

Phytochemicals Screening, and Synthesis of Silver Nanoparticles from Extract of *Nigella Sativa* L. Seeds and its Possible Application in Hypothyroid Treatment

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ABSTRACT

Phytochemicals are bioactive compound obtained from the plants and are widely applied in the traditional herbal medicine. These herbal medicines are used by the local people to cure the various diseases, such as diabetes, cancer, thyroids, HIV etc. The present study deals with the phytochemical screening of Nigella Sativa. L. Seeds, which are commonly known as black cumin seeds, are remedy for everything except death. It may provide an ideal treatment for auto immune system and has antithyroid quality. Phytochemical screenings were performed by qualitative and quantitative analysis of various phytochemicals by using standard method of Harborne. Phytochemicals separation were also performed by using TLC and HPLC method. Phytochemicals present in seeds may be used in future for preparation of herbal drugs. Nano Synthesis of phytochemicals extract were performed by using silver nitrate, and silver nanoparticles were prepared Nanoparticles were characterized by U.V spectroscopic method, SEM analysis and EDX spectra.

Keywords: *Nigella Sativa* .L, TLC, HPLC, Phytochemicals, Nano Synthesis.

I INTRODUCTION

The thyroid is a small butterfly –shaped gland is located in front of neck, just below the voice box (larynx).. This gland plays a very important role in controlling our body metabolism i.e., the rate at which our body uses energy and it does this by producing thyroid hormones like thyroxin or T4 and Triode thyronine or T3, chemicals that travel through our blood to every parts of our body. It produces chemicals that help the body to control metabolism. Thyroid hormone is normally produced in response to another hormone released by the pituitary gland.[1]Generally, thyroid problem are grouped in to two main categories i.e. hyperthyroidism (too much thyroid hormone),and hypothyroidism,(too little thyroid hormone) here we discuss about hypothyroidism, the most common symptoms of hypothyroidism includes fatigue ,depression , constipation ,weight gain ,slow heart rate etc.[2] *Nigella Sativa* Linn (kalaungi) seeds are extensively used for medicinal purpose in Asia, Middle East and far east countries for a long time. *Nigella sativa* may have antithyroid properties. *Nigella sativa* is a local annual dicotyledonous herb of Renunculaceae family ,maximum height of this plant is about 60 cm. There are different names of *Nigella sativa* in different languages in different region of the world eg kalaungi (Urdu) ,black cumin (English), shone (Persian) ,kalajira(Bengali) [3]the seeds of *Nigella sativa* are known as black cumin seeds and they are very important in many pharmacological studies for its immune modulatory and therapeutic properties [4] The most important compounds is due to which medicinal value of these seed increased are saponins flavonoids, volatile oils and trace elements[5] Seeds of *Nigella sativa* are being used for thousands of years as remedies for number of traditional diseases [6]*Nigella sativa* in traditional medicines as well as in recent years has been used for the treatment of microbial disease. In Egypt from a long time oil of *Nigella sativa*

has been used for severe cough and asthma [7].It is observed that many pharmacological activities such as antithyroid, antioxidants, anticancer, anti-inflammatory and anti-asthmatic activities are also shown by this miraculous medicinal plant.[8.].Black cumin seeds decrease the absorption of lipids which can lower the cholesterol and triglyceride level which can help in weight loss [9].

II MATERIAL METHODS

- (a) **Collection of Sample** -Seeds of *Nigella sativa* were collected from local market of Bilaspur district of Chhattisgarh and pulverized by using house hold electric grinder. Solvent extractions were performed to extract oil from seeds using sohxlet apparatus using methanol as solvent.
- (b) **Reduction of Sample** - The seeds were nicely cleaned and air dried. The dried sample were then powdered with the help of house hold griender. The powdered sample was kept in an air tight container. Extraction of seeds oil is done by using sohxlet apparatus using petroleum ether as solvent.

III METHODS OF EXTRACTION BY SOHXLET APPARATUS

50gms of powdered black cumin seeds was filled in thimble and extracted exhaustively by sohxlet apparatus (6hr) using methanol as solvent at 60 deg. Cent. The extract obtained was collected and passed through Wattmann No.1 filter paper to remove alldebris and unextracted matter. Filtered extract was concentrated using rotatory evaporator at 40 degree centigrade to obtain concentrated extract.

Qualitative and Quantitative analysis

Qualitative and Quantitative analysis Phytochemicals were done by using standard methods of Harborne. [10]

Methods of separation

Chromatography is a physical method of separation that distributes components to separate between two phases. In the present work TLC and HPLC technique are used for separation of phytochemicals. Thymoquinone is a bioactive constituent of *Nigella sativa* L. seeds which possesses considerable pharmacological properties. Thin layer Chromatographic elution of the separation of four spots with R_f value 0.81, 0.47, 0.38 and 0.07. The R_f value Thymoquinone was found to be 0.81 which is equivalent to Thymoquinone standard.

IV HPLC TECHNIQUE FOR SEPARATION

The chromatographic evaluation achieved at ambient temperature on a RP-C18 analytical column with a mobile phase composed of methanol: 0.1% trifluoroacetic acid (TFA) (80:20%) and isocratically with the flow rate 1ml min⁻¹. A small sample quantity of 20µL used for each run, the chromatogram monitored with UV detection at a wavelength of 254nm and analysis is performed in room temp, and its retention time is 15.04 min.

Nano synthesis of *Nigella sativa* seeds extract - Nano synthesis is performed by using silver nitrate solution, and nanoparticles is prepared. These nanoparticles are characterised by different methods.

Synthesis of AgNPs - The solution of silver nitrate salt (AgNO₃) 1mM was prepared and stored in amber colored bottle. Different concentrations of seed extracts (1 and 5 mL) were taken separately and 10 mL of 1 mM silver nitrate solution was added with constant stirring and exposed to different conditions like sunlight irradiation, UV irradiation. Change of the seed extract color from yellow to dark brown indicated that the silver nanoparticles were synthesized from the seeds.

Characterization of silver Nanoparticles

Characterization of prepared silver nanoparticles has done by UV spectrophotometer, SEM and EDS analysis.

V RESULT AND DISCUSSION

The preliminary phytochemical screening revealed the presence of Alkaloids, Flavonoids, Tannins, Steroids, Saponins and Terpenoids as shown in Table -1. The phytochemicals present in black Cumin seeds have many pharmacological applications

Table 1
Qualitative Analysis of extract of nigella sativa seeds

Alkaloids	Mayer's test	+
	Wagner's test	+
	Hager's test	+
Terpenoids	Salkowski test	+
Phenols	Ferric chloride	+
Steroids	Acetic anhydride+H ₂ SO ₄	+
Flavonoids	Alkaline reagent test	+
Saponins	Froth test	+
Diterpenes	Copper acetate	+
Coumarine	Sodium hydroxide	+
Carbohydrate	Molish test	+
E modol	25% NH ₃ solution	-

Table 2
Quantitative study of N. Sativa

Alkaloids	0.239 mg
Flavonoids	0.040 g
Saponins	0.37 mg
Phenols	0.043mg
Terpenoids	0.89mg

Table 3
TLC Analysis

S.No.	Mobile phase	No. of spots	R _f Value
1	Hexane 90ml: Ethyl acetate 10mi	2 275 U.V	0.81, 0.07

The R_f value of Thymoquinone was found to be 0.81 which is equivalent to Thymoquinone standard.

In the present study quantitative phytochemical analysis of methanol extract of Nigella Sativa shows the presence of alkaloids, Terpenoids, steroids, phenols, flavonoids, saponins, diterpenes, carboxylic acid, coumarins, carbohydrates while E -medol was not found in methanolic extract of Nigella sativa.

Quantitative analysis of nigella sativa shows alkaloids 0.239mg, flavonoids 0.040mg, saponins 0.37mg, phenols0.0215mg, terpenoids0.89mg. of phytochemicals. Sahib et al. (2016) suggested that due to presence of these phytochemicals the importance of Nigella sativa in medicinal fields has been increasing day by day. Peaks of many phytochemicals metabolites are seen in hplc chromatography but the peak at 9.067 retention time revealed the presence of Thymoquinone (TQ) in extract.

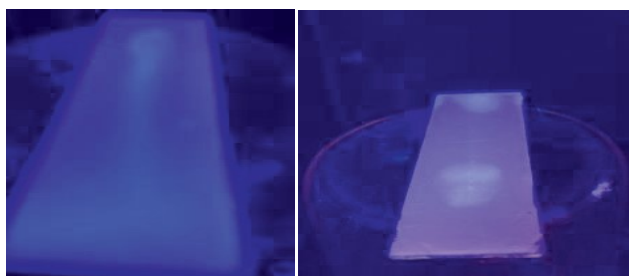


Fig. 1: TLC Chromatography of Nigella sativa seeds extract

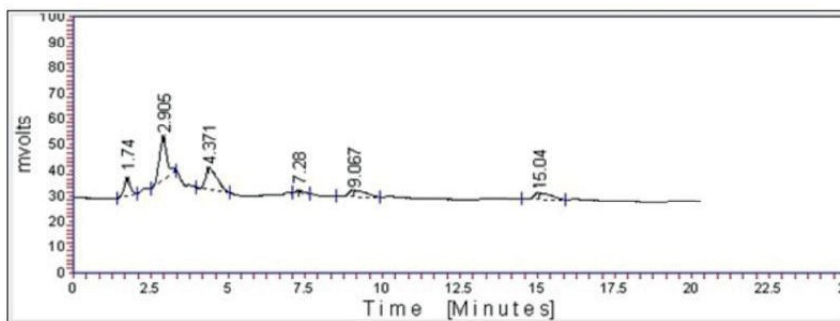


Fig. 2 : HPLC chromatography of Nigella Sativa seeds

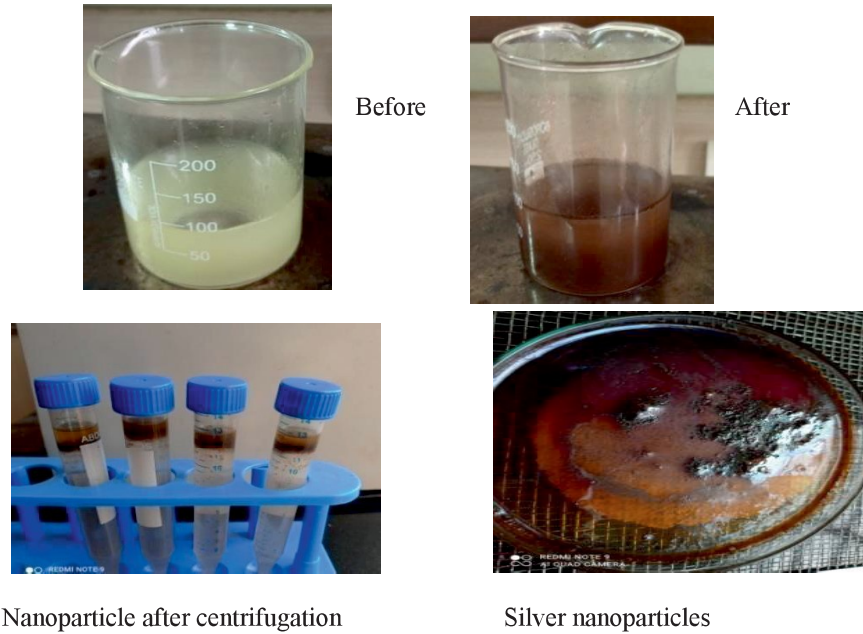


Fig. 3 : Nanosynthesis of NigellaSativa seeds

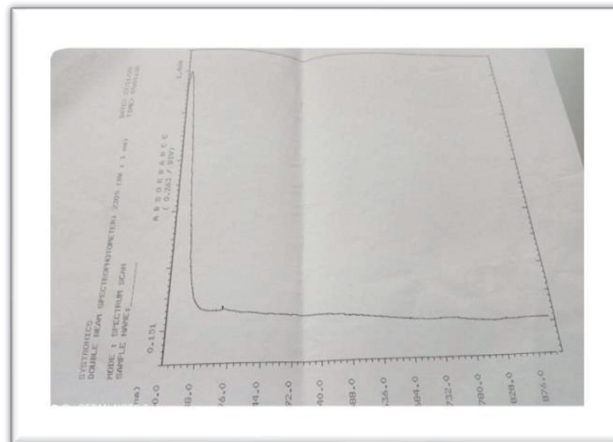


Fig. 4 U V spectroscopy of NigellaSativa seeds

VI CHARACTERIZATION OF SILVER NANOPARTICLES

UV-Vis spectroscopy is an important and simplest technique to confirm the synthesis of nanoparticles.

Maximum absorption peak of colloidal AgNPs were recorded at 348 nm due to surface plasma resonance caused by the free electrons of the silver nanoparticles

VII SEM AND EDS ANALYSIS

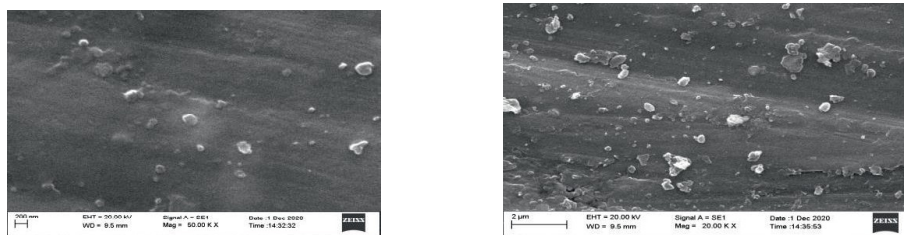


Fig. 5 SEM Analysis of nanoparticle of *Nigella sativa* seeds extract

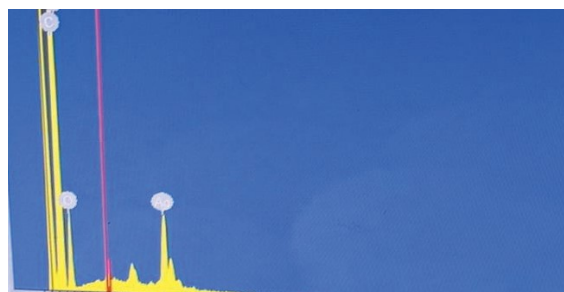


Fig. 6 EDS Analysis of nanoparticles of *Nigella Sativa* seeds extract

SEM and EDS analysis of *Nigella sativa* seeds were performed and found that silver nano particle were prepared in Nanosynthesis of range 67 nm to 78nm and its shape is like mosaic and irregular.

VIII CONCLUSION

Some of the herbal seeds are used as drugs for the treatment of thyroid dysfunction and which have a direct action on thyroid gland in condition of hypothyroidism. In the present study beneficial effect of *Nigella sativa* has demonstrated in hypothyroid. These seeds had a strong impact in reducing thyroid problems because of its major bioactive compounds like Thymoquinon, it is observed that black cumin seeds decreased the absorption of lipids which can lower the cholesterol and triglycerides level, which help in reducing body weight and constipation which is the major symptoms of hypothyroidism

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