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Review Article

Assessment of Antioxidant Activity of *Nigella sativa* seed: An *In Vitro* Approach

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ABSTRACT

Medicinal plants, vegetables, and food products are rich sources of natural compounds that may play an important role in human health, by maintaining it, or preventing and curing diseases. In medicinal plants, these compounds are considered as bioactive natural compounds that can be subsequently developed as new drugs.*Nigella sativa (N. sativa)* (Family Ranunculaceae) is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha.The aim of the present research is to assess the anti-oxidant activity of selected *Nigella sativa* plant seeds ethanolic extract.Ethanol was used to extract the plant powder three times using a Soxhlet apparatus. The anti-oxidant activity estimated by DPPH, ABTS, and H_2O_2 radicals scavenging assays.The IC_{50} values were determined by NSSE and ascorbic acid to be 192.25mL^{-1} (Y= 0.217x+1.028) and 185.65µg mL^{-1} (Y= 0.2332x+7.61).Results revealed that the pomegranate can be categorized as a leave with extremely high anti-oxidant potential.These bioactive substances could have extra nutritional properties and a central role in diet disease relationships. Further studies are required to disclose possible new bioactive constituents of the unsaponifiable fraction.

Key words: *Nigella sativa,* anti-oxidant, ABTS radical scavenging assay, DPPH radical scavenging activity.

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INTRODUCTION

In India, plants have been source of medicine in past centuries, since the ancient period the native plants are being used for prevention and curative of diseases. A medicinal plant plays a vital role in traditional system of medicine to keep the individual fit. Herbal plants are well known for their medicinal properties and have been used for millennia in traditional medicine [1, 2].

The process of extracting and identifying the chemical compounds produced by these herbs that have biological activity is known as isolation and characterization of bioactive compounds from herbal plants. The isolation and characterization of bioactive compounds from herbal plants involve a series of systematic steps aimed at identifying and understanding the chemical constituents responsible for the plant's therapeutic effects. This systematic approach helps in identifying and understanding the bioactive components of herbal plants, potentially leading to the development of new therapeutic agents. This enables further research, such as detailed characterization, toxicity assessments, and potential applications [2-4].

*Nigella sativa*in traditional medicine practices in Africa and Asia, there is insufficient high-quality clinical evidence to indicate that consuming the leaf, stem, flowers, seeds, or oil.More than 2000 years ago, *N. sativa* seeds and oil were used in traditional medicine, and Hippocrates and Discroides referred to the herb as "the Melanthion".The medicinal herb *Nigella sativa*of the Ranunculaceae family is utilized

extensively all over the World. It is widely used in several conventional medical systems, including Unani, Tibb, Ayurveda, and Siddha. Folklore has long used seeds and oil in a variety of food and medical systems. Numerous illnesses and disorders have been treated with *Nigella sativa*seeds in the past. The seed *Nigella sativa*, sometimes referred to as black caraway and "Kalonji," is well-known throughout the world [5-8].

Figure 1: *Nigella sativa* plant

Figure 2: *Nigella Sativa* Seeds

The aim of the present research is to assess the anti-oxidant activity of selected *Nigella sativa* plant seeds ethanolic extract.

MATERIALS AND METHODS

Collection of plant material

Plant material (seeds) was collected from Department of Botany, Osmania University, Hyderabad, Telangana, India. After carefully cleaning the plant to get rid of any dirt or earthy material, it was chopped into thin chips and allowed to dry at room temperature in the shade before being ground into a fine powder.

Chemical and reagents

All solvents and chemical compounds were purchased from Sigma Aldrich, Mumbai, India. Commercial organic solvents used were analytical grade (~99.5%).

Preparation of *Nigella sativa* extract

Seeds of *Nigella sativa* were washed with tap water and then with distilled water to remove dust and contaminants and shade dried. After the complete dryness, the samples were grinded into fine powder using electric grinder. Ethanol was used to extract the plant powder three times using a Soxhletapparatus. Throughout the solvent extraction process, a new solvent was added every 24 h. To produce crude ethanolic extract, the solvents from the pooled extracts were evaporated using a rotary evaporator operating at reduced pressure and 50–60°C. Then supernatant was filtered using double Whatman filter paper and the extract was stored at 4 °C for further use.The extracts underwent toscreen the biological activity testing [9-13].

In vitro anti-oxidant assessment

DPPH radical scavenging activity: To put it briefly, 0.2 mL of each sample solution was combined with 1 mL of freshly made DPPH (2,2-diphenyl-1-picrylhydrazyl) solution at 0.1 mM. The reaction mixture was vigorously shaken and the absorbance at 517 nm was measured after 20 min at room temperature.

Dimethyl sulfoxide, or DMSO, was utilized as a blank, and a control sample with the same volume but no test compounds or reference anti-oxidants was made. Butylated hydroxytoluene (BHT), a reference anti-oxidant, served as the positive control in each experiment. The decrease in DPPH absorbance was used to calculate the radical scavenging activity:

$$\text{Scavenging effect}\ (\%) = \left[\frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100\right]$$

Where;

$A_{control}$ is the absorbance of the control, and

A_{sample} is the absorbance of the extract or fractions or standard.

ABTS radical scavenging assay: The ABTS (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity is based on the estimation of ABTS radical cation formation, the colour of this ABTS radical compound is suppressed in the presence of anti-oxidant molecules, which have the ability of scavenging this radical.

ABTSis generated by mixing 7mM ofABTS solution with 2.45 mM of potassium sulphate then the mixture is stored at room temperature for 16h. The solution is diluted to get an absorbance of 0.7±0.05 at 734 nm. To assess the antiradical activity of seed extract andwe added 2 ml of ABTS solution to 0.3 ml of test samples with different concentrations, measurements were taking after 30 min at 734 nm. The antioxidant activity was estimated by calculating the percentage of the decrease in absorbance of different samples concentrations, using the following equation:

$$\%\ \text{Inhibition} = \left[\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\right]$$

Where;

$Abs_{Control}$ is the absorbance of the control reaction, and

Abs_{Sample} is the absorbance of the sample.

IC_{50} value indicated the concentration of sample required to scavenge 50% of ABTS radicals, low IC_{50} is equivalent of

high scavenging capacity, and it is calculated by plotting percentage inhibition against different concentrations.

H_2O_2 radical scavenging assay: The ability of our samples to trap hydrogen peroxide was determined according to the method developed. This method is based on the absorption of H_2O_2 in UV.The principle of the reaction is to neutralize hydrogen peroxide (H_2O_2) by an anti-oxidant which will facilitate its decomposition into molecules of water.

An H_2O_2 solution (40 mM) was prepared in phosphate buffer (0.02M) (pH = 7.4), 1.2 ml of this solution are added to 2 ml of standard or extract already prepared in methanol, after incubation for 10 min at room temperature, the absorbance was measured at 230 nm.The percentage of the scavenger activity of theH_2O_2radical is calculated according to the following formula:

$$\% \text{ Inhibition} = \left[\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\right]$$

Where;

$Abs_{Control}$ is the absorbance of the control reaction, and

Abs_{Sample} is the absorbance of the sample.

Statistical analysis

The results were expressed as mean ±SD. The data were subjected to one-way analysis of variance (ANOVA), where the difference between groups and standards were determined by Tukey's test, using Graph Pad program, p value ≤ 0.05 was regarded as significant [14-23].

RESULTS AND DISCUSSION

The yield percentage of the plant seed extract is 65.5%. The ethanolic seed extract is used to test the biological activity i.e. anti-oxidant activity.

DPPH radical evaluation

Due to their ability to donate hydrogen, anti-oxidants could change the stable radical DPPH (purple) into the non-radical form DPPH-H (yellow), acting as radical scavengers. The results of the DPPH scavenging activity for each test sample are shown in Table 1 and Figure 3. As the sample concentration (100-500µg mL^{-1}) increased, the scavenging activity of the NSSE(*Nigella sativa* seed extract) and ascorbic acid also increased. The IC_{50} values were determined by NSSE and ascorbic acid to be 192.25mL^{-1} (Y= 0.217x+1.028) and 185.65µg mL^{-1} (Y= 0.2332x+7.61). Based on these findings, the NSSE has been identified as an effective free-radical inhibitor as well as a primary anti-oxidant capable of limiting free-radical damage in the body.

Table 1: Percent inhibition of DPPH radical scavenging activity

Fractions	Conc. (µg/ml)	% Inhibition	Regression equation	IC_{50} (µg/mL)
NSSE	100	14.85 ± 1.10	y = 0.149x + 2.898	**192.25**
	200	35.51 ± 1.36		
	300	48.45 ± 2.21		
	400	64.23 ± 0.98		
	500	75.01 ± 2.25		
Ascorbic acid	100	22.21 ± 1.11	y = 0.1601x + 10.555	**185.65**
	200	47.82 ± 1.28		
	300	59.75 ± 5.36		
	400	74.01 ± 0.64		
	500	89.18 ± 2.21		

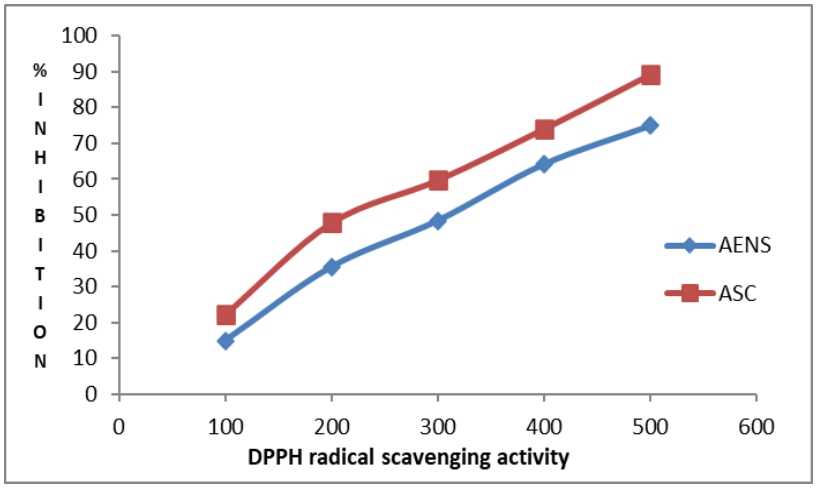

Figure 3: DPPH radical scavenging activity

Table 2: ABTS and H_2O_2 radicals scavenging activities

Fractions	IC_{50} for ABTS radical (mg/ml)	IC_{50} for H_2O_2 radical (mg/ml)
NSSE	0.602 ±0.006	0.39±0.002
α-Tocopherol	0.029 ±0	0.09±0.005

Each value is represented as mean±SD (n = 3), it has been reported that the lower IC_{50} indicated the higher activity, in this assay, the difference between radical scavenging capacities were statistically significant according to Tukey's test at $p < 0.05$.

In the present study, the anti-oxidant activity of seed extract of *Nigella sativa* was investigated.The anti-oxidant activity estimated by DPPH, ABTS, and H_2O_2 radicals scavenging assays. We can relate this capacity to (i) the important amounts of polyphenols and flavonoids, (ii) the diversity of its constituents and their synergism, such as sterols, tocopherols, higher aliphatic alcohols, pigments, and natural hydrocarbons, and (iii) the different kinetic behaviours of potential anti-oxidants.

The usage of herbal medicine has amplified dramatically for various diseases amongst general people over last few years not only because of their easy accessibility without prescription, low cost, and appointment to the health care specialists and more with the belief that natural remedies have less lethal effects as compared to synthetic medicines.

Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signalling, and immune function. Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids, and DNA which will lead to cell injury and can induce numerous diseases. Anti-oxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases, and inflammatory diseases. This activity is due to the ability of anti-oxidants to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation.

CONCLUSION

Nigella sativa is widely referred to as black seed. Its seeds and oil have been widely used in the treatment of various diseases Worldwide.From late summer to early autumn, this plant can be found in fields and grassy areas worldwide after rain. It is frequently found in small groups on lawns in suburban areas. Asia, Europe, northern Africa, Australia, New Zealand, and North America. The presence of medicinally active secondary metabolites, alkaloids, flavonoids, tannins, steroids, carbohydrates, proteins, and phenols were showing different pharmacological activities.

These findings revealed that the seed extract possessed intriguing anti-oxidant property, including DPPH, ABTS, and H_2O_2scavenging assay.Therefore, in further study selection of the doses and preparation of formulation for the evaluation of their pharmacological activities. Large scale clinical studies in patients are now indicated in those therapeutic areas that involve oxidative stress and/or inflammation as a core physiological component of the development and progression of their condition. Future research should explore the active fractions *in vitro* and compare their anti-oxidant activity in animal models before and after in-duction of diseases caused by oxidative stress. Further investigations are required to study the mechanism of actions of *N. sativa* seeds and its constituents by which they exert their therapeutic effects.

This study showed clearly the antioxidant potential of *Nigella sativa* unsaponifiable fraction and this capacity is explained by the diversity of its anti-oxidant components. This finding illustrates the utility of this fraction as a natural anti-oxidant for use in therapy and lipidcontaining foods. These bioactive substances could have extra nutritional properties and a central role in diet disease relationships. Further studies are required to disclose possible new bioactive constituents of the unsaponifiable fraction.

Author's contribution

All authors contributed to the design and implementation of the research.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Data availability

The data used to support the study are included within the article and are also available from the corresponding author.

Ethical approval

Not applicable.

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