



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 2213-2221
Received: 18-03-2019
Accepted: 19-04-2019

Divya KS
Department of Studies in
Microbiology, Pooja Bhagavat
Memorial Mahajana PG Centre,
Mysuru, Karnataka, India

Harshitha BS
Department of Studies in
Biochemistry, Pooja Bhagavat
Memorial Mahajana PG Centre,
Mysuru, Karnataka, India

Dilip Kumar R
Department of Studies in
Biochemistry, Pooja Bhagavat
Memorial Mahajana PG Centre,
Mysuru, Karnataka, India

Jyoti Bala Chauhan
Department of Studies in
Biochemistry, Pooja Bhagavat
Memorial Mahajana PG Centre,
Mysuru, Karnataka, India

In vitro investigation of antioxidant potentiality of methanol and silver nanoparticles extract from *Trigonella foenum-graecum*

Divya KS, Harshitha BS, Dilip Kumar R and Jyoti Bala Chauhan

Abstract

Trigonella foenum-graecum (Fenugreek) is an important spice, its dried seeds have wide application in food and beverages as a flavoring additive as well as in medicines. Crude extracts of fenugreek were prepared by soxhelt extraction method with methanol solvent. Methanol extracts and silver nanoparticle extracts were subjected for the measurement of phytochemical assays, total phenolic content by Folin-Ciocalteu method as well as flavonoid content, chelating activity, reducing power and antioxidant/radical scavenging activity [1,1-diphenyl-2-picryl-hydrazyl]. Results from these different parameters were in agreement with each other. The results revealed that the extracts of the fenugreek contains alkaloids, saponins, phytosterols, tannins, flavonoids, diterpens and exhibit antioxidant activity. These findings suggest that the fenugreek extracts could act as potent source of antioxidants. The anti-inflammatory effect of the silver nanoparticle and methanol extract of *Trigonella foenum-graecum* was investigated and even antimicrobial activity also was checked against six different strains of bacteria. Using these parameters antioxidant activity was assessed and the result revealed that fenugreek seed extract showed a inhibition of DPPH in lower concentrations.

Keywords: *Trigonella foenum-graecum*, silver nanoparticle, methanol extracts, antioxidant potential

Introduction

Fenugreek seeds are an annual legume crop mainly grown for use as a spice in many parts of the world. Scientific name of fenugreek seeds or methi seeds is *Trigonella foenum-graecum*, is known as one of the oldest medicinal plants. It is reported to have several compounds like alkoids, saponins, steroids, tannins, flavoids, amino acids and trigonillin. The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline (Umesh *et al.*, 2013) [12].

Previously, it was reported that fenugreek seeds possesses anticarcinogenic activities, hypocholesterolemic activities, hypoglycemic activities, antioxidant, antineoplastic agent, influence on enzymatic activities such as key enzymes in carbohydrate and protein metabolism, immunomodulatory effect, diabetes management, antiulcer, antihelminthic activity, it influences the digestive key enzymes. In pharmaceutical industry, commonly it is used as food stabilizer, food adhesive, food emulsifier and gum (Meghwal *et al.*, 2012).

Nanomedicine has become a leading research field. Scientists square measured concerned in synthesizing safe, effective, and most of all cheaper and less toxic drugs to combat diseases like diabetes, cancer and epilepsy. These nanoparticles have a site specific action attributable to which only a secure and a prescribed dosage of drug molecules have to be compelled to be administered and therefore helps in reducing the undesired toxicity (Roy *et al.*, 2015) [7]. These nanoparticles due to their targeted action increase the efficacy of the drug. Their small size gives them an edge where as evading the immune responses and also gives them the ability to cross relatively impermeable membranes (NethraDevi *et al.*, 2012) [5].

Nanotechnology is an achievement and has importance in a variety of fields such as food, health care and feed, cosmetics, biomedical science, environmental health, chemical industries, drug and gene delivery, power science, electronics, mechanics and space industries (Hassan Korbekandi *et al.*, 2012). It also has extensively been achieved for the treatments of diabetes cancer, allergy, inflammation and infection (Brigger *et al.*, 2012) [2].

The problem of drug resistance in microbes is increasing day by day (Teshager *et al.*, 2000) [9]. Continuous and overuse of synthetic antibiotics often produce tolerance in human and create resistance in microbes for a particular antibiotic. Being synthetic antibiotics, they have a number of side effects over agents of natural origin. So, researchers strive to find out a drug that could be therapeutically efficacious towards a wide range of microorganisms with

Correspondence

Divya KS
Department of Studies in
Microbiology, Pooja Bhagavat
Memorial Mahajana PG Centre,
Mysuru, Karnataka, India

minimal or no side-effects (Shrikanth *et al.*, 2015) [8]. The alarming increase in the rate of infectious diseases with antibiotic resistant microorganisms and side-effects of some synthetic antibiotics has led to an increasing interest towards medicinal plants as a natural alternative to synthetic drugs (Raghunath, 2008) [6].

The constituents in fenugreek that are thought to be responsible for its hypoglycemic effects include the testa and endosperm of the defatted seeds called the A subfraction, the 4-hydroxyisoleucine and the fibre. It is also thought that the saponins in the seeds are transformed in the gastrointestinal tract into saponinins and this is responsible for the lipid lowering effects. The fenugreek extract contains α -amylase inhibitory factors which probably interact with the active sites of the enzyme in a substrate specific manner. To inhibit the growth of *Pseudomonas* sp., *E. coli*, *Shigella dysenteriae* and *Salmonella typhi*, fenugreek is effective (Thirunavukkarasu *et al.*, 2003) [10].

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane *et al.*, 1995) [13]. It is a complex process, which is frequently associated with pain and involves occurrences such as the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy *et al.*, 2010) [11].

Materials and Methods

Seed extraction: The fenugreek seeds of about 250 gm were

taken and washed with distilled water and dried well in sunlight for 24 h. After drying, the seeds are grinded to get a fine powder. The fenugreek powder is subjected to extraction procedure.

Soxhlet extraction: Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. The fenugreek seed powder is subjected to soxhlet extraction procedure and methanol is used as solvent. Further, phytochemical analysis is done.

Synthesis of silver nanoparticles: 2 gm of fenugreeks seeds are soaked in 50 ml of deionised water for 24 h and filtered using whatman paper and then it is subjected to centrifugation at 2000 rpm for 20 min.

Firstly, in a 50 ml beaker, aqueous AgNO_3 solution of 10^{-3} M concentration was prepared in 25 ml of deionised Millipore filtered water. Secondly, 2.5 ml of concentrated fenugreek seeds extract was added slowly dropwise into the AgNO_3 solution and kept at room temperature for (7 h, 24 h, 7days and 21days) chemical reduction. During the chemical reduction, Ag^+ ions are converted into Ag nanoparticles. There is an electron transfer from the solution to the electrode, and this behaviour changes the electrical conduction of the colloid. However, reverse occurs in oxidation process, where electrons get lost during reaction (Figure 1a, b and c).

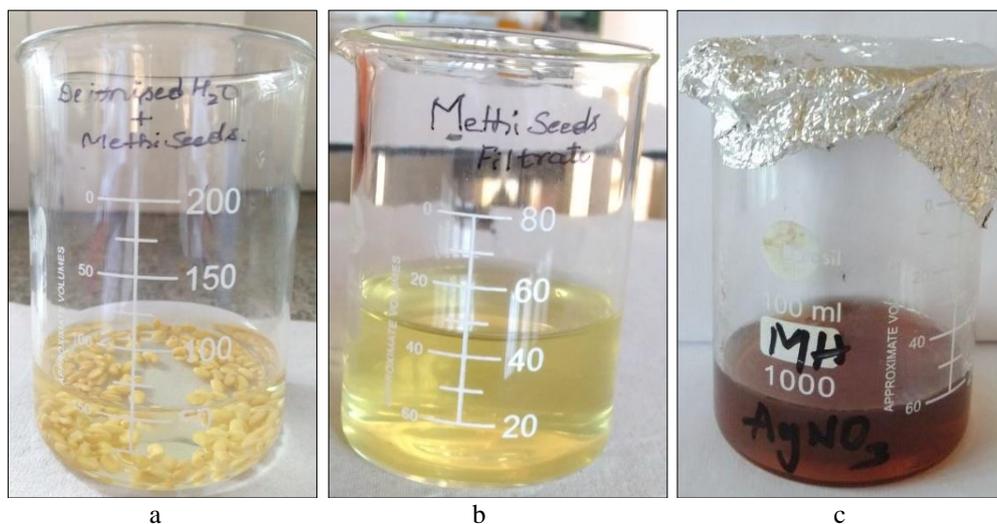


Fig 1a-2: gm of fenugreek seeds are soaked in 50 ml of deionised water for 24 h; b-The filtrate is subjected to centrifugation at 2000 rpm for 10 min; c-2.5 ml of concentrated filtrate is added to 25 ml of silver nitrate solution

Phytochemical analysis

Detection of alkaloids, carbohydrates, glycosides, Legal's Test, saponins, phytosterols, Phenols, tannins, flavonoids, proteins and amino acids and diterpenes were carried on using standard protocol.

Determination of total phenolic content

The amount of total soluble phenolic content of fenugreek seeds was determined according to Folin-Ciocalteu method. About 10 μl of methanol extract and methanol extract treated with AgNO_3 was prepared from the stock. The extracts were taken with different concentration (0,0.2,0.4,0.6,0.8,1.0). The volume is made-up to 1 ml with distilled water. 0.5 ml of Fc reagent is pipetted out to all the respective test tubes. After 3 min incubation at room temperature, 2 ml of 20%

Na_2CO_3 solution was added. The absorbance was measured at 650 nm using spectrophotometer against blank sample. The total phenolic content was measured using gallic acid with different concentration.

Determination of total flavonoids content

The content of flavonoids was determined by a pharmacopeia method using rutin as a standard compound. 10 μl of methanol extract of fenugreek and methanol extract of fenugreek treated with AgNO_3 was prepared from the stock. The extracts were taken with different concentrations (0,0.2,0.4,0.6,0.8,1.0). 20% aluminum chloride of 1 ml is pipetted out to all the respective test tubes. 2 drops of acetic acid was added to all the test tubes and incubated in boiling

water bath for 30 min. The absorbance was measured at 415 nm using spectrophotometer against blank sample.

Metal chelating assay

The chelation of ferrous ions by extracts was estimated by method of Dinis *et al.* (1994) [3]. Briefly, 0.5 ml of 2 mM FeCl₂ were added and 1.6 ml of distilled water were added to different concentrations (0.5,1.0,1.5 ml) of both extracts. The reaction was initiated by the addition of 0.1 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min and then it is absorbed under 562 nm against blank sample and ascorbic acid is used as a standard sample.

Ferric reducing antioxidant power (FRAP) activity

The FRAP assay was carried out according to Benzie and Strain (1996) [1]. Briefly, the working FRAP reagent produced by combination of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCL and 20 mM FeCl₃.6H₂O in 10:1:1 ratio prior to use and heated to 37°C in water bath for 10 min. Fenugreek seeds extract of various concentration (0,0.2,0.4,0.6,0.8,1.0) of both extracts were allowed to react with 0.5 ml of the FRAP reagent. The final volume of the reaction mixture was made up to 3 ml with distilled water. The reaction mixture was kept in dark for 30 min. The readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm against blank sample. Ascorbic acid is used as standard sample for the analysis. The FRAP values were determined as optical density readings. Higher optical density indicated the higher ferrous reducing power.

Radical scavenging activity using DPPH method

The ability of the plant extracts to scavenge the stable free radical DPPH was assayed by the method of Mensor *et al.* (2001) [4]. DPPH (2,2-diphenyl-2-picryl hydrazyl), a stable free radical, when acted upon by an antioxidant, is converted into diphenyl-picryl hydrazine with a colour change from deep violet to light yellow colour. This can be quantified spectrophotometrically at 518 nm to indicate the extent of DPPH scavenging activity by the seed extracts.

Calculating percentage growth inhibition:

$$\% \text{ Inhibition} = \frac{(\text{OD of Control} - \text{OD of Sample})}{\text{OD of Control}} \times 100$$

Total tannins of fenugreek seeds (Methanol extract)

1 mg of methanol extract and AgNO₃ is dissolved in 10 ml of methanol water (7:3), then 0.5 ml of Foli's phenol reagents is added and 5 ml of 3.5% sodium carbonate and incubated for 5 mins at room temperature and read at 640 nm in spectrophotometer and triplicate analysis is done.

Assay for α -amylase inhibition

The determination of α -amylase inhibition was carried out by quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions. The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated. A modified dinitrosalicylic acid (DNS) method was adopted to estimate the maltose equivalent. 1 ml of the methanol extracts and AgNO₃ treated methanol extract of the selected fenugreek seed extracts were pre-incubated with α -amylase 1U/ml for 30 min and thereafter 1 ml (1% w/v) starch solution was added. Then the reaction was stopped by adding 1 ml DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 ml of 2 M NaOH and 96 mM 3, 5- dinitrosalicylic acid solution) and

the contents were heated in a boiling water bath for 5 min. A blank was prepared without seed extracts and another without the amylase enzyme, replaced by equal quantities of buffer (20 mM sodium phosphate buffer with 6.7 mM Sodium chloride, pH 6.9 at 20 °C). The absorbance was measured at 540 nm. The reducing sugar released from starch was estimated as maltose equivalent from a standard graph.

In-vitro anti-inflammatory activity and inhibition of protein denaturation

Both extracts of different concentration was taken (0.4,0.8,1.2,1.6,2.0 ml) in test tubes and sample is made up to 1 ml with distilled water. 0.2 ml of bovine serum albumin and 2.8 ml of phosphate buffer is added to all the test tubes, respectively. After cooling the samples, 2.8 ml of phosphate buffer solution was added into each test tube. The test tubes were incubated for 15 min at 37 °C and 10 min at 70 °C in boiling water bath. Turbidity was measured spectrophotometrically at 660 nm against blank sample. The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = 100 - (O.D. of test – O.D. of product control) x 10/ O.D. of Control

The Percentage protection from denaturation is calculated by using the formulae and it is tabulated.

Protein estimation by Lowry's method

Reagents used for estimation are mentioned below

Reagent A

1. 2% sodium carbonate in 0.1% sodium hydroxide
2. 1% copper sulphate in 2% potassium sodium tartarate

Reagent B

1. FC reagent (1:1)
2. Protein standard

Preparation of reagents

Reagent A

Solution 1: Dissolve 0.4 g of NaOH crystals into 98 ml of distilled water in a standard flask and dissolve it thoroughly.

Solution 2: 0.1 g of potassium sodium tartarate was mixed with 20 ml of distilled water and to it 0.2 g of copper sulphate was dissolved thoroughly.

Procedure

Pipette out the amount of 0.0,0.4,0.8,1.2,1.6,2 ml of prepared standard protein solution into the clean and dry test tubes simultaneously methanol extract of fenugreek seed sample and fenugreek seeds extract treated with silver nitrate was taken in another set of test tubes. The volume was made up to 2 ml by adding respective volume of distilled water. 2 ml of the prepared reagent A was added to all the tubes including extract samples and mix well and kept aside for 10 min. 0.2 ml of reagent B was added to all the tubes including extract sample and mixed well and kept in dark for 30 min and after incubation, the absorbance was measured at 660 nm against blank.

Anti microbial assay

The isolated phytochemical fractions were assessed for their antibacterial activity against the pathogenic bacteria.

Test organisms

Six bacterial strains were used throughout the investigation namely *Klebsiella* sp., *Proteus vulgaris*, *E. coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Enterobacter*

aerogens. All the bacterial cultures were obtained from microbial type culture collection. Bacterial broth of 24 h old culture was used for screening procedure. Active cultures for the experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of nutrient broth and were incubated for 24 h at 37 °C.

Disc diffusion method

Procedure

Nutrient Agar plates were prepared and the test microorganisms were inoculated by the spread plate method. Filter paper discs approximately 6 mm in diameter were soaked with different concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 of both extracts and placed in the previously prepared agar plates. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they are no closer than 24 mm from each other, center to center. The agar plates were then incubated at 37 °C. After 24 h of incubation, each plate was examined. The resulting zones

of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disc where the chloramphenicol was used as control.

TLC for methanol extract

To analyze the functional groups of phytoactive compounds present thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) were carried out.

High-performance liquid chromatography

Samples: Test samples (10 mg/ml) were prepared from stock with HPLC grade Methanol, Chloroform and Hexane (based on solubility) and used for HPLC analysis.

Results

Phytochemical analysis of methanol extract and silver nanoparticle extract of fenugreek seeds are tabulated as follows (Table 1):

Table 1: Phytochemical analysis of methanol extract and silver nanoparticle extract

S. No	Phytochemicals	Result	
		Methanol extract	Silver nanoparticle extract
01	Alkaloids	Presence of Alkaloids	Presence of Alkaloids
02	Carbohydrates	Absence of carbohydrates	Absence of carbohydrates
03	Saponins	Presence of Saponins	Presence of Saponins
04	Glycosides	Absence of Glycosides	Absence of Glycosides
05	Phenols	Absence of phenols	Absence of phenols
06	Phytosterols	Presence of Phytosterols	Presence of Phytosterols
07	Tannins	Presence of Tannins	Presence of Tannins
08	Flavonoids	Presence of flavonoids	Presence of flavonoids
09	Proteins and amino acids	Absence of proteins and amino acids	Absence of proteins and amino acids
10	Deterepens	Presence of deterepens	Presence of deterepens

Both the extracts showed similar results; hence, giving the impression of having same phytochemical activity

Total phenol content (gallic acid as standard)

Phenolic content in the methanolic extract of Fenugreek seeds was estimated. Major role of phenols in scavenging the free radicals is due to the presence of hydroxyl groups. Antioxidant activity of the extract is proportional to the amount of phenol content present in the extract. The methanolic extract showed higher range than the silver nanoparticles extract, so the methanolic extract sample shows a tendency to have high phenolic content (Figures 2 and 3).

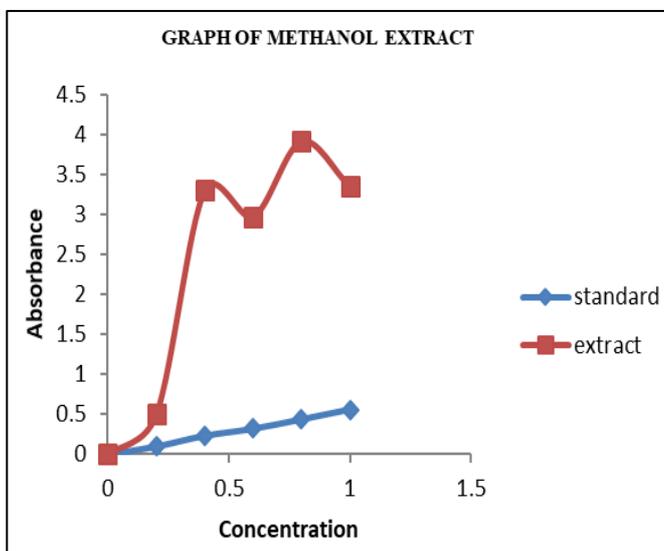


Fig 2: Estimation of methanol extract for total phenol

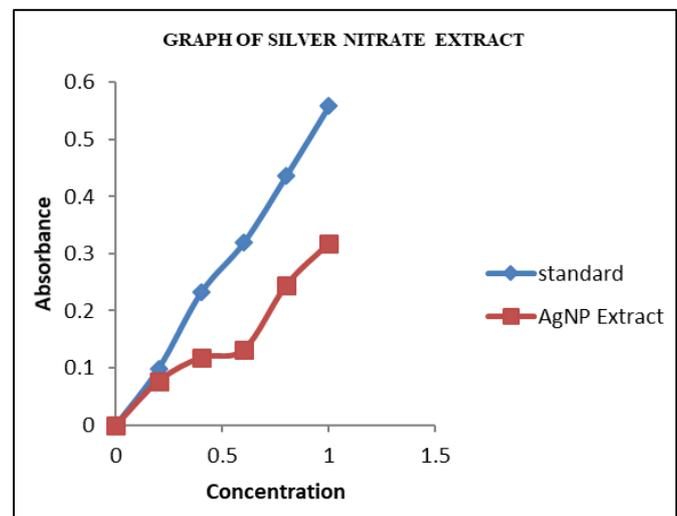


Fig 3: Estimation of silver nanoparticle extract

Total flavonoids (rutin as standard)

Total flavonoid content was determined using NaNO_2 and $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, and result was expressed as mg quercetin equivalents/g methanol extract content was determined using rutin reagent, and absorbance was recorded at 490 nm. The total methanol extract content was then determined from a rutin standard curve and methanolic extract and silver nanoparticles extract showed lesser range than the standard rutin, so the extract sample have low tendency of flavonoids (Figures 4 and 5).

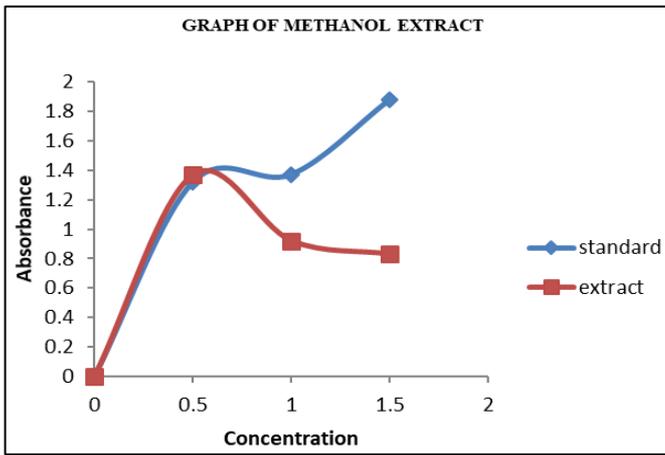


Fig 4: Estimation of methanol extract for total flavonoid

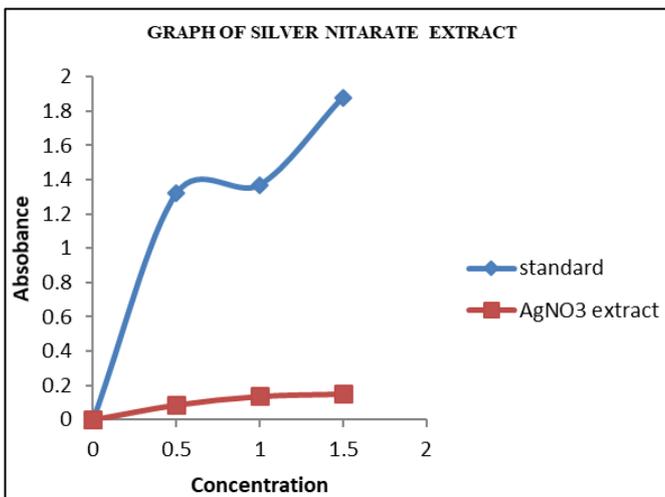


Fig 5: Estimation of silver nanoparticle extract

Ferric reducing antioxidant power (FRAP) activity (ascorbic acid)

The FRAP assay gives fast, reproducible results with methanol extract, with single antioxidants in pure solution and with mixtures of antioxidants in aqueous solution and added to methanol extract. The dose response characteristics of different antioxidants showed different activities, but the dose response of each individual antioxidant tested was linear, showing that activity is not concentration dependent, at least over the concentration ranges tested in this study. Quite a high range is observed with silver nanoparticle extract than the methanol extract but not as much as standard ascorbic acid (Figures 6 and 7).

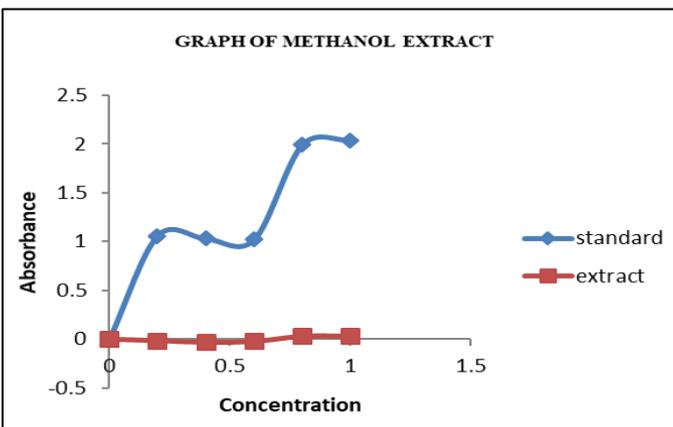


Fig 6: Estimation of methanol extract for FRAP

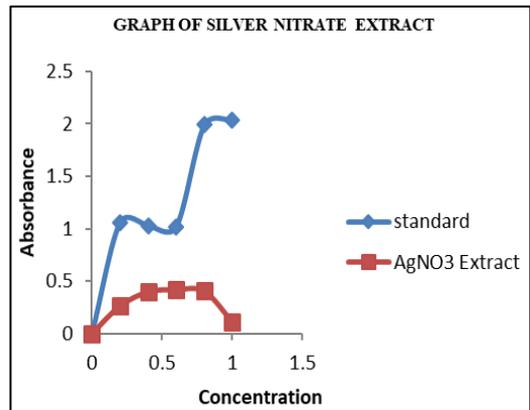


Fig 7: Estimation of silver nanoparticle extract

DPPH radical scavenging activity of standard ascorbic acid

The antioxidant capacity of the fenugreek extracts were analyzed by using the free radical scavenging (DPPH) and the ferric reducing antioxidant power (FRAP) methods. The DPPH test is the oldest indirect method for determining the antioxidant activity, which is based on the ability of the stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenols. Radical scavengers may directly react and quench with peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food product. The stable DPPH radical has been used to evaluate antioxidants for their radical quenching capacity and to better understand their antioxidant mechanism. Both the extracts were evaluated for radical scavenging activity against DPPH and silver nanoparticle extract showed higher absorbance than the methanol extract. Here, the nanoparticles extract have more antioxidant capacity than the methanol extract (Figures 8 and 9).

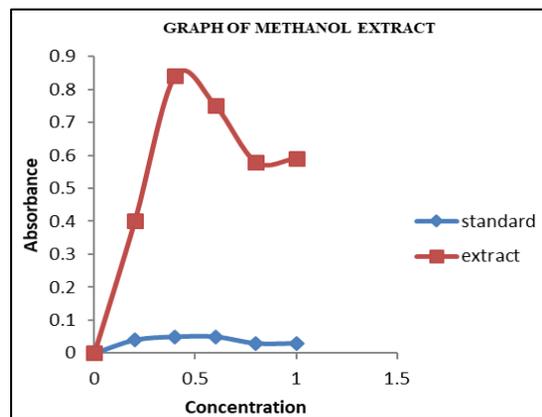


Fig 8: Estimation of methanol extract for DPPH

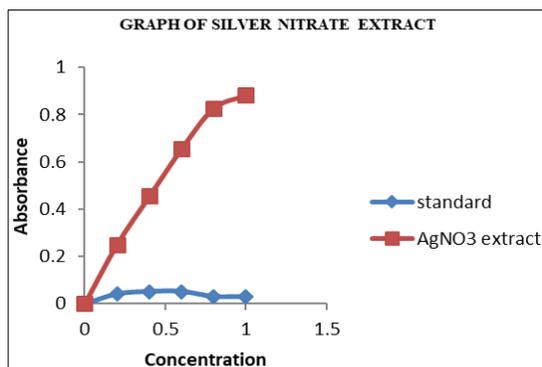


Fig 9: Estimation of silver nanoparticle extract

Alpha amylase inhibition assay (ascorbic acid as standard)

The carbohydrates digestive enzymes are hydrolyzed by pancreatic α -amylase and liable for the breakdown of oligosaccharides and disaccharides into monosaccharide suitable for absorption. The inhibition of these digestive enzymes is specifically useful for the treatment of non insulin diabetes because it will slow down the release of glucose in the blood. The results indicated that α -amylase was significantly inhibited in a concentration dependent manner following incubation with various concentrations of methanol extract and AgNP'S. The increasing concentration of methanol extract level, the enzymatic activity level was not reduced. When alpha-amylase is not inhibition by methanol extract of fenugreek seeds it is not used for the treatment of care diabetes (Figures 10 and 11).

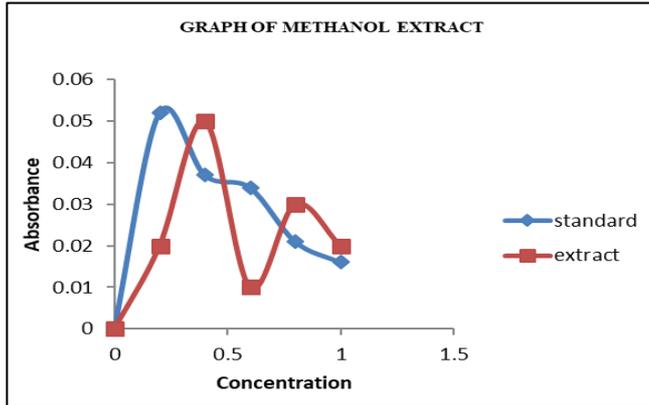


Fig 10: Estimation of methanol extract

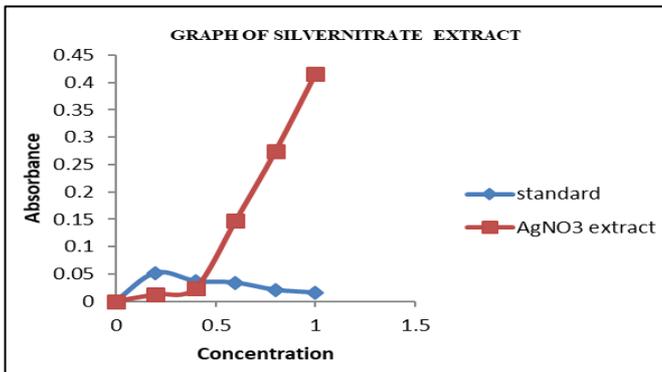


Fig 11: Estimation of silver nanoparticle extract for alpha amylase inhibition

Anti-inflammatory activity

Anti-inflammatory activity of the seed extracts were carried out. The methanol extract of the seed showed little activity than the silver nanoparticle extract but as not much as standard and it was evaluated on the basis of phytoconstituents present in it (Figures 12 and 13).

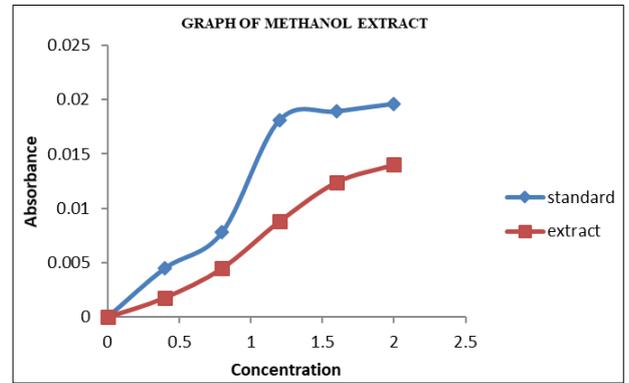


Fig 12: Estimation of methanol extract

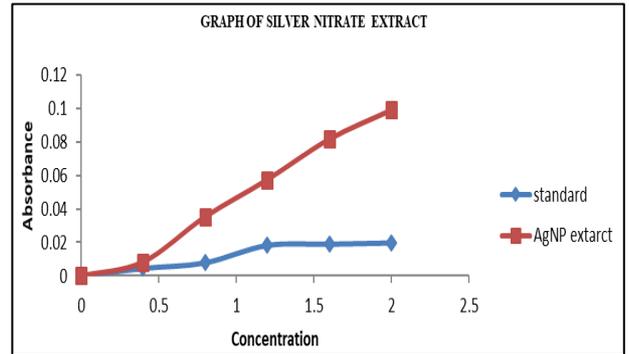


Fig 13: Estimation of silver nanoparticle extract for anti-inflammatory activity

Protein estimation by Lowry's method

Lowry's method enhances the sensitivity of the Biuret method. Protein reacts with Fc reagent to give a blue coloured complex. The colour so formed is due to the reaction of alkaline copper with the protein as in the biuret test and the reduction of phosphomolydic and phosphotungstic components in Fc by the amino acids of tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic acid present (Figures 14 and 15).

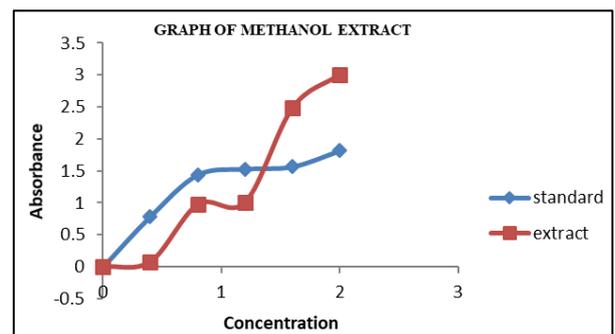


Fig 14: Estimation of methanol extract

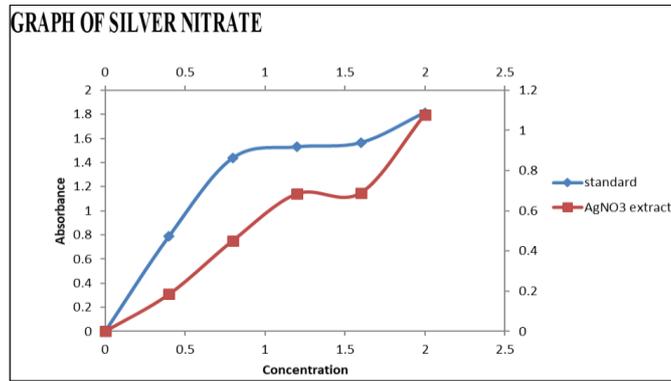


Fig 15: Estimation of silver nanoparticle extract for anti-inflammatory activity

Total tannins of fenugreek seeds (methanol extract)

Tube no.1	Tube no.2	Tube no.3
1.37	1.37	1.42

Total tannins of fenugreek seeds with silver nanoparticles

Tube no.1	Tube no.2	Tube no.3
0.152	0.153	0.156

By the obtained results, both the extract contains tannin. But silver nanoparticle extract showed higher range than the methanol extract.

Antimicrobial activity

The methanolic extract of the fenugreek did not showed zone of inhibition But in silver nanoparticle extract showed zone of inhibition against bacteria and results are tabulated (Table 2; Figures 16-21).

Table 2: Antibacterial activity in silver nanoparticles extract by disc diffusion method

Bacterial strains	Inhibition zone diameter in cm				
	0.2 ml	0.4 ml	0.6 ml	0.8 ml	1 ml
<i>Staphylococcus aureus</i>	0.3	0.4	0.4	0.4	0.4
<i>Proteus vulgaris</i>	0.1	0.2	0.2	0.2	0.2
<i>Enterobacter aerogens</i>	0.1	0.1	0.1	0.1	0.2
<i>Bacillus cereus</i>	—	0.1	0.2	0.2	0.2
<i>Escherichia coli</i>	0.4	0.4	0.4	0.4	0.3
<i>Klebsiella sp.</i>	0.1	0.1	0.2	0.2	0.2

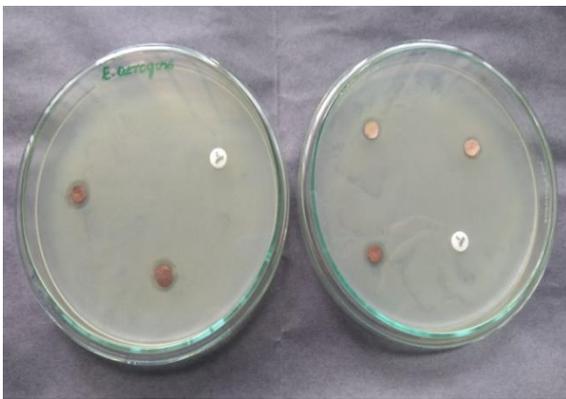


Fig 16: *E. aerogens*



Fig 18: *Proteus vulgaris*

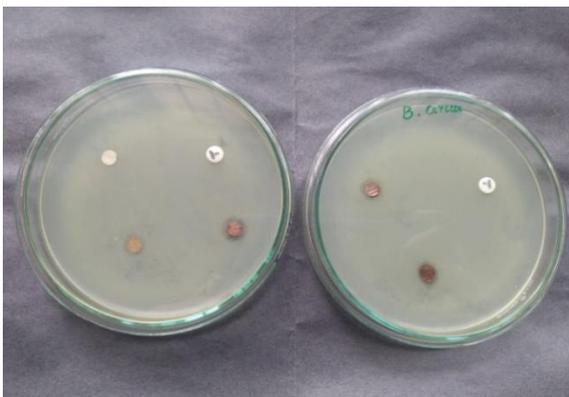


Fig 17: *Bacillus cereus*

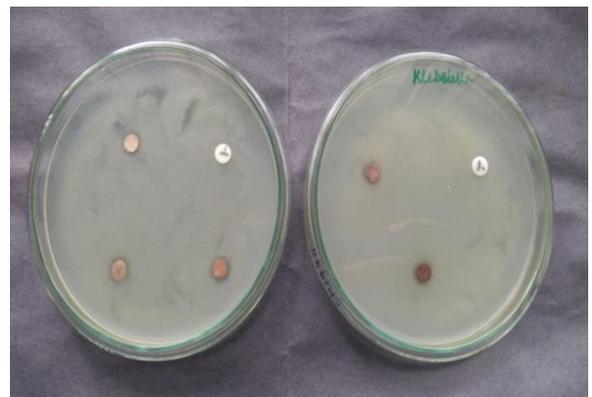


Fig 19: *Klebsiella sp.*

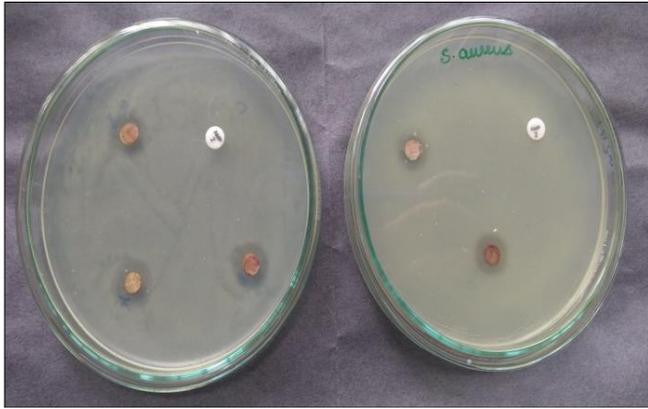


Fig 20: *Staphylococcus aureus*

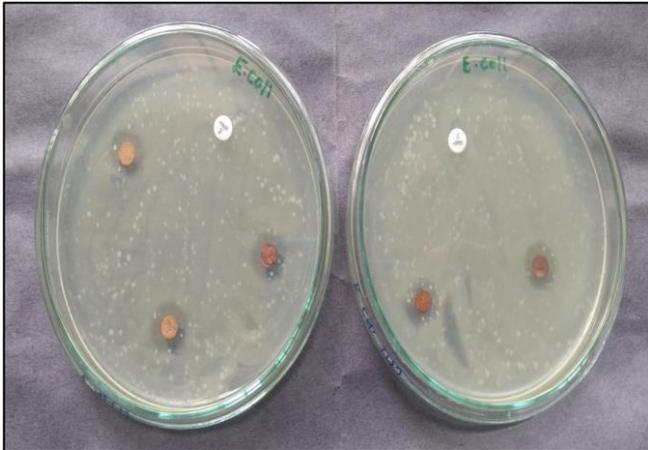


Fig 21: *Escherichia coli*

Thin layer chromatography



The total solvent run is 13.5cm and 12.6cm and the band length is 7.2 cm and 6.0 cm.

The Rf value is calculated using below formula:

$$R_f = \frac{\text{distance spot travels}}{\text{distance solvent travels}}$$

Band length	Rf values
13.5cm	0.53
12.6cm	0.44

Methanol extract of fenugreek and silver nitrate extract showed a band at 366 nm indicating the presence of unknown compound. Retention factor is a parameter that determined the polarity of compound based on Rf value 0.53 of one compound and another compound is 0.44. The Rf value generally ranges between 0 and 1.

High-performance liquid chromatography

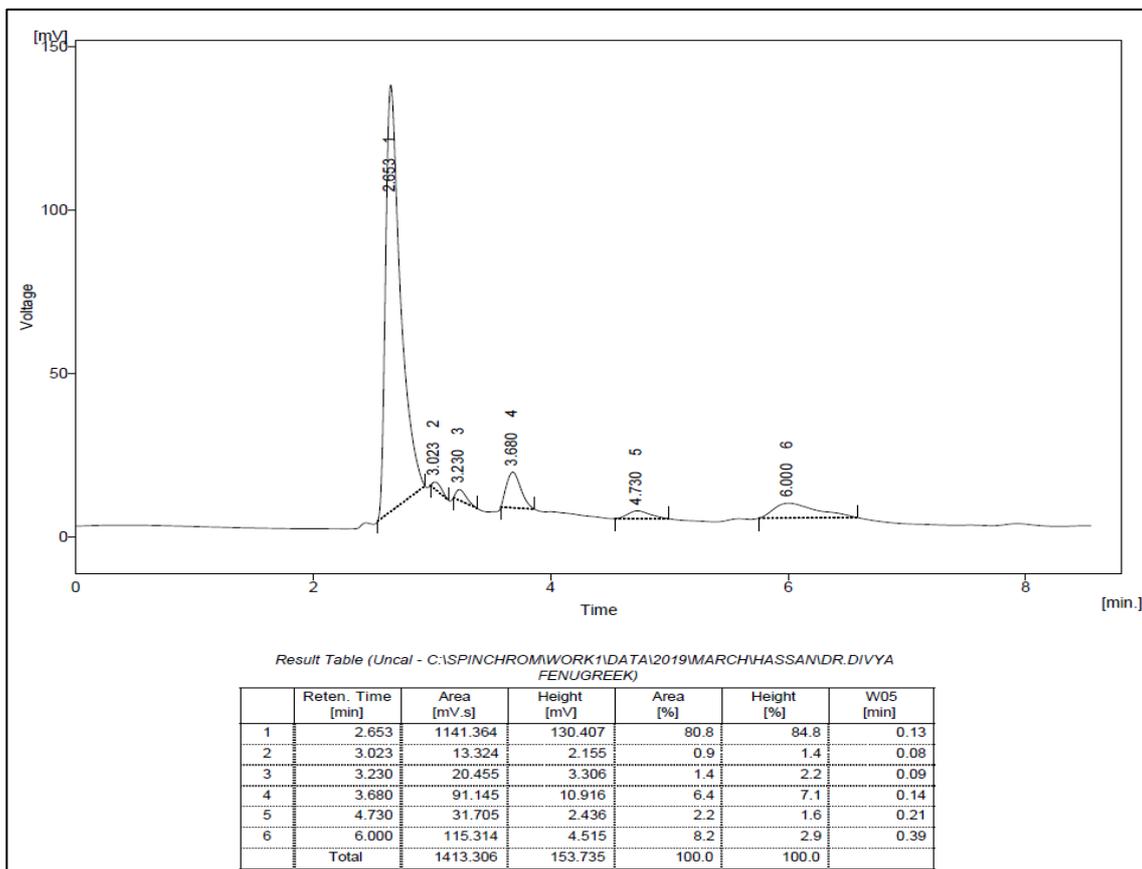


Fig 22: HPLC chromatogram of Fenugreek

Methanol extract of fenugreek has been analysed with a peak six of higher area is 80% purity with RT at 2.653, the area under peak gives us the RT of the compound in HPLC (Figure 22).

Conclusion

The phytochemical analysis revealed the presence of flavonoids, saponins, deterpens, tannins, phytosterols and antioxidants concentration is very low. Anti-inflammatory of methanol extract of the seed showed little activity than the silver nanoparticle extract. The results indicated that α -amylase was significantly inhibited in a concentration dependent manner following incubation with various concentrations of methanol extract and AgNP'S. With the increasing concentration of methanol extract level, the enzymatic activity level was not reduced. When alpha-amylase is not inhibited by methanol and silver nanoparticles extract of fenugreek seeds then it is not used for the treatment of diabetes.

Eco-friendly synthesis of silver nanoparticles is a low cost. It is shown that fenugreek extract is the easy, economic and environmentally friendly process for nano particles preparation. Fenugreek represents a considerable improvement in the synthesis of a nano silver because it is used as reducing, capping agent and better control for size and shape and silver nanoparticles is used for drug delivery. Silver nano particles are effective as antibacterial agent of the *Escherichia coli* and *staphylococcus aureus*, *proteus vulgaris*, *Enterobacter aerogens* and *Klebsiella* but the methanol extract showed activity against only *Proteus vulgaris*.

References

1. Benzie FFI, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry*. 1996; 239:70-76.
2. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. *Advanced Drug Delivery Review*. 2012; 64:24-36.
3. Dinis TCP, Madeira VMC, Almedia MLM. Action of phenolic derivatives as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Archives of Biochemistry and Biophysics*. 1994; 311:161-169.
4. Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy and Research*. 2001; 15:127-130.
5. Nethra Devi C, Sivakumar P, Renganathan S. Green synthesis of silver nanoparticles using *Daturametel* flower extract and evaluation of their antimicrobial activity. *International Journal of Nanomaterial and Biostructure*. 2012; 2:16-21.
6. Raghunath D. Emerging antibiotic resistance in bacteria with special reference to Indian Journal of Bioscience. 2008; 33:593-603.
7. Roy P, Abdulsalam F, Pandey D, Bhattacharjee A, Eruvaram N, Malik T. Evaluation of antioxidant, antibacterial and antidiabetic potential of two traditional medicinal plants of India: *Swertia cordata* and *Swertia chirayita*. *Pharmacognosy Research*. 2015; 7:57-62.
8. Shrikanth VM, Janardhan B, Dhananjaya BL, Muddapura UM, More SS. Antimicrobial and antioxidant activity of methanolic root extract of *Tabernaemontana alternifolia* L. *International Journal of Pharmaceutical Science*. 2015; 7:66-69.

9. Teshager T, Herrero IA, Porrero MC, Garde J, Moreno MA, Domínguez L. Surveillance of antimicrobial resistance in *Escherichia coli* strains isolated from pigs at Spanish slaughterhouses. *International Journal of Antimicrobial Agents*. 2000; 15:137-142.
10. Thirunavukkarasu V, Anuradha CV, Viswanathan P. Protective effect of fenugreek (*Trigonella foenum graecum*) seeds in experimental ethanol toxicity. *Phytotherapy Research*. 2003; 17:737-743.
11. Umopathy E, Ndebia EJ, Meeme A, Adam B, Menziura P, Nkeh-Chungag BN. An experimental evaluation of *Albucasetosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *Journal of Medicinal Plant Research*. 2010; 4:789-795.
12. Umesh B, Jagtap, Vishwas A, Bapat. Green synthesis of (Ag-Np) silver nanoparticles via *Artocarpus heterophyllus* Lam. Seed extract and its antibacterial activities. *Industrial Crops Production*. 2013; 46:132-137.
13. Vane JR, Botting RM. New insights into the mode of action of anti-inflammatory drugs. *Inflammatory Research*. 1995; 44:1-10.