IJPSR (2012), Vol. 3, Issue 04 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 November, 2011; received in revised form 14 January, 2012; accepted 28 March, 2012

ISOLATION OF β-SITOSTEROL & STIGMASTEROL AS ACTIVE IMMUNOMODULATORY CONSTITUENTS FROM FRUITS OF *SOLANUM XANTHOCARPUM* (SOLANACEAE)

S. Khanam* and R. Sultana

Department of Pharmacognosy, Al Ameen College of pharmacy, Hosur Road, Bangalore-560027, Karnataka, India

Keywords:
Solanum xanthocarpum, Phytochemical, β-sitosterol, Stigmasterol

Correspondence to Author:

S. Khanam

Department of Pharmacognosy, Al Ameen College of Pharmacy, Hosur Road, Bangalore-560027, Karnataka, India

ABSTRACT

General phytochemical screening of the aqueous extract of fruits of *Solanum xanthocarpum* (Solanaceae) revealed the presence of steroids, terpenes, phenolic compounds, saponins, fatty acids, alkaloids. Fractionation of aqueous extract and activity guided isolation resulted in isolation of two white crystalline powder which were subjected to physical, chemical and spectral identification using IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS. On the basis of the spectral data analysis and chemical reactions, the compounds have been established as β-sitosterol and Stigmasterol.

INTRODUCTION: *Solanum xanthocarpum* belonging to family Solanaceae commonly known as Indian night shade or yellow berried night shade is a perennial herbaceous weed. Different parts of this plant have been used traditionally for curing various ailments such as fever, cough, asthma and diabetes in Indian traditional medicines [1]. The hot aqueous extract of dried fruits is used for treating cough, fever and heart diseases [2]. The plant is extensively studied for the various pharmacological activities like antiasthmatic [3], hepatoprotective [4], cardiovascular [5], hypoglycemic [6], antiulcer [7] and other properties.

Scientific evidence in favor of the traditional use of the fruits of *Solanum xanthocarpum* for the treatment of diabetes mellitus has been reported [8]. The total extracts and steroidal saponins of *Solanum xanthocarpum*, have been reported to exhibit potent antistress-adaptogenic effects [9].

To prove traditional claim of this plant preliminary immunomodulatory evaluation of aqueous extract of fruits of *Solanum xanthocarpum* on cyclophosphamide induced immunosuppression was carried out and the results indicated good protection by showing increase in all the hematological parameters [10]. In continuation of the work, it was proposed to isolate active immunomodulating agents from the extract. The fruits are reported to contain several steroidal alkaloids like solanacarpine [11], solanacarpidine, solancarpine, solasodine, solasonine [12] and solamargine [13]. Other constituents like caffeic acid [14] coumarins like aesculetin and aesculin [15], steroids carpesterol, diosgenin, campesterol, daucosterol and triterpenes like cycloartanol and cycloartenol are also reported from the fruits [16].

In this paper, we report the isolation and characterization of bioactive principles namely β-sitosterol and stigmasterol from aqueous extracts of fruits of *Solanum xanthocarpum*.

MATERIAL AND METHODS:

Plant material. Fruits of the plant, *Solanum xanthocarpum* were collected from APRC Lab Chennai and authentication no APRC/78/22/08-09. The sample was shade dried and powdered to #22.

Extraction: Powdered plant material was defatted with petroleum ether (60-80^{o}C) in a soxhelet extractor. The marc was dried and refluxed with water for 8 hrs. The aqueous extract was filtered and concentrated using rotary vacuum evaporator and the dried extract was stored in an air tight container.

Chromatographic Separation:

TLC: The aqueous extract of fruits was subjected to thin layer chromatography using silica gel as stationary phase and petroleum ether: methanol (1:1) and petroleum ether: chloroform: methanol (5:2:1) as mobile phase. The chromatograms when developed yielded seven and eight spots respectively that showed zones for steroidal nucleus with Liebermann – Buchard visualizing reagent.

Isolation: Column chromatography of fruit aqueous extract (10g) was conducted using silica gel (Mesh 100-200) by wet packing method. The column was run using petroleum ether, ethyl acetate, methanol and water successive by gradient elution technique. TLC was used to monitor the eluates. A total of 200 eluates were collected. Similar fractions were pooled together to yield fifteen fractions. Eluates **A**, and **G** were worked upon to yield **Sx5**, and **Sx9** respectively.

Sx5 and **Sx9** yielded single spots when subjected to TLC using several solvent systems including petroleum ether: ethyl acetate (70:30), petroleum ether: methylene chloride (50:20) and Petroleum ether: Chloroform: Methanol (60:30:10) and it showed it to be homogenous compound.

Sx5 a white amorphous powder (8.3mg) with a melting point (139^{o}C) was subjected to thin layer chromatography using various solvent systems such as petroleum ether: chloroform: methanol (5:3:1), methylene chloride: petroleum ether (50:50) indicated it to be homogenous compound. **Sx5** was further subjected to IR, ^{1}HNMR, ^{13}CNMR and LCMS to ascertain the chemical structure.

Sx9 a white crystalline powder (9mg) with a melting point (137^{o}C) was subjected to TLC using various solvent systems such as petroleum ether: chloroform: methanol (5:3:1), methylene chloride: petroleum ether (50:50) indicated it to be homogenous compound. **Sx9** was further subjected to ^{1}HNMR, ^{13}CNMR and LCMS to ascertain the chemical structure.

The structures were simulated using ACD/NMR program to obtain the chemical shifts of both proton and carbon.

Phytochemical analysis (Salkowski's test and Lieberman-Burchard test) of both the compounds confirms its steroidal nature.

Spectroscopic Characterization: Different spectroscopic methods were used to elucidate the structure of isolated compounds. Among the spectroscopic techniques IR, ^{1}H-NMR, ^{13}C-NMR and LCMS were carried out. The infra red spectrum was recorded on FTIR (model Shimadzu 8700), ^{1}H-NMR and ^{13}C-NMR spectra were recorded using $CDCl_3$ as solvent on Bruker Advance II 400 NMR spectrometer and LCMS spectra were recorded at high resolution on a mass spectrometer (model Shimadzu) at Sophisticated Instrumentation centre and the data are given in m/z values.

Characterization of Sx5 and Sx9: The exact molecular mass for the Sx5 and Sx9 was found to be 414.7 and 412 respectively. Based on this the proposed molecular formula of the compounds could be tentatively: $C_{29}H_{50}O$ and $C_{29}H_{48}O$. From ^{13}CNMR and ^{1}HNMR the number of C and H were found to be near to the formula $C_{29}H_{50}O$ and $C_{29}H_{48}O$. Since, the compounds give positive test for steroids so all of the other structures other than steroids were rejected.

Based upon the functional group analysis it was found that the nature of oxygen was hydroxyl, also supported by IR spectroscopy. This implies presence of one double bond in the structure. So, the steroids with other functional groups were rejected. Also on considering the nature of oxygen as hydroxyl and presence of one double bond, the general formula for the compounds were C_nH_{2n-6}.

Therefore, they must be tetracyclic compounds. Based on the melting point and other related data (IR, NMR and Mass) the structure of the isolated compounds Sx5 and Sx9 were proposed as (**fig. 1**);

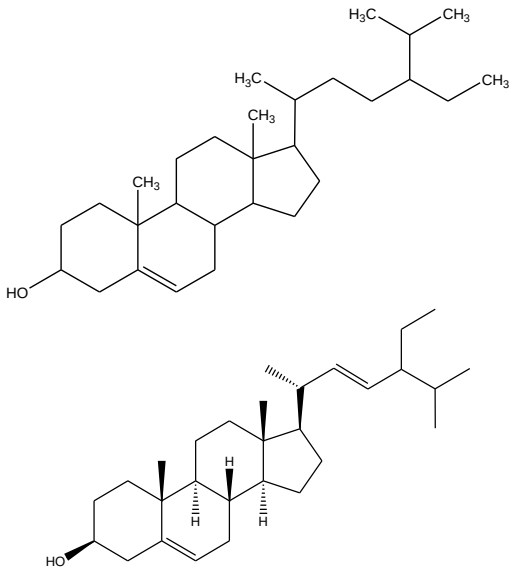

FIG. 1: Sx5 and Sx9

The compound **Sx5** is a white amorphous powder, m.p. 137^{o}C. IR absorptions bands appeared at 3549.99 cm-1 (OH), 2935.73 cm-1 (CH_2), 2867.38 cm-1 (CH), 1637.63 cm-1 (C=C), 1063.34cm-1 (C-O). Mass spectra of this compound suggested that its molecular mass is 414 (M.F. $C_{29}H_{50}O$) having characteristic fragments observed at m/z: 414, 396, 381, 329, 303, 289, 273, 255, 231, 213, 199, 173, 159, 119, 95, 81, 69. NMR spectrum of this compound resembled data published in previous studies [16-17].

This compound is having six methyl, eleven methylene and three quaternary carbons with a hydroxyl group. The carbons of alkenes conjugated are at 140.78 ppm (C5) and 121.72 ppm (C6) which was confirmed from the ^{13}CNMR. The above IR, ^{1}HNMR, ^{13}CNMR, LCMS spectal data and a comparison of the ^{13}CNMR signal with those described in the literatures [18-20] showed the structure of **Sx5** to be the β-Sitosterol.

The compound **Sx9** is a white crystalline substance with a melting point of 139^{o}C. IR absorptions bands appeared at 3384cm^{-1} (OH), 3218 cm^{-1} (cyclic olefinic –HC= CH- str), 3025 (=CH str) and 2868 cm^{-1} assigned to C-H str. Other absorption frequencies include 1665 cm^{-1} as a result of C=C absorption, however, this band is weak. 1462 cm^{-1} is a bending frequency for cyclic (CH2)n and 1382 cm^{-1} for –CH2 (CH3)2γ. The absorption frequency at 1332 cm^{-1} can be attributed to OH def. and at 1046 cm^{-1} signifies cycloalkane. These absorption frequencies resemble the absorption frequencies observed for Stigmasterol [20].

The Proton NMR has revealed the existence of signals for olefinic proton at 5.358 (br.,s.), Angular methyl proton at 0.68 (s), 0.699 (s) and 1.01 (s) corresponding to C_{18} and C_{19} proton respectively. The ^{13}CNMR has shown recognizable signals 140.8 and 121.7ppm, which are assigned C5 and C6 double bonds respectively as in Δ5 spirostene [19]. The δ value at 71.0 ppm is due to C3 β- hydroxyl group [20]. The signals at δ 19.4 and 11.9 ppm corresponds to angular carbon atom (C_{18} and C_{19} respectively). The above IR, ^{1}HNMR, ^{13}CNMR, LCMS spectal data and a comparison of the ^{13}CNMR signal with those described in the literatures [20-23] showed the structure of **Sx9** to be the Stigmasterol.

The structures were simulated using ACD/NMR program to obtain the chemical shifts of both proton and carbon. On comparison the experimental data matched with the simulated data which supports the proposed structure of this compound.

RESULTS AND DISCUSSION: *Solanum xanthocarpum* is widely recognized in Ayurvedic system of Indian medicine for treatment of respiratory problems. It has been reported as immunomodulatory agent. Preliminary immunomodulatory screening indicated the protective effect of the aqueous extract of fruits of *Solanum xanthocarpum* against Cyclophosphamide induced immunosuppression in mice. The study affirmed that aqueous extract of the fruits of *Solanum xanthocarpum* is effective immunomodulatory agent.

The extract potentiated the non- specific immune response by increasing the haematological parameter and neutrophil adhesion test, which may attributed to different phytoconstituents. Hence, to isolate the phytoconstituent it was subjected to fractionation and characterizations of isolated compounds.

Two compounds Sx5 and Sx9 showing positive tests for steroids and alcohols were isolated. The **Sx5** is white amorphous powder like substance with melting point 137^{o}C. Whereas, the **Sx9** is white crystalline substance with melting point 139^{o}C on subjection to IR spectroscopic analysis, the observed absorption frequencies resemble the absorption frequencies observed for β- sitosterol and Stigmasterol. The proton NMR showed the proton environment resembles the protons of β- sitosterol and Stigmasterol.

The ^{13}C-NMR has shown recognizable signals of β sitosterol and Stigmasterol.

The weak molecular ions were given at m/z 414 and 412.7. The molecular weight and fragmentation pattern indicate that the isolated compounds are β-sitosterol and stigmasterol.

The above I.R., ^{1}HNMR, ^{13}C-NMR and LCMS spectral data and their comparison with those described in the literatures showed the structure of isolated compounds are to be the β-sitosterol and stigmasterol [24].

CONCLUSION: β- sitosterol and Stigmasterol isolated from the aqueous extract of fruits of *Solanum xanthocarpum* these compounds may be the responsible for immunomodulatory activity.

ACKNOWLEDGEMENT: Authors are thankful to the Principal and Manager of Al-Ameen College of Pharmacy for providing the necessary facility to carry out the research work. One of the authors, Rokeya Sultana is thankful to IPA, for providing financial support.

REFERENCE:

1. Anonymous, The Wealth of India, *Solanum xanthocarpum*, Supplement series Raw Materials, Part-1st, A Dictionary of Indian Raw Materials and Industrial Products, Council of Scientific Industrial Research (New Delhi), 393.
2. Parmar S, Gangwal A and Sheth N. *Solanum xanthocarpum* (Yellow Berried Night Shade): A review, Der Pharmacia Lettre, 2010, 2(4): 373-383.
3. Parmar S, Gangwal A and Sheth N. Evaluation of antiasthmatic activity of a polyherbal formulation containing four plant extracts. J of Cur. Pharma Res. 2010; 2(1): 40-44.
4. Gupta AK, Ganguly P, Majumder UK, Ghosal S. Hepatoprotective and antioxidant effects of total extracts and stereoidal saponins of *Solanum xanthocarpum* and *Solanum nigrum* in paracetamol induced hepatotoxicity in rats. Pharmacologyonline 2009; 1(27) :757-768.
5. Samuel GY, Henry W. Comparative Cardiotonic activity of *Solanum xanthocarpum* with digoxin on isolated frog heart, IJPI's J of Pharmacog and Herbal Formul, 2011 ;1(5) :1-6.
6. Kar DM, Maharana L, Pattnaik S, Dash GK. Studies on hypoglycaemic activity of *S.Xanthocarpum* fruit extract in rats. J of Ethnopharmacol. 2006; 108: 251–256.
7. Yogendr B, Vijay J, Kuldeep G. Pharmacological evaluation of *Solanum Surattense* leaves for antiulcer activity, J of Pharm Res, 2008, 1(2):253- 259.
8. Gupta S, Mal M, Bhattacharya P. Evaluation of hyperglycemia potential of *S.Xanthocarpum* fruit in normal and Streptozin induced diabetic rats. *Eur.bull.Drug res*. 13, 51,2005,55.
9. Kumar AG, Gangulya P, Majumdera UK, Ghosalb S. Adaptogenic effects of total extracts and steroidal saponins of *Solanum xanthocarpum* and *Solanum nigrum.* J of Pharm Res. 2009, 2(8),1249-1254.
10. R Sultana, S Khanam and K Devi. Evaluation of Immunomodulatory activity of *Solanum xanthocarpum* fruits aqueous extract, Der Pharmacia Lettre, 2011, 3(1): 247-253.
11. Gupta MP, Dutt S, Chemical examination of the seed of *Solanum xanthocarpum* II. Constituents, J.Indian.Chem.Soc, 1938; 15: 95-100.
12. Siddiqui S, Faizi S, Shaheen B. Studies in the chemical constituents of the fresh berries of *Solanum xanthocarpum,* J of Chem society Pak. ,1983; 5(2): 99-122.
13. Tupkari SV, SAoji AN and Deshmukh VK, Phytochemical study of *Solanum xanthocarpum*. Planta medica, 1972; 22(2): 184-187.
14. Kumar A G, Ganguly P, Majumder UK, Ghosal S. Adaptogenic effects of total extracts and steroidal saponins of *Solanum xanthocarpum* and *Solanum nigrum*. J of Pharm Res. 2009, 2(8), 1249-1254.
15. Kusano G, Beisler J, Sato Y. Steroidal constituents of *Solanum xanthocarpum,* Phytochem: 1973, 12(2): 397-401.
16. Sato Y, Lantham JR. The isolation of diosgenin from *Solanum xanthocarpum*, J of America Chem Society; 1953:75, 60- 67.
17. Agarwal PK., Jain DC, Gupta RK., and Thakur RS. Carbon -13 NMR spectroscopy of steroidal sapogenins and steroidal saponins. Phytochem. 1985; 24: 2476-2496.
18. Agrawal, P.K., Jain, D.C, Gupta, R.K, and Thakur, R.S. "Carbon -13 NMR spectroscopy of steroidal sapgenins and steroidal saponins" Phytochem. Res., 1985; 24(11): 2476-2496.
19. Farlane MC. Application of Nuclear Magnetic resonance spectroscopy. in: Bentley KW., Kirby GW., Technique of chemistry vol. IV Elucidation of organic structures by physical and chemical methods 2nd Ed. Wiley interscience. 1972: 225-322.
20. Habib MR, Nikkon F, Rahman ME, and Karim MR. Isolation of stigmasterol and beta sitosterol from methanolic extract of root of bark of *Calotropis gigantean* (Linn). Pak. J. Biol. Sci. 2007; 10: 4174-4176.
21. Klaus Biemann. "Mass spectrometry-organic chemical Applications". McGraw-Hill Book Co. Pp 46-361"Annual report on NMR spectrorscopy" 1962; 8 : 199-226
22. Gamze Kokdil, Gulacti Topac, Ahmet C. Coren and Wolfgang Voelter Steroids and Terpenoids from *Ajuga relicta*: Z Naturforsch. 2002; 57: 957-960.
23. Smith, W.B "Carbon-13NMR Spectroscopy of Steroids" Annual reports on NMR spectrorscopy" Academic Press inc. London 1978; 8: 199-226.
24. Pateh UU, Haruna AK, Garba M, Iliya I, Sule IM, Abubakar MS, and Ambi AA. Isolation of stigmasterol, β-sitosterol and 2-Hydroxyhexadecanoic acid methyl ester from the rhizomes of Stylochiton Lancifolius Pyer and Kotchy (Aeaceae). Nigerian J of Pharm. Sci. 2009; 7(1): 19-25.

****