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Research Paper

# New Triterpene compound (Lup-20(29)-en-3β-3, 27-diol) isolate from extract of *Nigella sativa* (seeds)

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## ABSTRACT

One new pentacyclic triterpene compound named as lup-20(29)-en-3β-3, 27-diol isolate from hexane extract of *Nigella sativa* seeds. The compound structure was elucidated by spectroscopic IR, $^{1}H$ & $^{13}C$ NMR, EIMS analysis and other chemical evidences. This compound and their spectral data are reported first time hare.

**Keywords:** *Nigella sativa* (seeds); Hexane; Soxhlet extractor.

## INTRODUCTION

*Nigella sativa* (Family *Ranunculaceae)* commonly known "Kalaungi", is a nutritional plant and their seeds have medicinal importance and used as a natural remedy since ancient time. It is a spicy plant that bronchiodilatory, hypertensive, antibacterial and immune protecting activities (Hailat, et al., 1995). The seeds are commonly believed to have carminative, stimulatory and diaphoretic properties (Chopra, et al., 1995; El-Alfy, et al., 1975). The seeds contain an alkaloid like nigellidine (Atta-Ur-Rehman, et al., 1995), sterols like cholesterol, campestrol, stigmasterol, β-sitosterol, α-spinasterol (Salama, 1988), saponin (Ansar, et al., 1988), nigellone (Chakarvarti, 1993), nigellimine, nigellicine, nigellidine, α-spinasterol, saponin. The hexane extract of *N. sativa* seeds yielded two aliphatic compounds 16-triecosen-7-ol and 6-nanadecanone (Neelima, et al., 2000), new one steroid and two aliphatic esters (Mehta, et al., 2006).

Present work deals with the phytochemical investigation of the $C_6H_{14}$ extract of *N. sativa* seeds. *Nigella sativa* (*N. sativa*) seed has been an important nutritional flavoring agent and is aimed at summarizing the extremely valuable work done by various research scholars, studies carried out worldwide. Our data present *N. sativa* as a traditionally used herb with potent immunomodulatory, antibacterial etc.

Therefore we describe the isolation and structure elucidation of one new compound from unsaponifiable matter of hexane extract.

## MATERIALS AND METHODS

***Instrumentations*:** Melting points was uncorrected, $^{1}H$ NMR spectra recorded on 300 MHz Varian XL spectrometer and 400 MHz Brucker WM spectrometer, $^{13}C$ NMR spectra on a Varian XL 75 MHz spectrometer; IR spectra in KBr on a Perkin Elmer-

 

377 spectrometer, and EIMS on a Jeol-JMS D 300 mass spectrometer. The column chromatography was carried out on alumina Gr.III and TLC on silica gel G. Spots were visualized by iodine vapour or charring with $H_2SO_4$ vanillin spray.

***Plant Material:*** The seeds of *N. sativa* were collected from the nearby area of Ujjain city in the month of September year 2006, identified by School of studies in Botany,Vikram University, Ujjain

***Extraction:*** We have taken the seeds of *Nigella sativa*. The seeds (~16 kg) shade dried, cleaned, powdered and extracted with hexane in soxhlet extractor for 72 hours. The extract was concentrated by rotary evaporator under reduced pressure to afford oil (350ml). The oil was saponified by alcoholic potash method. Usual work up yielded (20g) unsaponifiable matter, which was separated by repeated column chromatography on alumina.

***Isolation of compound:*** The column was eluted by solvents of increasing polarity. This column afforded one compound with some impurity which on repeated crystallisation from $CHCl_3$.The fractions were collected in bulk and monitored by TLC. The hexane and benzene elute on repeated column chromatography yielded one compound in pure form designated as **NS-I**. Their structure was established by spectroscopic techniques: IR, $^1$H NMR, $^{13}$C NMR and Mass.

## RESULTS AND DISCUSSION

**NS-I:** $M^+$ 442, $C_{30}H_{50}O_2$, m.p. 134-135°C, isolated from hexane fraction of the column. It showed single spot on TLC using solvent system hexane: benzene (05:95 v/v).

**IR spectrum (λmax, cm$^{-1}$, KBr):** 3450, 3070, 1640 and 1390 cm$^{-1}$.

**$^1$H NMR (300 MHz, $CDCl_3$) δ:** δ 4.68, 4.58 (a pair of doublets –$CH_2$), δ 3.80, 3.50 (dd of -C=$CH_2$), δ 3.35 (m, –CH), δ 1.89 (s, $CH_3$ of vinyl group), δ 0.76, 0.79, 0.82, 0.89, 0.97(s, tertiary methyl group).

**$^{13}$C NMR (75 MHz, ) ppm:** 39.0, 41.1, 37.5, 42.2, 56.0 and 150.4 ppm (at $C_4$, $C_8$, $C_{10}$, $C_{14}$, $C_{17}$ and $C_{20}$), 78.9, 55.2, 50.3, 38.8, 48.7 and 47.7 ppm (at $C_3$, $C_5$, $C_9$, $C_{13}$, $C_{18}$ and $C_{19}$), 38.7, 25.2, 18.2, 34.2, 19.2, 27.0, 27.9, 34.2, 29.7, 37.3, 60.5 and 109.6 ppm (at $C_1$, $C_2$, $C_6$, $C_7$, $C_{11}$, $C_{12}$, $C_{15}$, $C_{16}$, $C_{21}$, $C_{22}$, $C_{27}$ and $C_{29}$) and 27.3, 15.3, 16.1, 14.7, 16.0 and 20.8 ppm (at $C_{23}$, $C_{24}$, $C_{25}$, $C_{26}$, $C_{28}$ and $C_{30}$).

**Mass Spectrum $M^+$ (m/z):** 442, 427, 391, 384, 302, 289, 279, 215, 203, 189, 167, 154, 136, 102, and 91. The mass spectrum showed $M^+$ at 442 and molecular formula $C_{30}H_{50}O_2$. (hexane: benzene). Its IR spectrum band in KBr showed the presence of hydroxyl group and unsaturation (at 3450, 1640 cm$^{-1}$). C-H stretching and bending vibration for gem dimethyl group (at 3070, 1385 cm$^{-1}$**).**

$^1$H NMR spectrum **(**300 MHz, $CDCl_3$, TMS,δ) has shown characteristic peaks for steroidal or terpenoidal molecule are follows carbinolic proton resonated at δ 3.35 as multiplet assigned to OH group present at C-3 position. The shielding of this signal indicating its α-orientation. The presence of a pair of doublet at δ 4.68 and 4.58 for two protons and a singlet at δ 1.89 for three protons indicates the presence of isopropylene group. The sharp singlets at δ 0.76, 0.79, 0.82, 0.89 and 0.97 each assigned to methyl attached at tertiary carbon atoms. The presence of doublets at δ 3.80 and 3.50 may be due to methyl alcohol attached at C-27 position.

$^{13}$C NMR spectrum showed the β-orientation of the C-3 hydroxyl group as the signal of carbinolic carbon appeared at 78.9 ppm. The signal at 60.5 ppm assigned to methylene carbon attached to hydroxyl group. The chemical shifts for the olefinic carbon atoms ($C_{20}$ and $C_{29}$) appeared at 150.4 and 109.6 ppm. The six methyl groups

 

appeared at 27.3, 15.3, 16.1, 14.7, 15.9, and 20.8 for $C_{23}$, $C_{24}$, $C_{25}$, $C_{26}$, $C_{28}$ and $C_{30}$ respectively. Thus the compound may be lupine series.

The position of hydroxyl group and unsaturation of olefinic bond was determined from its mass fragmentation pattern. The abundant fragments at m/z 427, 390, 307, 289, 215, 203, 189, 167, 154, 136, 102, 91 all are in agreement to the lupine series of compounds.

Seed extracts have antimicrobial activity. Seeds mixed with vinegar and honey to make the paste which is applied on insect's bites. A decoction of the seeds with other medicines is given to females for the delivery. *N. sativa* seed oil was found to be effective against gram positive and gram negative bacteria. The minimum inhibitory concentration was found against *Bacillus polymyxa* bacteria.

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## REFERENCE

Hailat, N., Bataineh, Z., Lafi, S., Rawcily, E., Aqel, M., Alkatib, M., Hanash, S., (1995): Effect of Nigella sativa volatile oil on Jorkat T cell leukemia polypeptides. *Int. J. Pharmacogn*, 33:16.

Chopra, R.N., Nayar, S.L., Chopra, I.C., (1995): *Glossary of Indian Medicinal Plants*, (CSIR Publication, New Delhi), pp. 134.

El-Alfy T.S., El-Fatatry H.M., Toama M.A., (1975): Isolation and structure assignement of an antimicrobial principle from the volatile oil of *Nigella sativa L. Pharmazie*, 30:109.

Atta-Ur-Rehman, Malik, S., Hasan, S.S., Choudhary, M.I., Chaq Z.N., Clardy, Z., (1995): Nigellidine a new indazole alkaloid from the seeds of *Nigella sativa*. *Tetrahedron Lett.*, 36:1993.

Salama R.B., (1988): Sterols in the seed oil of Nigella sativa. *Plant Med.*, 24:375.

Ansar A.B., Hassan, S., Kanne, L., Atta-Ur-Rehman, Wehler, T., (1988): Structural studies on a saponin isolated from *Nigella sativa. Phytochemistry*, 27:3977-3979.

Chakarvarti N., (1993): Inhibition of histamine release from mast cells by nigellone. *Ann. Allergy*, 70, 237.

Singh, N., Verma, M., Mehta, D., Mehta, B.K., (2005): Two new lipid constituents of *Nigella sativa* (seeds). *Ind. J. Chem.*, 44B:1742-1744.

Mehta B.K., Gupta M., Verma M., (2006): Steroid and aliphatic esters from the seeds of. *Nigella sativa*. *Ind. J. Chem.*, 45B:1567-1571.

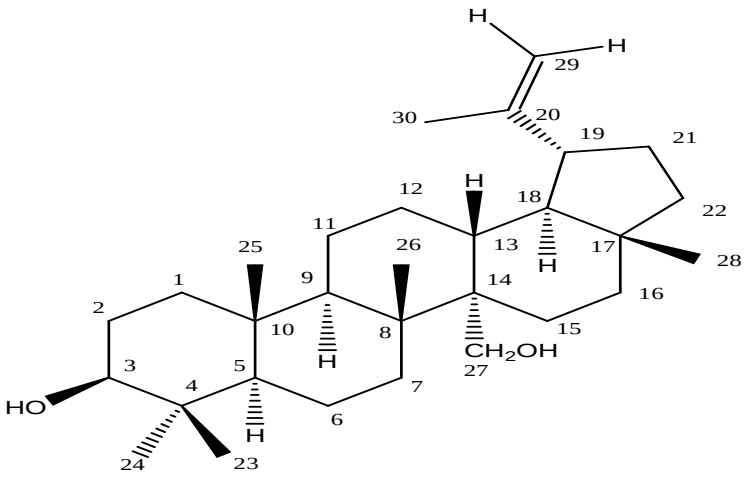


**Figure: NS-I; Structure of lup-20(29)-en-3β-3, 27-diol**

 