INVITRO INVESTIGATION OF METHANOLIC EXTRACT OF NIGELLA SATIVA (BLACK CUMIN)

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ABSTRACT

Black cumin botanical name is *Nigella sativa*. The most common name in Indian for this spice is kala jeera. Black cumin is considered as a miracle herb due to its wonderful power of healing. The black cumin seeds have been widely used for the treatmentof different diseases ailments. A seed exhibits a wide spectrum of biological and pharmacological activites, which include antihypertensive, antidiabetic, diuretics, anticancer immunomodulator, analgesic, antioxidant, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, pulmonaryprotective, nephro-protective, gastro-protective, antioxytocic and anticonvulsant properties. Due to its miraculous power of healing, *Nigella sativa* has got the place among the top ranked evindence based herbal medicine.

Keywords: *Nigella sativa*, Phytochemical, antioxidant, antimicribial activity

INTRODUCTION

The dried seeds of the herb are called as cumin seeds and locally knownas "jeera". Cumin seeds are oblong in shape, longitudinally ridged, and brown-black-grey incolor. Cumin seeds, in both whole and ground form, are used in the cuisines of manydifferent cultures from ancient time. The seeds of cumin have gained theirplace as main spice in Indian, African, Chinese, Cuban and Mexican cuisines, due totheir distinctive popular aroma. It has a spicy-sweet aroma with pungent, powerful, sharpand slightly bitter flavour. It is mainly used to spice and season variety of dishes likecurries, chutneys, masalas etc. It is extensively used in India to season dishes. Due to its numerous medicinal properties, jeera is used as an ingredient in manyhome remedies and ayurvedic preparations. The strong aroma of jeera or cumin seeds isdue to the presence of compound cuminaldehyde.

Cumin is widely used in Ayurvedicmedicine as a stimulant, carminative, and astringent and for the treatment of dyspepsia, diarrhea and jaundice. This spice has been praised as jeera, jarana and ajaaji for itsmedicinal qualities in ayurvedic texts. These names refer to its carminative and digestiveproperties. According ayurvedic principles these seeds balance vata and kapha. It also has stomachic, diuretic, emmanogogic and antispasmodic properties. Cumin shows several pharmacological actions. The anticarcinogenic property of cumin was investigated. The cumin seeds (*Cuminum cyminum* Linn) were reported to decrease the incidence of both neoplasia and hepatomas (Munde-Wagh et al., 2012). Cumin showed immunomodulatory properties in normal and Cyclosporine-A induced immune-suppressed animals. In both the groups *cumin* significantly increases T cells (CD4 and CD8) count and Th1 predominant immune response in a dose dependent manner. Hence, the immunomodulatory activity of *Cumin*was supposed through modulation of T lymphocytes expression (Aksoy et al., 2013). Methanolic extract of *Cumin*was reported to inhibit the ovarectomyinduced bone loss *i.e*., antiosteoporotic activity in rats (Patel *et al*., 2012). Extracellular application of the fruit essential oil of *Cumin* reduces the epileptic form activity induced by pentylenetetrazol (PTZ) in a dose dependentmanner (Li *et al*., 2011). The antibacterial and antifungal activity of cumin was greatly exploited in various studies. The activity was particularly highagainst the genera Clavibacter, Curtobacterium, Rhodococcus, Erwinia, Xanthomonas,Ralstonia, and Agrobacterium, which are responsible for plant or cultivated mushroomdiseases worldwide (Chandran *et al*., 2013). A biologically active compound 1-(2-Ethyl,6-Heptyl) Phenol (EHP), extracted by benzene from cumin shows inhibitoryactivity against a number of fungal pathogens. It also exhibited antitumor activity againstsix types of tumor cell lines *viz.*, HEPG2, HELA, HCT116, MCF7, HEP2 and $CACO_2$(Rajamanikandan *et al.* 2011).

Nigella sativa also known as “Black cumin” is one of the economically and medicinally important plant species. Black cumin essential oil is used in pharmaceutical, food sweetening, soft drink, food and hygiene industries. Ripe black cumin fruits contain an essential oil rich in monoterpene aldehydes with the main components as cuminaldehyde, p-mentha-1,3-dien-7-al and p-mentha-1,4-dien-7-al; terpene hydrocarbons are the main components of fruits collected in the wild or harvested unripe (γ-terpinene, p-cymene, β-pinene, limonene. Medicinal plants are the exclusive source of drugs for majority of the World’s population. Bioactive compounds extracted from these plants are used as food additives, pigments, dyes, insecticides, cosmetics, perfumes and fine chemicals (Motlhanka,

2008). These compounds belong to a group collectively known as secondary metabolites which represent an important source of pharmaceuticals (China et al., 2012).

The essential oil of *Cumin* decreased biofilm formation ability and plasmid integrity of *Klebsiella pneumonia*. Hence the essential oil of cumin seed may be useful to treat bacterial infections. The antibacterial property of cumin essential oil was postulated due to the cuminaldehyde (Karumari et al., 2014). Moreover, *cumin* oil exhibited higher antibacterial and antifungal activities with a high effectiveness against Vibrio spp. Strains (Padmalochana and Rajan 2014) and also bactericidal effects against *Bacillus cereus* (Powthong *et al*. 2013). The essential oil of Cuminum shows antioxidant activity and antimicrobial activity against *E. coli, S. aureus,* and *S. faecalis* (Wadood *et al*. 2013). Cumin oil and its isolated compound cumin aldehyde exhibited a significant antimicrobial activity (Oyaizu *et al*. 2002).

The free radical scavenging and antioxidant activity of *Cumin*was also exploited in various *in vitro* studies and shows good antioxidant activity (Braca et al., 2002). *Cumin*also shows good *in vivo* antioxidant activity in prolonged treated albino rats (Kunchandy and Rao, 1990). The anti-hyperglycemic activity of cumin and its use in secondary complications associated with diabetes mellitus was evaluated in various studies.

There was a significant reduction in plasma and tissue cholesterol, phospholipids, free fatty acids and triglycerides. It also prevented a decrease in body weight. Moreover, *Cumin*s upplementation was foundto be more effective than glibenclamide in the treatment of diabetes mellitus (Fang et al., 2002). The anti-hyperglycemic and hypolipidemic effect of cumin seeds was also studied in type 2 diabetic patients. Significant glycemic control was observed in patients with cumin seed therapy also there was a significant decrease in levels of cholesterol (47%), triglycerides (26%), plasma free fatty acids (4%), phospholipids (9%), LDL-cholesterol (5%), VLDL-cholesterol (26%) and atherogenic index (21%) while significantly increase in HDL-cholesterol (10%).

Cumin and glibenclamide improved antioxidant status in kidney and pancreas of diabetic rats. Diabetic rats arereported to show an increase in rat tail tendon collagen, glycated collagen, collagenlinked fluorescence and reduction in pepsin digestion. Treatment with Cumin significantly improved these parameters when compared to diabetic control andglibenclamide group. The cumin shows better effect in controlling oxidative stress andinhibiting the AGE formation, which are implicated in the pathogenesis of diabeticmicrovascular complications.

Phytochemical activity

Black cumin which is one of the micracuberus plant having multifarious roles in its phytochemical constituents and nutritional values, treating digestive tract conditions including gas, colic, diarrhea, dysentery, constripation and haemorrhoids. So powdered black cumin seed was used for crude oil extracts by using different solvents. In this manner, the result of investigation of qualitative phytochemical analysis conducted on the crude cumin seeds extract revealed the presence of bioactive compound in the methanol extracts which are known to exhibit medical as well as physiological activities. In identification and separations were taken by TLC and CC. Finally, four potentially active phytochemical have been obtained from methonal extracts are alkaloids, phenol, flavonids and steroids Black cumin is considered as a miracle herb due to its wonderful power of healing. The black cumin seeds have been widely used for the treatment.

Standardization is defined as best technical application consensual wisdom inclusive of processes for selection in making appropriate choices for ratification coupled with consistent decisions for maintaining obtained *standards*. This view includes the case of 'spontaneous standardization processes', to produce de facto standards. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in national health care programs because these drugs are easily available at low cost, safe and people have faith in them. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilise compounds with similar polarity. Phytopharmaceutical and secondary plant product of medicinal importance such as alkaloids, glycosides, terpenoids, flavonoids and lignans.

The medicinal and pharmaceutical properties of plants are due to the type of chemical substance they produce and store. These include compounds that are utilized as food by man and other animals and also other compounds that exert physiological effects on them. This second group of chemical substances often referred to as secondary metabolites, give plants their therapeutic properties. The usual term used to refer to these various chemical substances present in plant is "constituents". The constituent which possess pharmacological properties are called 'active constituents'. Phytochemistry is concerned with the chemical

study of these plant constituents (Wadood et al., 2013). The test used in phytochemical screening should be simple, standard and one should be aware of false positive result and hence the need for carrying out confirmatory tests. The chemical constituents that are of medicinal importance are mainly the secondary metabolites, and the examination of the chemical constituents of the plant can only reveal those compounds that have accumulated to some extent at a specific organ of a given plant. The presence or absence of such compounds depends largely on the extent of accumulation, the amount of plant material used and the analytical method employed.

The plants may be consisting of many chemical constituents like alkaloids, glycosides, carbohydrates, proteins, steroids, tannins, saponins, flavonoids etc. These chemical constituents are called as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of these secondary metabolites in aqueous, ethanol and acetone extracts was subjected to colored reactions of chemical tests and intensity of reactions. Various tests have been executed to find out the presence of phytochemical constituents in the different solvent derived leaf extract.

Antioxidant activity

Antioxidants have been recognized to exhibit protecting functions against oxidative damage and are associated with reduced risk of chronic diseases shows DPPH free radical scavenging activity of AgNPs at suitable concentration. When compared to standard (ascorbic acid) and *black cumin* extract, respectively.

Antioxidants can prevent undesirable oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as O_2 scavengers. Restriction in the use of same synthetic antioxidants is being imposed because of their carcinogenicity. There are some synthetic antioxidant compounds, such as butylatedhydroxytoluene (BHT) and butylated fruiting is almost throughout the year chiefly during hydroxyanisole (BHA), commonly used in processing foods. However, it has been suggested that these compounds have some side effects In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease. Herbal and natural products have been used for centuries in every culture all around the world. The search for natural antioxidants, especially of plant origin, had increased greatly in recent years. Plants have almost limited ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. In many cases, these substances serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive, protect

molecular damage and herbivores. Many scientists have investigated the chemical composition and antioxidant and anti microbial property of several tree bark samples. Some tree bark extracts have been used as analgestic, anti-fungal and anti-inflammatory medicines. Natural antioxidants can be phenolic compounds (Flavonoids, phenolic acids and tannins), nitrogen containing compounds (Alkaloids, chlorophyll, derivative amino acids, peptides and amino acids, peptides and amines), carotenoids, tocopherols or ascorbic acids and its derivatives. Karumari *et al.* (2014) reported that most of the antioxidant capacity of fruits and vegetables may come from total phenolics, anthocyanins, and flavonoids. Motlhanka *et al.* (2008) reported that, Total oxidant status (TOS) and total antioxidant status (TAS) were measured with commercially available kits. Methanol and acetone extracts of *T. turcica* were found to have a specific radical scavenging effect. This effect was found to be related to the total phenolic content of the extracts. Since the TTA had a higher phenolic content than the methanol extract, it had a stronger radical scavenging effect. In addition, the total antioxidant capacity of the methanol extract was observed to be higher than that of its acetone counterpart.

Antimicrobial activity

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infectionfighting strategies to control microbial infections.

MATERIALS AND METHODS

The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs, and has necessitated the search for new antimicrobials from alternative sources. Natural compounds are a source of numerous therapeutic agents. Recent progress to discover drugs from natural sources has resulted in compounds that are being developed to treat cancer, resistant bacteria and viruses and immunosuppressive disorders.

Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action are also very likely to differ.

There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Prachayasittikul *et al*., 2008; Nogueira *et al*.,2008).

Screening the active compounds from plants has lead to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases (Roy *et al*., 2009). The experimental procedure employed in the present study to analyze the antioxidant and antimicrobial properties from the seeds of black cumin.

Methanol extract

SEEDS MATERIAL PREPARATION**:** Fresh carum persicum boiss (black cumin seeds) were collected and cleaning wash and dried. They were shade dried at room temperature (26 ± 2°C) for 1-2 days.

Then the dried samples were fine powdered and soaked in methanol then keep for incubation for 24hours in shaker incubator. After incubation take out the sample mixer which contain methanol and filtered the sample mixer. After filtration the obtained extart were collected and allow to dried. The dried methonal extract stored in screw cap bottles until further analysis.

Phytochemical analysis:

Test for Alkaloids

To the 5 ml of both extract were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Test for tannins (Ferric chloride test):

To the 5 ml ofboth extract add few drops of 1 % ferric chloride solution and note the color of reaction. Formation of Green color precipitate indicates presence of tannins.

Test for saponins

About 5 ml of diluted both extracts were taken in a test tube and shaken vigorously and kept for 5 min. Formation of foamy layer indicates the presence of saponins.

Test for glycosides

Extracts were treated with Ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and addboth the extracted with equal volumes of Benzene. The benzenelayer was separated and treated with ammonia solution. Formation of rose-pink colour in theammoniac layer indicates the presence of glycosides.

Test for flavonoids

Extracts were treated with few drops of sodium hydroxide solution (0.1N) solution. Formation of intense yellow colour, which becomes colourless on addition of dil.Hcl, indicates the presence of flavonoids.

Test for protein

Both extract was treated with few drops of Con.Nitric acid. Formation of yellow colour indicates the presence of proteins.

Test for triterpenoids

The both extract were treated with chloroform and filtered. The filtrates were treated with few drops of conc.sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenoids.

Test for phenol

To the extracts add 3-4 drops of 5% ferric chloride solution and observed the formation of dark blue or blackish color which may indicates the presence of phenol in the extracts.

Test for steroids

To the both extract add few drops of acetic anhydride, warmed and cooled under tap water and add few drops of concentrated sulfuric acid and observe the color change violet to green color indicates the presence of steroids.

Test for terpenoids

About 5 ml of both extract was taken and add 2 ml of chloroform and 3 ml of concentrated sulfuric acid notice the formation of layer and color. A reddish brown coloration of the interface confirms the presence of terpenoids.

Antioxidant assay

DETERMINATION OF TOTAL PHENOLIC CONTENT

The amount of total soluble phenolic content was determined according to Folin-Ciocalteu method with slight modifications. Briefly, 10 µL of both methanol and silver nanopractical extract solution from the stock solution was mixed with 100 µL of Folin-Ciocalteu reagent. After 10 min of incubation, 300 µL of 20% Na_2CO_3 solution was added and the volume was adjusted to 1 Ml using distilled water. The mixture was incubated in dark for 2 hrs and the absorbance was measured at 765 nm using a U-Vis spectrophotometer against blank sample. The total phenolic content was measured as gallic acid equivalents (mg GAE)/gram of dry weight (dw) and thevalues were presented as means of triplicate analysis.

DETERMINATION OF TOTAL FLAVONOIDS CONTENT

The content of flavonoids was determined by a pharmacopeia method using rutin as a reference compound. For brief, One ml of both methanol and silver nanopractical aqueous extract (mg/ml) was mixed with1ml aluminium trichloride in methanol (20 g/l) and diluted with methanol to 25 ml. The absorption at 415 nm was read after 40 min at 20 C. Blank

samples were prepared from 1 ml plant extract and 1 drop acetic acid, and diluted to 25 ml. A standard graph was constructed using rutin as the reference standard using the above method.

METAL CHELATING ASSAY

The chelation of ferrous ions by extracts was estimated. Briefly, 50µl of 2 mM FeCl2 1.6 ml of deionised water were added to 0.5 ml of the both extract. The reaction was initiated by the addition of 0.1 ml of 5mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min and then it absorbed under 562 nm. The metal chelating activity % was calculated by using the formula.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ACTIVITY

The FRAP assay was carried out. 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. both methanol and silver nanopractical aqueousextract of various concentration 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 DW. The reaction mixture was kept in dark for 30 min. The readings of the colored product (ferrous tripyridyltriazinecomplex) were taken at 593 nm. 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 both seed extracts to scavenge the stable free radical DPPH was assayed. 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.

In this DPPH assay 2 mg of DPPH is dissolved in 100ml of methanol it is considered as standard then take a clean 7 test tubes for that add 1ml of DPPH then add 8ul , 16ul , 24ul, 32ul, 40ul, respectively then the concentration will 40-200 respectively then incubate for 30mit then OD at 517nm in spectrometer. Before using the test sample in the spectrophotometer first we should take methanol as a blank and made it to auto zero .after auto zero one side we kept methanol and another side the DDPH is kept and taken a reading .In that 2 test tube were considered as blank and control for the blank only the 1ml of methanol was added and for the control only the DPPH is added. Form the control what we get the reading is subtracted by the value which we get in other tubes.

Antimicrobial assay:

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

Test organisms

Of all, Six bacterial strains were used throughout investigation namely *Klebsiella* sp., *Proteus vulgaris, E. coli* and *Staphylococcus aureus*, Bacillus cereus, aerogens All the bacterial cultures were obtainedfrom microbial type culture collection. Fresh young bacterial broth cultures were prepared beforethe screening procedure.

Preparation of inoculum

Stock cultures were maintained at 4°C on slants of nutrient agar. 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 hours at 37°C.

Agar well-diffusion method

The agar well-diffusion method was conducted to evaluate the inhibitory spectrum of methanolic and silver nanopracticles extract against test microorganisms. A freshly grown culture was serially diluted, and 0.1 ml of diluted inoculum (106 CFU/mL) of test organism was spread on agar plates.

Nutrient agar was used for *E. coli, S. aureus, Klebsiella, P. vulgaris, B.cereus and aerogens*wells (6 mm in diameter) were made in agar using a sterilized stainless steel borer. Each well was filled with 50L of diluted extracts (100µl/well). The plates were kept at room temperature for 30 min to allow diffusion of materials in media. Methanol was used as positive controls. Plates were incubated at 30°C for 24h, until visible growth of test microorganisms was evident in control plates. Inhibition zones in mm (including well diameter) around wells were measured. The antimicrobial activity was expressed as the diameter of inhibition zones produced by methanol against test microorganisms.

RESULT AND DISSCUSSION

Phytochemical:

Natural phenolics exert their beneficial health effects mainly through their antioxidant activity. These compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, preventing 1stchain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non radical species and breaking chains to prevent continued hydrogen abstraction

fromsubstances. Phenolic compounds contribute to the overall antioxidant activities of the plant foods (Table 1).

Table 1: Phytochemical analysis of black cumin seed methanol extract

SL NO	Phytochemicals	Observation	Results
01	**Alkaloids**	Formation of Green colour	Presence of Alkaloids
02	**Tannins**	Formation of Green colour	Presence of tannins
03	**Saponins**	Formation of foamy layer	Presence of saponins
04	**Glycosides**	Formation of blood red color	presence of glycoside
05	**Flavonoids**	Formation of intense yellowcolour	Absent of flavonoids
06	**Protiens**	Formation of intense yellowcolour	Absent of protein
07	**Diterpenoids**	Formation of green color	Presence of diterpenoids
08	**Phenols**	Formation of dark blackish color	Absent of phenols
09	**Phytosteriods**	Formation of brown ring junction	Presence of phytosteriod
10	**carbohydrates**	Formation of orange red color	Absent of carbohydrates

By this phytochemical analysis we obtain results in which the methanol extract of black cumin seed contain alkaloids, phytostreiods, diterpenoids, glycosides, saponins and tannins.

Antioxidant:

Determination of total phenolic content:

Phenolic content in the methonolic extract of *Nigella sativa* was mg of Gallic acid equivalent per gram. 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. Several studies on polyphenolic compounds protecting from mutagenesis and carcinogenesis are reported (Fig. 1).

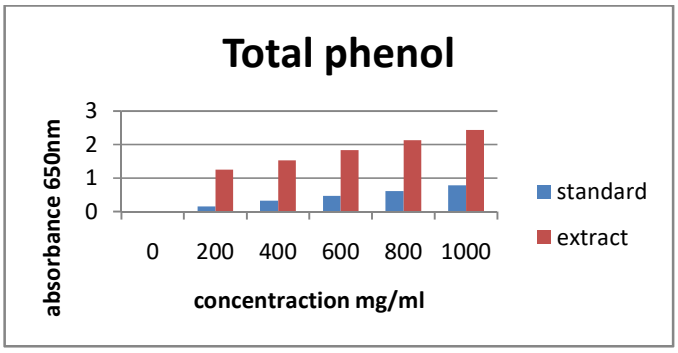

Fig. 1: Total phenol content in the *BLACK CUMIN* extract (Methanol extract)

Determination of total Flavonoids content

Total flavonoid content was determined using $NaNO_2$and $AlCl_3$.6H2O, and result was expressed as mg quercetin equivalents/g methanol extract was determined using rutin reagent, and absorbance was recorded at 490 nm. The total methanol extract was then determined from a rutin standard curve, and result was expressed as mg methanol extract equivalent/g DM (Fig. 2).

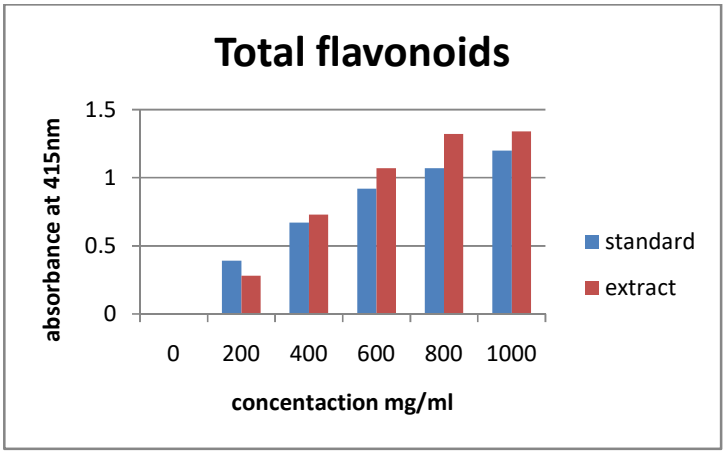

Fig. 2**:**Total Flavonoids content in the *black cumin* extract (Standard Rutin)

Metal chelating activities

As excess free irons have been implicated in the induction and formation of free radicals in biological systems, we tested our medicinal plant extracts in a metal chelating assay. Tested in the concentration range of 0.5 to 1.5 mg/mL, which showed strong chelating activities in concentration-dependent manners. Here the concentration of methanol extract and AgNP'S (*B.cumin)* increased the percentage of metal chelating assay more than standard ferrozine (Fig. 3).

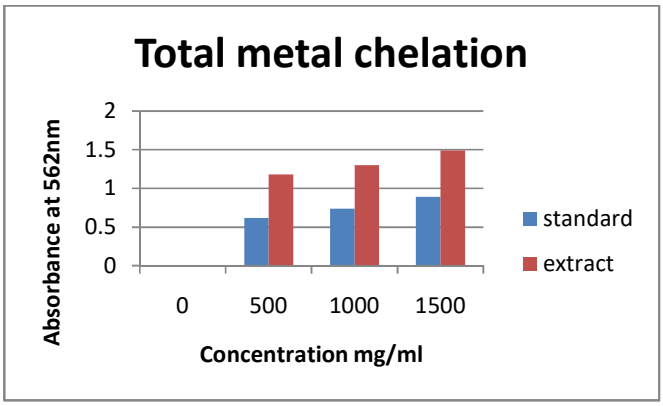

Fig. 3: % inhibition of metal chelation in *BLACK CUMIN* extracts (Standard Ascorbic acid)

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ACTIVITY

The FRAP assay gives fast, reproducible results with methanol extract and AgNP'S with singleantioxidants in pure solution and with mixtures of antioxidants in aqueous solution and added to methanol extract and AgNP'S. 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 (Fig. 4).

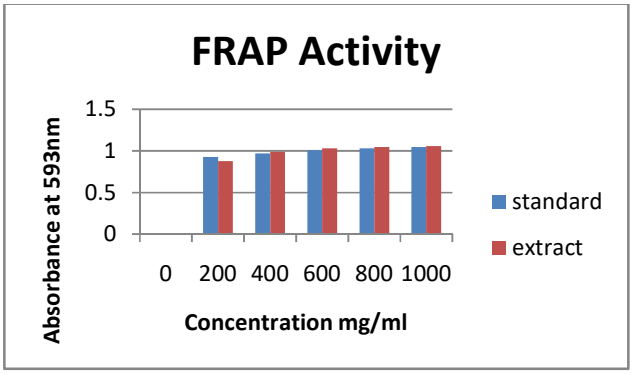

Fig. 4: Ferric reducing antioxidant power of *black cumin* (Methanol extract)

DPPH RADICAL SCAVENGING ACTIVITY

In our DPPH radical scavenging assay, all seed extracts exhibited scavenging activities in a concentration-dependent manner, in the range of 20 to 100 µg/mL. Here the concentration of methanol extract increase the scavenging activity (Fig. 5).

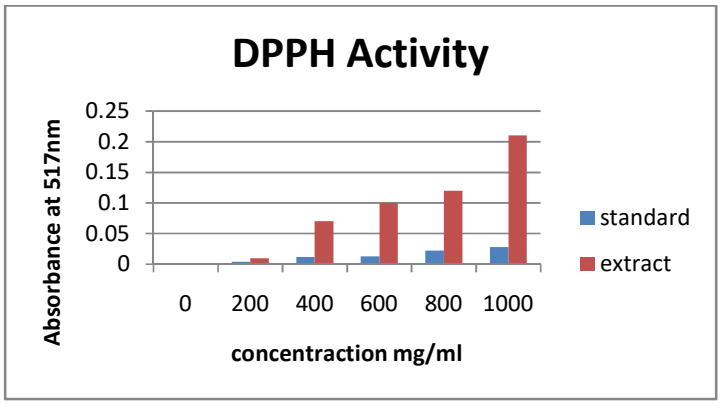

Fig. 5: DPPH radical scavenging activity for black cumin seed extract(methanol extract)

Antibacterial activity

Agar well-diffusion method

In this study, the methanolicand AgNP'S extract of *Nigella sativa* were tested for its antimicrobial effect against pathogens.There was formation of inhibition zone in methanol extract against the test organisms like *S.aureus, E.coli, P.vulgaris* where as AgNP'S shows zone of inhibtion against only klebsiella. As we obtained results from antimicrobial activity the methanol extract shows highest activity then compare to AgNP'S extract and it as shows that at higher concentration shows maximum activity and it purely dose dependent with lower concentration showing no activity at higher concentration high activity (Table 2).

Table 2:Antibacterical activity in methanol extract by well diffusion method.

Bacterical strains	**Inhibition zone diameter in cm**						
	0.2ml	0.4ml	0.6ml	0.8ml	1ml	1.5ml	2ml
Staphylococcus aureus	_	0.35	0.35	0.4	0.4	_	_
Proteus vulgaris	_	0.5	0.2	0.7	0.7	_	_
Aerogens	_	_	_	_	_	_	_
Bacillus cereus	_	_	_	_	_	_	_
Escherichia coli	_	_	0.2	0.3	0.35	_	_
Klebsilla	_	_	_	_	_	_	_

CONCLUSION

The phytochemical analysis of the both extract methanol and AgNP'S was evaluate we obtained alkaloids, tannins, saponins, glycosides and diterpenoid in the methanol extract.

The antioxidants activity of both the extract was measured by the ability to scavenging DPPH, Phenols, Flavanoids, Metal chelating FRAP are compared with suitable standards. These antioxidant assay revealed that the extract exhibit significant antioxidant activity. The highest percentage of inhibition was showed by methanol extract, it gives highest inhibition compared to standards.

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