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PHYTOCHEMICAL SCREENING AND ANTI-OXIDANT STUDIES OF *TRIGONELLA FOENUM GREACUM*

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ABSTRACT: The study of plants used in traditional medicine requires the effective integration of information on chemical composition of extracts, pharmacological activities of isolated compounds, as well as indigenous knowledge of traditional healers. This work was mainly concerned with the identification of phytochemicals and its anti-oxidant activity of *Trigonella foenum greacum* seed extract. From the results we identified that ethanolic extract has highest anti-oxidant activity. Ethanolic extract showed the highest scavenging activity followed by Aqueous extract. DPPH scavenging activity was significantly correlated with phenolics and flavonoids in different extracts.

KEYWORDS: *Trigonella foenum greacum*, Phytochemical screening, anti-oxidant property, pharmagonsy studies

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1.INTRODUCTION

Phytochemical screening of medicinal plants has exposed the presence of numerous secondary metabolites including alkaloids, flavonoides, steroids, glycosides, saponins and tannins etc. Secondary metabolite serves as defense mechanisim against predation by herbivores micro orgainisms and insects [1-3]. Phytochemicals are chemical compounds which can be isolated from plants. Phytochemicals have been in use, over a long history, as medicines. In common usage today, they are associated with health benefits [4]. Physicochemical studies verify the identity of plant and make certain the quality and purity of crude drug [5, 6]. Extraction is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures.

The main purpose of extraction process is to obtain the therapeutically active portions of crude drugs (medicinal plant/parts) and to discard the unwanted material by treating with a selective solvent known as menstruum. During extraction process, solvents diffuse slowly into the plant material and solubilize the compounds with similar polarity. Extractive values of different solvents are very useful parameters for the evaluation of crude drugs as they indicate the nature of the chemical constituents present in a crude drug [7]. Many drugs are used in powder form and almost all the crud drugs are dried and powdered for solvent extraction. The powder of plant tissues has specific fluorescence under UV light. This property is a useful tool for evaluation and for detection of adulteration, contamination as well as for identification of various chemical constituents of the crude drug [8, 9]. Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiations. The fluorescence analysis of powder drugs, treated with different chemical reagents, under visible & UV (254 & 366 nm) lights, show different color fluorescence, which help in evaluation of the drugs [10, 11]. Various complex organic and inorganic compounds (carbohydrates, fats, proteins, vitamins, and minerals) and water are needed by human beings in their diet to meet their body requirements [12]. Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments of the body. Plants are the rich source of all the elements essential for human beings and the curative effect of medicinal plants for the treatment of various diseases is based on the chemical compounds and different elements in them [13]. Metalic elements and their compounds have been used for therapeutic and cosmetic effects on skin, since earliest times. Solution of aluminum acetate, copper sulphate and lotion of zinc solution are used as antiseptic, cleansing agents, skin disinfectant, soothing and cooling agents. Calcium, magnesium and manganese are required for the formation of the collagen and connective tissue. Similarly sulpher and phosphorus are used for the treatment of leprosy and scabies [14-16]. Although herbal medicines have less side effects as compared to allopathic medicine but the presence of heavy metals in plants, which may be toxic when they are in higher amount, can cause health issues [17]. According to the recommendation of WHO (1998) medicinal plants must be checked for the presence of heavy metals and other contaminations. Excess doses or prolonged intake of medicinal plants can lead to accumulation of trace elements which cause various health problems [18]. In this perspective, elemental contents of the medicinal plants are very important and herbal preparations are needed to be screened for the presence of toxic heavy elements [19]. Recent studies on the elemental analysis of medicinal plants have enhanced the consciousness about the heavy elements in plant products [20-22].These studies showed that essential metals, when ingested is in high amounts, can result in toxicity whereas non-essential trace metals are toxic even in very low concentrations for human health.

2. MATERIALS AND METHODS

Collection of Plant Materials

The fresh and healthy seeds of *Trigonella foenum greacum* were collected and washed

Preparation of Seed Extract of *Trigonella foenum greacum*

The extraction of seeds of *Trigonella foenum greacum* was carried out using known standard procedures. The seeds were dried in shade and powdered in a mechanical grinder. The powder (10.0 g) was initially defatted with ethyl alcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The ethyl alcoholic seed extract yields a dark reddish residue weighing 4.50 g (45.0% w/w). This crude extracts of ethylalcohol was used for further investigation for potential of antimicrobial properties [23].

Preliminary Phytochemical Screening

The seed extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and seed powder was screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, stroids, glycosides, anthraquinones, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids [24].

Physico-chemical Constants

The procedures recommended in Indian Pharmacopoeia and WHO guidelines were followed to calculate the physico-chemical constants [25].

Physicochemical characterization of the plant extract

Physicochemical parameters such as color, consistency, pH and percent yield (% w/w) were determined for all plant extracts

Ash values

Total ash value

The total ash was determined by incinerating 2-3gms of accurately weighed air dried coarsely powdered drug in a tarred silica crucible which was previously ignited and cooled before weighing, at a temperature not exceeding 4500C. The ignition was repeated and the percentage of ash with reference to air-dried drug was calculated.

Water soluble ash

The total ash was boiled for 5min with 25 ml of water. The residue was washed with hot water, ignited for 15min at a temperature not exceeding 4500C, cooled and weighed. This weight was subtracted from the weight of ash, the difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to air-dried drug.

Acid insoluble ash

The ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5min and filtered through an ashless filter paper. The residue was washed with hot water, ignited, cooled in a dessiccator and weighed. The percentage of acid insoluble ash was calculated with reference to air dried drug.

Sulphated ash

The sulphated ash was determined by incinerating 1 gm of accurately weighed air dried coarsely powdered drug in a tarred silica crucible which was previously ignited and cooled before weighing at a temperature not exceeding 4500C. The residue was moistened with 1 ml of concentrated sulphuric acid, ignited at 800±250C until all black particles have disappeared. It was then cooled, again sulphuric acid was added and ignited. It was cooled and the percentage of sulphated ash was calculated with reference to air dried drug.

The preliminary phytochemical investigations were conducted employing various phytochemical tests and the presence of various phytochemical constituents were detected**.**

Test for carbohydrates

A small quantity of aqueous and hydroalcoholic plant extracts were dissolved in 5 ml of distilled water and filtered [26].

a. **Molisch's test**- The filtrate was tested with alcoholic solution of _ napthol and sulphuric acid. A purple coloured ring indicated the presence of carbohydrates.

b. **Fehling's test**- The filtrate was treated with equal quantity of Fehling A (Copper sulphate) and Fehling B (Sodium potassium tartarate) and solution was heated. Brick red precipitate indicates the presence of sugars.

c. **Barfoed's test**- Formation of red colour within 2 min after addition of the reagent indicates the presence of monosaccharides.

d. **Benedict's test**- The filtrate was heated with this reagent for 2 min. Formation of red precipitate indicates the presence of reducing sugars.

e. **Selwinoff's test-** The filtrate was heated with this reagent for 1-2 min. The formation of red colour of the solution indicated the presence of ketohexose like fructose.

2. Tests for non-reducing sugars

The aqueous and hydroalcoholic plant extracts which did not give response to Fehling's and Benedict's tests confirmed the presence of non-reducing sugars. The presence of nonreducing sugars was also indicated by positive Fehling's and Benedict's tests by the hydrolysed test solution.

3. Tests for non-reducing polysaccharides

In this test, 3 ml of test solution of extract was mixed with few drops of dilute iodine solution. The blue colour of the solution confirmed the presence of non-reducing polysaccharides.

4. Test for gums and mucilage:

About 1 ml of extract was added slowly to about 25 ml of alcohol with constant stirring. Formation

of a precipitate indicates the presence of gums and mucilage

5. Test for resins

The extract was dissolved in alcohol and diluted it 10 times with water, turbidity formed indicated the presence of resins.

6. Test for proteins [27]

Biuret test (General test): To 3 ml extract solution 4% sodium hydroxide and few drops of 1% copper sulfate solution was added. The appearance of violet or pink colour indicated the presence of proteins.

7. Tests for amino acids

a. Ninhydrin test (General test): The extract and 3 drops of 5% ninhydrin solution were heated in a boiling water bath for 10 min. Purple or bluish colour indicated the presence of amino acids.

b. Millon's reagent: The extract was heated with 3 drops of Millon's reagent. The dark red colour solution confirmed the presence of tyrosine.

8. Test for glycosides

A small portion of the extract was hydrolyzed by boiling with dilute hydrochloric acid for few min and hydrolysate was subjected to following tests [28].

a. Libermann-Burchard test - Chloroform solution of hydrolysate was treated with acetic anhydride and sulphuric acid. Formation of blue or blue–green colour indicated the presence of steroidal saponins whereas red, pink or violet colour indicated the presence of triterpenoids saponins.

b. Legal's test - The hydrolysate was dissolved in pyridine and solution of sodium nitroprusside was added to it and made alkaline. Formation of pink or red colour indicated the presence of cardiac glycosides.

c. Borntrager's test - An organic solvent like ether or chloroform was added to the hydrolysate and the contents were shaken. The organic layer was shaken and treated with solution of ammonia. The development of pink colour indicated the presence of anthraquinone glycosides.

9. Test for Saponin Glycosides

Foam test: About 1 ml of extract was diluted with water to 20 ml and shaken in a graduated cylinder for 15 min. A 1 cm layer of foam indicates presence of saponins.

10. Test for flavonoids

Shinoda test: A small piece of magnesium ribbon was added to the alcoholic solution of the extract followed by drop wise addition of concentrated hydrochloric acid. The green blue colour indicates the test is positive [29].

11. Test for alkaloids

A small portion of solvent free extract was stirred with few drops of dilute hydroalcoholic acid and filter. The filtrate was tested with following reagents.

a. Dragendrof reagent (Potassium bismuth iodide) - To 2-3 ml filtrate, few drops of the reagent

was added. Orange brown precipitate is formed.

b. Mayer's reagent (Mercury potassium iodide) – To 2-3 ml filtrate, few drops of the reagent added gives cream precipitate.

c. Hager's reagent (Saturated picric acid)- With 2-3 ml of filtrate the reagent gives yellow precipitate.

d. Wagner's reagent (Iodine reagent)-With 2-3 ml of filtrate the reagent gives reddish brown precipitate.

12. Test for phenolic compounds and tannins [30]

A small quantity of extract was diluted with water and tested with following reagent. a) Dilute ferric chloride (FeCl3) solution (5%) Intense blue, green, red or purple colour indicates the presence of phenolic compounds. An appearance of violet colour indicates the presence of tannins.

b) Acetic acid solution: Forms red colour solution indicating presence of phenolic compounds

c) Solution of gelatin (1%) containing 10% sodium chloride (NaCl)- Precipitate indicates positive test for tannins.

d) Lead acetate solution (10%)-Gives buff coloured precipitate for phenolic compounds.

Extractive values

Ethanol soluble extractive

5gms of dried coarse powder of plants were macerated with 100ml of 90% ethanol in a closed flask for 24hrs, shaken frequently during 6 hours and allowed to stand for 18hrs. Filtered immediately taking precautions against loss of ethanol. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. The residue was dried at 1050C and weighed. The percentage of ethanol soluble extractive was calculated with reference to air dried drug.

Water soluble extractive

5gms of coarse powder was weighed and dissolved in 100ml of water in a stoppered flask, heated at 800C, shaken well and allowed to stand for 10min. It was cooled, 2gms of kieselghur was added and filtered. 5ml of the filtrate was transferred to a tarred evaporating dish and the solvent was evaporated on a water bath. The percentage of water soluble extractive was calculated with reference to air dried drug.

Determination of volatile oil in drug

50gms of the drug was boiled with water in a Clavenger's apparatus. The process was continued till no more oil was collected in the graduated tube. The volume of oil was measured and expressed in percentage.

Determination of crude fibre content

About 2gms of the drug was accurately weighed and extracted with ether. Then 200ml of 1.25% sulphuric acid was added and boiled for 30min under reflux. It was filtered and washed with boiling water until free of acid. The entire residue was rinsed back into flask with 200ml of boiling 1.25%

sodium hydroxide solution and again boiled under reflux for 30min. The liquid was quickly filtered and the residue was washed with boiling water until neutral, dried at 1100C to constant weight. It was then ignited to 30min at 6000C, cooled and weighed. The percentage of crude fibre content was calculated with reference to the air dried drug.

Determination of loss on drying

Glass stoppered shallow bottle was weighed that had been dried in the same conditions to be employed in the determination. About 1gm of the sample was transferred to the bottle and distributed evenly by gently side wise shaking to a depth not exceeding 10mm. Place the loaded bottle in a drying chamber (the stopper was removed and left in the chamber). The sample was dried to a constant weight and allowed to cool. The bottle along with the

content was weighed. The process was repeated until the successive weights differed not more than 0.5mg (drying to constant weight). The percentage loss of weight was calculated with reference to the air dried drug.

Determination of foaming Index

1gm of the coarsely powdered drug was weighed and transferred to 500ml conical flask containing 100ml of boiling water. The flask was maintained at moderate boiling at 80-900C for about 30min. It was cooled, filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100ml.

Ten stoppered test tubes were cleaned (height 16cm, diameter 1- 6cm) and marked from 1 to 10. 1, 2, 3ml up to 10ml of the filtrate was measured and transferred to each tube and adjusted the volume of the liquid with water to 10ml. Then the tubes were stoppered and shaken lengthwise for 15sec uniformly, allowed to stand for 15min the length of the foam was measured in each tube.

If the height of the foam in each tube is more than 1cm, the foaming index is more than 1000. In this case, 10ml of the first decoction of the plant material is measured and transferred to a 100ml volumetric flask (V2) and the volume is made to 100ml and followed the same procedure.

Fluorescence Analysis

The fluorescence analysis of the drug powder as well as various extracts were carried out by using the method of Chase and Pratt. The behavior of the powder with different chemical reagents was also carried out.

Inorganic Mineral Analysis

Chemical analysis of higher plants in general has revealed the presence of 40 or more elements. Plant physiologists have proved that 18 of these elements are indispensable to plants and human beings require 28 or more elements. Of these elements, carbon, hydrogen, oxygen and nitrogen are present in larger quantities than others. Sulphur and phosphorous are present in protoplasm and has constituents of proteins or other important organic compounds. A study of inorganic constituents of plants is of interest to research workers in several fields, such as nutrition medicine and others

because plants constitute direct or indirect sources of many of the elements, which are essential to animals including man. Therefore, the plant material was subjected to inorganic mineral analysis.

Preparation of sample solution for inorganic mineral analysis

The plant material (10gms) was digested with 10 ml of nitric acid and left over night. It was then heated on a hot plate until the reddish brown colour ceased and cooled. A small volume of perchloric acid was added and transferred to a 50ml standard flask and made up to volume with double distilled water.

Determination of sodium and potassium by flame photometry

A series of standard solutions containing the element to be determined in increasing concentrations within the concentration range recommended for the instrument were prepared. Nitric acid and perchloric acid used for the preparation of sample solution of the plant material were also added in the same concentration to the standard solution. The appropriate filter was chosen, water was sprayed into the flame and the galvanometer reading was adjusted to zero. The most concentrated solution was then sprayed into the flame and the galvanometer reading was recorded.

Again, water was sprayed till the galvanometer reading was zero. Then the standard solution was sprayed into the flame and the procedure was repeated three times for each concentration. A calibration curve was prepared by plotting the mean of three readings of each standard against the concentration. The sample solution prepared as above was then aspirated into the flame three times, the galvanometer reading was recorded and the apparatus was washed thoroughly with water after each aspiration. Using the mean of three readings, the concentration of the element being examined was determined from the

calibration curve. To confirm the concentration thus obtained, the operation was repeated with the standard solution of the same concentration as that of the solution being examined.

Determination of calcium, cobalt, iron, copper, magnesium and manganese by atomic absorption spectroscopy

Three standard solutions of the element to be determined covering the concentration range recommended for the instrument were prepared. Nitric acid and perchloric acid used in the preparation of the substance being examined were also added to the standard solutions in the same concentration. After calibration of the instrument, each standard solution was introduced into the flame three times and the steady reading was recorded. The apparatus was thoroughly washed after each introduction. A calibration curve was prepared by plotting the mean of each group of three readings against concentration. The plant extract prepared above was then introduced into the flame and the reading was recorded. The sequence was then repeated twice. Using the mean of the three readings, the concentration of the element was determined from the calibration curve. The process was repeated for the determination of other elements using different lamps.

3. RESULTS AND DISCUSSION

Physicochemical characteristics of the crude drug

Various physicochemical characteristics of the powder drug of leaves were carried out.

Ash analysis and moisture contents

In the present study ash analysis for crude powder drug of seed was carried out. It was observed that highest value of total ash was recorded (4.23%). Acid insoluble ash was in the range of 0.58%, Water soluble ash was in the range of 29.39. Moisture contents was heighst (9.16%) in seeds 10.01 % (Table 1).

Plant species with medicinal properties have been playing a fundamental role in the efforts for drug discovery all over the world. 80% populations in developing countries are dependent on plants for their primary health care, and in spite of the significant progress in the field of synthetic organic chemistry of the twentieth century, more than 25% of prescribed medicines in developed countries are derived directly or indirectly from plant sources [31].

Table 1: Physico-chemical constants

S.N	Parameters	Percentage (%w/w)
1.	Total ash	4.23
2.	Acid insoluble ash	0.58
3.	Water soluble ash	29.39
4.	Sulphated ash	6.2
5.	Solubility	
	Water soluble extractive	13.67
	Alcohol soluble extractive	2.6
6	Crude fibre content	14.37
7	Loss on drying	4.29
8	Foaming index	Less than 100

Preliminary phytochemical screening is important and useful for isolation of pharmacologically active compounds present in the plants [32]. This serves as an important tool for the quality assurance of plant for future studies. Till now almost all investigated plants showed to contain different active constituents of pharmacological importance in the form of secondary metabolites [33]. It was found that ethyl alcoholic extracts of *Trigonella foenum greacum* roots contained tannins, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, reducing sugars, carbohydrates, proteins, and amino acids.

Table 2: Phytochemical studies

S.No	Secondary metabolites	Hexane	Ethyl acetate	Ethanolic	Aqueous
1	Steroids	-	+	++	++
2	Triterpenes	-	+	+	+
3	Saponins	+	-	-	+
4	Tri terpinoidal saponins	+	-	+	-
5	Alkaloids	+	+	++	+
6	Carbohydrates	++	+	+	++
7	Flavonoids	+	+	++	+
8	Tannins	+	+	+	+
9	Glycosides	+	+	++	+
10	Polyphenols	++	-	-	+

Fluorescence Analysis

Fluorescence study with UV light is a very useful tool for evaluation of crude drugs. Crude extract or powder drugs, when viewed under UV light give characteristic fluorescence [34]. The fluorescence phenomenon is the emission of light of different wave lengths, under the influence of UV light, due to different chemical constituents, otherwise not observable in ordinary visible light [35]. Fluorescence study helps for authentication and standardization of crude drugs. Saveral Crud drugs of plant origin have been authenticated though the fluorescence study technique e.g., *Morinda umbellatai*, *Holoptelea integrifolia* , root and stem of *Ichnocarpus frutescens*, leaves of *Catunaregum spinosa*, *Hygrophila auriculata* and *Crocus sativus [36]*. The fluorescence analysis of powder with various reagents and extracts are given in the Tables 3.

Table 3: Fluorescence analysis of powder

S.No	**Reagents**	**Day light**	**Short UV**	
	LongUV(365nm)			
1.	Powdered T.f.g	Yellow	pale Yellow	pale Yellow
2	Powder + 1 N HCl	Yellow	Green	dark brown
3	Powder + 1 N			
	NaOH	Yellow	Greenish yellow	dark brown
4	Powder + 50%			
	HCl	Yellow	yellow	brown

5	Powder + 50%			
	H2SO4	dark yellow	pale Yellow	dark brown
6	Powder +50%			
	HNO3	darkbrown	Brown	brown
7	Powder + Methanol	Yellow	Dark Brown	brown
8	Powder + Methanol +			
	1 NNaOH	yellow	brown	dark Green

Powder drug seed was such and after treatment with various solvents was subjected to fluorescence analysis. Observations were made under visible light and under UV light of short wave length and long wave length.

Table 4: Fluorescence analysis of various extracts

S.No	Extracts	Day light	UV light	
			Short (254nm)	Long (365 nm)
1.	n-Hexane	dark yellow	yellow	yellow
2.	Ethyl acetate	brown	dark green	dark green
3.	Ethanol	dark yellow	pale yellow	dark yellow
4.	Aqueous	yellow	dark yellow	light yellow

Inorganic Mineral Analysis

The amount of sodium and potassium present in 1 gm of *Trigonella foenum greacum* material was estimated by flame photometry. The amount of other metals present was estimated by Atomic absorption spectroscopy and the results are given in Table 5. Plants have the ability to accumulate such essential elements in their different parts, which are of highest importance in human nutrition. There are also some some toxic heavy metals like Co, Cd etc., which are not directly required by the plants,but still they are accumulated in some plants due to environmental pollutions which create serious health hazards, when the plants are consumed by the humans and animals. In human body, trace elements play a vital role both in prevention and treatment of different human diseases [37]. In the present study leaves were analyzed to determine the concentration of various heavy and toxic metals in these plants.

Table 5: Inorganic mineral analysis

S.No	Parameters	Amount present (mg/Kg)
1.	Total Iron	628
2	Cadmium (DB)	< 0.0005
3	Copper (DB)	84
4	Chromium (DB)	<0.02
5	Cyanide (DB)	<0.002
6	Cobalt (DB)	<0.003
7	Lead (DB)	<0.012
8	Manganese (DB)	439
9	Nickel (DB)	<0.002
10	Zinc (DB)	81
11	Sodium (WB)	224
12	Total phosphate (DB)	237

(DB-Dry basis WB –Wet basis)

Different fractions of *Trigonella foenum greacum* for free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) showed remarkable scavenging activities in Fig 1. Ethanolic extract showed the highest scavenging activity followed by Aqueous extract. DPPH scavenging activity was significantly correlated with phenolics and flavonoids in different extracts.

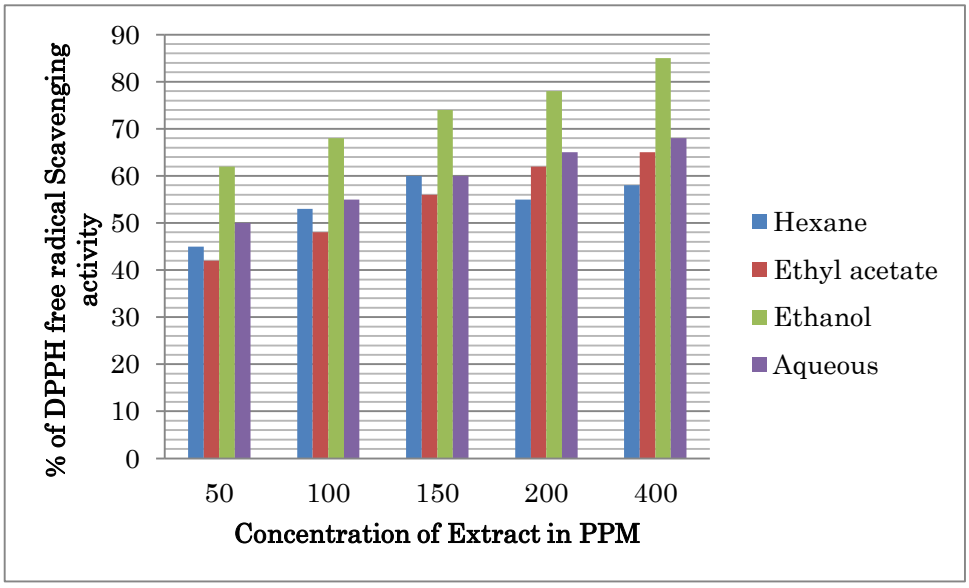

Fig 1: Antioxidant activity of *Trigonella foenum greacum* solvent extracts based on their polarity

4. CONCLUSION

In the present study ash analysis for crude powder drug of seed was carried out. It was observed that highest value of total ash was recorded (4.23%). Acid insoluble ash was in the range of 0.58%, Water soluble ash was in the range of 29.39. Moisture contents was heighst (9.16%) in seeds 10.01 %. Fluorescence study with UV light is a very useful tool for evaluation of crude drugs. The amount of sodium and potassium present in 1 gm of *Trigonella foenum greacum* material was estimated by flame photometry. Ethanolic extract showed highest anti-oxidant activity.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

1. Parthasarathy N, Selwyn M.A, Udayakumar M. Tropical dry evergreen forests of peninsular India: ecology and conservation significance. Trop Conserv Sci. 2008; 1: 89-110.
2. Maria John KM, Jung ES, Lee S, Kim JS, Lee CH. Primary and secondary metabolites variation of soybean contaminated with Aspergillus sojae. Food Res Int. 2013; 54(1): 487-494.
3. Kim MJ, Maria John KM, Choi JN, Lee S, Kim AJ, Kim YM. Changes in secondary metabolites of green tea during fermentation by Aspergillus oryzae and its effect on antioxidant potential. Food Res Int. 2013; 53(2): 670-677.
4. Maria John KM, Thiruvengadam M, Enkhtaivan G, Kim DH. Variation in major phenolic compounds and quality potential of CTC black tea elicited by Saccharomyces cercevisiae and its correlation with antioxidant potential. Ind Crops Prod. 2014; 55:289-294.
5. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A, et al. Indian medicinal herbs as sources of antioxidants. Food Res Int. 2008; 41(1): 1-15.
6. Wang H, Lu Y, Chen J, Li J, Liu S. Subcritical water extraction of alkaloids in Sophora flavescens Ait. and determination by capillary electrophoresis with field-amplified sample stacking. J Pharm Biomed Anal. 2012; 58: 146-151.
7. Upadhyay R, Jha A, Singh SP, Kumar A, Singh M. Appropriate solvents for extracting total phenolics, flavonoids and ascorbic acid from different kinds of millets. J Food Sci Technol. 2013; doi: 10.1007/s13197-013-0976-0.
8. Yao Y, Lin G, Xie Y, Ma P, Li G, Meng Q, et al. Preformulation studies of myricetin: a natural antioxidant flavonoid. Pharmazie. 2014; 69(1): 19-26.
9. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, in-vitro antioxidant and anti-inflammatory activity of flowers of Moringa oleifera. Asian Pac J Trop Biomed. 2013; 3(8): 623-627.

10. Cecchini S, Paciolla M, Caputo AR, Bavoso A. Antioxidant potential of the polyherbal formulation “immuplus”: a nutritional supplement for horses. Vet Med Int. 2014; 2014: 434239.
11. Kim IS, Yang MR, Lee OH, Kang SN. Antioxidant activities of hot water extracts from various spices. Int J Mol Sci. 2011; 12: 4120- 4131.
12. Settharaksa S, Jongjareonrak A, Hmadhlu P, Chansuwan W, Siripongvutikorn S. Flavonoid, phenolic contents and antioxidant properties of Thai hot curry paste extract and its ingredients as affected of pH, solvent types and high temperature. Int Food Res J. 2012; 19(4): 1581-1587.
13. Jang MH, Kim HY, Kang KS, Yokozawa T, Park JH. Hydroxyl radical scavenging activities of isoquinoline alkaloids isolated from Coptis chinensis. Arch Pharm Res. 2009; 32(3): 341-345.
14. Sardar AA, Akhan ZU, Perveen A, Farid S, Khan I. In vitro antioxidant potential and free radical scavenging activity of various extracts of pollen of Typha domigensis Pers. Pak J Pharm Sci. 2014; 27(2): 279-284.
15. Altintas A, Tabanca N, Tyihák E, Ott PG, Móricz AM, Mincsovics E. Characterization of volatile constituents from Origanum onites and their antifungal and antibacterial activity. J AOAC Int. 2013; 96(6): 1200-1208.
16. Al-Dissi, N. M, Salhab, AS, Al-Hajj, HA. Effects of Inula viscosa leaf extract on abortion and implantation in rats. J. of Ethnopharmacology. 2001; 77: 117–121.
17. Bhat, J.U, Nizami, S. Q, Parray, M, Aslam, N, Fahamiya, A, Siddiqui, M, Mujeeb, R, Khanam, M, Khan, MA. Pharmacognostical and phytochemical evaluation of Melissa parviflora and HPTLC finger printing of its extracts J. Nat. Prod. Plant Resour. 2012; 2 (1): 198-208.
18. Sathishkumar T, Baskar R, Aravind M, Tilak S, Deepthi S, Bharathikumar VM. Simultaneous extraction optimization and analysis of flavonoids from the flowers of Tabernaemontana heyneana by high performance liquid chromatography coupled to diode array detector and electron spray ionization/mass spectrometry. ISRN Biotech 2013; doi: 10.5402/2013/450948.
19. Shrivastava, S, Leelavathi, S. Preliminary phytochemical evaluation of leaf extracts of Catunaregum spinosa Thumb. Int. J. Pharma. Sci. Rev. & Res. 2010; 3(2): 114-118.
20. Barkathullah, Ibrar, M, Jelani, G, Rehamnullah, Ehsan, M. Phytosociological and ethnobotanical attributes of Skimmia laureola (dc.) siebold. & zucc. ex walp. and Zantoxylum armatum DC. Int. J. Biosciences. 2012; 2:46-56.
21. Evans, WC. Plants in medicine: the origins of pharmacognosy. In: Tease and Evans‘s Pharmacognosy.15th ed. W. B. Saunders, London, U. K. 2002; 2: 3 – 4.
22. Evans, CE, Banso, A, Samuel, OA. Efficacy of some medicinal plants against Salmonella typhi: an in vitro study. J Ethnopharmacol. 2002; 80: 21-24.
23. Gokhele, SB, Kokate, CK, Purohit, AP. A text book of Pharmacognosy. Nirali Prakashan, Punne India. 2008; 2: 12.
24. Balunas, M.J, Kinghorn, AD. Drug discovery from medicinal plants Life Sci. 2005; 78: 31-41.

25. Joy PP, Thomas J. Mathew S, Skaria BP. In Bose TK et al. (eds).Medicinal Plants. Naya Prokash publishers, Calcutta, Trop. Horticult. 1998; 2: 449-632.
26. Bhat JU, Nizami, SQ, Parray M, Aslam N, Fahamiya A, Siddiqui M, Mujeeb R, Khanam M, Khan MA. Pharmacognostical and phytochemical evaluation of Melissa parviflora and HPTLC finger printing of its extracts J. Nat. Prod. Plant Resour. 2012; 2 (1): 198-208.
27. Jeyaseelan EC, Jashothan PT. In vitro control of Staphylococcus aureus (NCTC 6571) and Escherichia coli (ATCC 25922) by Ricinus communis L. Asian Pac J Trop Biomed. 2012; 2(9): 717-721.
28. Rangari, V.D. Pharmacognosy and phytochemistry. 1st Edn., New Delhi: Carrier Publication. 2002; 1: 100-101.
29. Sarker SD. Pharmacognosy in modern pharmacy curricula. Phcog. Mag.2012; 8: 91-92.
30. Taylor, R.B.; Shakoor, O.; Behrens, R.H.; Everard, M.; Low, A.S.; Wangboonskul, J.; Reid, R.G.; Kolawole, J.A. Pharmacopoeial quality of drugs supplied by Nigerian pharmacies. Lancet. 2001; 357(9272): 1933-1936.
31. Ibrar M, Hussanin F, Amir S. Ethnobotanical studies on plant resources of Ranyal Hills. District Shangla, Pakistan. Pak. J. of Bot. 2007; 39(2): 329-337.
32. Igoli JO, Ogaji OG, Tor-anyiin TA, Igoli NP. Traditional medicine practice amongst the Igede people of Nigeria. Part II. Afr. J. Trad. 2005; 2: 134-152.
33. Sindhu RK, Vasudeva NS, Sharma K. Pharmacognostical and preliminary phytochemical investigations on Verbesina encelioides roots. Journal of Herbal Medicine and Toxicology. 2010; 4: 113-118.
34. Balakrishnan S, Natarajan B, Arumugam B, Hariram BV. Pharmacognostical Evaluation of Amaranthus spinosus L. Pharmacon. J. 2011; 3(19): 13-18.
35. Borris R.P. Natural productions research: perspectives from a major pharmaceutical company, J. Ethnopharmacol. 1996; 51: 29-38.
36. Patel PM, Patel NM, Goyal RK."Quality control of herbal products", The Indian Pharmacist. 2006; 5(45):26-30.
37. Shrikumar S, Maheshwari U, Sughanti A, Ravi TK. WHO guidelines for standardization of herbal drugs.Pharminfo.net. 2006; 2, 78-81.