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STUDIES ON ANTI-OXIDANT ACTIVITY OF *TRIGONELLA FOENUM* GRAECUM SEED USING *IN VITRO* MODELS

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ABSTRACT

Trigonella foenum graecum, *(Fenugreek)* (Family: Leguminosae) is used as a traditional medicine for anti-inflammatory, antiseptic, antidiabetic and antilipidemic. Hydroalcoholic extract of its seeds were subjected for *in vitro* antioxidant activity by different methods viz, 1- diphenylpicryl- hydrazyl radical (DPPH), hydroxyl, and ABTS radical cation assay. Total antioxidant capacity, Total phenol and total flavonoid, Total ascorbic acid assay also were estimated. It was observed that free radicals were scavenged by the extract in a dose dependent manner in all the models The content of total phenolics (expressed as mg of gallic acid equivalents/gm) and total flavonoids (expressed as mg of quercetin equivalent/gm) and ascorbic acid were determined along with antioxidant enzymes. The Hydroalcoholic extract of *Trigonella foenum graecum*, exhibited potent DPPH and ABTS radical scavenging activity with IC_{50} values of 350µg/ml, and 962.5µg/ml, respectively. The seeds of fenugreek showed significant total antioxidant capacity with IC_{50} value of 192 µg/ml and hydroxyl radical with IC_{50} value of 587.5µg/ml, respectively. Based on the results it can be concluded that hydroalcoholic extract of *Trigonella foenum graecum* may have potential antioxidant effects against several oxidants.

INTRODUCTION: Plants are used as a source of medicine in the past centuries and today scientists and the general public recognize their value as a source of new or complimentary medicinal products. Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal products to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases of elderly people [1].

Plant extracts has been attributed to possess multi-potent anti-oxidant, anti-microbial, anti-cancer, anti-ulcerative and anti-diabetic properties. Generally, anti-oxidants have been identified as major health beneficial compounds reported from varieties of medicinal plants and are sources for alternative medicines [2]. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents [3]. The protective effect of plant products are due to the presence of several components such as enzymes, proteins, vitamins [4,] carotenoids [5], flavonoids [6], and other phenolic compounds [7].

Free radicals or reactive oxygen species (ROS) are formed in our body as a result of biological oxidation. The over production of free radicals such as hydroxyl radical, super oxide anion radical, hydrogen peroxide can cause damage to the body and contribute to oxidative stress [8, 9]. Oxidative damage of proteins, DNA and lipid is associated with chronic degenerative diseases including cancer, coronary artery disease, hypertension, diabetes etc [10] and compounds that can scavenge free radicals have great potential in ameliorating these disease processes [11-13].

Most of the reactive oxygen species are scavenged by endogenous defense systems such as catalase, super oxide dismutase and peroxides-glutathione system[14]. The researchers have focused on natural anti-oxidants and numerous crude extracts and pure natural compounds have been recognized to have beneficial effects against free radicals in biological systems as anti-oxidants [15-17].

Trigonella foenum graceum is a widely used in folk and Ayurvedic systems of medicine. *Trigonella foenum graecum* (Linn.) belonging to the family Papilionaceae commonly known as Fenugreek is a aromatic, 30-60 cm tall, annual herb, cultivated throughout the country (Kirtikar and Basu) ("The Ayurvedic Pharmacopoeia of India") [18, 19]. Flavonoids of fenugreek extract have been observed to possess anti-oxidant activity [20- 22].

The objective of the present study was to determine the anti-oxidant activity of *T. foenum graceum seeds* extract using standard methods.

MATERIALS AND METHODS:

Plant Material and Extraction procedure: The fenugreek seed sample was collected from the local market of Chennai, Tamil Nadu, India. The sample *T. foenum graecum* seeds were identified by Dr. Sasikala Ethirajulu, Assistant director pharmacology, Siddha central Research institute, Chennai. Dry fenugreek seed (1kg) was cleaned and ground into coarse powder. 80%Ethanol and 20% water were used for extraction method. The extracts were filtered. The residue was re-extracted twice under the same condition to ensure complete extraction. The extracts were evaporated to dryness under reduced pressure at 60^{o} C by a rotary evaporator and used for further study

Chemicals: Ascorbic acid, 1, 1- diphenyl- 2- picrylhydrazyl (DPPH), sulfanilamide, N-(1-napthal) ethylenediaminedihydrochloride, 2-deoxyribose and ferrous sulphate were purchased from Sigma Chemical Co., USA. All other chemicals used of analytical reagent grade.

Determination of Total phenol: The total phenolic content was estimated according to the method of Makkar *et al*., [23.] The total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of extract.

Assay of Total Flavonoid Content: Total flavonoid content was measured by the aluminum chloride colorimetric assay Zhishen *et al*., [24]. Total flavonoid content was expressed as mg quercetin equivalents (QE) / g seed.

DPPH Free Radical Scavenging System: The effect of plant extracts on DPPH radical was estimated according to the method of Blois [25]. The absorbance of the resulting solution was measured spectro photometrically at 520 nm.

Determination of Total Antioxidant Capacity: The assay is based on the reduction of molybdenum (VI) to molybdenum (V) by the extract and the subsequent formation of a green phosphate Mo (V) complex at acid pH Priesto *et al*., [26]. The antioxidant activity was Expressed as the number of equivalent of ascorbic acid.

Hydroxyl Radical-Scavenging Activity: Hydroxyl radical scavenging activity of extract was measured according to the method of Halliwell *et al*., [27]. The color development was measured of 532 nm against a blank Containing phosphate buffer.

ABTS Radicals Scavenging Assay: The radical scavenging activity of PMTP against ABTS was measured using the methods of Re etal.[28] and Zhao et al [29] with some modifications. Ascorbic acid was used as standard. ABTS radicals scavenging effect was calculated as follows:

ABTS scavenging effect (%) = (Ao-At)/Ao ×100

Estimation of Ascorbic Acid Content (AAC): The ascorbic acid content of the extract was determined according to the method of Sadasivam *et al*. [30.] The values are expressed in mg/ g of the sample.

RESULT AND DISCUSSION: The hydroalcoholic extract of *Trigonella foenum graecum* was also evaluated for their *in vitro* antioxidant activity using three different methods such as, 1- diphenylpicryl- hydrazyl radical (DPPH), hydroxyl, ABTS radical cation assay. It was observed that free radicals were scavenged by the extract in a concentration dependent manner in all the models. The hydroalcoholic extract of *Trigonella foenum graecum* exhibited potent DPPH and ABTS radical scavenging activity with IC50 values 350 µg/ml,

and 585.7 µg /ml respectively. *Trigonella foenum graecum* showed significant antioxidant activity against ABTS with IC50 values 962.5 µg /ml. All the experimental values were compared with standards. Among the four methods *Trigonella foenum graecum* was found to be more active in inhibiting ABTS free radical with IC50 values 962.5 µg /ml. These details at various concentrations for different methods are given in the **Table 1 & 2**.

TABLE 1:

Model	IC_{50} values of *T. foenum graecum* (µg/ml)	Maximum scavenging concentration (mg/ml)	Model	Maximum scavenging activity (%)	Maximum scavenging concentration (mg/ml)
DPPH	350	1	DPPH	82.05	1
Hydroxyl	585.7	0.5	Hydroxyl	67.02	0.5
ABTS	962.5	1.5	ABTS	68.01	1.5

TABLE 2:

Model	Concentration (mg/ g of extract)
Total phenolic content (TPC) gallic acid Eq)	0.876
Ascorbic acid (AAC)Ascorbic acid Eq)	542.857
Total flavonoid content (TFC)(Quercetin Eq)	0.489
Total antioxidant capacity (TAC Ascorbic acid Eq)	192

The quantitative analysis of the extracts showed the presence of high amount of total phenols (0.876mg/g), flavonoids (0.489 mg/g) and ascorbic acid 542.857mg/gm). The DPPH radical has been used widely to test the potential of the compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts. The DPPH free radical scavenging activity is due to the neutralization of DPPH free radical by extract either by transfer of hydrogen or of an electron [31].

The result of DPPH scavenging activity assay in this study indicates that the seeds were active against free radical scavenging. This suggests that the seed extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity.

The ability of this seed extract to scavenge DPPH could also reflect its ability to inhibit the formation of ABTS+. The scavenging activity of ABTS+ radical by the seed extract was found to be appreciable; this implies that the plant extract may be useful for treating radical related pathological damage especially at higher concentration [32].

Trigonella foenum graecum seed with antioxidant activities have been reported to possess free radical scavenging activity [33]. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism [34].

Antioxidants are the compounds which help to delay or inhibit the oxidation of lipids and other molecules through the inhibition of either initiation or propagation of oxidative chain reactions [35]. According to [36], antioxidants can act as either reducing agents, or by free radical scavengers or singlet oxygen quenchers. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [37, 38].

Free radicals are often generated as by products of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented by [39]. Medicinal plants can protect against harmful effects of ionizing radiation. Natural plant extracts or pure compounds are safe ingredients, which do not have any toxic effects. According to [40] plant extracts can be characterized by polyvalent formulations and interpreted as additive, or, in some cases, potentiating.

First, the therapeutic benefit of medicinal plants is usually attributed to their antioxidant properties and oxidative stress is a prominent feature of these diseases [41, 42].

CONCLUSION: This study suggests that the *Trigonella foenum graecum* seed extract has antioxidant activity, which may be helpful in preventing or slowing the progress of diseases involved as a result of oxidative stress.

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