Preliminary Phytochemical Evaluation, In-vitro Antibacterial Activity and Antioxidant Activities of Nigella sativa

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ABSTRACT
Nigella Sativa, is an annual flowering plant, native to south and southwest Asia. It grows 20-30 cms tall, with finely divided, linear leaves. The flowers are delicate, pale blue or white in colour. The fruit is large and inflated capsule having 3-7 united follicle, each containing numerous seeds. The seed is used as spice. The present study was carried out to preliminary phytochemical evaluation, in-vitro antibacterial and anti-oxidant activities of the seed. The study involves preparation of extracts by successive solvent extraction.

Keywords: Nigella sativa, plant extract, anti bacterial activity, phytochemical evaluation.

1.1. MATERIALS AND METHODS

1.2. Collection and identification of plant seeds
The seeds of N. Sativa was purchased from local market of Chennai, during January 2013. The dried seeds were ground in a blender and stored in polythene bag and placed in dried place for further extraction.

1.3. Extract preparation
Soxhlet extraction assembly was used for this purpose. Each of 50g dried and powdered seed mixed with distilled water, methanol, ethanol, n-hexane successively and continuous extraction was done for 5 to 6 hours. After that extracts were filtered. Aqueous extracts were dried in room conditions.

Organic extracts were dried in a rotary evaporator.

2. PHYTOCHEMICAL ANALYSIS

2.1. Detection of phenolic compounds
The extract is dissolved in water. To this few drops of neutral 5% ferric chloride solution is added. A dark green colour indicates presence of Phenolic compounds.

2.2. Sodium Hydroxide test for Flavonoids
Few quantity of seed extract dissolved in water and filtered. To this 2ml of 10% aqueous NaOH is added. A yellow colouration is developed. On addition of HCl the colour disappears, indicating presence of Flavonoid.

2.3. Tannins
About 5g of seed extract is stirred with 10ml distilled water and filtered. To this 5% ferric chloride is added. Blue-green or green, blue-black precipitate obtained shows presence of Tannins.

2.4. Mayers test
To solvent free extract, few ml of HCl is added and filtered. To this filtrate, two drops of Mayers reagent is added. A white creamy precipitate shows presence of alkaloids.

2.5. Biuret test
The extract is dissolved in distilled water and filtered. To this filtrate, one drop of 2% copper sulphate and 1ml of 95% ethanol is added followed by addition of excess potassium hydroxide. Pink colouration ethanolic layer indicates presence of protein.

2.6. Foam test
The extract diluted with distil water, made upto 20ml, suspension shaken in graduated cylinder for 15mins. A 2cm layer of foam indicates presence of saponins.

2.7. Borntrager’s test
50mg of extract is hydrolyzed with conc. HCl for 2hrs in water bath and filtered. To 2ml filtrate, 3ml chloroform added and shaken. Chloroform layer was separated and 10% ammonia was added, pink colouration indicates presence of glycoside.
3. ANTIOXIDANT ACTIVITY

Antioxidant activity was tested using DPPH method.

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4. THIN LAYER CHROMATOGRAPHY

4.1. Three bands found in UV spec.
Distance travelled by solvent = 3.6cm
1. Distance travelled by first compound = 1.8
   Retardation factor = 0.5
2. Distance travelled by second compound = 2.7
   Retardation factor = 0.75
3. Distance travelled by third compound = 3
   Retardation factor = 0.83.

Figure 4.a. Bands under UV
4.2. Seven compounds separated in Iodine

1. Distance travelled by first compound = 0.6
   Retardation factor = 0.15

2. Distance travelled by second compound = 0.85
   Retardation factor = 0.2125

3. Distance travelled by third compound = 2
   Retardation factor = 0.5

4. Distance travelled by fourth compound = 2.25
   Retardation factor = 0.5625

5. Distance travelled by fifth compound = 2.8
   Retardation factor = 0.7

6. Distance travelled by sixth compound = 3.4
   Retardation factor = 0.85

7. Distance travelled by seventh compound = 3.6
   Retardation factor = 0.9

5. INVESTIGATION OF ANTIMICROBIAL ACTIVITY
5.1. Agar well diffusion method

It is used to examine antibacterial activity of spices fractions against two bacteria.

5.2. Bacterial culture

100µl of E. Coli (gram negative) and S. Aureus (gram positive) are mixed with 5ml broth. It is kept in shaker overnight.

5.3. Media preparation

200ml nutrient agar is prepared and autoclaved.

5.4. Subculture

The bacterial was spread on NA with a swab. Single well in centre was made, which is used as control. Four more wells are made around it, to which seed extracts of concentration 25µl, 50µl, 75µl, 100µl are added. The inoculated plates are incubated at 30°C for 24h. Antimicrobial activity was estimated by measuring the zone of inhibition against the test microorganisms in comparison to control.

6. RESULTS AND DISCUSSIONS
6.1. Phytochemical analysis shows the presence of Phenolic compounds, Flavanoids and Tannins.

Fig. 6.1.a: i. Mayers test shows negative; ii. phenol test shows positive, iii. Biuret test shows negative, iv. Sodium Hydroxide test shows positive, v. Foam test shows negative
6.2. The phytochemical extract of Nigella sativa shows potent antioxidant activity. The exact compound responsible is isolated using thin layer chromatography.

6.3. TLC results shows that the first compound in UV and fifth compound in iodine has Antioxidant properties.

Fig. 6.1.b: i. Tannin test shows positive, ii. Borntrager’s test shows negative

Fig. 6.3.a: Three bands found in UV
For E. Coli, at 75µg zone of inhibition of diameter 12mm and at 100µg zone of inhibition of diameter 14mm was observed.

S. Aureus (gram positive)

6.4. Antibacterial activity

E. Coli (gram negative)
For S. Aureus, only at 100µg zone of inhibition of diameter 13mm was observed.

7. REFERENCES
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