Phytochemical analysis of chloroform extract of leaves of *Skimmia laureola*

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**Abstract**- The air-dried and powdered leaves (3 kg) of *Skimmia laureola* were exhaustively defatted with light petroleum ether (60-80°C). The petroleum free mass extracted with 90% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water, which was washed with diethyl ether for several times and then partitioned with CHCl3:H2O:MeOH (6:4:4) in a separatory funnel. The chloroform layer was separated out and concentrated under reduced pressure to give CHCl3 extract (15g). The chloroform extract (10g) was subjected to repeated CC over Si-gel eluted with different proportions of n-hexane- CHCl3 and CHCl3:MeOH afforded β-stosterol (1), (E)-3’-(4-Hydroxyphenyl)-2’-propenoic methyl ester (2), (E)-3’-(4-Hydroxy-3-methoxyphenyl)-2’-propenoic methyl ester (3), Identified as bergapten (4), Identification of these compounds were made by the analysis of their chemical and spectral data.

**Key words:** *Skimmia laureola*, chloroform extract, coumarin, furanocoumarin.

**Introduction**

The genus *Skimmia* belongs to the family Rutaceae is a large genus of strongly scented unarmed shrubs, distributed chiefly in the shady moist localities of temperate and alpine region, up to 1500-3000m. It is distributed throughout the temperate Himalayases from Kashmir in the north to Mishmi and Khasia mountains in the south east1,2. The flowers are sweetly and leaves are strongly aromatic3. The leaves are often used as incense and burnt near small-pox patients for their supposed curative effects. The smoke produced by burning them is said to purify the air4 and are used in preparation of dhup and agarbatties. Skimmia species have been reported to possess antifungal, anti fertility, antiplatelete, and spasmylolytic, activity5-7. Phytochemical studies on *Skimia* species resulted in the isolation of flavonoids, terpenoids, iridoids, coumarins, alkaloids, and some fatty esters8-10. *S. laureola* is a strongly scented, evergreen, glabrous shrub, distributed in Northern India and China1. From *S. laureola* fatty ester, terpenoids and quinoline alkaloids have been isolated11-13. In the present study the phytochemical analysis of chloroform extract of air dried leaves of *S. laureola* was carried out using CC over Si-gel using various solvents afforded β-stosterol (1), (E)-3’-(4-
Hydroxyphenyl)-2'-propenoic methyl ester (2), (E)-3'-[4-Hydroxy-3-methoxyphenyl)-2'-propenoic methyl ester (3), and bergapten (4). Identification of these compounds was made by the analysis of their chemical and spectral data.

**Material and Method**

CC was carried out over silica gel (60-120 mesh, BDH) with gradient elution method using different solvent systems in the order of increasing polarity. TLC was conducted on Si-gel (E-Merck and BDH) coated on a thin glass plate (0.25 mm thickness containing 13% CaSO₄ as binder). Spots on TLC were detected by spraying with 5% H₂SO₄ followed by heating at 100°C, 5% methanolic KOH, Benedict’s reagent, iodine vapours, UV and alcoholic FeCl₃ solution. Preparative TLC was carried out on pre-coated reverse-phase TLC on Si-gel 60 HPTLC (Merck, 20 x 20 cm. and 0.25 mm thickness) developed with different proportion of CHCl₃:MeOH. PC was carried out on Whatman filter paper No. 1 using descending technique with n-BuOH-pyridine-H₂O (6:4:3) as solvent system and spots were detected by spraying with aniline hydrogen phthalate (AHP) followed by heating. M.Ps. were recorded in BOETIUS microscopic m.p. apparatus. UV-spectra (λ_max, nm) were recorded in MeOH on a SYSTRONIC spectrophotometer. IR-spectra (ν_max, cm⁻¹) were carried out on FT-IR-8100 Shimadzu spectrophotometer as KBr palettes. NMR spectra were recorded in BRUKER DRX-300 (300 MHz for ¹H and 75 MHz for ¹³C), BRUKER DRX-400 (400 MHz for ¹H and 100 MHz for ¹³C) spectrophotometer with CDCl₃ and Aetone-d₆ solvents. Chemical shifts are given in ppm scale with TMS as an internal standard.

**Plant Material**

The leaves of *S. laureola* were collected from Nachiketa Tal (at an altitude of 2450-2500m), District Uttarkashi, Uttarakhand, India (Garhwal Himalaya), in September 2017. The plant species was identified by Dr. Jai Laxmi Rawat Department of Botany, RCU Govt. PG College Uttarkashi, Uttarakhand. A voucher specimen (DOC 12/2009) was deposited in the Department of Botany, Govt. P.G. College, Uttarkashi and Uttarakhand, India.

**Extraction and Isolation**

The air-dried and powdered leaves (3 kg) of *S. laureola* were exhaustively defatted with light petroleum ether (60-80⁰). The petroleum free mass extracted with 90% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water, which was washed with diethyl ether for several times and then partitioned with CHCl₃:H₂O:MeOH (6:4:4) in a separatory funnel. The CHCl₃ layer was separated out and concentrated under reduced pressure to give CHCl₃ extract (15 g). The CHCl₃ extract (10g) was subjected to CC over Si-gel eluted with n-hexane-CHCl₃ (100:0→1:1) and then with CHCl₃:MeOH. The like fractions obtained on elution with n-hexane: CHCl₃ (6:4), (5:5) and (3:7) were mixed together and after evaporation of solvent afforded three fraction A, B, & C. Fraction A on CC over Si-gel eluted with n-hexane: CHCl₃ (3:7), afforded 3 (31 mg) and 4 (23 mg). Fraction B was subjected to CC over Si-gel eluted with n-hexane: CHCl₃ (3:7), afforded 4 (17 mg).
Results and Discussion

β-sitosterol: White amorphous solid
M.P.: 135-137°C
[α]D\textsuperscript{25} = -36° (c=0.1, CHCl\textsubscript{3})

(E)-3'-(4-Hydroxyphenyl)-2'-propenoic methyl ester (2): White amorphous solid
M.P.: 191-196°C
IR (v\textsubscript{max}\textsuperscript{KBr}): cm\textsuperscript{-1} 3405, 2858, 1705, 1608, 1326, 810 etc.
UV (λ\textsubscript{max}MeOH): nm 210, 311
\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}): \textit{δ} 7.74 (2H, brd, J = 8.6 Hz, H-2, 6), 6.80 (2H, brd, J = 8.6 Hz, H-3, 5), 6.21 (1H, d, J = 15.4 Hz, H-2'), 7.48 (1H, d, J = 15.4 Hz, H-3'), 3.77 (3H, s, OCH\textsubscript{3}) and 5.09 (1H, brs, OH);


UV (λ\textsubscript{max}MeOH): nm 217, 325

\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}): \textit{δ} 7.01 (1H, d, J = 1.9 Hz, H-2), 6.89 (1H, d, J = 8.0 Hz, H-5), 7.06 (1H, dd, J = 1.9, 8.0 Hz H-6), 6.28 (1H, d, J = 15.4 Hz H-2') 7.62 (1H, d, J = 15.4 Hz H-3'), 5.87 (1H, brs, -OH), 3.91 (3H, s, -OCH\textsubscript{3}) and 3.78 (3H, s, -OCH\textsubscript{3})

UV (λ\textsubscript{max}MeOH): nm 210, 260 and 310 nm
\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}): \textit{δ} 6.27 (1H, d, J=9.8 Hz, H-3), 8.14 (1H, d, J=9.8 Hz, H-4), 7.15 (1H, s, H-8), 7.59 (1H, d, J=2.5 Hz, H-2'), 7.02 (1H, d, J=2.5 Hz, H-3') and 4.27 (3H, s, OCH\textsubscript{3})

13C-NMR (100 MHz, CDCl\textsubscript{3}): \textit{δ} 160.33 (C-2), 112.59 (C-3), 139.56 (C-4), 149.74 (C-5), 113.96 (C-6), 158.02 (C-7), 93.78 (C-8), 152.66 (C-9), 106.44 (C-10), 145.02 (C-2'), 105.16 (C-3') and 60.24 (-OCH\textsubscript{3}).

13C-NMR (100 MHz, CDCl\textsubscript{3}): \textit{δ} 177.02 (C-1'), 147.03 (C-3), 148.21 (C-4), 145.02 (C-3'), 126.98 (C-1), 122.87 (C-2), 115.23 (C-2'), 114.68 (C-5), 109.26 (C-6), 56.17 (-OCH\textsubscript{3}) and 52.13 (-OCH\textsubscript{3}).

Figure-Isolated Compounds from \textit{S. laureola}
\(\beta\)-stosterol (1), was identified by Co-TLC and Co-MP with authentic sample\(^{14}\). Compound 2 showed strong absorption bands at 3405 for OH, 2858 for CH, 1705 for \(-\text{C}=\text{O}\), and 1608 cm\(^{-1}\) for \(-\text{C}=\text{C}\) in IR spectrum and two absorption band at 210, and 311 nm for \(\alpha, \beta\)-unsaturated carbonyl group in UV spectrum. The \(^1\)H-NMR spectrum displayed three proton singlet at \(\delta\) 3.77 corroborated with methoxy protons ester group. The two \(A_2B_2\)-type \(brd\) doublets (\(J = 8.6\) Hz) in the aromatic region at \(\delta\) 7.74 and 6.80, indicated presence of di-substituted phenyl ring and was further confirmed to be p-hydroxyphenyl by the \(^{13}\)C chemical shifts of carbon atom at \(\delta\) 130.09 (C-2, 6), 115.91 (C-3, 5), which was fairly corresponded with hydrogen carrying carbon of p-cresol\(^{15}\). Two olifinic protons were observed at \(\delta\) 6.21 (1H, d, \(J = 15.4\) Hz, H-2'), and 7.48 (1H, d, \(J = 15.4\) Hz, H-3') were assigned for H-2’ and H-3’ protons. The value of coupling constant (\(J = 15.4\) Hz) of olifinic protons indicated the trans-orientation of H-2’ and H-3’ protons. A broad singlet at \(\delta\) 5.09 indicated presence of OH group in the molecule. The \(^{13}\)C-NMR spectrum displayed eight carbon resonances (two of double intensity) indicated presence of nine carbon atoms in the molecule while DEPT spectrum displayed presence of one methyl, six methane (two of double intensity), and three quaternary carbon atoms. The presence of methoxy carbon at \(\delta\) 51.86, an ester carbon at \(\delta\) 168.14, olifinic carbons at 115.15 and 145.23 and other signals due benzene ring was displayed by \(^{13}\)C-NMR spectrum. On the basis of these spectral data compound 2 was identified as (E)-3'-(4-Hydroxyphenyl)-2'-propenoic methyl ester\(^{16}\).

The spectral data of 3 are similar to those of 2 except with additional methoxy group attached with benzene ring. The presence of three doublets, each for one proton at \(\delta\) 7.01 (1H, d, \(J = 1.9\) Hz, H-2), 6.89 (1H, d, \(J = 8.0\) Hz, H-5) and 7.06 (1H, dd, \(J = 1.9, 8.0\) Hz H-6) was assigned for H-2, H-5 and H-6 of benzene ring, respectively, indicated tri-substituted catechol type phenyl ring in the molecule\(^{17}\). The position of methoxy and OH group at C-3 and C-4 was determined by \(^{13}\)C-chemical shifts of C-3 at \(\delta\) 147.03 and C-4 at \(\delta\) 148.21. On the basis of these spectral evidences 3 was identified as (E)-3'(4-Hydroxy-3-methoxyphenyl)-2'-propenoic methyl ester\(^{16}\).

The IR spectrum of 4 exhibited strong absorption bands at 2923, for \(-\text{CH}, 1715\) for carbonyl carbon and 1612 cm\(^{-1}\) for double bond. The UV spectrum displayed characteristic absorption band for coumarin nucleus at 210, 260 and 310 nm\(^{18}\). The \(^1\)H-NMR spectrum displayed characteristic doublets at \(\delta\) 6.27 (1H, d, \(J=9.8\) Hz, H-3) and 8.14 (1H, d, \(J=9.8\) Hz, H-4), assignable for H-3 and H-4 protons of furanocoumarin nucleus\(^{18}\) which was confirmed by the \(^{13}\)C-chemical shift of C-3 at \(\delta\) 112.59 (C-3) and 139.56 (C-4). The two doublets (\(J = 2.5\)), for one proton each at \(\delta\) 7.59 and 7.02 were assigned for H-2’ and H-3’ protons of furan ring. \(^1\)H-NMR spectrum also displayed presence of methoxyl protons at \(\delta\) 4.27, which was confirmed by \(^{13}\)C-chemical shift of methoxy C-atom at \(\delta\) 60.24. The \(^{13}\)C-NMR spectrum also displayed presence of carbonyl carbon at \(\delta\) 160.33 and olifinic carbons at \(\delta\) 112.59 (C-3), 139.56 (C-4), and the carbon atoms of furan rings at \(\delta\) 145.02 (C-2’) and 105.16 (C-3’). The position of methoxy group was determined.
at C-5 position by the chemical shift of H-4 proton, which appeared downfield at δ 8.14 and presence of one proton singlet at δ 7.15, which was assigned for H-8 proton of phenyl ring of C-5 substituted furanocoumarins\textsuperscript{19,20}. On the basis of above discussed spectral data 4 was identified as bergapten\textsuperscript{21,22}.

**Conclusion**

The phytochemical studies on leaves of *S. laureola* led to the conclusion that the traditional claims for this plants in curing various diseases, may be due to the presence of β-sitosterol, (E)-3′-(4-Hydroxyphenyl)-2′-propenoic methyl ester, (E)-3′-(4-Hydroxy-3-methoxyphenyl)-2′-propenoic methyl ester, bergapten, and other metabolites which I am unable to isolate and identify. The extensive pharmacological work on isolated compounds may help in establishing mechanism of action, toxicity and side effects associated with the compounds as well as for generating new lead therapeutic molecules for treating various diseases.

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**Disclaimer Statement**

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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