An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

PHYSICOCHEMICAL AND PHYTOCHEMICAL INVESTIGATION OF DIFFERENT EXTRACTS OF *NIGELLA HISPANICA* L. SEED

Udaya Kumar Nagapatla[1*], P. Jaya Chandra Reddy[2], V. Raghu Moorthy[3]

[1]Research Scholar, Department of Pharmacy, Jawaharlal Nehru Technological University Kakinada, Kakinada, Andhra Pradesh, India.
[1]Department of Pharmaceutical Chemistry, Krishna Theja Pharmacy College, Tirupati, AndhraPradesh, India.
[2]Krishna Theja Pharmacy College, Chadalawada Nagar, Renigunta Road, Tirupati, Andhra Pradesh, India.
[3]Govt Polytechnic Womens, Hindupur, Anantapur, Andhra Pradesh, India

*Corresponding author E-mail: uday307@gmail.com

ARTICLE INFO

Key Words

Nigella hispanica L., miracle herb, Kala jeera, Phytoconstituents, seeds

ABSTRACT

Nigella hispanica L. used for several diseases. *Nigella hispanica* L. commonly known as Kala jeera, Nalla jeealkarra, Black seeds (or) Black cumin.It belongs to the family "Ranunculaceae"(Butter-cup family).It is distrubited and cultivated in Tirumala gardens and Srisilam Nallamala forest. This study was designed to perform the physicochemical and phytochemical standardization of *Nigella hispanica* L. seeds in order to establish the standard pharmacognostical parameters of this miracle herb. Different parameters like extractive values, total ash value, acid insoluble ash value and water soluble ash value, moisture content of *Nigella hispanica* L. seeds were performed. Preliminary phyochemical screening was done to detect different phytoconstituents by using different phytochemical methods. Phyochemical screening of the extracts in different solvent revealed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, lipids, sterols and tannins. Finally the outcome of this research may be utilized as substantial data for identification, purification and standardisation of *Nigella hispanica* L. seeds.

INTRODUCTION:

In the plant kingdom there is a remedy for every disease. Two hundred and fifty years ago, there were few or no synthetic medicines. The plants were the main source of drugs for the world's population[1]. Medicinal plants are the richest bio resource of drugs for traditional systems of medicine, nutraceuticals, food supplements, modern medicines. Pharmaceutical intermediates, folk medicines and chemical entities for synthetic drugs. World Health Organization (WHO) has suggested that medicinal plants would be the best source to obtain variety of drugs. Since the use of medicinal plant based drugs contain least or no side effects they are considered to be great importance to the health of individuals and communities.

WHO estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs[2]. The seeds of *Nigella hispanica* L. is known by many different names like black seeds (or) black cumin. In old Latin, it is calld as Panacea meaning cure all‟ while in Arabic it is termed as „Habbah Sawdada‟ or Habbat el Baraka‟ translated as seeds of blessing‟. In china it is reffered as Hak Jung Chou while in India it is called as Kalonji and in Persian, it is called as Shoneez. The plant belongs to the Ranunculaceae family of flowering plants and genus of about 14 species including *Nigella arvensis, Nigella ciliaris, Nigella damascence, Nigella hispanica, Nigella integrifolia, Nigella nigellastrum, Nigella orientalis* and *Nigella sativa* respsctively. Among these, *Nigella hispanica* is the species most exhaustively investigated for therapeutic purposes although other species have also been implicated for therapeutic uses[3].

The *N.hispanica* It is a spice that grows in the Mediterranean region and in western asian countries including India, Pakistan and Afghanistan. The species grow to 20-30 cm tall, with finely divided leaves where in the leaf segments are narrowly linear to threadlike. The flowers are white, yellow, pink, pale blue or pale purple with 5-10 petals. The fruit is a capsule composed of several united follicles, each containing numerous seeds while in some species(e.g. Nigella damascene), the capsule is large and inflated. The parts of the plant most commonly used for the therapeutic purposes in the "Alternative Medicinal" systems are the seeds which are contained in an inflated capsule formed from the united follicles containing considerable amount of oil having pungent and bitter taste. Commonly the seeds are used primarily as a spice and food preservative[3, 4]. Ethanobotany is a multidisciplinary science which is defined as the interaction between plants and people.Recent days most of the ethnobotanical knowledge has been fading away due to lack of interest in younger generation.Hence the traditional medicines used by the tribal communities belongs to Chenchu, Valmiki,Yanadhi's Kurnool district, A.P, India.The *Nigella hispanica* is available in Srisailam, forest of Nallamala ranges in Kurnool.And it also available in Tirumala gardens of Seshachela forest in Tirupati, Chittor district[5,6].

Taxonomic classification

Kingdom : Plantae
Subkingdom : Tracheobionta
Superdivision : Spermatophyta
Phylum : Magnoliophyta
Class : Magnoliopsida
Order : Ranunculales
Family : Ranunculaceae
Genus : Nigella
Species : N. Hispanica

Many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone, thymohydroquinone, dithymoquinone and thymol etc.Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; i.e isoquinoline alkaloids e.g. nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine [7].

MATERIALS AND METHODS:

I. Collection of plant material

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

II. Physicochemical evaluations:

1. Moisture content: Weighed quantity of the shade dried powder of *Nigella hispanica* L. (3g) was taken in a tared glass bottle and

initial weight was taken. The powder was heated in an oven at 105°C 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 by using formula [9,10]. % Moisture content = Loss in weight of the sample/ Weight of the sample x 100

2. Ash values

a) Determination of total ash

An accurately weighed quantity of the shade dried powder of *Nigella hispanica* L. (2g) was incinerated in a crucible at a temperature of 450°C in a muffle furnace until carbon free ash was obtained. Then cooled and weighed. The percentage of total ash was calculated with reference to the shade dried powder by using the following formula. % Total ash value = Weight of total ash/ Weight of the crude drug taken x 100

b) Determination of acid insoluble ash

The ash obtained was boiled with 25 ml of 2 M HCl for five minutes and it was filtered using an ash less filter paper. Insoluble matter retains on the filter paper and it was washed by using 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. % acid insoluble ash value= Weight of acid insoluble ash/ Weight of the crude drug taken x 100

c) Determination of water soluble ash

The ash above obtained, was boiled for 5min with 25 ml of water, cooled and the insoluble matter was collected on an ash less filter paper. Paper was washed with hot water and ignited at a temperature not exceeding 450°C, for 15min in a muffle furnace. The difference in the weight of ash and the 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. % Water soluble ash value = Weight of total ash-Weight of water insoluble ash/ Weight of the crude drug taken x 100

III. Preparation of extracts

Powdered *Nigella hispanica* L. seed was macerated in 400ml of petroleum ether, for 24 hrs with occasional shaking. The result was extracted and filtered by using filter paper (What Man No 1.5 WhaTMn Lted., England). The petroleum ether extract were evaporated to dryness in vacuum by using Rota vapor at 40^{o} C to yield 3.5 gm of crude extract (2.8 %W/W). The mark from petroleum ether extract would be socked with 400mL of ethyl acetate for 24hrs. The ethyl acetate would be filtered out connected by using Rota vapor at 40^{o} C to yield 7gm of crude extract(5.6%W/W).The mark from ethyl acetate extract would be socked with 400ml of methanol for 24 hrs. The methanol extract was filtered out and connected to Rota vapor at 40 centigrade to yield crude extract. The mark was socked again with the same amount of methanol for the same period of time. The total weight of crude methanol extract after evaporated using Rota vapor at 40^{o} C to yield 20gm of crude extract(16 %W/W). After dried and weighed the above three crude extract had been apply to phytochemical analysis (petroleum ether, ethyl acetate and methanol) [8].

IV. Phytochemical Evaluation:

The crude extract was tested for the presence of bioactive compounds by using following standard methods [11,12 &13].

1. Test for Alkaloids

a) Petroleum Ether Extract

To the 2ml of extract, 1.5ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Wagner's reagent was added. Formation of orange precipitated indicates the presence of alkaloid.

b) Ethyl Acetate Extract

To the 2mL of extract, 1.5ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Wagner's reagent was added. Formation of orange precipitated indicates the presence of alkaloid.

c) Methanol Extract: To the 2ml of extract, 1.5ml of 1% HCl was added. After heating

the solution in water bath, 6 drops of Wagner's reagent was added. Formation of orange precipitated indicates the presence of alkaloid.

2 .Test for Steroids

a)Petroleum Ether Extract

To 2 ml of extract, 5ml of chloroform and 2 ml acetic anhydride was added followed by concentrated H_2SO_4 reddish brown coloration of interface indicates the presence of steroids.

b) Ethyl Acetate Extract

To 2 ml of extract, 5ml of chloroform and 2 ml acetic anhydride was added followed by concentrated H_2SO_4 reddish brown coloration of interface indicates the presence of steroids.

c) Methanol Extract

To 2 ml of extract, 5ml of chloroform and 2 ml acetic anhydride was added followed by concentrated H_2SO_4 reddish brown coloration of interface indicates the presence of steroids.

3. Tests for Phenol

a) Petroleum Ether Extract

To 2ml of extract, 5% ferric chloride solution was added. Deep blue black colour indicates the presence of phenol.

b) Ethyl Acetate Extract

To 2ml of extract, 5% ferric chloride solution was added. Deep blue black colour indicates the presence of phenol.

c) Methanol Extract

To 2ml of extract, 5% ferric chloride solution was added. Deep blue black colour indicates the presence of phenol.

4.Test for Terpeniods

a) Petroleum Ether Extract

Treat extract in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, formation of yellow coloured lower layer indicate the presence of terpenoids.

b) Ethyl Acetate Extract

Treat extract in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, formation of yellow coloured lower layer indicate the presence of terpenoids.

c) Methanol Extract

Treat extract in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, formation of yellow coloured lower layer indicate the presence of terpenoids.

5.Test for Flavonoids

a) Petroleum Ether Extract

To the test solution, add few drops of ferric chloride solution, intense green colour was formed to show the presence of flavonoid.

b) Ethyl Acetate Extract

To the test solution, add few drops of ferric chloride solution, intense green colour was formed to show the presence of flavonoid.

c) Methanol Extract

To the test solution, add few drops of ferric chloride solution, intense green colour was formed to show the presence of flavonoid.

6. Test for Tannins

a) Petroleum Ether Extract

Some amount of extract was dissolved in distilled water to this solution 2 mL of 5% ferric chloride solution was added. Formation of blue green indicates presence of tannins.

b) Ethyl Acetate Extract

Some amount of extract was dissolved in distilled water to this solution 2 mL of 5% ferric chloride solution was added. Formation of blue green indicates presence of tannins.

c) Methanol Extract

Some amount of extract was dissolved in distilled water to this solution 2 mL of 5% ferric chloride solution was added. Formation of blue green indicates presence of tannins.

7. Tests for Saponins

a) Petroleum Ether Extract

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

b) Ethyl Acetate Extract

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

c) Methanol Extract

The extract was diluted with distilled water and shaken in graduated cylinder for

15 minutes. The formation of layer of foam indicates the presence of saponins.

8. Test for Cardiac Glycosides

a) Petroleum Ether Extract

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue colour in the acetic acid layer indicates the presence of Cardiac glycosides.

b) Ethyl Acetate Extract

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue colour in the acetic acid layer indicates the presence of Cardiac glycosides.

d) Methanol Extract: To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. 0.5 ml of concentrated sulphuric acid were carefully added by the side of the test tube. Formation of blue colour in the acetic acid layer indicates the presence of Cardiac glycosides.

The Phytochemical active compounds of black cumin (*Nigella hispanica* L.) were qualitatively analyzed for seeds and the results are presented in Table 3. In these screening process alkaloids, glycosides, saponins, phenol, tannins, sterols, flavanoids, and terpenoids shows different types of results in different solvents extracts. Among these phytochemicals, Alkaloids, Flavonoids, Glycosides and Phenols were absent in all solvent extracts except methanol, whereas saponins were absent in all solvent extracts. Tannins and Terpenoids are present in all solvent except methanol. Steroids were present in all solvent extract. So Alkaloids, Flavonoids, Phenol compounds, Glycosides and Steroids were present in methanol extract. In the present study phytochemical screening for all three extracts showed significant indication about the presence of metabolites; Alkaloids, Tannins, Flavanoids Terpenoids, Phenol and steriods were found to be present in the all the sequential extracts of Black cumin (*Negella hispanica* L.) *seed* whereas saponins were absent in all solvents, summarized in Table 3. These detected phytochemical compounds are known to have beneficial importance in medicinal as well as physiological activities. In this manner isolating and identifying these bioactive compounds, new drugs can be formulated to treat various diseases and disorders.

A. Alkaloids which are one of the largest groups of phytochemicals have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity[12].

B.Tannins are used medicinally in anti-diarrheal, haemostatic and anti-haemostatic and anti-haemorrhoidal compounds and may be responsible for its broad spectrum anti-microbial against bacteria, fungi and viruses.

C.Steroids possess antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones

D.Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection have been found to be antimicrobial substances against wide array of microorganism sin *vitro*. They also are effective antioxidant and show strong anticancer activities,

E. Terpenoids are essentially lipids, known for their aromatic qualities. Different function shave been described to terpenoids including growth regulating, colour, odorand anti-microbial activity and also responsible for anti-bacterial and anti-fungal activity.

F. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as anti-apoptosis, anti-aging, anti-inflammation, cardiovascular protection and improvement of endothelial function.

C.Glycosides *is* known to lower the blood pressure.

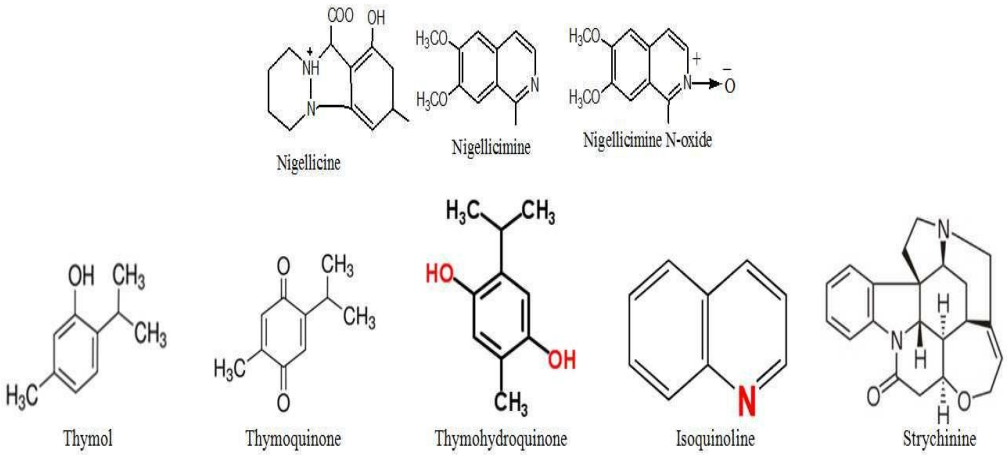

Fig.1 Chemical structures of some major compounds isolated from *Nigella hispanica* L.

Fig. 2. Seeds of *Nigella hispanica* L.

Fig. 3. Flower of *Nigella hispanica* L.

Table.1: The results of Physicochemical investigation of different extracts of *Nigella hispanica* L. seed

S. no	Quality Parameters		Results
1.	Moisture Content		0.51± 0.032%
2.	Ash Value		
	A	Total ash value	5.5±0.24%
	B	Acid insoluble ash value	0.41±0.02%
	C	Water soluble ash value	3.07±0.46%

Extracts of Black cumin seed were isolated successfully by using different solvents. The results are presented in Table 2.

Table 2: The Percentage yield of different extracts of *Nigella hispanica* L. seed

S.no	Solvent	Colour of Extract	Yield of Extract (gm)	Percentage yield(%w/w)
1	Petroleum ether	Light brown	3.5	2.8
2	Ethyl acetate	Light brown	7	5.6
3	Methanol	Dark brown	20	16

The phytochemical screening of the present study carried out in the black cumin (*Nigella hispanica* L.) revealed the presence of the following medicinal active constituents.

Table 3: Results of Phytochemical screening of petroleum ether, ethyl acetate and methanol seed extract of *Negella hispanica* L. seeds.

S.no	Name of the Phytoconstituents	Petroleum ether	Ethyl acetate	Methanol
1	Alkaloids	-	-	+
2	Flavonoids	-	-	+
3	Phenols	-	-	+
4	Tannins	+	+	-
5	Glycosides	-	-	+
6	Steroids	+	+	+
7	Saponins	-	-	-
8	Terpinoids	++	+	-

Key: + indicates presence of the Phytoconstituents, ++ indicates present in more quantity of the Phytoconstituent , – indicates absence of the Phytoconstituents

CONCLUSION:

In recent years, ethno-botanical and traditional uses of natural compounds, specially of plant origins received much attention as they are well tested for their efficiency and generally believed to be safe in human use. They obviously deserve scrutiny on modern scientific lines such as phytochemical investigations, biological evaluations, toxicity studies and investigations of molecular mechanism of actions of isolated phyto constituents. In the process of controlling food borne pathogens, antimicrobials from plant origin have more potential and effective[15]. In this manner, this study powdered *Negella hispanica* L. seed was used for crude extracts by using different solvents based on effectiveness of solvents . The crude extract was tested for the presence of bioactive compounds by using different standard methods. The results of investigation revealed the presence of medicinally important phytochemical constituents in the petroleum ether, ethyl acetate and methanol extracts of Black cumin (*Negella hispanica L.*) seeds, such as; alkaloids, Tannins, Steroids, Phenol, Terpenoids, Glycosides and flavonoids.

ACKNOWLEDGEMENTS:

The author wish thankful to the Chairman Dr. C. Sucharitha Madam garu, Krishna Teja Group of Institutions, Chadalawada Nagar, Renigunta Road, Principal Dr. P. Jaya Chandra Reddy sir, Krishna Teja Pharmacy College & Dr.V. Raghu Moorthy sir, HOD of Pharmacy Section, Govt Polytechnic Womens, Hindupur, Anantapur.

REFERENCES:

1. Majidul, Investigation of the Medicinal Potentials of Syzygium jambos (L.) Extract and Characterization of the Isolated Compounds. AJBS. Special Issue: Pharmacological and Phytochemicals Investigation. Vol.3, No. 2-1, 2015, pp. 12-18.
2. http://scholar.Lib.vt.edu./theses/available/edt.-2598-18137/unrestricted//body.pdf
3. Nadkarni , Meteria Medica Mumbai: Popular Prakashan. (1st Ed) 1979.
4. Louise MH. Encylopedia of Natural Remedies, Woodland Publishing,1995
5. Savithramma., 2013. Documentation of ethnobotanical knowledge of ethnic groups from Kurnool district, Andhra Pradesh, India. The journal of Ethnobiology and Traditional Medicine.Photon 118,295-305.
6. A book of Flowering plants of Chittoor (Dist) by Dr. K.Madhava Chetty,K.Sivaji,K.Tulasi Rao Page.No-3
7. Ali G.H, 2014. Optimization of Reflux Conditions for Total Flavonoid and Total Phenolic Extraction and Enhanced Antioxidant Capacity in Pandan

Using Response Surface Methodology. *Scientific World Journal vol(1).*

8. Azra Kamal, 2014. Phytochemical Screening of Syzygium Cumini Seeds. *Indian Journal Of Plant Sciences: 2319–3824. Vol. 3 (4). p.1.*
9. Khandelwal, Practical pharmacognosy, Nirali Publications, Pune, 1995, 1, 140 143.
10. Narsimha Reddy. Y et al, Determination of physicochemical and phytochemical parameters of different extracts of Glycyrrhiza glabra. *Journal of Global Trends in Pharmaceutical Sciences. 2017 8(2) P. 3845- 3851. (ISSN:2230-7346)*
11. Hamburger, 1991. Bioactivity in plants. The link between phytochemistry and medicine. *Phytochem., 30 (12): p. 3864-3874.*
12. Azra Kamal, 2014. Phytochemical Screening of Syzygium Cumini Seeds. *Indian Journal Of Plant Sciences: 2319–3824. Vol. 3 (4). p.1*
13. Madhukar. C, 2013. Phytochemical screening of cumin seeds extract. *Rep. Opinion, Karnataka, India. V. 5(1):57-58.*
14. Abdurohaman Phytochemical Extraction and Screening of Bio Active Compounds from Black Cumin (Nigella Sativa) Seeds Extract, *American Journal of Life Sciences*,2015; 3(5): 358-364
15. Sibi., Isolation and Characterization of Antimicrobial Alkaloids from Plumeriaalba Flowers against Food Borne Pathogens. *American Journal of Life Sciences.* Special Issue: Recent Developments in Health Care through Plants and Microbes Vol. 2, No. 6-1, 2014, pp. 1-6.