Enzymatic Extraction of Nigella Sativa Oil from their Seeds

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Abstract **- Nigella sativa is one of the most Useful medicinal seeds in history also important in developing agriculture sector nowadays."NigellaSativa(Kalonji) Can cure every disease except Death".Enzyme-assisted extraction is a safe and efficient oil extraction process. Seeds of Nigella sativa, a dicotyledon of the Ranunculaceae family, have been employed for thousands of years as a spice and food preservative. The oil and seed constituents, in particular thymoquinine (TQ), have shown potential medicinal properties in traditional medicine. In seeds a component Thymoquinine is present almost 50% of seed and having Anti carcenogenic Property. Nigella Sativa oil was extracted with different types of enzymes (Hemicellulase, Pectinase, Acid protease ,Protease ,Cellulase). Enzymes are useful for extraction give better yield and and catalysed the reaction by minimizing residence time. The temperature for the extraction process is maintained at 65-70 °C and 1 bar for the pressure. The oil extracts along with the control sample were further analyzed to determine their composition and yield.**

IndexTerms **- Dicotyledon, preservative, thymoquinine, Anticarcenogenic, Hemicellulase, Pectinase, Acid protease, Protease, Cellulase**

I. INTRODUCTION

Medicinal plants have been a major source of therapeutic agents since ancient times to cure human disease. India is considered as "Botanical Garden of the world" and more than 2200 species of medicinal and aromatic plants have been identified after studies. The revival of interest in natural drugs started in last decade mainly because of the wide spread belief that green medicine is healthier than synthetic products.Now-a-days, there is various increase in medicinal plant based industries due to the increase in the interest of use of medicinal plants throughout the world.

As per WHO, about 80% of the population in the world relays on the traditional medicine for the treatment of various diseases. Therefore, the evaluation of rich heritage of traditional medicine is essential. In this regard, one such plant is Nigella sativaLinn. which is a small elegant annual herb distributed and cultivated all over India. Nigella sativaLinn. (Family-Ranunculaceae) commonly known as Upakunchika, Ajaji, Kalvanjika, Kalika, Kunchika, Kalaunjiand Black cumin , is a small elegant herb, mostly found and cultivated in Punjab, Himachal Pradesh, Gangetic Plains, Bihar, Bengal, Assam, Maharashtra and also cultivated in Syria, Lebanon, Israel and Southern Europe. seeds are flattened, oblong, angular, funnelshaped, small, 0.2cm long and 0.1 cm wide, black in colour.

Fig 1 Nigella Sativa Seeds

Physical constants

Foreign matter, 2% w/w; total ash, 6% w/w; acid insoluble ash, 0.2% w/w; alcohol soluble extractive, 20% w/w; water soluble extractive, 15% w/w; total fixed oil, 25-32% w/w; volatile oil, 0.42% w/w; organic matter, 3.91% w/w; loss on drying, 4% w/w.

Main constituents of Nigella Sativa

The seeds contain numerous esters of structurally unusual unsaturated fatty acids with terpene alcohols (7%); furthermore, traces of alkaloids are found which belong to two different types Isochinoline alkaloids are represented by nigellimin and nigellimin- *N*-oxide, and pyrazol alkaloids include nigellidin and nigellicin. In the essential oil (avg. 0.5%, max. 1.5%), thymoquinone was identified as the main component (up to 50%) besides *p*-cymene (40%), α-pinene (up to 15%), dithymoquinone and thymohydroquinone. Other terpene derivatives were found only in trace amounts Carvacrol, carvone, limonene, 4-terpineol, citronellol. Furthermore, the essential oil contains significant (10%) amounts of fatty acid ethyl esters. On storage, thymoquinone yields dithymoquinonene and higher oligo condensation products (nigellone). The seeds also contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50 – 60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%) which is characteristic for the genus. Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. Commercial nigella oil ("Black Seed Oil", "Black Cumin Oil") may also contain parts of the essential oil, mostly thymoquinone, by which it acquires an aromatic flavour.

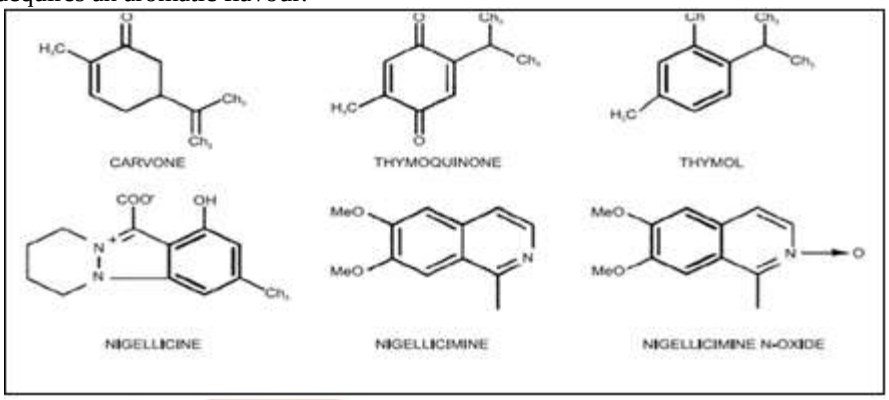

Fig 2 Chemical Structure of some major compound isolated Nigella Sativa

Enzymes

1)Trypsin

Trypsin is a serine protease found in the digestive system of many vertebrates , where it hydrolyses proteins. Trypsin is formed in the small intestine.Trypsin catalyzes the hydrolysis of Proteins bonds , breaking down proteins into smaller peptides. Trypsin is considered an endopeptidase , i.e., the cleavage occurs within the polypeptide chain rather than at the terminal amino acids located at the ends of polypeptides. Human trypsin has an optimal operating temperature of about 37°C.

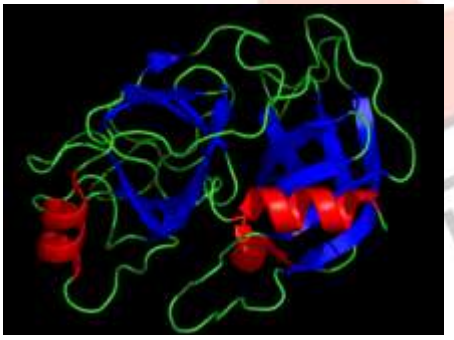

Fig 3 Structure of Trypsin

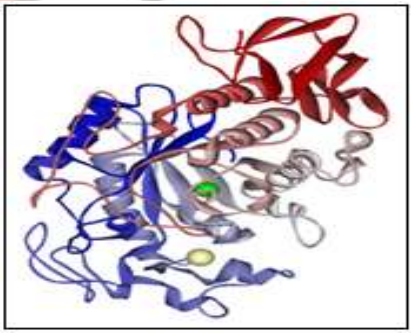

Fig 4 Structure of α-Amylase

2)α-Amylase

α-Amylase is a protein enzyme that hydrolyses alpha bonds of large, alpha-linked polysaccharides , such as starch and glycogen , yielding glucose and maltose .It is the major form of amylase found in humans and other mammals. It is also present in seeds containing starch as a food reserve, and is secreted by many fungi. Amylase is found in saliva and breaks starch into maltose. Optimum pH - 7.0.

II. MATERIAL AND METHOD

Nigella Sativa Seeds obtained from Ratnagiri local market and Crushed seeds were use for oil extraction. Enzymes α-amylase and Trypsin were obtain from Department of Biochemistry,Gogate Jogalekar College ,Ratnagiri .Solvents like Hexane,Iso-Propyl alcohol and Petroleum ether was purchased from Shree Chemicals,Ratnagiri.

Setup Used for Extraction of Nigella Sativa oil

Soxhlet Apparatus

A Soxhlet Apparatus is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally design for extraction of lipid from solid material. However a Soxhlet Apparatus is not limited to the extraction of lipids. Typically

Soxhlet extraction is only required where the designed compound has a limited solubility in a solvent, and the impurity is soluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration then used to separate the compound from the insoluble substance.

Fig 5 Experimental Setup

1.The source material containing the compound to be extracted is placed inside the thimble.
2.The thimble is loaded into the main chamber of the SoxhletApparatus.
3.The extraction solvent to be used is placed in a distillation flask.
4.The flask is placed on the heating element.
5.TheSoxhlet Apparatus is placed atop the flask.
6.A reflux condenser is placed atop the extractor.

Working of Soxhlet Apparatus

Normally a solid material containing some of desired compound is placed inside a thimble, which is loaded into the main chamber of the soxhlet Apparatus. Soxhlet Apparatus is placed into flask containing the extraction solvent. The soxhlet is then equipped with a condenser.

The solvent is heated to reflux. The solvent vapor travel up a distillation arm, and floods into the chamber housing the thimble of soild. The condenser ensure that any solvent vapor cools, and drips back down into the chamber housing the solid material. The chamber containing solid material slowly fills with warm solvent. Some of desired compound will then dissolve in the warm solvent.

When the soxhlet chamber is almost fill, the chamber is automatically emptied by siphon side arm, with solvent running back down to the distillation flask. This cycle may be allowed to repeat many time over hours.

During each cycle, a position of non volatile compound dissolve in the solvent. After many cycle desired component is concentrated in distillation flask. The advantages of this system is instead of many portion of warm solvent being pass through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of simple distillation . The non soluble of portion of extracted solid remain in the thimble , and is usually discarded.

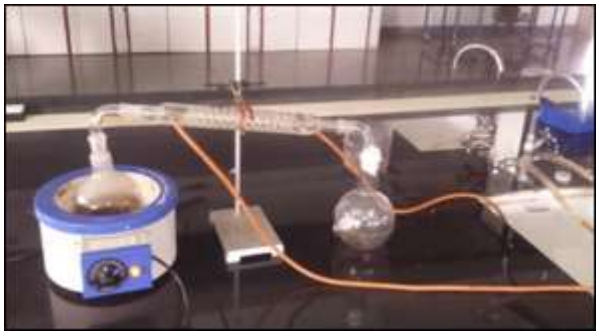

Fig 6 Simple distillation apparatus

Without Enzymatic Action

1. First of all Nigella sativa seeds are crushed in a mixer into the powder form (1.4mm)

2. Then powdered raw material of Nigella Sativa Seed are wrapped into the Whatmann filter paper are weight before placing into thimble.
3. The weight of powered seeds is taken about 50gm.
4. Material in filter paper placed in extractor of soxhlet apparatus for extraction operation.
5. Bottom flask of soxhlet is filled with solvent Hexane/ Petroleum ether/Iso-propyl alcohol around 500 ml.
6. Then heating is done by heating mantle.
7. Extraction process is carried out around 2h.
8 The main process begins when heating occurs; Hexane/ Petroleum ether/Iso-propyl alcohol vaporizes and goes in upward direction through side arm of thimble.
9. This vapors goes towards condenser in upward direction, the condensed is in a extractor.
10. Thus after condensation the liquid form is collected in the extractor where wrapped seeds are kept.
11. A liquid level goes on increasing such that wrapped seeds are dipped into the liquid and at certain point the liquid is refluxed through the reflux side.
12. This reflux is again collected in round bottom flask and again it vaporizes due to continuous heating treatment.
13. This process now it's time to separate the solvent and oil mixture, which is obtained in round bottom flask.
14. This separation is done by a simple distillation.
15. Now oil and solvent is separated on the basis of their difference in boiling point.
16. As solvent Hexane/ Petroleum ether/Iso-propyl alcohol has low boiling point than oil, thus oil is obtained at bottom of flask and solvent is at a top.

With Enzymatic action
1 . First of all Nigella Sativa seed are grinded in a mixer into the powdered form(1.4mm)
2. Enzymes solution is prepared by adding 500mg. of α-amylase/ trypsin in 100ml of buffer and then 50 gm of crushed seeds are socked in the prepared solution of enzyme and they are placed for incubation in incubator for 2 hours at 37 ^{0}C.
3. Shake the solution after every 5-10min. for proper mixing of enzyme and prepared solution.
4. Then soaked material of Nigella Sativa are wrapped into the whatmann filter paper are weighed first before placing it in thimble.
5. The weight of powdered seeds is taken about 50gm.
6. Material in filter paper placed in extractor of soxhlet apparatus for leaching operation.
7. Bottom flask of soxhlet is filled with hexane ether around 500ml.
8 Then heating is done, till around 5-6 hours.
9. The main process begins when heating occurs; hexane vaporizes and goes in upward direction through side arm of thimble.
10. This vapors goes to the extractor and towards condenser in upward direction, the condenser is placed at the top of extractor.
11. Thus after condensation the liquid form is collected in the extractor where wrapped seeds are kept.
12. A liquid level goes on increasing such that wrapped seeds are dipped into the liquid and at certain point the liquid is refluxed through the reflux side.
13. This reflux is again collected in round bottom flask and again it vaporized due to continuous heating treatment.
14. This process, separate the solvent and oil mixture, which is obtained in round bottom flask.
15. This separation is done by a simple distillation.
16. Now oil and solvent is separated on the basis of their difference in boiling point.
17. As solvent hexane has low boiling point than oil, thus oil is obtained at bottom of flask and solvent is recovered at a top.

III. Result and Discussion

Oil content in most Nigella seeds investigated is higher than 30% and possibly up to 40%. A comparison of solvent-extracted Nigella seed oil yields, without use of enzymes and enzymatic extraction are reported in table for various solvents respectively
Following table shows the comparison of oil yield obtained by using different solvents.

Table 1 Results obtained by using different solvents

Observation Parameter	Run No. 1	Run No. 2	Run No. 3
Solvent	Hexane	IPA	PE
Amount of Solvent(ml)	500	500	500
Time(h)	2	2	2
Oil extracted(ml)	6.5	3.7	3.4
Solvent Recovery(ml)	360	340	350
% oil yield	34.66	19.73	18.13
% Solvent Recovery	72.00	68.00	70.00

From above Table 1 ,we observed that hexane gives a higher percentage yield than the other two solvents. So we kept hexane constant and used different enzymes in enzymatic extraction. The results obtained are given in following Table 2 .From these results we observed that Trypsin (enzyme-2) gives better result than α-amylase (enzyme-1).

Table+ 2 Results obtained by Enzymatic extraction

Observation Parameter	Run No. 4	Run No. 5
Enzyme used	α-amylase	Trypsin
Amount of Solvent(ml)	500	500
Time(h)	4	4
Oil extracted(ml)	7.1	12.3
Solvent Recovery(ml)	380	390
% oil yield	37.86	65.6
% Solvent Recovery	76.00	78.00

IV. CONCLUSION

Enzymatic Pretreatment give better yield than without enzymatic pretreatment in same operating condition. We use hexane as solvent which give more yield as compare to Petroleum ether and isopropyl alcohol. By enzymatic treatment the oil yield increase up to 50 to 55% by treating seeds with solvent for approximately 4hr at 37^0 C.

Experimentally it is observed that if we soaked Nigella Sativa seed for longer period then oil is dissolved in the enzymatic solution and then it is difficult to recover the oil. To minimize solvent losses extra condenser need to be equipped. From FTIR and Density testing it is cleared that extracted oil is Nigella Sativa oil.

V. ACKNOWLEDGMENT

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