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Antioxidant and Antiproliferative Activity of the Methanolic Extract from *Nigella sativa* Seeds

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ABSTRACT

An investigation on the antioxidant and antiproliferative activities of *Nigella sativa* seeds was carried out. The methanolic extract from the dried sample of *Nigella sativa* seeds was prepared and examined for its phytochemical composition. The sample exhibited noticeable scavenging effects in DPPH free radical scavenging assay. It has also been evaluated the effect of methanolic extract of *Nigella sativa* seed on HepG2, MCF-7 and VERO cells proliferation using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. After 72 h incubation, HepG2 and MCF-7 cell proliferation was inhibited upto 50% by the methanolic extract. The phytochemical constituent appears to be responsible at least in part, for the observed antioxidant activity of the methanolic extract. The greater reducing activity of the extract reflected the generally greater phenolic content of the *Nigella sativa*. The DNA fragmenting pattern of the *Nigella sativa* seed extract was identified using DNA fragmentation assay. The number of DNA fragments were increased with the concentration of the seed extract. The results suggest that the *Nigella sativa* seeds could be a potential source of natural antioxidant.

Key words: *Nigella sativa*, DPPH, antioxidant, phenolic compounds, MTT assay

INTRODUCTION

In the aerobic life, different types of oxygen derivatives along with peroxides and transitionmetals are produced by the living organisms. These metaboliteshave proven to be having degenerative effects towards living cells. The oxidation of such oxygen derivatives were found to be having various ill effects (Decker, 1998). The oxidative derivatives of such molecular oxygen are principal cause for the qualitative decay of foods (Oxidative damage). Addition of antioxidants to food formulations is found to be an effective way to reduce the incidence of lipid oxidation in industrial food production. The search for antioxidant from natural sources is intensified to reduce the synthetic antioxidants (Duh *et al*., 1992).

Determination of effective natural antioxidant is important for evaluation of food protection against oxidative damage. Plants have recently received significant attention for their potential as natural antioxidants. The plant were possesing various industrial applications including uses in foods (Jimenez-Escrig and Sanchez-Muniz, 2000; Nagai and Yukimoto, 2003). Plants were also

known to possess several health protective compounds (Burtin, 2003). Among the different compounds, antioxidants are the most widely studied. Plants serve as the important resources for bioactive natural products (Leubner-Metzger, 2002).

Various antioxidant compounds such as fucoxantine and phylopheophytin were isolated from plants and algae (Kuda *et al*., 2005). Polyphenolic compounds are widely distributed in plants (Vahabzadeh *et al*., 2004). The antioxidant activity of a compound can be accessed by the assays in which capacity of the compound for expressing free Radical Scavenging Activity (RSA) is determined (Kuda *et al*., 2005).

Of the Indian traditional medicinal plants, *Nigella sativa* plays an important role due to its diverse therapeutic applications such as anti-rheumatic, anti-oxidant activities, etc. Several researchers analyzed the biological activities of *Nigella sativa* seeds including antioxidant activity, found to be having variation by several researchers (Parekh *et al*., 2006; Meziti *et al*., 2012). Against these backdrops, the present study aimed at the determination of antioxidant activity of methanolic extract of *Nigella sativa*by Radical Scavenging Assay using DPPH and *in vitro* anti proliferative activity against tumor cell lines.

MATERIALS AND METHODS

Sample and reagents: The *Nigella sativa* seeds were collected from the Centre for Advanced Studies in Botany, University of Madras, Chennai, India. All the chemicals used were of analytical grade and bought from Sigma and Merck. VERO, HepG2 and MCF-7 cell lines were procurred from National Centre for Cell Sciences (NCCS), Pune.

Plant extract preparation: The *Nigella sativa* seeds were rinsed with sterile water to remove any associated debris. Sample was kept for under sunshade for drying. The dried sample was grounded thoroughly to powder form. The powder was then used for the estimation of the antioxidant activity. The essential metabolites were extracted with the help of methanol for overnight a troom temperature. The extract was then filtered and concentrated and waswashed with an equal volume of hexane twice (Yan *et al*., 1999). The plant extract was solubilized in ethanol for use in the assays.

Antioxidant activity (DPPH assay): The antioxidant activity of the plant extracts on the DPPH radical scavenging activity was determined by a spectrophotometric assay (Kuda *et al*., 2005). About 0.2 mL of the plant extract was mixed with equal volume of methanol and 0.025 mL of DPPH methanol solution. The absorbance was measured at 550 nm after 30 min. The RSA was calculated as a percentage of DPPH discoloration using the equation:

$$\text{Percentage of RSA} = \left(1 - \frac{\text{Absorbance after incubation}}{\text{Initial absorbance}}\right) \times 100$$

Phytochemical composition assay: The phytochemical constituents of the mehanolic seed extract of *Nigella sativa* were determined using standard protocols according to APHA. The assay performed for the constituents including Tannins, Saponins, Flavanoids, Alkaloids, Proteins, Steroids, Anthraquinones, Terpenoids and Cardioglycosides.

Antimicrobial activity: The antibacterial and antifungal activities of the seed extract at different concentrations were analyzed using disc diffusion assay. The bacterial strains tested including

Escherichia coli MTCC 1687, *Staphylococcus aureus* MTCC 96, *Pseudomonas aeruginosa* MTCC 1688, *Vibrio cholera* ATCC 14035. The fungal strains *Candida albicans* MTCC 183, *Candida parapsilosis* MTCC 2509, *Candida tropicalis* MTCC 184 were analyzed for their susceptibility against *Nigella sativa* seed extract.

MTT assay (antiproliferative activity): The cells were allowed to grow separately in Minimal Essential Medium (MEM) with 10% Fetal Bovine Serum (FBS). Cells were seeded into 96-well plates and allowed to attach overnight in 300 μL medium. The methanolic extract of *Nigella sativa* at different concentrations were added to the wells containing VERO, HepG2 and MCF-7 cell lines and incubated for 72 h at 37°C and 5% CO_2 (Yuan *et al*., 2005). After incubation, traces of methanolic seed extract were removed by washing the cells twice with 200 μL Phosphate Buffered Saline (PBS) and applying 100 μL of fresh medium plus 10 μL of 12 mM MTT (di-Methyl Thiazolyl Tetrazolium) dissolved in PBS to determine the effects of the plant extracts on cell proliferation (Mosmann, 1983). Cells were then incubated for 4 h at 37°C, 5% CO_2. About 100 μL of isopropanol containing 0.04 N HCl was added to each well to solubilize the product of MTT cleavage. The absorbance was read at 570 nm and the percent inhibition of cell proliferation was calculated as follows:

$$\text{Percentage inhibition} = \left(\frac{\text{Absorbance of control at 570 nm - Absorbance of sample at 570 nm}}{\text{Absorbance of control at 570 nm}}\right)\times 100$$

DNA fragmentation assay: The aim of the assay is to determine the amount and pattern of DNA fragmented/degraded by the methanolic extract of *Nigella sativa* seeds. For the purpose, 1.5 mL of the VERO and HepG2 cell lines was allowed to centrifuge and the pellet was collected. The pellet was then mixed in Tris-Taps-EDTA (TTE) buffer, 0.7 mL ice cold isopropanol and 0.5 mL ice cold 1 M NaCl and incubated overnight at 20°C. After incubation, the mixture was purified by repeated centrifugation with 70% ice cold ethanol and the final pellet was dissolved in TE buffer. The fragmentation pattern of VERO and HEpG-2 cell lines by *Nigella sativa* extract was analyzed and compared with their cell lines without treatment with extract (control).

RESULT AND DISCUSSION

Natural products were found to be the major resource of drug development. Traditional knowledge of medicinal plants has always been traced to the occurrence of natural products with medicinal properties and has guided the search for new cures. A variety of microbes were examined for bioactive secondary metabolites since they were easy to cultivate (Firakova *et al*., 2007). Due to the various difficulties in chemical synthesis of drugs, natural bioactive products have a great impact on human health and are still needed to be explored.

The antioxidant activities of the crude extract on *Nigella sativa* seeds was determined in the present study. Accelerated solvent extraction method was used since it provides minimum solvent consumption while providing high recoveries. The radical scavenging activity of the methanolic extract of *Nigella sativa* was determined using DPPH. The results indicated the presence of reducing compounds in the plant extract in terms of hydrogen donation capacity (Fig. 1). In order to quench DPPH, the antioxidant compound present in the extract, fades the Purple color of the DPPH. For the same, the antioxidant donates either a hydrogen or electron to the DPPH (Amarowicz *et al*., 2004). The change in the absorbance of the DPPH solution upon the addition of plant extract was depicted.

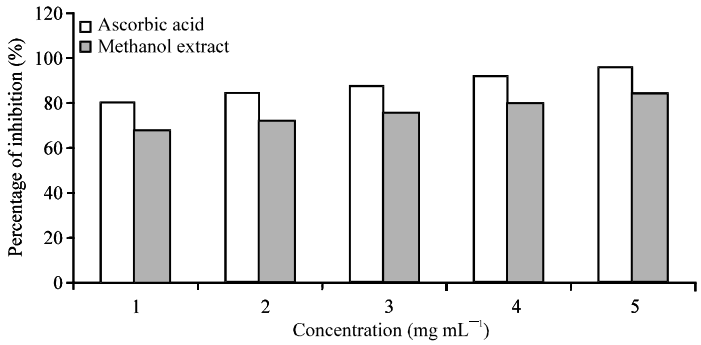

Fig. 1: DPPH assay of methanolic extract of *Nigella sativa*

Table 1: Phytochemical constituents present in methanolic extract of *Nigella sativa*

Phytochemical constituent	Methanolic extract
Tanins	Positive^{+++}
Saponins	Negative
Flavonoids	Positive
Alkaloids	Positive^{+++}
Proteins	Positive^{+++}
Steroids	Positive
Anthraquinones	Negative
Terpenoids	Positive
Cardio glycosides	Negative

From the results, it was shown that the RSA activity is directly proportional to the concentration of the plant extract. The level of interaction between the antioxidant and DPPH were depends on the structural confirmation of the antioxidant. These structural features help to differentiate the various phenolic compounds possessing antioxidant properties. The RSA on the basis of stable radical DPPH provides accurate and reliable results when compared to other free radicals (Huang *et al*., 2005). The results revealed that the methanolic extract of *Nigella sativa* acts as free radical inhibitor.

The phytochemical constituents such as tannins, terpenoids, saponins flavonoids, alkaloids, proteins, steroids, anthraquinons and cardioglycosides were screened from the seed extract and the results obtained were shown in Table 1. The results indicate the presence of tannins, proteins and alkaloids. The various phenolic compounds exhibit biological effects such as antioxidant activity (Rice-Evans *et al*., 1997). Surveswaran *et al*. (2007) found, there are positive correlations between antioxidant activities and total phenolic contents. The antioxidant activity of phenolics is mainly due to their redox properties which allow them to acts as reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelators (Morel *et al*., 1994).

The methanolic extracts of *Nigella sativa* were tested for their antibacterial and antifungal activity against the selected bacterial and fungal strains with the help of Amphotericin as standard antibiotic. All the tested strains were found to be sensitive to the extract. The zone of inhibition and minimum inhibitory concentration were determined and recorded. The result showed that the extract possess excellent antimicrobial activity and were expressed in Fig. 2.

The evaluation of radical scavenging activity using DPPH may not be complete picture of antioxidant activity as it relies on one aspect of the antioxidant activity (Decker, 1998). Hence, the present study also reported the antiproliferative activity of the methanolic extract of *Nigella sativa* against the breast cancer cell line (MCF-7) and human hepatocarcinoma cell line (HepG2 cells).

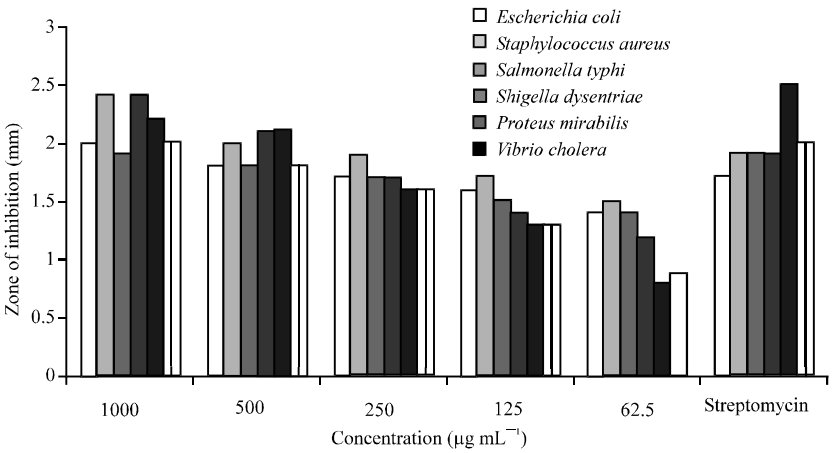

Fig. 2: Antimicrobial activity of methanolic extract of *Nigella sativa*

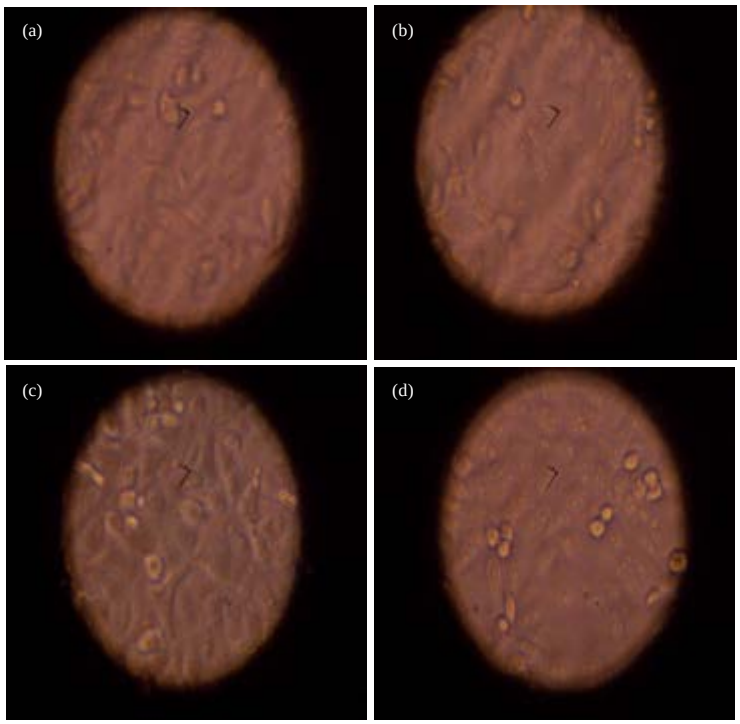

Fig. 3(a-d): Antiproliferative activity of *Nigella sativa* against VERO cell lines at different concentrations (a) 1000, (b) 500, (c) 250 and (d) 125 µg mL^{-1}

The antiproliferative activity of the crude extract of *Nigella sativa* against VERO (Fig. 3), HepG2 (Fig. 4) and MCF-7 (Fig. 5) cell lines were evaluated in terms of capacity to cellviability. The crude extract of the more polar methanol fraction showed considerable cytotoxic activity against MCF-7 and HepG2 cells. However, the methanol extract showed stronger dose-dependent cytotoxicactivity against cancer cell lines (Table 2).

The seed extract inhibited the cell proliferation of both MCF-7 and HepG2 cell lines. The percentage of inhibition of MCF-7 cell lines was higher when compared to HepG2

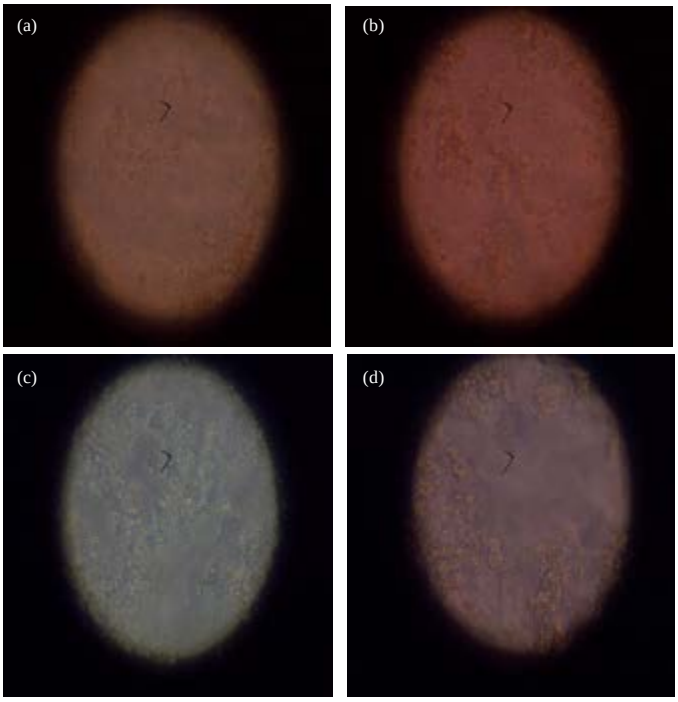

Fig. 4(a-d): Antiproliferative activity of *Nigella sativa* against HEPG2 cell lines at different concentrations (a) 100, (b) 500, (c) 250 and (d) 125 μg mL^{-1}

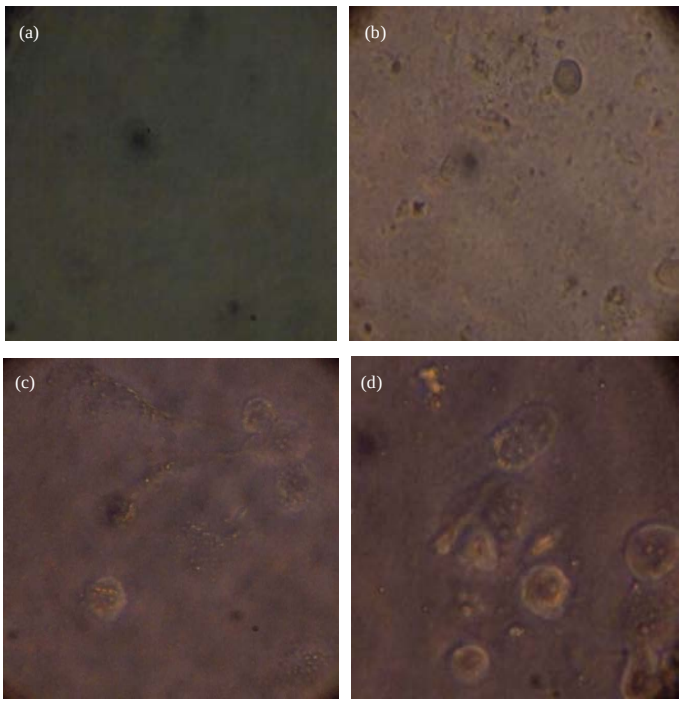

Fig. 5(a-d): Antiproliferative activity of *Nigella sativa* against MCF 7 cell lines at different concentrations (a) 100, (b) 500, (c) 250 and (d) 125 μg mL^{-1}

Table 2: Antiproliferative activity of methanolic extract of *Nigella sativa* against cell lines

Concentration (µg mL^{-1})	MCF-7	HEPG2	VERO
1000	7.6	21.6	22.8
500	12.3	38.2	35.9
250	32.3	40	41.2
125	44.6	52.8	57.0
62.5	63.0	66.3	64.0
31.2	75.3	75.1	76.3
15.6	81.5	89.2	86.8
7.8	96.9	88.1	89.4
Contr 0l	100	100	100

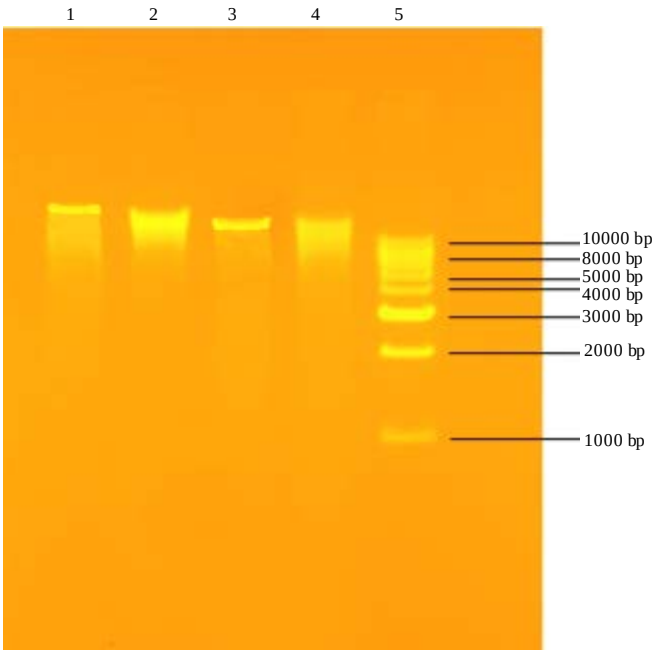

Fig. 6: DNA fragmentation assay. Lane 1: VERO control, Lane 2: Sample, Lane 3: HepG2 control, Lane 4: Sample and Lane 5: DNA ladder 1 kb (range 1000-10000 bp)

cell lines which elucidates that the themethanolic extract of *Nigella sativa* have greater anticancer activity against breast cancer (MCF-7) when compare with hepatoma cancer cell lines (HepG2).

The proliferation of cell involves various complex combinations of biochemical processes; flavonoids might influence different biochemical processes or stages in different manners (Yoshie-Stark *et al*., 2003). The results suggest strong antiproliferative properties and support the ethno medical claims forthe plant.

A study conducted by Tai *et al*. (2004), indicated that ethanolic extract of medicinal plants showed a concentration dependent antiproliferative effect on several tumor cell lines, with 50% inhibition (IC_{50}) of proliferation of MCF7 and HL60 cell lines. Chinkwo (2005) observed cytotoxicity induced by crude aqueous extract of the whole plant (leaves, stems and flowers) to neoplastic and CHO (Chinese Hamster Ovary) cell lines at different concentrations.

The DNA fragmentation assay results revealed that the *Nigella sativa* extract induced a good fragmentation of HepG2 cell lines and a moderate fragmentation was appeared in VERO MCF-7 cell lines (Fig. 6). The purpose of performing DNA fragmentation was to understand the inhibition

of the expression of genes responsible for the enzymes involved in cancer cell proliferation. Overall, all *Nigella sativa* methanolic extracts used in this study showed a concentration dependent growth inhibition effect on cancer cells. Further *in vivo* studies are needed to confirm the pharmacological efficacy and safety of *Nigella sativa*.

CONCLUSION

Plants have several active metabolites such as antioxidant compounds. In this research, the antioxidant and antiproliferative activities of *Nigella sativa* was investigated. Various organisms are currently undergoing investigations with the objective of isolating biologically active metabolites along with the quest for new compounds. Moreover,it was indicated that the plant is found to be a potential source of avariety ofbiologically active metabolites and it is hope that the present results will provide an initial point for investigations aimed at exploring new natural antioxidant substances present in the extracts of plant *Nigella sativa*.

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