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Quantitative determination of β-sitosterol in *Saraca asoca* leaves by HPLC method

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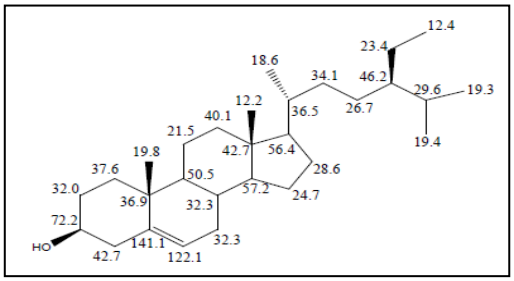
Abstract
Saraca asoca (Roxb.) is used as traditional herbal medicine. The plant contains phytoconstituents like flavonoids, steroids, glycosides, saponins, tannins. Among the phytosterols, β-sitosterol is major compound which has pharmacological activities such as antimicrobial, anticancer, immunomodulatory, anti inflammatory, hepatoprotective, antioxidant and also acts on various gynecological disorders. Due to its pharmacological importance, the main aim of present study is to determine the quantity of β-sitosterol in *Saraca asoca* leaves in methanol and aqueous extracts by HPLC technique. In this method, an isocratic binary system of acetonitrile and ethanol (70:30 v/v), a flow rate of 1ml/min with 198 nm UV detection and was maintained at 30 °C temperature. The retention time obtained for standard concentration of different dilutions of 0.05, 0.5 and 5 (mg/ml) were 25.793, 25.833 and 25.833 min respectively and for methanol and aqueous extracts of *Saraca asoca* leaves showed 25.860 and 25.793min respectively. The calibration curve constructed based on three dilutions of β-sitosterol standard at concentrations produced a linear regression (r = 0.999) and the concentration of were 3.37 X 10^{-4} mg/ml and 4.33 X 10^{-5}mg/ml respectively.

Keywords: *Saraca asoca* leaves, β-sitosterol, methanol and aqueous extracts of *Saraca asoca* leaves, HPLC

Introduction
Saraca asoca (Roxb.) Wilde belonging to Caesalpinaceae subfamily of the legume is one of the indigenous plants with lots of traditional significance. Qualitative determination of phytochemical analysis of *Saraca asoca* leaves showed the presence of carbohydrates, flavanoids, saponins, phenols and tannins, glycosides, steroids and phenolic compounds (Satpal Singh *et al.*, 2015; Mohan *et al.*, 2016; Nisha *et al.*, 2016) [1, 2, 3].
Leaves have sterol like β-sitosterol, steroidal glycoside like sitosterol glucoside (Yadav *et al.*, 2019) [4] which stimulate uterine contraction, useful in treating uterine bleeding, menorrhagia due to uterine fibroids, leucorrhoea, haemorrhoids and haemorrhagic dysentery (Amrita and Sarita, 2016; Borokar and Pansare, 2017) [5, 6]. So the present study is to determine the concentration of β-sitosterol in methanol and aqueous extracts of *Saraca asoca* leaves β-sitosterol (Fig 1) is a white, waxy powder with characteristic odour. It's molecular formula is $C_{29}H_{50}O$, molecular weight is 414.7gm, melting point is 139 to 142 °C (277 -284^{0}F; 409-413K), IUPAC name is 17-(5-Ethyl-6-methylheptan-2yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a] phenanthren-3-ol. It's absorption is at 208nm. It is hydrophobic, soluble in alcohols and is thermally unstable and converted to oxidized products (Sayeed *et al.*, 2016; Babu and Jayaram, 2020) [7, 8].



(Soodabeh *et al.*, 2014) [9]

Fig 1: Chemical shifts of 13C-NMR in the structure of β-sitosterol

Materials and Methods

Saraca asoca leaves were collected from around the premises of Rajendranagar, Hyderabad in the month February 2018. The leaves were dried in shade, powdered and stored in air tight containers.

Extraction: (Akshada and Magdum., 2012; Anjum Gahlaut *et al*., 2013) [10, 11]

Saraca asoca leaves were washed with deionized water and air dried and powdered. Methanol and aqueous extracts were prepared by taking 10 gm of powder in 10 ml methanol and 10 gm of powder in 10 ml of deionized water respectively in two conical flasks. They were extracted overnight by continuous shaking at 25 °C overnight. This step was repeated 3 times to ensure complete extraction. The extracts were centrifuged at 10,000 g for 10 minutes for complete removal of suspended particles. The extracts were lyophilized using lyophilizer and stored at -80 °C for further analysis. The extracts were reconstituted in ethanol (5mg/ml) and filtered through 0.22 µ filters.

HPLC

The experiment was performed on Agilent 1290 affinity series rapid resolution HPLC. A reversed phase column (C18, 4.5 mm X 250 mm) of 5 µm particle size was used. The column temperature was maintained at 30 °C. Mobile phase was comprised of acetonitrile and ethanol of 70:30 ratio with a flow rate of 1 min/ml. Standard solutions of β-sitosterol (ranging from 0.05, 0.5 and 5 (mg/ml) and a volume of 5 µl of sample extract were injected into HPLC to run. Detection of compound is at 198 nm. The corresponding peak areas were plotted against the concentration of standard β-sitosterol injected. Peak identification was achieved by comparison of both the retention time (RT) and UV absorption spectrum of standard.

Quantification of β-sitosterol from *Saraca asoca* leaves extracts:

A volume of 5 µl of standards with known concentration of 0.05, 0.5, 5 mg/ml and methanol and aqueous extracts of *Saraca asoca* leaves were injected into HPLC and was run one by one. The β-sitosterol was detected at 198 nm on a reverse phase column of C18, 45 mm X 250 mm at 30 °C with mobile phase comprised of acetonile and ethanol of 70:30 ratio and flow rate of 1 ml/min.

Results and Discussion

The retention time, areas and height of the β-sitosterol was as eluted and recorded as follows in the given Table 1.

Table 1: Retention time, average area, height of different β-sitosterol standards and extracts of *Saraca asoca* leaves

Standard concentration (mg/ml)	RT (min)	Average Area	Area%	Height	Height %
0.05	25.793	0.018580147	3.44	604004	0.96
0.5	25.833	1.31673457	15.79	4447894	4.17
5	25.833	11.56054484	76.09	43157007	50.27
Saraca asoca leaves powder					
Methanol extract	25.86	0.018227955	0.13	435237	0.04
Aqueous extract	25.793	0.0262254	0.03	322925	0.05

Calculation

Estimation of β-sitosterol in methanol extract of *Saraca asoca* leaves

Compound	Linearity	r^2 correlation co-efficient
β-sitosterol	y = 2.309x +0.026	r^2 = 0.999

$y = 2.309x + 0.026$ $r^2 = 0.999$
$0.0182 = 2.309 x + .026$
$2.309x = 0.0182 - 0.026$

$$x = \frac{0.0182 - 0.026}{2.309}$$

$= -0.00337$
$= 3.37 X 10^{-3}$ mg/ml

Concentration of β- sitosterol in *Saraca asoca* leaves methanol extract is $3.37 X 10^{-3}$ mg/ml.

Estimation of β- sitosterol in aqueous extract of *Saraca asoca* leaves

$y = 2.309x + 0.026$ $r^2 = 0.999$
$0.027 = 2.309 x + 0.026$
$2.309x = 0.027 - 0.026$

$$x = \frac{0.027 - 0.026}{2.309}$$

$= 4.33X 10^{-4}$ mg/ml

Concentration of β - sitosterol in *Saraca asoca* leaves aqueous extract is $4.33 X10^{-4}$ mg/ml.

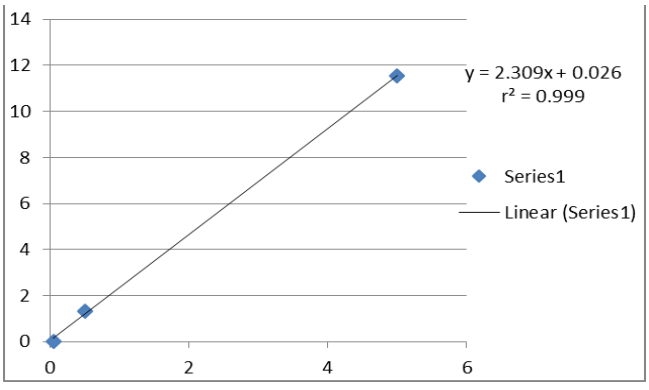
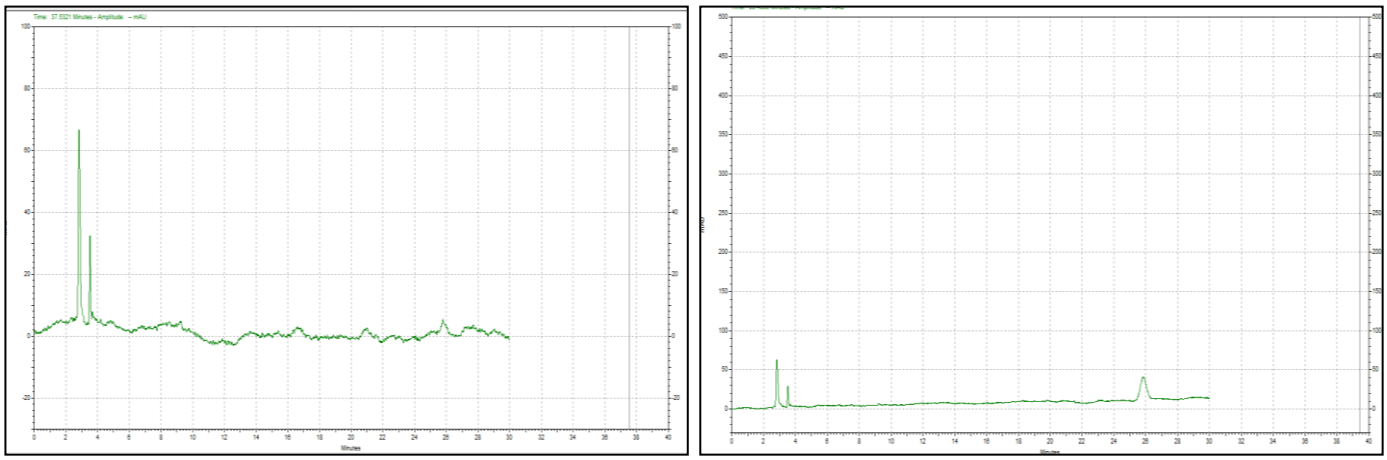


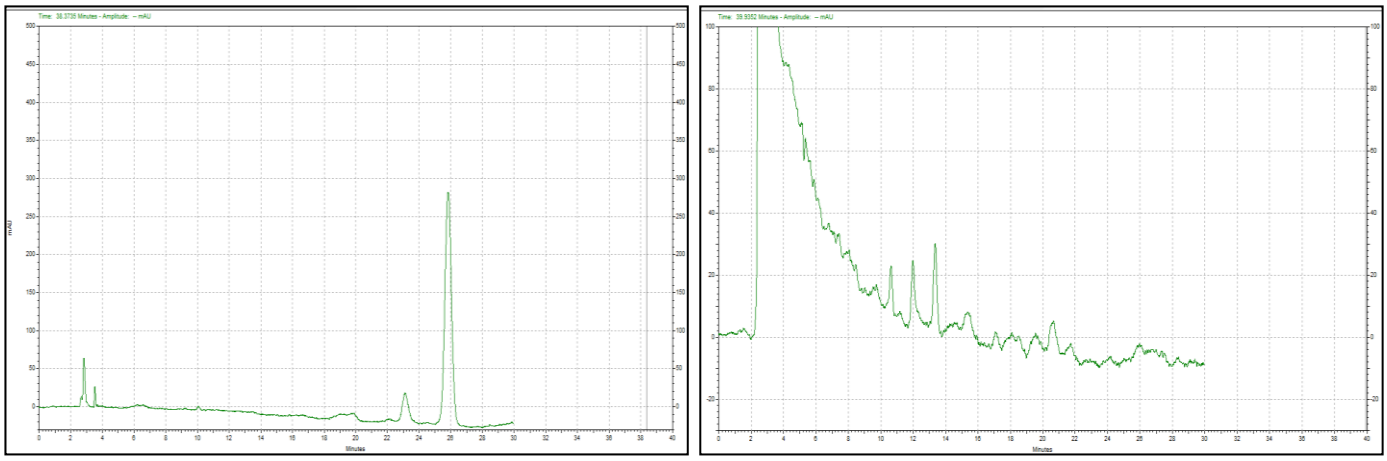
Fig 2: Linear regression curve for β-sitosterol of *Saraca asoca* leaves

Quantification of β-sitosterol in methanol and aqueous extracts of *Saraca asoca* leaves

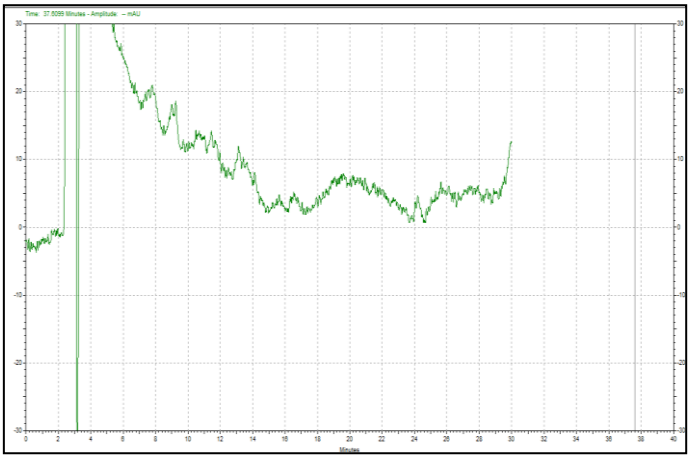
A satisfactory result with sharp well defined and resolved peak with minimum tailing was achieved using a mobile phase consisting acetonitrile: ethanol in the ratio of 70:30 v/v. Chromatogram from the HPLC analysis showed the peak area for β-sitosterol standard 0.05, 0.5 and 5 mg/ml were recognized at retention time (RT) 25.793, 25.833 and 25.833 min respectively (Fig. 3a, b, c), whereas the peak area for methanol and aqueous extracts were recognized at RT 25.86, 25.793 minutes (Figure 3d, e), both at 198 nm UV wavelength.



a. b.



c. d.



e.

Fig 3a, b, c: Total ion chromatogram of β-sitosterol with retention time for 0.05, 0.5 and 5mg/ml concentration; d, e Total ion chromatogram of β-sitosterol concentration with retention time in methanol extract and aqueous extract of *Saraca asoca* leaves

The retention time was on an average of 25.8 min. The results were similar as found with Yi Sheng and Xiao-Bin Chen, (2009) where retention time was on an average of 21.8 min with methanol as mobile phase. Retention time of β-sitosterol was found to be 35.596 min with mobile phase comprised of solvent A (water containing 0.1% formic acid) and solvent B (acetonitrile containing 0.1% formic acid) used in gradient mode (time/concentration [min/%]) and the combination for solvent B 8/5%; 15/10%; 22/45%; 30/65%; 35/90%; 40/5%, with a flow rate of 0.4 ml/min (Anjum et al., 2013) [11]. Retention time was 36.23min with 85% Acetonitrile and 15% ethanol (Akshada *et al.*, 2012) [10].

A calibration curve that was constructed based on three dilutions of β-sitosterol standard 0.05, 0.5 and 5 mg/ml produced a linear regression (r = 0.999) as shown in Fig 2. The concentration of β-sitosterol in *Saraca asoca* leaves in methanol and aqueous extracts as calculated using regression analysis is found to be 3.37 X 10^{-4}mg/ml and 4.33 X 10^{-5}mg/ml respectively. Anjum *et al.*, (2013) [11] obtained 123.5 ng/mL of β-sitosterol in aqueous extract of *Saraca asoca* leaves.

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References

1. Satpal Singh TH, Anantha Krishna, Subban Kamalraj, Gini Kuriakose C, Jinu Mathew Valayil, Chelliah Jayabaskaran. Phytomedicinal importance of *Saraca asoca* (Ashoka): an Exciting past, an emerging present and a promising future. Current science. 2015;109(10):1790-1801.
2. Mohan Ch, Kistamma S, Vani P, Narasimha Reddy. Biological activities of different parts of *Saraca asoca* an endangered valuable medicinal plant. Int J Curr Microbiol App Sci. 2016;5(3):300-308.
3. Nisha P, Athiralakshmy TR, Divyamol AS. Phytochemical screening of *Saraca asoca* and antimicrobial activity against bacterial species. Asian J Plant Sci Res. 2016;6(2):30-36.
4. Yadav SS, Ashwini A, Kamlesh Choure. Review of medicinally important plant speciec *Saraca asoca* (Roxb). Int J Adv Res. 2019;7(9):154-166.
5. Amrita Baranwal, Sarita Devi. Ashoka (*Saraca indica*) in Indian traditional medicine: A review. International J of Research in Applied Natural and Social Sci. 2016;4(5):137-140.
6. Borokar AA, Pansare TA. Plant profile phytochemistry and pharmacology of Ashoka (*Saraca asoca* (Roxb) De wilde - A comprehensive review. Intern J of ayurvedic and herbal medicine. 2017;7(2):2524-2541.
7. Sayeed MSB, Karim SMR, Sharmin T, Morshed MM. Critical Analysis on characterization systemic effect and therapeutic potential of Beta-Sitosterol: A plant-derived orphan phytosterol . Medicines. 2016;3:29.
8. Babu S, Jayaram. An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. Biomedicine and Pharmocotherapy. 2020;131:1-8.
9. Soodabeh Saeidnia, Azadeh Manayi, Ahmad R Gohari and Mohammad Abdollahi. The story of Beta-sitosterol - A Review. European J of Medicinal plants. 2014;4(5):590-609.
10. Akshada Kakade N, Magdum CS. HPLC analysis of β-sitosterol in herbal medicine and vegetable oils. Int J of Pharm & Life Sci (IJPLS). 2012;3:1666-1669.
11. Anjum Gahlaut, Amey Shirolkar, Vikas Hooda, Rajesh Dabur. β-sitosterol in different parts of *Saraca asoca* and herbal drug ashokarista: Quali-quantitative analysis by liquid chromatography - mass spectrometry. J Adv Pharm Technol Res. 2013 Jul-Sep;4(3):146-150.
12. Yi Sheng, Xiao-Bin Chen. Isolation and identification of an isomer of β-sitosterol by HPLC and GC-MS. Health. 2009;1(3):203-206.