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Screening the Antioxidant activity of *Trigonella foenum graecum* Seeds

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ABSTRACT:- This study was undertaken to examine the antioxidant activity of methanolic extract of seeds of Trigonella foenum-graecum seeds using DPPH free radical scavenging assay. The IC50 (The concentration of sample required to scavenge 50% of DPPH free radical) was calculated by plotting graph between % inhibition vs concentration. The Ascorbic acid was used as standard antioxidant in comparison to methanolic extract of Trigonella foenum-graecum. The IC_{50} value of extract and Ascorbic acid was found to be 3.24 µg/ml whereas IC_{50} value of methanolic extract was found to be 1.98 µg/ml. This suggests that methanolic extract of Fenugreek seeds had potent antioxidant activity.

Keyword: -*Trigonella foenum-graecum*, DPPH assay, IC_{50} value, Antioxidant activity

I. INTRODUCTION

Trigonella foenum-graecum (Family Fabaeace) is called methika in Ayurveda and used as medicine for the treatment of wounds, abscesses, arthritis, bronchitis and digestive disorders etc since oldest time. [4] It is also eaten in winters as to improve immunity and protects heart, brain and other vital organs of body through its medicinal properties. In traditional Chinese Medicine it is also used for kidney problems and conditions affecting the male reproductive tract. The recent researches have proved it beneficial for Atherosclerosis, Constipation, Diabetes, High cholesterol and Hyper-triglyceridemia.[2, 14]The seeds of fenugreek contain alkaloids, flavonoids, saponins, amino acids, tannins and some steroidal glycosides, proteins etc. [3]. Antioxidants or inhibitors of oxidation are the compounds which retard or prevent the oxidation in general and prolong the life of oxidizable matter. [9] Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as reactive species scavenger. Polyphenolic compounds like flavonoids and phenolic acids, commonly found in plants, have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activities of the individual compounds may depend on structural factors, such as number of phenolic, hydroxyl or methoxyl groups and other structural features. [12] Among the antioxidative compounds vitamin A, C, E, selenium, carotenoids, ascorbic acid show very strong intensity of antioxidative activities. [6]The molecule of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centred at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present). Representing the DPPH radical by $Z^{\bullet}$ and the donor molecule by AH, the primary reaction is

$$Z^{*} + AH = ZH + A^{*} \quad \ldots\ldots\ldots[1]$$

Where, ZH is the reduced form and $A^{\bullet}$ is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant. The reaction [1] is therefore intended to provide the link with the reactions taking place in an oxidising system, such as the auto-oxidation of a lipid or other unsaturated substance; the DPPH molecule $Z^{\bullet}$ is thus intended to represent the free radicals formed in the system whose activity is suppressed by the substance AH.[5,7]**Objective:** The objective of this study was to evaluate the IC50 value of Methanolic extract and standard ascorbic acid

II. MATERIAL AND METHODS

Plant material

The Trigonella foenum-graecum seeds were purchase from the R.R Herbs, Delhi and authenticated by the Dr. H.B Singh Taxonomist, National Institute of Science Communication and Information Resources (NISCAIR) New Delhi. A Voucher herbarium specimen (specimen no.RIT/MP/G/2010/279).

Preparation of extract

Dried coursed powder was taken and successively extraction was done by methanol solvent using soxhlet apparatus unit. Than solvent extract was concentrated under vacuum and stored at 2-8^{0}C in dark place.

III. PHYTOCHEMICAL INVESTIGATION

The plants may be considered as biosynthetic laboratory for compounds like alkaloids, glycosides, volatile oils and tannins etc that exert physiological effects. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. A weighed quantity of each of the part was extracted in a Soxhlet apparatus for 6 hrs. The extract was evaporated to dryness under reduced pressure and controlled temperature (40- 50 ºC).The following extracts of the plant material Petroleum ether (60^{0}C-70^{0}C) extract, Acetone extract, Chloroform extract, Methanol extract, Methanol: water (50:50) extract, aqueous extract were subjected to preliminary phytochemical screening for the detection of various plant constituents. The results were present in **Table 3.** The extract were subjected to preliminary phytochemical investigation for the detection of following phytoconstituents- Alkaloids, Glycoside, Saponins, Tannins, Flavonoids, Carbohydrates, Amino acids, Proteins, Starch, Mucilage, Steroids, Coumarin.[10]

IV. PREPARATION OF REAGENTS AND DILUTIONS

The 500µM Solution of DPPH was made by using 23 mg of DPPH (Assay 85%) of Hi Media Laboratories Pvt. Ltd. CAS No. 1898-66-4. 23 mg DPPH was dissolved in 100 ml methanol in a 100 ml volumetric flask and stored in dark.TRIS [2-amino-2 (hydroxy methyl)propane1-3di-ol] Buffer pH 7.4 was made by adding 0.605gm of TRIS Buffer 7.4 of Qualigens Fine Chemicals in 30 ml of water and adding 0.33 ml of concentrated HCl, diluted to 100 ml with distilled water. The use of TRIS buffer was to prevent the sudden pH change during the preparation of test dilutions (Anonymous, 2006).A series of 10 dilutions for Ascorbic acid (Standard antioxidant) in the range of 2.0 µg/ml to 20 µg/ml was prepared using Ascorbic acid. All dilutions were prepared in methanol. 2 ml solution from the above dilutions with concentrations 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/ml were further diluted by adding 2 ml DPPH, 0.5 ml TRIS, 0.5 ml methanol each so that the final concentration of the dilutions for measurement of absorbance by UV Visible spectrophotometer was in the range of 0.4 µg/ml to 4.0 µg/ml. For the preparation of various dilutions of methanolic extract of fenugreek seeds a series of 10 dilutions were prepared for methanolic extract in the concentration range of 0.4 µg/ml to 4.0 µg/ml in methanol. 1 ml from the above dilutions were taken and further diluted by adding 2 ml DPPH, 0.5 ml TRIS, 0.5 ml methanol each so that the final concentration of the dilutions for measurement of absorbance by UV Visible spectrophotometer was in the range of 0.4 mg/ml to 4.0 mg/ml. The 500µM solution of DPPH was prepared by dissolving 23 mg of DPPH in 100 ml of methanol.

V. DPPH RADICAL SCAVENGING ACTIVITY

The absorbance of final dilutions of Ascorbic acid prepared above after 30 minutes of addition of DPPH were measured at λ max 517 nm using methanol as blank and the readings were recorded.

Measurement of Antioxidant activity of Methanolic extract was prepared above after 30 minutes of addition of DPPH were measured at λ max 517 nm using methanol as blank and the readings were recorded along with the calculated % inhibition.

Statistical analysis

The percentage inhibition was calculated using:

$$\text{Percent Inhibition} = \frac{Ac-As}{Ac} \times 100$$

Where, Ac is absorbance of control, As is the absorbance of sample.

IC_{50} value (a concentration at 50% inhibition) was determined from the curve between percentage inhibition and concentration. All determinations were done in triplicate and the IC_{50} value was calculated by using the equation of line. (Papuc *et al.,* 2008)

VI. RESULT

In the **Phytochemical studies**, the various extracts of drugs were tested by different chemicals and reagents for the determination of significant chemical constituents The drug extract showed the presence of various types of important constituents- **Flavonoids, Carbohydrates, Tannins, Amino acids, Alkaloids, Saponins, Coumarins, Mucilage, Starch and Protein.** The results are present in

Table 1.

In the evaluation of **Antioxidant activity** it has been showed that fenugreek seeds had potant antioxidant activity as compared to that of standard antioxidant ascorbic acid. Absorbance of blank was found to be 2.036 at 517 nm, Absorbance of fenugreek extract and Standard ascorbic acid were taken and IC_{50}, % inhibition were calculated. The results are present in **Table 2, 3 and Figs. 1, 2.**

Calculations of IC_{50}

- Equation for the Standard curve for Ascorbic acid

$y = 12.52x + 9.411$

$R^2 = 0.979$

So $IC_{50} = 3.24$ µg/ml.

- Equation for curve of methanolic extract.

$y = 17.56x + 15.31$

$R^2 = 0.966$

So $IC_{50} = 1.98$ µg/ml.

VII. DISCUSSION

The antioxidant activity (in vitro) was performed by 1, 1-diphenyl-2-picryl- hydrazyl (DPPH) method. The methanolic extract of fenugreek seed had 4.938 µg/ml IC50 value while the standard Ascorbic acid had 8.10 µg/ml. This result showed fenugreek extract have potent antioxidant activity. The drug extract showed the presence of various types of important constituents- Flavonoids, Carbohydrates, Tannins, Amino acids, Alkaloids, Saponins, Coumarins, Mucilage, Starch and Protein. On the basis of the results the antioxidant activity was showed due to the presence of constituents like tannins, flavonoids and coumarins in the methanolic extract of fenugreek seeds.

VIII. ACKNOWLEDEMENT

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Table 1: Phytochemical screening of fenugreek seed's extracts.

Extract constituents	Tests		P:e	C	A	M	M:W	W
Alkaloids	Dregandorff test		-	-	+	+	+	+
	Wagnar's test		-	-	-	+	+	+
	Hager's test		-	-	+	+	+	+
Carbohydrates	Molisch Test		-	-	-	+	+	+
	Fehlings Test		-	-	+	+	+	+
	Benedict's Test		-	-	-	+	+	+
Glycosides	Cardiac Glycoside	Keller killiani Test	-	-	-	-	-	-
		Legal Test	-	-	-	-	-	-
		Liberma nn Test	-	-	-	+	+	-
		Baljet Test	-	-	-	-	-	-
	Anthraquinone Glycoside	Brontrager Test	-	-	-	-	-	-
		Mod. Brontrager	+	-	-	+	-	-
	Saponin Glycoside		-	-	+	+	+	+
Tannins & Phenols	5% $FeCl_3$		+	-	-	+	+	+
	Lead acetate		+	-	+	+	+	+
	5%HnO_3 Test		-	-	+	+	+	+
Flavonoids	Zinc chloride Test		-	-	-	+	+	+
	Shinoda Test		-	+	-	+	+	+
	Ammonia Test		-	+	-	+	+	+
Amino Acids	Ninhydrin test		-	-	-	+	+	+
	Cysteine test		+	-	-	+	+	+
Proteins	Xanthoprotein test		-	-	-	+	+	+

	Millon's test	-	-	-	+	+	+
	Biuret test	+	-	-	+	+	+
Mucilage	**KOH test**	-	-	-	+	+	+
	Ruthenium red test	-	-		+	+	+
starch	**Tannic acid test**	-	-	-	+	+	+
	Iodine test	-	-	-	+	+	+

P:e-Pet ether, C-Chloroform, A-Acetone, M-Methanol, M:W-Methanol:Water, W-Water extract

Table 2: Values of absorbance and percentage inhibition with increase in concentration of methanolic solution of Ascorbic acid (standard antioxidant).

Concentration (µg/ml)	Absorbance (nm)	% Inhibition
0.4	1.681	16.40
0.8	1.642	18.34
1.2	1.507	25.06
1.6	1.411	29.83
2.0	1.328	33.96
2.4	1.243	38.19
2.8	1.124	44.10
3.2	1.059	47.33
3.6	0.967	51.91
4.0	0.713	64.54

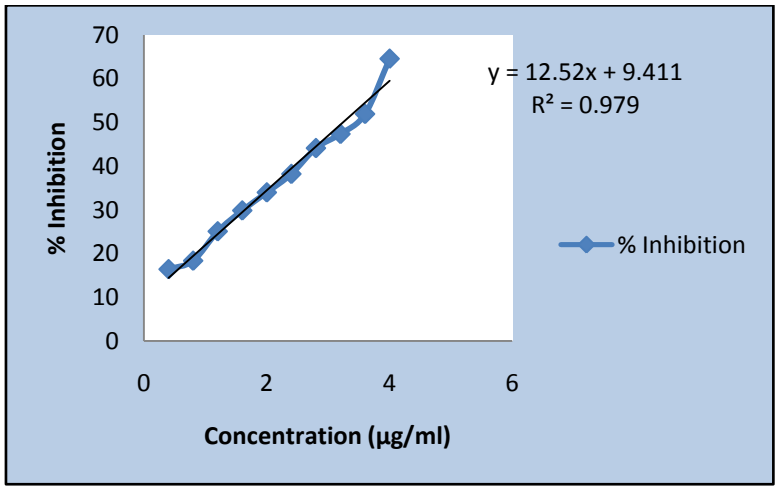

Fig.1: Graphical representation of concentration (µg) vs percentage inhibition of methanolic solution of Ascorbic acid (standard antioxidant)

Table 3: Values of absorbance and percentage inhibition with increase in concentration of methanolic extract.

S. No	Concentration (µg/ml)	Absorbance (nm)	% Inhibition
1	0.4	1.710	16.42

2	0.8	1.492	27.07
3	1.2	1.216	40.56
4	1.6	1.152	43.69
5	2.0	0.964	52.88
6	2.4	0.757	63.00
7	2.8	0.637	68.86
8	3.2	0.625	69.45
9	3.6	0.489	76.09
10	4.0	0.378	81.52

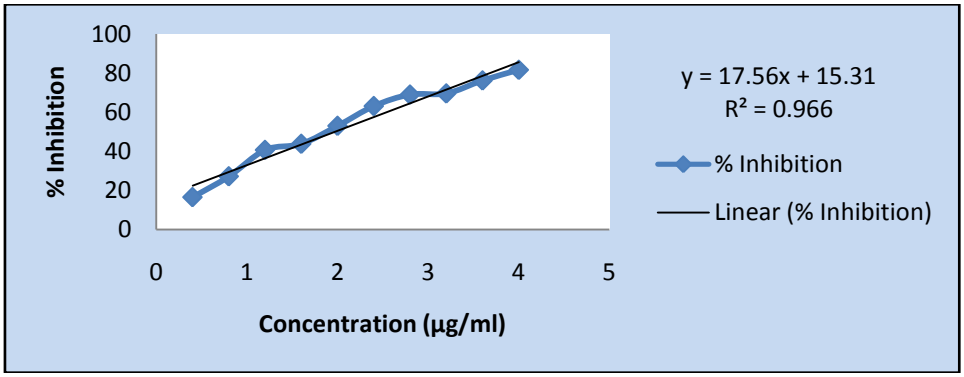

Fig.2: Graphical representation of concentration (µg) vs percentage inhibition of methanolic extract.