ISOLATION, CHARACTERISATION AND STRUCTURAL ELUCIDATION OF β-SITOSTEROL FROM *Abuliton indicum*

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Abstract: Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases and western medicine is trying to duplicate their successes. In this battle, the role of researcher is to provide thorough knowledge by experimental proofs for unknown plants. Thus, this paper determines standard methodology to isolate the bioactive constituent β- sitosterol from Abuliton indicum. The chromatographic analysis and spectral studies like Uv-vis, FTIR, NMR confirmed the pure isolation of β- sitosterol.

Keywords: A*buliton indicum*, β- sitosterol, NMR, GCMS, phytochemicals

Introduction

The relationship between humans and plants has existed since the existence of human beings, and currently with the global increase in the demand for plant-derived medicine, the relationship has become stronger and inseparable. Plants have a complex mixture of a variety of chemical constituents that can vary considerably, due to several factors such as genetic and environmental factors, method of cultivation, time of collection, post-harvest processing, etc. This inherent variability in the chemistry may adversely affect the efficacy of medicinal plants (Sivarajan and Balachandran., 1994). Thus, inspite of usage of plant derived products consistently as therapeutic agent, it is the necessity of each scientist and medical practioner tot ensure the quality of the herbal drugs and formulations (Samuelsson, 2004).

***Abutilon indicum*,** the Indian Abutilon, Indian mallow; is a small shrub in the Malvaceae family, native to tropic and subtropical regions and sometimes cultivated as an ornamental (Stone and Benjamin, 1970).This plant is often used as a medicinal plant and is considered invasive on certain tropical islands. The species occurs in a number of tropical and subtropical zones. Traditionally, the plant is used in inflammation, piles, gonorrhea treatment and as an immune stimulant. Root and bark are used as aphrodisiac, anti diabetic, nervine tonic, anddiuretic. Seeds are used in urinary disorders. The seeds are used as a laxative in piles and inthe treatment of cough. According to the Chinese in Hong Kong, the seeds are employed as anemollient and demulcent. The bark and the root are used as a diuretic, anthelmintic, pulmonarysedative and in fever (Kashmiri et al., 2009).

A*butilon indicum* L. has been used as a folk medicine in India. Many active compounds have been isolated from this plant through various extractions and these are pharmacologically active. Thus, this paper concentrates on the bioactive constituent of β- sitosterol, a phytosteroids isolated from Abuliton indicum and confirmed through various spectral analysis.

Materials and Methods

Chemicals

All solvents and chemicals employed for the present work were of analytical grade.

Collection and preparation of plant extract

The fresh green aerial parts of the plant *Abuliton indicum* were collected, authenticated and the leaves were dried at room temperature under shade for five days. This dried powdered was subjected to various extraction using different solvent in soxhlet apparatus for 24 hours. Then the extract were evaporated to dryness using Büchi Rotavapor (Switzerland) and ultimately dried in an oven.

Phytochemical tests

Phytochemical screening was carried out on the leaf extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids. General reactions in these analysis reveals the presence or absence of these compounds in the leaf extracts tested.

TLC

Commercially pre-coated TLC silica gel plate was used a line was drawn with a pencil 2cm at the bottom from one end of the plate. The sample(s) were dissolved in little ethyl acetate solution and was spotted on the line drawn on the plate by capillary tube and then allowed to dry. The dry plates were placed into the chroma tank contained (1:3) ratio of ethanol : hexane, the tank was covered. The solvent was set up on the plate by capillary action, when the solvent front was just about 2cm to the upper end of the plate, the plate was removed and a line was drawn to mark the position of the solvent front. The plates were allowed to dry and the spots were developed by spread with 5% sulphuric acid as spraying reagent. The Rf value of the spots were measured using meter rule.

Uv-Vis

UV-Vis spectral analysis was done by using diluting a small aliquot of the sample into distilled water and measuring at the range of 190- 1100 nm.

Structural elucidation by spectroscopy

The FTIR, ^{1}H-NMR and ^{13}C-NMR spectra were recorded. IR spectrum was recorded using KBr disk method on IR spectro-photometer (Shimadzu IR prestige -21). NMR spectra were recorded on FT-NMR Spectro-photometer (DRX 300, Bruker) at 300MHz using pyridine as solvent.

GCMS-MS

The column (30m× 0.25mm×0.25µm) with mobile phase: Helium gas (99.99% purity), flow speed was 0.8ml/min, split ratio was 10:1. Sample temperature: 280ºC. Column temperature: from 240o C and rose up to 265 oC at the rising speed of 10º C/min. Remained at 265 º C for 40 minutes. Ionization mode: EI+ . Electron energy: 70eV. Interface temperature: 250ºC. Ion source temperature: 200º C. Detection voltage: 350V. Sample loading: 0.5µl.

Results and discussion

β-sitosterol is one important kind of phytosterols isolated as bioactive component from Abuliton indicum by step by step processes. The collected leaves were powdered and extracted with ethanol using soxhlet apparatus and dried in evaporator to make a powder. The phytochemical analysis of this powdered extract showed the presence of carbohydrates, tannins, saponins, flavanoids, alkaloids, quinines, cardiac glycosides, terpenoids, phenols, coumarins, steroids, phytosteroids and absence of glycosides, phlobatannins, anthraquinones.The phytosteroids were isolated and identified by thin layer chromatography at a relative factor of 0.55.This spot was again obtained by slurry TLC plate and the scraped out portion was further subjected to spectroscopic analysis.

The UV-Vis spectral analysis shows maximum λmax of 257nm similar to the results obtained Padma sri and Sarada, 2011.

Different spectroscopic methods were used to elucidate the structure of isolated compound (1), including: IR, 1H NMR and 13C NMR. The infra red spectrum was recorded on FTIR Shimadzu IR prestige, 1H-NMR and 13C-NMR spectra were recorded using CDCl3 as solvent on DRX 300, Bruker NMR spectrometer. On subjection to IR Spectroscopic analysis (Figure no.2), the observed absorption bands are 3423.65 cm-1 that is characteristic of O-H stretching. Absorption at 2945.30 cm-1 is assumed to be due to cyclic olefinic –HC= CH- structure, and between 2376.30 to 2945.30 cm-1 assigned to CH structure. Nearly 15, Other absorption frequencies including in the range of 499.56 cm-1 to 1653.00 were observed as a result of C=C absorption, however, some bands are weak (Pretsch et al., 2000). 1460.11 cm-1 is a bending frequency for cyclic (CH2) n and 1375.25 cm-1 for –CH2 (CH3)2γ. The absorption frequency at 1056.06 cm-1 signifies cycloalkane.

1H NMR spectrum of compound Figure no.3A and B has revealed a one proton multiplet at δ 2.41, the position and multiplicity of which was indicative of 3H of the steroid nucleus. The typical 6H of the steroidal skeleton was evident as a multiplet at δ 5.39 that integrated for one proton. The spectrum further revealed signals at δ 1.47 and δ 1.19 (3H each) assignable to two tertiary methyl group at C- 18 and C-19 respectively. The 1 HNMR spectrum showed two doublets centered at δ 0.90 (J = 6.7Hz) and δ 0.89 (J = 6.7Hz) which could be attributed to two methyl groups at C-26 and C -27 respectively. The doublet at δ 1.62 (J = 6.5Hz) was demonstrative of a methyl group at C-21. On the other hand, the triplet of three proton intensity at δ 0.88 could be assigned to the primary methyl group at C- 29.

The 13C-NMR (Figure no. 4) has shown recognizable signals 140.77 and 121.7 ppm, which are assigned C5 and C6 double bonds respectively as in Δ5 spirostene. The value at 19.83ppm corresponds to angular carbon atom (C19). Spectra show twenty six carbon signal including six methyls, nine methylenes, nine methane and two quaternary carbons. The alkene carbons appeared at δ140.7 and 121.7 The structure was simulated using ACD/NMR program to obtain the chemical shifts of both proton and carbon. This is similar to the standard data matched with the simulated data which supports the proposed structure of this compound as β – Sitosterol (Bulama et al., 2015).

These absorption frequencies resemble the absorption frequencies observed for stigmasterol compared to the other literatures mentioned that β-sitosterol and Stigmasterol are always in a mixture form in which may have maximum portion of stigmasterol. It is very difficult to obtain stigmasterol in pure state. This statement is evident from the fact obtained by GCMS peaks and library search in this paper. From GCMS two peaks are obtained one in RT 18.290 and other in 19.910 as Hit 1 and Hit 2 in the figure no. 5 A and B.

The only difference between the two compounds is the presence of C22=C23 double bond in Stigmasterol and C22-C23 single bond in β-sitosterol. Furthermore, literatures have shown that sitosterol is difficult to be obtained in pure state Pollock and Stevem, 1965; Anjoo Kamboj and Ajay Kumar Saluja 2011; Pateh et al., 2008).

Stigmasterol and beta-sitosterol have the same Rf value 0.55 (EtAc/Hex: 1/3) despite the use of several solvent systems. Therefore, compound isolated is a mixture of β- sitosterol and Stigmasterol. β-sitosterol is colorless needle-like solid with a melting point of 147-149°c with a mass of 414g/mol and molecular formula of C29H50O.

Figures

Figure no. 1 UV-Vis

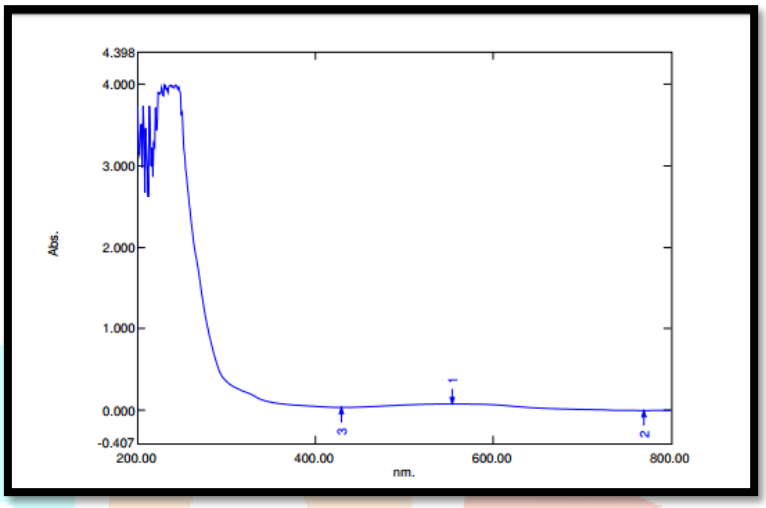


Figure no. 2 FTIR spectrum

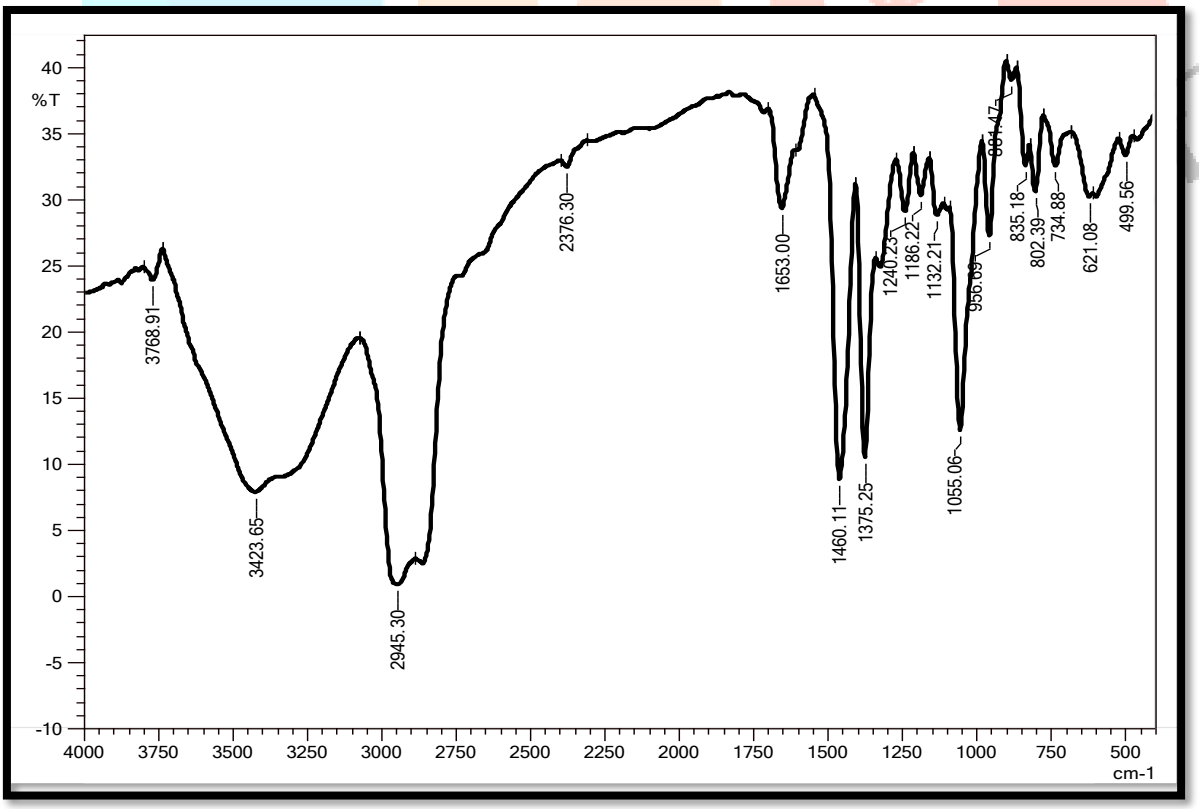


Figure no. 3 A ^{1}H-NMR spectra

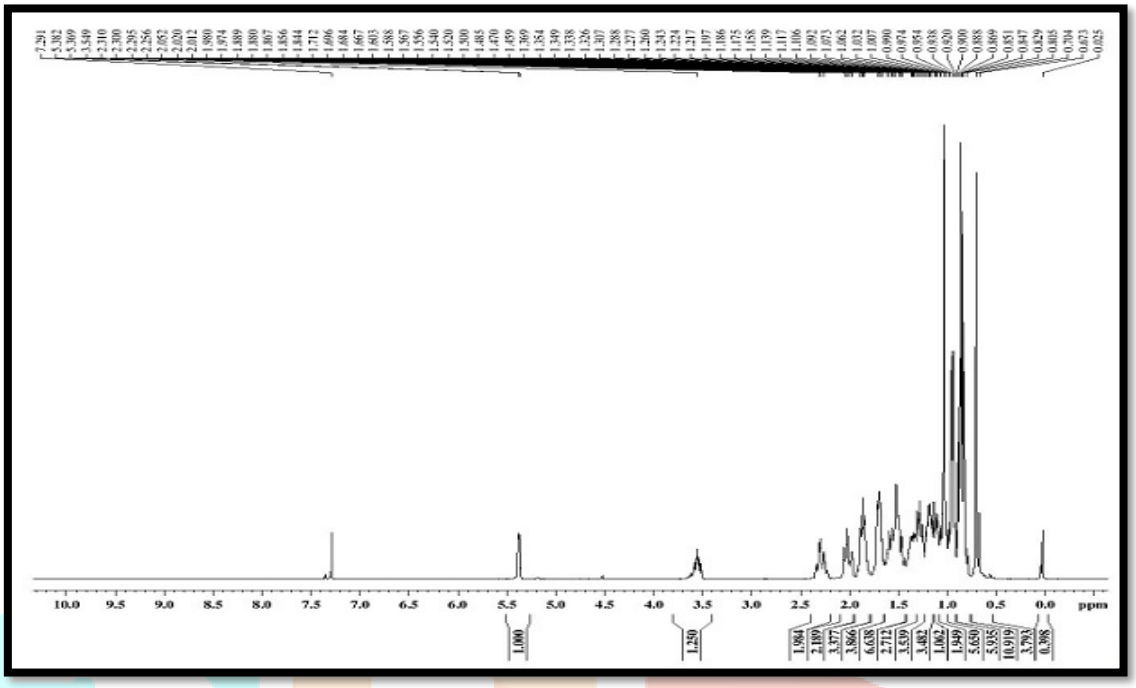


Figure no. 3 B ^{1}H-NMR spectra

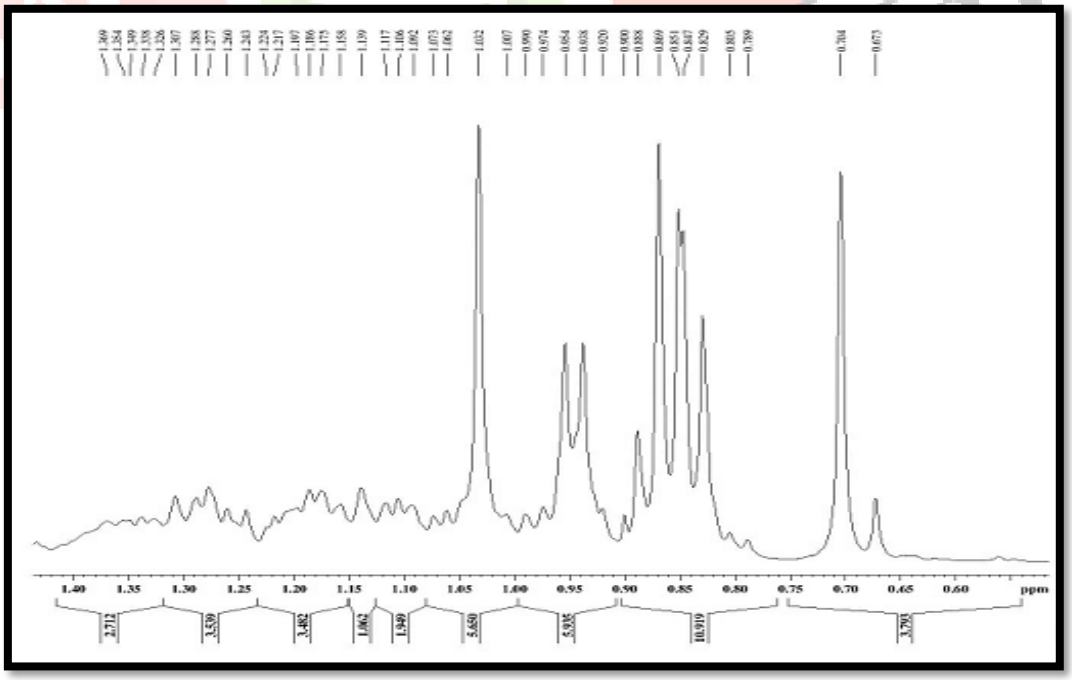


Figure no. 4 ^{13}C-NMR

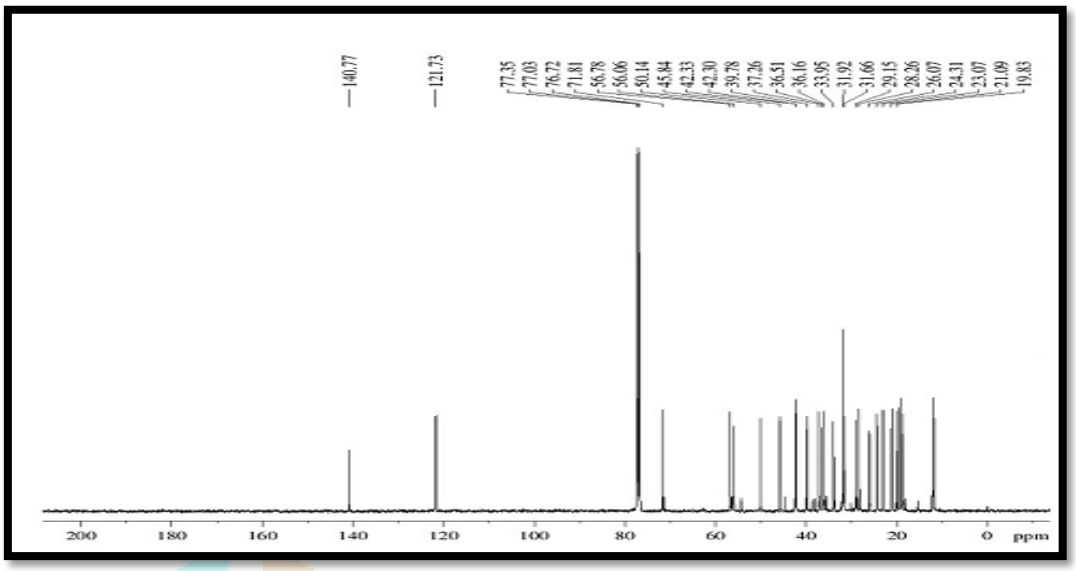
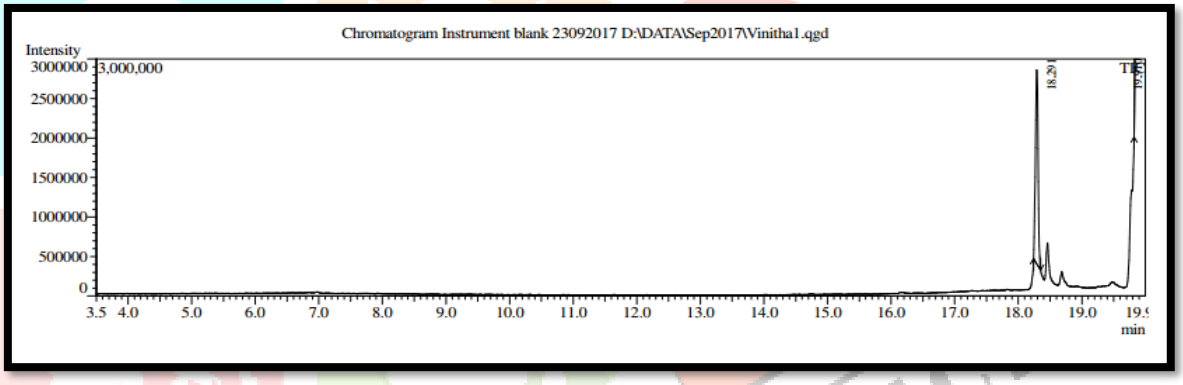
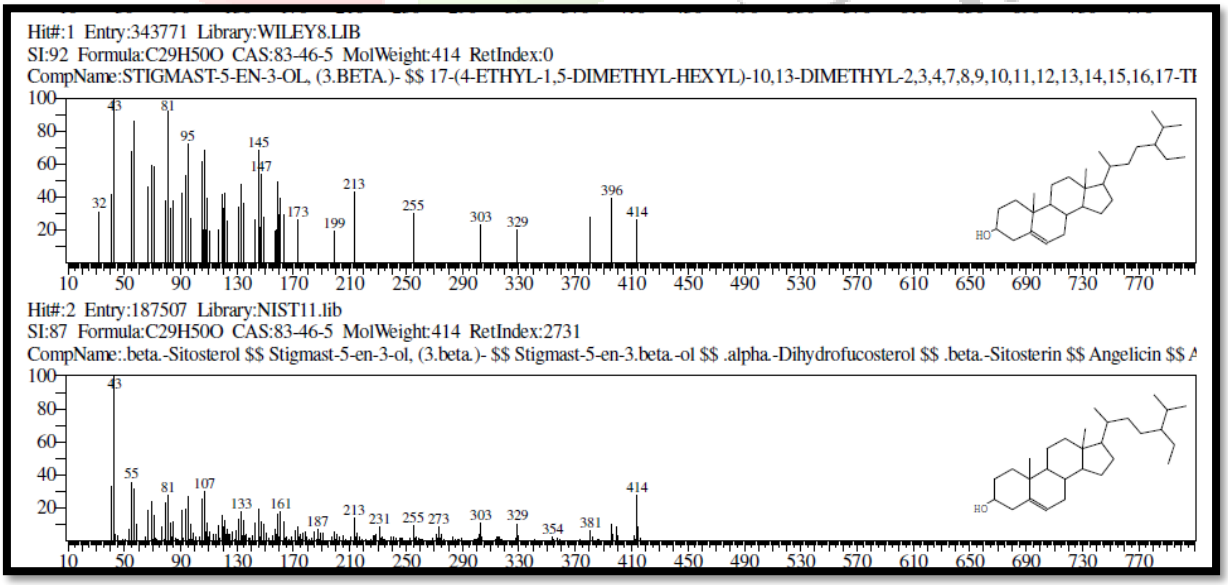


Figure no. 5

A. GCMS chromatogram



B. GCMS chromatogram library



Tables

Table no. 1 Phytochemical tests

Phytochemical Test	Samples
	Abuliton indicum
Carbohydrates	+
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Quinones	+
Glycosides	-
Cardiac Glycosides	+
Terpenoids	+
Triterpenoids	+
Phenols	+
Coumarins	+
Steroids	+
Phytosteroids	+
Phlobatannins	-
Anthraquinones	-

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Conflict of interest: None