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Methanolic extract of Nigella sativa oil conducted on bacterial strain and brine shrimp to detect its Antimicrobial activity and Cytotoxicity studies to determine the efficacy of Nigella sativa seed oil

Dilip kumar J[1], P.M.Yashaswini[2], Savitha N[3]
[1]*Assistant professor, Dept of Pharmaceutics, Gautham College of Pharmacy and Research Centre, Bangalore, India*
[2]*Assistant professor, Dept of Pharmaceutics, Gautham College of Pharmacy and Research Centre, Bangalore, India*
[3]*Assistant professor , Dept of Pharmaceutical Chemistry, Gautham College of Pharmacy and Research Centre, Bangalore, India*

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I. Introduction

Nigella sativa belongs to botanical family of Ranunculaceae commonly grows in Eastern Europe, the Middle East and Western Asia. Since ancient civilization natural sources especially plants are used as medicinal therapy because they contain several components which are believed to cure various infectious diseases. It is commonly called as "Black seed" and used as natural food additive. It is also known around the world by many other medicinal value. It is known as black caraway, roman coriander, carvi, kalongi, kezah, corek out etc. Black cumin seeds have been used to successfully keep people super healthy forever since from 3,300 years ago it was believed.

Scientific Classification
Kingdom : Plantae
Category : Angiopserms
Order : Ranunculaceae
Family : Ranunculaceae
Genus : Nigella
Species : N . sativa

Nigella sativa grows to 20-30cm tall, with finely divided linear leaves. The flower is delicate and usually colored, pale, blue and white, with five to ten petals. The black seeds taste like a combination of onion, black pepper and oregano. They have a pungent bitter taste and smwwl. The dry roasted Nigella seeds flavour curries, vegetables and pulses. It can be used as pepper in recipes with pod fruit, vegetables, salads and poultry. It is major ingredients in preparing pickles in most of Indian pickles it's found common ingredient to be added [1].

Chemistry

Nigella sativa oil contains linoleic acid, oleic acid, palmitic acid and trans-anethode with alkaloids, proteins, essentials oils; investigation reveals that isolation were succeeded in bringing out few active compounds like thymoquinone, tyhymohyderoquinone, dihydrothymoquinone, carvacol, nigelline-N- oxide, alpha-hedrine, thymol etc [1,2].

II. Experimental Methods

Plant Materials

Seeds of Nigella sativa were obtained from Sri Venkateswara University, Andhra Pradesh. The seeds were authenticated by Plant Botanist, Dr. Siddhamallya, from Regional Research Institute Of Ayurveda , Bangalore. The collect seeds were stored in polythene bags and maintained at 4ºC until extraction.

Extraction of Nigella seeds.

Seeds materials was dried and ground into a fine powder using laboratory mill. Further procedure carried under Supercritical fluid extraction method is followed on above milled powder which soaked into

methanol solvent and crushed and triturated in steel grinder. Super critical fluid extraction were conducted at pressures of 600 bar and temperature of 40ºC for the duration of 1 hour and liquid CO_2 was injected at approximately 150 L/ hour andcontrolled by an automatic back pressure regulator. Releasing crude oil as residue , filtered and stored [2, 3].

Phytochemical properties of Nigella sativa oil.

Peroxide value, free fatty acids content, saponification value and iodine value were established based on [3].The refractive index was calculated with Abbe refractometer at 20ºc. The viscosity of Nigella sativa oil was extracted using Supercritical fluid extraction was recorded at 25ºc with a Haake Rheometer. The rheometeris, in essence, as dynamically managed stress instrument with a standard senses system and a plate (PPPTi)[4].

Phytochemical analysis of Nigella sativa oil.

Test for alkaloids: A portion of the extract was made acidic with dilute sulphuric acid and the acidic was divided in to two parts. With Mayers reagent it gives while ppt for positive test. Dragendroffs reagent to give orange brown ppt for positive test[5].

Test for saponins: A small amount of the extract was boiled with water and allowed to cool . It was shaken vigorously in an attest tube and left for a few minutes. The formation of persistent honey comb like froth was taken as a positive test.

Test for sterols and terpenes: A small amount of the extract was evaporated to dryness and extract was dissolved in 3ml of chloroform. The filtrate was treated with three drops of a mixture of concentrated sulphuric acid and acetic anhydride. The production of different shade of color was recorded as a positive test.

Test for tannins: A small amount of the extract was treated with 5% ferric chloride solution and the production of green to blue color was taken as a positive test for tannins[6-8].

Test for carbohydrates: Molish test is positive when on treatment with alpha naphthol and concentrated sulphuric acid the extract gives purple color. Reduction of Fehling's solution is seen when in a solution of carbohydrates.

Test for flavonoids: There were two methods used to test for flavonoids. (A)A portion of the extract was heated with 10ml of ethyl acetate over a steam bath for 3min, the mixture was filtered and 4nl of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow coloration indicated the presence of flavanoids. (B)Dilute ammonia 5ml was added to a portion of an aqueous filtrate of the extract. Then, concentrated sulphuric acid 1ml was added. A yellow coloration indicated the presence of flavanoids.

Microbial Assay

Antimicrobial Test

Microbial growth inhibition under standard condition may be utilized for demonstrating therapeutic effects of the Nigella sativa oil. Any change in the methanolic extract of Nigella sativa will result in change of inhibition effect [9,10].

Cup Plate Method

Inoculate a previously liquefied medium appropriate to the assay with the requisite quantity of suspension of the micro organism. Add the suspension to the medium at the temp between 40ºC and 50ºC and immediately pour the inoculated medium into the Petridish or large rectangular plate to give a depth of 3 to 4mm. Ensure that the layer of medium are uniform in thickness by placing the dishes or plates or a level surface . Store the prepared dishes or plates in a manner so as to ensure that no significant growth or death of the test organism occurs before the dishes or plates are used and that the surface of the agar layer is dry at the time of use. Using appropriate buffer solutions prepare solutions of known concentration of the standard preparation and solution of the corresponding assured of the concentration the methanolic extract of Nigella sativa oil to beexamined. Apply the solution to the surface of the solid medium in cavities prepared in the agar. The volume of solution added to each cylinder or cavity must be uniform and sufficient almost to fill the holes when these are used. Leave the dishes or plates standing for 1 to 4 hours at room temperature or at 4ºC, as appropriate, as a period of pre incubation diffusion to minimize the effects of variation in time between the application of the different solutions. Inoculate them for about 18 hours at the required temperature. Accurately measure the diameter or areas of the circular inhibition zones and calculate the results [11,12].

Brine shrimp Lethality Test

Brine shrimp lethality test, was carried out to investigate the cytotoxicity of methanolic extract of Nigella sativa oil. Brine shrimp were hatched using brine shrimp eggs in a conical flask of 1 liter, filled with sterile artificial sea water prepared using seas salt 38gm and adjusted to p H 8.5 using 1N NaOH under constant

aeration for 48hours. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 3.5ml of brine shrimp solution. In each experiment, o.5ml of the methanolic extract of Nigella sativa oil was added along with 3.5ml of brine shrimp and maintained at room temperature for 24hours under the source of light and surviving nauplii were counted. Experiments were conducted along with control (brine shrimp with nauplii), different concentration of the extracts were prepared from 0.5mg/ml, 1gm/ml, 2gm/ml, 3mg/ml, 4gm/ml of the extracts in set were arranged and experiments continued12-15].

III. Results

Physiochemical properties of Nigella sativa oil

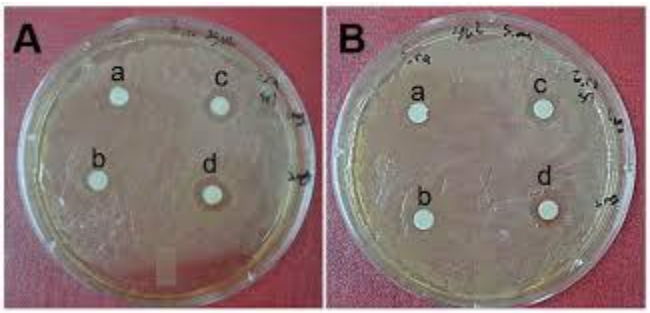
Physiochemical properties	Super critical fluid extraction value
Peroxide valve	6.01 ± 0.006
Saponification value	283.12 ± 0.2
Iodine value	110.2 ± 0.12
Refractive index	2.14 ± 0.86
Viscosity	6.62 ± 0.87

Phytochemical analysis of Nigella sativa oil

Phytochemicals	Test performed	Nigella sativa oil
Alkaloid	Mayers test/ Dragendroofs test	+
Saponin	Froth test	+
steroid	Libermen test	-
Tannin	Ferric chloride test	+
flavanoid	Alkaline reagent test	+
sterols	Fehlings test	-

Antimicrobial Activity

The anti bacterial effect of blackseeds oil was studied in a modified paper disc diffusion method. A clear inhibition of the growth of staphylococcus aureus was observed by concentration of 300mg/ml with distilled water as control, this inhibition was confirmed by using methanolic extract of Nigella sativa oil The positive inhibition may be attributed to the important phytochemical constituents present in methanolic extract of Nigella sativa oil.



Brine shrimp lethality test

Sl .No	Concentrationµg/ml	Number of surviving		Total number of survivor		% Mortality		
1	0.5 µg/ml	1	0	1	0	5	0	%
2	1 µg/ml	1	0	1	0	5	0	%
3	2 µg/ml	1	0	1	0	5	0	%
4	3 µg/ml	1	0	1	0	5	0	%
5	4 µg/ml	1	0	1	0	5	0	%

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