

Isolation & Characterisation of Beta-sitosterol from the rhizomes of *Arisaema utile* and its Evaluation for Antioxidant Activity

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Abstract- The aim of this study is isolation, identification and characterized the bioactive compounds from the rhizomes of *arisaema utile*. Preliminary Phytochemical screening of the rhizome extract of *arisaema utile* revealed the presence of Steroids, terpenoids, Flavonoids, Alkaloids, Saponins & Carbohydrates. The air dried rhizomes were pulverized to powder, subjected to Soxhlet extraction and compound Isolation. The isolated compound was colorless crystalline, which was further subjected to IR, ¹³CNMR and ¹HNMR for proper characterization and elucidation of the structure. The compound was concluded as β -Sitosterol. Antioxidant activity of the isolated compound was measured by DPPH assay under in-vitro condition. The isolated compound showed most promising radical scavenging activity at concentration of 10 μ g/ml.

Keywords: β -Sitosterol, Antioxidant activity, DPPH, *arisaema utile*, and Chromatography.

I. INTRODUCTION

A substantial part of all drugs are still based on compounds originally isolated from nature. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. *Arisaema* is a genus of about 150 species in the flowering plant family Araceae, native to eastern Africa, central Africa, Asia and eastern North America. Asiatic species are often called cobra lilies, while western species are often called jack-in-the-pulpit. It can be found growing on rocky slopes at an altitude of 2,400-4,600 meters. It grows in shady, moist, well-drained and fertile soil. *Arisaemas* are tuberous perennials that die back to the ground in winter. *Arisaema utile* emerges in spring. Most of the species from genus *Araceae* have a history of use in folk medicine for the treatment of various infectious diseases. Rhizomes of few species of *Arisaema* have long history of use in Traditional Medicine, especially in Asian countries like *Arisaema jacquemontiana* for Muscular strength and Skin infections, *Arisaema propinquum* for Skin eruption or rashes etc [1]. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide [2, 3, 4]. Further, plant phenolic compounds have been found to possess potent antioxidant [4-9], antimicrobial and anticancer activities [10, 11]. As an individual plant, *Arisaema utile* is used for treating various infections in the

blood, liver and bile which correlates to the signs and symptoms of parasitic and microbial infections, cancer and inflammatory conditions. A lectin was also purified from tubers of Himalayan cobra lily *Arisaema utile* [12]. One of the most exiting properties resulting out of the interaction of lectins with lymphocytes is mitogenicity, *i.e.* the triggering of quiescent, non-dividing lymphocytes into a state of growth and proliferation. The discovery of first mitogenic lectin Nowell [13] led to the detection of many other such lectins, most notably concanavalin A [14], Wheat germ agglutinin [15] and Pokeweed mitogen [16]. The crude extracts of this plant as mentioned above showed significant antimicrobial, antioxidant and prominent cytotoxic activities against few cancer cell lines. The plant has reports of being used in traditional medicines by the tribal people of Jammu and Kashmir for curing various diseases. Keeping in view global and national scenario of medicinal plants, Encouraged by these finding, we carried out in-depth phytochemical isolation and further investigate the antifungal and antioxidant activities of the isolated compound from *arisaema utile* especially existing at high altitudes of Jammu and Kashmir with proven folklore medicinal claim.

II. MATERIALS AND METHODS

Collection of Plant material and processing:

The *Arisaema utile* plant material was collected in the month of July from Gulmarg area of district Budgam of Jammu & Kashmir state, India. Voucher specimen of *Arisaema utile* bearing specimen no 27911, was Identified and deposited at KASH herbarium in

centre of biodiversity and plant taxonomy, University of Kashmir, Srinagar, J&K, India.. The rhizomes of the plant were shade dried, then pulverized into powder with the aid of grinder. The powder obtained from the plant was then used for the isolation of constituents using Soxhlet extraction and Column Chromatography.

Extraction and purification:

Eight hundred grams (800g) of powdered rhizome of *arisaema utile* was subjected to sequential extraction using soxhlet apparatus from non polar to polar solvents such as *n*-hexane < ethyl acetate < methanol. The solvent was recovered under reduced pressure using rotary evaporator under vacuum condition and the residue was stored in the refrigerator. Hexane extract was chromatographed on a silica gel column and eluted with solvent mixtures of increasing polarity, composed of hexane, ethyl acetate and methanol. All the fractions were monitored on TLC. Fractions collected with 20:80 ethyl acetate/Hexane were pulled together as these fractions showed a single spot on TLC. Further these combined fractions were kept in refrigerator overnight for crystallization which resulted in the formation of crystalline needle shaped **compound 1**. The structure of the isolated compound was established on the basis of elemental analysis and spectroscopic evidences (IR, ¹H-NMR, ¹³C-NMR). The structure was simulated using ACD/NMR program to obtain the chemical shifts of both proton and carbon.

Spectroscopic characterization of Compound 1

The various spectroscopic methods like FT-IR, DEPT, ¹H-NMR, and ¹³C-NMR were used to elucidate the structure of isolated compounds. The Fourier Transform-Infrared (FTIR) spectroscopy was carried out on a Perkin Elmer FT-IR fitted with Spectrum software version 10.3.2 using a liquid sampler. ¹H-NMR (400MHz) and ¹³C-NMR (400MHz) were recorded using CDCl₃ as solvent in **MeOD** on Bruker, Avance (400MHz) NMR spectrometer.

Determination of antioxidant activity

The in-vitro antioxidant potential of the isolated compounds was measured in terms of hydrogen donating or free radical scavenging ability using the stable radical DPPH according to the standard procedure [17]. 0.1mM solution of DPPH in Methanol was prepared and 1.0ml of this solution was added to 1.0 ml of the test solution in methanol at different concentrations of Isolated Compounds (2, 4, 8, 10 & 12µg/mL). The reaction mixture was incubated at 37°C for 30 min in darkness. The absorbance of the sample at 517 nm was measured and then compared with that of a control solution containing the reaction mixture amended methanol instead of Isolated Compounds. Ascorbic acid (2, 4, 8, 10 & 12µg/mL) was used as the standard reference compound, and the percentage of DPPH free radical scavenging activity was calculated using the following equation:

$$\% \text{ scavenging activity} = ((A_0 - A) / A_0 \times 100).$$

Where A₀ was the absorbance of the control (blank, without compound) and A was the absorbance of the reaction

mixture. All the tests were performed in triplicate and the graph was plotted with the mean values.

III. RESULTS AND DISCUSSION

A. Fourier Transform-Infrared (FT-IR) spectroscopy

The IR absorption spectrum of **Compound 1** showed absorption peaks at 3332.2cm⁻¹ (O-H stretching.); 2937.1 cm⁻¹ and 2870.1cm⁻¹ (aliphatic C-H stretching); 1632.6cm⁻¹ (C=C absorption peak); other absorption peaks includes 1461.1cm⁻¹ (CH₂); 1379.1cm⁻¹ (OH def), 1043.7cm⁻¹ (cycloalkane) and 797.7 cm⁻¹. (**Phg. 1**). The compound is a colourless crystalline compound, λ_{max} in CHCl₃: 220 nm. On subjecting to IR spectroscopic analysis, absorptions bands appeared between 3570.36 – 3186.51 cm⁻¹ that is characteristic of O-H stretching, 2870.1 cm⁻¹ is due to aliphatics or C-H stretching or (CH₃), 1632.6 cm⁻¹ due to double (C=C) stretching, 1043.7cm⁻¹ due to (C-O). Other absorption frequencies include 1379.1cm⁻¹ is a bending frequency for cyclic (CH₂)_n. The absorption frequency at 797.7cm⁻¹ signifies cycloalkane. These absorption frequencies resemble the absorption frequencies observed for β-sitosterol as resembled data published by Arjun patra and his co-workers. [18].

B. Nuclear magnetic resonance (NMR) spectroscopy

¹H-NMR (CDCl₃, 400MHz) of **compound 1** has given signals at δ 3.56 (1H, m, H-3), 5.38 (1H, m, H-6), 0.72 (3H, m, H-18), 1.05(3H, m, H-19), 0.80(3H, m, H-26), 0.90(1H, d, H-21), 0.84(3H, m, H-27), 5.08(1H, m, H-22), 5.18 (1H,m, H-23), 2.03 (m, H-10), 1.87 (2H, m, H-11), 1.90 (1H, m, H-14), 1.92 (2H, m, H-15), 2.27 (1H, m, H-17), 0.94 (1H, br., H-24) and 0.83 (3H, m, H-29), ppm. (**Phg. 2**). The ¹H-NMR spectrum (400MHz, CDCl₃) of **compound 1** 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 5.38 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 ¹H-NMR 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. This compound is having six methyl, eleven methylene and three quaternary carbons with a hydroxyl group. The above spectral features are in closed agreement to those observed for β - Sitosterol according to Manoharan *et al.*, 2005 and

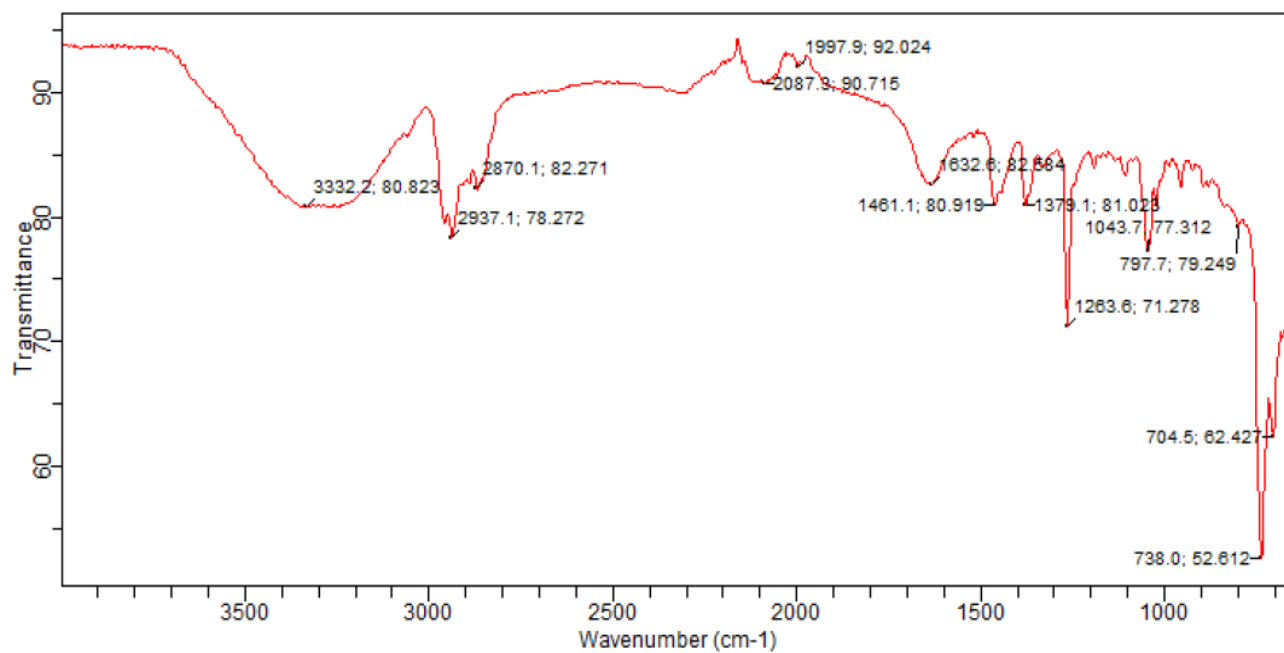
Escudero *et al.*, 1985 [19, 20]. (Table 1) shows the ^1H -NMR and ^{13}C -NMR values in comparison with the previous data available.

^{13}C -NMR and DEPT (Phg. 3 & Phg. 4) (CDCl_3 , 100MHz) of compound 1 has given signal at 140.8(C-5), 121.7(C-6), 71.8(C-3), 56.8(C-14), 56.09(C-17), 50.17(C-9), 45.87(C-24), 42.35(C-13), 42.34(C-4), 40.8(C-20), 39.8(C-12), 37.28(C-1), 36.5(C-10), 36.1(C-20), 33.97(C-22), 31.9(C-7), 31.7(C-8), 29.2(C-25), 28.26(C-16), 29.19(C-2), 24.32(C-15), 23.10(C-28), 26.12(C-23), 21.11(C-11), 19.83(C-26), 19.4(C-27), 19.06(C-19), 18.80(C-21), 11.89(C-18), 12.00(C-29)

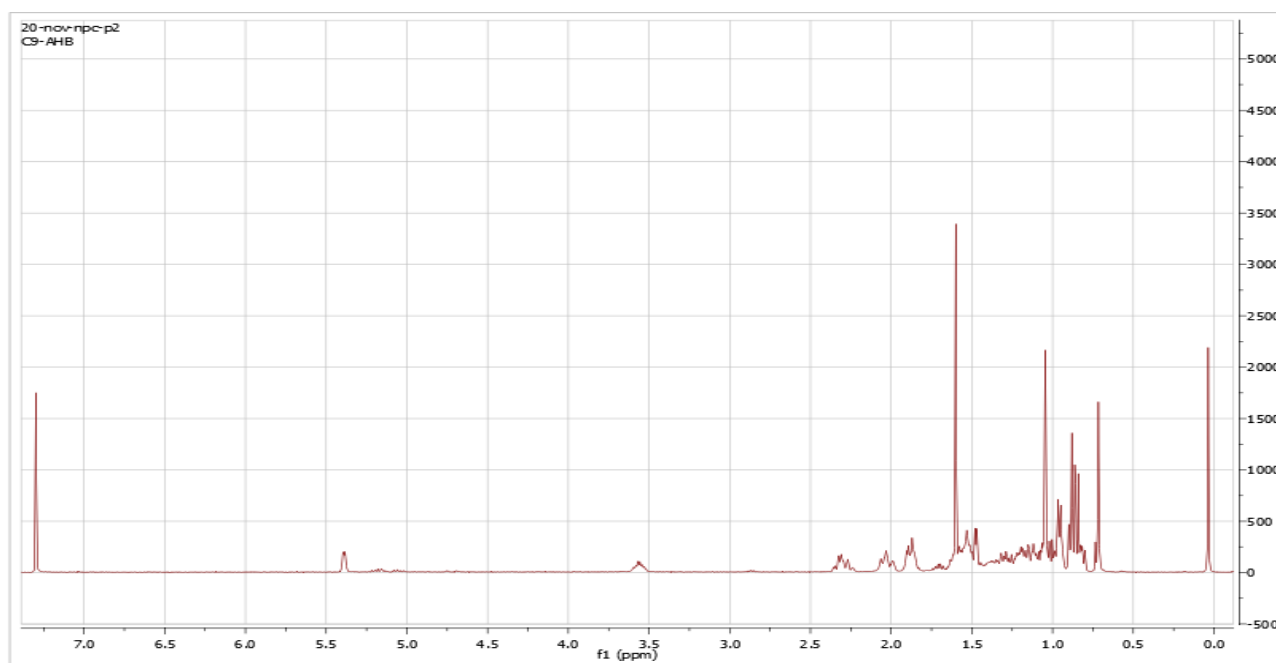
The ^{13}C -NMR of compound 1 has shown recognizable signals at 140.8 and 121.7 ppm, which are assigned C5 and C6 double bonds respectively. The value at 24.32 ppm corresponds to angular carbon atom (C-15). Spectra show twenty nine carbon signal including six methyls, nine methylenes, eleven methane and three quaternary carbons. The alkene carbons appeared at 140.8 and 121.7 ppm. The structure was simulated using ACD/NMR program to obtain the chemical shifts of both proton and carbon. The above spectral features are in closed agreement to those observed for β - Sitosterol according to Manoharan *et al.*, 2005 and Escudero *et al.*, 1985 [19, 20]. On comparison the standard data matched with the simulated data which supports the proposed structure of this compound as β - Sitosterol.

Table 1. ^1H , ^{13}C -NMR chemical shift values for compound-1 in comparison with those reported in literature

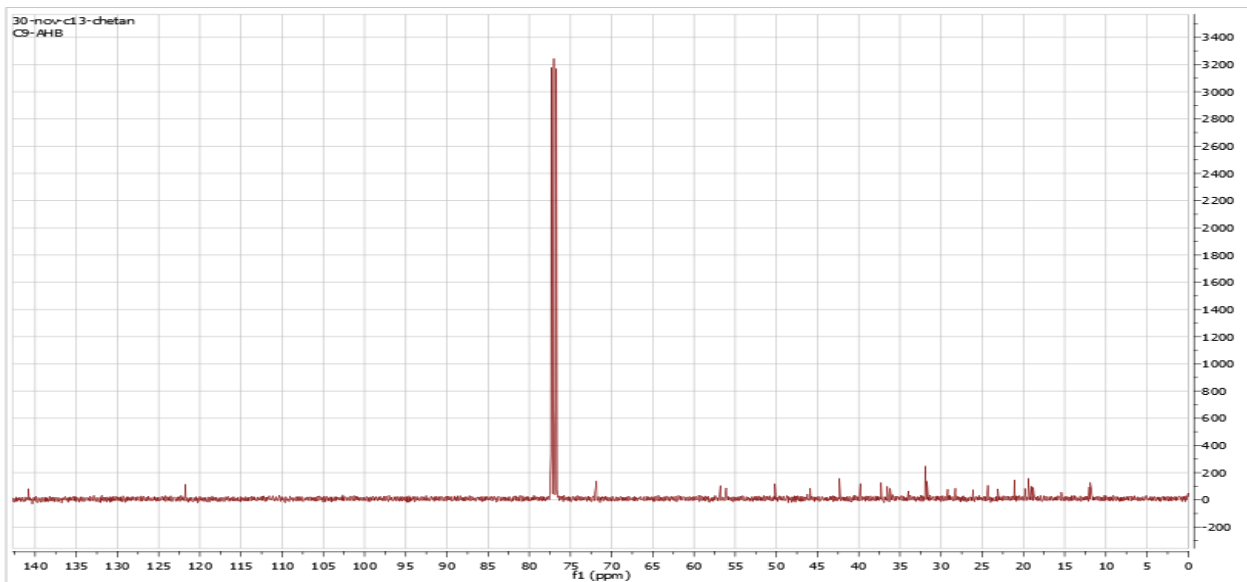
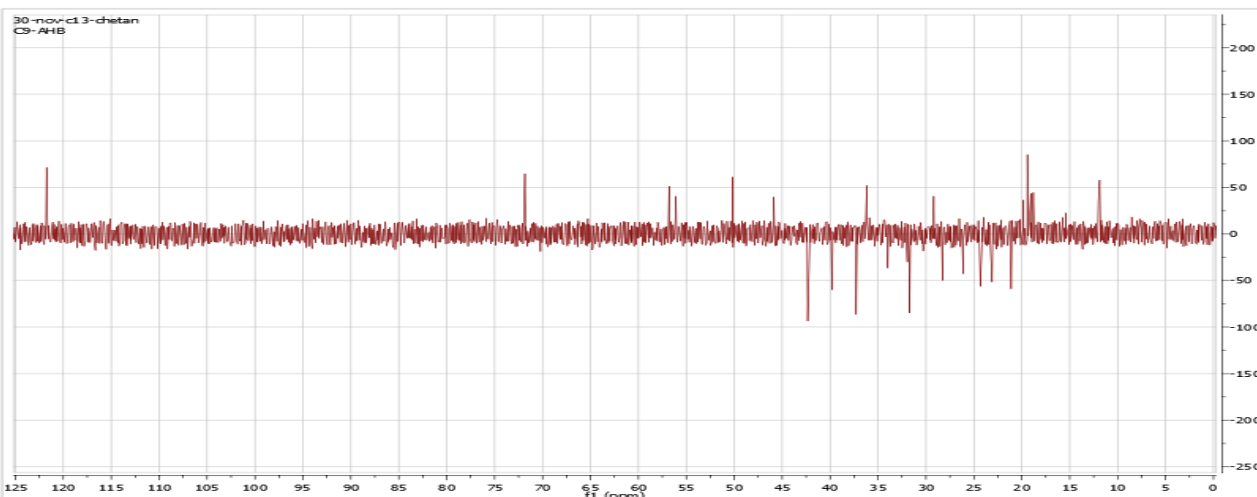
Carbon Atom	^{13}C Reported Literature	in	^{13}C Reported Literature	in	^{13}C NMR Experimental	^1H NMR Reported literature	in	^1H NMR Reported literature	in	^1H NMR Experimental	Nature of Carbon
C-1.	37.3		37.28		37.28						CH ₂
C-2.	30.76		29.19		29.19						CH ₂
C-3.	71.72		71.83		71.8	3.51 m, 1H		3.53 (tdd, 1H)		3.56 m	CH
C-4.	42.2		42.33		42.34						CH ₂
C-5.	140.8		140.7		140.8						C=C
C-6.	121.7		121.73		121.7	5.248 (br,s, 1H)		5.36 (t, 1H)		5.38 br s	C=CH
C-7.	31.9		31.93		31.9						CH ₂
C-8.	31.9		31.69		31.7						CH
C-9.	51.75		51.25		50.17						CH
C-10.	36.5		36.53		36.5			2.00 (m, H-2),		2.03	C
C-11.	21.35		21.22		21.11			1.80 (m, H -2)		1.87	CH ₂
C-12.	39.8		39.80		39.8						CH ₂
C-13.	42.3		42.33		42.35						C
C-14.	58.14		56.89		56.8			1.92 (m, H-1)		1.90	CH
C-15.	24.3		24.32		24.32			1.92 (m, H2)		1.92 s,	CH ₂
C-16.	28.3		28.26		28.26						CH ₂
C-17.	56		56.09		56.09			2.25 (m, 3H)		2.27	CH
C-18.	11.9		11.99		11.89	0.680 s		0.68 (s, 3H)		0.72 (s, 3H)	CH ₃
C-19.	19.4		19.05		19.06	1.07 s		1.08 (s, 3H)		1.05 (s, 3H)	CH ₃
C-20.	36.2		36.16		36.1						CH
C-21.	18.49		18.79		18.80	0.90 (d, 6.4)		0.91 (d, 3H)		0.90 (d, 3H)	CH ₃
C-22.	33.9		33.97		33.97			5.07 (m, 1H)		5.08 (m, 1H)	C=C
C-23.	26.1		26.12		26.12			5.20 (m, 1H)		5.18 (m, 1H)	C=C
C-24.	45.9		45.87		45.87			0.91 (br., H-24)		0.94	CH
C-25.	29.2		29.19		29.2						CH
C-26.	19.8		19.82		19.83	0.812 (d, 6.5)		0.80 (d, 3H)		0.80 (d, 3H)	CH ₃
C-27.	19.3		19.41		19.41	0.838 (d, 6.5)		0.83 (d, 3H)		0.84 (d, 3H)	CH ₃
C-28.	23.1		23.09		23.10						CH ₂
C-29.	12.2		12.25		12.00	0.812 (d, 6.5)		0.82 (t, 3H)		0.83 (t, 3H)	CH ₃



Phg. 1. IR absorption spectra of Compound-1



Phg. 2. ¹H-NMR absorption spectra of Compound-1

Phg. 3. ^{13}C -NMR absorption spectra of Compound-1

Phg. 4. DEPT absorption spectra of Compound-1

Tests for steroid

i). Salkowski reaction: A few crystals of **compound 1** were dissolved in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H_2SO_4). A reddish color was seen in the upper chloroform layer, indicative of steroid ring [21].

ii). Liebermann burchard reaction: A few crystals of **compound 1** 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 or green-blue coloured after a few minutes thus positive indication of steroid ring [21].

Phytochemical tests (Salkowski's test and Lieberman-Burchard test) of the **compound 1** confirm its steroidal nature. Since, the NMR machine indicated steroidal nucleus

and the compound gives positive test for steroids so all of the other structures other than steroids were rejected. Based upon the functional group analysis it was found that the nature of oxygen was hydroxyl, also supported by IR spectroscopy (Perkin Elmer FT-IR). This implies presence of one double bond in the structure. So, the steroids with other functional groups were rejected. Also on considering the nature of oxygen as hydroxyl and presence of one double bond, the general formula for the compound is $\text{C}_n\text{H}_{2n-8}\text{O}$. The exact molecular mass for the formula is found to be 414.37 and the chemical formula could be tentatively $\text{C}_{29}\text{H}_{50}\text{O}$. Therefore it must be a tetra cyclic compound. Based on the analysis of spectral data (IR, ^{13}C -NMR, ^1H NMR and DEPT) the structure of the isolated **compound 1** is proposed as (**Fig. 1**);

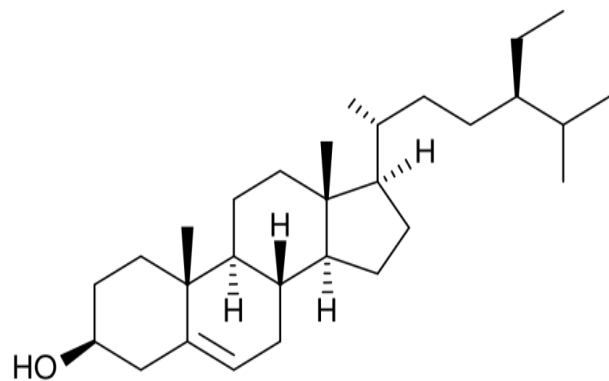


Fig. 1. β -sitosterol (Stigmast-5-en-3 β -ol) (Compound 1)

β -sitosterol is a natural micro-nutrient which is found in the cells and membranes of all oil producing plants, fruit, vegetables, grains, seeds and trees. It has been proven to be a safe, natural and effective nutritional supplement and has shown amazing potential benefits in many diverse applications. Presence of β -sitosterol has been reported in various plants, such as leaves of *Ocimum sanctum* [22], rhizomes of the *Stylochiton lancifolius* [23], fruits of *Corylus colurna* Linn [24] and *Solanum xanthocarpum* [25] as well as in the tissue cultures of *Adhatoda vasica* & *Ageratum conyzoides* [26] and cell suspension culture of *Chrysanthemum coronarium* L. [27].

4.1.1c. Antioxidant activity

DPPH free radical scavenging capacity of the compound 1 was measured by DPPH assay under in-vitro conditions. The

ability of the examined **Compound-1** and its derivatives to act as donor for hydrogen atoms in the transformation of DPPH radical into its reduced form DPPH₂ was investigated. The examined samples were able to reduce the stable purple coloured DPPH radical into yellow coloured DPPH₂ (**Table 2**). **Compound 1** showed most promising radical scavenging activity at concentration of **8 μ g/ml**. These results are plotted in the form of graph (**Fig. 2**). Similar type of results for antioxidant activity of Beta-sitosterol has been previously reported by various researchers. Several findings suggest that beta-sitosterol has antioxidant property [28, 29]. It has also been shown to modulate antioxidant enzymes and human estrogen receptor [30]. It has been reported from a study that beta-sitosterol reduced Oxygen free radical and Hydrogen Peroxide levels in Phorbol myristate acetate (PMA) stimulated RAW 264.7 cells but does not function as a radical scavenger [31]. Glutathione peroxidase (GSH) and Mn superoxide dismutase (SOD) activities are decreased significantly by beta-sitosterol treatment [32]. The efficacy of phytosterols especially Beta-sitosterol extracted from *Dioscorea alata* on antioxidant activities, plasma lipids and hematological profiles was assessed in postmenopausal women by Hsu *et al.*, 2017 [33]. Several other species are reported to possess Beta-sitosterol as an active antioxidant compound (Suhaj, 2006) [34]. These reports from previous literature further strengthen results of our experimental findings.

Table 2. Antioxidant activity of Compound-1

Concentrations	2 μ g/ml	4 μ g/ml	8 μ g/ml	10 μ g/ml	12 μ g/ml
%age inhibition of Comp. 1	30%	25%	41.66%	20.83%	28.33%
%age inhibition Asc. Acid	53.3%	58.3%	64.16%	68.33%	62.5%

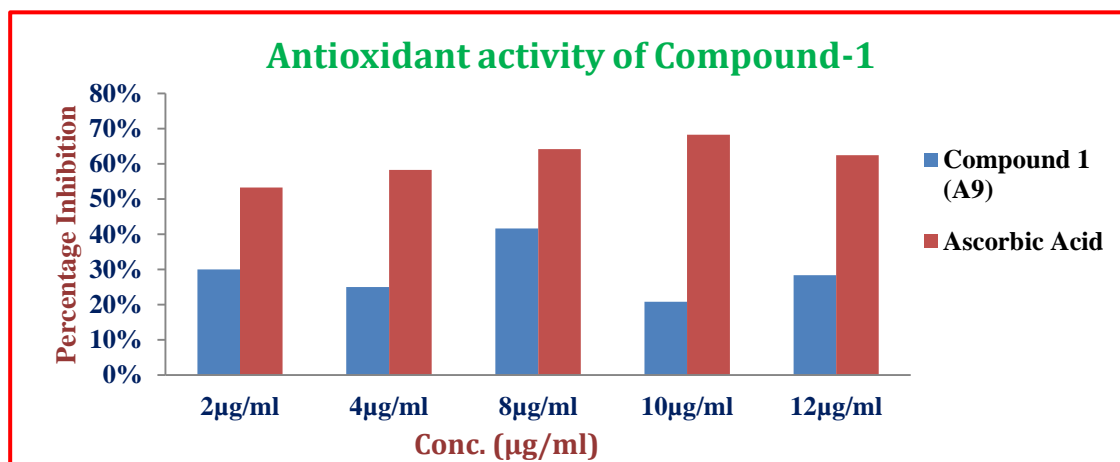


Fig. 2. DPPH radical scavenging activity of Compound-1

IV. CONCLUSION

In our study an attempt was made to isolate the compounds for the first time from rhizomes of *Arisaema utile* and highlight antioxidant potential of the isolated compounds. In our study a steroid was isolated and reported for the first time from rhizomes of *Arisaema utile* and its antioxidant potential was evaluated. The structure of the isolated compound was identified beta-sitosteroid on the basis of spectroscopic methods and by comparing their physical properties reported in the literature. Beta-sitosterol is mainly known and used for its cholesterol lowering property. But studies have shown that the phytochemical may have other health benefits: reducing risk of cancer, prevention of fungal infections and prevention of oxidative damage through its antioxidant activity. The results of the present study suggest that the isolated compound (Beta-sitosterol) from *Arisaema utile* shows promising antioxidant activity. Therefore this plant can hopefully be considered in future for more clinical evaluations and possible applications. We should maintain our efforts in considering and valorizing our natural patrimony as well as conducting more research on *Arisaema utile* with an aim to isolate some Novel compounds with promising pharmacological aspects.

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