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Investigation of Fenugreek (*Trigonella Foenum-Graecum* L.) Leaf for its Phenolic Composition and Antioxidant Activity

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Abstract

Fenugreek seed is an important source of steroidal sapogenins such as diosgenin which are used extensively by both pharmaceutical and nutraceutical industries. Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones. Biochemical estimation for phenolic contents and antioxidant potential were analyzed in leaves of five certified varieties of fenugreek namely: GM-2, RMT-305, Rajendra Kranti (RK), Pant Ragini (PR) and Hissar Mukta (HM). Variety GM-2 and HM exhibited highest phenol (5.2±0.20mg/gmdwt.) and flavonoid (21.38±0.17 mg/gmdwt.) contents. Among all the varieties RK exhibited highest (70.94±2.0%) percentage of DPPH scavenging activity followed by GM-2, HM, RMT-305 and poorest in PR. In our study no linear correlation was observed between total phenols and flavonoid contents and antioxidant activities in the leaf extracts of all the varieties of fenugreek. The data of our study showed that the phenolic compounds are higher in leaf extract of variety GM-2 and HM but variety RK exhibited strongest antioxidant capacity, percentage DPPH discoloration and lowest IC_{50} value.

Keywords Antioxidant, Fenugreek, Trigonella foenum- graecum, Fabaceae, Phenol, Flavonoid

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop and dicotyledonous plant belonging to the subfamily Papilionaceae, family Leguminacae (the Fabaceae). The plant is an aromatic herbaceous annual, widely cultivated in Mediterranean countries and Asia. In India, its cultivation is concentrated mainly in Rajasthan, which contributes 80% of the total area, as well as production. Fenugreek is also known as one of the oldest medicinal plants recognized in recorded history [1].Fenugreek seed is an important source of steroidal sapogenins such as diosgenin which are used extensively by both pharmaceutical and nutraceutical industries. Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids and progesterone [2], [3]. Mc Anuff et al. (2002) and Acharya et al.(2008) reported that steroidal sapogenins are effective agents for the treatment of hypocholesterolemia, a disorder often associated with diabetes [1], [4].

Fenugreek may be a viable alternative for production of diosgenin because of its shorter growing cycle, lower production costs, consistent yield and quality [5], [6]. The biological and pharmacological actions of fenugreek are attributed to the variety of its constituents, namely: steroids, polyphenolic substances, volatile constituents and amino acids. The aim of this study is to evaluate phenolic composition and antioxidant activity in leaf extract of five certified varieties of *Trigonella foenum- graecum*.

2. Material and Method

Fenugreek leaves were collected from National Research Centre for Seed Spices (NRCSS), Ajmer (26° 27' 0" North, 74° 38' 0" East). Biochemical estimation for phenolic contents and antioxidant potential were analyzed in five certified varieties of fenugreek namely: GM-2, RMT-305, Rajendra Kranti (RK), Pant Ragini (PR) and Hissar Mukta (HM). The methanolic extracts were prepared from leaf of all the varieties. The plant materials were collected from NRCSS field. The extracts prepared from fresh leaves were used for analyzing total phenols, flavonoids and antioxidant activity *in vitro*. 1 g of plant material was extracted in 10 ml of 80% methanol by maceration (10-15 min). The solvent was then centrifuged at 14,000 rpm for 30 min at room temperature. The extract obtained was used for analysis. All solvents used were of analytical grade. 1,1-diphenyl-2- picryl hydrazyle (DPPH) and quercetine were procured from

Sigma-Aldrich Inc., (St. Louis, USA); gallic acid and ascorbic acid were procured from Merck Co. (Darmstadt, Germany); Folin Ciocalteau reagent, aluminum chloride, methanol, sodium carbonate and potassium acetate were purchased from Qualigens Fine Chemical Co. (Mumbai, India).

Absorbance was measured on a Spectroscan-50, UV-VIS spectrophotometer (Biotech. Engineering Management Co. UK). Taking 0% inhibition the regression analysis was used to produce regression equation by plotting a graph between the concentrations of the extracts and percentage inhibitions of free radicals. The IC_{50} values (concentration of extracts required to scavenge 50% DPPH free radicals) were calculated by using regression equations.

2.1 Determination of Total Phenols

Total phenols were determined by the Folin Ciocalteau reagent method [7]. An aliquot of each plant extract (0.5 ml 1:10 mg l^{-1}) or gallic acid (standard phenolic compound) was added with Folin Ciocalteau reagent (5 ml 1:10 diluted with distilled water) and 4 ml of a 1M solution of Na_2CO_3. The mixture was allowed to stand for 30 min at room temperature and absorbance was measured at 710 nm. Total phenolic contents of extracts were expressed as mg gallic acid equivalent (GAE)/g dry weight. All samples were analyzed in triplicate.

2.2 Determination of Total Flavonoids

Total flavonoid content was analyzed by the aluminum chloride method [8]. Each plant extract (0.5 ml of 1:10 g l^{-1}) was mixed with 1.5 ml methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M potassium acetate and 2.8 ml distilled water. The mixture was allowed to stand for 30 min at room temperature and absorbance was measured at 415 nm. Total flavonoid content was expressed as mg quercitin equivalents (QE) g^{-1} dry weight. Samples were analyzed in triplicate.

2.3 Determination of DPPH-free Radical Scavenging Activity

Stable DPPH was used for *in vitro* determination of free radical scavenging activity of the extracts [9]. Different concentrations of each extract were mixed with a methanolic solution of DPPH (0.004%). The mixture was allowed to stand for 15 min. The scavenging of free radicals by each extract was evaluated spectrophotometrically at 517 nm against the absorbance of DPPH radicals. The percentage discoloration was calculated by following formula:

DPPH radical scavenging activity (%) = $[AC_{517} – AE_{517} / AC_{517}] \times 100$ where AC_{517} is the absorbance of the DPPH solution without extract, AE_{517} is the absorbance of the tested plant extract with DPPH. The degree of discoloration indicates the free radical scavenging efficiency of the substances. Ascorbic acid was used as a free radical scavenger reference compound.

2.4 Determination of IC_{50} Value

Taking 0% inhibition the regression analysis was used to produce regression equation by plotting a graph between the concentrations of the extracts and percentage inhibitions of free radicals. The IC_{50} values (concentration of extracts required to scavenge 50% DPPH free radicals) were calculated by using regression equations. Regression equations to derive the IC_{50} values showed an inverse

Table 1. Antioxidant capacity and DPPH free radical scavenging activity of methanolic extract of leaf of different varieties of *Trigonella foenum-graecum*.

relationship between IC_{50} value and percentage scavenging potential of a sample

2.5 Statistical analysis

The experiments were carried out in a completely randomized design with 10 replicates per treatment and each experiment was repeated three times. Mean values were subjected to analysis of variance (ANOVA) and statistical significances between means were assessed using new Duncan's multiple range test (DMRT) at $P < 0.05$ [10].

3. Results

Total phenol and flavonoid contents of methanolic extract of leaf were determined spectrophotometrically in five certified varieties [GM-2, RMT-305, Rajendra Kranti (RK), Pant Ragini (PR) and Hissar Mukta (HM)] of *Trigonella foenum – graecum.*

Variety GM-2 and HM exhibited highest phenol (5.2±0.20mg/gmdwt.) and flavonoid (21.38±0.17 mg/gmdwt.) contents. Significant difference was not observed in total phenols and flavonoids contents in the samples of RMT 305, RK and PR as they showed 4.5±0.21mg/ml, 4.63±0.3mg/ml and 4.02±0.2 mg/ml respectively (Fig. 1).

Leaf extracts were also analyzed in order to observe the comparative evaluation of antioxidative contents their scavenging activities and IC_{50} values in all the varieties of fenugreek. The variety RK exhibited strongest antioxidant capacity as this variety has maximum antioxidative contents 5.38±0.45mg/gm dwt. followed by GM-2, HM (4.89 mg/gm dwt.), RMT-305 (4.5±0.22mg/gm dwt.) and poorest in PR 1.81±0.16mg/gm dwt. (Table 1). Among all the varieties RK exhibited highest (70.94±2.0%) percentage of DPPH scavenging activity followed by GM-2, HM, RMT-305 and poorest in PR.

Regression equations to derive the IC_{50} values (concentration of extracts required to scavenge 50% DPPH-free radicals)

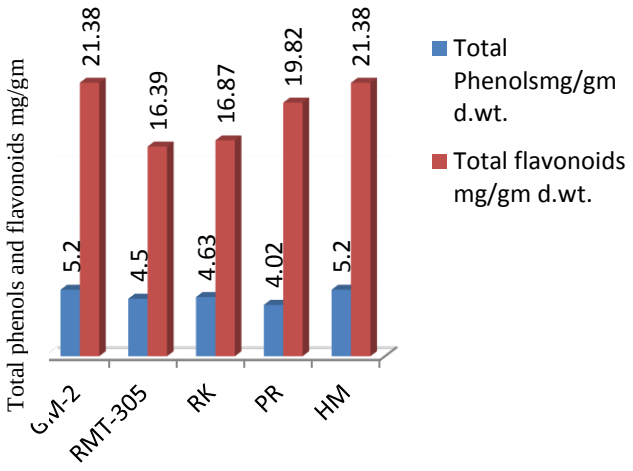

Fig. 1. Total phenol and flavonoid contents in the methanolic extract of leaf of different varieties of *Trigonella foenum-graecum*

showed an inverse relationship between IC_{50} value and percentage scavenging potential of a sample. The strongest DPPH radical scavenging activity was exhibited by the RK extract with IC_{50} = 0.14±0.08 mg ml^{-1}.

S.N	Fenugreek Variety	Antioxidant capacity mg/gm dwts.± SE	DPPH scavenging activity % ± SE	IC_{50} mg/ml ± SE
1.	GM-2	4.89±0.19	57.43±1.4	0.17±0.02
2.	RMT-305	4.5±0.22	46.6±1.25	0.21±0.06
3.	RK	5.38±0.45	70.94±2.0	0.14±0.08
4.	PR	1.81±0.16	21.62±1.04	0.46±0.02
5.	HM	4.89±0.2	57.43±2.5	0.17±0.08

4. Discussion

Recently researches have been focused to investigate natural antioxidants from plants directly as the synthetic antioxidants affects adversely. Natural antioxidants of plant origin are important in health, food and preventive medicine [11].

Antioxidant activities of aromatic plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them [12]. This study reports a comparative evaluation of phenolic contents and their antioxidant activities of leaf extracts of five certified varieties of fenugreek. Although many studies support that total phenols and flavonoids contribute significantly to the total antioxidant potential of many fruits, vegetables and aromatic plants [13] - [15]. Some publications also support that higher amount of phenolic compounds exhibit higher radical scavenging activities [16].Present investigation do not support these claims. In our study no linear correlation was observed between total phenols and flavonoid contents and antioxidant activities in the leaf extracts of all the varieties of fenugreek.

Various phenolic compounds respond differently in DPPH assay, depending on the number of phenolic groups they have [17]. However, some studies support that there is no correlation between phenolic contents and radical scavenging activity [18], observations of our investigation are accordance with these findings. The data of our study showed that the phenolic compounds are higher in leaf extract of variety GM-2 and HM but variety RK exhibited strongest antioxidant capacity, percentage DPPH discoloration and lowest IC_{50} value.

In conclusion, our study supports that antioxidant activity may not be correlated with the quantity of phenolic contents present in methanolic extract. Present investigation also recommends fenugreek leaves as a significant natural antioxidant supplement as it is commonly consumed in diet in India. In addition the observations of study likely to sensitize further isolation and characterization of bioactive compounds from extract of the plant under investigation.

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