



EVALUATION STUDY OF PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY IN FENUGREEK MICROGREENS (*Trigonella foenum-graecum*) METHANOLIC EXTRACT USING TLC ANALYSIS

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ABSTRACT:

According to Ayurveda plants have so many constituents which may be used for the treatment of so many diseases. Medicinal plants are of great importance to the health of individuals. Its various extracts possess reported number of pharmacological activities. Microgreens a hypothesized term used for the emerging food product that is developed from various commercial food crops, such as vegetables, grains and herbs. At present, studies have assessed the phytochemical composition and antioxidant activity of microgreens, mostly belonging to the Brassicaceae family and cultivated in the temperate areas. Microgreens, the tiny form of leafy vegetables are emerging as a culinary novelty ingredient. Fenugreek is easily available in our kitchen and use as a drug from oldest time. Fenugreek (*Trigonella foenum-graecum*) is a medicinal plant with numerous therapeutic benefits that belongs to the Fabaceae family. Fenugreek possess many pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anti-obesity, anti-arthritic and Gastroprotective properties. The methanolic extract of microgreens of fenugreek, shows the presence of high number of phytochemicals. It shows the presence of alkaloids, steroids, terpenoids, flavonoids, phenol, cardio glycosides, Saponins, and Tannins. TLC Analysis was carried out in the methanolic extract of Fenugreek sample was analysed

by TLC Plate has showed many variations. In this the Rf values was calculated and the peak values of the plant constituents was obtained to confirm the qualitative characterization of phytochemical screening and to determine the Purity and Quality analysis of the sample .

KEY WORDS: Fenugreek, *Trigonella foenum-graecum*, phytochemical , Tannins, Saponins, Flavonoids, Alkaloids, Quinones, Cardiac glycosides, Terpenoids, Phenols.

INTRODUCTION

In recent years, there has been a growing body of evidence showing edible greens to be a good source of dietary antioxidants. Consumption of dietary antioxidants is often associated with reduced risks of certain serious diseases. These dietary antioxidants include vitamin C, vitamin E, β -carotene, polyphenols and other bioactive components. Evidence from biochemical, clinical and epidemiological studies has suggested a dietary intake of 90–100 mg/day of ascorbic acid for the optimum reduction in the risks of chronic diseases. Another antioxidant present in high concentrations in green leafy vegetables is lutein, a lipid-soluble antioxidant pigment that protects against adverse effects of photochemical reactions.

The antioxidant activity of polyphenolic compounds has been of special interest as diets rich in polyphenols are associated with reduced risk of diseases. Chlorophylls are another major group of antioxidants in leafy vegetables and are reported to exhibit chemoprotective activity in carcinogenic conditions. (A. Vaishali, et al, (2006) .

Microgreens, the tiny form of leafy vegetables are emerging as a culinary novelty ingredient. They are young, tender greens having two fully developed cotyledon leaves with the first pair of true leaves emerging or partially developed. At present, studies have assessed the phytochemical composition and antioxidant activity of microgreens, mostly belonging to the Brassicaceae family and cultivated in the temperate areas. Phytochemical composition of four Brassicaceae microgreens and profiling of polyphenols of five Brassica microgreens species have been reported in earlier studies. Further (Xiao et al.) determined the phytochemical concentrations and antioxidant capacity in 30 microgreens belonging to the Brassicaceae family. (K.J. Hunter, et al, (2002).

Antioxidant properties of commercially grown broccoli microgreens have also been compared with farm-grown ones in another study. Recently, phytochemical composition of microgreens belonging to other families such as Chenopodiaceae, Malvaceae, Lamiaceae and Apiaceae have been evaluated by Kyriacou et al. and that from the Fabaceae family have been reported by Butkute et al, (2019).



Fig :Fenugreek Seed

Fenugreek (*Trigonella foenum-graecum*) is an annual plant belonging to the family *Fabaceae*. The green leaves and seeds of fenugreek are widely used in food and medicinal applications dating back to ancient times. However, the seeds are sour in taste due to the presence of bitter saponins, which limit their acceptability in foods. It has been possible to decrease the bitter taste of fenugreek seeds by using diverse household processes, such as soaking, germination, boiling, fermentation. Many extracts of each seed or leaf and its active components have been studied for their pharmacological effects and have been reported to have hypocholesterolaemic, antidiabetic, anti-inflammatory, antiulcer, analgesic, antipyretic, CNS-stimulant, antioxidant, wound healing and immune modulatory activity as well as gastro-protective and chemo-preventive activities (A.C. Carr, et al, 1999). Microgreens have piqued consumer interest, especially chefs' of high-end restaurants who use various microgreens, primarily as garnishing elements to enhance salads, soups, sandwiches, and other culinary inventories. However, due to their interesting quality traits, their use has been extended to enrich the diet of a particular group of demanding consumers. (K.B. Pandey, et al, 2009).



Fig:Microgreens of fenugreek

TAXONOMY:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: *Trigonella*Species: *Trigonella foenum-graecum*

MORPHOLOGY:

Microgreens are produced both at a small-scale level and along with large-scale production of commercial vegetables and edible flowers. Upon their meteoric rise and demand, various varieties of commonly grown vegetables were used to cultivate microgreens that belong to various families.

PHYTOCHEMICALS ANALYSIS

Phytochemicals are natural compounds that are found in plants and are responsible for many of the health benefits associated with a plant-based diet. These compounds are not considered essential nutrients, but they have been shown to have a positive impact on human health, including the prevention and treatment of chronic diseases such as cancer, heart disease, and diabetes (John T et al, 2013).

Some common types of phytochemicals include carotenoids, flavonoids, phenolic acids, and glycosylates. Phenolic acids, which are found in berries, nuts, and whole grains, have been shown to have anticancer and anti-inflammatory effects. Glucosinolates, found in cruciferous vegetables like broccoli and kale, are thought to have cancer-fighting properties (Teresa.MM.2011). Overall, phytochemicals are an important component of a healthy diet and can help to reduce the risk of chronic diseases when consumed as part of a balanced diet rich in fruits, vegetables, whole grains, and legumes (Andrzej KA 2011).

ANTIOXIDANT ACTIVITY

Antioxidants can protect against the cell damage that free radicals cause, known as oxidative stress. Trusted Source mitochondrial activity excessive tissue trauma due to inflammation and injury ischemia and reperfusion damage consumption of certain foods, especially refined and processed foods, trans fats, artificial sweeteners, and certain dyes and additives smoking environmental pollution radiation exposure to chemicals, such as pesticides and drugs, including chemotherapy industrial solvents ozone. Such activities and exposures can result in cell damage (Hamid AA ,et,al 2010)

THIN LAYER CHROMATOGRAPHY

TLC is an affinity-based method used to separate compounds in a mixture. TLC is a highly versatile separation method that is widely used for both Qualitative and quantitative sample analysis. TLC can be used to analyze virtually any substance class, including pesticides, steroids, alkaloids, lipids, nucleotides, glycosides, carbohydrates, and fatty acids. In TLC, the stationary phase is a thin adsorbent material layer, usually silica gel or aluminum oxide, coated onto an inert plate surface, typically glass, plastic, or aluminum. The sample is spotted onto one end of the TLC plate and placed vertically into a closed chamber with an organic solvent (mobile phase). The mobile phase travels up the plate by capillary forces and sample components migrate varying distances based on their differential affinities for the stationary and mobile phases. When the solvent reaches the top of the plate, the plate is removed from the developing chamber and dried. The separated components appear as spots on the plate and the retention

factor (Rf) of each component is assessed.

MATERIALS AND METHODS



Fig:FENUGREEK SPROUTS



Fig:FENUGREEK GREENS

PREAPARTION OF SAMPLE

Sample Growth and Preparation of Fenugreek (*Trigonella foenum-graecum* L.) microgreens were grown organically using vermin compost enriched soil under ambient conditions in a polyshade house. A 25g sample of fenugreek seeds (local market, Chennai) was soaked in potable water for 8 hours and sown in furrows of even depth (1inch) in soil filled plastic trays. Trays were kept in darkness in a high moisture (Relative humidity - $90 \pm 5\%$) tunnel located within the polyshade house at a temperature of $30 \pm 5^\circ\text{C}$ during the first 48 hours of germination.



Fig:AIR-DRIED FENUGREEK LEAVES

After germination, the trays were exposed to ambient light and watered thrice daily. Microgreens were harvested after 7-8 days at a height of 2-3 inches by cutting the mat the soil surface using a sharp sterile scissor. They were inspected for defects and unusable stems and leaves were discarded. Fenugreek Mature Leaves (FML), grown under the same conditions as microgreens, were harvested after 20 days of growth. The roots were excised, defected parts removed and the remaining stems and leaves were cleaned from any contaminating soil particles. Fresh and healthy leaves of fenugreek (*Trigonella foenum-graecum* Linn.) were collected. The leaves were washed thoroughly with tap water, and the surface water

has removed by air-drying under shade for 15 days. The leaves have subsequently dried in a hot air-oven at 50 °C for 4 h, homogenized to a fine powder and then stored at 4 °C.

Fig:DRIED FENUGREEK LEAVES **Fig:FENUGREEK GREENS POWDER**



EXTRACTION PROCEDURE OF SAMPLE

One hundred grams of each sample were extracted with 1 L (1:10w/v) % methanol for 24 hours at 220 rpm in orbital shaker. After filtration, extract condensed to dryness (resulting in crude extracts) using a rotary evaporator at 40 °C. The obtained residue has collected to calculate the yield and finally stored in the freezer for further biochemical and in-vitro biological analyses.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The qualitative analysis for the presence or absence of various bioactive compounds is commonly known as phytoconstituents was analyzed using standard protocols. By adopting the method of Harborne, (1999) and Jagadeesan et al. (2014) the following tests were carried out to identify the metabolites.

1. Test for Alkaloids

100 mg of the extract was dissolved and filtered in DMSO and 1ml of the filtered sample was added with 5ml of 1% hydrochloric acid followed by the addition of 2 to 3 drops Dragendorff's reagent resulting in the formation of Orangered precipitate that confirms the presence of alkaloids.

2. Test for Flavonoids

Microgreen extracts were dissolved in methanol on mild heating, a small piece of magnesium ribbon was added to each extract followed by the addition of few drops of Concentrated sulphuric acid. The color changes from orange, pink and red to purple indicating the presence of flavonoids.

3. Test for Alkaloids

100 mg of the extract was dissolved and filtered in DMSO and 1ml of the filtered sample was added with 5ml of 1% hydrochloric acid followed by the addition of 2 to 3 drops Dragendorff's reagent resulting in the formation of Orangered precipitate that confirms the presence of alkaloids.

4. Test for Alkaloids

100 mg of the extract was dissolved and filtered in DMSO and 1ml of the filtered sample was added with 5ml of 1% hydrochloric acid followed by the addition of 2 to 3 drops Dragendorff's reagent resulting in the formation of Orangered precipitate that confirms the presence of alkaloids. t for Alkaloids

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5. Test for Flavonoids

Microgreen extracts were dissolved in methanol on mild heating, a small piece of magnesium ribbon was added to each extract followed by the addition of few drops of Concentrated sulphuric acid. The color changes from orange, pink and red to purple indicating the presence of flavonoids.

6. Test for Tannins

200mg of extract was dissolved in 10 ml of distilled water and filtered. 2ml of filtered extracts were taken in a test tube and few drops of 1% alcoholic ferric chloride solution was added to the tubes and formation of blue green precipitate confirms the presence of tannins.

7. Test for Steroids

100mg of extract was dissolved in equal volume of acetic acid and chloroform mixture and the mixture was cooled for few minutes and the addition of few drops of concentrated sulphuric acid results in the formation of reddish brown or violet-brown ring indicating the presence of steroids.

8. Test for Terpenoids

500mg of extracts was dissolved in ethanol. Equal volume of extracts and acetic anhydride solutions were taken in a test tube and few drops of concentrated sulphuric acid was added to the tubes. The colour changes from pink to violet indicating the presence of terpenoids or the formation of blue green ring confirms the presence of terpenoids.

9. Test for Saponins

Vigorous shaking of 1ml of each extract in 3 to 5 ml distilled water for 10 minutes exhibit persistent foam for a period of one minute indicating the presence of saponins.

10. Cardiac glycosides (Keller-Killani test)

3ml of extracts were treated with 2ml of glacial acetic acid and few drops of 1% ferric chloride solution followed by 1ml of concentrated sulphuric acid and the brown coloured ring at the edges confirms the presence of cardiac glycosides.

THIN LAYER CHROMATOGRAPHY PRINCIPLE

Thin layer chromatography (TLC) is a type of planar chromatography. It is a convenient method for separation of many compounds based on polarity, binding tightly to the adsorbent and migrating less than others (Stahl, 1969). TLC is a chromatographic technique which is applied for the separation of mixture of compounds.

REAGENTS REQUIRED

TLC (Merck), all solvents used were of analytical grade.

PREPARATION OF SAMPLE

3 grams of methanol plant leaf extracts of microgreens was dissolved separately in the respective solvent and were used in the present study.

SOLVENT SYSTEM (MOBILE PHASE)

For extracts, different solvent systems were developed to run the TLC. The solvent system was made from methanol: chloroform: ethyl acetate and water in the ratio of 1:1.2:0.8:0.1 (v/v) for methanol.

PREPARATION OF TLC PLATE

The sterile silica coated plates with the size range of 2cm x 6cm were prepared and the bottom of the sheet is marked with 1cm margin for the application of sample.

The extract was spotted on the sheets separately with the help of capillary tubes and they were allowed to dry before introducing them into the running chamber where the respective mobile phase is present. The glass jars marked with specific label are filled with respective mobile phase upto 1cm height from the bottom and the chambers were closed to prevent the evaporation of solvents.

DEVELOPMENT OF TLC PLATES

With the help of sterile forceps, TLC plates were prepared and kept inside the saturated TLC chamber with the tops leaning against the chamber walls. The movement of solvents was continuously monitored. The plates were removed from chamber with forceps after the solvent reached the top of the plates and the solvent front was marked and dried.

The TLC plates were visualized for band formation with a portable UV lamp and the R_f values were calculated by measuring the distance moved by the solvent and distance moved by solute using a scale. Calculation R_f value can be calculated using the formula

$$\text{Distance moved by spot front} / \text{Rf} =$$

$$\text{Distance moved by solvent front}$$

ANTIOXIDANT ACTIVITY

DPPH Assay (2,2-diphenyl-1-picrylhydrazyl)

The antioxidant assay was estimated using DPPH as a free radical.

PRINCIPLE

2, 2- diphenyl 1- picrylhydrazyl is a stable free radical and synthesized nitrogen centered radical with three benzene rings. The paired function was lost by unpaired electron of centered nitrogen.

The scavenging reaction between antioxidant(H-A) and DPPH was written as, (DPPH) + (H-A) DPPH-H + (A)

The various concentration of plant extracts were taken from 20 g/ml to 200 g/ml and make upto 40 ul with DMSO. Then, 2.96 ml of DPPH solution (0.1mM) was added to the mixture and kept for incubation at dark room for 20min. After incubation, the optical densities were read at 517nm. For control, 3ml of DPPH was taken for the reaction. Radical scavenging activity was calculated using the below formula $\frac{\text{Abscontrol} - \text{Abssample}}{\text{Abscontrol}} \times 100$ Abs .

RESULTS

50 g of plant material extracted using cold maceration methods about 13.05 g of extract obtained after extraction.

PHYTOCHEMICAL ANALYSIS

Quantitative estimation of flavonoids, phenols and alkaloids were estimated using standard protocols and the units were represented respectively.



Fig: Qualitative analysis of phytochemicals present in methanol extract.

Table.1:Qualitative analysis of phytochemicals present in methanol extract.

S. no	Phytochemicals	Results
1	Alkaloids	(++)
2	Flavonoids	(+++)
3	Tannin	(+)
4	Glycosides	(+)
5	Cumarine	(+)
6	Terpenoids	(++)
7	saponins	(-)
8	Phenols	(+++)

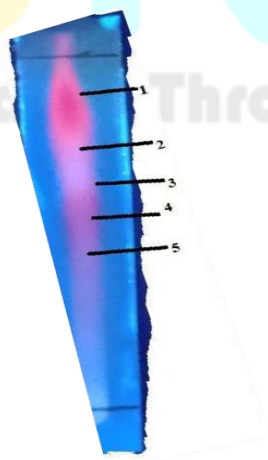
Table.2: Total phenolic, alkaloids and flavonoids of extracts of microgreen leaves

Extract	Flavonoids	TotalPhenolic	Alkaloids
Methanol extract	15.62 (mg/QE/g)	19.26content (mg/GAE/g)	11.32(mg/AT/g)

It is observed from the table 2 that the total phenolic content amounts to 15.62mg/GAE/g, in methanol extracts of leaves of microgreen. The analysis of table 2 also reveals the quantitative presence of flavonoids and the units were represented by 19.26 mg/AT/g. The presence of total alkaloids and presented in table-2 and the units were represented as 11.32mg/AT/g. The standard Atropine was used in the present study.

THIN LAYER CHROMATOGRAPHY

The methanol extract of microgreen shows the presence of five different bands when separated using Hexane: Ethyl acetate: Toluene solvent system, the fractions were identified with the R_f values of, 0.705, 0.696, 0.818, 0.848 and 0.909.

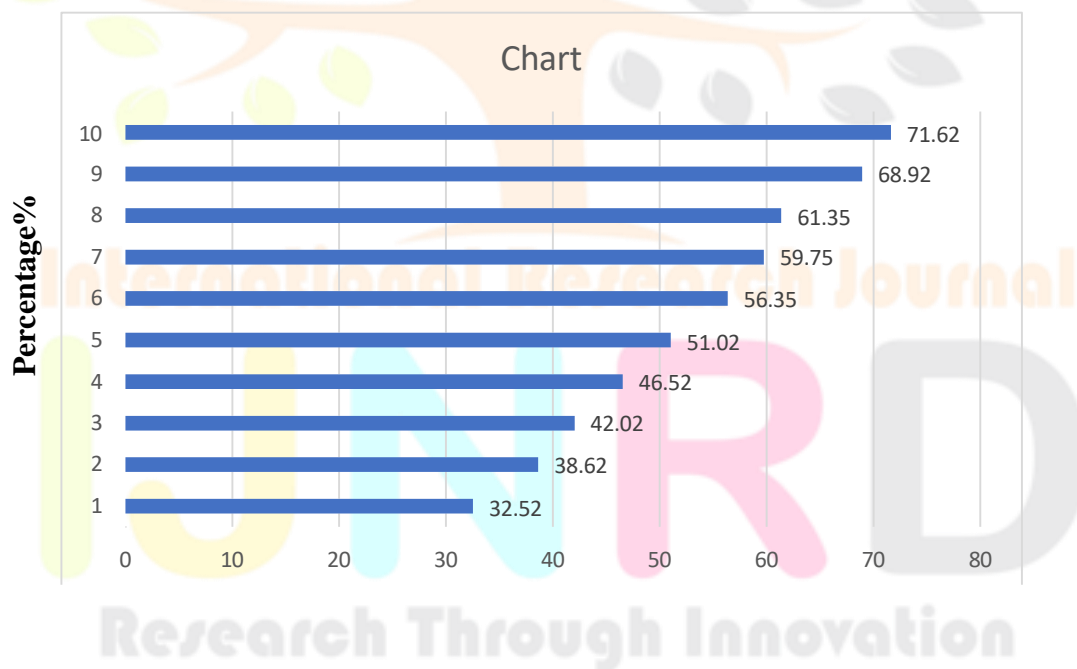


S.No	Solvent	Rf
1	Hexane:Ethyl acetate: Toluene	0.705,0.696, 0.818, 0.848 0.909

ANTIOXIDANT ACTIVITY

DPPH ASSAY

S.No	Concentration	Percentageofinhibition
1	10 µg/ml	32.52
2	20 µg/ml	38.62
3	30 µg/ml	42.02
4	40 µg/ml	46.52
5	50 µg/ml	51.62
6	60 µg/ml	56.35
7	70 µg/ml	59.75
8	80 µg/ml	61.35
9	90 µg/ml	68.92
10	100µg/ml(control)	71.62



Concentration

DISCUSSION

Plants have been used for medicinal purpose long before prehistoric period these medicinal plants are also used as foods and medicines. The qualitative phytochemical analysis of the fenugreek is summarized in the Quantification of important phytochemicals. The methanolic extract of microgreens of fenugreek, shows the presence of high number of phytochemicals. It shows the presence of alkaloids, steroids, terpenoids, flavonoids, phenol, cardio glycosides, Saponins, and Tannins. The microgreens of fenugreek shows the presence of terpenoids and they play a major role in the treatment of Anti-inflammatory activity. The microgreens of fenugreek show positive result for Flavonoids which can act as antioxidants. Phytochemicals have highest therapeutic efficiency in pharmaceutical field it helps to undertake further studies on isolation and identification of specific phytochemicals for pharmacological studies. Microgreens of fenugreek is rich Source of Antioxidants. This study shows that fenugreek Extract had the highest antioxidant activity. TLC is done in the microgreens of fenugreek to verify the presence of Flavonoids.

CONCLUSION

The present research work was aimed to do phytochemical screening. Antioxidant activity on the methanolic extract of microgreens of fenugreek leaves. The qualitative analysis showed the presence of Alkaloids, Flavonoids, Tannins, Cardiac Glycosides, saponins and Terpenoids. In quantitative analysis the microgreens of fenugreek shows highest flavonoids content. The microgreens of fenugreek has Antibacterial agents in the treatment of infectious organism, microgreens of fenugreek has its unique therapeutic efficacy. The plant could be utilized as an alternative source of useful antimicrobial drugs. The methanolic extract of microgreens of fenugreek frequently used as a natural remedy for many diseases. Phytochemicals have highest therapeutic efficiency in pharmaceutical field it helps to undertake further studies on isolation and identification of specific phytochemicals for pharmacological studies.

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