Detection Of Component Of Nigella Sativa Breeds Under The India Climate

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Abstract: This paper aims to provide information on experimental study on chromatographically activities of nigella sativa grows up in India climate when mixed with different aromatic solvent. Nigella Sativa commonly known as Black Cumin is an annual flowering plant found in India and Other Asian countries. It has been used for several years for curing many of the diseases because of its multiple medicinal properties. Nigella Sativa was mixed up with different aromatic compounds and found different un-saponifiable mixture. Afterwards these un-saponifiable mixtures were subjected to Thin Layer Chromatograph (TLC) examination. By using aluminium grade III material as absorbent for column chromatography, we get three compound in it name as NS-1,NS-2 and NS-3. The characterization of this components are done by the IR spectrum ,HNMR spectrum and mass spectrum . All the components show different properties.

Keywords: un-saponifiable mixture, angiosperm, TLC, appetizer

1. Introduction
Many of the plant drugs have not been investigated and their therapeutic values mentioned in ancient have literature have not been listed .hence systemic and meticulous investigation of these drugs has great future in today’s world. It is well known fact that people of India use the fresh juice of vegetable for the medicinal purposes. Common components of many vegetable juices were strongly effective in eliminating matagenity from the products formed in the sorbic acid/Nana2 reaction. The adequacy of the herb is dependent on the total effect of the herb. Moreover, the time of collection, storage, growth of plant, locality, occurrence, time of cultivation or environment, all of these influence the adequacy of drugs. The ease and success of finding the better drug for any disease depends upon, how best we can rationalise the design of the drug. The rational design of an agent with specific activity towards a selected target should be defined as precisely that it can hit selectively in the presence of other or similar targets. Nigella sativa often called black cumin or kalonji, to family rununculaceae .Nigella sativa grows to 20.30 cms tall, with pukle blue and white in color and also with 5 – 10 petals. This plant is a humongous source of new drugs[1]. Black cumin has been employed for thousands of years as the spice and food preservative as well as a protective and curative remedy for numerous disorders. The compounds are isolated from the plants are also used a natural insecticide and showed their utility in agriculture and industrial feed stocks. Now several institutions have been setup like CDRI, RRL, IIT etc., for the investigation and analysis of the medical plant and work on them is in progress. Now a day’s several physical methods and instrumental techniques are available to study the chemistry of natural product .The mass spectra Ultra Violet Spectrum (UV), Infra Red Spectrum (IR), Nuclear magnetic resonance (NMR) provide valuable information about the structure configuration and conformation of compounds. In these work we have also use these techniques to investigate the chemical components of black cumin which help to indentify the medical properties of this seed.

2. Medicinal applications
1) The seeds of Nigella sativa have strong pungent ,aromatic taste and used as carminative ,stimulant ,emmenagogue ,diuretic ,diaphoretic ,anti-bilious ,digestive ,appetizer ,an adjunct to purgative remedies[2].
2) In mild cases seeds are useful in puerperal fever (koman), in eruption of the skin the seed powder is mixed with sesame oil and used as an external agent.
3) Essential oil separated from the crude oil of nigella sativa yielded nigellone which was found active in protecting guinea pigs against histamine induced bronchospasm[3].
4) Seeds with other aromatic substances and bitters are used as a condiment in curries.
5) The seeds have shown significant action as a galactagogue. A decoction of the seeds with other medicines is given to pregnant females for the delivery of foetus, it stimulates uterine contraction. In doses of 10-20 grams seeds are useful in amenorrhoea and in larger doses causes’ abortion[4].
6) The seeds are mixed with water to form a paste, which is used to remove swelling from hands and feets.
7) For obesity, nigella sativa is used as a dose of half a drachm[5].
8) The seeds form a very useful remedy in worms with sweet oil in the decoction form. It also useful for the skin diseases, if applied as an externally.

3. Materials and methods
The seeds of nigella sativa were collected from the nearby area of Ujjain region. they were properly shade dried ,cleaned , powdered and then extracted with pure dried and distilled n-hexane for 62-72 hours in soxhlet extractor .the solvent was removed by rotator film evaporator and the concentrated extract was obtained in the form of oil which was first saponified to yield 100 grams unsaponifiable matter as yellow solid. The un-saponifiable matter thus obtained was subjected to TLC examination. TLC observation revealed the presence of 5-6 compounds.

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The extract was separated by column chromatography using alumina grade III as absorbent. The column was eluted with different solvents in their increasing order of polarity (Table No. 1). This column afforded three compounds NS-1, NS-2 and NS-3 with some impurity which on repeated crystallises from chloroform and methanol yielded three compounds in pure form and designated as NS-1, NS-2 and NS-3. In this work are detain with the NS-1.

**Table 1: Chromatography of un-saponifiable matter**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Fraction no</th>
<th>Eluent</th>
<th>Ratio of Eluent (v/v)</th>
<th>Volume collected (ml)</th>
<th>spots on TLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-4</td>
<td>Hexane: Benzene</td>
<td>50:50</td>
<td>1500</td>
<td>2-spots impurity</td>
</tr>
<tr>
<td>2</td>
<td>5-10</td>
<td>Hexane: Benzene</td>
<td>50:50</td>
<td>3000</td>
<td>NS-1</td>
</tr>
<tr>
<td>3</td>
<td>11-15</td>
<td>Hexane: Benzene</td>
<td>50:50</td>
<td>3000</td>
<td>NS-2</td>
</tr>
<tr>
<td>4</td>
<td>16-21</td>
<td>Hexane: Benzene</td>
<td>50:50</td>
<td>3500</td>
<td>NS-3</td>
</tr>
<tr>
<td>5</td>
<td>22-55</td>
<td>Benzene</td>
<td>100</td>
<td>4500</td>
<td>3-spots</td>
</tr>
<tr>
<td>6</td>
<td>36-43</td>
<td>Benzene: Ether</td>
<td>15:25</td>
<td>4000</td>
<td>2-spots</td>
</tr>
<tr>
<td>7</td>
<td>44-52</td>
<td>Benzene: Ether</td>
<td>50:50</td>
<td>3500</td>
<td>2-spots</td>
</tr>
<tr>
<td>8</td>
<td>53-59</td>
<td>Ether</td>
<td>50:50</td>
<td>5000</td>
<td>2-spots &amp; impurity</td>
</tr>
<tr>
<td>9</td>
<td>60-65</td>
<td>Methanol</td>
<td>50:50</td>
<td>3000</td>
<td>Streak</td>
</tr>
</tbody>
</table>

**4. Results**

4.1 Characterisation of NS-1

Melting point was found to be 136-138 degree Celsius. The mass spectral studies determine the molecular weight M 294 and Molecular formula C_{19}H_{34}O_{2}.

4.1.1 IR Spectrum

It showed band at 1737 cm^{-1} due to the presence of an ester group. Bonds at 2923 cm^{-1} and 2854 cm^{-1} were due to CH stretching vibration and band at 1460 cm^{-1} was due to C–H bending vibrations. Peak at 1617 cm^{-1} showed the presence of un-saturation in the molecule and bands at 722-732 cm^{-1} showed the long chain aliphatic nature of the compound [6,7,8].

4.1.2 ^1^HNMR Spectrum

It showed a triplet at δ 0.88 for 6 protons was due to terminal methyl group. Methylene protons adjacent to ester group were resonated as triplets at δ 2.30 and δ 4.07, while olefinic protons were resonated at δ 5.04 as triplet. A peak at δ 1.60 as singlet for 4 proton was due to methylene protons beta to ester group. The methylene protons alpha to both (HC=CH) group appeared at δ 1.66 as a multiplet for four protons. Rest of the methylene protons merged into a single at δ 1.25[9].

3.1.3 Mass spectrum

The mass spectrum showed the molecular ion peak at M/ Z 294. The abundant fragment at M/ Z 81 was due to allylic cleavage indicating the position of to un-saturation.

Form the above results the molecular formula of NS-1 is identified as shown in figure 1 and the isolation of the NS-1 shown in table 2.

**Table 2: Isolation of NS-1**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Properties</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chemical name</td>
<td>Methylene -14,16 octdecadienoate</td>
</tr>
<tr>
<td>2</td>
<td>Molecular Formula</td>
<td>C_{19}H_{34}O_{2}</td>
</tr>
<tr>
<td>3</td>
<td>Melting point</td>
<td>136-138 °C</td>
</tr>
<tr>
<td>4</td>
<td>Molecular weight</td>
<td>294</td>
</tr>
<tr>
<td>5</td>
<td>Solubility</td>
<td>Chloroform Methanol</td>
</tr>
<tr>
<td>6</td>
<td>Thin layer chromato graph (TLC)</td>
<td>Hexane : ether : Acetic acid, 9:5:5:5</td>
</tr>
<tr>
<td>7</td>
<td>coloured</td>
<td>white</td>
</tr>
<tr>
<td>8</td>
<td>State</td>
<td>Crystalline solid</td>
</tr>
<tr>
<td>9</td>
<td>Re-crystallisation</td>
<td>Methanol</td>
</tr>
</tbody>
</table>

**Figure 1. The molecular formula of NS-1**

5. Conclusions

1) Thin layer chromatography has been used as a diagnostic tool for testing the purity of sample and for qualitative analysis of extract. Silica gel G was used as an adsorbent. The visualization was carried out in iodine chamber. Sometimes chromatograms were developed by spraying the plate with concentrated sulphuric acid containing one percent vanillin followed by heating in an oven at 100 degree Celsius for half an hour.

2) All solvents were dried and distilled before use. Anhydrous CaCl_{2}, CaO, Na_2SO_4 were used for drying purpose.

3) Ether refers the solvent diethyl ether and alcohol (ethanol) refers to ethyl alcohol.

4) Aluminium oxide grade (III) was used for chromatography, aluminium oxide was made grade III of Brochmann scale by the addition of 7% of distilled water and mixing it thoroughly.

5) The taxonomic identification of plant material was conformed from the authority of school of studies in botany, Vikram University in Ujjain.

**References**


Author Profile

Dr. Meena Chouray did her Ph.D (Chemistry) from the Vikarm University, Ujjain in 2008. Recently she is working in Oriental College of technology Bhopal as Asst. Prof Chemistry. She is having 16 year teaching experience. Her research area is Photo Chemistry and Medicinal Chemistry.

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