Research Article

Phytochemical Screening and *in-vitro* Anti-inflammatory Activity of *Trigonella foenum-graecum* Leaves Extracts

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ABSTRACT

Phytochemicals are the dependable sources for the treatment of different health problems. Phytochemical screening is a process of identifying the presence of a certain phytochemical constituents in a plant. This process can give scientists the knowledge of the desirable constituents in plants. It tells not only the presence of therapeutic agent but also gives information on the presence of commercially important compounds such as tannins, oils, gums, precursors for the synthesis of complex compound etc. In the present work phytochemical screening of the extracts of *Trigonella foenum-graecum leaves* was carried out which revealed the presence of flavonoids, saponins, tannins, alkaloids, resins and glycosides. Methanolic extract showed the presence all the secondary metabolites screened. The anti-inflammatory activity of various extracts of *Trigonella foenum-graecum* leaves by *in vitro* HRBC membrane membrane stabilization method was also assessed. The percentage of membrane stabilisation for six different extracts and diclofenac sodium were done at a concentration of 2 mg/ml. Methanolic extract showed the maximum membrane stabilization activity and was found to be 72.22±1.11 %. Therefore, our studies support the isolation and the use of active constituents from *Trigonella foenum-graecum* leaves in treating inflammation.

Keywords: *Trigonella foenum-graecum,* Phytochemicals, inflammation.

INTRODUCTION

Traditional system of medicine continued to be widely practiced. Global estimate indicates that 80% of about 5 billion population cannot afford the products of the western pharmaceutical industry but they offered the uses of traditional medicines which are mainly derived from plant materials. In this modern world, now a day's plant based drags are widely used and many countries contributes 40-50% of their total, health budget in the population of novel drugs[1]. Phytochemicals are present in a variety of plants, and are utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables[2]. Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "Secondary metabolites" [3].

Membrane stabilization is a process of maintaining the integrity of biological membranes such as erythrocyte and lysosomal membranes against osmotic and heat-induced lyses[4, 5]. Biological membranes forming closed boundaries between compartments of varying composition consist mainly of proteins and lipids. They are asymmetric, fluid structures that are thermodynamically stable and metabolically active. The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of extracts on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane[6]. Temperature, ionic environments and fatty acid compositions of phospholipids and glycolipids and presence or absence of cholesterol can affect the general physical state of biological membrane. Presently many membrane stabilizers (enfenamic acid, phenyl butazone etc.,) and destabilizers (Vitamin A, bile salts etc.,) have been identified.

The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair which are aimed at host defense and usually activated in most disease condition. Currently much interest have been paid in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response in amplifying the disease process. Inflammations can be uncomfortable and painful. But using natural remedies for inflammation is much better than using any kind of chemical drugs to suppress the symptoms. This is because, like other symptoms, such as fever, they are actually a sign of the body healing itself of a problem, for example an infection. An area of the body is inflamed because the immune system is sending additional "combat forces" there to try to fight the enemies, so to speak. By using artificial and chemical means to suppress an inflammation, we are directly interfering with the body's attempts to tackle and repair the problem which it is facing. Such attempts to merely treat symptoms, without considering the deeper underlying issues and attempting to achieve true and ultimate healing, is a problem not just unique to anti-inflammatory drugs, but to chemical drugs in general [7].

Trigonella foenum-graecum L. (Legominosae) is one of the oldest medicinal plants. It is known commonly as fenugreek. Fenugreek is a legume, originally from

southeastern Europe and western Asia, but grown now mainly in India and also in certain parts of Asia, northern Africa, Europe and the United States[8]. It is known as methi (Hindi) in India and also as Fenugrec (France), Bockshorklee (Germany), Koroha (Japan), Hulba (Arab), Pazhitnik (Russia) and Ku-Tou (China). The seeds find extensive use in Indian cuisine to flavour many foods including curry powders, spice blends and tea. Its leaves are also used as a green leafy vegetable in the diet. Fenugreek seeds are aromatic but bitter with carminative, galactogouge, antibacterial and antiviral properties. The seeds contain a central hard yellow embryo surrounded by a corneous and comparatively large layer of white, semi-transparent endosperm[9]. Rheological properties and emulsions of galactomannans of fenugreek seed endosperm are reported[10]. Fenugreek seeds are traditionally used for the treatment of many diseases. Studies show that the seeds have antioxidant properties. Many medicinal properties are attributed to fenugreek seeds and leaves [11]. Fenugreek is known to have several pharmacologicalattributes such as hypoglycaemic [12, 13], hypercholesterolaemic [14], gastroprotective [15], chemo-preventive [16], antioxidant [17, 18], laxative and appetite stimulation [19]. The aim of this study was to evaluate the anti-inflammatory activity of *Trigonella foenum-graecum* leaves extracts by Human Red Blood Cell (HRBC) membrane stabilization method with preliminary phytochemical screening of the extracts.

MATERIALS AND METHODS

Preparation of plant extract

The whole plant of *Trigonella foenum graecum* was collected from Kholli hills. Leaves were taken for investigation. The leaves were shade dried, coarsely powdered and was extracted with solvents of increasing polarity viz., Petroleum Ether, Benzene, Chloroform, Ethyl acetate, Methanol and double distilled water.

Air dried powder was macerated with 100 ml of various solvents and stored for 72 hrs in ice cold condition. After 72 hrs the extracts were filtered through a Whatmann filter paper No. 42 (125 mm) and the organic layer was allowed to evaporate. The resulted dark green extracts were concentrated using a rotary evaporator with a water bath set at 40°C. The concentrated crude extracts were lyophilized into paste and used for further analysis.

Screening for secondary metabolites [20]

(a) Test for Alkaloids

(i) Dragendroff's test:

8g of Bi (NO3 .5H2 O was dissolved in 20 ml of Nitric acid and 2.72g of potassium iodide in 50 ml of water. They were mixed and allowed to stand, when potassium nitrate crystallizes out. The supernatant was decanted off and made up to 100ml with distilled water. The alkaloids were regenerated from the precipitate by treating with sodium carbonate followed by extraction of the liberated base with ether.

To 0.5 ml of extract, added 2.0 ml of HCl. To this acidic medium 1 ml of reagent was added. An orange red precipitate produced immediately indicates the presence of alkaloids.

(ii) Wagner's test

1.0 g of iodine and 2.0 g of potassium iodide were dissolved in 5 ml sulphuric acid and solution was diluted to 100 ml.

10 ml extract was acidified by adding 1.5% v/v HC1 and a few drops of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloids.

(iii) Meyer's

1.36 g Mercuric chloride was dissolved in 60 ml of distilled water and 5 g of potassium iodide in 10 ml of water. The two solutions were mixed and diluted to 100 ml with distilled water. To 1 ml of extract, few drops of reagent were added. Formation of white or pale precipitate showed the presence of alkaloids.

(b) Test for flavonoids

In a test tube containing 0.5ml of the extract, 5-10 drops of dilute hydrochloric acid and small piece of Zn or Mg was added and the solution was boiled for few minutes. In the presence of flavonoids, reddish pink or dirty brown colour was produced.

(c) Test for Tannins

To 1.2 ml of the extract, few drops of 1% solution of lead acetate was added. A yellow or red precipitate was formed, indicating the presence of tannins.

(d) Test for saponins

In a tube containing about 5.0 ml of extract, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 minutes. A honey comb like froth was formed and it showed the presence of saponins.

(e) Test for Glycosides

A small amount of extract was dissolved in 1.0 ml of water and then aqueous sodium hydroxide solution was added. Formation of a yellow colour indicates the presence of glycosides.

(f) Test for Resins

To 2.0 ml of extract, 5-10 ml of acetic anhydride was added, dissolved by gentle heating, cooling and then 0.5 ml of sulphuric acid was added. A bright purple colour was produced. It indicates the presence of resins.

(g) Test for Thoils

To about 0.5 ml of the extract enough ammonium sulphate was added to saturate the solution. 2-4 drops of 5% sodium nitroprusside was then added followed by one or more drop of concentrated nitric acid. Magenta colour was developed. This shows the presence of thiols.

(h) Test for Steroids

To 1.0 ml of extract, 1ml of concentrated sulphuric acid was added followed by the addition of 2.0 ml of acetic anhydride solution. A greenish colour developed and it turned blue. It indicates the presence of steroids.

Anti-inflammatory activity

Human Blood

The blood was collected from a healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and collected in heparinzed vacutainer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 minutes at 3000 rpm. The packed cells were washed with 0.9% saline and a 40% v/v suspension made using isotonic phosphate buffer which was composed of 154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4 used as Stock erythrocyte or RBC suspension.

Hypotonic solution –induced haemolysis or membrane stabilizing activity[21]

This test was done according to the method described with slight modifications. The test sample consisted of stock erythrocyte (RBC) suspension 0.030ml mixed with 5ml of hypotonic solution (154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4) containing Herbal Preparation (HP-4) ranging from concentration 100-500 µg/ml. The control sample consisted of 0.030ml RBC suspension mixed with hypotonic buffered solution alone. The standard drug acetylsalicylic was treated similar to test at 100 and 200 µg/ml concentrations. The experiment was carried out in triplicate. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

% Inhibition of haemolysis = 100 x [A 1 –A 2 /A 1]

Where: A 1 = Absorbance of hypotonic buffered solution alone

A 2 = Absorbance of test /standard sample in hypotonic solution.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Scientific analysis of plant component follows a logical pathway. Initial screening of plants for any activities begins by preparing the crude extracts. The preparation of extract is the most important step in the process of drug development from medicinal plant. There is a need to choose different solvent with varying polarity to prepare the extract so as to dissolve the different phytochemical present in the plant. Based on the above reasons and on the generally accepted concepts of phytochemical research six solvents of varying polarity namely Petroleum ether, Benzene, Chloroform, Ethyl acetate, Methanol, Water were used for the preparation of extracts. The phytochemical screening of the drug is a very sensitive aspect in the process of standardization and quality control because the constituents vary quantitative and qualitatively not only from plant to plant but also in different samples of the same species depending upon various atmospheric factors on storage condition[22] . The preliminary phytochemical screening of all six extracts shown the presence and absence of phytoconstituents according to the polarity of the solvent used for extraction. The results are shown in the Table: 1

Table 1: Preliminary phytochemical screening of *Trigonella foenum graecum linn* leaves

Solvents Phytoconstituents	Petroleum ether	Benzene	Chloroform	Ethyl acetate	Methanol	Double distilled water
Alkaloid	+	+	+	+	+	+
Flavonoid	+	-	+	-	+	+
Tannin	+	-	+	+	+	+
Saponin	+	+	+	+	+	-
Glycosides	+	+	-	+	+	+
Resin	-	-	-	-	+	-
Thiols	+	+	+	+	+	+
Steroid	+	+	+	+	+	-

(+) Presence of constituents; (-) Absence of constituents

Alkaloid found to be present in all the extract of *Trigonella foenum graecum* linn. Saponin present in all the extracts except distilled water, while tannin is present in all the extract except benzene. All the extracts showed positive results for glycosides except chloroform. The methanolic extract accounted for the presence of all the secondary metabolites screened viz. Flavonoids, Alkaloids, Tannins, Steroids, Saponin, Glycosides, Resins and Thiols. Steroids are present in all the extracts except distilled water.

The potential anti-inflammatory activity of *Justica gendarussa* was found to be due to the presence of compounds such as flavonoids and steroids[23]. The analgesic, anti-inflammatory and antipyretic effect of

Andrographis echioides have been attributed to presence of flavonoids [24]. There seen a positive correlation between the presence of polyphenols and anti-inflammatory activity of Syzygium cumini plant extract [25]. The latex of *Calotropis procera* containing tannins showed potent anti-inflammatory effects [26]. Resins, flavonoids and terpenoids containing plant extracts are known to produce anti-inflammatory and analgesic effects. Condensed tannin and polysaccharides are known for the anti-inflammatory effects of *Rumex acetosa* and *Rumex patientia* [27]. The qualitative analysis results of *Trigonella foenum-graecum* revealed the presence of number of secondary metabolites that is considered to be good candidate to pose therapeutic value of the plant selected for the present study. It holds very high effective medicinal properties and therapeutic application for curing different diseases and disorders.

Effect on erythrocyte membrane stability

The result of membranes stabilizing profiles for the various extracts of *Trigonella foenum-graecum* leaves on red blood cell exposed to hypotonic induced lyses provided in the Table 2. The methanolic extract exhibited a maximum membrane stabilization activity of 72.22% while chloroform extract showed the least activity i.e., 35.19%

The erythrocyte membrane resembles to lysosomal membrane and as such the erythrocyte could be extrapolated to the stabilization of lysosomal membrane [6] .The vitality of cells depends on the integrity of their membranes, exposure of RBC's to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin. An injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical induced lipid peroxidation[28, 29]. Phytochemical screening of methanolic extract revealed the presence of all the secondary metabolites screened. It is therefore expected that phytochemicals synergistic action as in *Trigonella foenum graecum* with membrane – stabilizing properties, should offer significant protection of the cell membrane against injurious substances [30, 31]. Compounds with membrane –stabilizing properties are well known for their ability to interfere with release of phospholipases that trigger the formation of inflammatory mediators[32]. Some of the NSAIDs are known to posses' membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is not known yet, hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components [33]. It was noted in this study methanolic extract showed highest membrane stability when compare to other extracts. This implied that the membrane stabilizing activities of this extract was aided by the presence of all secondary metabolites. On the basis of these results, it could be inferred that the extracts of *Trigonella foenum-graecum* contained principles that were capable of stabilizing red blood cells membranes against hypotonic- induced lyses. The plant leaves therefore could be regarded as a natural source of membrane stabilizers and was capable of providing an alternative remedy for the management and treatment of inflammatory related disorders and diseases.

Table 2: Effect of various extracts of *Trigonella foenum graecum linn* leaves on hypotonic solution induced hemolysis of erythrocyte membrane

Treatment	Concentration (mg/ml)	% Inhibition of haemolysis *
Petroleum ether	100 mg/ml	59.26±1.01
Benzene	100 mg/ml	68.52±0.09
Chloroform	100 mg/ml	35.19±0.81
Ethyl Acetate	100 mg/ml	53.07±0.21
Methanol	100 mg/ml	72.22±1.11
Distilled water	100 mg/ml	42.59±1.19
Diclofenac Sodium	100 mg/ml	74.07±0.56

*****Each value represents the mean ± SD*

CONCLUSION

Every plant had specific constituents which we can define it by phytochemical screening process. Medicinal plants are an important resource to traditional society's health care systems. In today's world the percentage of people using chemicals and drugs are increasing with their side effects. "The boon given to our earth is the herbs", which needs to be utilized in sustainable manner. Many of today's drugs are derived from plant sources. Plants have been used in medicine throughout the world and still continue to occupy an important place in traditional as well as modern system of medicine. A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant and anticancer properties. One such medicinal plant is *Trigonella foenim-graecum.* Phytochemical Screening revealed the presence of various medicinally important phytoconstituents in this plant. Methanolic extract of *Trigonella foenim-graecum* showed positive for all the phytochemical screened. Stabilization of the HRBCs membrane by hypotonicity induced membrane lysis was studied to establish the mechanism of anti-inflammatory action of *Trigonella foenum graecum.* Therefore, our present *in vitro* study on *Trigonella foenum graecum* extracts demonstrates the depression of inflammation. Due to the presence of active principles such as flavonoids and related polyphenols may responsible for this activity. Hence, *Trigonella foenum graecum* leaves can be used as a potent anti inflammatory agent.

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