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Development and optimization of bioanalytical parameters for the standardization of *Trigonella foenum-graecum*

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ABSTRACT

Objective: To develop a novel qualitative and quantitative method, which can pave the way for rapid and selective determination of different constituents of *Trigonella foenum-graecum* (*T. foenum-graecum*). **Methods:** Phytochemical analysis, solubility test, heavy metal analysis, antimicrobial study and quantitative analysis through HPTLC techniques were performed in the present investigation. **Results:** Phytochemical analysis showed to contain alkaloid, saponin, tannin, flavonoid, steroid, triterpenoid, glycoside and protein. pH of the 1% solution was found to be 5.65, loss on drying was 4.5%, total ash, solubility in water and in 50% alcohol was 7.11%, 81.00% and 84.25% respectively. Total flavonoid and total phenol content were found to be 1.8% and 0.60% (w/w). Level of different heavy metals and microorganisms were found to be under the limit. Content of quercetin and rutin in the *T. foenum-graecum* were found to be 0.879% (w/w) and 0.417% (w/w) respectively. **Conclusion:** In the future, these phytochemical parameters could be used as an important tool for the standardization of *T. foenum-graecum*.

1. Introduction

Trigonella foenum-graecum (*T. foenum-graecum*) L. is an annual spice and remunerative cash crop belongs to the family Leguminosae and found in Asia, Mediterranean and North African regions[1]. The seeds of *T. foenum-graecum* (Fenugreek) are commonly used in India and in oriental countries as a spice in food preparations due to their strong flavor and aroma. The seeds are reported to have restorative and nutritive properties and to stimulate digestive processes. Fenugreek seeds are used as a traditional remedy for the treatment of diabetes[2]. In India and China it has also been used to treat arthritis, asthma, bronchitis, improve digestion, maintain a healthy metabolism, cure skin problems, increase libido and male potency, treat sore throat and cure acid reflux. *T. foenum-graecum* has also been reported to exhibit antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive, antioxidant activity, hypoglycemic, hypocholesteremic, anticancer, and gastroprotective activity[3,4]. In Ayurvedic and Unani systems of medicine, fenugreek is used for the treatment of epilepsy, paralysis, gout, dropsy, chronic cough and piles[5]. *T. foenum-graecum* seeds contain protein, vitamin C, niacin, potassium, diosgenin, alkaloids, lysine, L-tryptophan, and steroidal saponins (diosgenin, yamogenin, tigogenin, and neotigogenin)[3]. Leaves are are a rich source of calcium, iron, β-carotene and other vitamins. Seeds of contain tannic acid, fixed oils, volatile oils, diosgenin, alkaloids trigonelline, trigocoumarin, trigomethyl coumarin, tannic acid, yellow colouring matter, gitogenin and traces of trigogenin and vitamin A[5,6]. Due to the rich sources of phytoconstituents and medicinal importance, plant extract must be standardized before consumption. So in the present investigation, *T. foenum-graecum* extract were taken for the standardization through both conventional and modern techniques such as high performance thin layer chromatography (HPTLC).

2. Materials and methods

Crude plant extract of *T. foenum-graecum* was procured from Garlico Herbal Concentrate (M.P.), India. Phytochemical screening was conducted on the *T. foenum-*

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graecum extract to conferm the presence of different phytoconstituents[7]. The presence of phytoconstituents in the extract was also analysed through TLC analysis[8]. Study of the parameters such as solubility in water, pH, moisture content, zingirol content, heavy metal analysis and microbiological analysis were also performed as per standard official methods[9,10]. Total phenol and flavonoid content were also determined according to the standard methods[11,12]. The quantification of rutin and quercetin in *T. foenum-graecum* were determined through HPTLC method (Table 1).

Table 1

Bioanalytical parameters of analysis.

Analysis	Estimation of rutin and quercetin in *T. foenum-graecum* extract.
Plate material	HPTLC Precoated plates Silica Gel Merck $60F_{254}$
Syringe	100 μL Hamilton (Bonadzu, Switzerland)
Application mode	CAMAG Automatic TLC Sampler III
Development mode	Ascending
Scanning	CAMAG TLC scanner 3 with Cats software
Experimental conditions	Temperature (25±2) ℃, relative humidity 40%

3. Results

Crude extract of *T. foenum-graecum* was screened for phytochemical analysis and was found to contain alkaloid, saponin, tannin, flavonoid, steroid, triterpenoid, glycoside and protein. TLC analysis showed seven spots with R_f (0.22, 0.41, 0.45, 0.54, 0.58, 0.80, 0.96) in ethyl acetate: methanol: H_2O (81:11:8) solvent system. pH of the 1% solution was found to be 5.65, loss on drying was 4.5%, total ash, solubility in water and in 50% alcohol was 7.11%, 81%, 84.25% respectively.

Table 2.

Fingerprint analysis of the *T. foenum-graecum* extract.

No of spot	Solvent system	R_f value	Maximum peak height	Peak area (%)
8	ethyl acetate : Formic acid : Glacial acetic acid : H_2O (100:11:11:26)	0.26	16.6	2.07
		0.35	32.3	6.19
		0.49	20.0	4.62
		0.60	67.7	18.49
		0.73	111.8	29.22
		0.89	73.5	14.20
		0.92	85.8	10.22
		0.97	140.4	14.99

The total flavonoid and total phenol content of *T. foenum-graecum* extract was determined and was found to be 1.8% and 0.60% (w/w). Moreover, fingerprint analysis through HPTLC was also performed in ethyl acetate: formic acid: glacial acetic acid: H_2O (100:11:11:26) solvent system and the respective data was presented in the Table 2. Heavy metal level (lead, arsenic, mercury, cadmium) were found to be under the limit. Microbiological assay showed that *E. coli* and salmonella was found to be absent whereas total bacterial count and yeast & moulds contents were found to be below the limit.

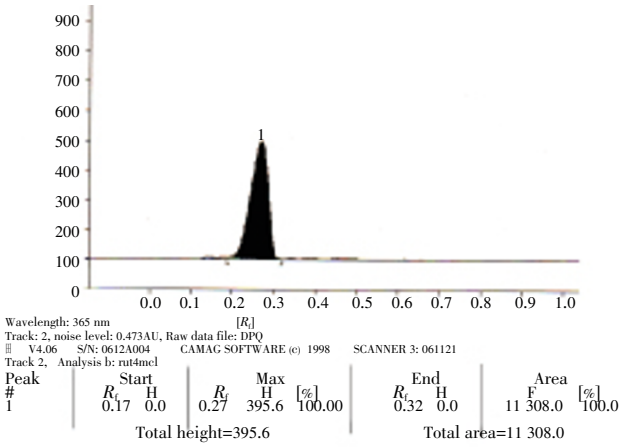

Figure 1. Standard HPTLC chromatogram of rutin.

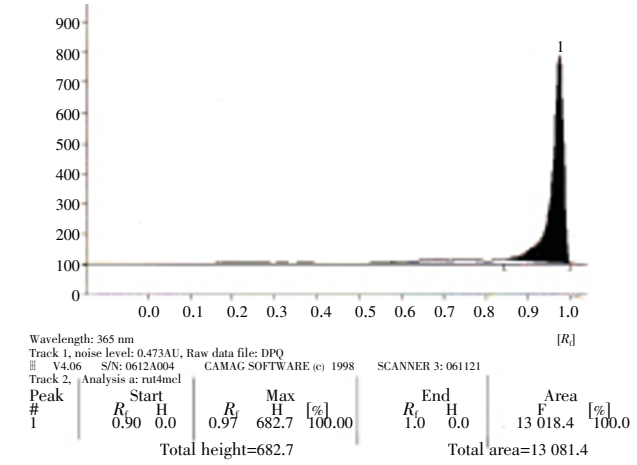

Figure 2. Standard HPTLC chromatogram of quercetin.

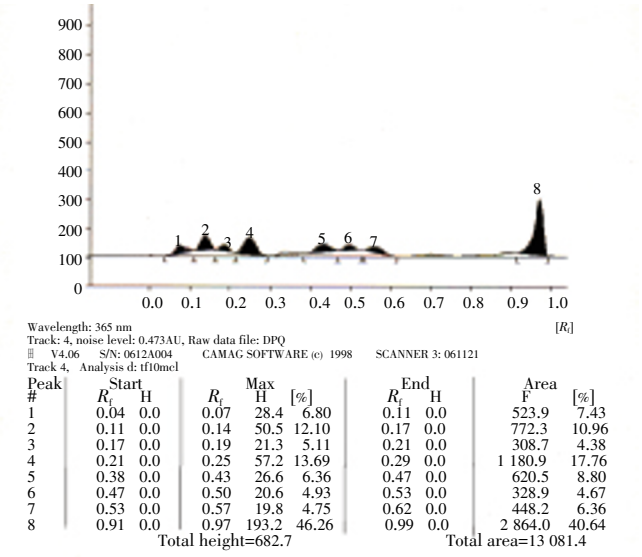

Figure 3. HPTLC chromatogram of *T. foenum-graecum* extract.

Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) was found to be suitable solven system for quantitative analysis of rutin and quercetin in the *T. foenum-graecum* extract through HPTLC methods. The content of quercetin and rutin in *T. foenum-graecum* were

found to be 0.879% (w/w) and 0.417% (w/w) respectively using above HPTLC method. The interpretation of results suggests that the sample contained considerable amount of flavonoids. The respective HPTLC chromatogram of rutin, quercetin and *T. foenum-graecum* extract were presented in the Figure 1 & 2 & 3.

4. Discussion

Development of various novel analytical techniques for the analysis of medicinally significant phytoconstituents has led to the resurgence in this area of research. *T. foenum-graecum* being a potent antidiabetic has been chosen for the present investigation. The overall objective has been to develop a novel qualitative and quantitative method, which can pave the way for rapid and selective determination of different phytoconstituents of *T. foenum-graecum*. Physicochemical and phytochemical analysis was generally performed to ensure the identity, purity and quality of the drug. These parameters are also used to check the genuine nature of the crude drug, thus it plays an important role in preventing the possible steps of adulteration. Phytoconstituents obtained from natural sources play an important role in the health care system due to the health promoting activity. So it is necessory to check the quality safety and efficacy inorder to its safety profile. HPTLC technique has gained much popularity for standardization of the herbal drugs and formulations in the last few decades due to analysis of several samples at a time using very small quantity of marker compound as well as solvent system. HPTLC techniques can be used to solve many qualitative and quantitative analytical problems in medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology and environmental science[13-16]. According to WHO guidelines, an herbal product needs to be standardized in order to its safe use for the treatment of different diseases[17,18]. So from the above results we can say that these phytochemical parameters can be used as standard tools for the simultaneous analysis of different phytoconstituents present in the *T. foenum-graecum* plant material. In future the information provided in the present investigation may be useful as standard tools for the identification of adultrants and other related species.

Conflict of interest

The authors declare they have no conflict of interests.

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