



PHYTOCHEMICAL ANALYSIS OF *NIGELLA SATIVA* LINN.(BLACK CUMIN) AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Nigella sativa L. belongs to the family Ranunculaceae is a popular plant in indigenous medicine all over the world used for treating various diseases. The present study was undertaken to analyze the phytochemical components and for screening the antimicrobial activity of seed extracts of *Nigella sativa* Linn. The extract is prepared using various solvents such as water, ethanol, and chloroform and physicochemical characters of *Nigella sativa* seed extract was carried out by standard procedures. The preliminary phytochemical screening of the extract showed the presence of alkaloid, flavanoids, phytosterol, saponins, steroids, tannins and terpenoids in ethanol extract. For this, the antibacterial properties of *Nigella sativa* L. seed extract were tested against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria and the antifungal activity of *Nigella sativa* L. seed extract were tested against *Candida albicans*, and *Aspergillus niger* by using different solvents namely chloroform, ethanol, and water. The ethanol seed extract of *Nigella sativa* L. showed significant antibacterial activity against *Escherichia coli* and antifungal activity against *Candida albicans*. Thus we can scientifically conclude that the black cumin seeds extract could be seen as an progressively valuable reservoir of bioactive compounds of substantial medicinal merit.

Key words: Physicochemical characters, *Nigella sativa*, antibacterial, antifungal.

1. Introduction



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Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and the green medicine is always supposed to be safe. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy availability, economic and less or no side effects.(SumitraChanda ,2013).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. According to World Health Organization (WHO), up to 80% of the people depend on traditional medicinal plants for their medicines (Arunkumaret al., 2009).

Plant products derived from barks, flowers, roots, leaves, seeds, fruits are the part of phytomedicines(Criagg et al., 2001). Bio active constituents of plants known as phytochemical components such as tannins, carbohydrates, alkaloids, terpenoids, phenolic compounds, steroids and flavonoids are responsible for various pharmacological activities of the plants. (Abbas et al., 2012a, b; Shah et al., 2011; Zaman et al., 2012)

1.1 Plant classification:

Kingdom : Plantae

Subkingdom : Tracheobionta

Superdivision : Spermatophyta

Phylum : Magnoliophyta

Class : Magnoliopsida

Order : Ranunculales

Family : Ranunculaceae



Genus : Nigella

Species N. sativa

1.2 Plant Description

N. sativa is an annual flowering plant grows at 20-90 cm tall, with finely divided leaves; the flowers are white, yellow, pink, pale blue or pale purple color, with 5-10 petals. The fruit is a large and inflated capsule consists of 3-7 united follicles, each containing several seeds (Sabira sultana et al., 2015). They are small dicotyledonous, trigonus, angular, regulose – tubercular, $2 - 3.5 \times 1 - 2$ mm, black externally and white inside. Odor slightly aromatic and taste bitter. Transverse section of seed shows single layered epidermic consisting of elliptical, thick walled cells, covered externally by papillose cuticle and filled with dark brown contents. Epidermis is followed by 2-4 layers of thick walled tangentially elongated parenchymatous cells, followed by a reddish brown pigmented layer composed of thick walled, rectangular elongated cells. Inner to the pigment layer, is present a layer composed of thick walled rectangles elongated or nearly columnar elongated. (SahaRajsekhar et al., 2011)



Fig.1 : Nigella sativa

The seeds of N. sativa (Fig. 1) are used in the treatment of various diseases like bronchitis, diarrhea, rheumatism, asthma and skin disorders. It acts as a liver tonic, anti-diarrheal, appetite stimulant, emmenagogue. It is used in digestive disorders, to increase milk production in nursing mothers to fight parasitic infections, and to strengthen immune system (Al-Ali A et al.,



2008). Seeds are also used in food like flavoring additive in the breads and pickles because it has very low level of toxicity (Yarnell et al., 2011). Seeds are useful in the treatment of worms and skin eruptions. Oil is used as an antiseptic and local anesthetic externally. Roasted black seeds are given internally to stop the vomiting (Morsi NM., 2000).

2. MATERIAL AND METHODS:

2.1. Sample Collection

Initially the black cumin (*Nigella sativa*) seed was purchased from local herb shop in Thuckalay, Tamilnadu. First the dirty part from black cumin seed was removed by using our hands and dried at room temperature, then grinded into fine powder by using electric grinder.

2.2. Extract preparation

Finely grinded *N. sativa* powder was subjected to aqueous and two organic solvent (chloroform & ethanol) separately with ratio 10:100 in a flask and then kept it on shaker at room temperature on continuous shaking for 24-48hrs. The solution was then centrifuged and the supernatant then collected and filtered through Whatmann filter paper 1. The filtrate then dried in a rotary evaporator at 50 – 60°C until all the solvents get evaporated and only crude extract left behind. The crude extracts were kept in a refrigerator for further research use.

2.3. Phytochemical Screening

Preliminary phytochemical screening of the black cumin seed extract was carried out for the detection of the various plant constituents. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure. (Abdurohaman Mengesha Yessuf, 2015).

2.3.1. Screening for Alkaloids (Wagner's test):



To 1 ml of extract, add few drops of dil.HCl mix well and stirred, after that filter the solution, and then add 2 drops of Wagner's reagent. Orange or Reddish brown precipitate indicates the presence of alkaloids.

2.3.2. Screening for Flavanoids (Alkaline Reagent Test):

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavanoids.

2.3.3. Screening for Phenol (Ferric Chloride Test):

Extracts were treated with 5 ml distilled water and 3-4 drops of ferric chloride solution. Formation of dark green colour indicates the presence of phenolic compounds.

2.3.4. Screening of phyto sterol:

To 1 ml of test solution a few drops of acetic anhydride and conc. H_2SO_4 was added and mix well. Allow the mixture to stand for some time; Bluish colour indicates the presence of phyto sterol.

2.3.5. Screening for Saponins (Foam Test):

0.5 g of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

2.3.6. Screening for steroids (Salkowski test):

To 1 ml of test solution 5 ml of chloroform was added and then few drops of conc. H_2SO_4 was added to the above mixture and mix well. Allow the mixture to stand for some time; brown colour in the lower layer indicates the presence of steroids, if bluish brown then indicates the presence of phyto steroids.

2.3.7. Screening for sugar:

To 1 ml of test solution add few drops of Molish reagent Violet colour indicate the presence of sugar.

2.3.8. Screening for Tannins:

Few drops of neutral $FeCl_3$ solutions and 5ml of distilled water were added to the test solution. Bluish green colour indicates the presence of tannins.

2.3.9. Screening for Terpenoids:



Treat the extract in chloroform with few drops of con.H₂SO₄ shaken well and allow to stand for some time, formation of yellow or reddish brown lower layer.

2.3.10. Screening for Xanthoproteins:

Extract is treated with conc.HNO₃ and excess NH₃. Formation of reddish orange precipitate indicates the presence of xanthoproteins.

2.4. Biological Activity

The biological activities of crude extract are tested against bacteria and fungi. These antimicrobials were grown in LB broth for 24 hrs. Approximately 20ml of molten and cooled Muller Hinton agar medium and the crude extract containing disks were kept over the medium using sterile forceps. Antimicrobial activity was evaluated by measuring the zone of inhibition for the test organisms. The diameters of zones were measured to the nearest millimeter with verniercalipers.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

In this study, the results of preliminary phytochemical screening of seed extracts of *Nigella sativa* L. are presented in **Table 1**. Investigations of the phytochemical screening of *Nigella sativa* ethanol extract revealed the presence of alkaloids, flavonoids, phytosterol, saponins, sugar, tannins, terpenoids. Whereas the metabolites like xanthoprotein and phenol were found to be absent in the ethanolic extract. In chloroform extract of seeds showed the presence of alkaloid, sugar, steroids, terpenoids, and xanthoprotein. Very few numbers of phytochemicals were found in aqueous extract like alkaloids, tannins and xanthoprotein.

Table 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF AQUEOUS, ETHANOL AND CHLOROFORM SEED EXTRACTS OF *NIGELLA SATIVA* L.

Sl.	Name of the	Aqueous	Ethanol	Chloroform
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No	Phytochemical	Extract	Extract	Extract
1	Alkaloid	+	+	+
2	Flavanoids	-	+	-
3	Phenol	-	-	-
4	Phytosterol	-	+	-
5	Saponins	-	+	-
6	Steroids	-	+	+
7	Sugar	-	+	+
8	Tannins	+	+	-
9	Terpenoids	-	+	+
10	Xanthoprotein	+	-	+

3.2.Antibacterial Activity

The antibacterial activity of the *Nigella sativa* L. in chloroform and ethanol extracts were tested against two bacteria viz., *Staphylococcus aureus* and *Escherichia coli*. Compare these two extracts, ethanol extract have higher activity than chloroform extract against *Staphylococcus aureus* and *Escherichia coli* (**Table 2**). Thus the *Nigella sativa* seed extract may have a potential use in the biomedical applications due to its antibacterial activity.

Table 2: ANTIBACTERIAL ACTIVITY OF *NIGELLA SATIVA* L.AGAINST HUMAN PATHOGENIC BACTERIA

Sl.No	Micorbes	Chloroform extract (mm)	Ethanol extract (mm)	Std. (Nystatin) (mm)
1.	<i>Escherichia coli</i>	8	12	12
2.	<i>Staphylococcus aureus</i>	-	9	25



3.3. Antifungal Activity

Antifungal Activity of the *Nigella sativa* L. crude extract is tested against *Candida albicans* and *Aspergillusniger*. The ethanolic extract of *Nigella sativa* L showed good antifungal activity against *Candida albicans* and *Aspergillusniger*. The chloroform extract of *Nigella sativa* L. was moderately active against *Candida albicans* and donot show any activity against *Aspergillusniger*(**Table 2**).The observed activity may be due to the presence of potent phytoconstituents in the extracts.

Table-3:ANTIFUNGAL ACTIVITY OF NIGELLA SATIVA L.AGAINSTFUNGAL SPECIES

Sl.No	Microbes	Chloroform extract (mm)	Ethanol extract (mm)	Std. (Nystatin) (mm)
1.	<i>Candida albicans</i>	10	17	29
2.	<i>Aspergillusniger</i>	-	15	16

From this study, we can conclude that the plant *Nigella sativa* possessed the highest antimicrobial activity and the observed activity may be due to the presence of phytoconstituents in the extracts. To promote proper conservation and sustainable use of such plant resources, awareness of local communities should be enhanced incorporating the traditional knowledge with scientific findings. Since *Nigella sativa* is used in various medicinal preparations. Hence the present study may be useful to supplement information in its identification, authentication and standardization. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Many



plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant.

4. CONCLUSION

The results clearly indicated that research works carried out on the medicinal plant, possessing traditional privileges of effectiveness in bacterial infections provided fruitful results. Therefore, ethanol extract of *Nigella sativa* L. possessing antimicrobial activity could be applicable in therapeutic procedure. The main focus of our work is on the antimicrobial potential of medicinal plants which we plan to study further with the ultimate objective of providing scientifically validated herbal remedies against bacterial and fungal infections. However, the present study of in vitro antimicrobial activity of *Nigella sativa* L. forms primary platform for further phytochemical and pharmacological studies.

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