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Comparitive assessment of the antioxidant, antidiabetic and anticancer activity of seed extracts from *N. Sativa* and *Cuminum cyminum*

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Abstract
The pharmacological effects in terms of anti-diabetic, anti-oxidant and anticancer properties of the methanolic seed extracts of *N. sativa* and *Cuminum cyminum* were studied extensively. An extensive phytochemical screening was done on the extracts obtained to acquire an understanding of the bioactivity of all compounds present. Standard DPPH assays were performed for evaluation antioxidant activity, where *N. sativa* showed a greater scavenging activity than *Cuminum cyminum*. MTT assays were employed to calculate the anti-proliferative nature of both seed extracts. The results showed an exponential increase in the cytotoxicity with increase in the extract concentration, against breast cancer cells. Unfortunately, the seed extracts did not show any promising anti-diabetic activity as inferred from the α-amylase assay. The comparative assessment carried out gave a comprehensive understanding of the pharmacological of these spices that are so commonly used in the standard Indian diet.

Keywords: pharmacological properties, phytochemical screening, anti-oxidants, cytotoxicity, anti-diabetic

1. Introduction
Spices are an integral part of any diet. They are predominantly composed of fiber, carbohydrate, fat, sugar, protein, gum, ash, volatile essential oils, and other nonvolatile components. These components impart each spice's particular flavor, color, nutritional, health, or preservative effects. When the spice is ground, cut, or crushed, the cells rupture, and it releases the volatile and non-volatile components [1].

Essential oils are a major flavoring constituent of all spices. They are soluble in organic solvents while only sparsely soluble in water. Essential oils lose their aroma with age. Essential oils are very concentrated, about 75 to 100 times more concentrated than the fresh spice. They do not have the complete flavor profile of ground spices, but they are used where a strong aromatic effect is desired.

Nigella sativa (N sativa) (black cumin) is an annual herb of the Ranunculaceae family, commonly used as a flavoring agent and natural health remedy in traditional folk medicine for the treatment of numerous disorders in ancient systems of Unani, Ayurveda, Chinese and Arabic medicine for thousands of years [2]. The *Nigella sativa* seeds contain ingredients, including nutritional components such as carbohydrates, fats, vitamins, mineral elements, and proteins, including eight of the nine essential amino acids. Pharmacological investigations of the seed extract reveal a wide spectrum of activities including anti-inflammatory, antibacterial, antifungal and antihelmenthic. The seeds are externally applied for eruptions on skin, the extracts of N sativa seeds have anti-inflammatory and antioxidant activities, and being used by patients to suppress coughs, disintegrate renal calculi, retard the carcinogenic process, Treat abdominal pain, diarrhea, flatulence and polio [3]. The seed of this plant, commonly known as black seed, are eaten alone or in combination with honey and in many food preparations and the oil prepared by compressing the seeds of N sativa is used for cooking. The seeds of N sativa contain both fixed and essential oils, proteins, alkaloids and saponin [4]. Therefore, the present study was designed to investigate the anticancer activity of seed extract (NSE) and seed oil (NSO) of N sativa against a human breast cancer cell line.

2. Materials and Methods
2.1 Plant Material and Extraction Procedure
Seeds of *N. sativa* and *Cuminum cyminum* were collected at maturity from cultivated plants. The seed powders (50 g) were extracted under reflux with absolute methanol for 1 h and then with 80% methanol for 3 h. Afterward, the different extracts were concentrated under vacuum.

2.2 Cell Culture
Human breast cancer cell line was grown in DMEM medium. The medium was supplemented with 10% fetal bovine serum, a solution of vitamins, sodium pyruvate, nonessential amino acids, penicillin and streptomycin (100mg/mL). Cells were cultured in a humidified atmosphere at 37^0C under 5% CO2.

2.3 Phytochemical Screening
Phytochemical examinations were carried out for all the extracts as per the standard methods [5].

1. **Detection of alkaloids:** Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids (Mayer's Test).
2. **Detection of anthraquinone:** About 0.1g of each portion

to be tested is shaken with 5ml of benzene and then filtered. 3ml of the 10% ammonia solution is then added to the filtrate and thereafter shaken. Appearance of a pink,red or violet colour in the ammonical(lower) phase was taken as the presence of free anthraquinones.

3. **Detection of Flavonoid:** To 0.1g of plant extract is boiled with distilled water and then filtered. To 2ml of the filtrate few drops of 10% ferric chloride solution is added. A green blue or violet coloration indicated the presence of a phenolic hydroxyl group.
4. **Detection of Coumarin:** To 2ml of the test solution a few drops of alcoholic sodium hydroxide are added. Appearance of yellow color indicates the presence of coumarin.
5. **Detection of Tannin:** About 0.1g each portion was stirred with about 2ml of distilled water and then filtered. Few drops of 1% ferric chloride solution are added to 2ml of the filtrate. Occurrence of blue-black precipitate indicates the presence of tannins.
6. **Detection of Saponin:** 200mg of extract is boiled with 3ml of distilled water and filtered to this about 3ml of distilled water is added and shaken for about 5 minutes. Frothing which persisted on warming is taken as an evidence for the presence of saponins.
7. **Detection of terpenoid:** The extract was mixed with 2ml of chloroform ad concentrated H_2S0_4(3ml) is carefully added to form a layer. A reddish-brown coloration of the interface is formed to show positive result of the presence of terpenoids.
8. **Detection of Steroids:** To 0.2g of each portion, 2ml of acetic acid is added, the solution is cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Colour development from violet to blue indicated the presence of a steroidal ring i.e. aglycone portion of cardiac glycoside.

2.4 Evaluation of *In Vitro* Antioxidant Activity through DPPH Assay

Each sample stock solution (1.0 mg ml_1) was diluted to final concentrations of 500, 250, 100, 50 and 10 mg ml_1, in ethanol. A total of 1 ml of a 0.3 mM DPPH ethanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. After 30 min, the Ab values were measured at 518 nm and converted into the percentage antioxidant activity using the following equation:

scavenging capacity %_/100_/[(Ab of sample_/Ab of blank)_/100/Ab of control].(1)

Ethanol (1.0 ml) plus plant extract solution (2.5 ml) was used as a blank, while DPPH solution plus ethanol was used as a negative control. The positive controls were DPPH solution plus each 1 mM flavonoid [6].

2.5 Evaluation of Antidiabetic Activity

Alpha-amylase activity was carried out by starch-iodine method. 10 µL of α- amylase solution (0.025 mg/mL) was mixed with 390 µL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37 °C for 10 min, 100 µL of starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 mL of 1% iodine solution was added, and after adding 5 mL distilled water, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions [7].

Inhibition of enzyme activity was calculated as

$$(\%) = (A\text{-}C)\ X100/\ (B\text{-}C)\ (2)$$

Where,

A= absorbance of the sample

B= absorbance of blank (without α-amylase)

C= absorbance of control (without starch).

2.6 Evaluation of Anticancer Activity

Exponentially growing cells were plated at a density of $5x10^{-3}$ cells per well, in 96-well microplates into 100 mL of culture medium and were allowed to adhere for 24 h at 37°C under 5% CO_2 before treatment. Then 100 mL of increasing concentrations of extracts dissolved in DMSO were added. The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Then the cells were incubated for 48 h in the presence or absence of extracts. The extracts were then treated with MTT reagent (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), incubated for 3 h and read at 620 nm after completely solubilizing it in isopropanol containing 37% HCl. Triplicate wells were used for each experimental condition [8].

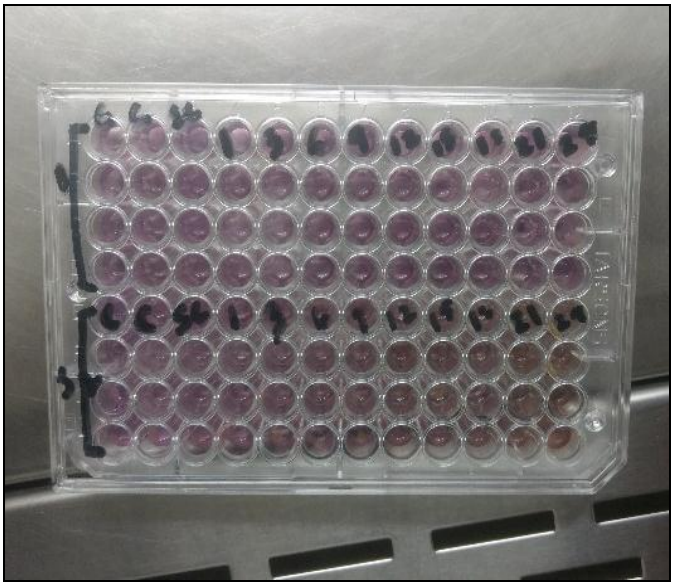

Fig 1: 96 well plate used for MTT assay

2.7 Data Analysis

Data were reported as mean values calculated from replicates (n P 3). Statistical treatments were performed using Sigma Stat software at 5% significance error level.

3. Results & Discussion

3.1 Phytochemical Screening

Phytochemical screening of the methanolic leaf extract of *N.Sativa* & *Cuminum cyminum*revealed they contain Flavonoids, Coumarin, Tannin, Saponin, Terpenoid, Steroid and phenol Alkaloids and Anthraquinone however was absent.

Table 1: Phytochemical Screening

Qualitative Test	*N. sativa*	*Cumimum cyminum*
Alkaloids	Negative	Negative
Anthraquinone	Negative	Negative
Flavonoid	Positive	Positive
Coumarin	Positive	Positive
Tannin	Positive	Positive
Saponin	Mildly Positive	Mildly Positive
Terpenoid	Positive	Positive
Steroids	Positive	Positive
Phenol	Positive	Positive

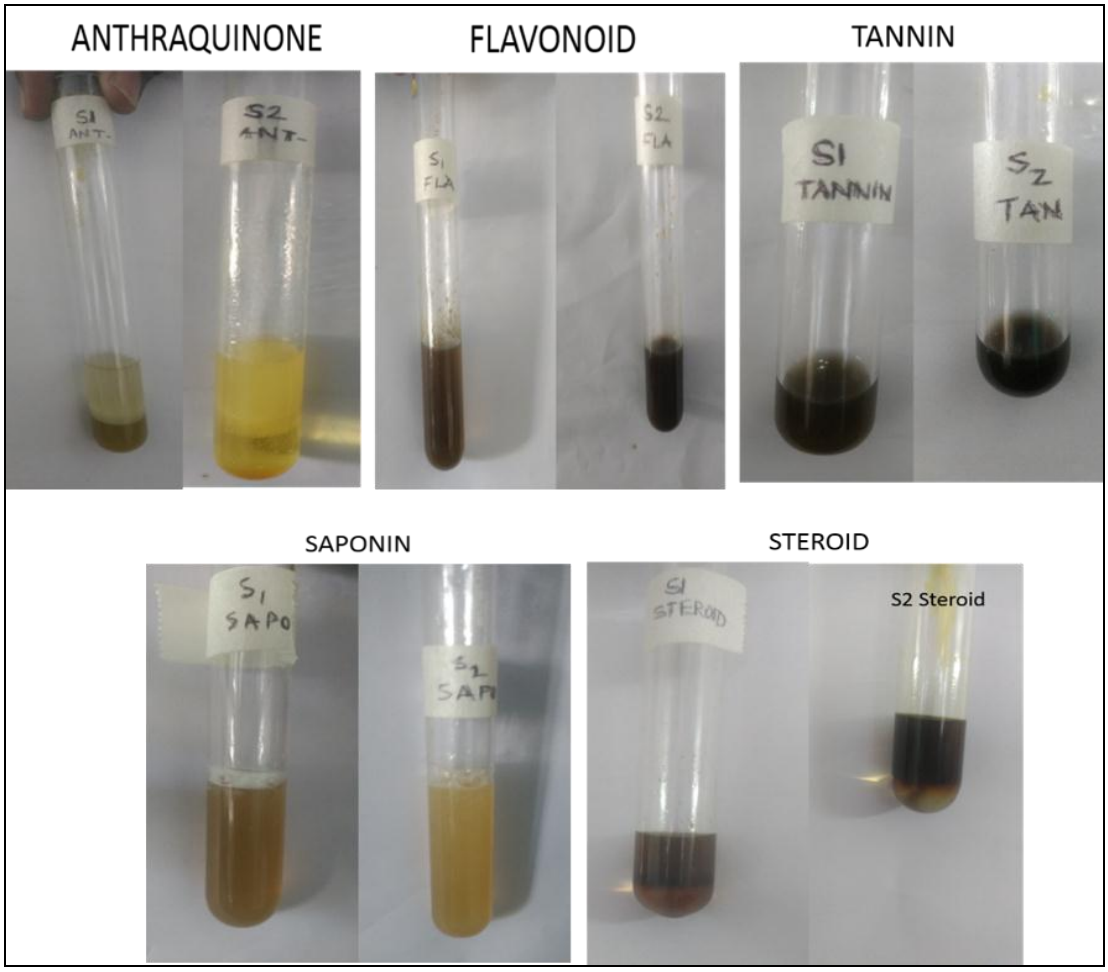

Fig 2: Results of Phytochemical tests

3.2 Evaluation of *In Vitro* Antioxidant Activity

The antioxidant potential of *N. sativa and Cuminum cyminum* seed extracts was assessed using the DPPH assay. The DPPH assay has been widely employed in the antioxidant capacity evaluation. Both the seed extracts exhibited important antioxidant activity and the *N. sativa* extract was found to be the more active with antioxidant activity of 90.16% compared to 32.2% activity shown by *Cuminum cyminum.* The values observed for *N. sativa* extracts are consistently close compared with those generally observed in extracts from plants known for their antioxidant properties such as bilberry, strawberry and oregano [9, 10]. Thus, *N. sativa* plant parts extracts have been demonstrated to be for the first time efficient in scavenging ROO**.** And to possess radical chain breaking antioxidant activity by H-atom transfer. Peroxyl is formed by a direct reaction of triplet oxygen with alkyl radicals in fatty acid oxidation. This radical produces hydroperoxide (ROOH) by abstracting oxygen froother molecules. Oxidation of unsaturated fatty acids and their esters to ROOHs cause deterioration of foods. Moreover, ROO**.** Has been proposed to be mediators of the ROOH-dependent oxidations related to human diseases [11].

In vitro and *ex vivo* antioxidant activity of *N. sativa* plant part extracts is likely because of the action of known *N. sativa* plant parts phenolic compounds. In fact, anti-peroxyl radical properties of natural extracts are generally attributed to redox reactions with some bioproducts present in the extracts, notably phenolics [12].

3.3 Evaluation of Antidiabetic Activity

The methanolic leaf extracts of both *N.sativa* and *Cuminum cyminum* did not show any promising anti-diabetic property. Though literature shows a known anti-diabetic effect, this observation indicates that leaf extracts are not ideal for anti-diabetic effects.

3.4 Evaluation of Anticancer Activity

In this study, the anticancer activity of *N. sativa* and *Cuminum cyminum* plant parts extracts was evaluated against breast cancer cell lines. The results presented in Table 2 indicates that the seed extract of *N. sativa* exhibited strong antiproliferative activity against the two cell lines.

Table 2: Antiproliferative effects of the seed extracts

	Nsativa	***Cuminum cyminum***
Control	0	0
Solvent control	12.4	12.4
c1	60.48	84.60
c2	47.62	72.72
c3	72.58	95.22
c4	57.23	98.91
c5	61.54	98.21
c6	69.92	98.32
c7	71.43	98.93
c8	58.17	90.04
c9	84.48	96.81

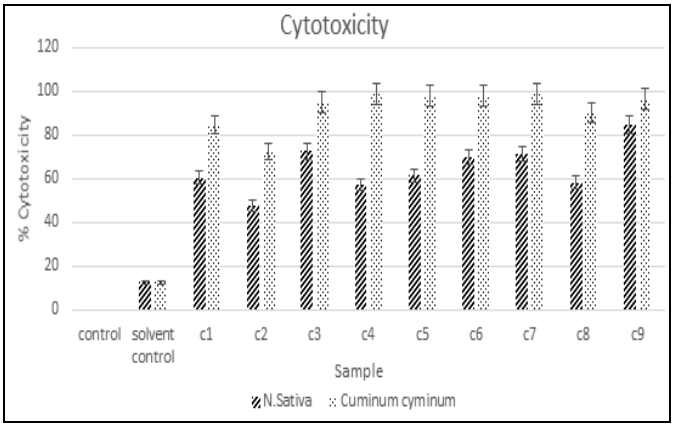

Fig 3: Cytotoxicity of different concentrations of the seed extracts

These results suggest that the anticancer activity of the seeds might be linked to the presence of active non-polar compounds. From the characteristic *N. sativa* compounds, thymoquinone was reported to exhibit a potent anticancer potential against four human cancer cell lines including A-549 [13].

4. Conclusion

Our work expatiates on the lesser known pharmacological benefits of *Nigella sativa* and *Cuminum cyminum.* It was observed that the above-mentioned samples showed promising levels of antioxidative and cytotoxic properties. The comparison between both samples shows that N.Sativa has more predominant cytotoxicity than *Cuminum cyminum.* Whereas, both the samples did not show any positive anti-diabetic activity.

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