Medicinal & Aromatic Plants
ISSN: 2167-0412

Katiyar et al., Med Aromat Plants 2015, 4:4
DOI: 10.4172/2167-0412.1000210

Research Article Open Access

N-Tetracosanyl Benzoate: A Novel Phytoconstituent of *Berberis aristata* DCs

Katiyar D[1]*, Dixit PK[2], Singh V[3], Gilani SJ[4] and Goel R[1]

[1]Department of Pharmacognosy, KIET School of Pharmacy, Ghaziabad, UP, India
[2]Department of Pharmacology, KIET School of Pharmacy, Ghaziabad, UP, India
[3]Department of Pharmacognosy, BBS Institute of Pharmacy, UP, India
[4]Department of Pharmaceutical Chemistry School of Pharmaceutical Sciences & Research, The Glocal University,Saharanpur, UP, India

Abstract

Numerous investigations have already been done on the roots and stems of *Berberis aristata* DC. var. aristata (Berberidaceae) and thus the present study involves the isolation and characterization of the ethanolic extract of the heartwood of *Berberis aristata*. The extraction of the drug (1.8 kg) was carried out in 95% ethanol using Soxhlet apparatus and the extract was concentrated. The slurry was prepared and packed in the column for isolating various phyto constituents using solvents of increasing polarity. The isolated phyto constituents were then characterized and their structures were elucidated using various spectral data analysis i.e., IR, 1HNMR, 13CNMR and +ve ion FAB MS. Elution of the column with chloroform gave the colourless crystals of a novel compound: n-tetracosanyl benzoate having Rf value 0.49 (Benzene: chloroform, 1:1) and m.p. 60°C-61°C.

Keywords: *Berberis aristata*, Heartwood; Soxhlet apparatus; Column; N-Tetracosanyl benzoate

Introduction

Daruharidra (in Sanskrit means 'the wood having yellow color') is one of the herbs mentioned in ancient scriptures of Ayurveda. Charaka has categorized Daruharidra as stanyasodhana (lactone purant), lekhana (a reducing herb), arsoghna (antihaemorrhoidal), kandughna (antipruritic), svedala (promotes sweating) and rasayana (rejuvenative). Susruta has mentioned it as ropana–a wound healer. Ayurvedic Pharmacopoeia of India correlates Daruharidra to *Berberis aristata* DC var. aristata (Berberidaceae), which is a spinous shrub native to northern Himalaya region. The plant is widely distributed from Himalayas to Srilanka, Bhutan and hilly areas of Nepal. It grows at the height of 2000-3000 m especially in Kumaon and Chammba region of Himachal Pradesh [1]. Phytochemical studies have shown that plant *B. aristata* contains berberine- the chief alkaloidal constituent, oxyberberine, berbamine, aromoline, a protoberberine alkaloid karachine, palmatine, oxycanthine, taxilamine, tannins, sugar and starch. E-caffeic acid, quercetin, chlorogenic acid, meratin, rutin have been isolated from the flowers of B. aristata [2]. Its various parts have been found to be beneficial as anti-inflammatory [3,4], anti-microbial [5-7], anti- tumor [8-10], anti-diabetic [11,12], antidiarrhoeal [13], anti-hepatotoxic [14], anti-pyretic [15], anti-oxidant [16] and anti-osteoporotic agent [17]. It has also shown to be effective in ear infections [1] and skin ageing [18].

Materials and Methods

The melting point of the compound was determined on Centigrade scale in one-end open capillary using Perfit melting point apparatus. IR spectra were recorded on Perkin Elmer spectrum RX 1 model. 1H and 13C-NMR spectra were scanned on Bruker DRX-300 NMR (300 MHz) instrument in $CDCl_3$ and D_2O using Tetramethylsilane (TMS) and $CDCl_3$ as the internal standard. The coupling constants (J values) are expressed in hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70 eV on a Jeol SX-102 (FAB) mass spectrometer equipped with direct inlet probe system. The m/z values of the more intense peaks are mentioned and the figures in brackets attached to each m/z values indicate relative intensities with respect to the base peak. The solvents used were of Qualigens LR grade. Silica gel (Qualigens 60-120 μm mesh) was used for column chromatography. TLC was performed on plates coated with silica gel G (Qualigens). Anhydrous sodium sulphate was used for drying all the solvents used during the research work.

Experimental

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, Ram-Eesh Institute of Technology, Greater Noida, and Uttar Pradesh. The plant material (1.8 kg) was air dried crushed to smaller pieces, coarsely powdered and was then exhaustively extracted with ethanol (95%) in a Soxhlet Apparatus for 72 hr. The ethanolic extract was dried and dark brown mass, 50 gm (2.77%) was obtained. 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 by mixing with a stainless steel spatula to obtain the desired consistency. 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. 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 silica gel slurry of the extract was packed in

***Corresponding author:** Katiyar D, Department of Pharmacognosy, KIET School of Pharmacy, Ghaziabad, UP, India, Tel: +91 8860132632; Fax: 01232-27978; E-mail: katiyar_deepti@yahoo.co.in

Received August 05, 2015; **Accepted** September 18, 2015; **Published** September 21, 2015

Citation: Katiyar D, Dixit PK, Singh V, Gilani SJ, Goel R (2015) N-Tetracosanyl Benzoate: A Novel Phytoconstituent of *Berberis aristata* DC. Med Aromat Plants 4: 210. doi:10.4172/2167-0412.1000210

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 identical fractions were combined and concentrated. Elution of the column with chloroform gave colourless crystals of a compound recrystallized from methanol: chloroform (1:1), yield 85 mg (0.17%).

Rf: 0.49 (benzene: chloroform, 1:1); **m.p.:** 600°C - 610°C; **IR υmax (KBr):** 2963, 2930, 2869, 1725, 1629, 1493, 1277, 1215, 1080, 970, 909, 756cm^{-1} ; ^{1}**HNMR(CDCl$_3$)**: δ 7.55 (2H, m, H$^{-2'}$, H$^{-6'}$), 7.36 (2H, m, H$^{-3'}$, H$^{-5'}$), 7.15 (1H, m, H$^{-4'}$), 4.27 (1H, d, J=11.6 Hz, H2 – 1a), 4.23 (1H, d, J=11.6 Hz, H2 – 1b), 1.57 (2H, m, CH2), 1.33 (14H, brs, 7 × CH_2), 6 1.29 (20H, brs, 10 × CH_2), 1.23 (8H, brs, 4 × CH_2), 1.12 (2H, brs, CH_2), 0.88 (3H, t, J=6.3 Hz, Me-24); +ve ion FAB MS m/z (rel. int.): 458[M]+ ($C_{31}H_{54}O_2$)(73.1), 443(14.2), 387(8.9), 345(12.6), 331(12.9), 317(11.2), 303(13.5), 289(13.7), 219(22.3), 205(18.6), 191(91.4), 135(20.3), 121(26.2), 105(71.6).

Results and Discussion

The Compound (Figure 1) was obtained as a colourless crystalline mass from chloroform eluents. Its IR spectrum (Figure 2) showed characteristic absorption bands for ester group at 1725 cm^{-1}, aromatic ring at 1629, 970 cm^{-1} and a long aliphatic chain at 756 cm^{-1}. The 1HNMR spectrum (Figure 3) exhibited signals for aromatic protons. The mass spectrum (Figure 4) displayed a molecular ion peak at m/z 458 corresponding to the benzyl ester, $C_{31}H_{54}O_2$. The ion fragments (Figure 5) arising at m/z 105(C_6H_5CO)+ and 121(C_6H_5COO)+ indicated that benzoyl function was present in the molecule. The ion peaks generating at m/z 135($C_6H_5COOCH_2$)+, 205($C_6H_5COO(CH_2)_6$)+, 219($C_6H_5COO(CH_2)_7$)+, 289($C_6H_5COO(CH_2)_{12}$)+, 303($C_6H_5COO(CH_2)_{13}$)+, 317($C_6H_5COO(CH_2)_{14}$)+, 331($C_6H_5COO(CH_2)_{15}$)+, 345($C_6H_5COO(CH_2)_{16}$)+, 387($C_6H_5COO(CH_2)_{19}$)+ and 443($C_6H_5COO(CH_2)_{23}$)+ supported the presence of the aromatic ring at one of the terminal carbon of the aliphatic chain. The ^{1}HNMR spectrum (Figure 3) exhibited signals for aromatic protons as multiplets at δ 7.55 (H-2', H-4'). Two one-proton doublets at δ 4.27 (J=11.6Hz) and 4.23 (J=11.6Hz) were ascribed to oxygenated methylene protons H2- 1'. A three-proton triplet at δ 0.88 (J=6.3Hz) was accounted to C-24 primary methyl protons. The remaining methyl protons resonate between δ 1.57-1.12. Alkaline hydrolysis of the compound yielded benzoic acid. On the basis of foregoing discussion the structure of the compound has been formulated as n-tetracosanyl benzoate.

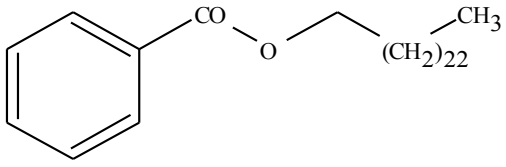

Figure 1: Structure of n-Tetracosanyl Benzoate.

Conclusion

A novel phytoconstituent "n-tetracosanyl benzoate" 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. B. aristata has been found to be beneficial as anti-inflammatory, anti-microbial, anti-tumor, anti-diabetic, anti-diarrheal, anti- hepatotoxic, anti-pyretic, anti-oxidant, anti-osteoporotic agent etc. due its diverse phyto constituents. So, n-tetracosanyl benzoate might also be an active constituent possessing some pharmacological activity. This compound can be useful object for some future research work.

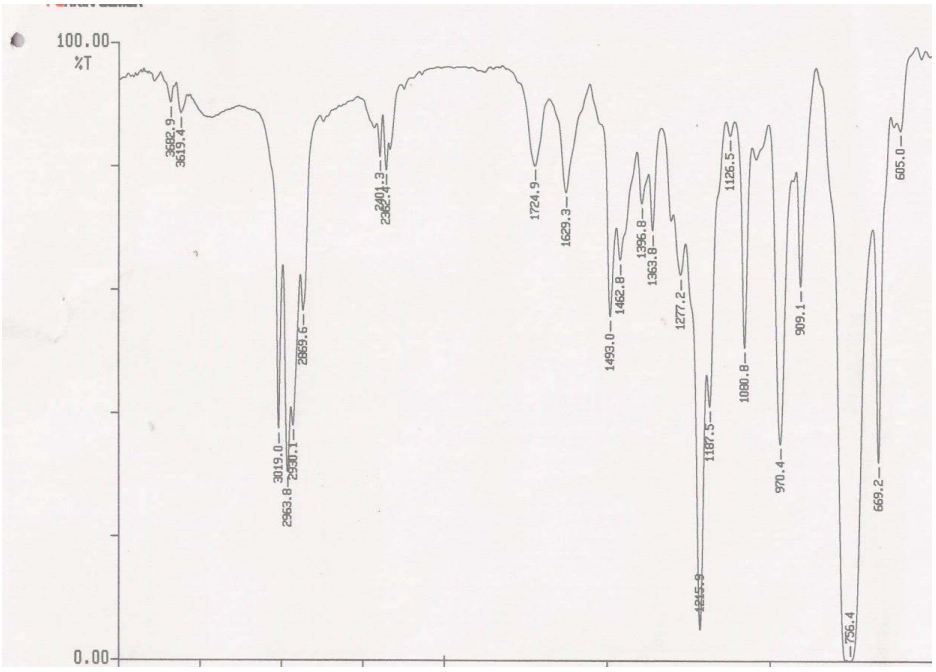

Figure 2: IR spectra of compound n-Tetracosanyl Benzoate.

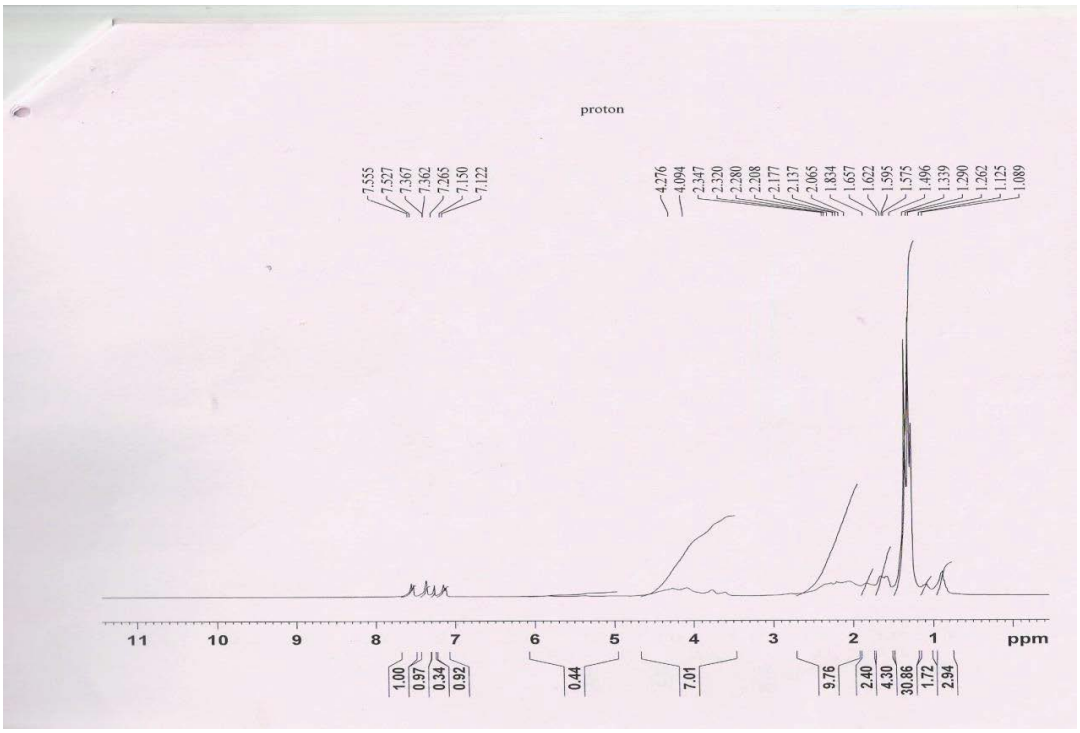

Figure 3: ^{1}H NMR spectra of compound n-Tetracosanyl Benzoate.

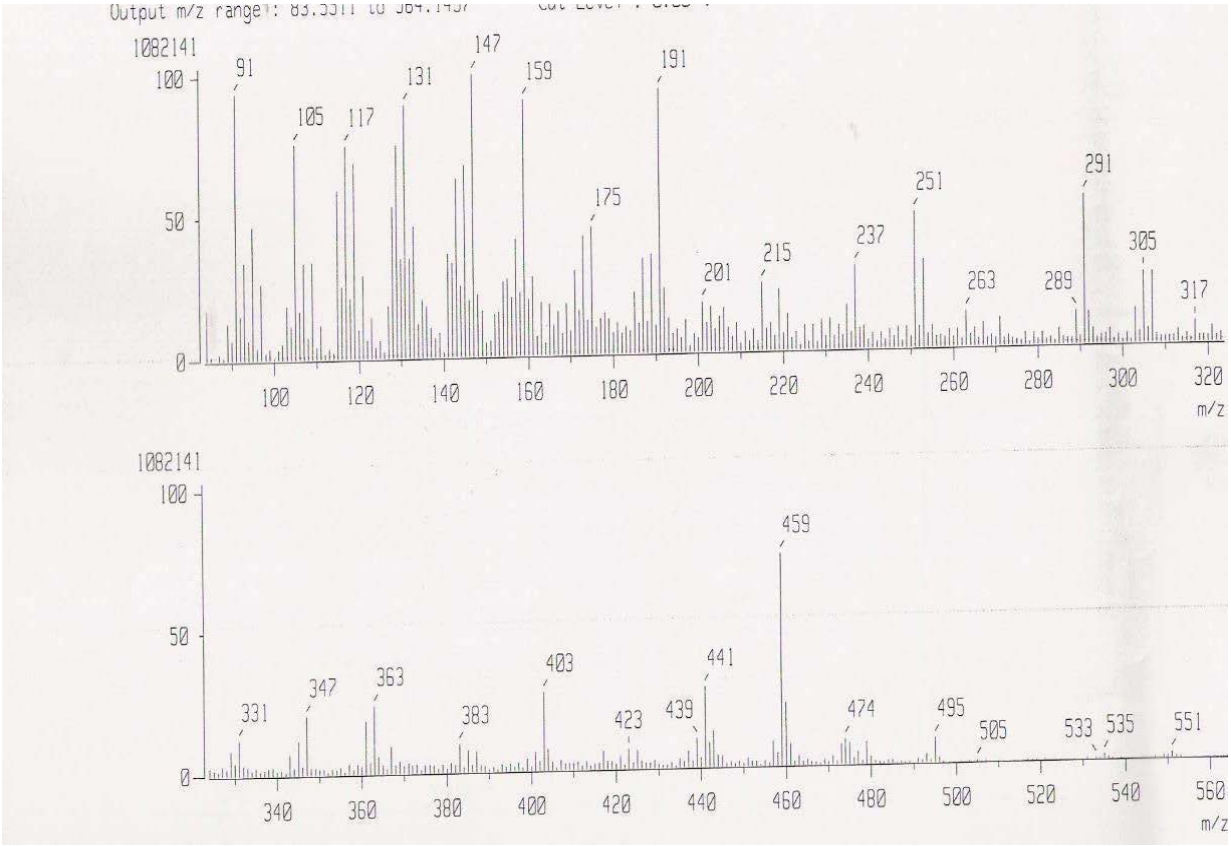

Figure 4: Mass spectra of compound n-Tetracosanyl Benzoate.-

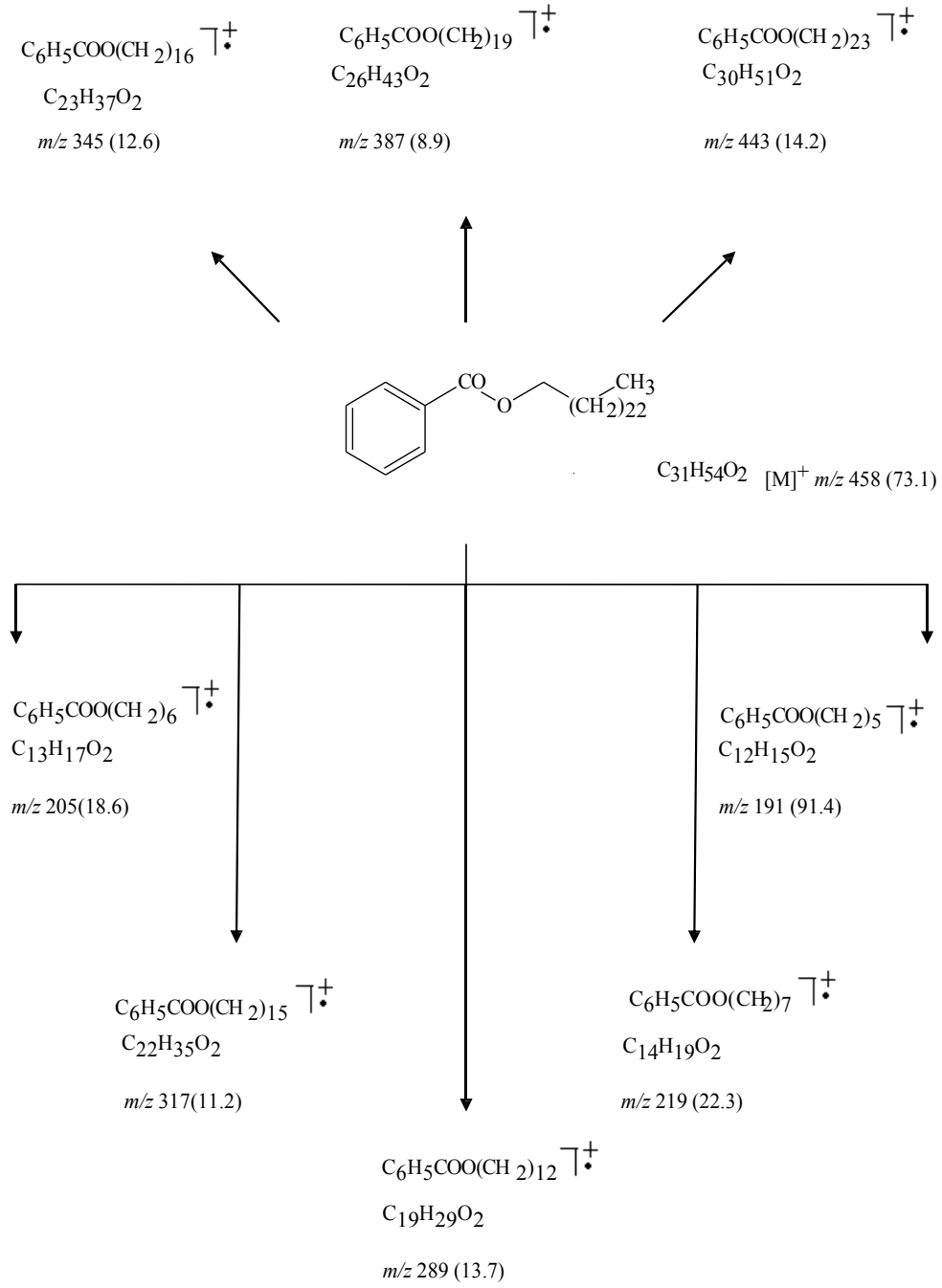

Figure 5: Mass Fragmentation Pattern of n-tetracosanyl benzoate.

Acknowledgments

The authors are thankful to the Principal, KIET School of Pharmacy for motivation. Authors are also thankful to CDRI, Lucknow for spectral analysis.

Conflict of Interest

None

References

1. Sharma C, Aneja KR and Kasera R (2011) Screening of Berberis aristata Dc. For Antimicrobial Potential Against the Pathogens Causing Ear Infection. International Journal of Pharmacology 7: 536-541.
2. Jha NK (2004) Berberis aristata: Daruharidra: Indian Barberry. Phytopharmaceuticals 5: 3-11.
3. Shahid M, Rahim T, Shahzad A, Latif TA, Fatma T, et al.(2009) Ethnobotanical Studies on Berberis aristata DC. Root Extracts. African Journal of Biotechnology 8: 55-563.
4. Sekhar S, Karmakar R, Ramchandra KK, Ramachandrappa SN, Prakash HS (2012) Potential Anti-inflammatory Bioactives from Medicinal Plants of Western Ghats, India. Pharmacognosy Communications 2: 2-12.
5. Rajasekharan RA, Pant J (2008) The Genus Berberis Linn.: A Review. Pharmacognosy Review 2: 369-385.

6. Singh B, Srivastava JS, Khosa RL, Singh UP (2001) Individual and combined effects of berberine and santonin on spore germination of some fungi. Folia Microbiol (Praha) 46: 137-142.

7. Sharma K, Bairwa R, Chauhan N, Shrivastava B, Saini NK (2011) Berberis aristata: A review. International Journal of Research in Ayurveda & Pharmacy 2: 383-388.

8. Mazumder PM, Das S, Das MK (2011) Cytotoxic Activity of Methanolic Extracts of Berbersis aristata Dc and Hemidesmus indicus R. Br. In MCF7 cell Line. Journal of Current Pharmaceutical Research 01: 12-15.

9. Das S, Das MK, Mazumdar PM, Basu SP (2009) Cytotoxicity Activity of Methanolic Extract of Berberis aristata DC on Colon Cancer. Global Journal of Pharmacology 3: 137-140.

10. Porwal KP, Kumar K, Jain N, Pathak KA, Jain P (2010) Evaluation of Antitumour Activity of Berberis aristata DC. Indian Drugs 47: 17-20.

11. Singh J1, Kakkar P (2009) Antihyperglycemic and antioxidant effect of Berberis aristata root extract and its role in regulating carbohydrate metabolism in diabetic rats. J Ethnopharmacol 123: 22-26.

12. Gupta JK, Mishra P, Rani A, Mazumder PM (2010) Blood Glucose Lowering Potential of Stem Bark of Berberis aristata DC in Alloxan-Induced Diabetic Rats. Iranian Journal of Pharmacology and Therapeutics 9: 21-24.

13. Akhtar MS, Sajid SM, Akhtar MS (2008) Hypoglycemic Effect of Berberis aristata Root, its Aqueous and Methanolic Extracts in Normal and Alloxan-Induced Rabbits. Pharmacology Online 2: 845-856.

14. Janbaz KH, Gilani AH (2000) Studies on preventive and curative effects of berberine on chemical-induced hepatotoxicity in rodents. Fitoterapia 71: 25-33.

15. Yesilada E, Kupeli E (2002) Berberis crataegina DC. root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats. See comment in PubMed Commons below J Ethnopharmacol 79: 237-248.

16. Ovas D, Reena L, Sikander D (2014) HPLC, Antioxidant and Antibacterial Activities of Methanolic Extract of Berberis aristata Stem. International journal of Science and Research 3: 1137-1141.

17. Yogesh HS, Chandrashekhar VM, Katti HR, Ganapaty S, Raghavendra HL, et al. (2011) Anti-osteoporotic activity of aqueous-methanol extract of Berberis aristata in ovariectomized rats. See comment in PubMed Commons below J Ethnopharmacol 134: 334-338.

18. Kim S, Kim Y, Kim JE, Cho KH, Chung JH (2008) Berberine inhibits TPA-induced MMP-9 and IL-6 expression in normal human keratinocytes. See comment in PubMed Commons below Phytomedicine 15: 340-347.