs on plants. Today the global movement towards a more natural life style has brought about resurgence of interest in herbs. 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 (A. M. 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* and *Cissus quadrangularis* commonly used as medicine in India.
Achyranthes aspera and *Cissus quadrangularis* are important medicinal plants. These plants have been used in traditional Indian medicine for thousands of years to treat various disorders. Several reviews about anti-obesity properties on these important medicinal plants have been appeared in literature. Considering, all the beneficial aspects and medicinal values of these plants for the present study, *Achyranthes aspera* and *Cissus quadrangularis* are taken for to investigate and to establish scientific data for its traditional claim.

Material and Methods
A. Plant collection
Achyranthes aspera (seeds) and *Cissus quadrangularis* (stem) was collected from various parks of Bikaner where it is cultivated as ornamental plant whereas seed samples of *Achyranthes* were purchased from the shop of herbal medicine. The fresh sample of *Cissus* stem and seeds of *Achyranthes* were dried separately and used for further analysis.

B. Extraction Procedure
To analyze the bioactive components of *Cissus quadrangularis* and *Achyranthes aspera* crude extract will be extracted using Benzene, Methanol, Distil water, Ethyl acetate. Crude extract formation is done under following steps of extraction

- **Crude Extract preparation using Benzene**

1. *Achyranthes aspera* (seeds) and *Cissus quadrangularis* (stem) were collected from various parts of Bikaner.
2. The fresh sample of *Cissus* stem and seeds of *Achyranthes* were dried separately at 100 ± 5 ^{0}C.
3. These samples were grounded finely and hydrolyzed with 30% (v/v) hydrochloric acid for 4 hours on water bath. The hydrolyzed test samples were washed separately with distilled water till the filtrate attained pH 7.0. Test samples so obtained were dried at 60 ^{0}C for eight hours
4. The known quantity was filled in the thimble. These sample containing thimbles were placed in the middle section of soxhlet apparatus and extracted in benzene (200 ml) for twenty four hours separately.
5. The extract was then concentrated using water bath set at 60 ^{0}C.
6. After that extract was weighed and the percentage extractive value was determined.

- **Crude Extract preparation using Methanol, Distil water and Ethyl acetate**

1. Fresh plant material was collected from various parts of Bikaner.
2. These samples were dried separately and grounded finely.
3. Then the known quantity of sample was filled in thimble directly and were placed in soxhlet apparatus and extracted separately time to time in ethyl actate, methanol, distil water etc.
4. After that the same steps of drying, concentration and weighing were followed.

(C). Preliminary phytochemical screening

Qualitative phytochemical analysis of *A. aspera* and *C. quadrangularis* extracts (methanol, ethyl acetate, benzene, aqueous) was done using standard procedures.

Preliminary qualitative phytochemical analysis

Compound Test & Reagents	
Carbohydrate	Felhing's
	Benedict's
Alkaloids	Mayer's
	Wagner's
	Drangondroff's
Flavonoids	Shinoda's
	Alkaline reagent test
	$FeCl_3$ Test
Phenolic compounds and Tannins	Lead Acetate
	$FeCl_3$ Test
	Gelatin
Amino Acid	Ninhydrin test
	Millon's test
Steroids and terpenoids	Libermann's test
	Salkowskis test
Fixed oil & Fats	Spot Test
Gum & Mucilage	Alcohol Precipitation
Saponins	Foam Test /Froth test
Phytosterol	L.B. Test

Test for Carbohydrate

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.

Benedict's test: : To a set of filtrates of various drugs' extracts, added equal volumes of Benedict's reagent and heated in boiling water bath for 5min. The appearance of green, yellow or red color indicated the presence of sugars.

Test For Alkaloids

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.

Mayer's test: To 1 ml of test solution was added a drop or two of the Mayer's reagent along the sides of the test tube. A white or a creamy precipitate confirmed the test as positive.

Wagner's test: Two drops of Wagner's reagent was added to 1ml of the each test solution along the the side of the test tube. The formation of yellow or brown precipitate confirmed the test as positive for alkaloids.

Test for Flavonoids

Shinoda's Test: A few Magnesium turnings and 5 drops of concentrated Hydrochloric acid was added drop wise to 1 ml of test solution. A crimson red color appeared after few minutes confirmed the test.

Alkaline reagent test Addition of 5 drops of 5% Sodium hydroxide to 1 ml of the test solution resulted an increase in the intensity of the yellow color which became colorless on addition of a few drops of 2 M hydrochloric acid which indicated the presence of flavonoids**.**

$FeCl_3$ Test To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of flavanoids.

Test for Phenolic Compounds and Tannins

Lead Acetate test: The extracts were treated with few drops of 10 % lead acetate solution. The formation of precipitate confirmed the presence of phenolic compounds and tannins.

Ferric Chloride test: To test solution added 10 ml distilled water, then filtered, in the filtrate 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.

Test for Amino Acids

Ninhydrin test to the test solution added 1 ml of 0.2 % ninhydrin solution, violet color indicate the presence of amino acids in sample.

Millon's test: Added 5 drops of millon's reagent to 1 ml of test solution and heated on a water bath for 10 min, cooled and added 1% sodium nitrite solution. Appearance of red color confirmed the test.

Steroids and Terpenoid

Libermann's test to the each test solution added 10 ml of chloroform then filtered. To the 2 ml filterate added 2 ml of acetic anhydride and con. H2So4. Blue green ring indicate the presence of steroids in the sample.

Salkowskis test Approximately 2 mg of dry extract was

shaken with 1 ml of chloroform and a few drops of concentrated Sulfuric acid were added along the side of the test tube. A red brown color formed at the interface indicated the test as positive for triterpenoids and steroids.

Gum & Mucilage

Alcohol Precipitation All the test solutions were mixed with absolute alcohol and dried in air and the residues were tested for swelling properties and didn't get positive results.

Fixed Oil & Fats

Spot Test Prepared spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil & fats.

Test for Saponins

Foam test or Froth 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

Haemolysis test: 1 ml of suspension of RBCs in normal saline was taken. In this equal volume of plant extract in normal saline was added. Solution was shaken gently. Clear red solution was obtained indicating haemolysis of RBCs (compared with blank).

Test for Steroids

Libermann test or LB test: To the test solution added 10 ml of chloroform then filtered. To the 2 ml filtrate added 2 ml of acetic anhydride and con. H_2SO_4. Blue green ring indicate the presence of steroids in the sample.

Result and Discussion

Phytochemical characteristics of the sequential extracts of *A. aspera* and *C. quadrangularis* were investigated and are summarized in table 2. The results reveal the presence of medicinally active phytoconstituents studied in the four kind of extracts. From table 2, different extracts of *A. aspera* seed and *C. quadrangularis* stem revealed the presence of various bioactive components of which alkaloids, saponins, sugars, tannins, steroids, flavonoids, terpenoids and amino acids were identified.

Table 2: Qualitative analysis of Phytochemicals of various extracts of *A. aspera* and *C. quadrangularis*

S. No.	Phytoconstituents	*Achyranthes aspera*				*Cissus quadrangularis*			
	Carbohydrate	**BEA**	**MEA**	**EEA**	**WEA**	**BEC**	**MEC**	**EEC**	**WEC**
1.	Felhing's	-	+	-	+	-	+	-	+
	Benedict's	-	++	-	++	-	++	-	++
2.	**Alkaloids**								
	Mayer's	-	++	-	+	-	++	-	+
	Wagner's	-	+	-	+	-	+	-	+
	Drangondroff's	-	+++	-	+	-	+++	-	+
3.	**Flavonoids**								
	Shinoda's	-	++++	-	++	-	+++	-	++
	Alkaline reagent test	-	+++++	-	++	-	+++++	-	+++
	$FeCl_3$ Test	-	++	-	+	-	+	-	+
4.	**Phenolic compounds and Tannins**								
	Lead Acetate	-	++	-	++	-	+	-	++
	$FeCl_3$ Test	-	+	-	+	-	+	-	+
	Gelatin	-	+	-	+	-	+	-	+
5.	**Amino Acid**	-	++	-	+++	-	+	-	++++
	Ninhydrin test	-	+	-	+	-	+	-	+
	Millon's test								
6.	**Steroids and terpenoids**								
	Libermann's test	-	+	+	+	-	+	+	+
	Salkowskis test	-	+	++	+++	-	+	++	+++
7.	**Fixed oil & Fats**								
	Spot Test	+	-	-	-	++	-	-	-
8.	**Gum & Mucilage**	-	-	-	-	-	-	-	-
	Alcohol Precipitation								
9.	**Saponins**								
	Foam Test /Froth test	-	+++	-	++++	-	+	-	++
	Haemolysis Test	-	++	-	+++	-	+	-	++
10	**Phytosterol**	+++	-	++	-	+++	-	++	-
	L.B. Test								

(++++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent. **Abbreviations:** BEA- Benzene extract of *Achyranthes aspera;* EEA- Ethyl acetate extract of *A. aspera;* MEA- Methanolic extract of *A. aspera*; WEA- Water (Aqueous) extract of *A. aspera* BEC- Benzene extract of *C. quadrangularis;* EEC- Ethyl acetate extract of *C. quadrangularis;* MEC- Methanolic extract of *C. quadrangularis;* WEC- Water (Aqueous) extract of *Cissus quadrangularis*

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 for obesity.

Conflict of interest: None

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