

Extraction and chemical tests on *Nigella Sativa* I. collected from Punjab region of India

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ABSTRACT

Nigella sativa is a wonder plant which has the potential to cure almost all diseases except death. The medicinal value of the plant has been reported quite long ago and finds its place in Holy Quran. *Nigella Sativa* of Family Ranunculaceae contains chemical components like Thymoquinone, alpha hederin which are anticancer in nature. So in this article emphasis on the different solvent extraction and its chemical tests have been shown from the seeds collected from Punjab, India.

Keywords: *Nigella sativa*, Thymoquinone, Anticancer

INTRODUCTION

Medicinal plants are used for curing diseases for several centuries in numerous indigenous systems of drugs also as folk medicines. Natural products are awesome success in our society. The utilization of plant and microorganism secondary metabolites has aided in doubling of our life within the twentieth century. they need reduced pain and suffering, and revolutionized medication by enabling the transplantation of organs. Since their chemical diversity is predicated on biological and geographical diversity, the complete globe is explored for bioprospecting by researchers (Khare 2004). Cancer is one among the foremost threats of recent life, that is taken into account because the second reason behind death when myocardial infarction. Variant individuals die per annum in numerous types of cancer despite tremendous efforts to search out strategies of management and cure within the last century, nice advances were created in trendy bioscience to regulate malady (Sharma, Yelne et al. 2000).

However several diseases like cancers aren't curable absolutely. To search out out new and authentic therapies, scientists square measure operating with ancient or people medications in parallel of recent medicine. *nigella sativa* has been used for healthful functions for hundreds of years. It originated from Southeastern Asia and also employed in ancient Egypt, Greece, geographic area and continent. In

Islam, it's considered one among the best forms of healing medication out there. it's a flowering plant, of that seed is employed as a spice. The seed is termed black cumin in English, whereas in Latin it absolutely was known as 'Panacea' that means 'cure all'; in Arabic it's termed as 'Habbah Sawda' or 'Habbat el Baraka' translated as 'Seeds of blessing'. it's conjointly referred to as 'Kalo jeera' (in Bangladesh), 'Kalonji' (in India) and 'Hak Jung Chou' in (China). Each seeds and oil extracted from this plant square measure employed in healthful functions.

Kalonji (*Nigella sativa*) could be a dicotyledonous of ranunculacea is an incredible herb with a non secular background and usually grows within the Japanese Europe geographic area and Western Asia (Al-Bukhari and Sahi 1976).

The seeds of *N. sativa* square measure the supply of the active ingredient of this plant. It is a black seed referred by Prophet mohammed as a panacea that's a remedy for all ailments however cannot stop ageing or death. The utilization of black seeds has been mentioned in numerous non secular and ethnic books. Black seeds square measure known because the curative black cumin within the holy Bible within the Unani-Tibb system of drugs that originate from medical man, his modern anatomist and Ibn- sina has regarded black seed as a valuable remedy within the viscus and biological process disorder. Through

thousands of years, till the time being, variant individuals within the Mediterranean region and much East countries use the oil of *N. sativa* seeds daily as a natural protecting and curative remedy.

PLANT CHARACTERISTICS

Taxonomic classification:

Kingdom - Plantae
 Subkingdom - Tracheobionta
 Superdivision - Spermatophyta
 Phylum - Magnoliophyta
 Class - Magnoliopsida
 Order - Ranunculales
 Family - Ranunculaceae
 Genus - *Nigella*
 Species - *N. sativa*

Common Names:

Black cumin, Fennel Flower, Nutmeg Flower, Black seed, Black Caraway, Roman Coriander, Damascena, Devil in-the-bush, Wild Onion Seed, Kalonji, Kalojeera, Habat ul- Bakarah

Habitat:

N. sativa is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia.

Plant morphology:

N. sativa is an annually flowering plant which grows from 20 cm to 90 cm in height, with finely divided leaf; the segments of the leaf are narrowly linear to threadlike. The flowers are delicate and are usually white, yellow, pink, pale blue or purple in colour. Number of petals present varies from 5-10. The fruit is large green n capsulated containing 3-7 united follicles, each having numerous black seeds.



Fig: *Nigella sativa* plant

Characteristics of seed and flower:

Macroscopically, seeds are small dicotyledonous, trigonus, angular, rugulose-tubercular, 2-3.5mm x 1-2 mm, black externally and white inside, odour slightly aromatic and taste bitter. Microscopically, transverse section of seed shows single layered epidermis consisting of elliptical, thick walled cells, covered externally by a 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 rectangular elongated or nearly columnar, elongated cells. Endosperm consists of thin walled, rectangular or polygonal cells mostly filled with oil globules. The powder microscopy of seed powder shows brownish black, parenchymatous cells and oil globules.



Fig: *Nigella* Flower

Fig: *Nigella* Seed

Chemical composition of black seeds

Many active compounds have been isolated, identified and reported in different varieties of black seeds. The most important active compounds are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpene longifolene (1%-8%) α -pinene 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.

Moreover, *N. sativa* seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent. Some other compounds e.g. carvone, limonene, citronellol were also found in trace amounts. Most of the pharmacological properties of *N. sativa* are mainly attributed to quinine constituents, of which TQ is the most abundant. On storage, TQ yields dithymoquinone and higher oligo condensation

products. The seeds of *N. sativa* contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fibre (8.4%) and total ash (4.8 %). The seeds are also containing good amount of various vitamins and minerals like Cu, P, Zn and Fe etc. The seeds contain carotene which is converted by the liver to vitamin A. Root and shoot are reported to contain vanillic acid. The seeds reported to contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50-60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%). Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. α -sitosterol is a major sterol, which accounts for 44% and 54% of the total sterols in Tunisian and Iranian varieties of black seed oils respectively, followed by stigmasterol (6.57-20.92% of total sterols).

Examples of various other reported chemical components includes nigellone, avenasterol-5-ene, avenasterol-7-ene, campesterol, cholesterol, citrostadienol, cycloeucalenol, gramisterol, lophenol, obtusifoliol, stigmastanol, stigmasterol-7-ene, β -amyrin, butyrospermol, cycloartenol, 24-methylene-cycloartanol, taraxerol, tirucallol, 3-O- $[\beta$ -D-xylopyranosyl(1 \rightarrow 3)- α -L-rhamnopyranosyl(1 \rightarrow 2)- α -L-arabino-pyranosyl]-28-O- $[\alpha$ -L-rhamnopyranosyl(1 \rightarrow 4)- β -D-glucopyranosyl(1 \rightarrow 6)- β -Dglucopyranosyl] hederagenin, volatile oil (0.5-1.6%), fattyoil (35.6-41.6%), oleic acid, esters of unsaturated fatty acids with C15 and higher terpenoids, esters of dehydrostearic and linoleic acid, aliphatic alcohol, β -unsaturated hydroxy ketone, hederagenin glycoside, melanthin, melanthigenin, bitter principle, tannin, resin, protein, reducing sugar, glycosidalsaponin, 3-O- $[\beta$ -D-xylopyranosyl-(1 \rightarrow 2)- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-11-methoxy-16,23-dihydroxy-28-methylolean-12 enoate, stigma-5, 22-dien-3- β -D-glucopyranoside, cycloart-23-methyl-7, 20, 22-triene-3 β , 25-diol, nigellidine-4-O-sulfite, N. mines A3, A4, A5, C, N. mines A1, A2, B1, and B2.

Traditional uses of folk remedies

N. sativa has been traditionally used for the treatment of a variety of disorders, diseases and conditions pertaining to respiratory system, digestive tract, kidney and liver function, cardio vascular system and immune system support, as well as for general well-being. Avicenna refers to black seeds in

the "The Canon of Medicine", as seeds stimulate the body's energy and helps recovery from fatigue and dispiritedness. Black seeds and their oil have a long history of folklore usage in Indian and Arabian civilization as food and medicine. The seeds have been traditionally used in Southeast Asian and the Middle East countries for the treatment of several diseases and ailments including asthma, bronchitis, rheumatism and related inflammatory diseases. Its many uses have earned *Nigella* the Arabic approbation 'Habbatul barakah', meaning the seed of blessing. A tincture prepared from the seeds is useful in indigestion, loss of appetite, diarrhoea, dropsy, amenorrhoea and dysmenorrhoea and in the treatment of worms and skin eruptions. Externally the oil is used as an antiseptic and local anesthetic. Roasted black seeds are given internally to stop the vomiting.

MATERIALS AND METHODS

Collection:

Seeds are collected from local market of Bathinda district of Punjab, India.

Extraction:

The seeds of *Nigella sativa* is crushed to coarse powder using electric grinder. The extraction is done using four different solvents namely Petroleum Ether, Chloroform, Methanol, Hydroalcoholic solution (20% DM Water in Methanol).

Equipments used are:

1. Speed Extractor: Grounded Powder is loaded in the four chambers along with purified sand. The lead taking solvent is put in pure covered solvent. After three cycles of automatic extraction the equipment (manufactured by B'U'CHI). The process continues for all four solvents. The extracts are stored in clean dried container.
2. Aspirator: Dried grounded powders are put in closed glass aspirator. Solvent in same order is poured over the powder so that all powder is in direct contact with solvent. The aspirator is kept overnight undisturbed with solvent. Next day the extract is collected and stored.

All the extracts are subjected to concentration using rotavapor equipment until only concentrated oil or sticky solid is left. The hydroalcoholic portion is fractionated against Petroleum ether, ethyl acetate and chloroform. The organic portion is collected and dried until minimum drop of oily substance is collected. They are mixed with parent extract and stored in cool and dry place. The only left water part is dried to form solid.

CHEMICAL TESTS:

1. Test for Alkaloid: The samples are added with 3ml of picric acid saturated solution. Samples giving yellow precipitate shows presence of alkaloid.
2. Test for Fixed oil: On a clean filter paper add 2 drops of each sample. If it leaves a translucent spot then it is the presence of fixed oil
3. Test for Volatile oil: All samples are added with alcoholic solution of Sudan III dye. If the samples become red then volatile oil is present.
4. Test for Tannins: Add FeCl_3 to all the sample. Yellow colour gives hydrolysable tannin whereas green colour gives condensed tannin.
5. Test for Flavanoid: The samples are added with NaOH solution producing yellow colouration. On adding dilute acid if the mixture goes colourless then presence of flavanoid is confirmed.
6. Test for Glycoside: Part A: Add dilute H_2SO_4 in the samples then add 5% NaOH neutralizing it. To it add equal volume of

Fehling solution 1 and solution 2. Red colour is produced.

Part B: Add DM Water to all the test tubes until it is diluted then add equal volumes of Fehling solution 1 and 2. Red colouration Compare the redness if part A has more intensity than part B then we can say that there is presence of reducing sugar

7. Test for Steroids and triterpenes: All samples are added with conc. Sulphuric acid. Yellow on top layer show steroid and green on bottom show triterpenes.

RESULT

Tests	Pet. Ether Ext.	CHCl_3 Ext.	Methanolic Ext.	Water Ext.
Alkaloid Test	+	+	+	+
Fixed oil Test	+	+	+	-
Test for tannin	-	-	-	-
Volatile oil Test	+	+	+	-
Flavanoid Test	+	+	+	+
Glycoside test	-	-	-	-
Steroid test	+	+	+	+
Triterpene test	+	-	+	+

CONCLUSION

From the above mentioned result we can conclude that all proportion of extract contains alkaloids and flavanoids stark absence of glycoside and tannins rest are present in some and absent in some.

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↓ REFERENCES

1. Al-Bukhari, M. and A. Sahi (1976). "The collection of authentic sayings of Prophet Mohammad (peace be upon him), division 71 on medicine." Hilal Yayinlari, Ankara, Turkey.
2. Khare, C. (2004). "Encyclopedia of Indian Medicinal Plants: Rational Western therapy, Ayurvedic and other Traditional usage." Springer, Germany, ISBN 3: 540-20033.
3. Sharma, P., et al. (2000). Database on medicinal plants used in Ayurveda.