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Comparative Phytoconstituents study in Two Varieties of *Trigonella* i.e. *Trigonella foenum graceum* and *Trigonella monantha*

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Abstract

The present study deals with the preliminary phytochemical analysis of ethanolic extract of various plant parts (Leaf, Stem, Root and Seed) of Trigonella foenum-graecum & Trigonella monantha Qualitative and Quantitative analysis of phytochemical constituents' viz. Carbohydrate, Starch, Amino Acid, Protein, Lipid, Chlorophyll, Phenol and Alkaloid were performed by well-known test protocols available in the literature. These observations would be of immense value in standardization of the drug in crude form & would help distinguish the drug from its other species.

Key Words: *Trigonella foenum-graecum*, *Trigonella monantha*, Qualitative analysis, Quantitative analysis, ethanolic extract, phytochemical constituents.

INTRODUCTION

Medicinal plants as a group comprise approximately 8000 species and account for about 50% of all the higher flowering plant species of India. The term "phytochemicals" refers to a wide variety of compounds produced by plants. They are found in fruits, vegetables, beans, grains and other plants. They are non-nutritive and have protective or disease preventive properties. The plants of medicinal values are major sources of natural products used as pharmaceuticals, flavor and fragrance ingredients and food additives, (Balandrin and Klocke 1988).

Primary metabolites are substances widely distributed in nature, occurring in one form or another in virtually all organisms. Secondary metabolites that are used commercially as biologically active compounds (pharmaceuticals, flavors, fragrances and pesticides) are generally higher value lower volume products than the primary metabolites.

Herbs and spices have been extensively used as food additives for natural antioxidants. Spices and aromatic herbs are considered to be essential in diets or medical therapies for delaying aging and biological tissue deterioration (Frankel EN 1996).

Legumes are rich in nutrients such as digestible protein with good array of amino acids and minerals. Leguminous seeds have been reported to be excellent sources of energy in animal and human diets. This explains why considerable research has been directed to harnessing the potential of these seeds in animal or human diets (Kelloff et al 1992).

Fenugreek (Trigonella foenum-graecum L.), plant is widely distributed throughout the world and which belongs to the family Fabacecae. It is reported as a cultivated crop in parts of Europe, northern Africa, west and south Asia, Argentina, Canada, United States of America (USA) and Australia (Petropoulos GA 2002; AAFRD

1998; Edison S 1995; Fazil & Hardman 1968). India is the leading fenugreek producing country in the world (Edison S 1995).

Trigonella monantha is the name of a species, part of the genus Trigonella. This species has been described by C.A.Mey. under the rules of the International Code of Nomenclature for algae, fungi, and plants (ICN / commonly called the botany code).

This study looks into the fundamental scientific bases for the use of some medicinal plant such as Trigonella graceum foenum and Trigonella monantha will be investigated to better understand their properties and efficiency by determining the crude phytochemical constituents present in these plants. The use of plant extracts and phytochemicals can be of great significance in therapeutic treatments. In the present study efforts will be taken to assess the qualitative estimation of phytochemical compounds as well as quantitative analysis in two species of Trigonella such as Trigonella graceum foenum and Trigonella monantha.

MATERIALS AND METHODS

Study Site

The study site is situated in Rajasthan. It is an agricultural field having a vast array of biodiversity. The experimental work was done in Department of Botany, Rameshwari Devi Girls College, Bharatpur (Raj.).

Sample Collection

The samples were collected from the Agricultural field of Bharatpur. The plant was transferred in department for further investigation and processing for the research.

Sample preparation

The plants were separated into leaf, stem, fruit and root. After this the plant parts (Leaf, Stem, Fruit & Root) were washed under running tap water and shade dried, after this the material were kept in hot air oven at 40-50˚C. After that the dried plant materials were grinded into a fine powder with the help of a suitable grinder. Approximate half of the powdered sample was stored in air tight container for phytochemical estimation.

The plant extracts were prepared using the solvent ethanol. For the extraction of dried powered sample parts (each 30g) weighed and put in the soxhlet thimble using Whatman filter paper No. 1 and 300 ml of ethanol (50%) in soxhlet flask. After that these sample were extracted at 50-60^0. After extraction the solvents were removed under pressure using rotary vacuum evaporator. All extracts were stored in air tight bottles in freeze at 40C for evaluation the phytoconstituents.

QUALITATIVE ESTIMATION

Test for carbohydrates (Molisch H 1937)

To 2 ml of the plant extract, 1 ml of Molisch's reagent was added. A purple or reddish change in colour indicated the presence of carbohydrates.

Test for Starch (Sawhney SK 2008)

Take 1g of dry powder in 50 ml of water boil for one minute and cool, thin and cloudly mucilage is produced, which gives thick and more transparent mucilage. To 10

ml of the mucilage add 0.05ml of 0.01 M Iodine, a dark blue colour is produced, which disappears on heating and reappears on cooling.

Ninhydrin Test (Richardson 1969)

In the pH range of 4-8, all α- amino acids react with ninhydrin (triketohydrindene hydrate), a powerful oxidizing agent to give a purple colored product (diketohydrin) termed Rhuemann's purple. All primary amines and ammonia react similarly but without the liberation of carbon dioxide. The amino acid proline and hydroxyproline also react with ninhydrin, but they give a yellow colored complex instead of a purple one. Besides amino acids, other complex structures such as peptides, peptones and proteins also react positively when subjected to the ninhydrin reaction.

Biuret Test (Richardson 1969)

Under alkaline conditions substances containing two or more peptide bonds form a purple complex with copper salts in the reagent. In sample 5-6 drops of dilute Copper sulphate, 40% NaOH solution was added and observe the change.

Test for Lipids (M. Weindlmayar-Goettel 1993)

Sample with equal amount of water was added with Sudan Dye then colour change was observed.

Test for alkaloids - Dragendorff's test (Harborne 1973)

To a few millilitre of the filtrate, 1 or 2 ml of Dragendorff's reagent was added. A prominent red or orange precipitate indicated a positive result for alkaloids.

QUANTITATIVE ESTIMATION

Carbohydrate Estimation (Dubois et al 1956)

0.5g of sample (Stem, Leaf, Fruit and Peel) were homogenized with 10mL of 80% ethanol and centrifuged at 2000rpm for 20 minutes. 1mL of the supernatant was added with 5% phenol followed by the addition of 5mL H_2SO_4. Mixture was agitated and allowed to stand in water bath at 26-30°C for 20 minutes to develop color. The absorbance of the solution was read at 490nm wavelength using spectrophotometer (Systronics Model no. 2205). Standard solutions of dextrose were prepared at 0, 0.2, 0.4, 0.6, 0.8, 1mg/g respectively with the same treatment. Calibration curves of the absorbance values versus concentration of the standard were constructed and the value of carbohydrate in the sample was calculated.

Starch Estimation (McCready et al 1950)

0.5g of fresh plant tissue were homogenized with 10mL of 80% ethanol and centrifuged at 2000rpm for 20 minutes. After discarding the supernatant, Pellet was suspended in 5mL of distilled water by subsequently adding 6.5mL Perchloric acid (52%) to residue. The mixture was centrifuged for 20minutes at 2000rpm. Supernatant was decanted and collected by repeating this step thrice. Supernatant was transferred to 100mL volumetric flask and made up to 100mL mark with the distilled water. 1mL of this filtrate was analyzed followed by same procedure as that in carbohydrate estimation. Quantity of starch was calculated as glucose equivalent was used to convert the value of dextrose for starch estimation.

Protein Estimation (Lowry et al 1951)

0.5g of fresh weight of plant tissue was extract with 5mL of 5% TCA. The homogenized material was centrifuged at 2000rpm for 20 minutes. Pellet was dissolved in 10mL of 0.1N NaOH after discarding the supernatant. 0.1mL of this solution made up to

1mL by adding distilled water. By adding alkaline copper reagent residue was dissolved and allowed to stand for 10minutes followed by the addition of 0.5mL folin-ciocalteau reagent (50%). The optical density was measured at 750nm in spectrophotometer. The standard curve was prepared by using 0-1mg/ml solution of BSA in 0.1N NaOH and values of protein in the sample were calculated.

Lipid Determination (Folch et al 1957)

1g of the fresh tissue was taken and homogenized with 10 mL CHCl3 and methanol in 2:1 ratio. The crushed material was transferred to screw capped tubes and were kept overnight. The contents were filtered through sintered through glass funnel. Washing of mixture was done by $CHCl_3$ and methanol twice in times. Crude extract was added by one fifth of its volume of 1% NaCl to remove water soluble impurities. Now centrifugation step has taken at low speed, lower $CHCl_3$ layer containing lipids was then withdrawn by Pasteur pipette.

Aliphatic Amino Acid Determination (Richardson 1969)

0.5g of sample was crushed in 80% ethanol. This mixture was making up to 10mL and centrifuged at 2000rpm for 20minutes. 1mL of the supernatant is collected followed by the addition of 2mL ninhydrin. The tubes were kept at 100°C for 15minutes. Tubes were cooled at room temperature and absorbance taken at 575nm wavelength

Phenol Determination (Bray and Thorpe 1954)

1 ml of sample (1 mg/ml) was mixed with 1 ml of FolinCiocalteu's phenol reagent. After 5 min, 10 ml of a 7% Na2CO3 solution was added to the mixture followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample.

Determination of Alkaloids (Harborne 1984)

5 g of the dried powder of each sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The mixture is covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath until it reaches to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried, weighed and percentage was calculated.

RESULTS & DISCUSSION

Table 1

Phytoconstituents	*Troigonella foenum graceum*				*Trigonella monantha*			
	Leaf	Stem	Root	Seed	Leaf	Stem	Root	Seed
Carbohydrate	++	++	+	++	++	++	++	++
Starch	+	+	++	++	++	++	++	++
Amino Acid	+	+	+	+	+	+	+	+
Protein	++	++	++	++	+	+	++	++
Lipid	+	+	++	++	+	+	+	+
IAA	+	++	++	++	++	++	++	++
Phenol	++	+	++	++	++	++	++	++
Alkaloid	+	+	++	++	+	++	++	++

- Absence; + Low Presence; ++ Moderate Presence; Maximum Presence

In Qualitative analysis root of Trigonella foenum graceum contain low presence of Carbohydrate otherwise it was present maximum in all other parts of Trigonella foenum graceum & all plant parts of Trigonella monantha. Leaf & Stem of Trigonella foenum graceum contain low presence of starch while Amino acid was only highest in stem of Trigonella foenum graceum. Leaves & Stem of Trigonella monantha contain low presence of Protein while lipid was present maximum in root & seed part of Trigonella foenum graceum. IAA was present maximum qualitatively in all plant parts of both plants except leaves of Trigonella foenum graceum. Phenol was present low in stem of Trigonella foenum graceum otherwise it is highest in both plant parts. Alkaloid content was qualitatively maximum present in root & seed of Trigonella foenum graceum & stem, root & seed of Trigonella monantha.

Quantitative analysis revealed that Roots of both plants contain maximum amount of (0.387 mg/g & 0.390 mg/g) carbohydrate in root part while minimum (0.240 mg/g) content of same was present in seed of Trigonella foenum graceum. Starch content was present maximum (0.415 mg/g) in seed of Trigonella monantha & minimum (0.208 mg/g) in leaves of Trigonella foenum graceum. Maximum & Minimum (0.230 mg/g & 0.112 mg/g) amount of protein was present in root of Trigonella monantha & leaves of Trigonella foenum graceum while lipid content was highest (0.142 mg/g) in root of Trigonella monantha & minimum (0.040 mg/g) in stem of Trigonella foenum graceum. Minimum Chlorophyll was present (0.783 mg/g) in stem of Trigonella foenum graceum and maximum (1.580 mg/g) was observed in leaves of Trigonella foenum graceum. IAA was maximum (0.354 mg/g) in root of Trigonella monantha while it was minimum (0.154 mg/g) in leaves of Trigonella foenum graceum. Maximum (0.289 mg/g) & minimum (0.122 mg/g) phenolic content was observed in root of Trigonella monantha & leaves of Trigonella foenum graceum. Root of Trigonella foenum graceum contain maximum amount (0.384 mg/g) of alkaloid while minimum (0.135 mg/g) alkaloid was observed in leaves of Trigonella monantha.

Results are shown in Mean±S.E.

Table 2

Phytoconstituents	*Troigonella foenum graceum*				*Trigonella monantha*			
	Leaf	**Stem**	**Root**	**Seed**	**Leaf**	**Stem**	**Root**	**Seed**
Carbohydrate	0.310± 0.13	0.280± 0.16	0.390± 0.42	0.240± 0.14	0.293± 0.16	0.324± 0.08	0.387± 0.78	0.312± 0.54
Starch	0.208± 0.27	0.243± 0.22	0.285± 0.33	0.324± 0.16	0.245± 0.20	0.296± 0.23	0.335± 0.34	0.415± 0.42
Protein	0.112±0 .54	0.140± 0.34	0.164± 0.68	0.136± 0.23	0.122± 0.31	0.129± 0.45	0.230± 0.18	0.153± 0.07
Lipid	0.065± 0.64	0.040± 0.07	0.110±0 .25	0.087± 0.74	0.086± 0.08	0.096± 0.29	0.142± 0.09	0.120± 0.16
Chlorophyll a	0.870± 0.15	0.412± 0.57	-	-	0.740± 0.21	0.581± 0.89	-	-
Chlorophyll b	0.710± 0.09	0.371± 0.31	-	-	0.624± 0.38	0.425± 1.02	-	-
Total Chlorophyll	1.580± 0.21	0.783± 0.14	-	-	1.364± 0.46	1.006± 0.54	-	-
IAA	0.154± 0.23	0.160± 0.52	0.245± 0.36	0.169± 0.33	0.195± 0.09	0.184± 0.36	0.354± 0.32	0.208± 0.55
Phenol	0.122± 0.32	0.154± 0.18	0.289± 0.29	0.182± 0.07	0.170± 0.17	0.192± 0.44	0.289± 0.69	0.196± 0.09
Alkaloid	0.187± 0.47	0.192± 0.09	0.384± 0.52	0.210± 0.44	0.135± 0.24	0.154± 0.11	0.243± 0.09	0.180± 0.28

CONCLUSION

Among the qualitative and quantitative phyto-chemical tests carried out, most of the important phyto-chemicals i.e. Phenol, Alkaloid, IAA, Carbohydrate, Chlorophyll, Lipid, Protein erc. were present in extract. The qualitative and quantitative distribution of these metabolites was differed from plant to plant & part to part. The higher phytoconstituents was estimated in Trigonella monantha C.A. Mayer comparative to Trigonella foenum graceum. The results revealed the presence of medicinally important constituents in plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Extract of these plants could be good source for useful drugs. Further analysis of phytoconstituents and extraction of these constituents can be too much useful for remedial action to specific disease and to explore this plant.

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