Introduction

Berberis aristata DC var aristata (Berberidaceae), commonly known as Rasaut or Chitra in Hindi, Darhald in Urdu and Darvi in Sanskrit\(^1\), is an erect, glabrous, spinescent, deciduous shrub, 3-6m in height\(^2\) with obovate to elliptic, subacute to obtuse, entire or toothed leaves, yellow flowers in corymbose racemes and oblong-ovoid to ovoid, bright-red berries\(^3\) mostly found in Nepal, grown in Nilgiris at an altitude of 1000-2400m and temperate Himalayas at an altitude of 1000-3000m\(^4\).

Berberine, an alkaloid is the chief constituent of the roots and stem bark of Berberis aristata\(^5\). Other constituents include berbamine, aramoline, palmatine and oxycanthine. Phytoconstituents isolated from the flowers of Berberis aristata include E-caffeic acid, quercetin, chlorogenic acid, meratin and rutin\(^6\) while those from roots showed the presence of alkaloids, flavonoids, glycosides, saponins and sterols and the absence of terpenoids\(^7\). New protoberberine alkaloids-karachine and taxilamine have been isolated and characterized\(^8\).

Anti-carcinogenic\(^9\), anti-diarrhoeal\(^8\), anti-hepatotoxic\(^10\), anti-inflammatory\(^9\), anti-microbial\(^11\), anti-pyretic\(^12\), anti-hyperglycaemic\(^13\), anti-oxidant\(^14\), anti-malarial\(^15\), immunomodulatory\(^16\) and tuberculostatic activities\(^17\) have been studied on various parts of Berberis aristata.

Majority of studies have been performed on roots and stems of Berberis aristata, so the present study...
involves the phytochemical investigation of ethanolic extract of the heartwood of *Berberis aristata*.

**Materials and Methods**

All melting points were determined in Centigrade scale in one-end open capillary on Perfit melting point apparatus and are uncorrected. IR spectra were recorded on Perkin Elmer spectrum RX 1 model. $^1$H NMR and $^{13}$C-NMR spectra were scanned on Bruker DRX-300 NMR (300MHz) instrument in CDCl$_3$ and D$_2$O using Tetramethylsilane (TMS) and CDCl$_3$ as the internal standard and coupling constants ($J$ values) are expressed in hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70eV on a Jeol SX-102 (FAB) mass spectrometer equipped with direct inlet prob system. The m/z values of the more intense peaks are mentioned and the figures in bracket attached to each m/z values indicated relative intensities with respect to the base peak. The solvents used were of Qualigens LR grade. Silica gel (Qualigen 60-120 µm mesh) was used for column chromatography. TLC was performed on plates coated with silica gel G (Qualigen). Anhydrous sodium sulphate was used for drying all the solvents used during the research work.

**Plant Material**

The plant material was procured from AIMIL Pharmaceuticals, New Delhi. It was authenticated as *Berberis aristata* by Dr. M.P. Sharma, Reader, Department of Botany, Jamia Hamdard, New Delhi and a voucher specimen is preserved in the herbarium section of Department of Pharmacognosy, R.I.T., Greater Noida, Uttar Pradesh.

**Extraction**

The plant material (1.8kg) was air dried, crushed to smaller pieces, re-dried, coarsely powdered and was then exhaustively extracted with ethanol (95%) in a Soxhlet apparatus for 72 hours. The ethanolic extract was dried and dark brown mass, 50gm (2.77%) was obtained.

**Preparation of Slurry**

The concentrated extract of the drug was taken in a china dish and heated continuously on a water bath, gradually adding methanol in small portions with constant stirring till desired consistency was obtained. Weighed quantity of silica gel (60-120 mesh) was added slowly with mixing with a stainless steel spatula until a desired consistency was obtained. It was dried in air; the larger lumps were broken-up and finally passed through a sieve (No. 8) to get a uniform particle size.

**Packing of Column**

The lower end of a clean dry column was plugged with adsorbent cotton. The column was then half filled with petroleum ether. Silica gel was added in small proportions and allowed to settle down gently until the necessary length of the column was attained. All the air bubbles were allowed to escape by running the column blank thrice with solvent. The dried silicagel slurry of the extract was packed in the column and plugged with the adsorbent cotton and then eluted successively in the order of increasing polarity with different solvents. The development and elution of the column was carried out with successive series of solvents in various combinations, viz., petroleum ether, chloroform in petroleum ether (0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%) chloroform(100%), and methanol in chloroform. The fractions collected were subjected to thin layer chromatography. Chromatographically

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identical fractions were combined and concentrated.

**Isolation of Phytoconstituents**

Elution of the column with chloroform (100%) afforded a colourless amorphous powder which was recrystallized from chloroform:methanol (1:1), yield 125mg (0.25%);Rf: 0.62 (petroleum ether: chloroform: methanol, 5:2:2); m.p.: 137°C; IR \( \nu_{\text{max}} \) (KBr): 3465, 2955, 2845, 1640, 1475, 1365, 1210, 1105 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300MHz): \( \delta \) 6.50 (1H, d, \( J=5.5 \text{Hz}, \text{H-6} \)), 3.51 (1H, brs, \( w_{1/2}=16.5 \text{Hz}, \text{H-3a} \)), 1.01 (3H, brs, Me-19), 0.97 (3H, d, \( J=6.5 \text{Hz}, \text{Me-21} \)), 0.86 (3H, d, J=6.0 Hz, Me-29), 0.85 (3H, d, J=6.0 Hz, Me-27), 0.83 (3H, t, J=6.2 Hz, Me-26), 0.67 (3H, brs, Me-18); \(^{13}\)C NMR (CDCl\(_3\), 300MHz): 37.33 (C-1), 31.63 (C-138), 0.97 (3H, d, J=6.0 Hz, Me-29), 0.85 (3H, d, J=6.0 Hz, Me-27), 0.83 (3H, t, J=6.2 Hz, Me-26), 0.67 (3H, brs, Me-18); FAB MS \( m/z \) 55(C\(_{11}\)O, 10), 21.66 (C-11), 39.80 (C-12), 41.98 (C-13), 56.04 (C-14), 24.19 (C-15), 28.60(C-16), 55.41(C-17), 11.36 (C-18), 19.30 (C-19), 36.74(C-20), 18.75(C-21), 33.00(C-22), 25.73(C-23), 45.14(C-24), 29.15(C-25), 20.37(C-26), 19.30(C-27), 23.56(C-28), 11.03 (C-29); FAB MS \( m/z \) 414 (M\(^+\) (C\(_{29}\)H\(_{50}\)O) (22.3), 399 (21.6), 397 (33.1), 395 (35.2), 381 (16.5), 365 (11.3), 339 (12.6), 371 (17.8), 273 (12.5), 255 (13.2), 239 (9.6), 213 (15.6), 198 (17.9), 159 (39.7), 145 (52.6), 119(62.6),105 (100).

**Results**

The Compound, a phytosterol, was obtained as a colourless powder from chloroform eluants. It responded positively to Liebermann-Burchardt test for steroids. Its IR spectrum showed absorption band for hydroxyl group (3465 cm\(^{-1}\)) and unsaturation (1640 cm\(^{-1}\)). Its mass spectrum had a molecular ion peak at \( m/z \) 414 corresponding to a steroidal formula, C\(_{29}\)H\(_{50}\)O. It indicated five double bond equivalents; four of them were adjusted in the steroidal carbon skeleton and one in the olefinic linkage. The other diagnostic peaks were generated at \( m/z \) 399 (M-Me\(^+\)), 395(M-H\(_2\)O\(^+\), 273(M-side chain\(^+\)), 213(255-ring D fission \(^+\)), 255(273-H\(_2\)O\(^+\) and 198(213- Me\(^+\)). These fragments suggested that it was a C\(_{29}\) sterol possessing one double bond in the steroidal skeleton and a C\(_{10}\) saturated side chain. The ion fragments at \( m/z \) 55(C\(_{11}\)O, 10) \( \alpha \)-fission +, 69(C\(_{2,3}\)C\(_{5,10}\)C\(_{6,7}\) fission\(^+\)), 83(C\(_{2,3}\)C\(_{10}\)C\(_{7,8}\) fission\(^+\)), indicated that the hydroxyl group was located in ring A which was placed at C-3 on biogenetic grounds. The mass spectrum indicated the presence of an ethyl group in the side chain which was placed at C-24 on the basis of biological analogy as well as similarities in chemical shifts of the protons and carbons of the side chain with related compounds. Therefore, it had identical framework to that of \( \beta \)-sitosterol.

The \(^1\)H NMR spectrum exhibited a one proton doublet at \( \delta \) 5.30 (J=5.5 Hz) assigned to H-6 proton. A broad one-proton multiplet at \( \delta \) 3.51 with \( w_{1/2} = 16.5 \text{Hz} \) was ascribed to \( \alpha \)-oriented H-3 methine proton (axial) interacting with C-2 equitorial, C-2 axial and C-4 equitorial protons. Three doublets, integrating three protons each, at \( \delta \) 0.97 (J=6.0 Hz), 0.86 (J=6.0 Hz) and 0.83 (J=6.0 Hz) were due to C-21, C-26 and C-27 secondary methyls, respectively, and a three-proton triplet at \( \delta \) 0.82 (J=6.2 Hz) was ascribed to C-29 primary methyl protons. The remaining two tertiary C-18 and C-19 methyl signals appeared as singlets at \( \delta \) 0.67 and 1.01 respectively. The presence of all the methyl in the region \( \delta \) 0.67 - 1.01 suggested that these functionalities were attached to saturated carbons. The remaining methylene and methine protons resonated in the region \( \delta \) 2.28-1.03. Further evidence for the structure was provided by its \(^{13}\)C NMR spectral data, which showed the
presence of 29 carbon atoms in the molecule. The signals at δ 141.17, 119.94 and 69.51 were assigned to C-5, C-6 unsaturated and C-3 carbinol carbons, respectively. The β configuration of the ethyl group was confirmed by comparison of chemical shifts of carbons and protons of the side chain in the $^1$H and $^{13}$C spectra with β-sitosterol and other related sterols, e.g., stigmast-4-en-6β-ol-3-one and lawsonitol. The $^3$-29 resonance of 24-R configuration (δ 0.83) was more upshifted as compared to 24S resonance (0.86). Based on these evidences the structure of the compound has been formulated as stigmat-5-en-3β-ol.

**Discussions**

The result summarizes that Stigmast-5-en-3β-ol (β-Sitosterol), a phytosterol was isolated and characterized from ethanolic extract of the heartwood of *Berberis aristata*. The chemical structure was elucidated by means of various physical (solvent extraction, TLC, Column chromatography) and spectral techniques. β-sitosterol is beneficial in heart disease and high cholesterol (inhibits cholesterol absorption in the intestine). The liver function activity (GDP, GOP) can be improved with β-sitosterol, and this can reduce prostate cancer and colon-cancer cell growth. It exhibits anti-pyretic, anti-inflammatory, anti-ulcer, anti-arthritis, insulin releasing and oestrogenic effects. It is also used for boosting the immune system and shows beneficial effects in HIV/AIDS, tuberculosis, psoriasis, allergies, cervical cancer, fibromyalgia, systemic lupus erythematosus (SLE), asthma, hair loss, bronchitis, migraine headache, and chronic fatigue syndrome. In conclusion, β-sitosterol obtained from ethanolic extract of the heartwood of *Berberis aristata* in our investigation appear to be beneficial for various human ailments.

*Figure 1: Mass Fragmentation Pattern of stigmat-5-en-3β-ol.*

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Figure 2: IR spectra of stigmat-5-en-3\(\beta\)-ol

Figure 3: \(^1\)H NMR spectra of stigmat-5-en-3\(\beta\)-ol.

Figure 4: \(^{13}\)C NMR spectra of stigmat-5-en-3\(\beta\)-ol
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