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

[*1]D.P. Pandey and [2]Shikha Saxena

[1]Department of Chemistry, Govt. Degree College Dehradun Uttarakhand, India

[2]Department of Chemistry, DAV (PG) College, Dehradun, Uttarakhand, India

[*]E. mail: Pandeydp_123@rediffmail.com

DOI 10.51129/ujpah-2022-33-2(6)

Received – November 19, 2022
Revised – November 26, 2022
Accepted – November 28, 2022
Published – December 24, 2022

Abstract- The air-dried and powdered leaves (3 kg) of *Skimmia laureola* were exhaustively defatted with light petroleum ether (60-80^0). 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 $CHCl_3$:H_2O:MeOH (6:4:4) in a separatory funnel. The chloroform layer was separated out and concentrated under reduced pressure to give $CHCl_3$ extract (15g). The chloroform extract (10g) was subjected to repeated CC over Si-gel eluted with different proportions of n-hexane- $CHCl_3$ and $CHCl_3$: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 Himalayas from Kashmir in the north to Mishmi and Khasia mountains in the south east[1,2]. The flowers are sweetly and leaves are strongly aromatic[3]. 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 air[4] and are used in preparation of dhup and agarbatties. Skimmia species have been reported to possess antifungal, anti fertility, antiplatelete, and spasmolytic, activity[5-7]. Phytochemical studies on *Skimia* species resulted in the isolation of flavonods, terpenoids, iridoids, coumarins, alkaloids, and some fatty esters[8-10].

S. laureola is a strongly scented, evergreen, glabrous shrub, distributed in Northern India and China[1]. From *S.* laureola fatty ester, terpenoids and quinoline alkaloids have been isolated[11-13]. 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% $CaSO_4$ as binder). Spots on TLC were detected by spraying with 5% H_2SO_4 followed by heating at 100^0C, 5% methanolic KOH, Benedict's reagent, iodine vapours, UV and alcoholic $FeCl_3$ 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 $CHCl_3$:MeOH. PC was carried out on Whatman filter paper No. 1 using descending technique with n-BuOH-pyridine-H_2O (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 ^{1}H and 75 MHz for ^{13}C), BRUKER DRX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrophotometer with $CDCl_3$ and Aetone-d_6 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 ($60\text{-}80^0$). 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 $CHCl_3$:H_2O:MeOH (6:4:4) in a separatory funnel. The $CHCl_3$ layer was separated out and concentrated under reduced pressure to give $CHCl_3$ extract (15 g). The $CHCl_3$ extract (10g) was subjected to CC over Si-gel eluted with n-hexane- $CHCl_3$ ($100:0 \rightarrow 1:1$) and then with $CHCl_3$:MeOH. The like fractions obtained on elution with n-hexane: $CHCl_3$ (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: $CHCl_3$ (5:5), provide β-stosterol (1), and several other fractions. First few fractions were subjected to preparative TLC using n-hexane: $CHCl_3$ (3:7) solvent afforded (2) (31 mg) and (3) (23 mg). Fraction B was subjected to CC over Si-gel eluted with n-hexane: $CHCl_3$ (3:7), afforded 4 (17 mg).

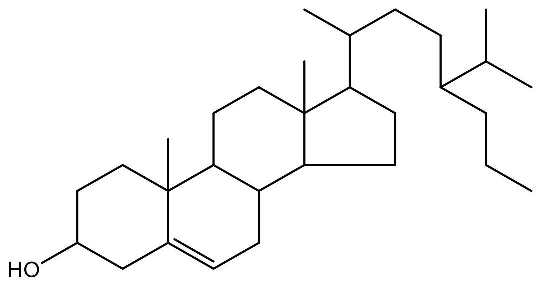
Results and Discussion

β-sitosterol: White amorphous solid
M.P. :135-137^{0}C
$[\alpha]^{25}_{D}$: -36^{0} (c=0.1, $CHCl_3$)

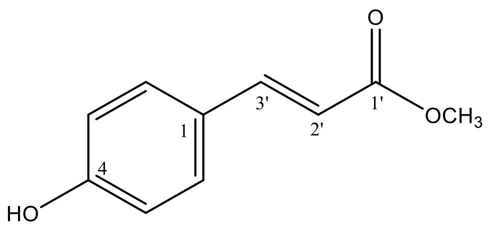
(E)-3'-(4-Hydroxyphenyl)-2'-propenoic methyl ester (2): White amorphous solid
M.P. :191-196^{0}C
IR (ν_{max}^{KBr}): cm^{-1} 3405, 2858, 1705, 1608, 1326, 810 etc.
UV (λ_{max}MeOH): nm 210, 311
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.74 (2H, brd, *J* = 8.6 Hz, H-2, 6), 6.80 (2H, brd, *J* = 8.6 Hz, H-3, 5), 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_3) and 5.09 (1H, br*s*, OH);
^{13}C-NMR (100 MHz, $CDCl_3$): δ 126.93 (C-1), 130.09 (C-2, 6), 157.97 (C-4), 115.91 (C-3, 5), 168.14 (C-1'), 115.15 (C-2'), 145.23 (C-3'), 51.86 (-OCH_3).

(E)-3'-(4-Hydroxy-3-methoxyphenyl)-2'-propenoic methyl ester (3): Amorphous solid,
M.P. : 191-196^{0}C
IR (ν_{max}^{KBr}): cm^{-1} 3718, 2915, 1709, 1608, 1597 etc.
UV (λ_{max}MeOH): nm 217, 325
^{1}H-NMR (400 MHz, $CDCl_3$): 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_3) and 3.78 (3H, s, -OCH_3)
^{13}C-NMR (100 MHz, $CDCl_3$): 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_3) and 52.13(-OCH_3).

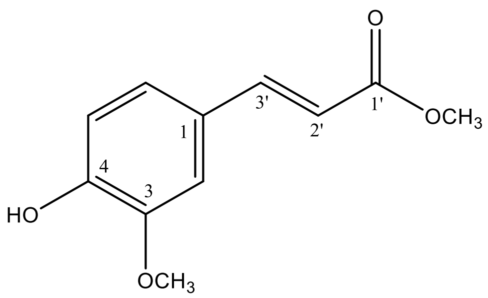
Bergapten (4): White crystalline solid
M.P.: 189-191^{0}C
IR (ν_{max}^{KBr}): cm^{-1} 2923, 1715, 1612, 1590 etc.
UV (λ_{max}MeOH): 210, 260 and 310 nm
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.27 (1H, d, *J*=9.8 Hz, H-3), 8.14 (1H, d, *J*=9.8 Hz, H-4), 7.15 (1H, s, H-8), 7.59 (1H, d, *J*=2.5 Hz, H-2'), 7.02 (1H, d, *J*=2.5 Hz, H-3') and 4.27 (3H, s, OCH_3)
^{13}C-NMR (100 MHz, $CDCl_3$): 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_3).



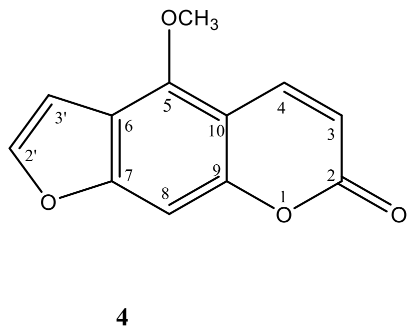
1



2



3



4

Figure-Isolated Compounds from *S. laureola*

β-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 -C=O, and 1608 cm^{-1} for –C=C- in IR spectrum and two absorption band at 210, and 311 nm for α, β-unsaturated carbonyl group in UV spectrum. The ^{1}H-NMR spectrum displayed three proton singlet at δ 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 δ 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 δ 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 δ 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 δ 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 δ 51.86, an ester carbon at δ 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 tese spectral data compound 2 was identified as (E)-3'-(4-Hydroxyphenyl)-2'-propenoic methyl[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 δ 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 δ 147.03 and C-4 at δ 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 –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 δ 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 δ 112.59 (C-3) and 139.56 (C-4). The two doublets (J = 2.5), for one proton each at δ 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 δ 4.27, which was confirmed by ^{13}C-chemical shift of methoxy C-atom at δ 60.24. The ^{13}C-NMR spectrum also displayed presence of carbonyl carbon at δ 160.33 and olifinic carbons at δ 112.59 (C-3), 139.56 (C-4), and the carbon atoms of furan rings at δ 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[19,20]. On the basis of above discussed spectral data 4 was identified as bergapten[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.

Acknowledgement

Author acknowledge RCU Govt. PG College Uttarkashi for the infrastructure, constant support and encouragement, Dr. Jai Laxmi Rawat Department of Botany, RCU Govt. PG College Uttarkashi for identification of plant species, and also thankful to the MRI Centre, AIIMS, New Delhi, India for the NMR facility.

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.

References

1. Gaur, R. D. *Flora of District Garhwal*, Trans Media, Srinagar Garhwal. 1999; pp. 381.
2. Chopra, R.N. and Chopra, I.C. Bimal Kumar Dhur of Academic Publisher Kolkata. *Indigenous Drugs of India*, 2006; 411-412.
3. Genders. R. St. Martin's Press, New York. *Scented Flora of the World*, 1977; 445.
4. Singh, G. and Kachroo. P. *Forest Flora of Srinagar.* Bishen Singh Mahendra Pal Singh, Dehradun, 1976.
5. Hmad, K.F. and Sultana, N.*J. Chem. Soc. Pakistan*, 2003; 25(4):328.
6. Tsai, I.L.; Lin, W.Y.T.; Che-Ming, I.T., Doong, S.L.; Huang, M.W.; Chen, Y.Ch. and Chen, I.S. *Planta Medica*, 2000; 66(7):618 .
7. Atta-Ur-Rahman; Asaad, K.; Nighat, S.; Ghayur, M.; Nabeel Mesaik, M.A.; Khan, M.R.; Gilani, A.H. and Choudhary, I. M. *Journal Enzy. Inhibi. Med. Chem.*, 2006; 21(6):703.
8. Kostova, I.; Simeonov, M.; Iossifova, T.; Tappe, R.; Pardeshi, N. and Budzikiewicz, H. *Phytochemistry*, 1996; 43(3):643-648
9. Razdan, T.K.; Harkar, S.; Qadri, B.; Qurishi, M.A. and Khuroo, M.A. *Phytochemistry*, 1988;27(6): 1890-1892
10. Boyd, D.R. and Grundon, M.F. *Tetrahedron Letters*, 1967; 8(28):2637-2638.
11. Sultana, N. and Khalid, A. *Nat Prod Res*., 2008; 22(1):37-47.
12. Atta-ur-Rahman; Khalid, A.; Sultana, N.; Ghayur, M.N.; Mesaik, M.A; Khan, M.R.; Gilani, A.H. and Choudhary, M.I. *J. Enzyme Inhib. Med. Chem.*, 2006; 21(6):703-10

13. Atta-Ur-Rahman; Sultana, N.; Khan, M.R. and Choudhary, M.I. *Nat Prod Lett.*, 2002; 16(5):305-13
14. Sati, O.P. and Pant, G. *Pharmazie*, 1983; 38:353
15. Pouchart, C.J. and Compbell, J.R. *The Aldrich Library of NMR Spectra*, 1979; IV, 144.
16. Sanro, T. and Masashi S. Holzfoschung, 1980;34(4), 131.
17. Porte, L.J.; Newmann, R.H.; Foo, L.Y.; Weng, H. and Hemingway, R.W. *J. Chem. Soc. Perkin Trans.*, 1982;1217.
18. Stek, W. and *Can. Journal of Chem.*, 1972; *50*;443.
19. Rajdan, T.K. and Kachro, H.B. *Tetrahedron*, 1964; 20:2431.
20. Nielsen, B. E. *Coumarins of Umbelliferous Plants.* The Royal Danish School of Pharmacy, *Copenhagen*, 1970.
21. Murphy, E. M.; Nahar, L.; Byres, L.M.; Shoeb, M.; Siakalima, M.; Rahman, M. M.; Gray, A.I. and Sarker S. D., *Biochem. Syst. Ecol.*, 2004; 32:203–207.
22. Muller, M.; Byres, L. M.; Jaspars, M.; Kumarasami, Y.; Middleton, M.; Nahar, L.; Shoeb, M. and Sarker S. D. *Acta. Pharm.*, 2004; 54:277–285.