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Preliminary Phytochemical Screening and *In vitro* Antioxidant Activity of *Nigella sativa* and *Allium cepa* Oil

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Abstract

Many oxidative stress related diseases are as a consequence of accumulation of free radicals in the body. The various researches are going on globally directed towards finding natural antioxidants of plants origins. We planned to investigate the preliminary phytochemical screening and *in vitro* antioxidant activity of *Nigella sativa* and *Allium cepa* oil. The yield of oil of *Nigella sativa* and *Allium cepa* were 21.5% and 27.3%, respectively. Phytochemical screening was performed by using standard methods. The *in vitro* antioxidant study was done for *Nigella sativa* and *Allium cepa* oil by using hydrogen-donating activity, superoxide scavenging activity, hydrogen peroxide scavenging assay and reducing power assay. The result of phytochemical screening demonstrated the presence of flavonoids, steroids and fats in *Nigella sativa* and *Allium cepa* oil. The findings of DPPH, superoxide, reducing power and H_2O_2 radical scavenging activity exhibited that the oil of *Nigella sativa* and *Allium cepa* showed moderately to strongly free radical scavenging activity. Hence *Nigella sativa* and *Allium cepa* oil can play a major role in alleviating the number of oxidative stress by reducing the oxidative damage to cellular component caused by Reactive oxygen species (ROS).

1 Introduction

Plants are the backbone of life on Earth and an essential resource for humans. Plants produce several secondary metabolites that have many biological functions, as well as provide defence against insects, fungi and anthophagous mammals. Ever since times immemorial plants are accustomed to cure illness or strengthen physical health in humans[1-4]. Medicinal plants continue to play a central role in the healthcare system of large proportions of the world's population. In spite of great advances of modern scientific medicine, traditional medicine is still the primary form of treating diseases of majority of people. Increasing knowledge of metabolic process and the effect of plants on human physiology has enlarged the range of application of medicinal plants. Nearly 50% of medicines in the market are made of natural basic materials[5].

Common reasons for use of herbal drugs include health promotion, disease prevention and limited treatment options for a serious illness, significant side effects or risks associated with conventional medicine, belief that herbal and natural products are better or safer. Whereas side effects of allopathic medications vary wildly from mild to severe and there are many[6]. Recently, WHO survey showed that 80% of population worldwide depends on natural medicines at some stages of their primary health care concerns. According to report generated by WHO around 21,000 plant species have the high capacity for being employed as medicinal plants for treatments. Treatment with herbal plants is safe as there are less side effects. The outstanding fact is that, use of natural medicine treatments is referred to any age groups and the sex[7].

Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and

antiviral activities. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing[8,9].

Nigella sativa (Family Ranunculaceae) is a widely used medicinal plant throughout the world. *It is* commonly known as black seed or Kalonji because when the seeds are exposed to air, they turned black. It has been recognized as a "miracle cure" for its ability to treat various diseases and assist the body in its own natural healing process[10]. It has been recorded that *N. sativa* seeds were prescribed to treat headache, nasal congestion, toothache and intestinal worm, as well as a diuretic to promote menstruation and milk production. The seeds are given with butter-milk to obstinate hiccups and are also used in loss of appetite, vomiting, dropsy, obesity and dyspnoea. The seeds contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activity of the seeds has been shown to be due to thymoquinone, the major component of the essential oil[11].

Allium cepa (Alliaceae) is commonly known as onion, has been important coniferous plant in ayurvedic and indigenous medicinal systems. Onion is a versatile and strong antioxidant that quenches free radicals in the human organism. Pharmacological studies found that onions have antimicrobial and antifungal properties, and may also be of benefit in preventing or treating heart disease and atherosclerosis, diabetes, cancer, and possibly asthma. Clinical trials and animal research support the use of *Allium cepa* for anti asthmatic, anti diabetic, anti viral, anti thrombotic, hypo cholestremic, anti inflammatory, anti oxidant, aphrodisiacs, cardiotonic, diueretic, expectorant, stimulant, anti cancer, platelet aggregation inhibitor and insecticidal properties. It is also used in osteoporosis treatment. The major biochemical constituents of onion extraction are identified as quercetin, allicin, alliin, diallyl disulfide, S-methyl L-cysteine S-oxide, propanethial S-oxide (thiopropanal S-oxide) and 3-mercapto-2-methypentan-1-ol[12,13].

The present study was aimed to assess for preliminary phytochemical screening and *in vitro* antioxidant activity of the seeds of *Nigella sativa* and *Allium cepa*.

2 Materials and Methods

2.1 Collection and identification of plant material

The seeds of *Nigella sativa* and *Allium cepa* were purchased from the local market of Bhopal, Madhya Pradesh, India. The plant material were identified and authenticated by Dr. Shoukat Saeed Khan, Department of Botany, Safia Science College, Bhopal (MP), India. The seeds were reduced to coarse powder and stored in airtight container till further use.

2.2 Preparation of oil

The hydraulic press is equipped with a hydro-electric power connected to a jack screw which compresses the sample inside a metallic cylinder surrounded by a resistance controlled by a thermostat. A thermocouple is installed in this press for the recording of the temperature value inside the ground seeds. Oil extraction is carried out from 600 g of ground seeds of *Nigella sativa* and *Allium cepa* packed separately in cloth; steel plates were placed between each pair of cloth. The ground sample is compressed at various pressures 120 bars and temperatures 60 °C. The oil yield was the ratio between the mass of oil extracted and the mass of the sample.

2.3 Preliminary phytochemical tests of oil

Qualitative chemical tests were performed to determine the presence of alkaloids, carbohydrates, cardiac glycosides, polyphenols, saponins, tannins and terpenoids[14-17].

2.3.1 Test for alkaloids

(a) Dragendorff's test: To 1 ml of the oil, add 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) Mayer's test: To 1 ml of the oil, add 1 ml of mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

(c) Hager's test: To 1 ml of the oil, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.

(d) Wagner's test: To 1 ml of the oil, add 2 ml of wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Test for Proteins

(a) Biuret test: Added 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color was produced, and then added to the 1ml of the oil. Formation of pinkish or purple violet color indicated the presence of proteins.

(b) Ninhydrin test: Added two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of oil solution and heated. Development of blue color revealed the presence of proteins, peptides or amino acids.

(c) Millon's test: 1ml of test solution was made acidify with sulphuric acid and added Millon's reagent and boiled this solution. A yellow precipitate was formed indicated the presence of protein.

(d) Xanthoproteic test: To 1ml of the oil, added 1ml of concentrated nitric acid. A white precipitate was formed, it is boiled and cooled. Then 20% of sodium hydroxide or

ammonia was added. No formation of orange color indicated the absence of aromatic amino acids.

2.3.3 Test for Glycosides

(a) Legal test: Dissolve the oil in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

(b) Baljet test: To 1ml of the test oil, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

(c) Keller-Killiani test: 1gm of powdered drug is oiled with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) Borntrager's test: Add a few ml of dilute Sulphuric acid to 1ml of the oil solution. Boil, filter and oil the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

2.3.4 Test for carbohydrates and sugars

(a) Molisch's test: To 2ml of the oil, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

(b) Fehling's test: To 1ml of the oil, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars

(c) Benedict's test: To 5ml of Benedict's reagent, add 1ml of oil solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.3.5 Test for tannins and phenolic compounds

(a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

(b) To 1ml of the oil, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

(c) The little quantity of test oil is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

2.3.6 Test for flavonoids

(a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.

(b) Little quantity of oil is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids.

(c) Shinoda's test: The alcoholic oil of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

(d) The oil is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.

(e) The oil is treated with concentrated H_2SO_4, formation of yellow or orange colour indicates flavones.

2.3.7 Test for steroids

(a) Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

(b) Salkowski test: Dissolve the oil in chloroform and add equal volume of conc. H_2SO_4 . Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested oil.

2.3.8 Test for fixed oils and fats

(a) Spot Test: Press a small quantity of oils between the filter paper. Oil stains on paper indicates the presence of fixed oils.

(b) Saponification test: To 1ml of the oil, add few drops of 0.5 N alcoholic Potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

2.4 In vitro antioxidant activity of oil

2.4.1 Hydrogen-donating activity

In this experiment methanolic solution of DPPH (100 mM, 2.95 ml), 0.05 ml of each extracts dissolved in methanol was added at different concentrations (50-250 µg/ml). Reaction mixture

was shaken and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (% AA). Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation:

$$\% AA = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{DPPH}\}$$

2.4.2 Superoxide scavenging activity

Superoxide scavenging was carried out by using alkaline Dimethyl sulfoxide (DMSO). Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. Filtrate (200 ml) was added to 2.8 ml of an aqueous solution containing nitroblue tetrazolium (56 mM), EDTA (10 mM) and potassium phophate buffer (10 mM, pH 7.4). Sample extract (1 ml) at various concentrations (50-250 µg/ml) in water was added and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO[18-20].

2.4.3 Hydrogen peroxide scavenging assay

The ability of oils to scavenge hydrogen peroxide was estimated by hydrogen peroxide scavenging assay. A solution of hydrogen peroxide (43 mM) is prepared in phosphate buffer (1 M pH 7.4). Different concentration of sample (50-250 µg/ml) was added to a hydrogen peroxide solution (0.6 ml, 43 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard[21]. The free radical scavenging activity was determined by evaluating % inhibition as mention below:

$$\% \text{ Scavenged } [H_2O_2] = [(A_C - A_S)/A_C] \times 100$$

Where A_C is the absorbance of the control and A_S is the absorbance in the presence of the sample of oil or standards.

2.4.4 Reducing power assay

The relative reducing activity in terms of antioxidant activity of extracts was determined by using individual extracts (5 mg) as well as its combination with equal amount of ascorbic acid The extracts and ascorbic acid were dissolved separately in 1.0 mL of deionized water with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL, 10% w/v) were added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm by making 500 µg mL^{-1} extracts aliquot. Increased absorbance of the reaction mixture indicated increased antioxidant activity via reducing power with reference to equal amount of standard ascorbic acid[18-20]. Similar procedure was repeated to know combination antioxidant power of extracts with ascorbic acid.

3 Results and Discussions

3.1 Phytochemical screening

Presence of classes of secondary metabolite may be a useful indicator of both efficacy and potential toxicity; hence test for the presence of phytochemical classes with known bioactivity was done. The yield of oil of *Nigella sativa* and *Allium cepa* were 21.5% and 27.3%, respectively (Table 1).

Table 1: Characteristics of various oil

Oil	Yield (% w/w of powdered drug)	Physical appearance
Nigella sativa	21.5	Liquid
Allium cepa	27.3	Liquid

From the result of phytochemical screening, flavonoids, steroids and fats were found in *Nigella sativa* and *Allium cepa* oil (Table 2). Now oil of *Nigella sativa* and *Allium cepa* were selected for further *in vitro* antioxidant activity evaluation as this oil revealed the presence of flavonoids and phenolic compounds.

Table 2: Phytochemicals present in *Nigella sativa* oil

Phytoconstituents	*N. sativa oil*	*A. cepa* oil
Alkaloids	-	-
Glycosides	-	-
Carbohydrates	-	-
Tannins and Phenolic compound	+	+
Flavonoids	+	+
Steroid test	+	+
Protein	-	-
Fat and oil test	+	+

+ = Present, – = Absent

3.2 *In vitro* antioxidant activity

The oil of *Nigella sativa* and *Allium cepa* were subjected to *in vitro* antioxidant studies to determine and compare the antioxidant activities of extracts.

3.2.1 Hydrogen-donating activity

DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colour stoichometrically with the number of electrons consumed, which is measured spectrophotometricallty at 517

nm[19,20]. As shown in table 3, *Nigella sativa* and *Allium cepa* of oil strongly scavenged DPPH radical with the IC_{50} being 65.35 and 131.02 µg/ml, respectively (Fig 1 and Fig 2). The scavenging was found to dose dependent. The standard drug ascorbic acid scavenged DPPH radical was found to be 91.38.

Table 3: Free radical scavenging capacity of oil of *Nigella sativa* and *Allium cepa*

Conc.	DPPH Scavenging %		
(µg/ml)	*N. sativa oil*	*A. cepa* oil	Ascorbic Acid
50	42.13±0.32	26.14±0.83	91.38±0.73
100	65.47±0.63	41.63±0.43	-
150	73.18±0.81	55.29±0.65	-
200	88.35±0.53	69.81±0.37	-
250	102.61±0.79	84.73±0.93	-
IC_{50}	65.35	131.02	-

Values are mean ± SEM of six determinations

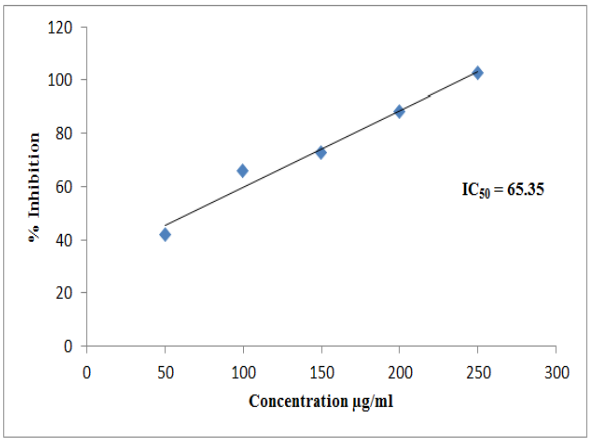

Fig 1: IC_{50} values of oil of *Nigella sativa* for free radical scavenging capacity

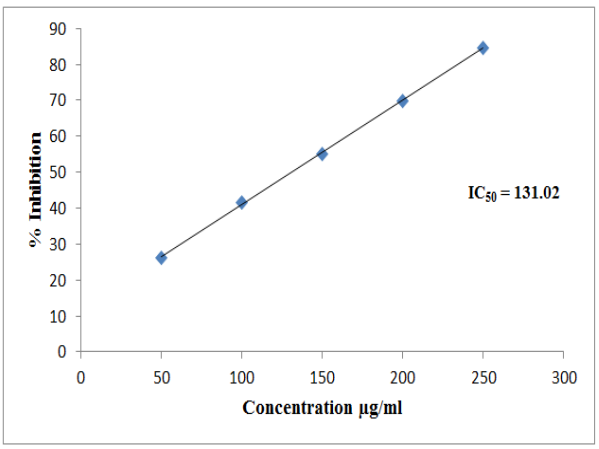

Fig. 2: IC_{50} values of oil of *Allium cepa* for free radical scavenging capacity

3.2.2 Superoxide scavenging assay

Superoxide radical scavenging activity is measured by Nitro blue tetrazolium (NBT) reduction. The method is based on generation of superoxide radical by auto oxidation of riboflavin in presence of light. Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive species. Superoxide is biologically important as it can form singlet oxygen and hydroxyl radical. Overproduction of superoxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. The superoxide radical reduces NBT to a blue colored formazan that can be measured at 560 nm. The decrease of absorbance at 560 nm with extracts and antioxidants indicates the consumption of superoxide anion in the reaction mixture[19,20].

Superoxide free radical scavenging activity was performed with the oil of *Nigella sativa* and was expressed as IC_{50} value. The IC_{50} value for *Nigella sativa* oil and *Allium cepa* oil were 117.64 µg/ml and 149.63 µg/ml, respectively (Table 4, Fig 3 and Fig 4). The superoxide radical scavenging activity was found to dose dependent. The standard drug ascorbic acid scavenged superoxide radical with the IC_{50} being 89.42.

Table 4: Superoxide scavenging capacity of oil of *Nigella sativa* and *Allium cepa*

Conc.	Superoxide Scavenging %		
(µg/ml)	*N. sativa oil*	*A. cepa* oil	Ascorbic Acid
50	29.32±0.82	21.19±0.83	89.42±0.71
100	43.17±0.39	32.63±0.47	-
150	59.48±0.52	48.34±0.62	-
200	78.12±0.93	67.12±0.78	-
250	91.40±0.27	81.26±0.55	-
IC_{50}	117.64	149.63	-

Values are mean ± SEM of six determinations

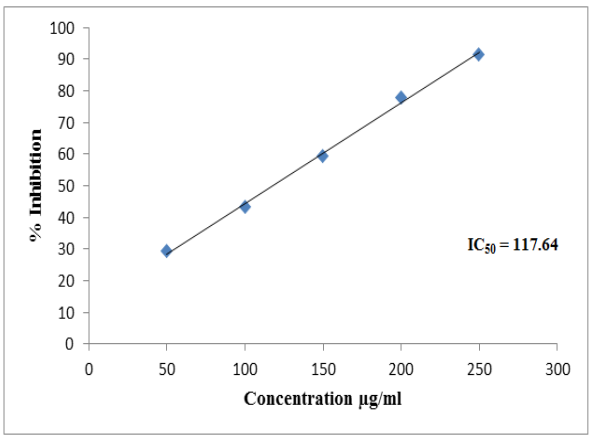

Fig 3: IC_{50} values of oil of *Nigella sativa* for Superoxide scavenging capacity

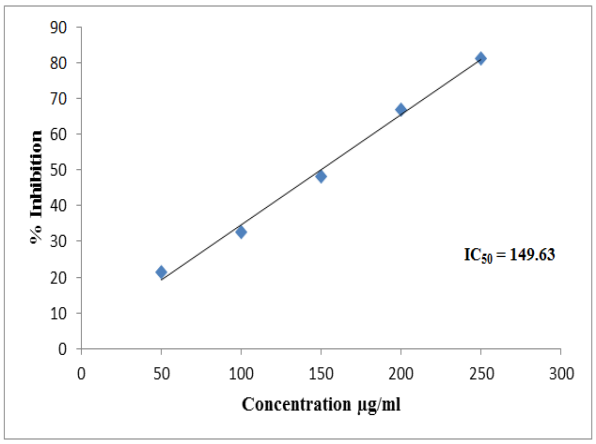

Fig 4: IC_{50} values of oil of *Allium cepa* for Superoxide scavenging capacity

3.2.3 Hydrogen peroxide scavenging assay

Hydrogen peroxide (H_2O_2) scavenging activity of natural antioxidants present in plant extracts has been determined widely by measuring decrement of H_2O_2 in an incubation system containing H_2O_2 and the scavenger using the classical UV-method at 230 nm. A simple and rapid colorimetric assay was developed to determine H_2O_2 scavenging activity of plant extracts and standard antioxidants based on the reaction system where H_2O_2 rapidly reacts with phenol and 4-aminoantipyrine in the presence of horseradish peroxidase (HRP) to produce a pink coloured quinoneimine dye. H_2O_2 scavengers will eventually result in decreased production of this particular chromophore[22,23]. This method was applied to standard antioxidants ascorbic acid, gallic acid and tannic acid in addition to selected oil to determine their hydrogen peroxide scavenging abilities.

H_2O_2 free radical scavenging activity was performed with the oil of *Nigella sativa* and was expressed as IC_{50} value. The IC_{50} value for *Nigella sativa* and *Allium cepa* oil were 140.11µg/ml and 172.15µg/ml, respectively (Table 5, Fig 5 and Fig 6). The H_2O_2 radical scavenging activity was found to dose dependent. The standard drug ascorbic acid scavenged H_2O_2 radical with the IC_{50} being 95.14.

From results, it was found that the oil of *Nigella sativa* and *Allium cepa* showed moderately to strongly free radical scavenging activity. The oil donated their electrons to the superoxide and scavenge them to prevent their further interaction with NBT followed by inhibition of formation of blue colored formazan product. The findings of results revealed that the oil of *Nigella sativa* displayed high content of flavonoids, which was significantly correlated with the superoxide radical scavenging activity.

H_2O_2 is highly important because of its ability to penetrate into biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radicals in the cells. Scavenging of H_2O_2 by oil may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water[24,25]. The results show the oil had potent H_2O_2 scavenging activity which may be due to the antioxidant compounds. As the antioxidant components present in the oil are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O.

Table 5: Hydrogen peroxide scavenging capacity of oil of *Nigella sativa* and *Allium cepa*

Conc. (µg/ml)	Hydrogen peroxide scavenging%		
	N. sativa oil	***A. cepa* oil**	**Ascorbic Acid**
50	20.32±0.69	15.58±0.64	95.14±0.28
100	35.12±0.52	26.91±0.29	-
150	49.42±0.37	39.42±0.53	-
200	73.37±0.28	60.54±0.37	-
250	89.43±0.61	74.17±0.83	-
IC_{50}	140.11	172.15	-

Values are mean ± SEM of six determinations

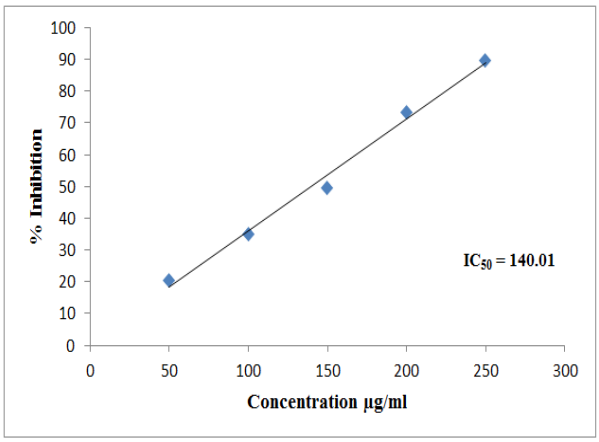

Fig 5: IC_{50} values of oil of *Nigella sativa* for hydrogen peroxide scavenging capacity

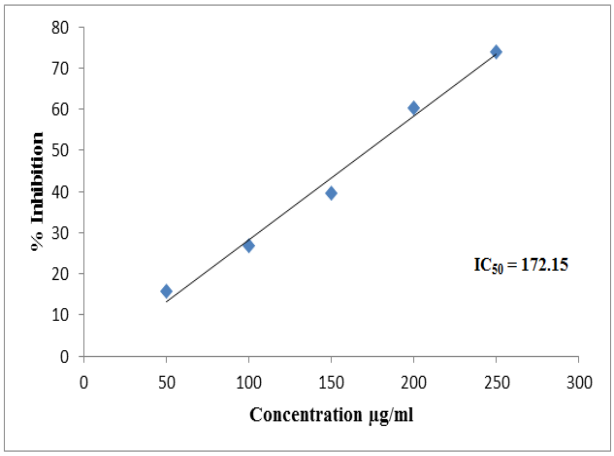

Fig 6: IC_{50} values of oil of *Allium cepa* for hydrogen peroxide scavenging capacity

From the result of DPPH, superoxide and H_2O_2 radical scavenging activity it was observed that the oil of *Nigella sativa* showed highest DPPH radical scavenging activity and maximum superoxide radical scavenging activity. It indicates the presence of different character of antioxidant components in crude oil of *Nigella sativa***.** From these results it can be concluded that antioxidant activity of extracts depends on the presence of quality of active constituents, because each *in vitro* antioxidant model has different mechanism to reduce free radicals.

3.2.4 Reducing power assay

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. In this assay, the ability of extracts to reduce Fe^{3+} to Fe^{2+} was determined. The presence of antioxidants in the extracts resulted into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). In reducing power assay, antioxidants cause the reduction of the Fe^{3+} into Fe^{2+}, thereby changing the solution into various shades from green to blue, depending on the reducing power of the compounds. Strong reducing agents, however, formed Perl's Prussian blue colour and absorbed at 700 nm.

The absorbance value of ascorbic acid was considered to be 100% antioxidant activity. The higher the absorbance of the reaction mixture, the higher would be the reducing power. Table 6 revealed that the antioxidant activity of oil *Nigella sativa*. The reducing power of the oil of *Nigella sativa* and *Allium cepa* were found to be 42.87% and 32.03%, respectively (Table 6).

Table 6: Reducing power assay of *Nigella sativa* and *Allium cepa* oil

Particulars	Absorbance at 700 nm	Antioxidant activity (%)
Ascorbic acid	0.821±0.53	100.00
Nigella sativa oil	0.352±0.41	42.87
Allium cepa oil	0.263±0.87	32.03

Values are mean ± SEM of triplicate determinations

The reducing power of ascorbic acid was found to be higher than oil of *Nigella sativa* and *Allium cepa*. It has been reported that the reducing power of substances is probably because of their hydrogen donating ability[33]. The oil of *Nigella sativa* might, therefore, contain high amount of reductions than *Allium cepa* oil. The result indicates that oil act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions. During study it was found that antioxidant activity was produced due to the presence of phenolic compounds.

The reducing power assay is generally used to estimate the ability of an antioxidant to donate an electron which is an important mechanism of phenolic antioxidant action[34]. Earlier many researchers have reported that the antioxidant activity of extracts is directly proportional to the phenolic and flavonol contents of certain plant extracts[26]. So here reducing power assay justify that oil of *Nigella sativa* contain the maximum amount of the total polyphenol and flavonol.

From the results of antioxidant, it can be concluded that oil of *Nigella sativa* produces higher antioxidant activity compared to *Allium cepa* and could alleviate the number of oxidative stress.

4 Conclusion

The information obtained from the preliminary phytochemical screening will reveal the useful findings about chemical nature of the drugs. The seeds are rich in secondary metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. From the findings of *in vitro* antioxidant study, it was concluded that the oil of *Nigella sativa* and *Allium cepa* can play a major role in alleviating the number of oxidative stress by reducing the oxidative damage to cellular component caused by ROS.

5 Conflicts of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

6 Author's contributions

AAR carried out literature review and experimental work of the present study. KJ was responsible for statistical work and calculations in addition to manuscript proofing. Both authors read and approved the final manuscript.

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