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ISOLATION AND CHEMICAL CHARACTERIZATION OF POTENTIAL BIOACTIVE COMPOUNDS FROM *ALBIZIA STIPULATA* BARK

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ABSTRACT: The present research effort was undertaken to isolate phytoconstituents from pharmacologically active extracts of stem bark *of Albizia stipulata* based on in-vitro pharmacological screening and their subsequent characterization. **Methods:** Crude extracts of bark of *Albizia stipulata* were prepared using various solvents such as methanol, hydro alcohol, and water. These extracts were screened for *in-vitro* pharmacological activities like antioxidant, anti-inflammatory, and anti-diabetic activities. The active extract was subjected to column chromatography through a mixture of solvents to get fractions and eluted fractions were run in TLC using various mobile phases with different solvent ratios. The isolated compounds were subjected to IR, 1H NMR, 13CNMR and LC-MS spectral analysis for chemical characterization. **Results:** The methanol extract of bark of *Albizia stipulata* was potent compared to other extracts. This extract was subjected to column chromatography to get fractions, and eluted fractions were run in TLC. The fractions with similar R_f values to standard were united and crystallized. The spectral analysis confirmed that the isolated compounds were found to be β-Sitosteryl-3-O-β-D-glucoside and 3 -O methyl D- Chiro Inositol. **Conclusion:** Various extracts from the bark of the plant *Albizia stipulata* were prepared. B-sitosteryl-3-O-β-D-glucoside and 3-O methyl D-Chiro Inositol were isolated from the methanol extract of bark and characterized.

INTRODUCTION: Herbs used in Ayurveda offer biologically active molecules and lead structures for modification. Derivatives with improved activity and reduced toxicity hereafter, research to stagger on the technical evidence for assumes of plants used for the Indian Ayurvedic system of medicine has accentuated. Furthermore, these preparations are evaluated and distributed properly; our indigenous population can be given better access to effective drug treatment and enhanced health status. Thorough research on the chemistry and pharmacology of plant origin products is significantly necessary and may eventually lead to

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the discovery of new medications and used in the treatment of numerous diseases [1, 2]. *Albizia* is a large genus that belongs to the family Leguminosae comprising 150 species of tropical and subtropical trees and shrubs. These plants have a short lifespan and are scattered in India, Mauritius, China, East Africa, South Africa, Australia, America, Mexico, West Indies and Brazil [3].

Albizia stipulata is a potent medicinal plant that has medicinal values and traditional uses. Common name for *A. stipulata* are nalla regi, bandi chindugu, konda-chiragu. *A. stipulata* is a deciduous tree, up to 20 m tall, crown broad, spreading, and flat-topped. The bark is pale grey or greenish-grey, smooth, horizontally furrowed. Leaves are 11-18 cm long, bipinnate, tomentose: petiole 4.5 cm, gland just below the middle. Flowers are pinkish-white, light yellow, or reddish. This plant occurs naturally in India, Myanmar, Thailand, Indo-China, China, Java, and the Lesser Sunda Island. In India, this plant is seen in Anantagiri, Sunkarimetta, Borra caves, and Molachintapally and is using in traditional medicine for treating inflammation and ulcer effects [4]. This plant is selected from the book “Flora of the presidency of Madras” Vol.1 Adlard & Sons Ltd., London. Rep. Ed. 1997, Dehradun [5]. *Albizia* species are rich in alkaloids, anthraquinones, glycosides, sapogenin, aromatic acids. These species also have triterpenoids, phenolic compounds, flavonoids, tannins and saponins [6, 7].

Bioactive compounds that have antioxidant, anti-microbial and anti-inflammatory properties for treatment of transmittable diseases are searched from medicinal plants by Microbiologists, Natural product chemists and Ethno Botanists. *Albizia* species has a record of use in folkloric medicine, particularly for asthma and allergic disorders and, also practiced in Ayurveda for the treatment of bronchitis, asthma, leprosy, eczema, pruritus, paralysis [8] and has been reported to retain antibacterial, and antioxidant, anti-microbial, Anti-cancer [9, 16,] analgesic anti-inflammatory, and antipyretic [17] and also anti-allergic activities [18]. The therapeutic activity against Parkinson’s disease also has been published [19]. The medicinal plants of this species have been reported for hepatoprotective and antioxidant, acetylcholinesterase inhibitory [20, 21,] anti-diabetic [22,] ant-idiarrheal, antispasmodic, and Bronchodilator effects [23]. *A. stipulata* is a conventionally significant plant and is reported to possess enormous medicinal properties such as anti-ulcer, anti-proliferative, antioxidant, anti-diabetic, anti-microbial and anti-inflammatory, spermicidal, thrombolytic, analgesic, anti-diarrheal, CNS depressant activities of various extract from different parts of the plant and its gum used as a polymer for control released tablets [24, 34,] which exposed that it has been contained terpenoids, steroids, flavonoids, phenols, saponins, inositol, and quinone. Moreover, previous phytochemical studies revealed the presence of several cytotoxic triterpenes, saponins, glycosides, flavonoids such as luteolin, quercetin, steroids namely Stigmasterol, β-Sitosterol, alkaloids, and phenols [26, 35].

The exploration for obviously occurring β-Sitosterol and Inositol has a tremendous concern in manufacturing with scientific research as these compounds have several pharmacological properties. β- Sitosteryl-3-O-β-D-glucoside is known as Daucosterol, has hepatoprotective, hypoglycemic anticancer, antimicrobial, antioxidant activities [36, 42]. It also has anti-allergic and anti-inflammatory properties [43]. 3-O-methyl-D-Chiro-Inositol is also called D-Pinitol, has substantial pharmacological importance; it has extensively used in the treatment of cancer, Alzheimer’s disease, Type-2 Diabetes, and hepatoprotective activity [44, 47].

D-Pinitol also Increases Insulin secretion and regulates hepatic lipid metabolism [48]. It also has antioxidant and anti-inflammatory activities [49, 50]. Therefore, Inositol as a pharmaceutical ingredient is in great require worldwide in developing countries. The availability of allopathic medicine is tedious and is expensive. Phyto constituents are isolated from various herbs can be used to a greater extent due to their fewer side effects. Very little qualitative and quantitative information has on the effective isolation of these bioactive compounds from medicinal plants. It is necessary to explore their content in the *A. Stipulata* stem bark. Thus, considering the vast potentiality of the medicinal plant as a source of antimicrobial, anti-Inflammatory and antioxidant compounds, an attempt has been made to investigate phytochemicals from the bark, two antioxidant, anti-inflammatory principles, namely β-sitosteryl-3-O-β-D-glucoside and 3 -O methyl D- Chiro Inositol have been fruitfully isolated by column chromatography from methanol extract of *A. Stipulata* bark and characterized by spectral analysis. It is the first report on the isolation of bioactive compounds from the bark of *A. Stipulata*.

FIG. 1: PICTURE OF *ALBEZIA STIPULATA* BARK

MATERIALS AND METHODS:

Plant Collection and Authentication: During the vegetative stage, the stem bark of *Albizia stipulata* was collected from a tree grown without pesticide in the fields from Papikondalu, East Godavari district. The herbarium specimen was recognized and authorized by Dr. K. N. Reddy; Dept. of taxonomy, Laila Impex R&D Centre, Vijayawada. The stem bark was collected in March 2011 and placed in a raw drug museum. The voucher no. of bark material was [33, 18]. The Stem bark was cut, cleaned, and dried completely under shade to eliminate moisture content. It was powdered mechanically to a coarse or fine powder for further investigational use.

Preparation of Plant Extract: The extracts of bark were prepared in a chronological procedure. At first by soaking each 100 g of dried bark powder in 600 ml of methanol, hydro-alcohol (60% methanol in water) and 80 g of powder in 600 ml of water for about 48 h to obtain methanol, hydroalcoholic and aqueous extracts of bark of *A. Sipulata*. At last, the plant extracts were filtered using a Whatman filter paper. The filtrates were then concentrated under reduced pressure in a vacuum at 40 °C for 25 min using a rotary evaporator. Codes have been given for the above extracts shown below. ASBM - *Albezia Stipulata* bark methanolic extract. ASBH - *Albezia Stipulata* bark hydroalcoholic extract. ASBW- *Albezia Stipulata* bark water extract.

Pharmacological Screening: The obtained various extracts of stem bark of *A. Stipulata* were screened for *in-vitro* antioxidant, anti-inflammatory, and ant diabetic activities. The ASBM was found to have significant antioxidant and anti-inflammatory activities, and ASBW was found to be potent anti-diabetic activity. In consideration of extractive value and activity, ASBM was forwarded to fractionation for isolation of phytoconstituents.

Column Chromatography: 30 g of methanol bark extract was adsorbed on 90 gm of silica gel, 120 gm of the adsorbed extract was placed over 360 gm of silica gel (100-200 mesh) (Merck) and then eluted with ethyl acetate, a mixture of ethyl acetate & methanol and methanol of rising polarity to get portions. The solvents bed volume for the elution was 750 ml. Elution first starts using 100% ethyl acetate and then using a blend of ethyl acetate and methanol in the proportion of 99:1, 95:5, 90:10, 80:20, 50:50, and finally 100% methanol. All the obtained fractions were dart for TLC. Based on the TLC profile, fractions with similar R_f values were pooled into fractions 1-8 and filtered through a Whatman number-1 filter paper. The filtrates were concentrated under reduced pressure in a rotary vacuum evaporator and air-dried to a constant weight at room temperature. The collected fractions (F1-8) were screened for DPPH radical scavenging activity and alpha-glycosidase assay. Fractions 5, 6, and 7 have been found good antioxidant activity compared to the remaining fraction. Fraction-6 was column chromate graphed for isolation.

Isolation of Bioactive Compounds from Fraction: 3 g of fraction 6 (F-6) was adsorbed on 9 g of silica gel finally, 12 g of silica gel adsorbed extract was packed over 100 g of the silica gel column and chromate graphed. The bed volume of solvents to run the column was 150 ml. At initial the extract was eluted with chloroform and then a mixture of chloroform and methanol in a ratio of 98:2, followed by 95:5, then 90:10 and finally the ratio was altered to 80:20.

Thin Layer Chromatography (TLC) Procedure: The TLC progress was put as twin through chamber were scrutinized in a variety of solvent systems such as chloroform, methanol, hexane, and ethyl acetate. The best probable solvent system for the recognition of compounds was determined by varying the ratios of solvents.

These fractions were run on a silica gel 60 F254 pre-coated aluminum plate of 0.2 mm thickness for the observation of spots after collection. Visualization was accomplished through the UV chamber. The retardation factor (R_f) was considered using the following formula.

R_f = Distance traveled by the solute/Distance moved by the solvent.

Characterization of Bioactive Compounds: Isolated bioactive compounds were imperiled to spectral study *via* FT-IR, NMR, and LC-MS for identification.

FT-IR spectra were recorded on Bruker Alpha TKBR and ATR spectrophotometer functioning at 500-4000cm^{-1} using the KBr pellet method that shows peaks at diverse wavenumber ranges.

1H and 13C-NMR spectra were run on a Bruker AV NMR instrument equipped with 5 mm 1H and 13 °C operating at 400 MHZ and 100MHZ respectively with tetramethylsilane (TMS) as an internal standard.

An Agilent 6400 series Triple, Quad (QQQ) LC-MS (Agilent, Beijing, China) apparatus qualified with an electrospray ionization source (ESI) was operated to analyze the isolated compounds. The optimized detection variables were as follows:

Chromatographic conditions: Wavelength: 190 to 400 nm, Flow rate: 0.4 ml/min, sampling volume: 5µl, and column temperature: 30 °C.

Mass spectrometry conditions: negative ion mode, atomization gas pressure: 40 psi, dry gas, velocity: 9l min, drying temperature: 350 °C, ionization voltage: 3,000V, electron spray ionization (ESI), detection of anion way-auto MSN, scanning range: 200-800 m/z.

RESULTS AND DISCUSSION:

Total Yield Extracts: The plant's bark was collected, dried, and extracted with various solvents. The final yield of extracts in different solvents was determined and listed in **Table 1.**

TABLE 1: FINAL YIELD OF VARIOUS EXTRACTS IN DIFFERENT SOLVENTS

Extract Code	Quantity (g)	Solvents used	Volume (ml)	Yield (g)
ASBM	100	Methanol	600	9.60
ASBH	100	Hydro alcohol	600	13.52
ASBW	80	Water	600	10.32

The three extracts were screened for *in-vitro* radical scavenging, anti-inflammatory and hypoglycemic activities. The methanol extract of bark was established to be a powerful antioxidant, anti-inflammatory, and hypoglycemic activity; hence it has been exposed to column chromatography for fractionation.

Fractionation: About 10 fractions were isolated from methanol extract using several solvents (%) of improving polarity. Based on the TLC visibility, fractions with similar R_f values were united into fractions 1-8 (F-1 to 8) and crystallized. This process was repeated several times to get desired quantities. The weight (g) of various fractions isolated with different solvents and the mixing pattern are given in **Table 2.** The isolated fractions were also evaluated for antioxidant and anti-inflammatory activities. Fractions- 5, 6, and 7 found good DPPH radical scavenging activity compared to the remaining fraction. Fraction-6 was further isolated by chromatography.

TABLE 2: MIXING PATTERN OF ASBM COLUMN

Name of the Solvent	Fractions	Weight (g)
Ethyl acetate	F-1	2
Ethyl acetate	F-2	0.5
Ethyl acetate	F-3	0.6
1% methanol in ethyl acetate	F-4	0.3
5%methanol in ethyl acetate	F-5	0.1
5%,10% and 20% methanol in ethyl acetate	F-6	3.79
50% methanol in ethyl acetate	F-7	6.74
Methanol	F-8	0.8

Isolation of Bioactive Compounds: About 5 subfractions were isolated (SF-1-5) from F-6 based on the TLC profile. The SF-4 was isolated with 10% methanol in chloroform and the spot was identified in the TLC had mobile phase chloroform: methanol in a ratio of 8:2 showed an R_f value of 0.8 equal to that of standard Sitosteryl-3-O-β-D-glucoside. The SF-4 genuine compound crystals were produced, filtered, and cleaned with methanol.

The crystals were named Compound-1. The SF-5 was isolated with 20% methanol in chloroform and the spot was recognized in the solvent system chloroform: methanol 6:4 showed an R_f value of 0.5 equal to that of reference Methyl Inositol.

The SF-5 pure crystalline compound was filtered from the mother liquor and washed with hexane named Compound -3.

Mixing patterns for all subfractions of fraction 6 are shown in **Table 3.**

TABLE 3: MIXING PATTERNS FOR SUBFRACTIONS OF F-6

Name of the solvent	Volume(ml)	SubFractions	Yield (g)
Chloroform	4×150	SF-1	0.6
2% methanol in chloroform	5×150	SF-2	0.01
5% methanol in chloroform	5×150	SF-3	0.10
10% methanol in chloroform	8×150	SF-4	0.5
20% methanol in chloroform	2×150	SF-5	0.8

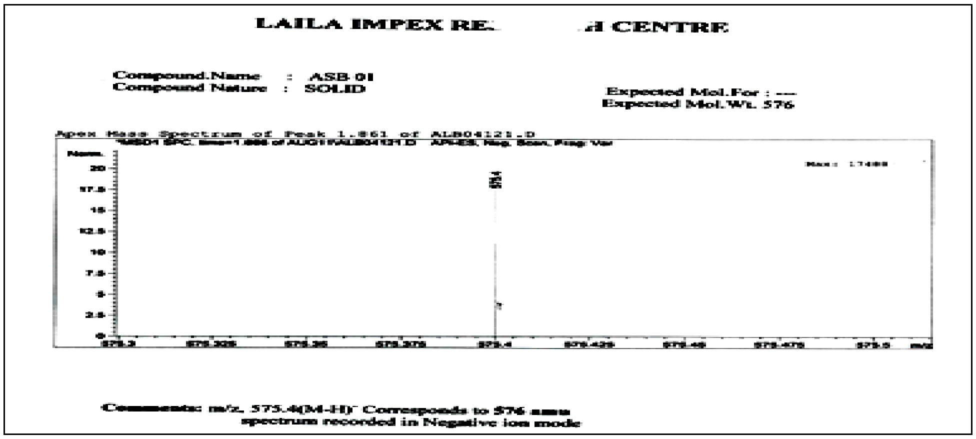


FIG. 2: MASS SPECTRUM OF ISOLATED COMPOUND-1

FT-IR Analysis: Fourier transmission Infra-red (FT-IR) spectrum of compound-1 was represented in **Fig. 2.** showed absorption bands at 3407 cm-1 for -OH stretching, at 2934 and 2871 cm^{-1} were due to the presence of C-H (aliphatic) asymmetric and symmetric stretching, at 1462 and 1373 cm^{-1} showed the presence of methyl ($-CH_3$) and methylene ($-CH_2-$) group. The absorption frequency at 1067 and 1024 cm^{-1} indicated (>C-O-C<) the structural feature of the compound. The absorption band at 1632 cm^{-1} (C=C stretching) along with the band at 800 cm^{-1} were due to the presence of a trisubstituted double bond (>C=CH) as in the spectrum.

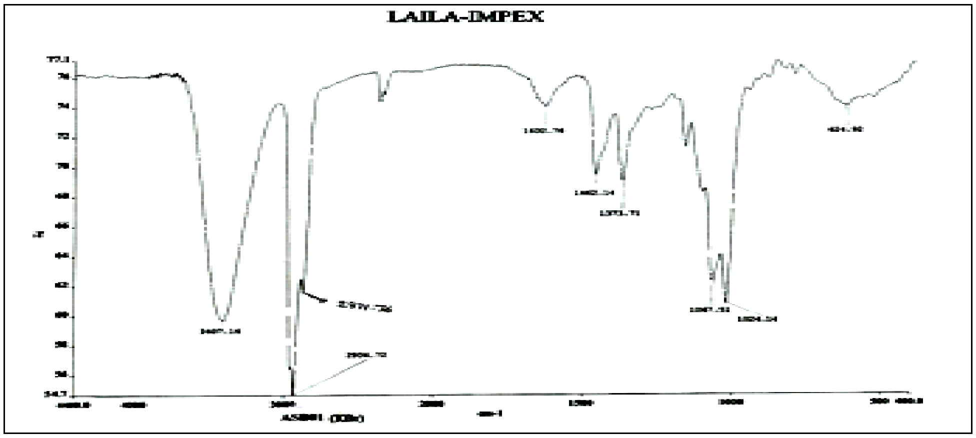


FIG. 3: FT-IR SPECTRUM OF ISOLATED COMPOUND-1

NMR Analysis: 1H NMR spectrum [400 MHz, CDCl3] of compound-1 represented in Figure 3 shows signals corresponding to protons. The anomeric proton of sugar residue appeared as a doublet (J=7.76) at 4.409 ppm. The olefinic proton resonated as a broad singlet (J=4.8) at δ 5.362 ppm indicated the presence of >C=C< in the ring system. The spectrum further reveals two singlets at δ 0.695 and 0.909 ppm each of three proton intensities, assigned to two tertiary methyl groups at C-18 and C-19, respectively. A doublet (J=6.5) at δ =0.93 ascribes to methyl protons at C-21. The spectrum also displayed two doublets (J = 6.4) at δ0.83 and 0.80ppm, which correspond to two methyl protons at C-26 and C-27, respectively. Oxygenated methyne and methylene protons of sugar residue were observed as multiplet in the region of 3.23 to 3.64 ppm and were attributed to. On the other hand, a triplet (J = 7.2) at δ 0.84 attributed to a three protons intensity of primary methyl group at C-29, which was attached to the methylene group. The sugar moiety hydroxyl groups were appeared resonance at 3.56-3.6 and were assigned to C- 2´, C-3’ C-4 and C-6’ respectively as in the spectrum. The spectrum revealed the presence of different types of neighboring H atoms and their splitting. 1H NMR Chemical shift values are shown in **Table 4.**

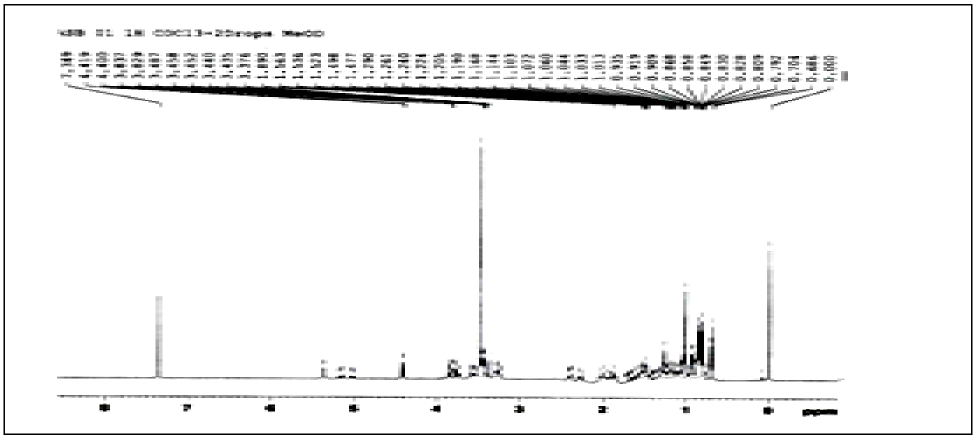


FIG. 4:1H NMR SPECTRUM OF ISOLATED COMPOUND-1

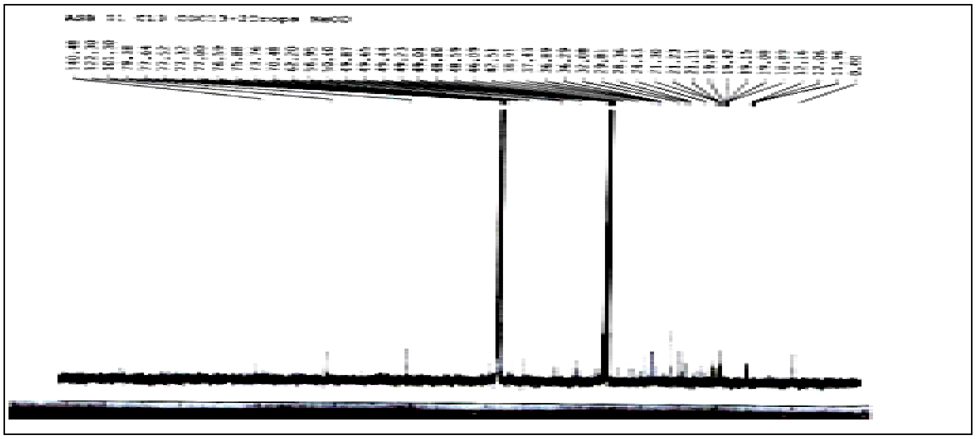


FIG. 5:13C NMR SPECTRUM OF ISOLATED COMPOUND-1

TABLE 4: 1H NMR CHEMICAL SHIFT VALUES OF B- SITOSTERYL-3-O-B-D-GLUCOSIDE

Position	Type of proton	δ (ppm)
5 and 6	CH=CH	5.362 (s, J=4.8Hz)
8,9	CH,CH	1.36(m, H),0.85 (m, H)
11	CH2	1.42 (m, 2H)
18,19	CH3,CH3	0.695(s, 3H), 0.909(s, 3H)
21	CH3	0.93(d, 3H, J=6.5Hz)
26	CH3	0.83(d, 3H, J = 6.4 Hz)
27	CH3	0. 80 (d, 3H J= 6.4 Hz)
29	CH3	0.84 (t, 3H, J = 7.2 Hz)
2,3,4, 5	CH	3.23, 3.36, 3.26,3.28
6’	CH_2	3.87, 3.64 (m, H)
1´of sugar	Anomeric	4.409(d1H, J=7.76 Hz)
2,3	OH	3.56(d,J=4.5),3.56(d, J=4.5)
4,6	OH	3.4 (d, J=4.5), 3.6 (t, J=4.5)

The 13C NMR spectrum [100 MHz, CDCl3] of compound-1 in Figure 4 revealed the presence of 35 carbons [29]. Signals were due to skeleton and 6 signals for a sugar residue. A signal at δ 101.09 ppm corresponding to anomeric carbon (C1) of the sugar residue, linked to the steroid aglycone part by ß-linkage. Three quaternary carbons C5, C10, and C13 were assigned by the signals at δ 140.48, 36.89, and 42.51, respectively as in the spectrum. The C 18 and C29 carbons have appeared at 12.0 and 12.16ppm. The signals at 19.0, 19.15, and 19.42 ppm are attributed to C19, C21 and C 27 carbons, respectively. The C 26 carbon resonated at 19.87. The 13C-NMR data of the sugar residue was identical to that of the reported signals of glucose residue. The 13C signals for each carbon atom of the identified compound-1 have been recorded by numbering the carbon atoms. The 13C NMR chemical shift values are shown in **Table 5.**

TABLE 5: 13 C NMR CHEMICAL SHIFT VALUES OF β- SITOSTERYL-3-O-β-D-GLUCOSIDE

Position	Type of proton	δ (ppm)
1’	Anomeric carbon of sugar	101.09
5	Quaternary Carbon	140.48
10	Quaternary Carbon	36.89
13	Quaternary Carbon	42.51
18	CH3	12.0
19	CH3	19.0
21	CH3	19.15
26	CH3	19.87
27	CH3	19.42
29	CH3	12.16

The structural assignment of compound-1 was performed based on spectral analysis and comparisons with the literature [51, 53].

The data are resemblance very well with those reported, and the structure was identified as β-sitosteryl-3-O-β-D-glucoside. Chemically, it is 3-O beta glucopyranosyl-24-ethyl cholest-5-en-3β-ol.

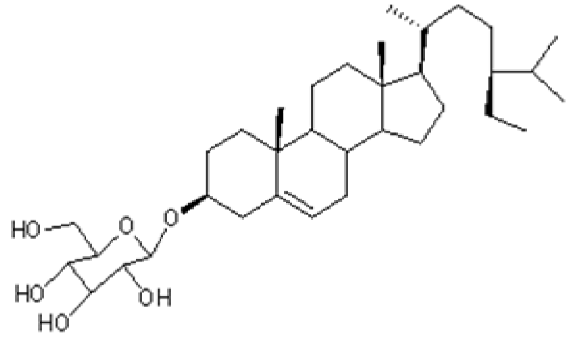
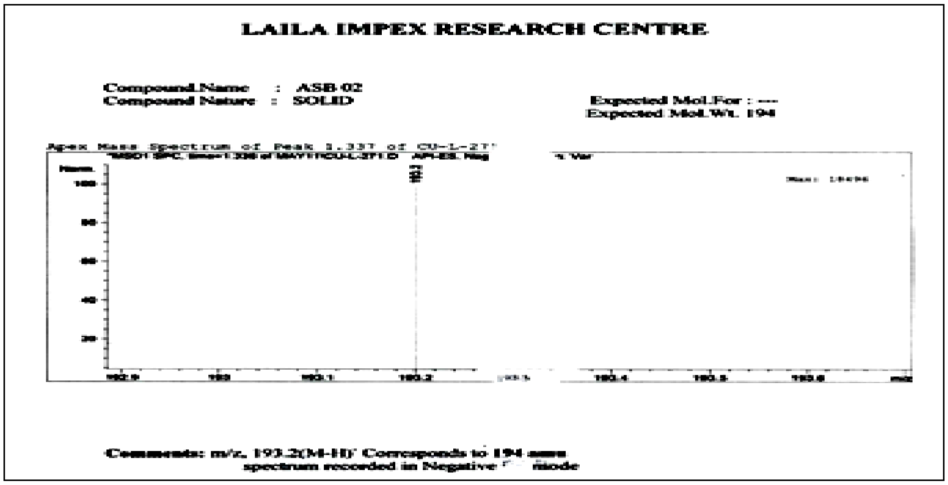


FIG. 6: CHEMICAL STRUCTURE OF B-SITOSTERYL-3-O-B -D-GLUCOSIDE

Compound-2: The pure compound ASB-2 was obtained as a colorless white crystalline solid having a melting point 180-182 °C.

Mass Analysis: The molecular weight of pure compound-2 was established by LC-MS analysis. Based on the presence of negative ion [m/z 193(M-H)-] confirmed that the molecular weight is 194 as in **Fig. 6.** The empirical formula was shown to be $C_7H_{14}O_6$.


LAILA IMPEX RESEARCH CENTRE

Compound.Name : ASB-02
Compound Nature : SOLID

Expected Mol.For : ---
Expected Mol.Wt. 194

Apex Mass Spectrum of Peak 1.337 of CU-L-27'

Comments: m/z. 193.2(M-H)' Corresponds to 194 amu
spectrum recorded in Negative mode

FIG. 7: MASS SPECTRUM OF ISOLATED COMPOUND-2

FT-IR Analysis: Fourier transmission Infra-red (FT-IR) spectrum of compound-2 was represented in **Fig. 7** showed characteristic absorption bands at 3403.65, 3318 cm^{-1} due to stretching of O-H group, peaks at 2951.77 and 2907.38 cm^{-1} due to C-H stretching, absorption peaks between 1452.43-1341.83 cm^{-1} due to CH_3 of methoxy group. The absorption peak at 1452.43 cm^{-1} due to asymmetric bending deformation of CH_3 and at 1381.44 cm^{-1} due to bending vibration of the CH_3 group.

The bending vibrations of H-O were observed between 1280.73- 1192.87 cm^{-1}, C-O stretching appeared at 1129cm−1and absorption peaks at 1072.47, 1002, and 961 cm^{-1} due to C-O-C stretch.

The C-C and C-H bending vibrations were observed between 901-750 cm^{-1} and 661-437 cm^{-1} respectively.

The pure compound ASB-2 was obtained as a colorless white crystalline solid melting 180-182 °C.

Mass Analysis: The molecular weight of pure compound-2 was established by LC-MS analysis. Based on the presence of negative ion [m/z 193(M-H)-] confirmed that the molecular weight is 194 as in **Fig. 6.** The empirical formula was shown to be $C_7H_{14}O_6$.

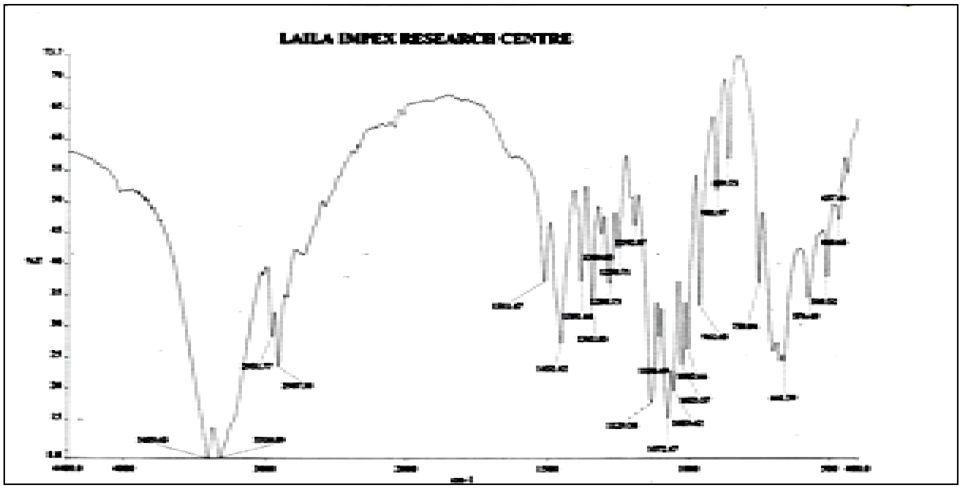


FIG. 8: FT-IR SPECTRUM OF ISOLATED COMPOUND-2

TABLE 6: 1 H NMR CHEMICAL SHIFT VALUES OF 3 -O-METHYL -D- CHIRO INOSITOL

Position	Type of Proton	δ (ppm)
3	OCH_3	3.536 (s,3H)
3	CH	3.276(t,1H, J=10.0Hz)
4	CH	3.574(t,1H, J=9.6Hz)
2	CH	3.670(dd,1H, J2,3=10.0, J2,1=2.8Hz,)
5	CH	3.756(dd,IH, J5,4=10.0, J5,6=2.8Hz)
1 and 6	CH	3.974 (m,2H)

TABLE 7: 13C NMR CHEMICAL SHIFT VALUES OF 3 -O-METHYL -D- CHIRO INOSITOL

Position	Type of Carbon	δ (ppm)
3	OCH3	59.70
4	CH	69.91
2	CH	70.66
6	CH	71.55
1	CH	71.77
5	CH	72.18
3	CH	82.81

NMR Analysis: 1H NMR spectrum [D2O, 400 MHz,] of compound-2 represented in **Fig. 8** shows signals corresponding to protons. The methoxy protons appeared as a singlet at 3.536 ppm and the six oxygenated methylene protons are resonated with different chemical shift values. Two protons appeared as triplets (J=10Hz, J=9.6Hz) at 3.276 and 3.574 ppm assigned to protons at C-3 and-4. A doublet of doublet (J2, 3=10.0, J2, 1=2.8Hz) at 3.670 ppm ascribed to proton at C-2.

The spectrum also displays a doublet of doublet (J5, 4=10.0, J5, 6=2.8Hz) at 3.756ppm corresponding to proton at C-5 and multiplet at 3.974 ppm two protons at C-1 and C-6, respectively. The spectrum disclosed the occurrence of various types of neighboring protons and their splitting. The 1H NMR delta (δ) values are shown in **Table 6.**

The 13CNMR spectrum [D2O, 100 MHz] of compound-2 in **Fig. 9.** shows the presence of methoxy group resonating at 59.70ppm, and six oxygenated methylene carbons are resonated at various chemical shift values. The C-4 is at 69.91, C-2 is at 70.66, C-6 is at 71.55, C-1 is at 71.77, C-5 is at 72.18, and C-3 is at 82.81 ppm. These signals indicated the presence of the Inositol skeleton. The 13CNMR chemical shift values are shown in **Table 7.**

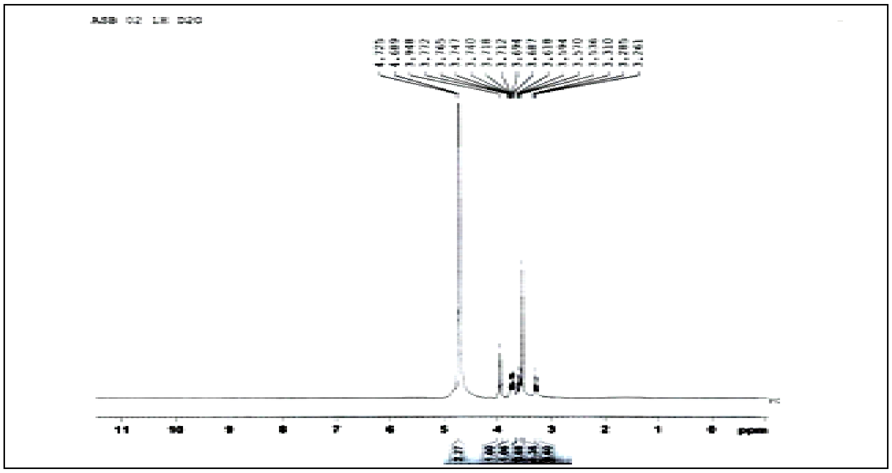


FIG. 9: 1H NMR SPECTRUM OF ISOLATED COMPOUND-2

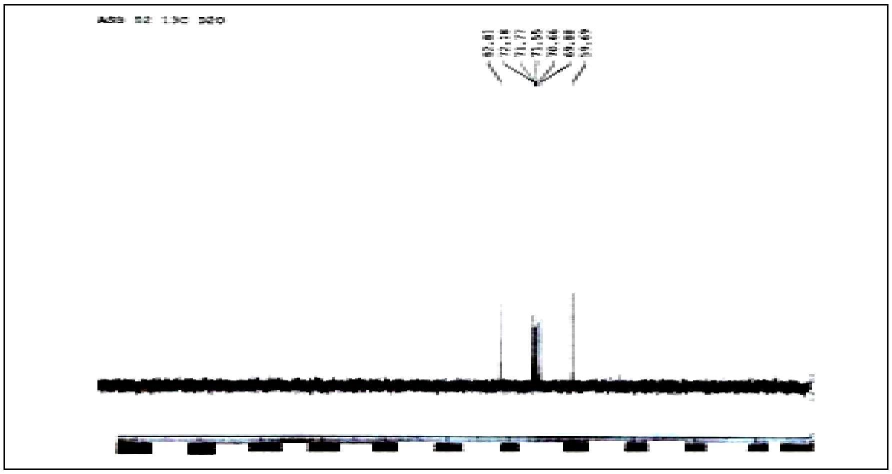


FIG. 10: 13C NMR SPECTRUM OF ISOLATED COMPOUND-2

The structural assignment of compound-2 was performed based on spectral analysis and comparisons with the literature [54, 55]. The data are corroborating well with those reported, and the structure was identified as Methyl Inositol. Chemically, it is 3-O-Methyl-D-chiro-Inositol.

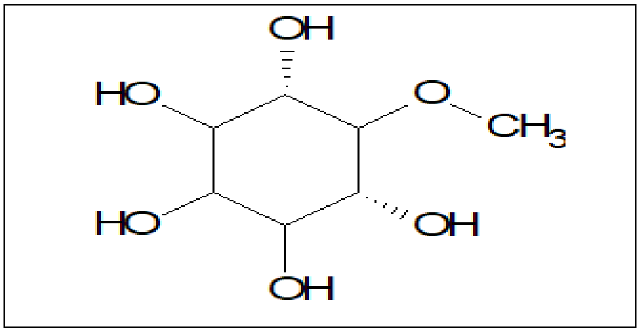


FIG. 11: CHEMICAL STRUCTURE OF 3 -O-METHYL-D-CHIRO INOSITOL

CONCLUSION: From the present work, the methanol extract of bark of *A. Stipulata* showed significant antioxidant and anti-inflammatory activities, hence forwarded to fractionalize and column chromatography for isolation of phytoconstituents. β -sitosteryl-3-O-β-D-glucoside and Methyl Inositol have been isolated fruitfully with various solvents. It is initiated that the solvent plays an important role in the extraction of phytochemicals. B-sitosteryl-3-O-β-D-glucoside was isolated using 10% methanol in chloroform compared to the 5% methanol in chloroform and Methyl Inositol was isolated with 20% methanol in chloroform. The identification of β-sitosteryl-3-O-β-D-glucoside and Methyl Inositol was endeavored by direct comparison with its retardation factor. The isolated compounds were recognized through FT-IR, NMR, and mass spectral data. This method is a simple, rapid, and highly efficient extraction method for isolating potent bioactive components from the *Albizia stipulata* plant.

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