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Antioxidant and Anticancer Potential of *Nigella sativa* Seeds

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ABSTRACT

To determine the phytochemical composition, anticancer activities and antioxidant of *Nigella sativa* methanol and ethyl acetate seed extracts. Phytochemical screening was characterized using standard qualitative and quantitative methods. The antioxidant activity was determined using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and nitric oxide scavenging process whereas the *in vitro* cell viability and cytotoxicity was determined by using Breast Cancer Cell Line (MCF-7) cancer cells. Phytochemical screening of the extracts showing that they were rich in secondary class metabolite compounds in all three different solvents such as quonones, cardiac glycosides, terpinoides and phyosteroides and absence of saponins, phlobatannines and glycosides whereas tannins, flavonoids, phenols, coumarins were present only in Ethyl Acetate (EAC) and Methanol (MCE). Total phenolic and whole flavonoid composition were quantified and showed more in Methanol Extract (ME) than Ethyl Acetate Extract (EAE). *In vitro* antioxidant activity of DPPH radical scavenging showed 63.32 µg/ml in methanol extracts than in ethyl acetate extracts which found about 37.41 µg/ml. The nitric oxide scavenging activity of *N. sativa* showed 66.59 µg/ml in the methanolic extract and 36.66 µg/ml were found in EAE. Cytotoxic activity of 100 µg/ml concentrations of EAC and MCE exhibited 69.28% and 82.01% of cancer cell death respectively. The results show that *N. sativa* was a promising antioxidant and anticancer agent.

Keywords: *Nigella sativa*, Phytochemical screening, Antioxidant, Anticancer

INTRODUCTION

Plants are the natural industrial units for the production of chemical compounds, many of which are used as therapeutics for diseases and some of them are marketed as herbal medicines. Herbal medicines have long been viewed as a source of restorative medication based on religious and cultural traditions. The medicinal value of plants lies in their chemical constituents that produce a definite physiological action on the human body. The most important bioactive compounds of plants include flavonoids, tannins, alkaloids, and phenolic compounds [1]. Medicinal plants have been used for curing ailments for many centuries in different aboriginal systems of medicine as well as folk medicines. Moreover, medicinal plants are also used in the preparation of herbal medicines as they are considered to be safe as compared to contemporary allopathic medicines [2]. Scientists are focusing on medicinal plants, only a few plant species have been thoroughly investigated for their medicinal properties, potential, mechanism of action, safety evaluation and toxicological studies others are yet to be explored for scientifically evaluation in detail.

Nigella sativa Linn. (family Ranunculaceae), commonly known as black cumin, is an annual plant that has been conventionally used in the Indian subcontinent and also widely used medicinal plant throughout the world [2]. The seeds of *N. sativa* and their oil have been extensively used for centuries in the treatment of a wide range of ailments all over the world. It is also a key medicinal component in the Indian traditional system of medicine like Unani and Ayurveda [3]. Literature search has revealed that a lot more studies have been recently carried out related to the anticancer activities of *N. sativa*. High Performance Liquid Chromatography (HPLC) analysis of showed that there are atleast four main chemicals such as Dithymoquinone (DTQ), Thymohydroquinone (THQ), Thymoquinone (TQ) and Thymol (THY) are present in *N. sativa* seed oil. The seeds also contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activities of the seeds have been shown to be due to thymoquinone. TQ is considered as potent antioxidant [4,5], anticarcinogenic and antimutagenic agent [6]. Alpha (α)-hederin, a pentacyclic triterpene saponin isolated from the seeds of *N. sativa*, was also evidenced to have effective *in vivo* antitumor activity [7]. In addition, TQ is a relatively safe compound, particularly when given orally to experimental animals [8]. The seeds of *N. sativa* have been widely used in the treatment of different diseases and ailments. The present study is to understand the chemical profile, anticancer potential and antioxidant properties of *N. sativa* seeds.

MATERIALS AND METHODS

Crude extract preparation

The *N. sativa* seeds were collected from organic supermarket and which is authenticated by botanist. The collected seeds were ground into powder using electric grinder. About 300 g of the powder was soaked in 900 ml of petroleum ether, methanol and ethyl acetate separately. After 72 h, it was filtered using Whatman filter paper no.1 and filtrate was concentrated under reduced pressure using rotary vacuum evaporator. The final dried extract was collected and quantified.

Qualitative phytochemical screening

The different solvent extracts of *N. sativa* seeds such as petroleum ether extract (PE), Ethyl Acetate Extract (EAE) and Methanol Extract (ME) were qualitatively estimated for various phytochemicals such as The following tests viz. carbohydrate, tannins, saponins, alkaloids, flavanoids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, phytosteroids, phlobatannins, anthroquinones [9].

Quantitative phytochemical analysis

Total Phenolic Content (TPC)

Total phenolic content for EAE and ME was assessing according to the Folin–Ciocalteu method [10] with some modifications. Briefly, 0.1 ml of the *N. sativa* seed extracts at concentrations of 200, 600 and 1000 µg/ml were taken in separate tubes. About 1.9 ml of distilled water and 1 ml of Folin–Ciocalteu's reagent were seeded in a tube, and then 1 ml of Na₂CO₃ (100 g/l) was added. The reaction mixture was incubated at 25°C for 2 h and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was collected. The results were compared to a catechol calibration curve and the total phenolic content of *N. sativa* was represented as mg of catechol equivalents per gram of extract.

Estimation of total flavonoid content

The EAE and ME extracts were analyzed for the total flavonoid content by colorimetric method [11]. Various concentrations (200, 600 and 1000 µg/ml) of EAE and ME extracts were prepared separately. Briefly, 500 µl of extract was added with 2 ml of distilled water and subsequently with 0.15 ml of a NaNO₂ solution (15%). After 6 min, 0.15 ml of an AlCl₃ solution (10%) was mixed and allowed to stand for 6 min, then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was uniformly stirred and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm. Quercetin was used as positive test compound for the assay. The results were represented as quercetin equivalents (mg quercetin/g dried extract).

Assay for total tannins

The analysis of condensed tannins (proanthocyanidins) in EAE and ME extracts of *N. sativa* seeds was carried out according to the method of Sun et al. To 50 µl of EAE at concentration of 200 µg/ml, 3 ml of 4% methanol vanillin solution and 1.5 ml of concentrated hydrochloric acid were added. The mixture was allowed to stand for 15 min, and the absorption was measured at 500 nm against methanol as a blank. The same procedure was followed for other concentrations of EAE and all ME sample. The amount of total condensed tannins was expressed as mg (+)-catechin/g⁻¹. The calibration curve range was 0-400 µg/ml⁻¹.

Testing for antioxidant activity

DPPH free radical scavenging activity

The ability of the EAE and ME extracts to annihilate the 1,1-Diphenil-2-Picrylhydrazyl (DPPH) radical was investigated by the method described [12]. Briefly, DPPH was added to the solutions prepared with EAE and ME at concentrations of 200, 600 and 1000 µg/ml and standard compound Butylated Hydroxy Toulene (BHT) and stirred. The reaction mixture was incubated for 30 min at room temperature; then absorbance was recorded at 517 nm against a blank. The annihilation activity of free radicals was calculated in % inhibition according formula.

$$\% \text{ of Inhibition} = \frac{\text{control} - \text{Test}}{\text{control}} \times 100$$

Nitric oxide scavenging activity assay

The assay was performed according to procedure described by Kadhum et al. In this assay, Griess Illosvoy reagent was customized using naphthylethylene diamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). About 500 µl of EAE and ME at concentration of 1000 µg/ml were added separately to mixture containing 2.5 ml of sodium nitroprusside (10 mM) and 0.5 ml of phosphate buffer saline and incubated at 25°C for 150 min. After incubation, 0.5 ml of reaction mixture was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand. After 5 min, 1 ml of 1% naphthylethylene diamine dihydrochloride was added, mixed and kept for 30 min. Then the absorbance of these solutions was measured at 540 nm against the corresponding blank. The above procedure was followed for 200 and 600 µg/ml concentrations of EAE and MCE separately. Vitamin C was used as positive control. The scavenging activity was calculated using the formula:

$$\% \text{ of Inhibition} = \frac{\text{control} - \text{Test}}{\text{control}} \times 100$$

Detection of cell viability and cytotoxicity of extracts

The 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide (MTT) assay was used to detect the cell viability and cytotoxicity in MCF-7 cancer cells. Briefly, the cancer cells which cell line were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum (FBS), at 37°C in humidified atmosphere with 5% CO₂. The cells were plated in 96-well flat bottom tissue culture plates at a density of approximately 1.2 × 10⁴ cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated separately with three extracts at different concentrations of 100, 200 and 300 µg/ml for 24 h. After the incubation, medium was discarded and 100 µl fresh medium was added with 10 µl of MTT (5 mg/ml). After 4 h, the medium was discarded and 100 µl of Dimethyl Sulfoxide (DMSO) was added to dissolve

the formazan crystals. Then, the absorbance was read at 570 nm in a microtitre plate reader. Cyclophosphamide was used as a positive control [13,14].

RESULTS AND DISCUSSION

Quantity of extracts obtained from *N. sativa* seeds

N. sativa seeds revealed the presence of chemical constituents that have enormous pharmacological properties. In our study, we have found three extracts (PCE, EAC and MCE) showed the presence of quinones, cardiac glycosides, terpenoids and phyosteroides and absence of saponins, phlobatannins and glycosides. Other phytochemicals such as tannins, flavonoids, phenols, coumarins were present only in EAC and MCE but absent in PCE. The presence of alkaloids was found only in EAC and not in PCE ad MCE (Table 1). Yessuf et al., reported that methanol extracts of *N. sativa* seeds showed the presence of alkaloids. Sameera et al., revealed that aqueous extract of *N. sativa* contain alkaloids, flavonoids, tannins, steroids, reducing sugar, phenolic compounds and higher saponin content. This may, due to seasonal variations that can affect the chemical composition of the plants and thus biological activity [15-18].

Table 1: Phytochemical analysis

| Phytochemical constituent | PE | EAE | ME |
|---------------------------|----|-----|-----|
| Tannins | - | + | + |
| Saponins | - | - | - |
| Alkaloids | - | + | - |
| Flavonoids | - | Wk+ | Wk+ |
| Quinones | + | + | Wk+ |
| Glycosides | - | - | - |
| Cardiac glycosides | + | + | + |
| Terpenoids | + | + | + |

The geographical location of a plant can affect its active constituents, which may be induced by many factors like climate, soil, propagation method, etc. [19]. Collection time of plant also affects its efficiency [20]. The combination of the above mentioned factors may attribute to the results, hence further studies are recommended. The results of TPC content in *N. sativa* were represented in Table 2. The phenolic content of *N. sativa* seed extracts obtained from Ethyl Acetate extract (EAE) and Methanol extract (ME) was quantified as 40.57 $\mu\text{g}/\text{mg}$ and 121.92 $\mu\text{g}/\text{mg}$ respectively as shown in (Figure 1).

Table 2: Percentage of cell viability and cytotoxicity of extract

| Test | Treatment (μg) | | | | | | P | C |
|-------------------|-----------------------------|--------|--------|--------|--------|--------|--------|-----|
| | EAE | | | ME | | | | |
| | 100 | 200 | 300 | 100 | 200 | 300 | | |
| % of Viability | 30.714 | 35.192 | 46.363 | 17.988 | 25.654 | 33.118 | 76.651 | 100 |
| % of Cytotoxicity | 69.285 | 64.807 | 53.636 | 82.011 | 74.345 | 66.881 | 23.348 | 0 |

EAE-Ethyl acetate extract; ME-Methanol extract; P-Positive, C-Control

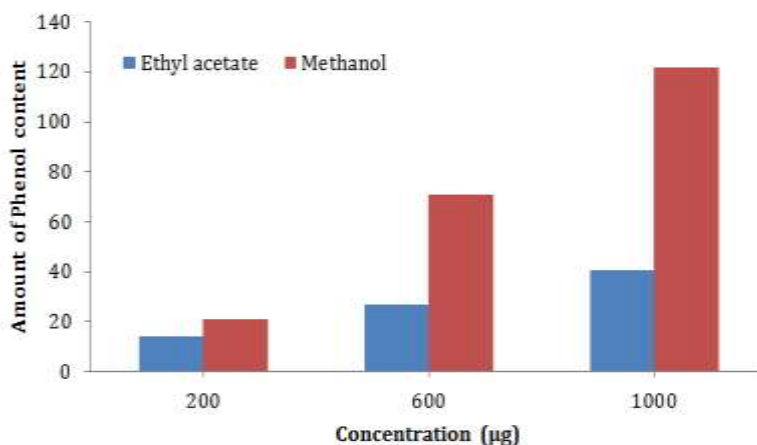


Figure 1: The phenolic content estimation of *N. sativa* seed extracts obtained from Ethyl Acetate extract (EAE) and Methanol extract (ME)

The flavonoid content was showed in ethyl acetate is 202.69 $\mu\text{g}/\text{g}$ and in methanol extract 373.61 $\mu\text{g}/\text{g}$ (Figure 2). It was shown that ME have

more phenolic and flavonoid content than EAE. Alimohammadi *et al.* [21], showed that hydro alcoholic extract of *N. sativa* is rich in polyphenolic compounds.

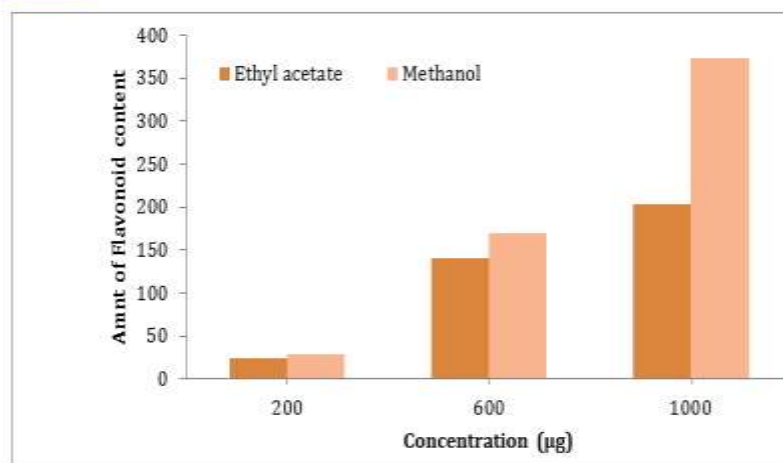


Figure 2: The flavonoid content estimation of *N. sativa* seed extracts obtained from Ethyl Acetate extract (EAE) and Methanol extract (ME)

The presence of phenols and flavonoids may have been responsible for the observed antioxidant activities. The total tannin content in EAE and ME were summarized Figure 3. The result was showed that total tannin contents in the seed extracts of *N. sativa* was found 862.94 µg in EAE and 345.82 µg in ME (Figure 3).

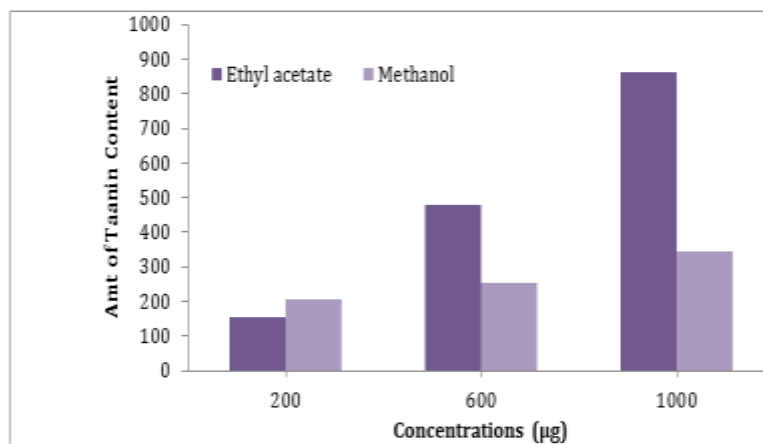


Figure 3: The tannin content estimation of *N. sativa* seed extracts obtained from Ethyl Acetate extract (EAE) and Methanol extract (ME)

About 63.32 µg/ml of DPPH radical scavenging activity with reference to ascorbic acid was found in ME than in ethyl acetate extracts which showed about 37.41 µg/ml concentration (Figure 4).

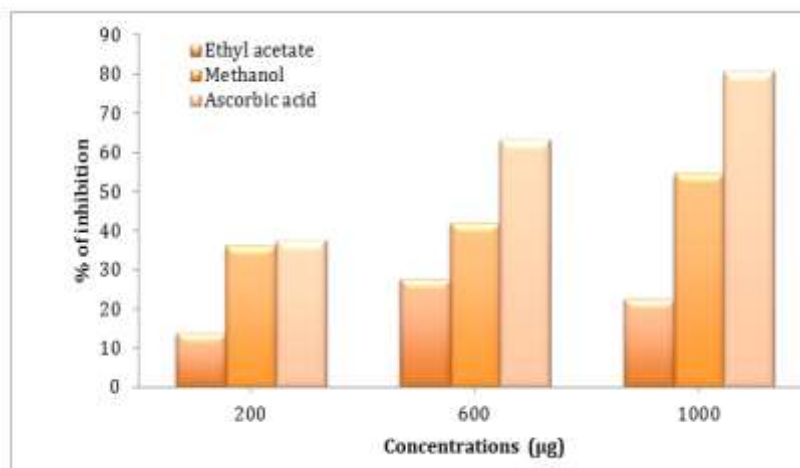


Figure 4: DPPH radical scavenging activity with reference to ascorbic acid was found in Ethyl Acetate extract (EAE) and Methanol extract (ME)

Our study showed various concentrations of *N. saliva* seed extracts effectively reduced the generation of nitric oxide from sodium nitroprusside (Figure 5).

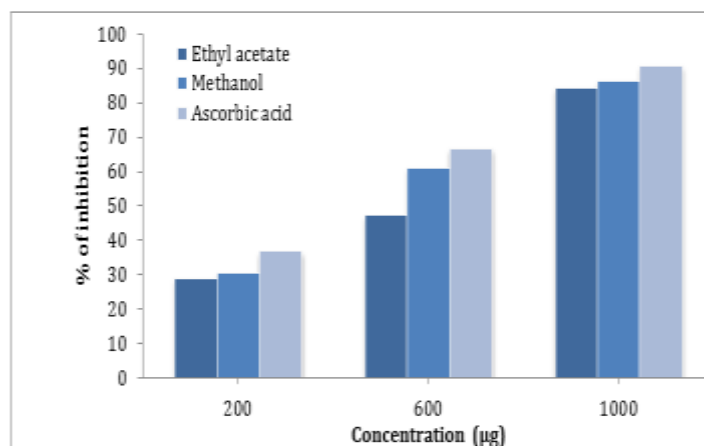


Figure 5: NO₂ scavenging assay

The scavenging percentage of nitric oxide was noticed increasing in increasing concentration of the plant extracts. Our results found that 1000 µg/ml of extract ME showed 66.59 µg/ml concentration of NO₂ scavenging activity and lower activity at concentration of 36.66 µg/ml was found in EAC. The results of cytotoxic activity of MCE and EAC of *N. saliva* on MCF-7 cancer cell were represented in Table 2 and Figure 6. The results showed that 100 µg/ml concentrations of EAE and ME exhibited 69.28% and 82.01% of cancer cell death respectively.

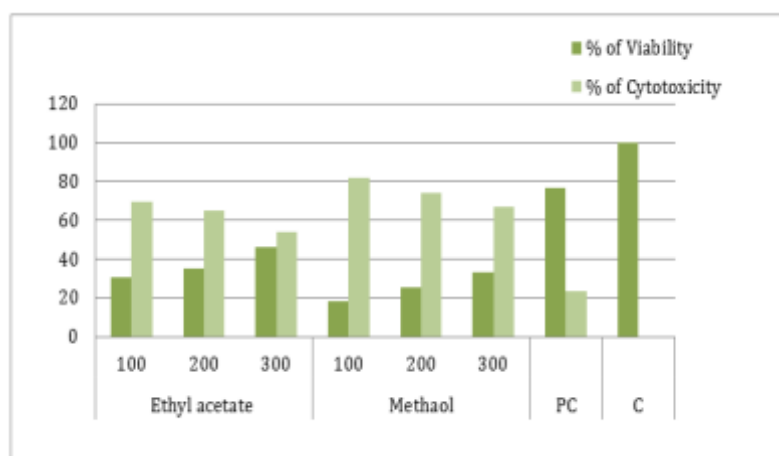


Figure 6: Effect of cell viability and cytotoxicity of extracts in MCF-7 Cancer cells
C-Control; P-Positive

CONCLUSION

The study results demonstrated that the seed extract of *N. saliva* could have potent antioxidant potentials to prevent or control free radical and also possess anticancer property to cure various types of cancers in humans. So these data confirm the traditional use of this plant with its phenolic, flavonoid and tannins compounds. Hence, we believe that the seed extract could be used to treat many inflammatory diseases and cancer with further more scientific justification are required in *in vivo* and *in vitro* evaluations.

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