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PHYTO CHEMICAL STUDIES AND ELEMENTAL ANALYSIS OF *TINOSPORA CORDIFOLIA* AND *TRIGONELLA FOENUM-GRAECUM*

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ABSTRACT: Plants are used medicinally in different countries and are a source of many potent and prevailing drugs. Natural products have been a major source of novel drugs. Medicinal plants are finding used as pharmaceuticals, neuraceuticles, cosmetics and food supplements. To evaluate the preliminary physico-phyto chemical studies, fluorescence studies and detects the amount of elements in the *Tinospora Cordifolia* (TC) and *Trigonella foenum graecum* (TFG). The determination of several elements like calcium (Ca), cobalt (Co), chromium (Cr), copper (Cu) , iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), titanium (Ti) and zinc (Zn) in plant samples TC and TFG using inductively coupled plasma–optical emission spectrometry (ICP-OES) are evaluated.

KEYWORDS: *Tinospora Cordifolia, Trigonella foenum-graecum,* elemental analysis, physico – phyto chemical properties**.**

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1.INTRODUCTION

The use of plants as medicines goes back to early man. Certainly the great civilizations of the ancient Chinese, Indians, and North Africans provided written evidence of man's ingenuity in utilizing. Plants for the treatment of a wide variety of ailments [1] .It is estimated that 70-80 % of people world-wide rely on traditional herbal medicine to meet primary health care needs [2]. Herbs may be defined as the dried leaves of aromatic plants used to impart flavour and odour to foods. The leaves are commonly traded separate from the plant stems and leaf stalks. *Tinospora Cordifolia* (TC) commonly named as shindila kodi in Tamil belonging to family Menispermaceae is a

genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude [3-4]. In racemes or racemose panicles, the male flowers are clustered and female are solitary. The flowering season expands over summers and winters. A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides [5]. Aqueous *Tinospora Cordifolia* extract has been also reported to influence the cytokine production, mitogenicity, stimulation and activation of immune effector cells [6]. *Trigonella foenum graecum* (TFG) Family Fabaceae, popularly known as fenugreek, is an annual herbaceous aromatic leguminous crop. It is widely cultivated in Mediterranean countries and asia, the fenugreek seeds that are rich in protein (30% dry matter) and have a pleasing appetizing aroma [7]. Fenugreek seeds and leaves have been used as part of traditional medicinal purposes [8]. References for utilization of fenugreek as far back as 1578 have been reported [9] and uses of fenugreek seeds as incense and to embalm mummies were documented in ancient Egypt [8]. TFG are known to have several pharmacological effects such as hypoglycaemia, hypocholesstrolemia, antioxidation, laxation, apetite stimulation, antiulcer, and immunomodulatory [10-13]. Metals and minerals present in biological system play a vital role in the metabolism and developments in the field of metals and trace elements research and their clinical, biochemical and immunological areas is fast growing [14]. Metals are not biodegradable and when accumulated by the human beings, they undergo biochemical changes ultimately transforming into various chemical species [15]. Good quality control of medicinal plants is important, since they are normally consumed without any limitation. It is almost a consensus that, being a natural product, they do not pose any harm to health. Most of these plants are found to be rich in one or more individual elements, thereby providing a possible link to the therapeutic action of the medicinal property of these plants [16]. In this study, both plants powders are used to examine the presence of elements by ICP-OES, preliminary physico-chemical studies, phytochemicals and fluorescence studies.

2. MATERIALS AND METHODS

Scientific classification of *Tinospora cordifolia* and *Trigonella foenum- graecum*

Classification	***Tinospora Cordifolia***	***Trigonelle foenum graecum***
Kingdom	Plantae	Plantae
Division	Magnoliophyta	Magnoliophyta
Class	Magnoliopsida	Magnoliopsida
Order	Ranunculales	Fabales
Family	Menispermaceae	Fabaceae
Genus	Tinospora	Trigonella
Species	*T. Cordifolia*	*T. foenum graecum*
Botanical Name	*Tinospora Cordifolia*	*Trigonelle foenum graecum*

Collection and Preparation of Plant Extract

Healthy, disease free entire plants of TC (leaves) are collected from near Kollidam River side, Thirumanur, Thanjavur, Tamil Nadu, India. TFG seeds are collected from local market, Thiruvaiyaru, Thanjavur, Tamil Nadu, India. The collected specimens are authenticated by Dr. S. John Brito, The Director, The Rapinat Herbarium and center for Molecular systematics, St. Joseph's college, Trichy, Tamil Nadu, India. The fresh TC leaves are washed in tap water for 10 minutes and are dried using blotting papers. The washed plants are air and shade dried for two weeks and pulverized to powder using Mortar. The Voucher specimen and the powder form of *Tinospora Cordifolia* (TC) and *Trigonella foenum graecum.* (TFG) are shown in Fig. 1(a), 1(b), 1(c) and 1(d). The dried and powdered plant material of TC (25 g) is extracted using 250 ml of Chloroform, Ether, methanol, Ethanol and water individually using soxhlet extractor for 18 Hrs, at a temperature not the boiling point of the respective solvent. The extracts are concentrated in vacuum at 40 using rotatory evaporator. The residues obtained are stored in a freezer until further test. Similarly TFG seeds are extracted by above solvents.

Fig 1: (a) Voucher specimen of TC and (b) TFG. (c) Powder form of TC and (d) TFG

Pharmacognosy Studies

a. Organoleptic Characters

The various organoleptic characters such as taste, odour, texture and color of TC and TFG are noted [17].

b. Fluorescence Studies

The Fluorescence Studies of the TC and TFG powder are observed in day light and UV light (254 nm and 365 nm) using different chemical reagents [18-19].

c. Physico-Chemical Studies

Physico-Chemical test and different extractive values are carried out on the powdered specimens using standard procedures [20-22].

d. Qualitative Phyto-Chemical Analysis

Phytochemical screenings for the different extracts are carried out according to standard methods [23-24].

ICP-OES Equipment

ICP-OES (Inductively coupled plasma - optical emission spectrometry) is a technique in which the composition of elements in (mostly water-dissolved) samples can be determined using plasma and a spectrometer. PERKIN ELMER OPTIMA 5300 DV ICP-OES is used for present elemental analysis study.

Estimation of Elements

1 g of leaves powder of TC and seeds of TFG are digested individually is 10 ml of ultrapure metal free nitric acid in a microwave digestee. After digestion, the content is diluted to 25 ml with distilled water. Estimation of elements is performed using inductively coupled plasma with optical Emission Spectroscopy (ICP-OES) Perkin Elmer Optima (5300 DV). The microwave digested samples are aspirated into ICP-OES to estimate elements viz. Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ti, Zn, and P [25-27]. The calibration standards are prepared by diluting the stock multi elemental standard solution (1000 mg of lit) in HNO_3.

3. RESULTS AND DISCUSSION

The leaves of TC extracts and seeds of TFG extracts are observed and also results discussed. The organoleptic character of TC leaves possess Green colour with astringent taste and rough texture whereas TFG seeds appear Yellowish brown colour, bitter taste and fibrous texture (Table 1).The two powders of the TC and TFG are treated in Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Nitric acid (HNO_3), Sulphuric acid (H_2SO_4), Ferric chloride ($FeCl_3$), Iodine Solution, Ammonia (NH_3), Sodium Nitro Prusside, 5% KOH solution, Picric acid and Acetic acid. The fluorescence analysis of two sample powder are observed under ordinary visible light and also under UV light at 254 nm and 365 nm the results are recorded in Table 2(a) and Table 2 (b). The fluorescence studies of the both plant powders exhibit very interesting colour variations with different reagents; The TC powder appears dark green colour under normal light and greenish brown colour, light brown colour

Table 1: Organoleptic characters of TC leaves and TFG seeds powder

Characters	TC	TFG
Color	Green color	Yellowish brown
Taste	Astringent	Bitter
Odor	Not specific.	Not discernible
Texture	Rough	Fibrous

Table 2 (a): Fluorescence studies of TC leaves

S.No	Reagents Used	Leaves		
		Day Light	254 nm	365 nm
1	TC Powder	Dark green	Greenish brown	Light brown
2	TC + 1N NaOH	Blackish green	Greenish brown	Greenish brown
3	TC + 1N HCl	Greenish Brown	Dark brown	Dark green
4	TC + 1 N H_2SO_4	Greenish Brown	Light yellowish green	Green
5	TC + Conc. HNO_3	Green	Dark blackish brown	Greenish green
6	TC + 5 % $FeCl_3$	Chocolate brown	Reddish brown	Green
7	TC + Iodine solution	Greenish Black	Dark green	Black
8	TC + 1N NH_3	Dark yellowish brown	Blackish brown	Dark brown
9	TC + Sodium nitro prusside (SNP)	Green	Light Greyish green	Blackish brown
10	TC + 5% KOH solution	Dark black	Brown	Green
11	TC + Picric acid	Dark brown	Brown	Pale green
12	TC+ Acetic acid	Dark yellowish brown	Light yellowish brown	Chocolate brown

Table 2 (b): Fluorescence studies of TFG seeds

S.No	Reagents Used	TFG Seeds		
		Day Light	254 nm	365 nm
1	TFG Powder	Yellow	Greenish Yellow	Brown
2	TFG + 1N NaOH	Dark Yellow	Yellowish Brown	Dark Brown
3	TFG + 1N HCl	Greenish Yellow	Dark Yellow	Dark Red
4	TFG + 1 N H_2SO_4	Yellow	Black	Dark Brown
5	TFG + Conc. HNO_3	Pale Yellow	Brown	Brown
6	TFG + 5 % $FeCl_3$	Yellow	Blackish Brown	Black
7	TFG + Iodine solution	Yellow	Yellowish Brown	Brown
8	TFG + 1N NH_3	Yellowish Brown	Brown	Dark brown

9	TFG + Sodium nitro prusside (SNP)	Pale Yellow	Brown	Black
10	TFG + 5% KOH solution	Yellow	Yellowish Brown	Dark brown
11	TFG + Picric acid	Yellowish Brown	Brown	Brown
12	TFG + Acetic acid	Dark Yellow	Yellow	Brown

Table: 3(a) Physico-Chemical parameters of TC leaves and TFG seeds

Parameters	**TC (g)***	**TFG (g)***
Moisture content	0.026	0.030
Total ash	0.184	0.175
Acid insoluble ash	0.032	0.029
Water soluble ash	0.078	0.087

*For 1 g of sample; values are average of three readings

Table: 3(b) Extractive Values of TC leaves and TFG seeds

Extracts	**Yield (% w/w)***	
	TC (%)	**TFG (%)**
Water extractive	22.13	28.85
Ethanol extractive	24.56	29.73
Methanol extractive	17.89	21.05
Chloroform extractive	5.28	19.24
Ether extractive	4.75	17.21

* Values are average of three readings

Table: 4(a) Preliminary phytochemical analysis of TC leaves

S. No	**Chemical reagents**	**Name of the test**	**$CHCl_3$**	**Ether**	**Methanol**	**Ethanol**	**Water**
1	Alkaloids	Mayer's test	+	+	+	+	+
		Wagner's test	+	+	+	+	+
		Dragendorff's test	+	+	+	+	+
2	Carbohydrates	Molisch's test	-	+	+	-	+
		Beneticts test	-	+	+	-	+
		Fehling's test	+	+	+	-	+
3	Glycosides	Legal's test	-	+	+	+	-
4	Saponins	Froth test	+	+	+	-	+
5	Phytosterol	Salkowski's test	-	+	-	+	+

6	Phenol	Ferric chloride	+	+	+	+	+
7	Fixed oil	Stain test	-	+	+	-	-
8	Tannins	Ferric chloride	+	+	+	+	+
9	Diterpenes	Copper acetate	+	+	+	+	+
10	Protein and Amino acids	Xanthoprotectic test	-	-	+	+	-
		Ninhydrin test	-	+	+	-	+
11	Flavonoids	Alkaline reagent	+	+	+	+	+
		Ammonia test	-	+	+	+	+
12	Resins	Zinc HCl test	+	+	+	+	+

Table: 4 (b) Preliminary phytochemical analysis of TFG seeds

S. No	Chemical reagents	Name of the test	$CHCl_3$	Ether	Methanol	Ethanol	Water
1	Alkaloids	Mayer's test	-	+	-	+	+
		Wagner's test	-	+	+	+	+
		Dragendorff's test	-	+	-	+	+
2	Carbohydrates	Molisch's test	-	+	+	+	+
		Beneticts test	-	+	+	+	+
		Fehling's test	-	+	+	+	+
3	Glycosides	Legal's test	+	-	+	-	+
4	Saponins	Froth test	+	+	+	-	+
5	Phytosterol	Salkowski's test	-	+	+	+	+
6	Phenol	Ferric chloride	-	+	+	-	+
7	Fixed oil	Stain test	-	+	+	-	+
8	Tannins	Ferric chloride	-	+	+	-	+
9	Diterpenes	Copper acetate	-	-	-	-	+
10	Protein and Amino acids	Xanthoprotectic test	-	+	+	-	+
		Ninhydrin test	-	-	+	-	+
11	Flavonoids	Alkaline reagent	+	+	+	+	+
		Ammonia test	+	+	-	+	+
12	Resins	Zinc HCl test	-	-	-	-	-

Table 5: Elemental analysis of TC leaves and TFG seeds

Elements Symbol	Wavelength (nm)	Concentration. in ppm	
		TC leaves (mg/L)	TFG seeds (mg/L)
Ca	317.933	175.7	52.94
Co	228.616	0.079	0.166
Cr	267.716	0.054	0.217
Cu	327.393	1.554	1.568
Fe	238.204	10.21	5.363
K	766.490	97.42	143.7
Mg	285.213	34.19	33.71
Mn	257.610	0.616	0.390
Na	589.592	47.14	61.26
Ti	334.940	274.1	128.6
Zn	206.200	9.814	13.62
P	213.617	-	4.766

under UV light 254 nm, 365 nm respectively. The fluorescence studies of TFG powder shows yellow colour under normal light and greenish yellow, brown colour under UV light 254 nm and 365 nm respectively. The physico-chemical properties of TC leaves and TFG seeds powders are shown in the Table 3 (a). Moisture content of TFG is 0.030 g and TC is 0.026 g. Generally if moisture content value is high then the sample is more prone to microbial contamination. The total ash contents of TC are 0.184 g and TFG 0.175 g. It High ash value it denotes the presence of more organic matter in the plant. Extractive values of TC and TFG are noted at different solvents (Table 3 b). The TC ethanolic extractive value is 24.56 % and TFG ethanolic extract value is 29.73 %. In both cases ethanolic extractive values are higher than other extractive values. Higher extractive value indicates that ethanol has more capacity to extract the biologically active constituents in the plant. Table 4(a) and 4(b) show the phytochemical qualitative analysis of TC and TFG which indicates the presence or absence of major constituents. The analysis of TC indicates the presence of alkaloids, flavonoids, carbohydrates, phenols, tannins and saponins. Similarly the TFG indicates the presence of alkaloids, flavonoids, carbohydrates, glycosides, protein and amino acids. The majority of the constituents are shown their presence in methanol, ethanol and water extracts than chloroform and ether extracts. The quantification values of various elements such as Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ti, Zn and P are shown in Table 5. Calcium contents of TC are 175.7 mg/L and TFG 52.94 mg/L. Calcium play important role in building and maintaining strong bones and teeth. Calcium contents are necessary for normal functioning of cardiac muscles, blood coagulation, milk clotting and regulation of cell permeability [28]. Certain chemicals of brain are controlled by

presence or absences of iron, it is essential for formation of hemoglobin, carry oxygen around the body [29]. Iron contents of TC are 10.21 mg/L and TFG 5.63 mg/L. Potassium works with sodium to maintain the body's water balance, thus helping to maintain the blood pressure. Thus Potassium has a protective effect against hypertension [30]. Potassium is present in TC (97.42 mg/L) and TFG (143.7 mg/L). Magnesium plays important role in formation and function of bones, muscles and prevents high disorders, high blood pressure and depression [31]. Zinc maintains various reactions of the body which help to construct and maintain DNA, required for growth and repair of body tissues, important element of ligaments and tendons. Zinc is the regulation of insulin levels in the blood and has been reported to improve the sensitivity of insulin in the management of diabetes [32-33]. Zinc values of TC are 9.814 mg/L and TFG value 13.62mg/L. Manganese is an antioxidant nutrient, the breakdown of amino acids and production energy. It is need for the metabolism of vitamins B1, C and E for the activation of various enzymes which are important for digestion and regulation of immune system [34]. In the present study, Mn is found in appreciable amounts in TC (0.616 mg/L) and in TFG (0.390 mg/L). All elements present in the both plants are very much needed for normal metabolism and they are present within permissible limit which are normally require for daily requirement of human.

4. CONCLUSION

TC leaves possess Greenish colour and TFG seeds appear Yellowish brown colour.The fluorescent study using different chemical reagents showed different colouration under visible and UV light. Generally fluorescence characters are used for assessing the quality and purity of raw drug powder. The present study revealed that both plants possess many bioactive components like alkaloids, flavonoids, carbohydrates, tannins, proteins and amino acids etc. These active constituents present in the plants are found to give synergistic effect in curing ailments. The majority of plant elemental analyses are now performed by techniques based on ICP spectrometry such as inductively coupled plasma-optical emission spectroscopy (ICP-OES). TC leaves and TFG seeds study has to be extended for animal studies in future to know the curing capacity of various diseases.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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