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Isolation and Characterization of a Steroid Resembling Bioactive Compound from *Saraca Indica* Bark

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A B S T R A C T

The present investigation was intended to isolate and characterize a steroid like bioactive compound from the bark of *Saraca indica*. Moisture free powder of *Saraca indica* bark was extracted succesively using soxhlet. Hexane extract was checked by thin layer chromatography (TLC) and subjected to column chromatography for the elution of bioactive compounds. Fractions having same R_f value are combined to form a product which was analyzed by various analytical procedures. Among the three spots eluted, third spot in the fractions 250 to 290 (4% ethyl acetate: pet ether) yielded a quantity of 1.5g. Analysis of isolated compound revealed the prersence of alkyl, keto, aldehyde and alkenic functional groups with two carbonyl carbons ketone moiety, and a carbon bearing a single proton likely located next to the ketone moiety. The structural details for the *Saraca indica* compound obtained suggested that the metabolite was likely to be a steroid and the molecular weight was found to be 328.0040 with molecular formula $C_{21}H_{28}0_3$.

Keywords: *Saraca indica*, Soxhlet, Hexane extract, Steroid

A R T I C L E I N F O

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1. Introduction

Medicinal products had up to the beginning of the 20th century mainly been produced by using extracts or powder of medicinal plants as the main active ingredient. The chemical and biological achievements were amongst those that were important for the development of more modern medicines based on pure compounds isolated from plants or micro-organisms. After a plant has been identified as a source for new products, the bioactive compounds are located in the relevant fractions using so called "Bioassay guided isolation".

When the bioactivity has been located to a specific fraction, further separation is performed by a combination of chromatographic methods based on separation by size, charge and hydrophobicity. When a pure compound has been obtained, structural elucidation is performed using a variety of spectroscopic methods like NMR, IR and MS and in certain cases x-ray crystallography (Aksel Berhoft, 2010).

The present study is intended to extract, purify and characterize the steroid like bioactive compound from bark of *Saraca indica. Saraca indica* alias Ashoka tree belond to family Caesalpinaceae (Biswas *et al.*, 1972, web 4). The *Saraca indica* tree contains glycosides, non-phenolic, sapogenetic glycoside, aliphatic alcohols, tannin, catechol, sterol, and organic calcium compounds. Its methanol fraction contains haematoxylene, tannin, and water-soluble glycoside (Web 5). Bark of Saraca tree is known for its medicinal value having spasmogenic, oxytocic, uterotonic, antibacterial, anti-implantation, anti-tumor, anti-pro gestational, anti-estrogenic activity against menorrhagia and anticancer. (Web 4)

2. Materials and method

Plant material

The bark of *Saraca indica* was collected from Public garden, Nampally, Hyderabad, Andhra Pradesh, India. The plant was authentified by Prof. Ram reddy, Department of Botany, Osmania University, Hyderabad, Telangana, India. The voucher specimen of the same was deposited in the herbarium of Botany Department, Osmania University, Hyderabad, Telangana, India.

Successive solvent extraction

50 g of moisture free bark powder of the *Saraca indica* was placed in a thimble-holder and 400 ml of hexane added to the solvent flask. The temperature was raised to 45°C for 16 hours (using heating mantle) and cooled to room temperature, separated the pet ether layer. Then added 400 ml of ethyl acetate and ran the soxhlet at 55°C for 16 hours. At the end, the ethyl acetate layer was separated. To the above residuary gum 400 ml of methanol was added and repeated the above procedure. Finally the extracted samples were concentrated under reduced pressure with rotary evaporator (Heidolph) to get the residues.

Isolation of bioactive compounds

The solvent extractions were checked in different mobile phases like 2%, 4%, 6%, 10% and 15% ethyl acetate: pet ether. It was observed that 4% ethyl acetate: pet ether was the best eluent to separate the components in hexane extraction and other extractions didn't contain any compound. Pet ether extracted brown gum was checked by TLC, where three bands were observed under UV and Iodine. The above (pet ether extracted) gum was subjected to column chromatography, eluting with ethyl acetate: pet ether and 300 fractions each 20 ml were collected and examined with thin layer chromatography (TLC).Fractions were collected and fractions having same R_f value are combined and concentrated to get pure compound.

Characterization of bioactive compounds

The analysis of the sample was done using FTIR (recorded on JASCO FT/IR-5300- sample was prepared by dissolving 1 mg of compound in 10 micro liters of the dichloro methane (DCM), ^{1}H, ^{13}C NMR recorded on Bruker 400MHz NMR spectrometer (in CDCL3 with TMS as internal reference), DEPT, COSY and NOSY were recorded on Bruker 500 MHZ NMR spectrometer. LCMS were recorded on VG7070H mass spectrometer using EI technique or Shimadzu-LCMS-2010.LC-MS data were obtained using electrospray ionization (positive mode) on a C-18 column at a flow rate 0.2 mL/min using MeOH/water (90:10) as eluent. Further, for accurate molecular weight identification high resolution mass spectrophotometer (HRMS) technique was used. For this, sample was dissolved in methanol and submitted to the analysis.

3. Results and Discussion

Isolation of bioactive compounds

The column packed with hexane extracted gum (containing three spots) is eluted with the solvents whose polarity gradually increased serially from 1- 4% of ethyl acetate: pet ether mixture. The first spot was obtained in fractions 100 to 120 at 2% ethyl acetate: pet ether with an yield of 0.5g, and second spot was eluted in the fractions 170 to 200 (3% ethyl acetate: pet ether) with an yield of 1.0g and finally third spot was eluted in the fractions 250 to 290 (4% ethyl acetate: pet ether) with an yield of 1.5g and the results of which are depicted in **figure 1a-d.**

Characterization of bioactive compounds

The functional groups of the purified natural product were identified using standard FT-IR values. The reference values ~ 1697 and 1751 cm^{-1} corresponds to carbonyl groups and peak around 1637 cm^{-1} corresponds to alkene functionality. From this reference values, the purified compound is having keto, aldehyde and alkenic functional groups as illustrated in **figure 2a.** ^{1}H-NMR spectrum of a more purified sample has displayed chemical shifts in the aliphatic region (0.5-2 ppm), a deshielded resonance at 2.8 ppm, and an olefinic resonance between 5-6 ppm. There were no aromatic resonances in the spectrum as there is no peak in the aromatic region (7-8 ppm). This spectrum clearly showing that there is a presence of aldehyde functionality by a peak at 10 ppm as visualized in **figure 2b.**

The ^{13}C spectrum contained resonances primarily in the aliphatic region from 10-40 ppm, but the spectrum also contained resonances indicative of carbons attached to

oxygen or otherwise deshielded by the electronegative environment (-80 ppm), olefinic carbons between 100-150 ppm, and two carbonyl carbons at 170 and 200 ppm. From the envisaged results we can infer that the purified compound is having one carbon attached to electronegative environment, two alkenic carbons and two carbonyl carbons as pictured in figure 2c. To confirm the structure of purified compound further we have carried out the 2D-NMR that is DEPT, COSY and NOESY. The ^{13}C NMR and DEPT135 spectra revealed that there are two carbonyl carbons in the compound that was analyzed. The proton singlet at 2.8 ppm that integrates to one proton in a deshielded signal that is consistent with a proton near to oxygen in the molecule. These data suggest that the *Saraca indica* compounds contains at least one ketone moiety. DEPT135 analysis confirmed the 50.1 ppm carbon to be a CH bearing carbon as shown in **figure 3a**. Together, the NMR data put forward that the *Saraca indica* compound contains two olefinic carbons, a terminal bond as well as one involving a quaternary center and a ketone moiety, and a carbon bearing a single proton likely located next to the ketone moiety.

Additional structural details of the *Saraca indica* metabolite was mined from the aliphatic region of the spectroscopic data. The ^{1}H NMR spectrum contains two singlet resonances at 1.1 ppm and 1.2 ppm that integrated to 3 H and 7 H respectively. These resonances suggested the presence of three methyl group on the compound. The COSY and DEPT135 spectra justified that the 1.1 ppm singlet was linked to a CH/CH_3 carbon at 24.6 ppm. The 7 H singlet at 1.2 ppm was found to be linked to two CH/CH_3 carbons at 22.5 and 32.8 ppm. Because these three methyl groups were all singlets and not split by any other proton signals .So, they all appeared to be attached to quaternary centers. Furthermore, the deshielded aliphatic carbon resonance at 32.8 ppm linked to a non-deshielded resonance at 1.2 ppm is characteristic. These resonances are consistent with Vicinal-dimethyl groups attached to a quaternary center.

The ^{1}H NMR spectrum also showed a number of signals with CH_2 splitting patterns between 1-2 ppm. Analyzing the DEPT and COSY spectrum, it revealed that in addition to the three methyl carbons on the major component of *Saraca indica* there were six additional carbons bearing hydrogens. All of these carbons (18.1, 23.0, 26.9, 29.6, and 40.5 ppm) showed multiple proton interaction suggesting these carbons had at least two attached protons. The DEPT135 confirmed that these six carbon signals were CH_2 signals for the *Saraca indica* compound. The rigid and sharp shapes of the CH_2 peaks suggested that they were involved in a ring system. All the spectroscopical structural analysis of the *Saraca indica* compound obtained elucidated that the metabolite was likely to be a steroid as shown in **figure 3b.** The reason was based on the aliphatic nature of the compound, the non-aromatic ring system, the olefinic bonds, methyl groups as well as the carbonyl and hydroxyl/keto moieties that all are the common components of steroid compounds. Further analysis of the COSY data revealed that one olefinic proton at 6.8 ppm is not coupled with another olefinic proton suggesting this olefinic bond must contain a quaternary carbon as shown in **figure 3c**. An olefinic bond containing a quaternary center is further supported by the presence of a quaternary carbon in the olefinic region of 100-150 ppm in the DEPT135 (Pretsch *et al.,* 2000).

Identification of individual steroid components was made based on the mass spectral data obtained for our compound, and by comparison with literature information. The major components of the *Saraca Indica* (purified compound) retained the common Steroid core. Fragment peak at m/z 329 corresponding to the [M-l] as shown in **figure 3d.** High resolution mass spectroscopy spectrum revealing the molecular weight of the purified natural product is 328.0040 displayed in **figure 3e.** The isolated product was identified and its molecular formula is found to be $C_{21}H_{28}0_3$.

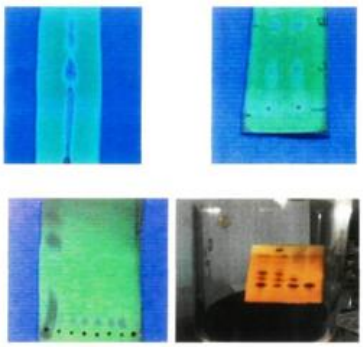


Figure 1: a) TLC profile at 2% EA:PE. b) TLC profile at 3% EA: PE c) TLC profile at 4% EA:PE d) TLC profile of all the extrations under iodine vapour

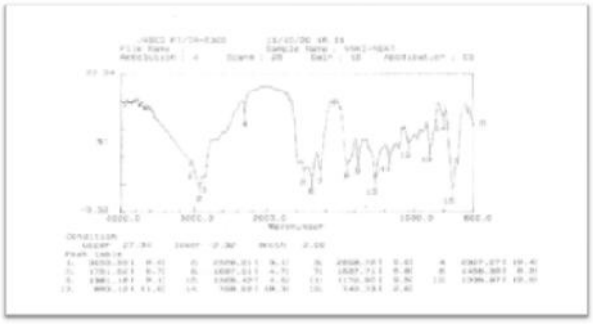


Figure 2a: Fourier transformed infrared spectra

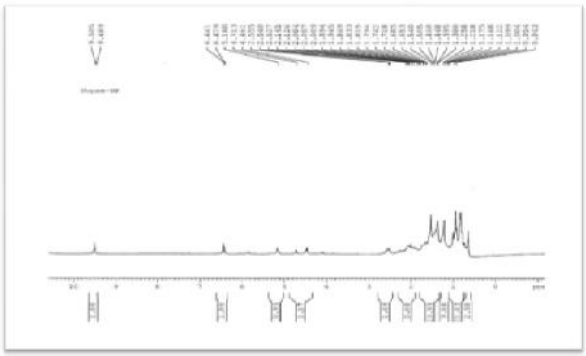


Figure 2b: 1H-NMR spectra

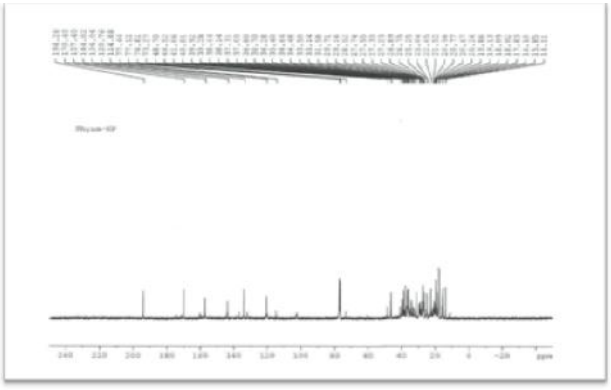


Figure 2c: ^{13}C NMR spectrum

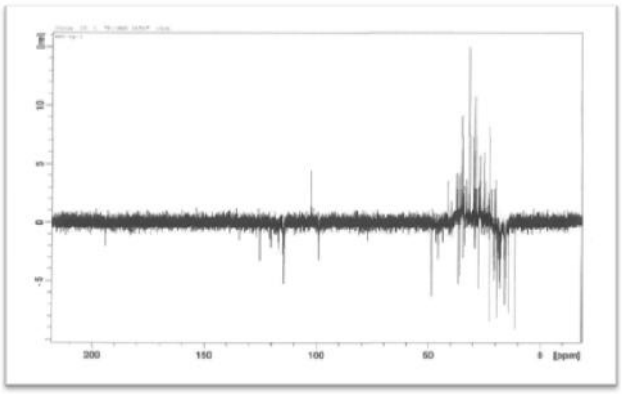


Figure 3a: Distortion less enhancement of polarization transfer spectrum.

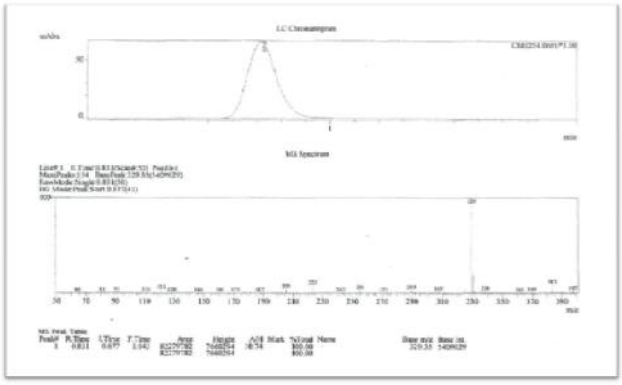


Figure 3b: Correlation spectroscopy spectrum

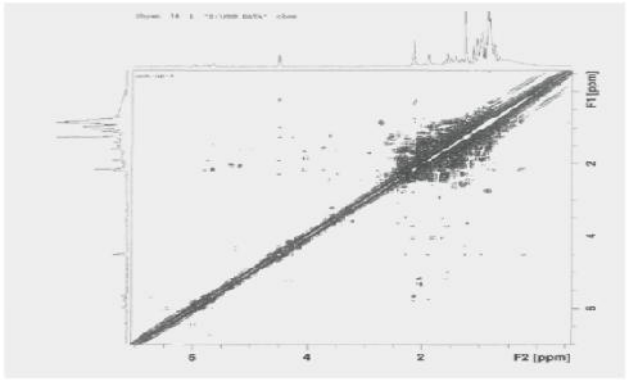


Figure 3c: Liquid chromatography mass spectrometry spectrum

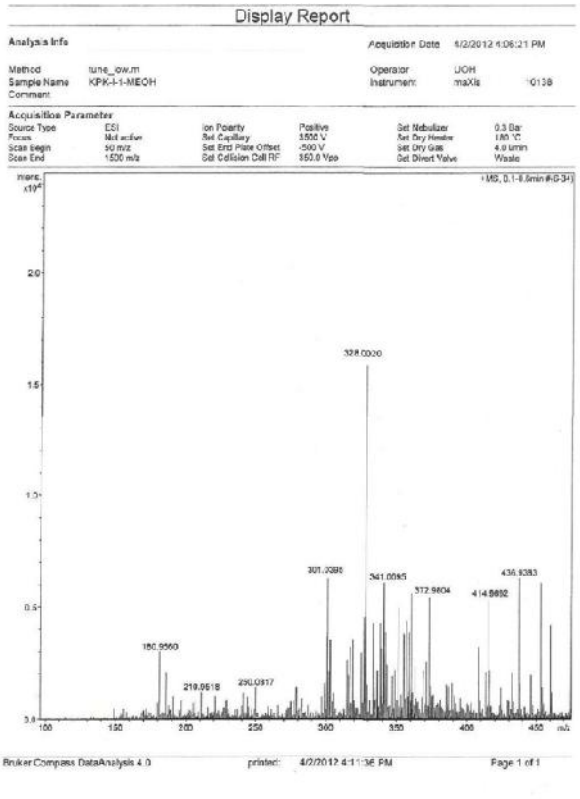
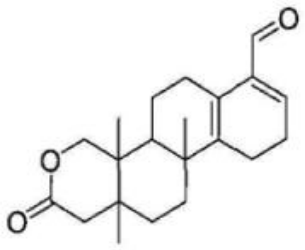


Figure 3d: High resolution mass spectroscopy spectrum



Chemical Formula: $C_{21}H_{28}O_3$

Exact Mass: 328.2

Molecular Weight: 328.004

m/z: 328.20 (100.0%), 329.21 (23.1%), 330.21 (3.2%)

Elemental Analysis: C, 76.79; H, 8.59; O, 14.61

Figure 3e: Chemical formula & molecular weight of bioactive compound

4. Conclusion

Based on the above biochemical experiments, it suggests the molecular structure of extracted ketosterol from the bark of *Saraca indica* as elucidated by ^{1}H, ^{13}C and 2D NMR having steroid ring, two carbonyl groups, two alkenic moieties, one carbon attached oxygen and three methyl groups similar to steroid moiety shown above. This is further confirmed by the LC-MS and ESI-MS as molecular weight 328 is matching with structure of ketosterol with a molecular formula $C_{21}H_{28}0_3$ as proposed from our observations.

5. References

[1] P. Pradhan, L. Joseph, and V. Gupta, "Saraca asoca (Ashoka): a review," Journal of Chemical and Pharmaceutical Research, vol. 1, no. 1, pp. 62–71, **2009**.

[2] http://www.daacademy.org/dagardens_saraca1.html

[3] http://www.saraca-indica.com/Ayurvedic Pharmacopoeia of India. 2001. Vol. I; Part-I:17-18

[4] http://commons.wikimedia.org/wiki/Category:Saraca_indica

[5] TK Biswas; PK Debnath. Ind J Hist Sci, **1972**, 7(2), 99-114.

[6] BL Sharma. Dravyaguna Hasthamalaka, 1st edition, Publication scheme, Jaipur, **1957**; 420.

[7] http:www.nandanbiomatrix.com/newsletter.htm

[8] PK Warrier; VPK Nambier; PM Ganpathy. Some important medicinal plants of the western ghats, India: A Profile. International Development Research Centre, New Delhi. **2000**, 343-360.

[9] http://www.chakrapaniayurveda.com/ashoka.html

[10] M Ali. Pharmacognosy, CBS Publishers & Distributors, New Delhi. **2008**, 668-669.

[11] VD Rastogi. Pharmacognosy & Phytochemistry, Career Publication, Nashik, **2003**, 269-270.

[12] SK Jain. Medicinal Plants. National Book Trust, New Delhi, **1968**; 124.

[13] SK Sadhu; A Khatun; P Phattanawasin; T Ohtsuki; M Ishibashi. J. Nat. Med., **2007**, 61, 480-482

[14] BN Dhawan; GK Patnaik; RP Rastogi; KK Singh; JS Tandon. Indian J Exp Boil., **1977**, 15, 208-219.

[15] SC Pal; AP Maiti; BP Chatterjee; A Nandy. Ind. J Med. Rec., **1985**, 82(2), 188-189.

[16] SR Jain; SN Sharma. Planta Med., **1967**, 15(4), 439-442.

[17] A Singh; S Singh; BK Sarma; UP Singh; R Srivastava; KP Singh. Internet J Alternative Med., **2009**, 6(2), 1-20.

[18] R Dabur; A Gupta; TK Mandal; DD Singh; V Bajpai; AM Gurav; GS Lavekar. Afr. J. Trad. Cam, **2007**, 4(3), 313-318.

[19] S Rajan; J Johnson; J Selvichristy. J. Sci. Trans. Environ., **2008**, 1(3), 149-151.

[20] J Annapurna; UT Bhalerao; DS Iyengar. Fitoterapia., **1999**, 70, 80-82.

[21] N Seetharam; H Sujeeth; G Jyothishwaran; A Barad; G Sharanabasappa; P Shabana. Ind J P Sci., **2003**, 65(6), 658-659.

[22] N Mathew; MG Anitha; TSL Bala; SM Sivakumar; R Narmadha; M Kalayansundaram. Paracitol Res., **2009**, 104, 1017-1025.

[23] MJ Bhandary; KR Chandrasekhar; KMK averiappa. J Ethnopharmacol, **1995**, 47(3): 149-158.

[24] Carvalho ACB, Nunes DSG, Baratelli TG, Shuqair NSMSAQ, Machado Netto E. Aspectos da legislação no controle dos medicamentos fitoterápicos. T&C Amazônia. **2007**, 5(11): 26-32.

[25] Funari CS, Ferro VO. Ethical use of the Brazilian biodiversity: necessity and opportunity. Rev Bras Farmacogn. **2005**, 15(2):178-82.

[26] Niero R. Fármacos, fitofármacos e fitoterápicos: abordagem econômica e de mercado. In: Bresolin TMB, Cechinel Filho V, editors. Fármacos e medicamentos. Uma abordagem multidisciplinar. São Paulo: Editora Santos, **2010**. pp. 1-15.

[27] Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. **2000**, 31(1): 247-56.

[28] Sakagami Y, Kajimura K. Bactericidal activities of disinfectants against vancomycin-resistant enterococci. J Hosp Infec. **2002**, 50(2): 140-4.