World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

EVALUATION OF PHYSICOCHEMICAL AND PHYTOCHEMICAL PARAMETERS OF DIFFERENT EXTRACTS OF *NIGELLA SATIVA*

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Article Received on 19/04/2017 **Article Revised on 08/05/2017** **Article Accepted on 29/05/2017**

ABSTRACT

Nigella sativa has been used for different coditions in ayurveda and is used for several diseases. *Nigella sativa* traditionally known as “Black seed”, "Black caraway" etc which belongs to family Ranunculaceae. The present study comprises physico-chemical and phytochemical evaluation of different extracts of *Nigella sativa* by using standard methods. The physical evaluation was carried out for the determination of ash values, extractive values and moisture content. Whereas phyto chemical evaluation was carried out for the presence of carbohydrates, flavonoids, tannins, phytosterols, glycosides and phenolic compounds in different extracts of *Nigella sativa.* Results revealed the presence of carbohydrates, tannins, phytosterols, flavonoids, glycosides and phenolic compounds. This study will help in determining the quality and purity of a crude drug and laying down pharmacopoieal standards for *Nigella sativa.*

KEY WORDS: Black seed, *Nigella sativa*, Phytochemical Evaluation, Flavonoids.

INTRODUCTION

Nigella sativa belonging to family Ranunculaceae and is commonly known as Black seed. The other names are roman coriander, black caraway, fennel flower, Kalonji and black cumin.[1] It is annual flowering plant, and is native to south west asia. The plant grows upto 25 to 35 cm tall. The leaves are linear and the flowers are blue and white colour. Fruit of this plant is a capsule and it consists of 3 to 7 follicles.

The *Nigella sativa* seed extracts is used to suppress cough.[2] It is also has anticarcinogenic activity.[3,4] The extracts of the seeds are also used to treat abdominal pain, flatulence, diarrhea and polio.[5] The entire plant is also having uricosuric activity,[6] antioxidant activity[7] and antiinflammatory activity.[8,9] The oil from the seeds of *Nigella sativa* was used for cooking. It is also having antihelminthic activity,[10] antiviral activity[11] and antimicrobial activity.[12] The seeds are having properties like carminative, diaphoretic and stimulatory. It is also used to treat eczema and asthma.[13,14] Previously reported chemical constituents of *Nigella sativa* are linoleic acid, thymoquinone, nigellone, melanthin, nigilline, damascennine, anethole and dithymoquinone.[15,16] The present objective of the study is to determine the physiochemical parameters and the phytochemical screening of the different extracts of *Nigella sativa.*

MATERIALS AND METHODS

A. Collection of plant material

The seeds of *Nigella sativa* were collected from local market of Tirupati. They were verified taxonomically and authenticated in the Department of Botany, S.V. University, Tirupati. By using a rotary grinder the seeds were coarsely powdered and stored in airtight plastic containers. This powder was used for all phytochemical analysis.

B. Preparation of extracts

The freshly collected plant material was washed, dried at room temperature for 15-20 days and under shade and was subjected for size reduction. The fine powder was used for preparation of extracts. The plant material (100 g) was extracted with Soxhlet apparatus by using 400 ml petroleum ether for 48h. After defatting, the marc was dried in hot air oven at 50°C and it is packed in Soxhlet apparatus for further extraction with 400 ml of 95% ethanol until it does not show the presence of any residue on evaporation. The aqueous extract was prepared by cold maceration with 3% methanol-water for 7 days with

occasional shaking. The solvents were removed from the extracts by using rotary vacuum evaporator.

C. Physicochemical evaluations

1. Moisture content

An accurately weighed quantity of the shade dried powder of *Nigella sativa* (3 g) was taken in a tared glass bottle and initial weight was taken. The crude drug was heated at 105°C in an oven and is weighed. This procedure was repeated till the constant weight was obtained. The moisture content of the sample was calculated in the percentage with reference to shade dried plant powder using formula.[17]

$$\% \text{ Moisture content} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

2. Ash values[18]

a) Determination of total ash

An accurately weighed quantity of the shade dried powder of *Nigella sativa* (2 g) was incinerated in a crucible at a temperature of 450°C in a muffle furnace till carbon free ash was obtained. It was then cooled and weighed. The percentage of total ash was calculated with reference to the shade dried powder by using the following formula.

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of the crude drug taken}} \times 100$$

b) Determination of acid insoluble ash

The ash obtained was boiled for 5min with 25 ml of 2 M HCl and was filtered using an ash less filter paper. Insoluble matter retains on filter paper and it was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the shade dried plant powder by using the following formula.

$$\% \text{ acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

c) Determination of water soluble ash

The ash above obtained, was boiled for 5min with 25 ml of distilled water, cooled and the insoluble matter was collected on an ash less filter paper. Paper was washed with hot water and ignited for 15min at a temperature not exceeding 450°C in a muffle furnace. The difference in weight of ash and weight of water insoluble matter gave the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the shade dried plant powder by using the following formula.

$$\% \text{ Water soluble ash value} = \frac{\text{Weight of total ash-Weight of water insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

3. Extractive values[19]

Extractive values of shade-dried powder of *Nigella sativa* were determined using following methods.

a) Determination of alcohol soluble extractive

An accurately weighed quantity of the shade dried powder of *Nigella sativa* (5 g) was macerated with 100 ml of alcohol (Ethanol) in a closed flask for 24 h, shaking frequently during the first 6 h. It was then allowed to stand for 18 h and it was filtered rapidly to prevent any loss during evaporation. Evaporate 25 ml of the filtrate to dryness in a porcelain dish and dried at 105°C and weighed. The percentage of alcohol (Ethanol) soluble extractive was calculated with reference to the shade dried plant powder.

b) Determination of water soluble extractive

Weighed quantity of the shade dried powder of *Nigella sativa* (5 g) was macerated with 100 ml of water in a closed flask, shaking frequently for the first 6 hrs and allowed to stand for 18 hrs. Thereafter it was filtered taking precaution against loss of water. Evaporate 25 ml of filtrate to dryness in a tared flat bottom shallow dish dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the shade dried plant powder.

c) Determination of petroleum ether soluble extractive

Weighed quantity of the shade dried powder of *Nigella sativa* (5 g) was macerated with 100 ml petroleum ether in a closed flask for 24 h, shaking frequently for the first 6 hrs and allowed to stand for 18 hrs. Thereafter it was filtered rapidly taking precaution against loss of petroleum ether due to its volatility. Evaporate 25 ml of filtrate to dryness in a porcelain dish and dried at 105°C and weighed. The percentage of petroleum ether soluble extractive was calculated with reference to the shade dried plant powder.

D. Phytochemical Evaluation

The freshly prepared petroleum ether, methanolic and aqueous extracts of *Nigella sativa* were qualitatively analyzed for the presence of major phytochemical constituents using the standard procedures.

1. Detection of Carbohydrates[20]

100 mg of extracts were dissolved in 10 ml of water and filtered. The filtrate was used to test the presence of proteins and amino acids.

(a) Molisch's Test: To the 1 ml of filtrate add 2 drops of Molisch's reagent in a test tube and 2 ml of concentrated sulphuric acid were added carefully along the sides of the test tube. Formation of violet color at the interface indicates the presence of carbohydrates.

(b) Fehling's Test: To the 1 ml of filtrate add 4 ml of Fehling's reagent (2 ml Fehling A and 2 ml Fehling B solutions) in a test tube and heated for 10 minutes

in a water bath. Formation of red precipitate indicates the presence of reducing sugar.

(c) **Barfoed's Test:** 1 ml of Barfoed's reagent is heated with 5 drops of filtrate in a test tube on boiling water bath. Formation of a brick-red precipitate within 5 min indicates the presence of monosaccharides. Disaccharides generally don't give any reaction even for 10 min

2. Detection of Proteins and Amino acid[21]

100 mg of extracts were dissolved in 10 ml of water and filtered. The filtrate was used to test the presence of proteins and amino acids.

(a) **Millon's Test:** 2 ml of filtrate was treated with 2 ml of Millon's reagent in a test tube and was heated in a water bath for 5 min, cooled and add few drops of NaNO2 solution. Formation of white precipitate and turns to red upon heating indicates the presence of proteins and amino acids.

(b) **Ninhydrin Test:** To the 2 ml of filtrate add 2-3 drops of Ninhydrin reagent in a test tube and boiled for 2 min. Formation of blue colour indicates the presence of amino acids.

(c) **Biuret Test:** 2 ml of filtrate was treated with 2 ml of 10% sodium hydroxide solution in a test tube and heated for 10 min, add a drop of 7% of copper sulphate in the above solution. Formation of violet colour confirms the presence of proteins.

3. Detection of Glycosides[22]

0.5 g of extract was hydrolyzed with 20 ml of dilute hydrochloric acid (0.1N) and filtered. The filtrate was used to test the presence of glycosides.

(a) **Legal Test:** To 1 ml of filtrate add 3 ml of sodium nitropruside in pyridine and methanolic alkali (KOH) in a test tube. A blue colour in the alkaline layer indicates the presence of glycosides.

(b) **Keller-killiani Test:** 1 ml of filtrate was shaken with 1 ml of glacial acetic acid containing traces of ferric chloride. Add 1 ml of concentrated sulphuric acid along the sides of the test tubes. A blue colour in acetic acid layer and red colour at the junction of the two liquids indicates the presence of glycosides

(c) **Modified Borntrager Test:** To the 1ml of filtrate add 2 ml of 1% ferric chloride solution in a test tube and heated for 10 min in boiling water bath. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of pink colour in the ammonical layer indicates the presence of glycosides.

4. Detection of Alkaloids[23]

0.5 g. of extract was taken and it was dissolved in 10 ml of dilute hydrochloric acid (0.1N) and filtered. The filtrate was used to test the presence of alkaloids.

(a) **Dragendorff's Test:** 2 ml of filtrate was added with 2-3 drops of Dragendorff's reagent. Appearance of reddish brown colored precipitate indicates the presence of alkaloids.

(b) **Hager's Test:** To the 2 ml of filtrate, Hager's reagent was added. Formation of yellow colored precipitate indicates the presence of alkaloids.

(c) **Mayer's Test:** To the 2 ml of filtrate add 2-3 drops of Mayer's reagent, formation of cream colored precipitate indicates the presence of alkaloids.

(d) **Wagner's Test:** To the 1 ml of the extract, add 2 ml of Wagner's reagent, formation of reddish brown precipitate indicates the presence of alkaloids.

5. Detection of Flavonoids[24]

(a) **Shinoda Test:** 100 mg of extract was added with few fragments of magnesium metal in a test tube. To the test tube add 3 to 4 drops of concentrated hydrochloric acid. Formation of magenta colour or light pink colour indicates the presence of flavonoids.

(b) **Alkaline Reagent Test:** To the 100 mg of extract add few drops of sodium hydroxide solution in a test tube. Intense yellow colour is appeared. Add few drops of dilute hydrochloric acid, then yellow colour becomes colourless which indicates the presence of flavonoids.

(c) **Fluroscence test:** To the 100 mg of extract, 0.3 ml boric acid solution (3 %w/v) was added and then add 1 ml oxalic acid solution (10 %w/ v) and evaporated to dryness. The residue obtained was dissolved in 10 ml of ether. The ethereal layer shows greenish fluorescence under UV light indicates presence of flavanoids.

6. Detection of Phenolic Compounds and Tannins[25]

100 mg of extract mixed with 1 ml of distilled water and it was boiled and filtered. The filtrate was used for the following test.

(a) **(a) Ferric Chloride Test:** Take 2 ml of filtrate in a test tube and add 2 ml of 1% ferric chloride solution. Appearance of bluish to black colour indicates the presence of phenolic nucleus.

(b) **Lead Acetate Test:** To the 2 ml of filtrate add few drops of lead acetate solution in a test tube. Appearance of yellowish precipitate indicates the presence of tannins.

7. Detection of Fats and Oils[26]

Oily Spot Test

1 drop of extract was placed on filter paper and the solvent was allowed to evaporate. Appearance of oily stain on filter paper indicates the presence of fixed oil.

8. Detection of Saponins[27]

Foam Test

To 1 ml of extract add 20 ml of distilled water and shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

9. Detection of Phytosterols[28]

To 0.5 g of extract, 10 ml of chloroform was added and filtered. The filtrate was used to test the presence of phytosterols and triterpenoids.

(a) Libermann's Test: To the 2 ml of filtrate in hot alcohol, few drops of acetic anhydride were added. Formation of brown precipitate indicates the presence of sterols.

(b) Salkowski Test: To the extract, few drops of concentrate sulfuric acid were added, shaken and then allowed to stand. Appearance of red colour in lower layer indicates the presence of sterols.

Statistical Analysis

The values are represented as mean ± S.D. (n=3) and results were analyzed using ANOVA, followed by Dunnett's test where $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Nigella sativa was subjected to systematic physicochemical and phytochemical screening by extracting with various organic solvents of increasing polarity to determine the soluble constituents in a given amount of plant material. The present investigation is helpful in determining the quality and purity of a crude drug. In this study the parameters used for the evaluation of *Nigella sativa* were moisture content, extractive values by different solvents (includes petroleum ether, methanol and water), ash values (total ash, water soluble and acid insoluble ash) (Table 1). On incineration, crude drugs leave an ash which consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The determination of ash value is useful for detecting low-grade products, exhausted drugs and excess of sandy matter which is especially applicable to powdered drugs.

Table 1: Physico-chemical investigation of *Nigella sativa.*

S.No.	Quality parameters		Results
1	**Moisture content**		6.4
2	**Ash value**		
	A	Total ash value	6.9
	B	Acid insoluble ash value	0.8
	C	Water soluble ash value	2.5
3	**Extractive values**		
	A	Petroleum ether soluble extract	5.8
	B	Methanol soluble extract	6.7
	C	Aqueous soluble extract	7.9

Phytochemical analysis was performed on the petroleum ether, methanol and aqueous extracts of *Nigella sativa*. Petroleum ether extract was found to contain proteins and aminoacids, tannins, saponons, phytosterols, fats and oils. Methanolic extract contains carbohydrates, proteins and aminoacids, glycosides, alkaloids, flavonoids, phenolic compounds and tannins. Aqueous extract contains carbohydrates, proteins and aminoacids, glycosides, flavonoids, phenolic compounds and tannins. (Table 2).

Table 2: Phyto-chemical investigation of *Nigella sativa.*

S. No	Tests	Petroleum ether extract	Methanolic extract	Aqueous extract
1	Carbohydrates	-	+	+
2	Proteins and aminoacids	+	+	+
3	Glycosides	-	+	+
4	Alkaloids	-	+	-
5	Flavonoids	-	+	+
6	Phenolic compounds	-	+	+
7	Tannins	+	+	+
8	Saponins	+	-	-
9	Phytosterols	+	-	-
10	Fats and oils	+	-	-

CONCLUSION

Herbal remedies have been common place in different cultures throughout recorded history, and still serve as the vital means of therapeutic medical treatment. The recent years have witnessed a resurgence of interest in natural drugs world over as people are turning to use herbal plants and phyto-chemicals in health care. India has one of the oldest cultural traditions of use of its herbal flora since vedic period. Ayurveda, Unani, Siddha and other traditional systems of medicine are the oldest systems of medicine and utilize large number of medicinal plants. Phytochemical screening, massive biological screening of randomly collected plants and phytochemical examination of plants have proved to be very helpful in discovering new drugs.

Nigella sativa, traditionally known as black seed, is very important medicinal plant belonging to family Ranunculaceae. The present study concluded that the plant *Nigella sativa* contains variety of phytoconstituents. The physicochemical evaluation of *Nigella sativa* revealed that the standard quality and purity of drug. The objective of ashing vegetable drugs is to remove all traces of organic matter and to prevent interference in an analytical determination. On incineration, drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. Phytochemical studies on the extracts of *Nigella sativa* showed presence of phytosterols, carbohydrates, glycosides, flavonoids, proteins and amino acids, tannins & phenolic compounds. This information may be further used for isolation of various compounds from *Nidella seed* for treatment of diseases in human beings.

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