Research Article

Anjoo Kamboj et al. /BioMedRx 2013,1(3),235-238

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Isolation and Characterization of Bioactive Compounds from the Petroleum Ether Extracts of Leaves of *Xanthium Strumarium* Linn.

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Received on:17-12-2012; Revised on: 19-01-2013; Accepted on:10-02-2013

ABSTRACT

Objective: *Xanthium strumarium* Linn. belonging to the family compositae, commonly known as 'burweed' and chotagokhru in hindi contains a number of phytoconstituents such as alkaloids, phytosterols, glycosides, bioflavonoids, phenols, terpenoids and sugar. The objective of the present study was to isolate and characterize phytoconstituents from the petroleum ether extract of leaves of *Xanthium strumarium* Linn. **Methods:** Petroleum ether extract was saponified with alcoholic KOH and then subjected to column chromatography and eluted with different solvents of increasing polarity, composed of hexane, chloroform, ethyl acetate to isolate the constituents. Isolated compounds were purified by PTLC, CC and recrystallization method. The structure of the isolated compounds was established on the basis of spectroscopic evidences (IR, UV, ^{1}HNMR, ^{13}CNMR, MS). **Results:** Phytosterol such as stigmasterol, ß-sitosterol and two long chain alcohols pentatriacontan-1-ol and tritriacontanol isolated from the petroleum ether extract of leaves of the plant. **Conclusions:** *Xanthium strumarium* contains stigmasterol, ß-sitosterol, pentatriacontan-1-ol and tritriacontanol which are responsible for various pharmacological activities of the plant.

Keywords: *Xanthium strumarium*, stigamasterol, ß-sitosterol, compositae, phytosterols, tritriacontanol.

INTRODUCTION

Xanthium strumarium L. (Family: Compositae) a medicinal plant commonly found as a weed, is widely distributed in North America, Brazil, China, Malaysia and hotter parts of India. Extract of the whole plant, especially leaves, stems, roots , fruits & seeds have been applied traditional medicine for treatment of leucoderma, poisonous bites of insects, epilepsy, salivation, long- standing cases of malaria, rheumatism, tuberculosis, allergic rhinitis, sinitis, urticaria, constipation, diarrhea, leprosy, lumbgo, bacterial and fungal infection. Most of the pharmacological effects can be explained by the constituents like sesquiterpene lactones, glycoside, phenols, polysterols present in all plant parts. The main objective of the present study was to isolate and characterize the bioactive principles from the petroleum ether extract of *Xanthium strumarium* leaves. [1-3]

MATERIALS & METHODS

Collection, Authentication and Preparation of Plant material

Xanthium strumarium L. were collected from Landran, Mohali (Punjab) in month of October. The plant was originally authenticated by Dr. H. B. Singh, Chief Scientist and Head, Raw Materials Herbarium and Museum (RHMF), NISCAIR, New Delhi. A herbarium sample of this plant is preserved in the department.

The plant parts was separated manually and dried under shade, coarsely powdered, sieved, weighed and stored in airtight container and subsequently referred as powdered drug and was used for the extraction.

Extraction and Isolation

Powdered leaves of *Xanthium strumarium* was defatted with petroleum ether (60^0-80^0C) in a soxhlet apparatus. The solvent was recovered under pressure to obtained dark green mass. The petroleum ether extract of leaves of the plant was saponified using 1M alcoholic KOH, to remove fatty material and then subsequently picked up in petroleum ether and the solvent was evaporated to yield unsaponified matter. This fraction contains lesser number of components than the unsaponified extract [4].

Chromatographic Separation

A small quantity of unsaponified matter was dissolved in the petroleum ether and this solution was spotted on TLC plates using precoated aluminium with silica gel 60 F_{254}. Then the TLC plates were run in specific solvent system and viewed individually under UV light and also (5%) sulphuric acid in methanol reagent. Through several

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pilot experiments it was found that the compounds of unsaponifiable fraction were separated by the solvent system of chloroform and ethanol in the proportion of 9.8: 0.2. The chromatograms when developed in iodine chamber yielded six to seven spots respectively and three spots appears reddish brown soon turns to purple or violet indicate zones for steroidal nucleus. Column chromatography of petroleum ether extract was conducted using silica gel (mesh 60-120) that was packed using wet packing method in hexane. The column was run with using hexane and ethyl acetate by gradient elution technique. TLC was used to monitor the eluates. 68 fractions each of 25ml were collected. Similar fractions were pooled together and concentrated. Further purification is carried out using preparative TLC. Spots were identified, scraped and elutes using petroleum ether and chloroform as a solvents. Fraction 8-15 (hexane: ethyl acetate; 95:5) were pooled together, concentrated and purified as compound A, fraction 16-20 (hexane: ethyl acetate; 90:10) were pooled together, concentrated and purified as compound B and fractions 30-50 (hexane: ethyl acetate; 85:15) were pooled together, concentrated and two compounds were isolated named as compound C and D. finally purification is carried out by recrystallization method [4-6].

Finally eluates yielded a single spot when subjected to TLC using several solvents systems including chloroform: ethanol (9.8:0.2), chloroform: ethyl acetate (9.8: 0.2), hexane. These four compounds were further subjected to IR, ^{1}H NMR (400MHz), ^{13}C-NMR (100MHz) and MS to ascertain the chemical structure.

Test for Alcohol

4g of cerric ammonium nitrate was dissolved in 10ml of 2N HNO_3 on mild heating. A few crystals of isolated compounds were dissolved in0.5ml of dioxane. The solution was added to 0.5ml of cerric ammonium nitrate reagent and diluted to 1 ml with dioxane and shaken well. The developed yellow to red color indicates the presence of an alcoholic hydroxyl group.

Tests for Steroid

Salkowski reaction: A few crystals were dissolved in chloroform and a few drops of concentrated sulphuric acid were added to the solution. Reddish color in the upper layer indicates the presence of steroid.

Liebermann-burchard reaction: A few crystals were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it followed by addition of 2-3 drops of acetic anhydride. Solution turned violet blue and finally green.

Spectroscopic Characterization:

Among the spectroscopic techniques IR, ^{1}H-NMR, ^{13}C-NMR and MS were carried out. The infrared spectrum was recorded on FTIR Perkin Elmer, ^{1}H-NMR and ^{13}C-NMR spectra were recorded using $CDCl_3$ as a solvent on Bruker Advance 400NMR Spectrometer SAIF Panjab University, Chandigarh.

Compound A

Compound A is a white needle shape crystalline powder (250 mg), melting point: 156^0-160^{0}C, λ_{max} in ethanol: 253. In hexane R_f is 0.29 and chloroform: ethanol (9.8:0.2) R_f is 0.46. The IR spectra showed absorption peaks at 3426.42cm^{-1}(O-H stretching); 2939.31 cm^{-1} (=C-H Str.), 2926.23 cm^{-1}and 2867.84 cm^{-1} (asymmetric and symmetric -C-H stretching of CH_2 group); 1641.6 cm^{-1}(C=C absorption); other bands includes 1463.65 cm^{-1} (CH_2 bending); 1389.71 cm^{-1} (CH_3 bending) and 1039.29 cm^{-1}(C-C stretching of cycloalkane). Mass spectra of this compound suggested that its molecular mass is 412 (M.F $C_{29}H_{48}O$) having characteristic fragments observed at m/z: 367, 271, 255, 229, 189, 175, 161, 133, 121, and 105. Peak at m/z 273 is due to loss of $C_{10}H_{19}$ (M-139). The dehydration of fragment m/z 273 yield m/z 255, which on successive dealkylation would yield ions at m/z 203, 149, 133, 121, 105.

^{1}H- NMR has given signals at δ 5.34 (1H, br (S), H-6) due to H-6 olefinic protons, δ 5.14 (1H, q, H-23), 5.02 (1H, q, H-22), 3.52 (1H, tdd, J=4.66, H-3) and six methyl protons also appeared at δ 0.697, 0.786-0.822 (2 CH_3), 0.9127, 0.9843 and 1.014.

Compound B

The compound B is obtained as colorless needles (100 mg), melting point: 136^0-138^{0}C, λ_{max} in ethanol: 208. In hexane R_f 0.23 and chloroform: ethanol (9.8:0.2) R_f 0.58. The IR spectra showed absorption peaks at 3432 cm^{-1}(O-H stretching); 2937 cm^{-1} (=C-H Str.), 2849 cm^{-1} and 2844 cm^{-1} (aliphatic C-H stretching); 1642 cm^{-1}(C=C absorption); other includes 1465 cm^{-1} (CH_2 bending for cyclic $(CH_2)_n$); 1381.6 cm^{-1}(CH_3 bending) and 1050 cm^{-1}(C-C stretching of cycloalkane). 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, 329, 273, 255, 243, 203,149,121 and 105. ^{1}H- NMR has given signals at δ 5.277 (1H, br, S, H-6), 4.077 (1H, m, H-3), and six methyl protons appeared at d 1.06-0.97 (6H, m, H-18, 19), 0.924-0.801 (9H, m, H-26, 27, 29), 0.67 (3H, s, H-21).

Compound C

The compound C is white amorphous compound (100 mg), melting point: 88^0-91^{0}C. In chloroform R_f 0.34 and chloroform: ethyl acetate (3:2) R_f 0.59. The IR spectra showed absorption peaks at 3306.57 cm^{-1}(O-H stretching); 2917.35 cm^{-1} and 2848.79 cm^{-1}(aliphatic C-H stretching); other includes 1473.13,1462.65 cm^{-1} (CH_2 bending vibration); 1071.93, 1061.67 cm^{-1} (C-O stretching) and 730.33, 719.27 cm^{-1}(a long aliphatic chain). Mass spectra of this compound suggested that its molecular mass is 480 (M.F $C_{33}H_{68}O$) having characteristic fragments observed at m/z: 480, 453, 338, 321,284, 242, 213, 155, 141 and 112. ^{1}H- NMR has given signals at δ 3.64 (2H, t, J=6.6, H-1), 1.52 (8H, m, 4xCH2), 1.25 (54H, br s, 27xCH2), 0.88 (3H, m, H-33).

Compound D

The compound D is white amorphous compound (100 mg), melting point: 84^0-89^{0}C, λ_{max} in ethanol: 288. In chloroform R_f 0.43 and chloroform: ethyl acetate (3:1) R_f 0.62. The IR spectra showed absorption peaks at 3306.57cm^{-1}(O-H stretching); 2848.94 cm^{-1} and 2917.40 cm^{-1}

(aliphatic C-H stretching); other includes 1473.04 cm^{-1}, 1462.66 cm^{-1} (CH_2 bending); 1061.76 cm^{-1} (C-O stretching) and 730.13, 719.25 cm^{-1} (long aliphatic chain). Mass spectra of this compound suggested that its molecular mass is 508 (M.F $C_{35}H_{72}O$) having characteristic fragments observed at m/z: 507, 475, 435, 361, 321, 285, 257, 227, 210 and 133. ^{1}H- NMR has given signals at δ 3.62 (2H, t, j=6.6, H-1), 2.03 (1H, s,-CH_2OH), 1.67 (1H, s), 1.25 (64H, br s, 32xCH_2), 0.88 (3H, m, H-33).

RESULT & DISCUSSION :

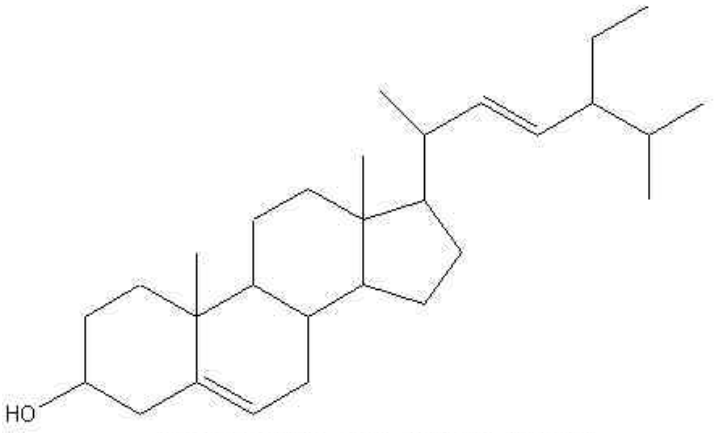
Four compounds isolated from the petroleum ether extracts of leaves of *Xanthium strumarium* by increasing polarity of solvent system (hexane: ethyl acetate) using silica gel. The ^{1}H- NMR and other spectral data for these compounds revealed that compound A and B belong to sterol group. Compounds C and D were identified as long chain alcohols from their physical constants and spectral data.

Compound A and B isolated as white powder. The melting point of A and B were 156^0-160^0C & 134^0-138^0C respectively and both showed positive results in alcohol test and in all the tests of steroid. Mass spectrum of A and B showed a parent molecular ion peak $[M+H]^+$ at m/z 412 and 414 respectively which corresponds to the molecular formula $C_{29}H_{48}O$ and $C_{29}H_{50}O$ [6-10].

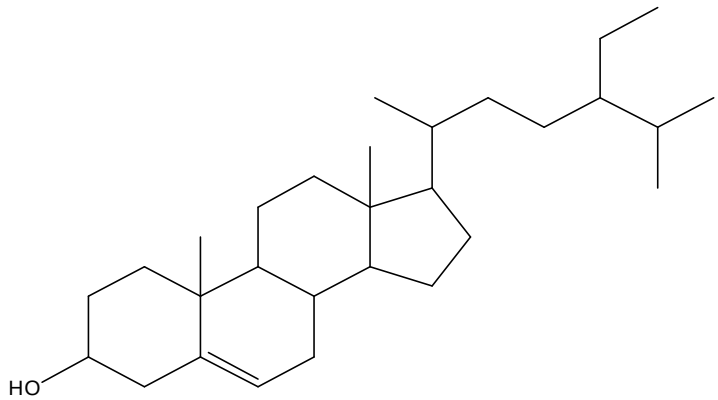
The compounds were characterized on the basis of spectroscopic analysis and comparison with reported data in literature. The melting point of compound A and B were in good agreement with melting point given for stigmasterol and ß-sitosterol in the literature. The molecular formula was established as $C_{29}H_{48}O$ by high resolution mass spectrometry which indicated M+ peak at m/z 412 (calculated for $C_{29}H_{48}O$, 412.3926). The fragmentation pattern was in line with that of sterols. The IR spectrum showed absorption bands at 3426.42 cm^{-1} that is characteristic of O-H stretching. Absorption at 2939.31 cm^{-1} is due to =C-H Stretching, 2926.23 $cm^{-1}$and 2867.84 cm^{-1} is due to C-H stretching, frequency at 1641.6 $cm^{-1}$is due to C=C stretching; other bands includes 1463.65 cm^{-1} due to CH_2 bending; 1389.71 cm^{-1} CH_3 bending and 1039.29 cm^{-1} signifies C-C stretching of cycloalkane.

In ^{1}H-NMR spectrum of compound A, H-3 proton appeared as a triplet of a double doublet (tdd) at δ 3.53 and H-6 olefinic protons showed a broad singlet at δ 5.34. Two olefinic protons appeared downfield at δ 5.14(m) and d 5.02 (m) which was identical with the chemical shift of H-22 and H-23, respectively of stigmasterol. Six methyl protons also appeared at δ 0.697, 0.786-0.822 (2CH3), 0.9127, 0.9843 and 1.0104. The nature of oxygen was shown to be hydroxyl group as depicted by IR spectrum (3426.42cm^{-1}) [6-10].

Similarly from ^{1}H-NMR spectrum of compound B it was seen that H-3 proton appeared at δ 4.077 triplet of a double doublet (tdd) and H-6 olefinic protons showed a broad singlet at δ 5.277. Moreover, six methyl protons appeared at δ 0.67, 0.801-0.924 (3 CH_3), 0.97-1.06 (2 CH_3). These assignments are in good agreement for the structure of ß-sitosterol. The molecular ion peaks were given at m/z 414 and the characteristic ion peak at m/z 273 because of loss of $C_{10}H_{21}$ (M-141). The dehydration of fragment m/z 245, which on successive dealkylation would yield ions at m/z 243, 203, 149,121, 105 respectively. The IR spectra showed absorption peaks at 3432 cm^{-1}(O-H stretching); 2937 cm^{-1} (=C-H Str.), 2849 cm^{-1} and 2844 cm^{-1} (aliphatic C-H stretching); 1642 cm^{-1}(C=C absorption); other includes 1465 cm^{-1} (CH_2 bending for cyclic $(CH_2)_n$); 1381.6 cm^{-1}(CH_3 bending) and 1050 cm^{-1}(C-C stretching of cycloalkane) [6-10].



Stigmasterol ($C_{29}H_{48}O$, Mol. Wt. 412.69)

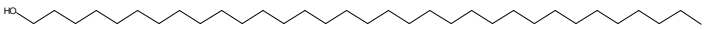


Sitosterol ($C_{29}H_{50}O$; Mol.Wt.414.71)

Compound C was isolated as white amorphous powder. The mass spectral data of the compound gave a molecular formula $C_{33}H_{68}O$ [m/z 480]. IR spectra showed absorption peaks at 3306.57 cm^{-1} indicate presence of alcohol group and band at 730.33, 719.27 cm^{-1} was due to long aliphatic nature of molecule. ^{1}H- NMR spectra showed presence of triplet at δ_H 0.88 for the three end protons while the methylene group α to the hydroxyl group or carbinolic proton (CH_2OH) as a triplet at δ_H 3.64. The rest of methylene protons merged into a broad singlet at δ_H 1.25. The methylene protons ß- to carbinolic group resonated at δ_H 1.52 with OH proton.

Compound D was isolated as white amorphous powder. The mass spectral data of the compound gave a molecular formula $C_{35}H_{72}O$ [m/z 508]. IR spectra showed absorption peaks at 3306.57cm^{-1} indi-

cate presence of alcohol group and band at 730.33, 719.27 cm^{-1} was due to long aliphatic nature of molecule. ^{1}H- NMR spectra showed presence of triplet at δ_H 0.86 for the three end protons while the methylene group α to the hydroxyl group or carbinolic proton (CH_2OH) as a triplet at δ_H 3.62 (J=6.6Hz). The rest of methylene protons merged into a broad singlet at δ_H 1.27. The methylene protons ß- to carbinolic group resonated at δ_H 1.51 with OH proton.



Tritriacontan-1-ol

Pentatriacontan-1-ol

CONCLUSION

The isolation and identification of compound A-D from the petroleum ether extract leaves of *Xanthium strumarium* was the first ever reported from this plant. From the physical, chemical and spectral evidences compound A-D were confirmed as stigmasterol, beta-sitosterol, tritriacontanol and pentatriacontan-1-ol respectively.

REFERENCES

1. Kamboj A, Saluja AK, Phytopharmacological review of Xanthium strumarium L. (Cocklebur), International j of Green Pharmacy, 2010, 129-139.
2. Gill LS. In 'The ethnomedical uses of plants in Nigeria.' Published by University of Benin Press, University of Benin, Benin city, Edo State, 1992, 46: 43.
3. Sofowora A, In 'Medical plants and traditional medicine in Africa.' Published by Spectrum books Ltd. Ibadan, 1993, 156-158.
4. Stahl E, Thin Layer Chromatography: A Laboratory Handbook. 2nd edition, Springer, New York, 1969. ISBN: 0387047360.
5. Harborne JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd Edn., chapman and Hall: London. ISBN: 0-412-57270-2 (1998) 302.
6. Peach K, Tracey MV, Modern methods of plant Analysis. 1st Edn, Springer Varlag, Berlin, 1955, 328-373.
7. Pateh UU, Haruna AK, Garba M, Iliya I, Sule IM, Abubakar MS, Ambi AA, Isolation of stigmasterol, ß-sitosterol and 2-Hydroxyhexadecanoic acid methyl ester from the rhizomes of *Stylochiton Lancifolius* Pyer and Kotchy (Aeaceae), Nigerian journal of Pharmaceutical Sciences, 2009, 7(1), 19-25.
8. Jamal AK, Yaacob WA, Din LB, A Chemical study on *Phyllanthus Columnaris*, European Journal of Scientific Research, 2009, 28(1), 76-81.
9. Habib MR, Nikkon F, Rahman ME, Karim MR, Isolation of stigmasterol and beta sitosterol from methanolic extract of root of bark of *Calotropis gigantea* (Linn), Pak. J. Biol. Sci., 2007, 10, 4174-4176.
10. Rao GV, Rao KS, Annamalai T, Radhakrishnan N, Mukhopadhyay T, Chemical constituents and mushroom tyrosinase inhibition activity of *chloroxylon swietenia* leaves, Turk J Chem, 2009, 32, 521-526.

Source of support: Nil, Conflict of interest: None Declared