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RESEARCH ARTICLE

ASSESSMENT OF ACETYLCHOLINESTERASE INHIBITORY AND ANTIOXIDANT ACTIVITIES OF *NIGELLA SATIVA* EXTRACTS *IN VITRO*

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ABSTRACT: Alzheimer disease is a neurodegenerative disorder, age related, progressive, and irreversible; which is marked by severe memory lapses, abnormal behaviour in patients, personality changes and cognitive deterioration. Nevertheless, after a long research and the efforts of pharmaceutical industries, there is still little effective treatment of the Alzheimer disease, and even the existing medications have serious side effects. It is the reason why the interest in the plant-based therapies increases, since natural compounds are typically linked to lower toxicity. Plant-derived cholinesterase inhibitors hold one of the brightest perspectives of slowed down development of Alzheimer disease. Antioxidant and anti-cholinesterase effects of different extracts of *Nigella sativa* were determined in this study. The antioxidant activity was evaluated in terms of DPPH assay and hydrogen peroxide radical scavenging activity whereas anti-cholinesterase activity was measured in terms of Ellman colorimetric method. The extracts included were most active with the ethanolic extract exhibiting IC_{50} of 25 µg/ml (DPPH assay) 54 µg/ml (hydrogen peroxide scavenging assay), and 115 µg/ml (AChE inhibition). These findings suggest a great in vitro antioxidant and anti-Alzheimer potential of *Nigella sativa* extracts showing that their use in in vivo research and eventual formulation as therapeutic agents is feasible.

Keywords: Alzheimer disease, *Nigella sativa*, Anti-oxidant, Anti cholinesterase activity.

INTRODUCTION

Of all cases of dementia, roughly 6070% are caused by a neurodegenerative disorder known as Alzheimer disease (AD) which is characterized by progressive and gradual impairment of cognitive abilities and memory. The illness normally progresses gradually and becomes exacerbated in the long run [1, 2]. AD was initially described in 1906 by a physician named Dr. Alois Alzheimer and subsequently named Alzheimer disease [3]. He described pathological alterations of a thin cerebral cortex, which had abnormal brown deposits and atypical fibrillary structures inside brain cells which are now known as amyloid plaques and neurofibrillary tangles. The development of these two abnormal protein aggregates of misfolded proteins are the brain characteristics of the individuals with AD. The illness impacts both sexes and it is common in people who are above 65 years [4]. It is estimated that there were about 26.6 million people with AD above 65 years of age in the world in the year 2006 and it is estimated that there will be 1 in every 85 persons with AD in the world by the year 2050 [5]. The clinical presentation of AD differs significantly across individuals [6]. AD is mainly diagnosed using behavioral evaluations, cognitive functions test and neuroimaging techniques [7].

Activity-dependent neuroprotective protein (ADNP) is recently found as one of the possible biomarkers of evaluating the risk of developing Alzheimer disease in the elderly. Illana Gozes, the lead researcher in Tel Aviv University reported that blood concentration of ADNP is associated with the formation of amyloid plaque in the brain a characteristic of AD.

There is a number of biochemical and pathological processes that can lead to the emergence of AD and its progression [8]. These include:

(i) An imbalance between the production and clearance of amyloid- B (A1), which causes it to accumulate in the form of senile plaques (amyloid hypothesis). These plaques are capable of triggering neuroinflammation and the formation of reactive oxygen species [9].

(ii) Cholinergic malfunction caused by the heightened action of acetylcholinesterase, and butyrylcholinesterase, which is a component of the cognitive deficiency existing in AD (cholinergic hypothesis) [10].

According to these assumptions, a variety of anti-amyloid agents [11] and cholinesterase inhibitors [12] have been examined as possible disease-modifying agents.

In the past, medicinal plants have been an excellent source of therapeutic agents. The world health organization reports that almost 80 percent of the population in the developing world utilizes plant based medicines as their primary healthcare [13]. Myriads of plants are known to have medicinal qualities and researches are currently underway in this field.

Nigella sativa (*N. sativa*) is a medicinal plant that is widely used in the world and is a plant in Ranunculaceae family. It plays a significant role in the traditional medical system like Unani, Tibb, Ayurveda and Siddha. The seeds, as well as the oil of N. sativa, have a folklore history of use both as a dietary supplement and in folklore medicine. The seeds have been used traditionally in curing several ailments and diseases. *N. sativa* is considered to be among the most effective healing plants in Islamic literature, and is prescribed on a regular basis in Tibb-e-Nabwi (Prophetic Medicine). Historically, N. sativa has a large variety of uses, including as an antihypertensive, liver tonic, diuretic, digestive, anti-diarrheal, appetite stimulant, analgesic, antibacterial agent, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renoprotective, gastroprotective, and antioxidant.Recent studies have greatly expanded the scope of pharmacological activity of N. sativa to include antidiabetic, antic These results substantiate huge therapeutic potential of the plant [14].

MATERIALS AND METHODS

Plant Material

The *Nigella sativa* seeds were purchased from Vatika Agro Shop in Jaipur, 302020, in the Indian state of Rajasthan. A well-known botanist confirmed that the plant was real. Janta Postgraduate College, A.P.S. University, Rewa (486001), M.P. India, is where the specimen was deposited at the university's herbarium house. J/Bot/2022APS-019.

Extraction

Shade-drying and powdering the plant material into a rough powder took place. To extract *Nigella sativa*, a Soxhlet extractor was used in Ethanol and hydroalcoholic solvents to bring out respective extracts. Thereafter, the filtrate was concentrated by evaporation in a water bath. To measure the yield of the soluble constituents, we took the weight of the drained extracts, and computed it [15].

% = Weight of dry extract/Weight of dry seeds powder ×100

In Vitro Antioxidant Activity

The antioxidant potential of the extracts was evaluated using the following assays:

1. DPPH radical scavenging assay
2. Hydrogen peroxide scavenging assay

DPPH Radical Scavenging Assay

A 0.1 mL portion of different concentrations of the extract was combined with 0.4 mL of 0.3 mM DPPH solution that had been prepared in ethanol. Shaking the mixture followed by incubation in the dark at room temperature over 30 minutes were done. At 517 nm, the absorbance was taken at a UV-visual spectrophotometer [15]. The DPPH radical scavenging activity was expressed as a percentage by the following formula:

$$\% \text{ Scavenging Activity } = \frac{\text{Abs}_{control} - \text{Abs}_{sample}}{\text{Abs}_{control}} \times 100$$

Butylated hydroxytoluene (BHT) was used as the standard. All experiments were performed in triplicate, and IC_{50} values were determined.

Hydrogen Peroxide Scavenging Assay

Phosphate buffer (pH 7.4) was prepared with Hydrogen peroxide solution (2 mM). The extracts were put in diverse concentrations (10-100 µg/mL) to 0.6 mL of hydrogen peroxide solution. The incubation was done at 10 minutes and absorbance was recorded at 230 nm using a blank solution of phosphate buffer without hydrogen peroxide. The reference standard was ascorbic acid [16].

Anti-Acetylcholinesterase Assay

The Ellman method of acetylcholinesterase inhibitory was assessed (1961). The source of enzyme was electric eel acetylcholinesterase, the substrate was acetylthiocholine iodide and the chromogenic reagent was 5,5 -dithiobis-2-nitrobenzoic acid (DTNB) [17].

This reaction mixture was made by adding 150 0.1 M sodium phosphate buffer (pH 8.0), 10 0.1 U/mL enzyme solution and 20 0.1 U/mL test extract to the reaction mixture and incubation at a temperature of 25 ° C was done over 15 minutes. It was then followed by the addition of 10 µL of DTNB and 10 µL of acetylthiocholine iodide and incubation of further 10 minutes. The absorbance was measured at 410 nm. Physostigmine was used as the control inhibitor.

$$\% \text{ Enzyme Inhibition } = \frac{(\text{OD}_{test} - \text{OD}_{blank})}{(\text{OD}_{standard} - \text{OD}_{blank})} \times 100$$

Statistical analysis:

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). IC_{50} values were calculated using standard statistical methods.

RESULT AND DISCUSSION:

Antioxidant Activity

The role of oxidative stress in the pathogenesis of age-related neurodegenerative diseases such as Alzheimer disease is significant. It has been confirmed in many studies that antioxidants are capable of reducing the damage of neurons by counteracting the reactive oxygen species. The *Nigella sativa* extracts were also tested on the basis of the antioxidant ability of DPPH scavenging activity and hydrogen peroxide scavenging activity.

DPPH Radical Scavenging Activity

The DPPH radical scavenging assay was used to determine the antioxidant activity of different extracts of *Nigella sativa*.

Results are shown in Table 1 which show that the free radical scavenging activity is concentration-dependent.

TABLE 1: DPPH RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS

S. no.	Conc. (µg/ml)	% Inhibition		
		Standard (Butylated hydroxyl Toulene)	Ethanol	Hydroalcoholic Extract
1.	20	53.95	45.94	19.34
2.	40	64.81	59.86	29.34
3.	60	76.62	68.18	30.34
4.	80	85.21	76.95	34.32
5	100	91.45	88.87	37.45
IC_{50} (µg/ml)		10	25	147

DPPH Radical Scavenging Activity

DPPH is a free radical with a deep purple colour because of its unpaired electron and is extensively utilized in assessment of the free radical scavenging ability of antioxidant substances. DPPH is reduced on taking up a hydrogen atom or an electron by the proton-donating substance leading to a drop in absorbance at 517 nm and a colour change to yellow. The *Nigella sativa* ethanolic extract had a high DPPH radical scavenging ability of 25 µg/mL with an IC_{50} and 147 µg/mL with the hydroalcoholic extract having a very high antioxidant activity. The regular antioxidant, butylated hydroxytoluene (BHT) had an IC_{50} of 10 µg/ml. The ethanolic extract of *Nigella sativa* is one of the extracts tested that possessed a significant free radical scavenging capacity and a promising antioxidant potential in comparison to the standard.

Hydrogen Peroxide Radical Scavenging Activity

The hydrogen peroxide radical scavenging of both ethanolic and hydroalcoholic extracts of Nigella sativa was determined and the findings are shown in Table 2. Hydrogen peroxide, being a weak reagent can react to produce highly reactive hydroxyl radicals within the cells, which lead to oxidative stress and cell damage. Both extracts had concentration-dependent hydrogen peroxide scavenging activity. The ethanolic extract was found to scavenge better than the hydroalcoholic extract at all concentrations used. The IC_{50} of the ethanolic extract was 54 µg/ml, as compared to a much higher value of 131 µg/ml of hydro alcoholic extract. The ascorbic acid (the conventional antioxidant) had IC_{50} of 47 µg/ml. These results denote that the ethanolic extract has a strong hydrogen peroxide scavenging activity that is comparable to the standard antioxidant.

TABLE 2: HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACT

S. no.	Conc.	% inhibition		
		Standard (Ascorbic acid)	Ethanol	Hydroalcoholic Extract
1	20 µg/ml	32.23	40.43	12.34
2	40 µg/ml	38.13	45.65	24.54
3	60 µg/ml	56.34	50.81	28.45
4	80 µg/ml	73.32	57.65	32.54
5	100 µg/ml	88.34	65.56	38.56
IC_{50} (µg/ml)		47	54	131

Anti-Acetylcholinesterase Activity

An *in vitro* acetylcholinesterase inhibitory test was done to determine the potential of the enzyme inhibition of different extracts, and which extract would be the most active to be used in the future pharmacological study. All tested extracts were determined to obtain the percentage inhibition of acetylcholinesterase and also IC_{50}values were calculated. Reduced values of IC_{50} denote higher enzyme inhibitory potential. Of the extracts assessed, the ethanolic extract of *Nigella sativa* had the lowest IC_{50} which implies that it has better acetylcholinesterase inhibitory properties than other extracts. The ethanolic extract was chosen on these results to be marketed *in vivo* as part of the pharmacological studies. Table 3 is a summary of the results of the acetylcholinesterase inhibition assay and Figure 1 shows the results.

TABLE 3: ACETYL CHOLINESTERASE ASSAY OF VARIOUS EXTRACT

Extract	IC_{50} value
Ethanol extract	115 µg/ml
Hydroalcoholic Extract	950µg/ml

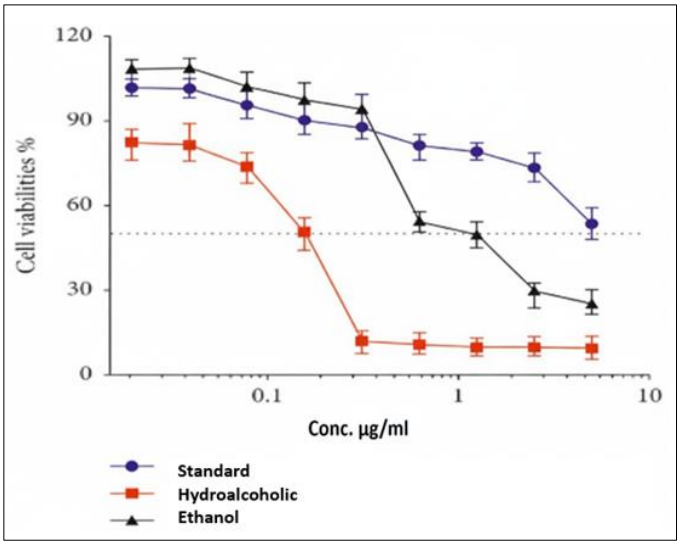

FIG. 1: ACETYL CHOLINESTERASE ASSAY OF VARIOUS EXTRACT

Amnesia has an important biomarker, enzyme acetylcholinesterase (AChE). The various extracts of *Nigella sativa* were used to measure the percentage inhibition of AChE in this study. The IC_{50} of the ethanolic extract was 115 µg/ml, and that of the hydroalcoholic extract was 950 µg/ml. This shows that even though all extracts had some AChE inhibitory activity, ethanol extract had the highest AChE inhibitory activity, which is in agreement with earlier studies, since with ethanol as a solvent, it is believed that it extracts stronger AChE inhibitors than the hydroalcoholic solvents. It is perhaps the increased amount of bioactive compounds extracted by the ethanolic extract with strong AChE-inhibitory properties, which have made the ethanolic extract more active. Thus, ethanolic extract is a good prospect in future research.

CONCLUSION

In the given study, the *in vitro* antioxidant and AChE inhibitory properties of different extracts of *Nigella sativa* were assessed. The ethanolic extract had a considerable radical scavenging action in a dose-dependent way. The modification of Ellman method to determine in vitro AChE was used to determine that maximum inhibition was obtained using the ethanolic extract, in comparison with other extracts in terms of IC_{50} values. Such results represent the idea that the extracts have significant antioxidant and AChE inhibitory effects, which argues in favor of their use as alternative therapeutic agents. Additional *in vivo* research might be carried out to investigate their effectiveness in the natural systems.

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CONFLICT OF INTEREST: Nil

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