

A rapid and pollutionless microwave assisted efficient extraction from seeds of *Nigella sativa*

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ABSTRACT

A simple, rapid and precise microwave-assisted extraction process was developed and optimized for preparing n-hexane extract from shade dried seeds of *Nigella sativa*. This newly developed process was compared with conventional soxhlet extraction. The major parameters were studied and showed effect on extraction efficiency including processing time, strength of radiation, moisture content, mesh size and irradiated with and solvent free conditions. The most favourable condition was obtained by using plant material of coarse-powdered for 10 min at 340 W with n-hexane solvent under moisture. Observation was suggested that no change in composition of constituents of both extracts; under microwave and conventional soxhlet extraction which examined by TLC. The extraction rate observed first order kinetics under microwave radiation. A new compound was isolated and identified as dotriaconta-2, 9, 25-triene from unsaponifiable matter of n-hexane extract of seeds on basis of spectral and literature studies. © 2016 Trade Science Inc. - INDIA

KEYWORDS

Nigella sativa;
Seeds;
Ranunculaceae;
N-Hexane extract;
Microwave and soxhlet
extraction;
Extraction rate constant;
Dotriaconta-2, 9, 25-triene.

INTRODUCTION

Nigella sativa L., (Ranunculaceae), seeds also known as black seed/ kalaungi, annual herbaceous spicy plants, has many uses in indigenous system of medicine. It has been used in many Middle Eastern countries as a natural remedy for 2000 yrs. The seeds are believed to have carminative, diuretic, lactogoge, stimulatory and diaphoretic properties and recommended in Ayurveda in the treatment of bronchial asthma, cough, fever and eczema^[1-2]. The various extract and isolated compounds from seeds have been shown wide spectrum biological activities including anti-inflammatory, antidiabetic, analgesic, antibacterial, antifungal and anti-helminthic^[3]. The effects

of the black cumin seeds have also been evaluated in clinical and animal studies^[4]. Various bioactive alkaloids viz. nigellamine, nigellicine were isolated from seeds^[5-10]. Thymoquinone and nigellone are main active phytoconstituents of volatile oil of its seeds which inhibited the synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats and showed cytotoxic, antibacterial and antifungal activities^[11]. Bioactive principle α -hederin had been isolated from seeds and reported in vitro anti tumor activity^[12].

The present work report on useful solvent extraction fast technique via microwave radiation for preparing n-hexane extract from seeds of *Nigella sativa* at optimum conditions which give an idea

about new cheaper and eco-friendly process than conventional soxhlet extraction, hence it was advanced in further phytochemical analysis. Analysis was reported a new compound from unsaponifiable matter of n-hexane extract from seeds and identified as dotriaconta-2, 9, 25-triene.

Conventional techniques like percolation, liquid solvent extraction with different solvents is the most currently applied techniques with time required several hrs or days^[13]. Therefore, microwave assisted extraction (MAE) is an alternative to conventional solid-liquid extraction method, especially in case of natural product field where phytochemical investigation and other activities required much time attention.

Ganzler et al in 1986, first reported use of microwave heating for organic extraction^[14].

In Microwave-assisted solvent extraction (MASE), Chemical compounds absorb microwave energy roughly in proportion to their dielectric constants; the higher the value of the dielectric constant^[15-17], the higher the level of absorption of microwave energy. Hence microwave radiation depends on the dielectric susceptibility of both solvent and matrix. Disruption of hydrogen bond, resulting from dipole moment of the molecules and migration of dissolved ions facilitate the penetration of solvent molecules in the matrix and allow the salvation of component to be extracted^[18].

In the non-microwave-absorbing solvent approach, sometimes called microwave-assisted process (MAP)^[19-20], the sample and solvent are placed in an open vessel and solvent does not become hot because it cannot absorb the microwave radiation, due to non polar nature. However, the sample sorbs the microwave radiation and can release the energy heated analytes into the surrounding cool liquid, chosen for its solubility characteristics. MAP process more successfully applied on extraction of essential oils from plant materials, lipids from fish and organochloropesticides from sediment samples^[21].

In MASE less solvent is used than in conventional soxhlet or liquid-liquid extractions. The extraction solvents can be selected based on their microwave-absorbing ability to that there is little or no heating, a great deal of heating, or anything in

between. In addition, the extraction solvent power for the analytes of choice can be important selection criteria. In the non-microwave-absorbing solvent extraction approach, for sample matrices that have little absorption of microwaves such as dehydrated or dry materials, the addition of a solvent of high dielectric constant such as water or methanol to the sample can aid in the extraction process by rehydrating the matrix.

EXPERIMENTAL

General : mps, uncorr, ¹H NMR was recorded on 300 MHz Varian XL spectrometer, ¹³C NMR spectra were recorded on Varian XL 75 MHz spectrometer, IR spectrum was recorded in KBr on Perkin Elmer-377 spectrometer, EIMS on Jeol - JMS D 300 mass spectrometer. The conical flask shaking method was carried out on alumina Gr. III and TLC on silica gel G. Spots were visualized by iodine vapour or charring with H₂SO₄ - vanillin spray. The seeds of *N. sativa* were collected from the nearby area of Ujjain city, identified from IEMPS, Vikram University, Ujjain.

Optimum Condition for extraction by microwave radiation

We have taken accurate weighed 2.0 g of shade dried seeds on analytical balance and mixed with 0.2 ml of water in 100 ml of beaker. The sample was irradiated by microwave energy without heating solvent and each set of cycle was irradiated by microwave radiation by varying the time from 5, 10, 15 and 20 min at three wattage 160 W, 320 W and 480 W. After then 10 ml of n-hexane was added and shake well about 10 min., then decanting the first leaching. This procedure is run in three cycles and last cycle is rinsed by 2 ml of solvent. Same condition is applied on all of the sets and observed optimum condition (TABLE 1).

Nature and shape of sample

In another set, we have taken three sets of whole seeds, coarse powdered seeds and germinated seeds. Each set was run at optimal condition (320W/10 min) and prepared hexane extract by microwave radia-

Full Paper

TABLE 1 : Without hexane in sample: Experimental condition: 2.0 g/ 0.4 ml Water, 3 cycle/ 10 ml hexane

Set No.	Wattage/ time duration	I 5 min.	II 10 min.	III 15 min.	IV 20 min.
1	160 W	0.032 g	0.040 g	0.040 g	0.040 g
2	320 W	0.040 g	0.046 g	0.040 g	0.040 g
3	480 W	0.035 g	0.038 g	0.037 g	0.036 g

TABLE 2 : Without hexane in sample: Experimental condition: 2.0 g / 0.4 ml Water, 320W/10 min., 3 cycle/ 10 ml hexane

S. No.	Whole seeds	Coarse powder	Germinated seeds	Coarse powder With hexane	Conventional Soxhlet ext. 10 hr.
1	0.041 g	0.060 g	0.072 g	0.2850 g	0.040 g
2	0.048 g	0.061 g	0.077 g	0.2790 g	0.044 g
3	0.049 g	0.059 g	0.075 g	0.2950 g	0.042 g

TABLE 3 : Observation table for rate of extraction: Optimum condition (2 g, 320 W/10 Min/10 ml hexane) irradiation/cycle

S.No.	Time (Min)	Dry extract in g V_t	Conc. of extract ($V_g - V_t$)	K
1	10	0.1009	0.3391	0.0260
2	20	0.2400	0.2000	0.0394
3	30	0.2800	0.1600	0.0337
4	40	0.3306	0.1094	0.0348
5	50	0.33082	0.1091	0.0278
6	60	0.3400	0.1000	0.0246
7	70	0.4000	0.004	0.0300
8	infinite	0.4400 (V_g)		

tion. The process was repeated in three cycles as mentioned above. Results suggested that extraction from coarse seeds (3.0 %) for n-hexane was more than whole seeds (2.5%). Germinated seeds showed better yield (3.8 %) than nongerminated in same condition (TABLE 2).

Extraction rate followed Ist order kinetic

Further 2.0g crushed seed sample containing moisture and 10 ml n-hexane are irradiated in three cycle/ 10 min at 320 W and observed that it gave good extraction yield (5.2%), hence sample with taking solvent gave good result than without solvent. The whole experiment was irradiated till complete extraction after definite interval of time. In experiment observed that rate of extraction followed first order kinetic, since rate constant observed constant value in each extraction (TABLE 3). Hence it was also proved that as increase the number of time irradiation increases rate of concentration of extract.

Saponification of n-hexane extract by standard diethyl ether method

The seeds (100g) were shade dried, cleaned, powdered and extracted with hexane in soxhlet extractor for 72 h. The extract was concentrated on a rotary evaporator to afford 4.0 ml oil. The oil was saponified by alcoholic potash method. Usual workup yielded 1.75 g unsaponifiable matter, which was checked by TLC (Hexane: Benzene: Ether, 4.5:4.5:1v/v). The unsaponifiable matter was also prepared by microwave radiation. The method consists of the microwave assisted treatment of 4g of oil with 40 ml of an ethanolic KOH solution inside a 200 ml closed reactor in which sample was irradiated for 3.0 min at 40% power level of an exit power of 650 W. After usual work carried out same as conventional method and yielded unsaponified matter^[25]. There is no change in composition of the components and their R_f values of the compounds obtained by conventional and MASE method while saving a considerable time.

Theory

The fatty material is converted into soap by

ethanolic alkali saponification, the solution was diluted with water and the unsaponifiable matter was extracted with diethyl ether.

Reagents

Ethanolic potassium hydroxide solution 1N, diethyl ether, potassium hydroxide solution 0.5 N, ethanol 95 % neutralized and freshly distilled.

Apparatus

R. B. flask (100 ml), fitted with a ground joint in the air reflux condenser. Separating funnels: two 250 ml.

Procedure

Approximately 4.0 gm oil (concentrated hexane extract of *N. sativa* seeds) was weighed into the 250 ml of R. B. flask with an accuracy of ± 0.01 gm and about 400 ml ethanolic potassium hydroxide solution (approximately, 1N) was added to it. Then the reflux condenser was fitted to the flask and the flask was heated on boiling water bath for two hr., with constant shaking from time to time. The content were transferred to a 500 ml separating funnel and the flask was first rinsed with 100 ml water and then with 100 ml diethyl ether. The water and diethyl ether were added to the contents of the separating funnel. Then the contents were shaken which were still slightly worm. The separating funnel was allowed to stand until the layers have been separated. Drained off ethanol water layer into the flask in which saponification has taken place and the diethyl ether layer was transferred to a second 500 ml separating funnel containing 40 ml water. The ethanol water layer was shaken twice more, as before each time with 100 ml diethyl ether and the three quantities diethyl ether solution were collected. This was washed twice with 40 ml water in separating funnel.

Then the diethyl ether solution was transferred to 500 ml R.B. flask. Most of the residual solvent was evaporated and the concentrated unsaponifiable matter was collected in beaker. In this way, all the oil was saponified and unsaponifiable matter was obtained as yellow solid.

Methylation of unsaponifiable matter

Sodium metal (0.2g) was added to freshly distilled and dry methanol (25mL) while stirring in an ice bath. When all the sodium metal was dissolved, unsaponifiable matter (4.5 mg) was added and left overnight at room temperature under nitrogen. Methanol was removed and yellow residue thus obtained, was diluted with water, acidified with dilute HCl and the organic layer was extracted with ether. It is washed several times till it attained neutral pH .

The resulting methyl esters of the both unsaponifiable matter of hexane extract were studied and were analyzed by CO-TLC of both. There was observed that no change in chemical composition of methyl esters.

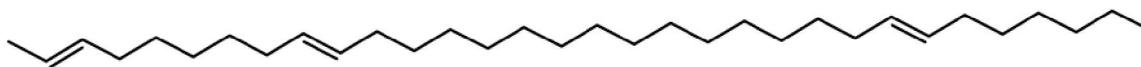
Hence we have obtained two type of unsaponifiable matter, one by conventional extraction and other by microwave extraction. Both gave comparable yield but conventional extraction gave result in 3-4 days while by microwave extraction we got results within a day, hence it was more efficient, less solvent consuming. Besides this new compound (1) was isolated from unsaponifiable matter of n-hexane extract of the plant seeds.

Dotriaconta-2, 9, 25-triene (1). White amorphous (32 mg): $C_{32}H_{60}$; m/z : 444; mp : 151-153 °C; R_f = 0.49 (benzene : ether: acetic acid, 9:1:1); IR ν_{max} (Smear on KBr, cm^{-1}): $\nu = 3072, 1667, 1523$ ($C=C$), 2927, 2855, 1465, 1215, 1410, 970, 758 and 722 ($C-H$); 1H -NMR (200MHz, $CDCl_3$, TMS): $\delta = 0.75$ (t, $^3J(H,H) = 6.0$ Hz, 6H; $2XCH_3$), 1.25 ppm (s, 48H, $24XCH_2$), 1.90 (br s, CH), 5.0, 4.9, 4.7 (t, 6H; 3-CH=CH); EI-MS: M^+ 444(3.9), 394(5.3), 393(3.6), 392(4.0), 350(2.1), 349(2.0), 254(17.1), 212(6.5), 198(2.3), 184(5.6), 170(4.9), 169(5.6), 156(5.5), 154(3.5), 152(2.5), 144(3.3), 140(2.6), 138(3.6), 128(19.6), 96(18.1), 84(43.1), 83(23.7), 82(10.8), 73(37.8), 72(31.1), 71(73.1), 56(100) and 43(60.6).

RESULT AND DISCUSSION

In the current research a study of extraction method from shade dried seeds of *N. sativa* in moisture with organic solvents and solvent free conditions were studied under microwave radiation and compared with conventional extraction method by

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Dotriacont-2, 9, 25-triene

TLC examination. The fatty acid methyl esters and unsaponifiable matter was examined by TLC and observed that there were no changes in their chemical composition of both type of extraction.

For this purpose, we have taken 2.0 g of whole seeds and mixed 0.2ml/g of water in a 100 ml of beaker. The sample of each set were irradiated under microwave energy without taking solvent and monitoring by varying the time and power of microwave radiation at definite interval of time in different sets. The optimum condition of extraction was observed at 320W /10min/cycle. The same conditions were applied on crushed seeds and germinated seeds. We observed that as increase the wattage hexane extract yield also increases up to a limit but at very high wattage the seeds were burned. Germinated seeds showed more and good yield than that of non germinated seeds and crushed seeds gave good yield than whole seeds.

Further in another set crushed seeds are irradiated under moisture with n-hexane solvent and observed that yield was increase in good extant. The concentration of extract increased after each irradiation at definite interval of time and observed that extraction rate followed first order kinetic.

Besides these, it was observed that R_f values and shape of spots of both extracts were no change. Both types of extracts were saponified by alkali potash method and free fatty acids were methylated^[22]. During all these processes, we observed by TLC there were no changes. Hence microwave assisted extraction was efficient, it completed within an hour and yield was also increased in good extent, with consumption of less amount of solvent in eco friend condition.

Further new aliphatic compound (1) was isolated from unsaponifiable matter of hexane extract of the plant by simple conical flask shaking method. The structure was established by spectroscopic and chemical means.

The mass spectrum showed the molecular ion peak at m/z 444 corresponding to the molecular formula $C_{32}H_{60}$. IR spectrum showed strong absorption

for olefinic bond (1667 cm^{-1}) and long chain aliphatic nature ($1096, 730-720\text{ cm}^{-1}$) of the molecule and absorption around 970 cm^{-1} indicated trans geometry of the olefinic bonds^[23-24].

In its ^1H NMR spectrum a triplet at δ 0.75 ($J = 7.5\text{ Hz}$) for six protons was due to the presence of terminal methyl groups. The triplet at δ 5.0, 4.9 and 4.7 each for two protons revealed the presence of three double bonds in the molecule^[20]. The methylene protons α to the double bond were resonated at δ 1.9 as a broad singlet and rests of the methylene proton was resonated at δ 1.25. The α - cleavage from the double bond followed the transfer of a hydrogen yielded fragments at m/z 374, 347, 333, 291, 179 and 85 revealed the position of double bonds at C-2, C-9 and C-25^[22].

Thus on the basis of above evidence the compound was identified as dotriacont-2, 9, 25-triene.

ACKNOWLEDGEMENT

Authors are thankful to UGC, New Delhi for the financial assistance and RSIC, CDRI, Lucknow for spectral analysis.

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