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Phytochemical estimation of medicinal plant achyranthes aspera root

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Abstract
Achyranthes aspera root having much therapeutic importance due to presence of various types' chemical constituents. In this study root of achyranthes aspera was subjected in different extracts of various solvent (ethanol, hexane, ethyl acetate and chloroform). Phytochemical analysis of achyranthes aspera root was carried out to various chemical test and found alkaloids, glycosides, flavonoids, carbohydrate, phytosteroides, phenols, tannins, protein, diterpenens and saponin like compound in different extracts. Percentage of extractive is useful for estimation of crude drug and nature of chemical constituents present in crud drugof different solvents used for Achyranthes aspera root extracts.

Keywords: Root, Achyranthes aspera, Medicinal

Introduction
The medicinal plant Achyranthes aspera territory has been the greatest and most important source of medicinal Preparations. Herbal medicine used to treat disease and promote health. Achyranthes aspera is used from many years for curing the disease. Plant contains different types of substances like carbohydrates, lipids, proteins, glycosides, alkaloids, Tannins, flavonoids etc. responsible for theirs pharmacological activity. Achyranthes aspera are the richest source of bioactive compound which is responsible for their therapeutically effectiveness. Phytochemical analysis is the useful for discovery of new medicines form the herbal source. Plants have their different pharmacological and therapeutic importance. Different parts of plant which contain biologically active ingredients like root, bark, stem and leaf are used for treatment of acute and chronic ailments like Asthma, fever, hypertension, malaria, fungal, bacterial infection and heart disorder. In many developed country, they are also used plant related foodstuffs for pregnant and nursing mothers for medicinal purpose. Herbal medicines are also available in the form of ointment for skin related disease. These medicines are safe and effective to the proper treatment of different types of disease compare to synthetic drugs. Most of the people depend upon herbal medicines substance which is isolated form plants to be less expensive than synthetic drugs pharmaceutical products isolated from plants are at times found to be less costly than Synthetic drugs. Products are atropine; digoxin and morphine are isolated form plants. Ordinary produces may also be used for the formation of synthetic and semi synthesis drugs like procaine and local anesthetics

Material & Method
The plant of Achyranthes aspera root was collected from the Botanical garden of Shekhawati College of Pharmacy, Dundlod, Rajasthan in the November 2016. The identification & authentication of the plant was confirmed at department of Botany, University of Rajasthan Jaipur. After collection washed carefully and dried to constant weight at 45°C. The plants roots were stored in an air-tight container. All chemicals and solvents were of analytical grade purchased from local source.

Determination of extractives value
Roots of plant achyranthes aspera were separated and converted in the coerce powder after drying. Then shaded the plant part through sieve no.40 and successively extracted with different solvent by soxhlet extraction. Around 20 g of crushed air dried powders were extracted with ethanol, hexane, chloroform and ethyl acetate solution in a soxhlet apparatus for 72 hrs. The obtained solutions were filtrated and evaporated through rotary flash evaporator and kept in a refrigerator. All extracts were received through same procedure.

Test for alkaloids
A little portion of the dissolvable free petroleum ether, chloroform, ethyl acetic acid derivation, ethanol and water extricates independently with a couple of drops of dilute Hydrochloric, acid and clear out. The filtrates were attempted with extraordinary alkaloid reagents for instance, Mayer's reagent (cream encourage), Wagner reagent (reddish brown Precipitate) and Dragendorff's reagent (orange brown precipitate).

Dragendorff's Reagent
1 ml of Dragendroff's reagent while introduced with few drops of test sample pattern brick purple ppt showed the presence of alkaloids.

Wagner Reagent: When few drops of Wagner reagent have been delivered in filtrates formation of the red colored ppt. indicated the presence of alkaloids.

Hager's test: 2-3 ml of filtrates when added with saturated picric acid solution then yellow precipitate indicated the presence of alkaloids.

Mayer's Reagent: filtrated test sample was added with few drops of potassium mercuric iodide (Mayer's reagent) and white or creamy precipitates and indicated the presence of alkaloids.

Test for flavonoids

Ammonia Test: Filter paper strips had been dunked inside the different extracts and 5ml of diluted ammonia solution added then introduced con. H2SO4.The filter paper converted its colour to yellow which indicated the presence of flavonoids.

Pew test for flavonoids: To 1ml of the each concentrates, a bit of steel magnesium/zinc was added and then 2 drop of concentrated hydrochloric acids added and heated development brown red colour indicated the presence of flavonoids.

Fluorescence Test

Plant drugs were extracted in 15 ml methanol for on a boiling water bath and filtered while hot and vaporized extracts till dryness. The dry residue was added in 0.3 ml boric acid (3%w/v) and 1 ml of oxalic acid (10%w/v).The mixture was evaporated to dryness and the residue was dissolved in 10 ml of ether. The ether solution showed greenish fluorescence under UV light indicating presence of flavonoids.

Test of saponins

Foam test: 10 ml of water and 1 ml diluted extracts of plant shaked for 20 minutes and detected a one centimeter layer of foam shows the presence of saponin.

Sodium bicarbonate test: To the couple of milligrams of concentrate couple of drops of sodium bicarbonate were included and shaken well. Arrangement of honeycomb like foaming shows positive test for saponins.

Hemolytic zone: 0.5 ml of blood was mixed with gelatin solution in 18% v/w NaCl solution was shaken in six test tubes at 60°C. Ethanol, methanol and chloroform were added in three test tube and extracts were added in remaining test tube. Taken on a glass slide and observed under microscope. A clear hemolytic zone was formed showed the Presence of saponins.

Test for phenolic compound and tannins

Taken very few amounts of different concentrates individually in water and test for the Phenolic mixes and tannins with weaken ferric chloride arrangement (5%) and lead acetic acid test. 2-3 ml of plant extract, 10 % ferric chloride answer changed into delivered. If dark blue or greenish grey color appear inside the solution indicated the Presence of tannins inside the drug

Ferric chloride test: when some drops of 5% ferric chloride solution were added the presence of tannin and phenols conformed if green/ blue colour appear in the test tube.

Lead acetic test: When few drops of lead acetic acid (5%) were added to the specific extract of the root the appearance of white ppt exhibited the presence of phenolic components.

Test for glycosides

Baljet's test: plant extracts give yellow to orange colour with sodium picrate show the vicinity of glycosides.

Legal's test: when plant extracts were added with 1 ml pyridine and 1 ml sodium nitro prusside converted in pink and red showed the presence of glycosides.

Kellar Killani test: glacial acetic acid, 5% FeCl3 and 1ml concentrated sulphuric acid were added with plant extracts and observed disappearance of reddish brown colour at junction and bluish green in upper layers shows the presence of glycosides in plant extracts.

Libermann's test (For bufadenolids): plant extracts added with 3 ml acetic anhydride and after that heated and then cooled. After that introduced few drop of concentrated H_2SO_4 detecting the blue color in sample exhibited the presence of glycosides.

Borntrager's test: To the test tubes containing 2 ml of dilute H_2SO_4 was added with 2 ml of extract and become boil for 5 min after that filtered the filtrates and equal volumes of chloroform was added and mixed well. Organic layers were separated and ammonia was added to this. Pinkish red colour of the ammonia layer showed the presence of anthraquinone glycosides.

Test for proteins and free amino acids

Added a couple of drops of various extracts in a couple ml of refined water and subjected to ninhydrin and million's and biuret tests.

Million's test: when 3 ml of extract added with few drops of million's reagent and heated. If white ppt acquired which converted in to brick red colour after heating. It indicated the presence of proteins.

Biuret test: 3 ml plant extracts added with 4% w/v sodium hydroxide solution and added few drops of 1% copper sulphate solution. The presence of free amino acids and proteins conformed due to appearing a violet or pink color.

Ninhydrin test: To each of the extract sample was added with 5% lead acetate solution and heated on water bath. If changed in colour of solution in purple blue indicated the presence of amino acids. The Filtrate was spotted on a paper chromatogram, splashed with 5% ninhydrin reagent and dried at 11º C for 5 minutes. Violet spots if seen affirmed the vicinity of proteins and free amino acids

Test for phytosterol and triterpenes

Liebermann-Burchard's test: The 2ml concentrate extracts was added with acidic anhydride and shaken with chloroform and added the few drops of concentrated sulphuric acid and allowed to stand. Blue to block red shading indicated the

presence of sterol and triterpenes in test sample.

Hesse's Response: The deposit was broken down in chloroform (4 ml) and equivalent amount of concentrated sulphuric acid was added in the sample. The formation of the pink hued ring, which is on shaking diffused in both the layers, demonstrated the vicinity of sterols in the concentrate.

Salkowaski test: When add 5 drops of conc. sulphuric acid with in test solution, shaken and kept at room temperature and observed the green blue colour indicates the presence of triterpenoids.

Results

Root

Table 1

S. No	Tests	Hexane	CHCl3	Ethyl acetate	Ethanol
	Alkaloids				
1	Mayer's test :	-	-	+	+
	Wager's test:	-	+	+	+
	Hager test:	-	+	+	+
	Carbohydrates:				
2	Molish's test:	+	+	+	-
	Benedicts test:	-	-	+	-
	Fehlings test:	+	-	-	-
3	Glycosides	-	-	-	+
	Cardiac glycosides:				
	a. Baljet's test:				
	b. Legal's test:				
	B. Anthraquinone glycosides:				
	a. Borntrager's test:				
	C. Saponin glycosides:				
	a. Foam test:				
	b. Froth test:				
4	Saponins	+	-	+	+
5	Phytosterols	-	+	+	-
6	Phenols	-	-	-	+
7	Tannins	-	+	+	+
8	Flavonoids	+	+	+	+
	a. Shinoda test:				
	b. Alkaline reagent test				
9	Proteins	-	-	+	+
	a. Millon's test:				
	b. Ninhydrin test:				
10	Diterpenens	+	+	+	+

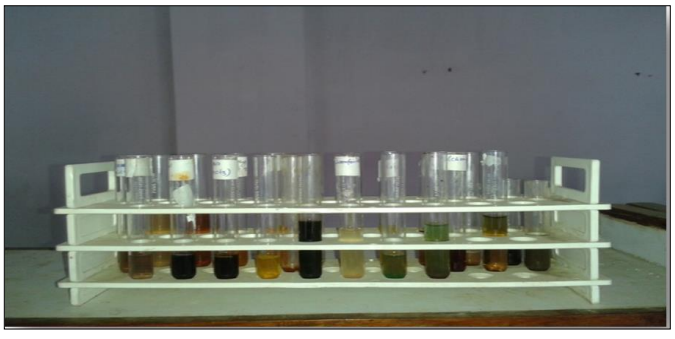

Fig 1

Alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins like chemical constituents found in *Achyranthes aspera* extraction in different solvent and exhibited different type's activity. Ethanol exatrcts of root part of plant exhibited the presence of tannin, proteins, saponin, flavonoids, phenols, diterpenes and Alkaloids. Chloroform extracts contain the alkaloids, carbohydrates, phytosteroide, phenols, tannin and diterpenes also presented in ethyl acetate and ethanol extracts of root part of plant contains alkaloids, glycosides, saponin, phytosterols, flavonoids and carbohydrates were also found in root extracts of *Achyranthes aspera*.

Conclusion

In the present study the root of achyranthes aspera were collected, air dried and converted in powder material. Obtained the various extracts by using various solvent and subjected to various chemical tests. The study gives the various promising results to phytochemical analysis and soluble extractive percentage of achyranthes aspera roots using four different solvents. The achyranthes aspera roots show the presence of different bioactive phytochemical constitute such as Alkaloids, phytosteroles, proteins, carbohydrates, glycosides, saponins, diterpenens, steroids, flavonoids, phenols, tannins. These types bioactive constituent is responsible for the rapetical and pharmacological properties. This may give the idea to develop a new drug from the Achyranthes aspera plant.

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