ASSESSMENT OF DIFFERENT THERAPEUTIC POTENTIAL EFFECTS OF CORIANDRUM SATIVUM METHANOLIC EXTRACT AND ITS FRACTIONS USING IN VITRO MODEL SYSTEMS

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Abstract

Antioxidants are vital substances that have an ability to protect the body from various damages caused by free radical-induced oxidative stress. Plants are the source of a wide variety of natural free radical scavenging antioxidants. The aim of this research was to investigate the antioxidant activity of *Coriandrum Sativum* seeds, including its effect on inhibition of lipid peroxidation, Hemoglobin-induced lipid peroxidation system. The objective of the present study is to carry out in vitro antioxidant tests to evaluate the antioxidant ability of Methanolic extracts of selected mangrove plans i.e., Coriandrum Sativum L. In this study, *In vitro* antioxidant activity was estimated by total antioxidant activity methods. The *in vitro* antioxidant studies revealed that, the extract of Coriandrum Sativum L has the highest antioxidant potential. The present study investigated the antimicrobial activity of methanol (MeOH) extract of figs against bacteria. The MeOH extract showed a strong antibacterial activity against bacteria.

Keywords: Antioxidant, Coriandrum Sativum.

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1.INTRODUCTION

Anti oxidant activity:

Antioxidants became a vital part of our lives today since antioxidants neutralizes or destroys "reactive oxygen species" (ROS) or free radicals before they damage cells. The oxidation induced by ROS results in cell membrane disintegration, membrane protein damage, and DNA mutations, which results in aging and further initiates or propagates the development of many diseases such as arteriosclerosis, cancer, diabetes mellitus, liver injury, inflammation, skin damages, coronary heart diseases, and arthritis.

The chemical compounds, which decrease the rate of lipid oxidation reaction in food systems, are called antioxidants. By definition, a substance that opposes oxidation or inhibits reactions promoted by oxygen or peroxides; many of these substances being used as preservatives in various products are antioxidants. Biologically antioxidants are defined as synthetic or natural substances added to products to prevent or delay their deterioration by the action of oxygen in air. For example, enzymes or other organic substances such as vitamin E or β-carotene.

Antioxidants are chemical compounds which bind to free oxygen radicals and prevents these radicals from damaging healthy cells.

By the normal use of oxygen[1], free radicals are produced continuously by the body. Oxygen is an element indispensable for life. When cells use oxygen to generate energy, free radicals are produced by the mitochondria. These by-products are generally ROS as well as reactive nitrogen species (RNS) that result from the cellular redox process. The free radicals have a special affinity for lipids, proteins, carbohydrates, and nucleic acids.[2]

A free radical is a chemical species, capable of independent existence possessing one or more unpaired electron. The free radicals are less stable than non-radicals and are capable of reacting indiscriminately with molecules. Once radicals are formed, they can either react with another radical or with another non-radical molecule by various interactions. When two radicals collide with their unpaired electron, forms a covalent bond. The most molecules found in vivo are non radicals. A radical donates its unpaired electron to the other molecules, or takes one electron from it, thus transforming its radical character. At the same time, a new radical is formed [3,4]. ROS/RNS are present in the atmosphere as pollutants and can be generated (i) during ultraviolet (UV) light irradiation, by X-rays and gamma rays; (ii) during metal catalyzedreactions; (iii) by neutrophils, esinophils and macrophages during inflammatory cell activation [5,6]; (iv) as by-products of mitochondrial catalyzed electron transport reactions; (v) by cytochrome P450 metabolism and the enzyme xanthine oxidase, which catalyzes the reaction of hypoxanthine to xanthine and xanthine to uric acid.[7]

Oxidative stress is defined as an imbalance between the production of free radicals and reactive metabolites, so-called oxidants or ROS, and their elimination by protective mechanisms referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism [8,9,10,11]. The harmful effects of ROS are balanced by the action of antioxidants, example like enzymes present in the body [12]. Despite the presence of the cell's antioxidant defense system to counteract oxidative damage from ROS, oxidative damage accumulates during the life cycle and has been implicated in diseases, aging and age-dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders, and other chronic conditions [13].

ROS is classified into oxygen-centered radicals and oxygen-centered non-radicals.

i. Oxygen-centered radicals are superoxide anion (·O2–), hydroxyl radical (·OH), alkoxyl radical (RO·), and peroxyl radical (ROO·). Other reactive species are nitrogen species such as nitric oxide (NO·), nitric dioxide (NO2·), and peroxynitrite (OONO–).
ii. Oxygen-centered non-radicals are hydrogen peroxide (H2O2) and singlet oxygen (1O2), hypochlorous acid and ozone [14,15].

Role of Antioxidants

An antioxidant is a molecule capable of inhibiting the oxidation of another molecule (Fig. 1). It breaks the free radical chain of reactions by sacrificing their own electrons to feed free radicals, without becoming free radicals themselves[16,17] (Fig. 2).

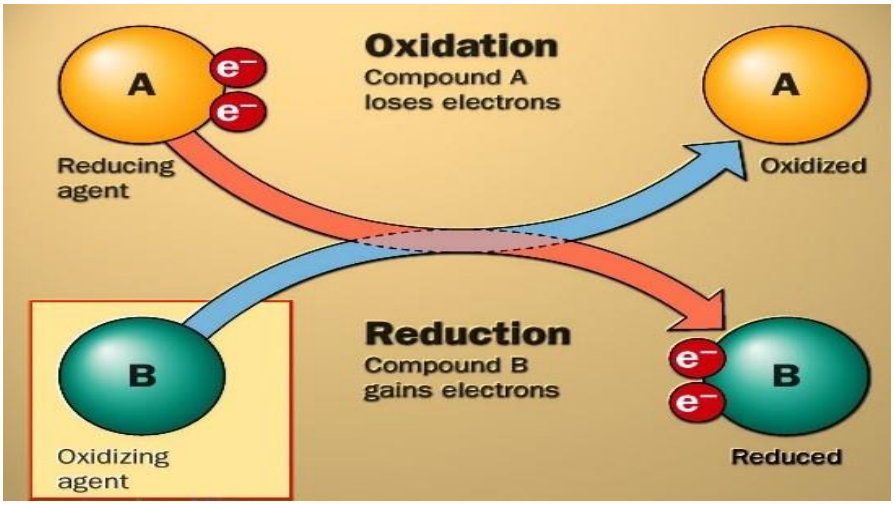

Fig. 1: Oxidation and reduction process

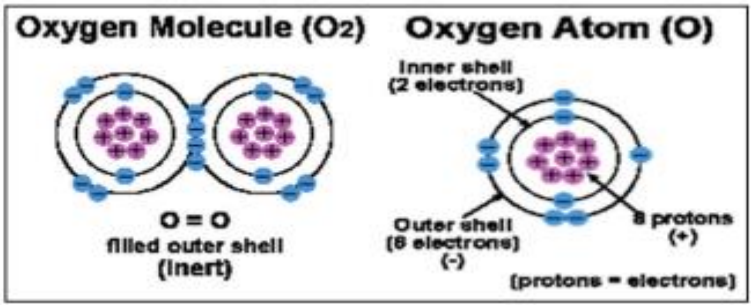

Fig. 2: Electrons in the outer shell

Anti microbial activity:

In general, humans have a large number of microorganisms which usually do not cause disease (normal flora). But on the way, some bacteria that are important causes of disease generally originate from normal flora, resulting in an infection. Staphylococcus aureus and Escherichia coli are the two main causes of various infections in humans, such as bloodstream infections (BSI). Staphylococcus aureus is part of the normal flora that lives on the skin and mucosa of the human body. Staphylococcus aureus causes infections of the skin and soft tissue, surgery marks, infections of the bones and joints, and causes of hospital-acquired bacteraemia (HAB) and respiratory infections that are obtained from hospitals. Staphylococcus aureusis the most isolated pathogenic bacteria from patients. Escherichia coli, a gram negative bacterium in the form of bacilli, is a normal flora that generally colonizes the large intestine. Escherichia coli is the main pathogen that causes about 90% of urinary tract infections, gastrointestinal infections, and systemic infections in humans. The high incidence of antimicrobial resistance (AMR) due to antibiotic abuse has led to new discoveries of antibacterial agents becoming very important and are considered to be one of the pillars of modern medical science in preventing millions of premature deaths caused by infectious diseases, especially bacterial infections [18].

Anti Proliferative Activity:

The potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is still enormous. The antitumor area has the greatest impact of plant derived drugs, where drugs like vinblastine, vincristine, taxol, and camptothecin have improved the chemotherapy of some cancers. Plants have an almost unlimited capacity to produce substances that attract researchers in the quest for new and novel chemotherapeutics. The continuing search for new anticancer compounds in plant medicines and traditional foods is a realistic and promising strategy for its prevention. Numerous groups with antitumor properties are plant derived natural products including alkaloids, phenylpropanoids, and terpenoids. The present study was conducted to evaluate the antiproliferative activity of extracts from coriander plant[19].

Anti-Inflammatory Activity

Inflammation is the response of the human or animal body and the complex interactions between immune cells and mediator molecules . This is a common problem in society as a result of lifestyle

and health problems. Inflammation can play an important role in the pathogenesis of various types of acute and chronic diseases such as cardiovascular disease, neurodegenerative diseases, obesity, and type 2 diabetes mellitus. Pharmacotherapy for inflammatory conditions is still dominated by the use of non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs comprise a group of non-selective and selective NSAIDs. Non-selective NSAIDs work to inhibit the cyclooxygenase enzymes (COX-1 and COX2) which decrease prostaglandin production. The inhibitor group works by inhibiting the COX-2 enzyme. NSAIDs are commonly used to relieve pain and inflammation caused by various types of arthritis and musculoskeletal disorders. Although effective in reducing pain and inflammation, NSAIDs cause side effects including serious gastrointestinal toxicities such as gastric ulcers and bleeding. Some NSAIDs that work to inhibit COX-2 induce an increase in blood pressure, increasing the risk of blockages that lead to heart failure, clotting, and heart muscle death[20].

Anti Diabetic Activity

Diabetes-mellitus is a chronic disease characterized by elevated blood glucose levels and disturbance in carbohydrate, fat and protein metabolism. These metabolic abnormalities result, in part, from a deficiency of the blood sugar-lowering hormone insulin. This deficiency in insulin results in type 1 diabetes or insulin dependent diabetes mellitus (IDDM). Type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM) is a result of hyperglycemia caused by overproduction of glucose at the hepatic level or because of abnormal E - cell function or insulin resistance at target cells. Currently available synthetic antidiabetic agents produce serious side effects like hypoglycemic coma and hepatorenal disturbances. The present study was aimed to investigate the anti-diabetic activity of extract of coriander leaves in alloxon induced diabetic rats[21].

MATERIALS AND METHODS

2. MATERIALS

Procurement of sample

A sample of coriander seeds was taken from the whole lot purchased from wholesale spice supplier of Local market.

Chemicals

All the chemicals and solvents were of analytical grade obtained from SRL, E- Merck and Sigma–Aldrich.

Samples preparation

Seeds of coriander plants were dried and grinded in a blender. The powder of each sample was kept in a polyethylene bags and preserved in deep freezer until use.

Extraction of the methanolic extract

About 250 grams of each milled plant seeds of coriander samples were macerated in 500 mL of methanol overnight at room temperature, then filtered and the methanolic crude extract was collected. Another portion of 500 mL of methanol were added to the plant residue and homogenized in a blender for five minutes and filtered. The filtrate was collected to the previous crude extract. The residue was subjected to additional 500 mL of methanol and left at room temperature overnight, then filtered. The filtrate was added to the previous crude extract to form the methanolic extract. The solvent was evaporated under vacuum using rotary evaporator. The crude methanolic extract was obtained, kept in dark bottles and stored in a deep freezer until use.

3. METHODS

3.1 ANTIOXIDANT ACTIVITY

Total phenolic contents of the seeds of coriander extracts.

The FolinCiocalteu procedure (Ghafoor& Choi, 2009) was used to measure the total phenolic contents of the seeds of coriander extracts. This method depends on the reduction of Folin's reagent by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm. A 200 µL properly diluted sample or a standard solution of varying concentrations were mixed with 400 µLFolinCiocalteu reagent. The deionized water was used for dilution and control. The solution was diluted to a total volume of 4.6 mL using deionized water then thoroughly mixed. After incubation for 10 minutes at room temperature, 1 mL of 20% Na_2CO_3 solution was added then immediately mixed and incubated for 2 h. The absorbance was read at 765 nm on a spectrophotometer. Measurements were recorded in triplicates.

Gallic acid of 1 mg/mL was used as the standard and the total phenolic compounds of the samples were expressed in grams gallic acid equivalent (GAE) per 100 g extract (g GAE/100 g).

Reducing activity

Reducing activity of the methanol extracts of the seeds of coriander was determined according to the method of Yen and Chen (1995). The capacity

of coriander herb extract to reduce the ferric–ferricyanide complex to the ferrous-ferricyande complex of Prussian blue was determined by recording the absorbance at 700 nm after incubation. Reducing activity of coriander extract, on an equivalent phenolic content basis, was expressed as percentages (%) of ascorbic acid (1 mM) equivalent activity.

Assessment of antiperoxidative effect (inhibition of lipid peroxidation) in:

β-carotene linoleate model system (β -carotene bleaching assay)

The antioxidant activity of the test sample was evaluated by the inhibition of peroxidation of linoleic acid in terms of inhibition of bleaching of β -carotene using β carotene-linoleate model system (Hidalgo et al., 1994) with slight modification. β - carotene (0.2 mg), 20 mg of linoleic acid and 200mg of Tween-40 (polyoxyethylene sorbitan monopalmitate) were mixed in 0.5 mL of chloroform and chloroform was removed at 400C under vacuum using a rotary evaporator. The resulting mixture was immediately diluted with 10mL of triple-distilled water and was mixed well for 1-2 min. The emulsion was further made up to 50 mL with oxygenated water. Aliquots (4 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of test sample at various concentrations (100-500µg/mL) in ethanol. Butylated hydroxy anisole (BHA) was used as positive control for comparative purposes. A control, containing 0.2 mL of ethanol and 4 mL of the above emulsion was prepared. The tubes were placed at 500C in a water bath. Absorbance of all the samples at 470 nm was taken at zero time (t = 0). Measurement of absorbance was continued until the colour of β -carotene disappears in the control reaction (t=180 min) at 15 min intervals. A mixture prepared as above without β carotene served as blank. All determinations were carried out in triplicate. Dose-response relationships of antioxidant activity were determined at different concentrations. The antioxidant activity (AA) of the test samples was evaluated in terms of inhibition of bleaching of β -carotene using the following formula (Hidalgo et al., 1994).

AA = 100[1–(A0-At)/(A'0-A't)] where A0 and A'0 are the absorbance values measured at zero time of the incubation for test sample and control, respectively, At and A't are the absorbance measured in the test sample and control, respectively, after incubation for 180 min.

Linoleic acid emulsion system

Antioxidant activity in terms of lipid peroxidation inhibitory effect was determined using the thiocyanate method (Kikuzaki and Nakatani, 1993). 0.5 mL of test sample at different concentrations (100-500µg/mL) in 0.5mL of absolute ethanol was mixed with 0.5mL of 2.51% linoleic acid in absolute ethanol, 1mL of 0.05M phosphate buffer (pH 7), and 0.5mL of distilled water and placed in a screw capped tube, incubated in dark at 400C in an oven. The mixture prepared as above without test sample served as control. Aliquots of 0.1mL were taken at 12hrs during incubation and the degree of oxidation was measured by sequentially adding 9.7mL ethanol (75%), 0.1mL ammonium thiocyanate (30%) and 0.1mL ferrous chloride (0.02M in 3.5% HCl). After the mixture was rested for 3 min., the peroxide value was determined by monitoring absorbance at 500nm until the absorbance of the control reached the maximum. The antioxidant activity was calculated as percentage inhibition of peroxidation relative to the control using the formula:

$$\% \text{ inhibition} = 100 \quad \frac{\text{Sample abs at 48hr-sample abs. at 0 hr}}{\text{Control abs at 48hr-control abs. at 0hr}} * 100$$

Abs.= absorbance

Liver homogenate model system

The degree of lipid peroxidation was assayed by estimating the thiobarbituric acid-reactive substances (TBARS) as given by Yue et al., (1995). In brief, different concentrations of the test sample (100-500µg/mL) in water was added to the goat liver homogenate. Lipid peroxidation was initiated by adding 100µl of 15mM $FeSO_4$ solution to 3 mL of liver homogenate (final concentration is 0.5mM). After 30 min, 1mL of this reaction mixture was taken in a tube containing 1.5 mL of 10% TCA. After 10 mins tubes were centrifuged 3500 rpm for 10 mins, supernatant was separated and mixed with 1.5 mL of 0.67% TBA in 50% acetic acid. The mixture was heated in a hot water bath at 85°C for 30 min to complete the reaction. The intensity of pink colour complex formed was measured at 532nm. The % inhibition of lipid peroxidation was calculated by comparing the results of the test with that of controls not treated with the extracts. Quercetin was used as a positive control. The extent of inhibition of lipid peroxidation in liver homogenate by the test sample was calculated by the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control}) - (\text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Hemoglobin-induced lipid peroxidation system

Effect of the extract on hemoglobin-induced linoleic acid oxidation was determined by the method of Kuda et al., (2005). To 0.1 mL of test sample at different concentrations (100-500µg/mL), 0.025 mL linoleic acid in ethanol (0.1M) and 0.075 mL phosphate buffer (0.2 M, pH 7.2) were added, oxidation was initiated by addition of 0.05 mL of 0.08% hemoglobin, followed by incubation for 60 min at 37°C and oxidation stopped by the addition of 5 mL of 0.6% HCl/ethanol. The peroxide value of the reaction mixture (2mL) was measured by mixing with 0.02 mL of 20 mM $FeCl_2$ and 0.01 mL of 30% ammonium thiocyanate and reading the absorbance at 490 nm using Cyberlab double beam spectrophotometer. The percentage of inhibition of lipid peroxidation was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control}) - (\text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

3.2 ANTI MICROBIAL ACTIVITY

Agar well diffusion method Test microorganisms:

Four pathogenic micro-organisms, two bacteria- S.aureus, E.coli, and two fungi

P.notatum and F.oxysporum were obtained from Defence Food Research Laboratory (DFRL), Mysore. The micro-organisms were subcultured on the specific media recommended for different micro-organisms such as nutrient agar and incubated at 37°C. Antimicrobial activity of fractions of methanolic extract of coriander seeds was determined by following the agar well diffusion method (Okeke et al., 2001).

In this method, pure isolate of bacteria was subcultured on the nutrient agar medium for each microorganism at 37°C for 24 hrs, in case of bacteria and in case of fungi, it was subcultured in potato dextrose agar at 30°C for 48 hrs in case of fungi. A plate of each microorganism was taken and a minimum of four or five colonies were touched with the sterile loop and transferred into normal saline (0.9% NaCl) under aseptic conditions. A 100µl volume of both test sample and standard (100µg/mL) was propelled directly into the wells (in duplicates) of the inoculated nutrient agar media plates for each test organism. The plates were allowed to stand for 10min for diffusion of the extract to take place and incubated at 37°C for 24 hrs in case of bacteria and 30°C for 48 hrs in case of fungi (Aneja et al., 2009). Sterile DMSO served as negative control, ampicillin and nystatin served as positive controls. The antimicrobial activity, indicated by the formation of an inhibition zone surrounded the well containing the test sample. The experiments were performed in triplicate and the mean values of the diameter of inhibition zones (mm) with ± standard error of means were calculated.

3.3 ANTI PROLIFERATIVE ACTIVITY

Cell culture and treatment

The cell lines used in this study were maintained in monolayer in tissue culture petri dishes. Medium for all the cell lines was RPMI- 1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 µg/mL streptomycin and 2 mM L-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO2 at 37° C. The cultured cells were passaged twice a week, seeding at a density of 5 x 103 cells per well in 96 well plate before the day of experiment. Before the treatment with test compound cells were washed with PBS and fresh medium was added.

Cell proliferation assay

Cell proliferation was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide (MTT) staining as described by Mosmann (1983). Since the MTT colorimetric technique was developed, it has been applied to the quantification of anti tumor activity mediated by macrophages, LAK cells and NK cells against tumor cell targets as well as cell proliferation and drug sensitivity to tumor cells. The advantages, which it offers, include sensitivity, simplicity, rapidity and the avoidance of radioactivity. The MTT assay is based on the reduction of the tetrazolium salt, MTT, by viable cells. The NADH or NADPH generated in the living cells, convert the yellow form of the MTT salt to insoluble, purple formazan crystals. The absorbance of the formazan solution was measured spectrop-hotometrically after dissolving the crystals in an organic solvent (DMSO). Cells (5 x 103 cells per well) were incubated in 96-well plates in the presence or absence of methanolic extract of coriander seeds for 24 h in a final volume of 100 µl. At the end of the treatment, 20 µl of MTT (5 mg/mL in PBS) was added to each well and incubated for an additional 4 hours at 370C. The purple-blue MTT formazan precipitate was dissolved in 100 µl of DMSO. The activity of the mitochondria, reflecting cellular growth and viability, was evaluated by measuring the optical density at 570 nm using □l Quant Bio-tek Instruments, Inc. micro titer plate reader. Each concentration was tested in three different experiments run in four replicates.

Reverse phase high performance liquid chromatography (RP-HPLC) technique (pilot study):

Methanolic extract Sample cleanup was done to remove the impurities using a C18 Sep-Pak cartridge and 20 µl aliquots were analyzed by HPLC.

Preparation of standard solutions

Standard solutions of caffeic acid and chlorogenic acid were prepared by dissolving 10mg of the standards in methanol in 25mL volumetric flasks, sonicated and volume was made up to 25 mL with respective solvents to give concentration of 400ppm.

Standard stock solutions of two flavonoids, rutin and quercetin were prepared in methanol, at concentrations of 100ppm (10 mg of the standards were dissolved in 25mL methanol in volumetric flasks, sonicated and volume made up to 25 mL with methanol to give 400ppm, 2.5mL of stock solution was taken and made up to 10mL with methanol to give concentrations of 100ppm).

Procedure

Reverse phase high-performance liquid chromatography with C18 columns is the most popular technique for the analysis of polyphenols of the different foods. A UV- vis multiwavelength detector (SPD-20A) was used because all phenolic compounds show intense absorption in the UV region of the spectrum. This method used for the separation of caffeic acid and chlorogenic acid (320nm), rutin and quercetin (370nm) included mobile phase 0.5% formic acid: acetonitrile (ACN)(70:30) at a flow rate 0.9mL/min; column (VARIAN Pursuit XPs- C18 dimension 250 x 4.6mm) at 40^{o}C temperature. The identification of polyphenols was based on the comparison of their retention times with those of the standards [Defence Food Research Laboratory (DFRL), Mysore].

High performance thin layer chromatography (HPTLC)

High performance thin layer chromatography studies were carried out (Anchrom enterprises (l) P. Ltd., Mumbai) for methanolic extract of coriander seeds and the fractions to confirm the presence of different flavonoids.

Procedure

The sample was applied on Aluminium plate (E. Merck) pre-coated with Silica gel 60 F254 of 0.2 mm thickness. 5µL of the sample was applied on aluminium plate pre-coated with silica gel (60 F254 of 0.2 mm thickness) in a band-shaped of 1cm and then was air dried and run for 8cm height using a standardized solvent (ethylacetate: methanol: water 77:15:8) in a glass chamber. After air drying, the plate was visualized in UV 254 and 366 nm and comparison was done using different standards (apigenin, quercetin, rutin, chlorogenic acid and caffeic acid) with respect to the no of spots and the length of elution (Rf).

3.4 ANTI-INFLAMMATORY ACTIVITY

Inhibition of bovine serum albumin (BSA) denaturation

The *in vitro* anti-inflammatory activity of the test samples was determined using bovine serum albumin denaturation assay. To 1mL of various test samples of coriander seeds at different concentrations (100- 500 µg/mL), 1 mL of 1mM albumin solution in phosphate buffer (0.2M, pH 7.4) was added, incubated at 72° ± 1°C in a water bath for 10 min, cooled and the turbidity was measured at 660 nm. Percentage inhibition of denaturation was calculated from control where no test sample was added. Butylated hydroxy toluene (BHT) was used as a positive control. The percentage of inhibition was calculated from the following formula.

% Inhibition = $100(1-V_t/V_c)$

Where V_t = absorbance of test solution; V_c = absorbance of control solution

Inhibition of soybean lipoxidase (LOX) activity

Inhibition of lipoxygenase was studied using linoleic acid as substrate and soybean lipoxidase as enzyme using the method given by Shinde et al., (1999). To various test samples of coriander seeds at different concentrations (100-500 µg/mL) made up to 0.5 mL with 2M borate buffer (consisting of boric acid and NaOH, pH 9.0), 0.25 mL of lipoxidase enzyme solution (20,000U/mL) was added, incubated for 5 min at 25°C, 1.0 mL of linoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234nm. Control had all the reagents except the test sample. Quercetin was used as a positive control. The per cent inhibition was calculated from the following equation:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control}) - (\text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Inhibition of xanthine oxidase (XO) activity

Xanthine oxidase inhibitory activity was determined by the method given by Bondet et al.,

(1997). To 1mL of test sample at different concentrations (100-500 µg/mL), 2.9mL of 50mM phosphate buffer solution (pH 7.0), 100 µl of xanthine oxidase solution (0.4U/mL in phosphate buffer) and 100 µM xanthine in phosphate buffer were added, incubated at room temperature (24oC) for 3 minutes, uric acid produced was determined by measuring the absorbance at 295nm. The blank used was buffer and the control used was a solution containing xanthine and xanthine oxidase. Allopurinol was used as a positive control. The percent inhibition of xanthine oxidase activity was calculated using the formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control}) - (\text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

3.5 ANTI DIABETIC ACTIVITY

α-amylase inhibitory assay

α-amylase inhibitory assay was performed using the chromogenic 3,5-dinitro salicylic acid (DNSA) method (Miller, 1959). To 500 µl of test samples of coriander seeds at different concentrations (100-500 µg /mL), 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 500 µl Porcine Pancreatic Amylase (PPA) solution were added and incubated at 37°C for 10 min. After pre-incubation, 500 µl of 1% (w/v) starch solution in the buffer was added and incubated at 37°C for 15 min. The reaction was terminated with1.0 mL DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature, diluted and the absorbance was measured at 540 nm. The control PPA represented 100% enzyme activity and did not contain any test samples. To eliminate the absorbance produced by test samples, appropriate test sample controls with the sample in the reaction mixture except for the enzyme were also included. Quercetin was used as a positive control. The α-amylase inhibitory activity was calculated as follows:

% Relative enzyme activity = (enzyme activity of test/enzyme activity of control) x100; percentage inhibition of α-amylase activity = (100 − % relative enzyme activity).

α-glucosidase inhibitory assay

α-glucosidase inhibitory assay was performed according to Worthington Enzyme Manual (1993) with some modifications (Mc Cue et al., 2005). To 500 µl of various test samples of coriander seeds at different concentrations (100-500µg/mL), 1000µl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1 U/mL) was added and incubated at 250C for 10 min. After pre-incubation, 500 µl of 5mM p- nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added and incubated at 250C for 5 min. Before and after incubation, absorbance was recorded at 405 nm using double beam spectrophotometer and compared to a control containing 500 µl buffer instead of the test sample. Quercetin was used as a positive control. The α-glucosidase inhibitory activity (%) was calculated as follows:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control}) - (\text{Absorbance of test})}{\text{Absorbance of control}} * 100$$

4. RESULTS AND DISCUSSION

4.1 ANTIOXIDANT ACTIVITY

Total phenolic contents of the seeds of coriander extracts

The total phenolic contents in extracts obtained from the seeds of coriander are shown in Table 1. The highest content (0.91 g GAE/100g) was observed in extract of coriander seeds followed by coriander leaves (0.82 g GAE/100g). It was noticed that the seeds have higher amounts of phenolic compounds than the leaves. These results are consistent with that obtained by Al-Juhaimiand Ghafoor (2011.)

Reducing activity

The reducing activities of both the leaf and seeds of coriander are given in Table 1. No significant difference in reducing activities was detected between the herbal two anatomical parts extracted with methanol. However, a significant ($p < 0.05$) lower reducing activity of methanolic coriander seeds extracts was detected and this paralleled. The higher total phenolic content. The different phenolic compositions of the coriander herb likely accounts for the different reducing activities, obtained from methanolic extracts of coriander extract.

Table 1: The total phenolic contents in extracts obtained from the seeds and leaves of coriander in g GAE/100 g extract.

Extract	Phenolic contents (g GAE/100g)	Reducing activity
Coriander seeds	0.91 ±0.08	21.2 ±2.0
Coriander leaves	0.82 ±0.02	25.6 ±2.2

Each value is the Mean ± SD

Antiperoxidative effect (inhibition of lipid peroxidation) in:

β-carotene linoleate model system

Dose dependent inhibition of peroxidation in β carotene linoleic acid model system measured in terms of inhibition of bleaching of β carotene in the model system by methanolic extract and fractions of methanolic extract of coriander seeds was as presented in Table 6. Almost all the fractions exhibited good inhibition of bleaching of β-carotene in β-carotene linoleate model system. Among fractions, ethylacetate fraction exhibited maximum percent inhibitory activity (45-83%) as compared with all the other fractions at the concentrations ranging from 100 to 500 µg/mL. The inhibitory activity of ethyl acetate fraction was followed by hexane fraction (IC50 255 µg/mL), methanolic extract (IC50 260 µg/mL), benzene (IC50 270 µg/mL), n-butanol (IC50 277 µg/mL) and aqueous (IC50-350 µg/mL) fractions. Ethyl acetate fraction showed slightly lesser per cent inhibition (IC50112µg/mL) than the synthetic antioxidant BHT (IC50 102µg/mL). The anti-lipid peroxidative effect of methanolic extract and its fractions in β-carotene model system was positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.848$), total flavonoids ($r=0.721$), total flavonols ($r=0.773$) and tannins ($r=0.203$).

The mechanism of bleaching of β carotene is a free radical mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. β-carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β-carotene molecules (Jayaprakasha et al., 2001).

As β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and characteristic orange color, which can be monitored spectrophotometrically. The presence of different compounds can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system (Jayaprakasha et al., 2001).

The inhibitory effect of methanolic extract and its fractions on lipid peroxidation in β-carotene linoleate model system is actually the inhibition of bleaching of β- carotene by the lipid peroxides formed from linoleic acid. In the present investigation, the maximum inhibition exerted by ethyl acetate fraction followed by hexane fraction and other fractions of methanolic extract of coriander seeds indicates inhibition of lipid peroxidation in β-carotene linoleate model by the phytochemicals present in ethyl acetate fraction (phenolics, sterols and terpenoids and flavonoids and hexane fraction (essential oil components and flavonoids). The bioactive components present in coriander seeds by inhibiting and scavenging the radicals and/or inactivating the radicals by donating hydrogen or by donating an electron have controlled lipid peroxidation, thereby controlled bleaching of β-carotene as observed in this investigation.

Linoleic acid emulsion system

The inhibition of peroxidation of linoleic acid by methanolic extract and its fractions is given in Table 7. All fraction showed significant antiperoxidative capacity in a concentration dependent manner (100-500 µg/mL). Among all the fractions, ethyl acetate showed very good anti-peroxidative activity (47-79%) with the least IC50 value of 105 µg/mL, followed by benzene (IC50 179 µg/mL), n-butanol (IC50 194 µg/mL), methanolic extract (IC50 349 µg/mL), aqueous (IC50 367 µg/mL) and hexane (IC50380µg/mL) fractions. The synthetic antioxidant quercetin showed highest anti- peroxidative activity with a least IC50 97 µg/mL. The anti-lipid peroxidative effect of methanolic extract and its fractions in linoleic acid emulsion system was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.596$), total flavonoids ($r=0.793$), total flavonols ($r=0.138$) and tannins ($r=0.219$).

The antioxidant effect of various extracts in preventing peroxidation of linoleic acid can be measured by thiocyanate method. Linoleic acid gets oxidized generating linoleic acid hydroperoxides, which decompose to many secondary oxidation products (Hua-Ming et al., 1996). The oxidised products react with ferrous sulphate to form ferric sulphate, then to ferric thiocyanate of blood red colour. After incubation (120 hrs), the formation of peroxides will be stopped, due to non- availability of linoleic acid. Also, the intermediate products may be converted to stable end products. The non- availability of hydroperoxides, results in the stoppage of oxidation of ferrous sulphate resulting in decreased absorbance. In the presence of antioxidants, oxidation of linoleic acid will be slow. Hence, the colour development, due to formation of thiocyanate, will be slow.

In the present study, the compounds present in coriander seeds that are extracted into methanolic extract and fractions of methanolic extract of coriander seeds appear to inhibit the peroxidation of linoleic acid in the reaction system thereby, inhibited oxidation of ferrous sulphate caused by the peroxides. In the process, the maximum action exhibited by ethyl acetate fraction is a reflection of maximum amount of phenolics, flavonoids, steroids and terpenoids present in ethyl acetate fraction than all the other fractions as evidenced by the results of qualitative and quantitative (Table 2 and 3, Chapter 1) analyses of coriander seeds.

Table 2: Antiperoxidative effect of methanolic extract and its fractions in β-carotene linoleate model system

Concentration (µg/ml)	Me	He	Be	Ea	nBu	Aq	BHT
100	34.3±0.8	36.0±0.5	25.4±0.8	**44.7±0.7**	44.7±1.5	33.3±0.5	**48.6±0.6**
200	45.4±0.5	47.4±0.3	33.8±1.4	**56.3±0.1**	49.8±2.3	45.6±0.6	**55.8±0.8**
300	57.7±0.3	58.8±0.2	55.6±0.9	**67.5±0.4**	54.2±1.5	46.7±0.8	**67.7±1.4**
400	61.9±1.6	63.8±1.3	64.3±1.3	**79.5±0.7**	63.6±0.6	57.2±0.7	**72.9±1.6**
500	72.4±1.4	68.9±3.2	67.3±1.5	**82.8±1.5**	69.2±1.6	69.4±1.4	**88.3±0.6**
IC50 (µg/ml)	**260**	**255**	**270**	**112**	**277**	**350**	**102**

Values are mean ± SEM of three replicates, $p<0.001$
(Comparison among concentrations & among samples)

Table 3: Antiperoxidative effect of methanolic extract and its fractions in linoleic acid emulsion system

Concentration (µg/ml)	Me	He	Be	Ea	nB	Aq	Quercetin
100	22.4±0.2	39.6±0.8	44.3±1.4	**47.4±0.9**	45.3±2.8	36.2±0.6	**51.5±0.4**
200	34.5±1.5	43.7±0.5	55.7±0.4	**54.2±0.1**	51.4±1.0	42.4±0.1	**58.2±0.5**
300	46.3±2.4	47.3±0.8	57.0±0.1	**62.1±0.2**	64.5±1.2	48.3±0.4	**59.1±2.4**
400	57.2±1.4	52.6±0.5	58.8±0.7	**72.4±1.9**	68.1±1.1	54.5±0.5	**64.3±2.2**
500	62.8±0.3	56.8±0.2	69.2±0.3	**79.1±0.2**	72.5±0.1	59.2±0.6	**78.4±1.3**
IC50 (µg/ml)	**349**	**380**	**179**	**105**	**194**	**367**	**97**

Values are mean ± SEM of three replicates, $p<0.001$ (comparison among concentrations & among samples). The values are % inhibition of lipid peroxidation.
Me-methanolic extract; He-hexane fraction; Be-benzene fraction; Ea- ethyl acetate fraction; nBu-n-butanol fraction and Aq-aqueous fraction.

Liver homogenate model system

Fig. depicts the anti-lipid peroxidative effects of methanolic extract and its fractions in FeSO4 - induced lipid peroxidation in goat liver homogenate as a model. Methanolic extract of coriander seeds and its fractions significantly ($p<0.05$) inhibited FeSO4-induced lipid peroxidation in a concentration dependent manner (100-500 µg/mL). The highest inhibitory activity was shown by ethyl acetate fraction (50 to 73%) (IC50 100 µg/mL) followed by benzene (IC50 296 µg/mL), n-butanol (IC50 364 µg/mL), hexane (IC50475 µg/mL) fractions, methanolic extract (IC50 501 µg/mL) and aqueous fraction (IC50 507 µg/mL).

Positive correlation ($p<0.01$) was observed between anti-lipid peroxidative effect of methanolic extract and its fractions in liver homogenate with the levels of total phenolics ($r=0.723$), total flavonoids ($r=0.786$), total flavonols ($r=0.537$) and tannins ($r=0.123$) in the methanolic extract of coriander seeds and fractions (Table 3). Mammalian cells have evolved interrelated antioxidant defense mechanisms, which minimize the injurious events that result from toxic chemicals and normal oxidative products of cellular metabolism (Khajuria, 1997). Biological membranes are a rich source of polyunsaturated fatty acids (PUFA) that are susceptible to lipid peroxidation in the presence of metal ions and other pro-oxidants (Gutteridge and Halliwell, 1994). Lipid peroxidation (LPO) has been identified as one of the basic reactions involved in free radical-induced cellular damages. A number of toxic compounds are generated during this process viz. TBARS which are produced as by- products of lipid peroxidation that occurs in the hydrophobic core of biomembranes (Fraga et al., 1987).

In the present investigation, maximum inhibition of TBARS that are formed during peroxidation of lipids, exerted very efficiently by ethyl acetate fraction, followed by benzene fraction indicates inhibitory effect of bioactive compounds (polyphenolics, flavonoids and flavonols) in these two fractions of methanolic extract of coriander seeds as evidenced by qualitative and quantitative

analysis. This is further strengthened by the highest inhibitory effect of ethyl acetate fraction having maximum amount of phenolics (Table 1 and 3, Chapter 1). By inhibiting lipid peroxidation, coriander seeds proved themselves to be antiperoxidative which can protect biological membranes from the effect of pro-oxidants as well as oxidative deterioration of polyunsaturated fatty acids present in biomembranes.

Hemoglobin-induced lipid peroxidation system

Fig. 9 depicts inhibition of hemoglobin induced LPO by methanolic extract and fractions of coriander seeds. All the fractions exhibited moderate ($p<0.001$) inhibitory activity ranging from 31% to 78%. Among these fractions, ethyl acetate fraction exhibited the maximum inhibitory activity with IC50 value 95 µg/mL. Methanolic extract and n-butanol fraction, though inferior to ethyl acetate fraction, exhibited good inhibitory activity against LPO with IC50 value 179 µg/mL and 181µg/mL respectively. The lowest inhibition was exhibited by aqueous fraction of methanolic extract of coriander seeds with IC50 value 307 µg/mL. Inhibition of hemoglobin-induced lipid peroxidation of methanolic extract and its fractions was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.790$), total flavonoids ($r=0.885$), total flavonols ($r=0.561$) and tannins ($r=0.005$).

Peroxyl radicals are formed by a direct reaction of oxygen with alkyl radicals, and these radicals attack biomolecules such as lipids to initiate free radical chain reactions and cause lipid peroxidation (Upston et al., 2002). In the present study, hemoglobin catalyzed peroxidation was inhibited by all the fractions and methanolic extract of coriander seeds in individualized and concentration dependant manner. However, ethyl acetate fraction exhibited better inhibition of LPO as compared to all other fractions and methanolic extracts of coriander seeds. As ethyl acetate fraction contained maximum amount of phenolic compounds, inhibition of LPO by ethyl acetate is attributed to the polyphenolic compounds present in coriander seeds that are extracted by ethyl acetate. Owing to the lesser quantity of polyphenols, other fractions showed decreased per cent inhibition of LPO, while methanolic extract and n-butanol fraction, though lesser in polyphenol content, contains other phytochemicals which could contribute to the inhibition of hemoglobin-induced lipid peroxidation.

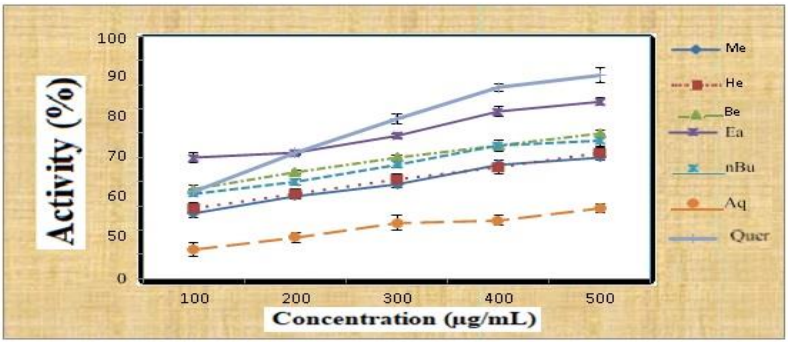

Fig 1: Antiperoxidative effect of methanolic extract and its fractions in liver homogenate model
Values are mean ± SEM of three replicates, $p<0.05$ (comparison among concentrations & among samples

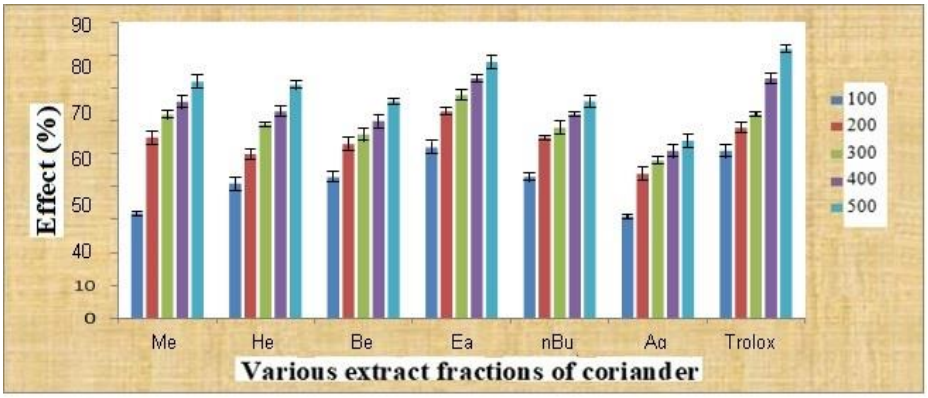

Fig 2: Antiperoxidative Effect (%) of methanolic extract and its fractions in Hb-induced model system
Values are mean ± SEM of three replicates, $p<0.001$(comparison among concentrations & among samples)
Me-methanolic extract; He-hexane fraction; Be-benzene fraction; Ea- ethyl acetate fraction; nBu-n-butanol fraction and Aq-aqueous fraction

DPPH radical-scavenging activity

Very few studies have described the radical-scavenging activities of coriander towards the hydrophobic DPPH radical. In general, the seed component of coriander herb scavenged significantly ($p < 0.05$) more DPPH radical than did the other parts of herb. Coriander seed methanol extract gave significantly ($p < 0.05$) greater DPPH radical-scavenging activity. The greater DPPH radical-scavenging activity observed from seed extracts was not directly related to total content of phenolic compounds.

4.2 ANTI MICROBIAL ACTIVITY

General Components of *Coriandrum Sativum* have antibacterial activities, noted against both foodborne Gram-positive and Gram-negative bacteria, as well as yeasts and molds. It has been suggested that antimicrobial activity of *Coriandrum Sativum* herb is due to the presence of phenolic compounds containing isopropyl functional a polar group.

The findings depicted in Table and Fig. indicated that methanolic extract and its fractions possessed inhibitory effect against tested bacteria viz. E. coli and S. aureus and both tested fungal strains P. notatum and F.oxysporum. The ethyl acetate fraction showed larger zones of inhibition, viz. 17.2 mm and 15.4 mm against E. coli and S. aureus respectively indicating bacteriostatic activity.

Table 4: Agar well diffusion test for antibiotics, methanolic extract and its fractions

Micro- organisms	Me	He	Be	Ea	nBu	Aq	Positive control
							Ampicillin
E. coli	6.8±0.03	4.9±0.07	5.5±0.07	**17.2±0.04**	7.9±0.12	5.9±0.17	**18.3±0.05**
S. aureus	6.9±0.02	4.7±0.05	5.8±0.04	**15.4±0.03**	8.4±0.05	6.7±0.13	**16.7±0.07**
							Nystatin
F.oxysporum	7.8±0.07	5.7±0.02	6.5±0.07	**10.9±0.06**	8.3±0.09	6.7±0.06	**12.6±0.09**
P.notatum	7.5±0.06	5.6±0.04	6.3±0.22	**11.5±0.03**	9.2±0.16	6.9±0.12	**13.1±0.05**

Values, including diameter of the well (8mm), are means of the three replicates ±SEM, ($p<0.001$) (comparison among concentrations & among samples) indicates significantly different from control.
Me-methanolic extract; He-hexane fraction; Be-benzene fraction; Ea- ethyl acetate fraction; nBu-n- butanol fraction and Aq-aqueous fraction

The inhibition zone produced against F.oxysporum was 10.9 mm and 11.5 mm against P.notatum. Among the fractions of methanolic extract of coriander, ethyl acetate fraction exhibited significant antibacterial and antifungal activity by means of inhibiting the growth of

respective organisms. However, the inhibitory effects of ethyl acteate fraction was lesser than that of standard drugs. A positive correlation ($p<0.01$) was observed between the levels of total phenolics ($r=0.740$), total flavonoids ($r=0.606$), total flavonols ($r=0.593$) and tannins ($r=0.117$) in the methanolic extract of coriander seeds and fractions with the antimicrobial activity.

The antimicrobial activity exhibited by methanolic extract and its fractions is attributed to phenolic compounds such as flavonoids, tannins as reported by Edeoga et al. (2005) that the bioactive constituents in plants such as phenolic compounds- tannins, flavonoids possess antimicrobial properties.

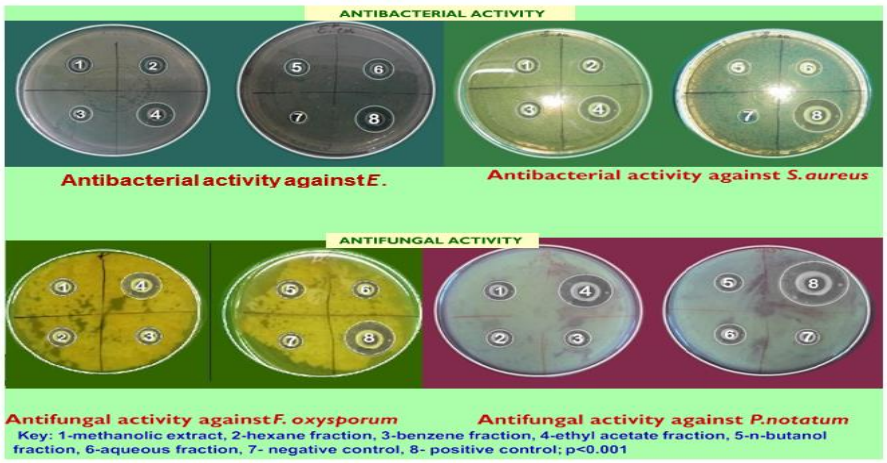

Fig. Plates showing agar well diffusion test for antibiotics, methanolic extract and its fractions

4.3 ANTIPROLIFERATIVE EFFECT (pilot study)

The antiproliferative effect of methanolic extract of coriander seeds was depicted in Fig . Methanolic extract of coriander seeds exhibited antiproliferative effect in HEP-G2 cell lines in a concentration dependent manner (5-100 µg/mL) with IC50 35 µg/mL. The results obtained in the present study indicated ethyl acetate fraction of methanolic extract of coriander seeds to possess maximum therapeutic potential. Hence, methanolic (multicomponent) extract and the ethyl acetate fraction were sub- fractionated using column chromatographic technique. Totally about 50 fractions were collected from methanolic extract and ethyl acetate fraction. Among all the sub- fractions collected, four major sub-fractions of methanolic extract giving single compound over TLC plates were designated as compound M1, M2, M3, M4 and the sub-fractions of ethyl acetate fraction were designated as E1, E2, E3 and E4 respectively and tested for therapeutic potential.

Therapeutic potential of sub-fractions (M1-M4, E1-E4) (*in vitro*)

DPPH radical scavenging activity, iron chelating capacity and α-glucosidase inhibitory activity respectively of the sub-fractions M1-M4 of methanolic extract and E1-E4 of ethyl acetate fraction of coriander seeds. With respect to all the mentioned parameters, E2 exhibited better response as compared to the sub-fractions of ethyl-acetate fraction E1-E4 as well as sub-fractions of methanolic extract M1-M4. E2 exhibited lesser percent performance.

4.4 ANTI-INFLAMMATORY ACTIVITY

Inhibition of bovine serum albumin (BSA) denaturation

Methanolic extract and all the other fractions of coriander seeds exhibited moderate anti-inflammatory activity measured in terms of inhibition of denaturation of BSA, which is depicted in Fig.. All the fractions exhibited significant anti- inflammatory activity, among which, ethyl acetate fraction exhibited the highest activity (IC50 184µg/mL) but lesser than that of the synthetic antioxidant Trolox (IC50 95µg/mL). The other fractions exhibited lesser per cent inhibition (<70%) at all the concentration especially at 500 µg/mL concentration with the IC50 values ranging from 293 µg/mL to 587 µg/mL, showing less anti-inflammatory activity, among which hexane fraction exhibited the lowest activity. A positive correlation ($p<0.01$) was noticed between inhibition of bovine serum albumin denaturation with the levels of total phenolics (r=0.714), total flavonoids (r=0.777), total flavonols (r=0.664) and tannins (r=0.469).

The use of *in vitro* anti-denaturation (stabilization) effects of heat tested (immunogenic) bovine serum albumin is one of the methods used to test the anti- inflammatory activity of the test compound. When BSA is heated and is undergoing denaturation, it expresses antigens associated to type III hypersensitive reaction and which are related to diseases such as serum sickness, glomerulo nephritis, rheumatoid arthritis, systemic lupus erythematosus etc. The inhibitory activity involves stabilization of denaturation process and thereby prevents the occurrence of the above mentioned degenerative disorders (Kannan, 2006). In the present study, methanolic extract of coriander seeds and all the fractions protected BSA against denaturation (though to a lesser extent). Ethyl acetate fraction ranking first in this assay is because of maximum polyphenolics (Table 3, Chapter 1) in ethyl acetate fraction as polyphenols are reported to possess such activity (Williams et al., 2008).

Inhibition of soyabean lipoxidase (LOX) activity

The present study revealed concentration dependent ($p<0.001$) inhibitory effect of soyabean lipoxidase activity by methanolic extract of coriander seeds and fractions, ethyl acetate being the most effective (IC50 97µg/mL), followed by n-butanol (IC50 105 µg/mL), benzene (IC50 195 µg/mL), methanolic extract (IC50 362 µg/mL), hexane (IC50 369 µg/mL) and aqueous (IC50 390 µg/mL) fractions. Ethyl acetate fraction of coriander seeds exhibited higher inhibition than the synthetic antioxidant, quercetin (IC50 99 µg/mL) (Fig. 11). The inhibition of soyabean lipoxidase activity was positively correlated ($p<0.01$) with the levels of total phenolics (r=0.520), total flavonoids (r=0.754), total flavonols (r=0.436) and tannins (r=0.410).

Lipoxygenases (LOX) are members of a class of non-heme iron-containing dioxygenases that catalyze the addition of molecular oxygen to fatty acids containing a cis-1, 4-pentadiene system to give unsaturated fatty acid hydroperoxides. LOXs are sensitive to antioxidants as most of the antioxidants inhibit formation of lipid hydroperoxides by scavenging lipidoxy or lipid peroxy radicals. In mammals, lipoxygenases carry out the first step in the arachidonic acid cascade (Gaffney, 1996). 5- and 15-LOXs lead to biologically active lipoxins, whereas 5-LOX leads

to 5, 6- epoxy-leukotrienes which are involved in a variety of inflammatory responses, including neutrophil chemotaxis, vascular permeability, and smooth muscle contraction (Samuelsson et al., 1987).

In the present investigation, coriander seeds very effectively inhibited the activity of soyabean lipoxidase. Antioxidants present in coriander seeds (Table 1 to 3, Chapter 1) can scavenge lipidoxy and lipidperoxy radicals and inhibit the formation of lipid hydroperoxides unavailable to the catalytic activity of lipoxidases which catalyses a cascade of reaction in lipid peroxidation resulting in a variety of inflammatory responses. In the present study, by inhibiting lipoxidase activity, the phytochemicals proved themselves to be effective anti-inflammatory agents. This can be supported by the maximum inhibitory activity exhibited by ethyl acetate fraction, rich in phytochemicals as well as inhibition of lipid peroxidation shown by ethyl acetate fraction in various model systems in our study.

Inhibition of xanthine oxidase (XO) activity

Methanolic extract of coriander seeds and all the fractions showed concentration dependent (100-500 µg/mL) xanthine oxidase inhibitory activity ($p<0.001$). Out of the samples examined, n-butanol fraction (IC50 80µg/mL) showed maximum (89%) inhibition at 500µg/mL whereas, all the other fractions showed lesser inhibition (42- 77%) with IC50 values ranging from 206 to 591 µg/mL and aqueous fraction is the least effective. n-butanol fraction, was not only found to be most effective among others, but also had lesser IC50 value than the commercial xanthine oxidase inhibitor, allopurinol (IC50 106µg/mL), indicating more efficiency than allopurinol in inhibiting xanthine oxidase (Table 8). There was a positive correlation ($p<0.01$) between inhibition of xanthine oxidase activity and the levels of total phenolics ($r=0.577$), total flavonoids ($r=0.802$), total flavonols ($r=0.618$) and tannins ($r=0.483$).

Xanthine oxidase, a cytoplasmic enzyme, is a flavoprotein, which catalyses the oxidation of hypoxanthine to xanthine and generates superoxide and uric acid (Cheng et al., 2003). Studies have shown that xanthine oxidase inhibitors may be useful for the treatment of gout, which is caused by the generation of uric acid and superoxide anion radical (Lin et al., 2001). Hence, phytochemicals or plant extracts, which inhibit the formation of uric acid and superoxide anion regeneration by the enzymatic pathway, would be beneficial in the prevention/treatment of gout.

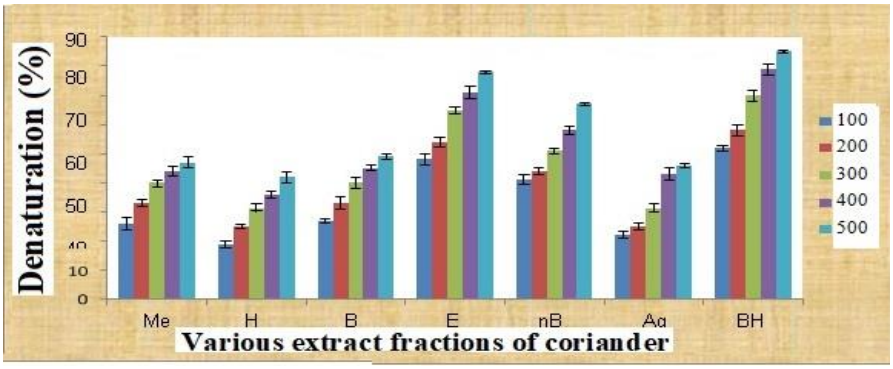

Fig. Inhibition of bovine serum albumin Denaturation (%) by methanolic extract and its fractions

Values are mean ± SEM of three replicates, $p<0.05$(comparison among concentrations & among samples)

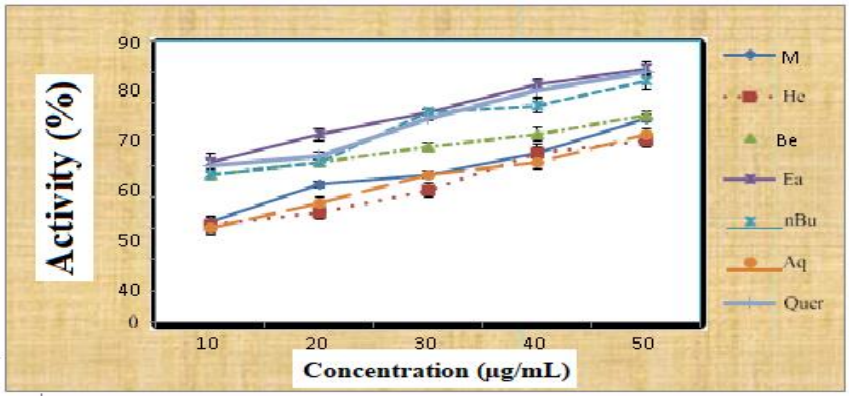

Fig. Inhibition of soyabean lipoxidase Activity (%) by methanolic extract and its fractions

Values are mean ± SEM of three replicates, $p<0.001$(comparison among concentrations & among samples)

Me-methanolic extract; He-hexane fraction; Be-benzene fraction; Ea- ethyl acetate fraction; nBu-n-butanol fraction, Aq-aqueous fraction and Quer-quercetin.

Table 5: Inhibition of xanthine oxidase activity (%) by methanolic extract and its fractions

Concentration (µg/ml)	Me	He	Be	Ea	nBu	Aq	Allopurinol
100	15.8±0.4	25.8±0.2	22.1±0.1	30.2±0.7	**62.5±0.5**	7.2±0.1	**47.2±0.1**
200	28.8±0.8	32.9±0.5	30.1±0.4	48.4±0.2	**74.3±1.2**	16.8±0.5	**56.8±0.5**
300	34.1±0.6	41.5±0.4	42.6±0.9	59.4±0.3	**77.5±0.2**	18.3±0.8	**68.3±0.8**
400	44.1±0.5	52.2±0.9	51.5±0.4	68.2±0.7	**82.7±0.6**	26.6±0.4	**76.6±0.4**
500	58.4±0.7	55.3±0.6	68.2±0.2	77.3±0.5	**89.3±0.2**	42.3±1.6	**82.3±1.6**
IC50 (µg/ml)	**428**	**383**	**388**	**206**	**80**	**591**	**106**

4.5 ANTI DIABETIC ACTIVITY

α-amylase and α-glucosidase inhibitory activities

Methanolic extract of coriander seeds and fractions inhibited significant ($p<0.05$) α-amylase and α-glucosidase, the carbohydrate hydrolysing enzymes in a concentration dependent manner (Table 9 and Fig. 12) with the inhibition ranging from 12% to 87%. Among the different fractions, ethyl acetate fraction displayed the highest α-amylase inhibitory activity with IC50 value of 81µg/mL and hexane fraction showed the lowest activity with IC50 value of 587µg/mL. Besides, ethyl acetate fraction showed better inhibition than the synthetic antioxidant, quercetin (IC50 118µg/mL). At 500 µg/mL concentration the sequence of inhibition is as follows:

Ethyl acetate (87%) > n-butanol (72%) > benzene (70%) > methanolic extract (68%) > aqueous (46%)> hexane fraction (43%). The α-amylase inhibitory activity was positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.740$), total flavonoids ($r=0.885$), total flavonols ($r=0.554$) and tannins ($r=0.242$).

Inhibition of α-amylase activity is considered to be an effective strategy for controlling diabetes, by diminishing the absorption of glucose (Hara and Honda, 1990) thereby decreasing post prandial hyperglycemia (Shobana et al., 2009).

In this investigation, ethyl acetate fraction also inhibited the activity of α- glucosidase (IC50 72µg/mL) higher than the other fractions and even quercetin (IC5093µg/mL) indicating the presence of inhibitors of the enzyme in coriander seeds.

Table 6: α-amylase inhibitory activity (%) of methanolic extract and its fractions

Concentration (µg/mL)	Me	He	Be	Ea	nBu	Aq	Quercetin
100	12.4±0.2	19.0±0.4	37.1±0.2	**61.8±0.6**	48.6±0.1	12.2±0.1	**42.5±0.7**
200	20.3±0.9	25.2±0.2	43.8±0.4	**67.4±0.2**	50.8±1.3	19.1±0.2	**54.5±1.2**
300	33.7±0.7	31.8±0.4	50.6±0.4	**72.9±0.1**	65.2±0.2	24.3±0.7	**60.5±0.6**
400	44.7±0.2	36.4±2.3	55.3±1.4	**81.2±0.7**	68.7±0.1	32.4±0.6	**62.4±1.2**
500	68.2±1.2	42.6±0.1	69.5±2.4	**86.8±1.5**	72.1±0.9	46.2±1.7	**69.7±0.3**
IC50	**447**	**587**	**296**	**81**	**197**	**541**	**118**

Values are mean ± SEM of three replicates, $p<0.001$ (comparison among concentrations & among samples)
Me-methanolic extract; He-hexane fraction; Be-benzene fraction; Ea- ethyl acetate fraction; nBu-n-butanol fraction and Aq-aqueous fraction

α- glucosidase inhibitory activity was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.684$), total flavonoids ($r=0.841$), total flavonols ($r=0.466$) and tannins ($r=0.295$). Presence of good amount of phenolics, flavonoids, tannins, sterols, terpenoids and saponins in ethyl acetate fraction of coriander seeds (Table 1 to 3, Chapter 1) as seen by qualitative and quantitative analyses support the role of these phytochemical in influencing the activity of the enzyme.

Coriander seeds can be good therapeutic agents as they possess phytochemicals viz. flavonoids that efficiently inhibit the activity of α-amylase and α-glucosidase. Our observation can be supported by Dukes database indicating the presence of phytochemicals like carvacrol and minerals like copper and zinc reported to possess anti-diabetic activity. Inhibition of carbohydrate hydrolysing enzyme has been used to treat type 1 and type 2 diabetes(David and Bell, 2004) and hence, coriander seeds may contribute to the treatment of diabetes possessing inhibitors of α- amylase and α- glucosidase enzymes.

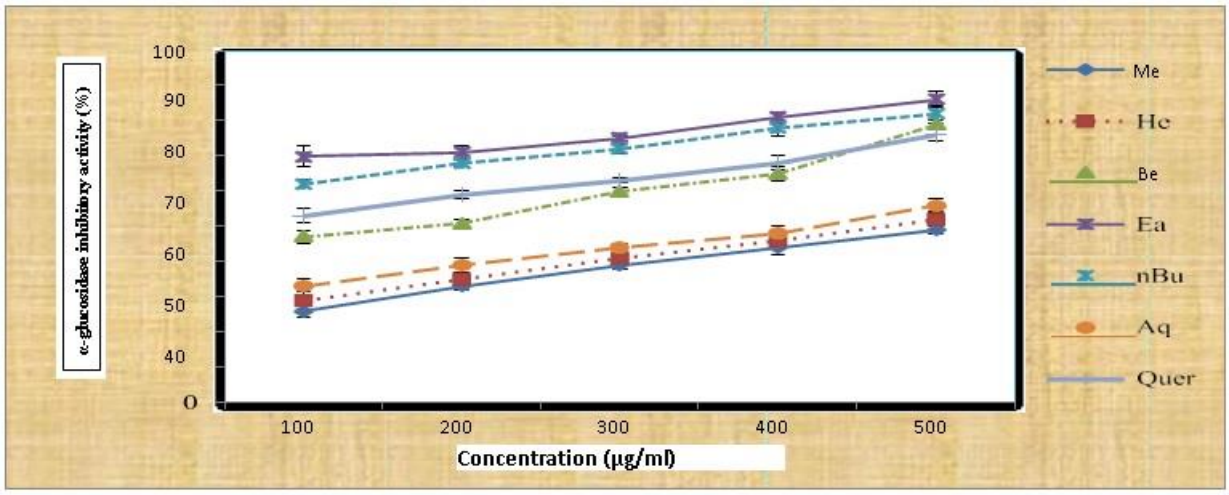

Fig. α-glucosidase inhibitory activity (%) of methanolic extract and its fractions

Values are mean ± SEM of three replicates, $p<0.05$ (comparison among concentrations & among samples).

5. CONCLUSION

In order to characterize and analyze antioxidant activity of plant extracts, it is desirable to subject the extracts for the tests that evaluate the range of activities. The antioxidant activity of the test sample was evaluated β-carotene linoleate, Linoleic acid emulsion, Liver homogenate and Hemoglobin-induced lipid peroxidation system.

Antioxidant rich plant extracts serves as sources of nutraceuticals that reduce the oxidative stress and therefore prevent or slow down the degenerative diseases. In the present study, an effort has been made to explore the antioxidant property of the methanol extracts of selected *Coriandrum Sativum L* plants.

All the extracts demonstrated significant antioxidant properties as determined by the scavenging assay, reducing power assay and total antioxidant capacity. However, the methanol extract of *Coriandrum Sativum L* exhibited antioxidant potential. The present study indicates the potential of the extracts as a source of natural antioxidants with potential applications to reduce oxidative stress with consequent health benefits.

In this present study, the antibacterial activity of the herb coriander seeds has been planned to prove its activity against various species of microbes by spread plate method. This study has been very beneficial as it has given an evidence of utilizing this species against the particular microbes satisfactorily.

The antiproliferative effect of methanolic extract of coriander seeds was exhibited antiproliferative effect in HEP-G2 cell lines in a concentration dependent manner (5-100µg/mL) with IC50 35µg/mL. The results obtained in the present study indicated ethyl acetate fraction of methanolic extract of coriander seeds to possess maximum therapeutic potential.

Coriander seeds can be good therapeutic agents as they possess phytochemicals viz. flavonoids that efficiently inhibit the activity of α-amylase and α-glucosidase.

6. AUTHOR CONTRIBUTION STATEMENT

Ala Vishnu Priya, had completed this work under the supervision of Santhosh Kumar. All authors together contributed to this research work.

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