WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 10, 882-892. Research Article ISSN 2277– 7105

LIPID PEROXIDATION AND ANTI-OBESITY ACTIVITY OF *Nigella Sativa* SEEDS

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Article Received on
06 July 2017,

Revised on 27 July 2017,
Accepted on 16 Aug. 2017

DOI: 10.20959/wjpr201710-9389

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ABSTRACT

Nigella sativa which belongs to the Ranunculaceae family is a traditional medicinal plant used to treat various ailments in earlier days. The seeds of this plant has high potential effect on diseases like cancer and it shows high antioxidant and antilipid activities due to the presence of phytochemical thymoquinone. It also has potent activity against diseases like bronchitis, asthma and skin diseases. The methanol extract of these seeds shows great antibacterial against *E.coli* and *B.subtilis.* In the present study, lipid peroxidation and anti-obesity activity of *N.sativa* seeds was well studied and it can shed more lights on different dimension to the researchers. It also deals with the Quantitative & Qualitative determination of phytochemicals and its pharmacological activities.

KEYWORDS: *Nigella sativa,* lipid peroxidation, anti-obesity, antioxidant, DPPH, invitro.

INTRODUCTION

Nigella sativa (black cumin) is an annual herb which belongs to the Ranunculaceae Family and it is widely present throughout the world. Especially, in the Southwest Asia and North Africa regions (Al-Ghamdi MS 2001). It is an important medicinal herb which is used for the treatment of cancer (Malik MA *et al*., 2016). It is also used as a traditional medicine in Ayurveda, Unani and Siddha to treat various ailments (Ahmad A *et al*., 2013). The seeds of

Nigella sativa were used to treat diseases such as diarrhea, bronchitis, asthma, rheumatism and skin disorders (Gilani AH *et al*., 2004).

N. sativa has been studied for itsvarious biological activities and therapeutic potential.It showed that, the seeds have some pharmacological activities such as anticancer, antihypertensive, antioxidant, anti-inflammatory, anti-cholesterol, diuretic and spasmolytic activities due to the phytochemicals present in it (Ahmad A *et al*., 2013). It also possess hepato-protective and renal protective properties. Due to its high healing activity, *N. sativa* has got the separate unique standard in the field of herbal medicine (Hussain AS and Hussain MM 2016). The above activities were regulated by the phytochemical thymoquinone(Staniek K and Gille L 2010., Nagi MN and Mansour MA 2000) present in the seeds. In food, it is used as additives for the enhancing of flavor (Duncker SC *et al*., 2014). For the reason that of the low toxic level it is used in breads and pickles. In the present study, our investigation is mainly focused on the antibacterial, lipid peroxidation and anti-obesity activities of *N. sativa.*

MATERIALS AND METHODS

Plant collection

Nigella sativa plants have been collected from Thanjavur, Tamilnadu, India. All the chemicals used were purchased from Thermo fisher scientific with analytical grade and the glassware used were fully sterilized & the suitable environment was maintained throughout the experiment.

Preparation of extract

The powder of *Nigella sativa* (50 g) was extracted with 96% methanol for 24 h in soxhlet apparatus. The extract was boiled in a heating mantle until a viscous liquid is obtained.

Qualitative determination of the chemical constituents

It was performed to determine the presence of alkaloids, terpenoids, flavanoids, saponins, tannins, steroids, glycosides, phlobatannins, proteins, coumarins,etc., in the methanolic extract of *Nigella sativa* seeds (Kamal A and Ahmad IZ 2014).

Table.1: Qualitative determination of the phytochemicals.

S.no	Phytochemical	Reagent mixture	Amount of Methanol extract (ml)	Confirmation
1	Terpenoids	2 ml of chloroform + conc.H_2SO_4 (after the addition of *N. sativa*)	2	Appearance of reddish brown color.
2	Flavanoids	3 drops of 10% lead acetate	1	Appearance of yellow color.
3	Saponins	5 ml of distilled water + 3 drops of olive oil (shaked vigorously)	5	Formation of oil emulsion.
4	Tannins	2 ml distilled water + 3 drops of 0.1% ferric chloride (after the addition of *N. sativa*)	2	Appearance of green color.
5	Alkaloids	3 drops of Hager's reagent	2	Formation of yellow precipitate.
6	Steroids	2 ml of chloroform + 5 drops of conc.H_2SO_4	2	Reddish brown ring was formed.
7	Glycosides	2 ml of chloroform + 2 ml of acetic acid	2	Color change from Violet to blue to green.
8	Phlobatannins	2 ml of1% HCl	2	Formation of red precipitate.
9	Proteins	1 ml of conc.H_2SO_4	2	Formation of white precipitate.
10	Coumarins	3 ml of 10% NaOH	2	Appearance of yellow color.

Quantification of Phytochemical compounds

It was performed to determine the presence of Phytochemical such as tannins, flavanoids, alkaloids and total phenolics with Quantification measures.

Determination of tannins

For the quantification of tannins 0.2 g of *N. sativa* was added to 20 ml of methanol and in a water bath for 1 hour at 80ºc. The *N. sativa* was filtered with the No.1 whatmann filter paper in the volumetric flask. 20 ml of distilled water, 2.5 ml of folin's reagent and 10 ml of sodium carbonate were added to the filtrate and made to 100 ml. Absorbance was taken at 725 nm.

Determination of flavanoids

To a 0.5 ml of extract 0.5 ml of aluminium chloride-methanol mixture was added and left for 1 hour. After 1 hour absorbance was taken at 430 nm.

Determination of alkaloids

To a 5 g of extract 50 ml of acetic acid-ethanol solution was added and left for 4 hour. Then filtered and kept for some time to evaporate ethanol in the filtrate.0.1 N NaOH was added and

precipitate was formed. The precipitate was dried and reweight for the determination of amount of alkaloids and the absorbance was taken at 435 nm.

Determination of total phenolics

To a volume of 0.5 ml of extract, 2.5 ml of folin's reagent and 2 ml of sodium carbonate was added and incubated for 30 minutes. Appearance of bluish green was regarded as positive for total phenolics. Then absorbance was taken at 650nm.

Pharmacological activities

Invitro antioxidant assays

A stock solution (20µg/ml) of the methanol extract was prepared for various antioxidant and reducing assay. Antioxidant power of each assay was compared with efficacy of standard chemicals (Alenzi FQ *et al.*, 2013).

DPPH radical scavenging activity

The antioxidant potential of the extract was assessed by using 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) assay. Stock solution of the extract was prepared at various concentrations. 1 ml of DPPH was added to each concentration of the extract and incubated in dark for 30 minutes. Absorbance was taken at 520 nm. Ascorbic acid was used as standard for the *Nigella sativa*. It is calculated by the following formula,

$$\text{Percentage inhibition} = \frac{(\text{control absorbance- Seed sample absorbance})}{(\text{Control absorbance})} \times 100$$

Total antioxidant assay (TAC)

0.1 ml of extract at various concentrations was taken and mixed with 1 ml of reagent (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) and incubated at 95˚c in a water bath for 90 minutes. Absorbance was recorded at 680 nm When it attains room temperature. Ascorbic acid was used as standard. The total antioxidant capacity was calculated by using the following formula,

$$TAC = \frac{C \times v}{M}$$

Where, V= volume of plant extract.M=concentration of plant extract.

C=x.

Reducing power assay

To perform this assay, 2.5ml phosphate buffer and 2.1 ml of potassium ferricyanide were added to the test tubes containing various concentration of the extract. Test tubes were kept at 50˚c in a water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes. Then to the upper layer (2.5 ml), 2.5 ml of distilled water and 0.5 ml of ferric chloride were added. Absorbance was recorded at 700 nm.

In vitro anti-lipid peroxidation

A stock solution (20µg/ml) of the methanol extract was prepared for various anti-lipid and reducing assays. Anti-lipid power of each assay was compared with efficacy of standard chemicals.

Lipid inhibition assay

The lipid peroxidation effect of the extract in liver homogenate was determined (Ohkawa *et al.,* 1979) with a minor modification (Kumar *et al*., 2000). A freshly excised goat liver was processed to get 10% homogenate in cold phosphate buffer saline at 7.4 pH using a glass rod and was filtered to get clear homogenate. The degree of lipid peroxidation was assessed by estimating the thiobarbutaric acid reactive substance (TBARS).different concentrations of extract were prepared (10-500µg/ml) and were added to liver homogenate. Lipid peroxidation was initiated by adding 100µl of 15mM ferrous sulphate to 3 ml tissue homogenate. After 30 minutes 100µl of that reaction mixture was taken in a tube which contains 1.5 ml of TCA. After 10 minutes, tubes were centrifuged at 6000 rpm for 10 min. The supernatant was separated and mixed with 1.5 ml of 0.67% TBA in 50% acetic acid. This mixture was heated for 30 minutes in boiling water bath. Pink colored complex was formed and measured at 535nm. The Anti-lipid peroxidation was assessed by using a following formula,

$$\% \text{ inhibition} = \frac{(\text{abs control} - \text{abs } \textit{N. sativa}) \times 100}{\text{Abs control}}$$

Phenol red plate assay

It is a pH indicator dye showing difference in color at various pH ranges differing from acidic to basic (yellow to orange-below 7, magenta to pink-above pH 8.). This assay includes 2% of agar, 1% olive oil, 0.01%phenol red (10mg/ml), Tween 80, distilled water, 2.5mM phosphate buffer. Media was prepared by adding 2% agar and autoclaved at 121˚c for 15 min. Then 1% olive oil, few drops of Tween 80, 0.01% phenol red was added in flask containing 2% agar when it attains 60ºc. The pH of media was set at neutral by adding few drops of 1 N NaoH

which gives media the blood red color. The medium was plated and wells have been made with the help of pre-sterilized 5mM core-borer. Lipase (100mg/ml in 2.5mM phosphate buffer) was prepared and 60µl of lipase was added to the eppendorf tubes containing 110 µl of different concentration of extract. This mixture was incubated at 37˚c for 30 minutes. After incubation, 35µl of above reaction mixture was added in each well along 30µl of lipase as control in separate well in the Petri plates. Again it is incubation at 37˚c for 24-48 hours. The color changes were observed on the next day.

Anti-microbial activity

Antimicrobial activity of the *N. sativa* seeds were studied by the standard protocol (Hanafy MS and Hatem ME., 1991) Nutrient broth was prepared by adding 0.6g Beef extract, 1g Peptone, 1g Nacl and 7gof Agar in 200ml of distilled water. The broth was autoclaved at 121˚c for 15 min and poured on Petri plate. After solidification, the culture was added (*E.coli* and *B.sutilis* pour plate method) (Saranya *et al*., 2017). The different concentration of extract (200, 100, 50, 25, 12, 6) were placed on the plate by paper disc method (Sundaramoorthy M*et al*., 2014).

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical analysis of methanol extract of *Nigella sativa* seeds indicated the existence of terpenoids, saponins, tannins, alkaloids in the *N. sativa*.

Table. 2: Phytochemical analysis of methanol extract of *Nigella sativa* seeds.

S.no	Phytochemical	Presence/ Absence
1	terpenoids	+
2	flavanoids	+
3	saponins	+
4	tannins	+
5	alkaloids	+
6	steroids	+
7	glycosides	-
8	phlobatannins	-
9	proteins	-
10	coumarins	-

+ represent Present - represent Absent.

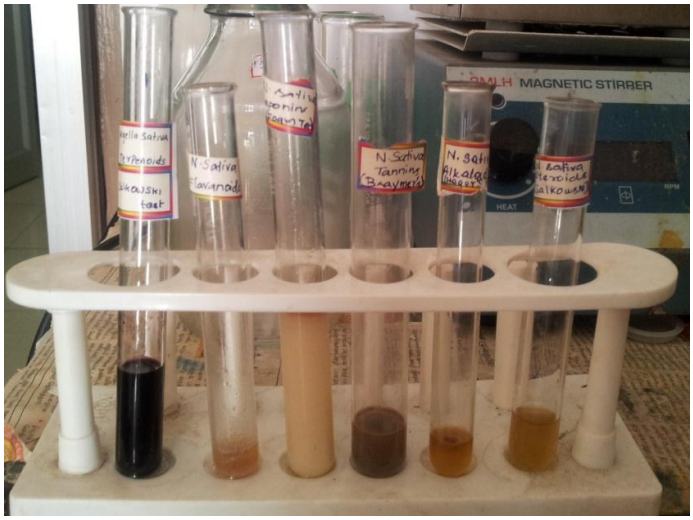

Fig 1: Phytochemical analysis of methanol extract of *Nigella sativa* seeds.

DPPH radical scavenging activity

The results revealed that the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of *Nigella sativa* seeds was high at larger concentrations of the extracts. The absorbances were measured at 570nm with Ascorbic acid as standard.

Table 3: DPPH radical scavenging activity of methanol extract of *Nigella sativa* seeds.

S.no	Concentration (µg/ml)	Absorbance at 570nm	
		Ascorbic acid	*N. sativa*
1	Blank	0.0	0.0
2	0.2	84.90	34.4
3	0.4	86.79	39.78
4	0.6	88.67	51.61
5	0.8	90.56	58.06
6	1.0	92.43	63.44

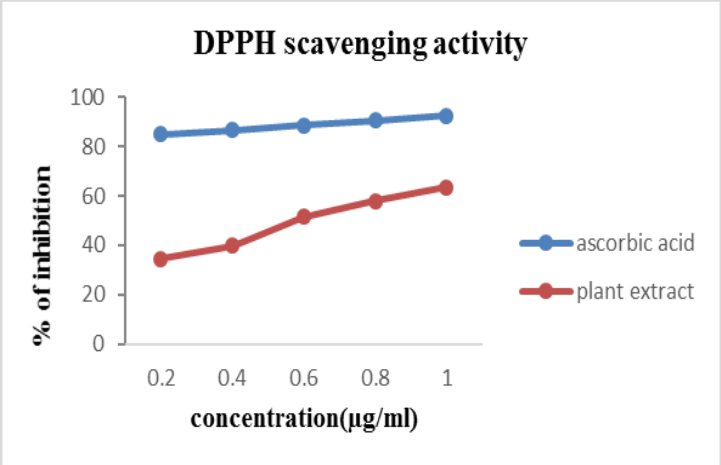

Fig.2: DPPH radical scavenging activity of methanol extract of *Nigella sativa* seeds.

Total anti-oxidant assay

The anti-oxidant assay was performed and Absorbance was taken at 570nm using ascorbic acid as standard and graph was plotted.

Table 4: Determination of Total anti-oxidant activity of the *N. sativa* seeds.

S.no	Concentration(µg/ml) (µg/ml)	Absorbance at 570nm	
		Ascorbic acid	*N. sativa*
1	Blank	0.0	0.0
2	0.2	0.21	0.42
3	0.4	0.23	0.44
4	0.6	0.31	0.52
5	0.8	0.64	0.85
6	1.0	0.72	0.93

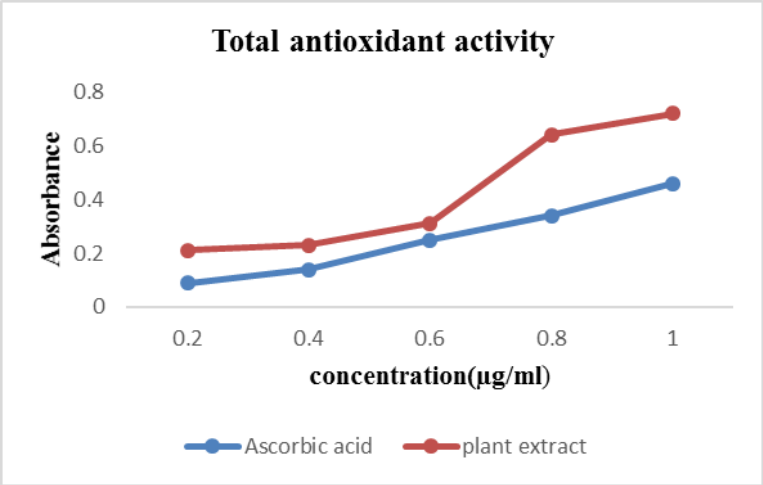

Fig. 3: Determination of Total anti-oxidant activity of the *N. sativa* seeds.

Reducing power assay

The reduction of ferric cyanide complex to ferrous form by donating an electron indicates the presence of reductants in the plant extract.

Table 5: Determination of reductants presents in the *N. sativa* seeds.

.no	Concentration (µg/ml)	Absorbance at 570nm	
		Ascorbic acid	*N. sativa*
1	Blank	0.0	0.0
2	0.2	0.4	0.46
3	0.4	0.58	0.48
4	0.6	0.85	0.49
5	0.8	1.00	0.53
6	1.0	1.2	0.73

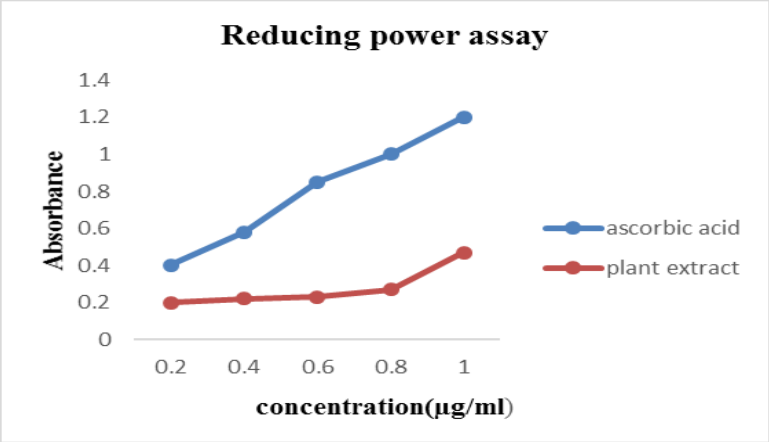

Fig. 4: Determination of reductants in the *N. sativa* seeds.

In-vitro anti-lipid peroxidation

The result reveals that the *N. sativa* seed extract have better anti-lipid peroxidation property which can be used for the control of cholesterol.

Table 6: Anti-lipid peroxidation effect of methanol extracts of *N. sativa* seeds.

S.no	Concentration (µg/ml)	Percentage inhibition	Absorbance at 570 nm	Vitamin E % of inhibition
1	Blank	0.0	0.0	0.0
2	0.2	15.38	0.22	42.86
3	0.4	23.07	0.20	50.00
4	0.6	30.76	0.18	64.29
5	0.8	42.30	0.15	71.43
6	1.0	50	0.13	85.71

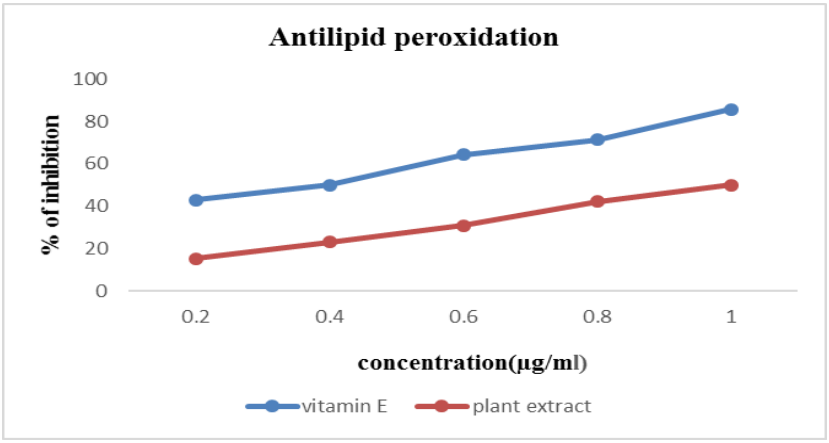

Fig. 5: Anti-lipid peroxidation effect of methanol extracts of *N. sativa* seeds.

Phenol red plate assay

The plate was analyzed for the antilipid effect of *N. sativa* seeds. It shows a positive result which is determibed by the zone of inhibition in the culture plate.

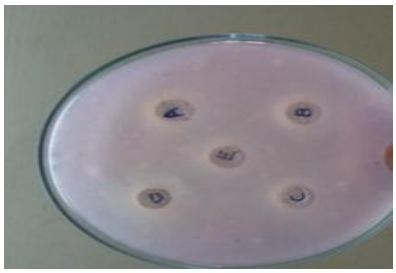

Fig.6: Phenol red plate assay plate of *N. sativa* seeds.

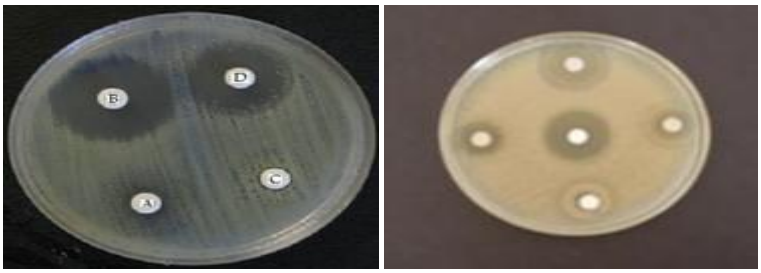

Fig.7: Antibacterial effect of *N. sativa seeds* on the *E. coli* bacteria (Gram -ve) and *B. subtilis* bacteria (Gram +ve).

Antibacterial activity

The methanol extract of *N. sativa* seeds has effective antibacterial property which is observed by the zone of inhibition in both gram positive bacteria and gram negative bacteria such as *Bacillus subtilis* and *Escherichia coli* respectively. 1µg/ml of the *N. sativa* was tested against the culture plates.

CONCLUSION

The present study reveals that the qualitative and quantitative determination of phytochemicals present in the *N. sativa* seeds. It also deals with the antioxidant, antilipid and antibacterial effect of the seeds. Due to its effective pharmacological activities, In future it may be used for the treatment of many diseases. As we are keenly interested in the study of traditional medicinal plants and our team have also have published our articles in the plants such as Allium sativum (Rathnasamy S *et al*., 2014), Solanum trilobatum (Balakrishnan P et al., 2015), *Leucas aspera* (Nagarasan S, Boominathan M 2016), *Adhatoda vasica* (Nagarasan S, Boominathan M 2016).

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