Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014; 6(2); 379-382

ISSN: 0975-4873

Research Article

Phytochemical Analysis of a Fodder Crop *Trifolium alexandrium* L.

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Available online: 1st June 2014

ABSTRACT

Trifolium alexandrium is a winter legume fodder crop with high nutritional value. Leaf, stem and roots of *Trifolium alexandrium* were screened for its phytochemical analysis. Different extracts of the Leaf, stem and roots of *Trifolium alexandrium* revealed the presence of alkaloids, carotenoids, flavonoids, steroids, phenolics, Volatile oils, anthracene glycosides, amino and Chlorogenic acid, Gums and mucilage.

Keywords: *Trifolium alexandrium*, Phytochemicals, flavonoids, alkaloids.

INTRODUCTION

The wealth of India is stored in the enormous natural flora which has been gifted to her by nature. India has a wide diversity of agro-climatic conditions. Therefore, it has large number of plants and is virtually called herbarium of the world. Due to the rich biodiversity India has rare and useful herbs and forage crops which are used in medicine and as a cattle feed, such as *Curcuma, Hedychium, Euphorbia, Boerhaavia* etc. and forage crops like *Medicago, Dichanthium, Digiteria, Cynodon, Trifolium* etc. The cultivation of these medicinal plants and its extractions is for the sustainable development of human in India. The crude drugs are obtained from the leaves, roots, flowers, barks, fruits etc of the plants.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect itself but recent research demonstrate that they can also protect humans against diseases.

Trifolium alexandrium L. commonly known as Berseem or Clover is an important leguminous winter fodder crop. In comparison to other legumes and grasses, Clover provides forage with high nutritional value and quality.

MATERIALS AND METHODS

Healthy plants of *Trifolium alexandrium* were collected for phytochemical screening. The plant parts used were leaves (L), stems (S) and roots (R). The plant material was washed thoroughly and chopped into small pieces and shade dried. The dried material was grounded to fine powder and stored in polythene bags at room temperature.

Preliminary phytochemical screening of plant was done according to the standard procedure adopted by different pioneer workers [1, 2,3,4,5,6,7,8].

Extract Preparation: For preliminary detection of phytochemical constituents, 15gm of powdered plant material was taken in thimble of Whatman No.-1 filter paper and soxhelated with petroleum ether for about 12-16 hrs. The ether extract (1a) was distilled off and the residue (1b) was dried over night. The petroleum ether extract (1a) was collected and tested for the presence of alkaloids, carotenoids, coumarins, emodins, fatty acids, flavonoids, steroids, phenolics, triterpenoids and volatile oils. The residue (1b) was soxhelated with chloroform until complete decolouration. The chloroform extract (2a) was collected and the residue (2b) was kept overnight for drying. The chloroform extract (2a) was tested for the presence of the alkaloids, coumarins, flavonoids, phenolics, steroids, and triterpenoids. The residue (2b) was soxhelated with acetone for 5-6 hrs and the acetone extract (3a) was collected. The residue (3b) was kept overnight. The acetone extract (3a) was tested for the presence of alkaloids, coumarins, flavonoids, phenolics, steroids, and triterpenoids. The residue (3b) was soxhelated with ethyl alcohol for 10-12hrs. The methanol extract (4a) was collected and the residue (4b) was dried till next day. The methanol extract (4a) was tested for the presence of alkaloids, anthracene glycosides, anthocyanins, anthocyanidins, coumarins, cardiac glycoside, flavonoids, phenolics, steroids, tannins, and triterpenoids. The residue (4b) was finally soxhelated with distilled water for about 10-12 hrs. The water extract (5a) was tested for the presence of alkaloids, Anthracene glycosides, anthocyanins, anthocyanidins, amino and chlorogenic acid coumarins, flavonoids, gums and mucilage**,** phenolics, phlobatanin, steroids, saponins, tannins, and triterpenoids. The residue (5b) was discarded. Fresh material was used for the screening of aucubins (diterpenoids) and iridoids (monoterpenoids), anthraquinones, cardiac glycosides, cyanogenic glycosides**.**

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Table-1 Phytochemical constituents of *T. alexandrium*

Solvent	Compounds/part → ↓	*Trifolium alexandrium* Leaf	Stem	Root
Petroleum	Alkaloids	+	+	+
Ether	Carotenoids	+	+	-
Extract	Coumarins	-	-	-
(1a)	Emodins	-	-	-
	Fatty acids	-	-	-
	Flavonoids	+	+	+
	Steroids	+	+	+
	Phenolics	+	+	+
	Triterpenoids	-	-	-
	Volatile oils	+	+	-
Chloroform Extract	Alkaloids	+	+	+
(2a)	Cumarins	-	-	-
	Flavonoids	+	+	+
	Phenolics	+	+	+
	Steroids	+	+	+
	Triterpenoids	-	-	-
Acetone	Alkaloids	+	+	+
Extract	Cumarins	-	-	-
(3a)	Flavonoids	+	+	+
	Phenolics	+	+	+
	Steroids	+	+	+
	Triterpenoids	-	-	-
Alcohol Extract	Alkaloids	+	+	+
(4a)	Anthracene glycosides	-	-	-
	Anthocyanins	-	-	-
	Anthocyanidins	-	-	-
	Coumarins	-	-	-
	Cardiac glycosides	+	-	-
	Flavonoids	+	+	+
	Phenolics	+	+	+
Alcohol Extract	Steroids	+	+	+
(4a)	Tannins	-	-	-
	Triterpenoid	-	-	-
	Alkaloids	+	+	+
	Anthracene glycosides	-	-	-
	Anthocyanins	-	-	-
	Anthocyanidins	-	-	-
	Amino acid	+	+	+
	Chlorogenic acid	+	+	+
	Coumarins	-	-	-
Water	Flavonoids	+	+	+
Extract	Gums and mucilage	+	+	+
(5a)	Phenolics	+	+	+
	Phlobatannins	-	-	-
	Steroids	+	+	+
	Saponins	+	+	+
	Tannins	-	-	-
	Triterpenoids	-	-	-
Powdered	Aucubins/Iridoids	-	-	-
Plant material	Anthraquinones	-	-	-
	Cyanogenic glycosides	-	-	-

- Alkaloids: 2ml of each extract (1a, 2a, 3a, 4a and5a) was taken separately with 5ml of 1.5%V/V aqueous hydrochloric acid and filtered. The resulting acidic solution was divided into four parts. Three parts were tested with Mayer's, Wagner and Dragendroff's reagent and the fourth served as blank. A brown flocculent precipitate was observed on addition of Wagner's reagent, indicated the presence of alkaloids. A faint turbidity, light opalescence or yellowish white precipitate on addition of Mayer's reagent was the

positive test of alkaloids. Development of orange precipitation on addition of Dragendroff's reagent is the positive test for alkaloid.

- Anthracene Glycosides: The alcohol and water extracts (4a and 5a) were heated on water bath till the liquid extract is evaporated and residue is left. The residue of 4a and 5a mixed with 5ml ether. These ethereal solutions of extracts were treated with 25% ammonium hydroxide. The development of red colour indicates the presence of anthracene glycosides.
- Anthocyanins and Anthocyanidins: The alcohol and water extracts (4a and 5a) were tested for the presence of anthocyanins and anthocyanidins. Red colour in acidic aqueous solution of extracts at pH 3-4 indicates the presence of anthocyanins and change of colour with pH modification (pH 8-9) indicates the presence of anthocyanidins.
- Amino acid: When 0.1% Ninhydrin in acetone solution was added to water extract (5a) violet purple colour indicated the presence of amino acid.
- Emodins: Ethereal solution (1a) was evaporated and residue dissolved in benzene, followed by addition of 25% ammonium hydroxide. The development of red colour indicated the presence of emodins (Borntrager's reaction).
- Carotenoids: Concentrated hydrochloric acid and phenol (1:1 ml) was added to the petroleum ether extract (1a). Development of blue/green colour indicate the presence of carotenoids
- Chlorogenic acid: Few drops of ammonia is added to water extract (5a), which turns green and indicates the presence of chlorogenic acid.
- Coumarins: All the extracts (1a,2a,3a,4a and 5a) were dried and the residues were extracted with ether. The resulting extracts were taken in test tubes. The test tubes, covered with filter paper moistened with dilute sodium hydroxide solution, were placed in water bath at boiling temperature for 20- 30 minutes. The filter paper was removed and observed in UV light. Yellow green fluorescence indicated the presence of coumarins.
- Cardiac glycosides: 1ml of glacial acetic acid was added to 2ml of methanol extract (4a) in a test tube. In this mixture few ml of ferric chloride followed by 2drops of concentrated H_2SO_4 were added. Green blue colour indicated the presence of cardiac glycosides (Killer-Kiliani tests).
- Flavonoids: The solutes of all the extracts (1a,2a,3a,4a and 5a) were evaporated and residue dissolved in etanol. On addition of magnesium powder and concentrated hydrochloric acid, the development of yellow/ red colour indicated the presence of flavonoids [Shinoda's reaction[9]].
- Saponins: 2 ml of the water extract (5a) was shaken vigorously for 10 seconds and allowed to stand. The formation of persistent honeycomb like froth is the positive test for the presence of saponins [6, 10].
- Steroids: Salkowski reaction – All the five extracts (1a, 2a, 3a, 4a and 5a) were separately evaporated on water bath and residue was formed. A few mg of residue was taken in 2ml of chloroform. To this 2ml of concentrated H_2SO_4 was added by the side of the testy tube. The test tube was shaken for few minutes. A red colour developed in the chloroform layer and lower layer of acid gave greenish yellow fluorescence. This colorization and fluorescence is due to presence of steroids.
- Libermann- Burchard reaction – All the five extracts were separately evaporated on water bath and residue was formed. A few mg of the residue was dissolved in chloroform. To this was added few ml of acetic anhydride and 2 drops of concentrated H_2SO_4 from the side of the tube. The transient greenish colour indicates the presence of steroids.
- Tannins: 0.5 ml of alcoholic and water extracts (4a and 5a) were diluted with 1.0 ml of water and 2-3 drops of dilute ferric chloride solution was added. Development of a blue black/green colour indicates the presence of tannin.
- Triterpenoids: All the five extracts were separately evaporated on water bath and residue was formed. A few mg of the residue was dissolved in chloroform. To this was added few ml of acetic anhydride and two drops of concentrated H_2SO_4 from the sides of the test tube. The red/ violet colour indicates the presence of triterpenoids (Libermann- Burchard reaction)
- Gums and mucilage: To 5ml of water extract (5a), 15ml alcohol was added and stirred. Formation of mucilaginous texture of precipitation was the test of gums and mucilage.
- Fatty acids: A portion of the petroleum ether extract (1a) was evaporated on a piece of filter paper. Observation of a translucent spot on the filter paper indicates the presence of fatty acids.
- Phenolics: The dried residue of each extract (1a, 2a, 3a, 4a and 5a) was dissolved in methanol. Methanolic extract was tested for the presence of phenolics [11]. A few drops of acidified ferric chloride (5%) solution were added to the extract. The presence of blue, green or brown coloration indicate the presence of phenolics compound in the sample.
- Phlobatanins: Water extract (5a) was screened for phlobatanins. Addition of 1ml of 1% aqueous HCl to the extract gives red precipitation on boiling indicates presence of phlobatanins.
- Aucubins/ Iridoids: Fresh material was chopped and treated with 5ml of 1% V/V aqueous Hydrochloric acid. After 3-6 hrs, the extract was treated with 1ml of Trim Hill reagent and heated on boiling water bath. The development of blue colour indicates the presence of aucubins (Diterpenoids) while green or red colour indicates the presence of Iridoids (monoterpenoids).
- Cyanogenic Glycosides: 2mg of dried powdered material was taken in a test tube. This material was moistened by addition of few drops of chloroform. Sodium picrate solution (5gm sodium carbonate, 0.5gm picric acid and 100ml distilled water) was prepared and strips of filter paper were moistened in this solution. These strips were removed, dried and inserted in test tube containing the reaction mixture. The test tube

contents were warmed at 30-35°C for 30 minutes, the development of red colour on paper indicates the presence of cyanogenic glycosides.

RESULTS AND DISCUSSION

Trifolium alexandrium is rich in phytochemical constituents, as shown in Table 1. The present study reveals the presence of alkaloids, flavonoids, steroids, saponins, phenols and glycosides in *Trifolium alexandrium.* All these are important in animal nutrition and they have both beneficial and toxic properties. Plants and animals have coevolved. As plants have developed the enzymatic means to synthesize defensive chemicals, animals have evolved detoxification mechanisms to overcome the plant defenses[12].

Present author found the presence of alkaloids, flavonoids, steroids, saponins, phenols, glycosides in *T. alexandrium*. In in aerial parts of *Trifolium pratense*, alkaloids, flavonoids and saponins are present [13].Glycosides and phenols are present in leaves of *Trifolium pratense*[14].While steroid is present in root of *Medicago sativa* (Leguminosae plant)[15]. Present research revealed the absence of tannin in *T. alexandrium*. Tannin is also absent in aerial parts of *Trifolium pratense* [13].

ACKNOWLEDGEMENT

We are thankful to the Principal, Hislop College, Nagpur. (Ms.) Dipti Christian, for availing all facilities required for this research work.

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