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Isolation of B-Sitosterol from methanol extract of stems of *Atylosia Barbata* baker

Bharat V Jain*, Md Rageeb Md Usman
Smt Sharadchandrika Suresh Patil College of Pharmacy, Chopda, Maharashtra, India

Abstract
Natural products and herbal remedies used in traditional folklore medicine have been the source of many medically beneficial drugs because they elicit fewer side effects, relatively cheap, affordable and claimed to be effective. However, in order to make these remedies acceptable to modern medicine, there is a need to scientifically evaluate them to identify the active principles and to understand their mechanism of action. *Atylosia barbata* Baker. (Fabaceae). is a medicinal plant widely used as a folk medicine in India. The present study deals with the isolation and partial purification of bioactive compounds from the crude methanol extracts of the leaves of *Atylosia barbata* Baker. The quantification and the identification of compounds in the crude extract and active bands isolated by preparative TLC were accomplished using spectroscopic analysis. The most important compounds β-sitosterol identified in the crude extract appreciable amounts may account for its various biological activities.

Keywords: isolation, stems, β-sitosterol, plant extraction, *Atylosia barbata*

Introduction
Standardization of plant based medicine is a difficult task; because plants synthesize not only single compounds but it may vary even up to hundreds of compounds may be present in plant. Hence it is difficult to standardize herbal medicines as compared to other medicines. Correct identification and quality assurance of the starting material is therefore an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy [1-3].

The quality and quantity of safety and efficacy information on traditional medicines are not sufficient to meet the criteria to support its use worldwide. The reason behind lack of research data are not only due to health policies but also due to lack of methodologies for the evaluation of herbal medicines. The plants posses many active therapeutically active chemical constituents associated with many inert substances such as cellulose, lignin and coloring agents etc. The active constituents are extracted from plants and purified for their pharmacological utility. So the quality control of herbal drugs is important for their active chemical constituents in modern system of medicine. To meet new thrust of inquisitiveness, standardization of herbal drug is mandatory [4-8]. *Atylosia barbata Baker*. has many medicinally active compound in it hence, focus of this paper is on the analytical methodologies, which include the extraction, isolation and characterization of active ingredients in leaves of *Atylosia barbata* Plant. β-sitosterol is reported to exhibit a spectrum of pharmacological activities against various disease conditions. These include conditions such as inflammation, arthritis, diabetes, cardiovascular ailments, renal disorder, hepatic toxicity, microbial infections and cancer [9]. The available literature suggests that β-Sitosterol is a non toxic agent and does not cause any systemic toxicity in animals at doses ranging from 30 to 2000 mg/kg [10].

Materials and Methods
Plant material
The plant *Atylosia barbata Baker*. is widely found throughout India. For my work the plant was collected from in the deep forest of Satpuda hills with the help of forest officers of Chopda Tahsil, Dist. Jalgaon, (M.S.) India and authenticated by Dr. C. R. Jadhav, scientist, BSI, Pune (M.S.). The leaves of the plant were dried under shade and then coarsely powdered with help of mechanical grinder. The powder was passed through sieve no. 40 and stored in an airtight container for further studies. Extraction was carried out by continuous soxhlet extraction process for 72 hr [11-14].

Qualitative estimations
Preliminary phytochemical screening of extracts. The above extracts obtained from the leaves were subjected for the various chemical test for the identification of active phytoconstituents groups by following standard procedure [15-16].

Thin layer chromatography and preparative TLC
For thin layer chromatography and preparative TLC analysis, the method used was taken from quality standards of Indian medicinal plants ICMR for β-sitosterol. Improvements were made to the sample preparation and standard preparation.

Identification of separated compound
Pinch of sample was added in clean and dry test tube and dissolve in chloroform. Acetic anhydride (1 ml) was added in test tube. Few drops of sulphuric acid solution were added from wall of the test tube, solution shows violet color indicates presence of triterpenoids.

Results and Discussion

For thin layer chromatography, improvements were made to the sample preparation 0.25g of *Atylosia barbata* methanolic extract was diluted with 10ml methanol and standard preparation dissolve 10 mg of β-sitosterol (available from total herb solution) in 10 ml of methanol. TLC plates developed with toluene: ethyl acetate (70: 30) showed a violet spot at a Rf value of 0.58 (Fig. 1) when sprayed with 1% anisaldehyde-sulfuric acid reagent, heat to 105ºC for 15 minutes. The spot coinciding with the standard was marked and then scraped from the plate and scraped silica was sonicated in methanol for 15 minutes then the solution was filtered and β-Sitosterol was obtained by evaporating methanol. Further analysis was done for the isolated compound. By repetitive preparative TLC the β-sitosterol separated was about 25 mg [17-18].

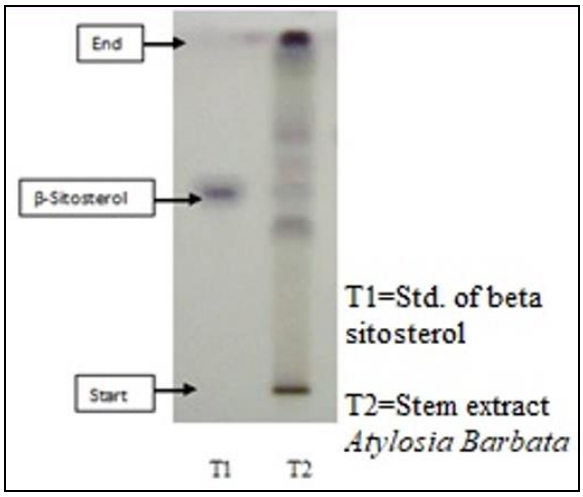

Fig 1: TLC of extract with standard β-Sitosterol

Isolation of β-sitosterol was done using preparative thin layer chromatography (Prep TLC)

Prep TLC is an alternative technique to column chromatography much faster than classic column chromatography. For sample preparation for preparative TLC methanolic extract 25 g was partitioned with 50 ml portions of petroleum ether to separate the non polar terpenoids. Petroleum ether extract was concentrated and used for isolating β-sitosterol by preparative thin layer chromatography. Silica gel $60F_{254}$ pre-coated TLC plates (Merck) 20 X 20 cm developed with toluene: ethyl acetate (70: 30) showed a violet spot at a Rf value of 0.58 (Fig. 2) when sprayed with 1% anisaldehyde-sulfuric acid reagent, heat to 105ºC for 15 minutes.

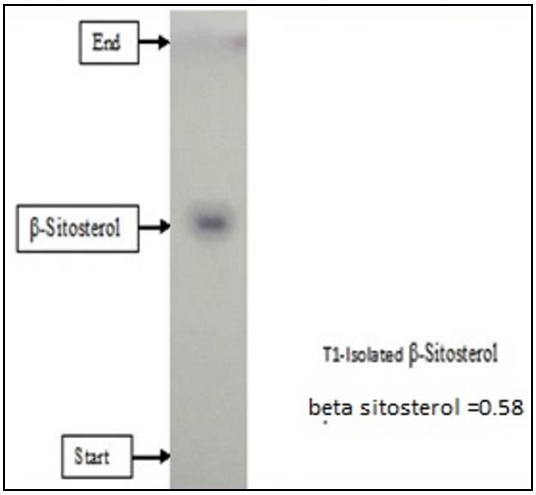

Fig 2: TLC of standard β-Sitosterol

IR spectrum

IR spectrum was taken as KBr pellets on Perkin-Elmer IR spectrometer. 3426.3 (stretching, O-H), 2936.9, 2864.2 (stretching, C-H), 1648, 1636 (stretching, C=C) results were showed in (Fig. 3) and (Table 1).

Table 1: Functional group ranges for IR spectrum of compound

Sr. No.	Functional group	Range	
		Actual	Observed
1.	OH	3200-3600	3426.3
2.	C-H Strech	2850-2970	2936.9, 2864.2
3.	C=C Strech	1500-1680	1648, 1636
4.	C-H Bend	1340-1470	1461.8
5.	C-O	1050-1300	1056.1

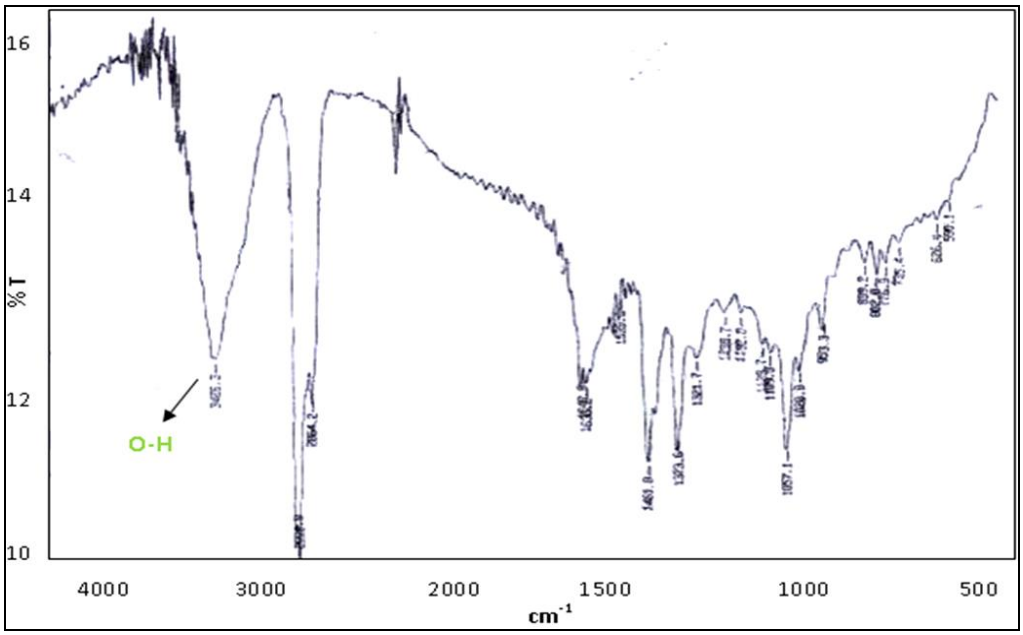

Fig 3: IR spectrum of compound

H^1 NMR spectra of compound H^1 NMR ($CDCl_3$, 400 MHz): H- 3-3.52 (1H, m), H-6-5.34 (1H, m), H-18-1.14 (3H,s), H-19-1.26 (3H, s), H-21-0.91 (3H, s), H-26-1.01(3H, s), H-27-0.97 (3H,s), H-29-0.93 (3H, s) (Fig. 4 and 5) and (Table 2).

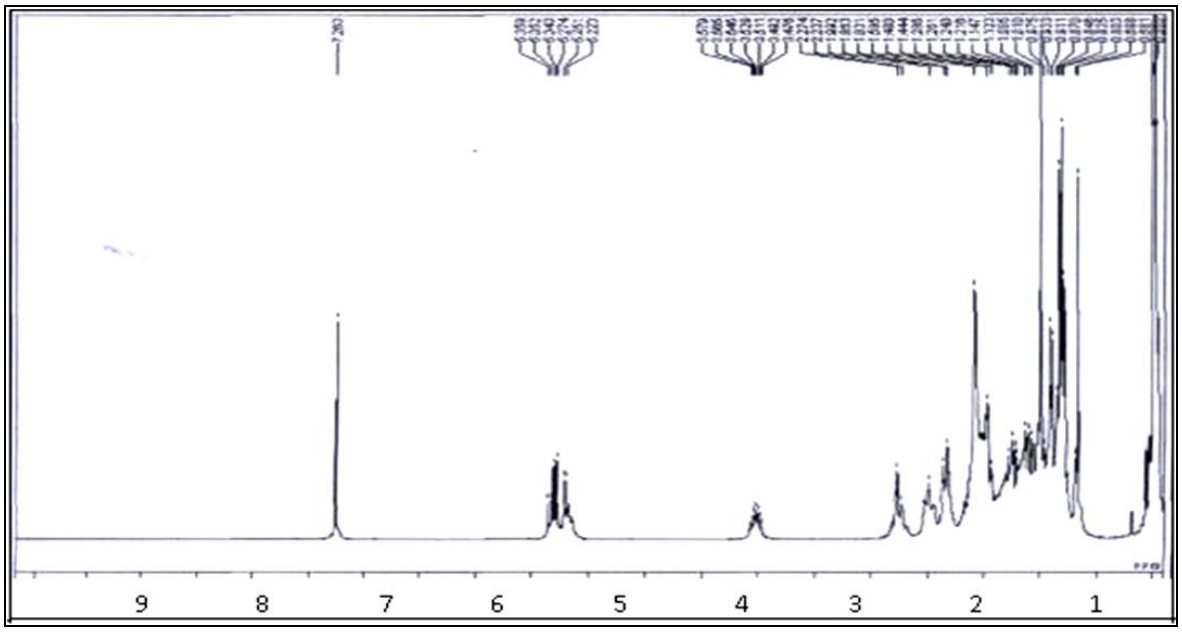

Fig. 4: H^1 NMR spectra of compound recorded in $CDCl_3$, 400 MHz

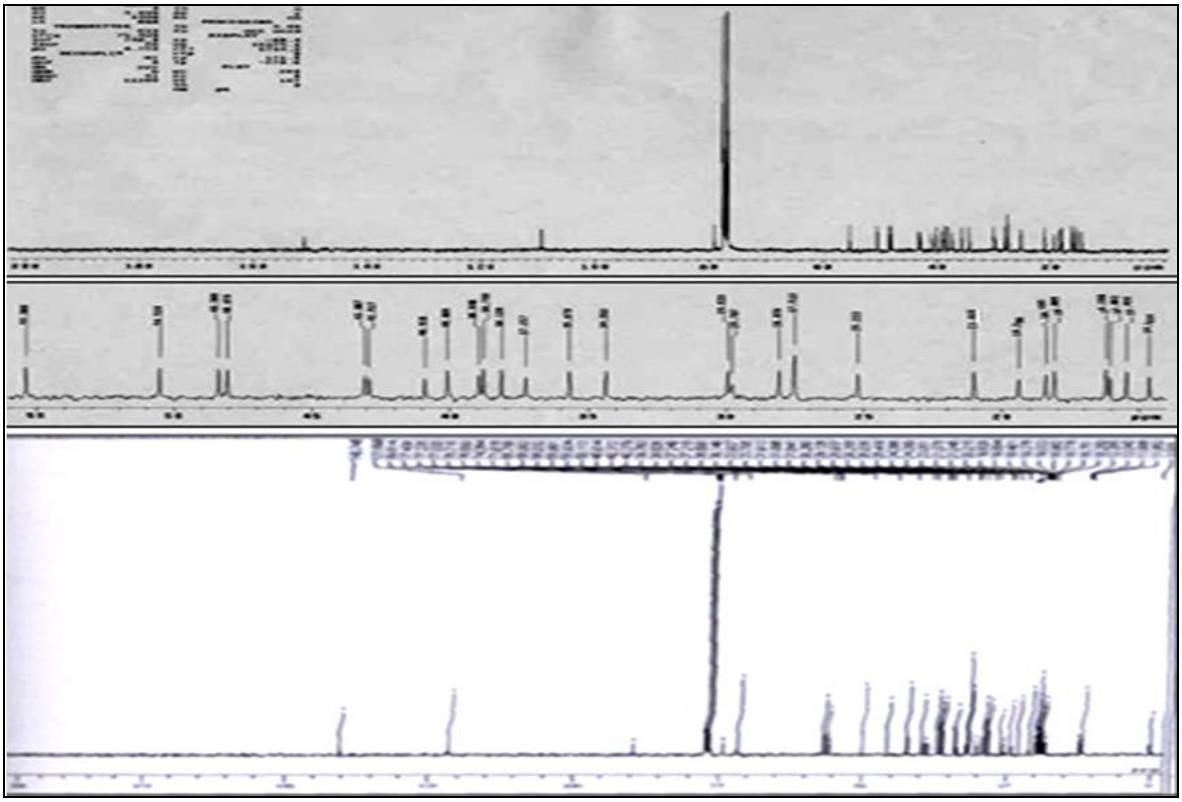

Fig 5: C^{13} NMR spectra of compound recorded in $CDCl_3$, 400 MHz

Table 2: ^1H NMR and ^{13}C NMR spectra for compound

Position	δ_H NMR	δ_C NMR
1		31.9
2		36.1
3	3.52 (1H,m)	71.8
4		42.2
5		140.7
6	5.34 (1H,m)	121.6

7		31.9
8		28.2
9		42.3
10		39.7
11		21.0
12		31.8
13		42.3
14		45.8
15		20.2
16		21.0
17		50.1
18	1.14 (1H,s)	20.2
19	1.26 (3H,s)	19.4
20		30.2
21	0.91 (3H,s)	18.9
22		33.9
23		28.9
24		45.8
25		29.1
26	1.01 (3H,s)	19.8
27	0.97 (3H,s)	19.8
28		23.0
29	0.93 (3H,s)	11.9

High performance liquid chromatography (HPLC) for β-sitosterol

The data obtained from chemical test, physical tests and spectral studies of the isolated compound is matching well with that of reported for β-sitosterol and on the basis of which the isolated compound is characterized as β-sitosterol (Fig. 7).

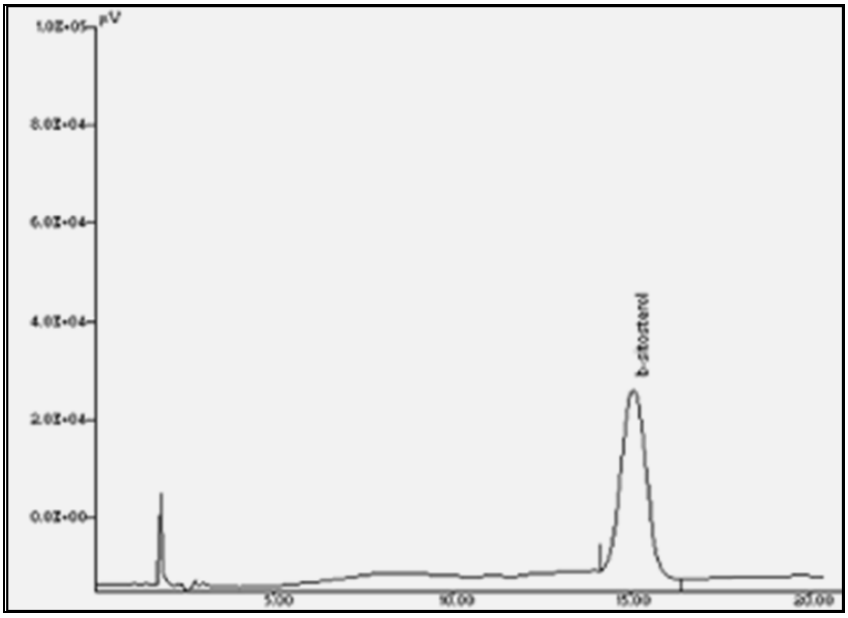

Fig 6: HPLC for β-Sitosterol

Conclusion

Isolation of β-sitosterol was done using preparative thin layer chromatography, chemical formula for β-sitosterol $C_{29}H_{50}O$, synonym 22, 23-Dihydrostigmasterol, Stigmast-5-en-3-ol, β-sitosterin, description colourless crystals, solubility Freely Soluble in chloroform, petroleum ether and solubilised only upon sonication and heating in methanol, melting point 147-146°C, purity 95.36%. β-sitosterol IR (KBr) 3426.3 (stretching, O-H), 2936.9,2864.2 (stretching, C-H), 1648, 1636 (stretching, C=C) results were showed in (Fig. 3) and (Table 1). Mass (m/z): 415.13 (Fig. 6) and (Table 3). H^1 NMR ($CDCl_3$, 400 MHz) H- 3-3.52 (1H, m), H-6-5.34 (1H, m), H-18-1.14 (3H,s), H-19-1.26 (3H, s), H-21-0.91 (3H, s), H-26-1.01(3H, s), H-27-0.97 (3H,s), H-29-0.93 (3H, s). HPLC purosphere RP 18 column, mobile phase Methanol: water: Acetic acid (70:30:1), flow rate 1ml/min, detector UV/VIS, λmax 205nm and retention time 15 minute. Estimation of β-sitosterol in *Atylosia barbata* Baker. Stems chromatographic technique column Microsorb-MV 100-5 C18 250 X 4.6 mm, Mobile Phase acetonitrile: methanol: glacial acetic acid (80: 20:0.01%), flow rate 1ml/min, detection 205 nm, injection volume 20 µL and run time 3.5 minutes. The percentage of β-sitosterol was found in *Atylosia barbata* Baker. stems 2.08 %.

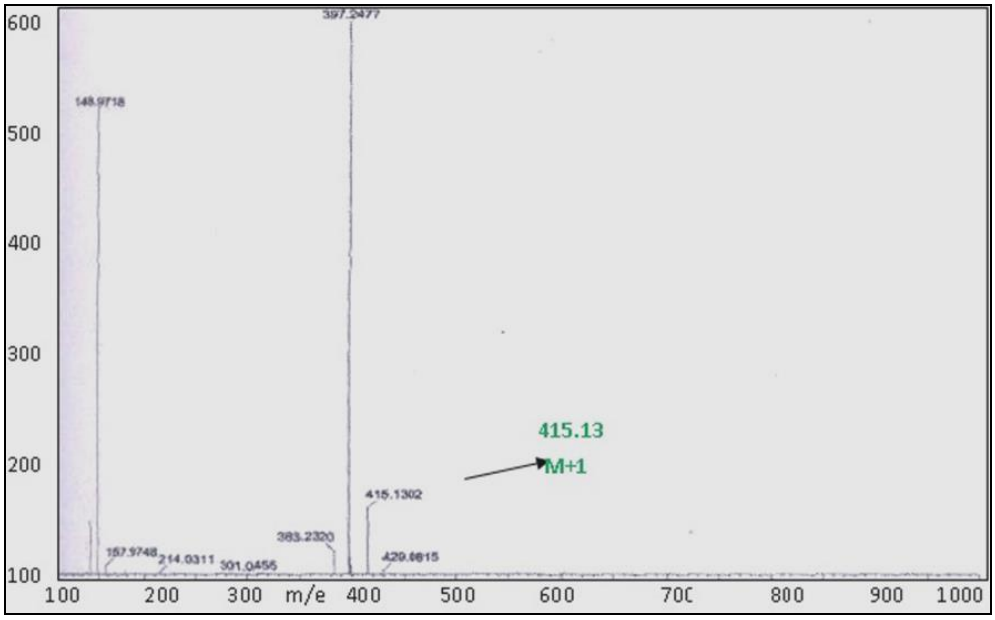

Fig 7: Mass spectra of compound

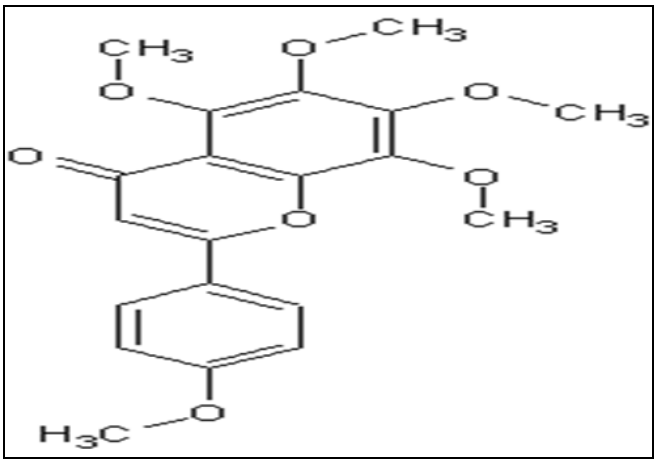

Fig 8: Chemical structure of β- Sitosterol

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