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ANTI-DIABETIC ACTIVITIES OF ISOLATED COMPOUND BETA-SITOSTEROL FROM THE ETHANOLIC EXTRACT OF STEM OF *ANDROGRAPHIS ECHIOIDES*

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Keywords:

Andrographis echioides stem, betasitosterol, Anti-oxidant activity, alpha amylase and alpha glucosidase inhibitory activity

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ABSTRACT: To study the anti-oxidant and anti-diabetic activities of isolated compound beta-sitosterol from the ethanolic extract of stem of *Andrographis echioides* under the *in-vitro* model. The isolation was done using column chromatography using gradient elution with different mobile phases. Structural elucidation was carried out on the basis of spectral analysis. Anti-oxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl scavenging assay. The anti-diabetic activity was evaluated by the inhibitory potential of isolated compound beta-sitosterol against alpha-amylase and alpha-glucosidase assays. The study revealed that the beta-sitosterol exhibited significant α-amylase (58.57±0.29) and α-glucosidase (43.85±0.032) inhibitory activities respectively and well compared with standard acarbose drug. The beta-sitosterol showed the best scavenging activity (74.46±0.036) against the tested radicals like 1,1-diphenyl-2-picrylhydrazyl, The infra-red spectrum specific absorption bands for beta-sitosterol *viz*: 3427.78 cm^{-1} (O-H stretching.); 2937.40 cm^{-1} (aliphatic C-Hstretching); 1640.58 cm^{-1} (C=C absorption peak); other absorption peaks includes 1464.31cm^{-1} (CH_2); 1381.56 cm^{-1} (OH def), 1054.11 cm^{-1} (cycloalkane) and 800.97 cm^{-1}. Structural elucidation of beta-sitosterol was done by spectrum analysis such as 13C and 1H depth nuclear magnetic resources. Therefore, it is concluded that beta-sitosterol is a potential source for natural anti-oxidant and anti-diabetic compounds and could have potential use in the management of diabetes mellitus.

INTRODUCTION: Diabetes mellitus is a chronic metabolic disorder, and it also affects the metabolism of carbohydrates, protein, and fat. The main reason is the production of the low amount of insulin by pancreas [1]. Type I diabetes occurs due to low amount of insulin production by **β** cells, while type II diabetes occurs due to **β** cell dysfunction [2].

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Diabetes mellitus type 1 and type 2 are associated with microvascular complications and macro-vascular complications. Microvascular compli-cations: Hyperglycemia and hypertension is the major reason for microvascular complications. Diabetic nephropathy is the leading cause of mortality [3, 4].

The enzymes alpha-glucosidase are responsible for the breakdown of oligo- and disaccharides to monosaccharides. α -amylase and α- glucosidase inhibitors is useful for lowering the process of glucose absorption and decreases glucose level in blood [5, 6]. Diabetes mellitus patients suffer from a high blood sugar level, unusual thirst, frequent

urination, extreme hunger, loss of weight, blurred vision, nausea and vomiting, extreme weakness and irritability, tiredness and mood change [7]. Inhibitors of amylase and α glucosidase are responsible for the high amount of glucose in the blood [8]. Now day's herbal medicines are more effective than synthetic medicines. There is no side effect while using herbal medicine [9, 10].

Andrographis echioides belong to the Acanthaceae family, and its Tamil name is Gopuramthangi [11, 12]. It is an ayurvedic herb plant used in the treatment of many ailments such as anti-inflammatory, anti-arthritic, antimicrobial, anti-ulcer, anti-oxidant activity, hair problems, *etc*., [13-14].

Their phytochemical constituents like flavonoids, tannins, phenol, glycosides, terpenoids, saponins, steroids, *etc., Andrographis echioides* is a medicinally valuable species widely distributed in the tropical region in South Asian countries [15-19].

Therefore, in the present study, the anti-oxidant and anti-diabetic activities of beta-sitosterol isolated from the ethanolic extract of stem of *Andrographis echioides* were evaluated employing *in-vitro* assay methods.

MATERIALS AND METHODS:

Collection of Plant Material: The stem of *Andrographis echioides* was collected from Manarkkudi, Mayiladurai, Nagapattinam district, Tamil Nadu, India, in December 2019. It was authenticated by Dr. N. Ravichandran, Research associate, Centre for advanced research in Indian system of Medicine, (CARISM) and SRC; SASTRA Deemed to be University, Tirumalaisamuthiram, Thanjavur (Voucher no. SRC SASTRA 0005).

Chemicals and Reagents: All the chemicals, including solvents such as ethyl acetate, n-hexane, chloroform, methanol, anisaldehyde sulphuric acid reagents (0.5 ml p-anisaldehyde in 50 ml glacial acetic acid and 1 ml conc. sulfuric acid was of analytical grade and was procured from E. Merck, India. Alpha (α)-Glucosidase, porcine pancreas alpha (α)-amylase, p-nitrophenyl-α-D-gluco-pyranose (p-NPG), 3,5-dinitrosalicylic acid (DNS), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid and acarbose were purchased from Sigma Chemical Co. (St. Louis, MO, USA) was purchased from Sigma-Aldrich, New Delhi.

Preparation of Ethanol extracts: The stems of *Andrographis echioides* were washed in running water, cut into small pieces and then shade dried for a week at 35-40 ºC, after which it was grinded to a uniform powder of 40 mesh size. The ethanol extracts were prepared by soaking 1.5 kg of the dried powder plant materials in 1 L of ethanol by continuously using a soxhlet extractor for 10 hr. The extracts were filtered through Whatman filter paper No. 42 (125mm). The filtered extract was concentrated and dried by using a rotary evaporator under reduced pressure. The final dried samples (998 g) were stored in labeled sterile bottles and kept at -20 ºC. The filtrate obtained was used as a sample solution for further isolation [20].

Isolation of Beta-sterol by Column Chromatography: The condensed methanol extract of the stem (500 g) of the sample was subjected to column chromatography over TLC grade silica gel. Elution of the column first with n-hexane, an increasing amount of ethyl acetate in n-hexane and finally with methanol yielded a number of fractions. The preparation of solvent systems used to obtain beta-sitosterol (234 mg/500 g) was ethyl acetate-methanol (60:40) from fraction 7. The compounds were detected on TLC plates by spraying with Libermann Burchard reagent and heated at 100 °C for 10 min [21].

Purification of Isolated Compounds Beta-sitosterol by HPTLC and High-Performance Liquid Chromatography:

High-Performance Thin-layer Chromatography (HPTLC): The isolated pure compound was dissolved in appropriate solvents. 5 µl of isolated compounds (beta-sitosterol) were applied to silica gel plates, Merck (Germany) 20 × 20 cm, 0.25 mm in thickness. Plates were developed using the solvent system n-hexane: ethyl acetate (8:2 v/v). The separated zones were visualized with freshly prepared Libermann Burchard reagent and heated at 100 °C for 10 min. Chromatograms were then examined under daylight within 10 min [22].

High-performance Liquid Chromatography (HPLC): The analytical HPLC system (Shimadzu) was equipped with a diode array detector, a 20 µl

loop, 200 × 4.6 mm C18 column, methanol (HPLC grade, 0.2 mm filtered) used as a mobile phase. The isolated beta-sitosterol compounds were separated using a mobile phase of n-hexane: ethyl acetate (8:2 v/v) at a flow rate of 1.0 ml/min, column temperature 30 °C. Injection volume was 40 µl, and detection was carried out at 346 nm [23].

Structural Elucidation Study of Isolated Compound: Different spectroscopic methods including U.V., FTIR, 1H NMR, 13C NMR were used to elucidate the structure of isolated compounds. The U.V.–visible spectrum of the isolated compounds in methanol was recorded using a Shimadzu 160A U.V.–visible spectro-photometer. The Fourier Transform Infrared (FTIR) spectra were recorded with a nominal resolution of 4 cm^{-1} and a wavenumber range from 400 to 4000 cm^{-1} using the KBr pellet technique.

1H and 13C NMR spectra were acquired on Bruker WP 200 SY and AM 200 SY instruments (1H, 200.13 MHz; 13C, 50.32 MHz) using TMS as internal standard and $CDCL_3$ as solvent. GC-MS analysis of the extract was performed using a Perkin-Elmer G.C. Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with an Elite-I, fused silica capillary column (30 mm × 0.25 mm 1D × 1 µMdf, composed of 100% Dimethylpolysiloxane) [24, 25].

Anti-oxidant Activity (DPPH Free Radical Scavenging Activity) Determination: The anti-oxidant activity of the isolated compound beta-sitosterol was examined on the basis of the scavenging effect [26]. Ethanolic solution of DPPH (0.05 mM) (300 µl) was added to 40 µl of isolated compound with different concentrations (20 – 100 µg/ml). The freshly prepared DPPH solution was kept in the dark at 4 °C. 96% (2.7 ml) of ethanol was added and shaken vigorously. The mixture was kept constant for 5 min, and absorbance was measured at 517 nm spectrophotometrically. Ethanol was used to set the absorbance at zero. A blank sample was also prepared, which contains the same amount of ethanol and DPPH. All the determinations were performed in triplicate. The radical scavenging activities of the tested samples expressed are calculated as a percentage of inhibition according to the equation,

DPPH activity (% inhibition) = [(A – B) / A] × 100

Where A and B are the absorbance value for the test and blank sample, respectively.

***In-vitro* Antidiabetic Activity of Beta-sitosterol from the Stem of *Andrographis echnoides*:**

Alpha-Amylase Inhibitory Assay: The alpha-amylase inhibitory assay was carried out by using the isolated compounds beta-sitosterol from the stem of Andrographis echioides. A total of 250 µL of beta-sitosterol compound (20-100 µg/ml) was placed in a tube and 250 µL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) was added. This solution was pre-incubated at 25 ºC for 10 min, after which 250 µL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at regular time intervals and then further incubated at for 25 ºC for 10 min. The reaction gets terminated by the addition of 500 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled at room temperature. The reaction mixture was diluted by adding 5 mL distilled water, and the absorbance was measured at 540 nm using a spectrophotometer. A control was prepared during the same procedure by the replacement of the extract with distilled water. The α-amylase inhibitory activity was calculated in terms of percentage inhibition [27].

%Inhibition = [(Abs control – Abs beta-sitosterol) / Abs control] × 100

Alpha-glucosidase Inhibitory Assay: The activity of isolated compound beta-sitosterol on α-glucosidase was determined by using α-glucosidase from Saccharomyces cerevisiae. P-nitro phenyl glucopyranoside (p-NPG) was prepared in 20mM phosphate buffer as a substrate solution and pH 6.9. 100 µL of α- glucosidase (1.0 U/mL) was pre-incubated with 50 µL of the different concentrations (20-100 µg/ml) of the isolated compound for 10 min. Then 50 µL of 3.0 mM (pNPG) substrate was dissolved in 20 mM phosphate buffer (pH 6.9) were added to start the reaction. The reaction mixture was incubated at 37 ºC for 20min and stopped by adding 2 mL of 0.1 M sodium carbonate. The α-glucosidase activity was determined by measuring the yellow-colored p-nitrophenol released from pNPG at 405 nm. The results were expressed in the percentage of the

blank control. The α-glucosidase inhibitory activity was calculated by percentage inhibition [28].

% Inhibition = [(Abs control – Abs beta-sitosterol) / Abs control] × 100

Statistical Analysis: All assays were conducted in triplicate. Statistical analyses were performed with SPSS 16.0 for an analysis of variance (ANOVA) followed by Duncan's test. Differences at $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION:

Structural Elucidation of Isolated Compounds: Beta-sitosterol melting point 136 °C, M.W.: 414.7 g/mol, which corresponds to the molecular formulae $C_{29}H_{50}O$. The U.V. λ_{max} value of compound beta-sitosterol was 257 nm **Fig. 1**.

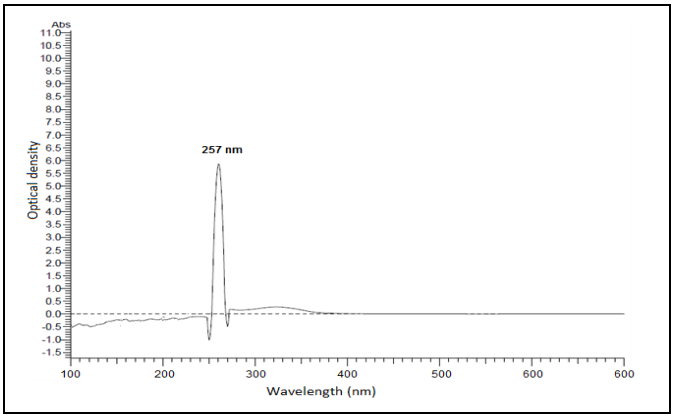

FIG. 1: UV SPECTRA OF THE ISOLATED COMPOUND BETA-SITOSTEROL

The IR absorption spectrum showed absorption peaks at 3427.78 cm^{-1} (O-H stretching.); 2937.40 cm^{-1} (aliphatic C-Hstretching); 1640.58 cm^{-1} (C=C absorption peak); other absorption peaks includes 1464.31 cm^{-1} (CH2); 1381.56 cm^{-1} (OH def), 1054.11 cm^{-1} (cycloalkane) and 800.97 cm^{-1} **Fig. 2**.

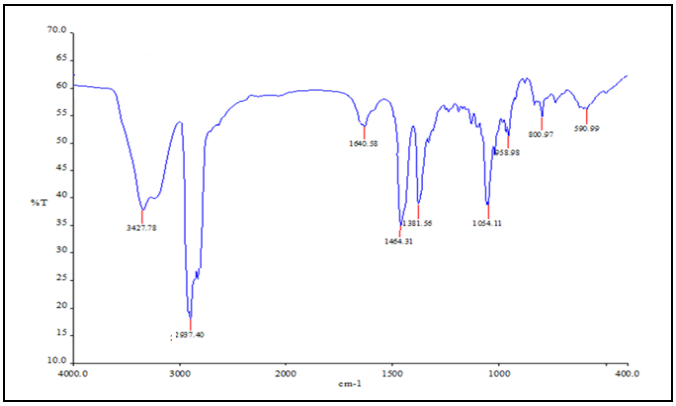

FIG. 2: IR SPECTRA OF THE ISOLATED COMPOUND BETA-SITOSTEROL

In the proton 1H NMR spectra of beta-sitosterol **Fig. 3** δ 2.28 (1H, m, H-3), 5.36 (1H, m, H-6), 5.34 (1H, m, H-23), 5.34 (1H, m, H-22), 2.28 (1H, m, H-3), 2.27(1H, m, H-20), 1.8-2.0 (5H, m) ppm. Other peaks are observed at δ 0.76-0.89 (m, 9H), 0.91-1.07 (m, 5H), 1.28-1.43 (m, 4H), 0.69-0.79 (m, 3H), 1.81-2.00 (m, 5H), 1.07-1.12 (m, 3H), 1.28-1.58 (m, 9H) ppm.

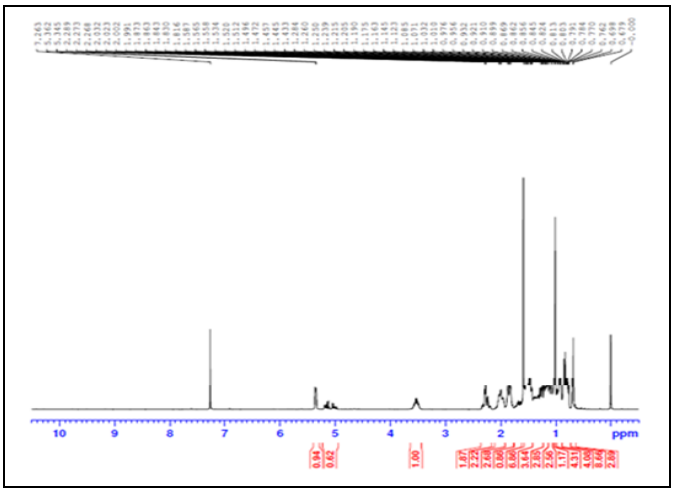

FIG. 3: 1H NMR SPECTRA OF THE ISOLATED COMPOUND BETA-SITOSTEROL

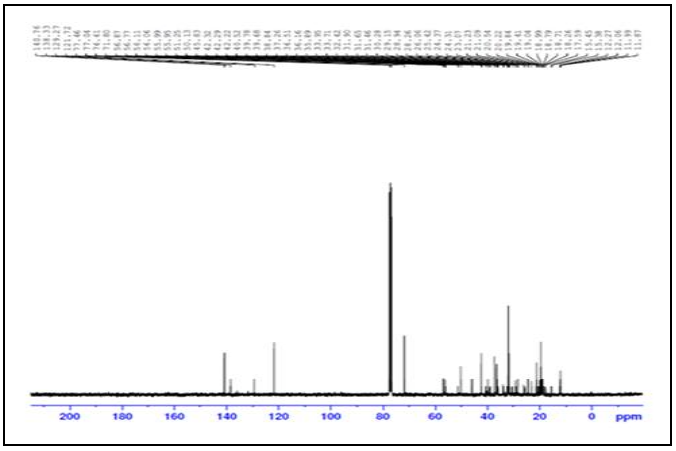

FIG. 4: 13C NMR SPECTRA OF THE ISOLATED COMPOUND BETA-SITOSTEROL

In the proton 13C NMR spectra of beta-sitosterol **Fig. 4** showed 140.7 (C-5), 138.3 (C-22), 121.7, 129.2 (C-6), 77.4 (C-3), 55.9(C-14), 55.9(C-17), 50.1 (C-9), 45.8 (C-9), 40.5 (C-20), 39.7(C-12), 39.6 (C-13), 38.8 (C-4), 38.8 (C-12), 37.2 (C-1), 37.2 (C-10), 36.5(C-8), 35.8(C-20), 33.9 (C-22), 33.9(C-7), 32.4 (C-8), 29.1 (C-25), 28.9 (C-16), 28.26 (C-2), 28.2 (C-15), 26.0 (C-28), 26.0 (C-11,26), 21.2 (C-27), 19.8 (C-19), 17.5 (C-21), 15.4 (C-18, 29). The structure was confirmed by comparison with literature data.

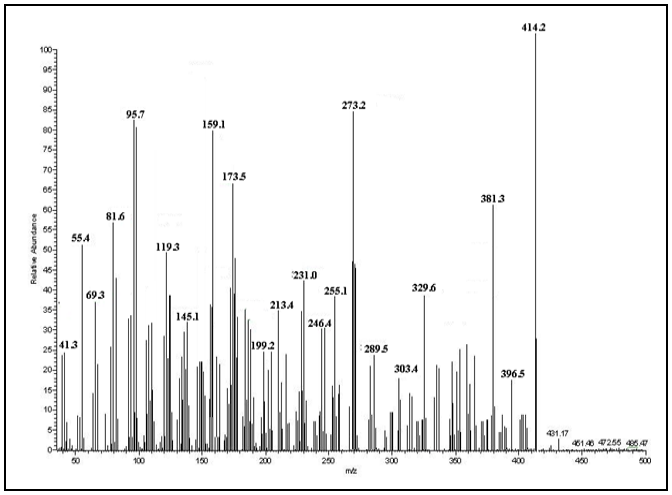

FIG. 5: MASS SPECTRA OF THE ISOLATED COMPOUND BETA-SITOSTEROL

Mass spectrum of isolated compound beta-sitosterol showed parent molecular ion [M+] peak at mlz 414, which corresponds to the molecular formula $C_{29}H_{50}O$.

The GCMS spectra of these isolated compounds revealed the characteristic fragments m/z with % abundance 414.2, 396.5, 381.3, 329.6, 303.4, 289.5, 273.2, 255.1, 231.0, 213.4, 199.2, 173.5, 159.1, 145.1, 119.3, 95.7, 81.6, 69.3, 55.4.

The molecular weight and fragmentation pattern indicate that the compounds were presenting beta-sitosterol, respectively **Fig. 5**.

Purification of Isolated Compound by HPTLC and HPLC: HPTLC fingerprint patterns have been therefore evolved to check the purity of isolated compound from methanolic extract of the sample.

The R_f value of standard beta-sitosterol 0.70 was matched with the R_f value of isolated compound beta-sitosterol was about 0.70 was shown in peak **Fig. 6**.

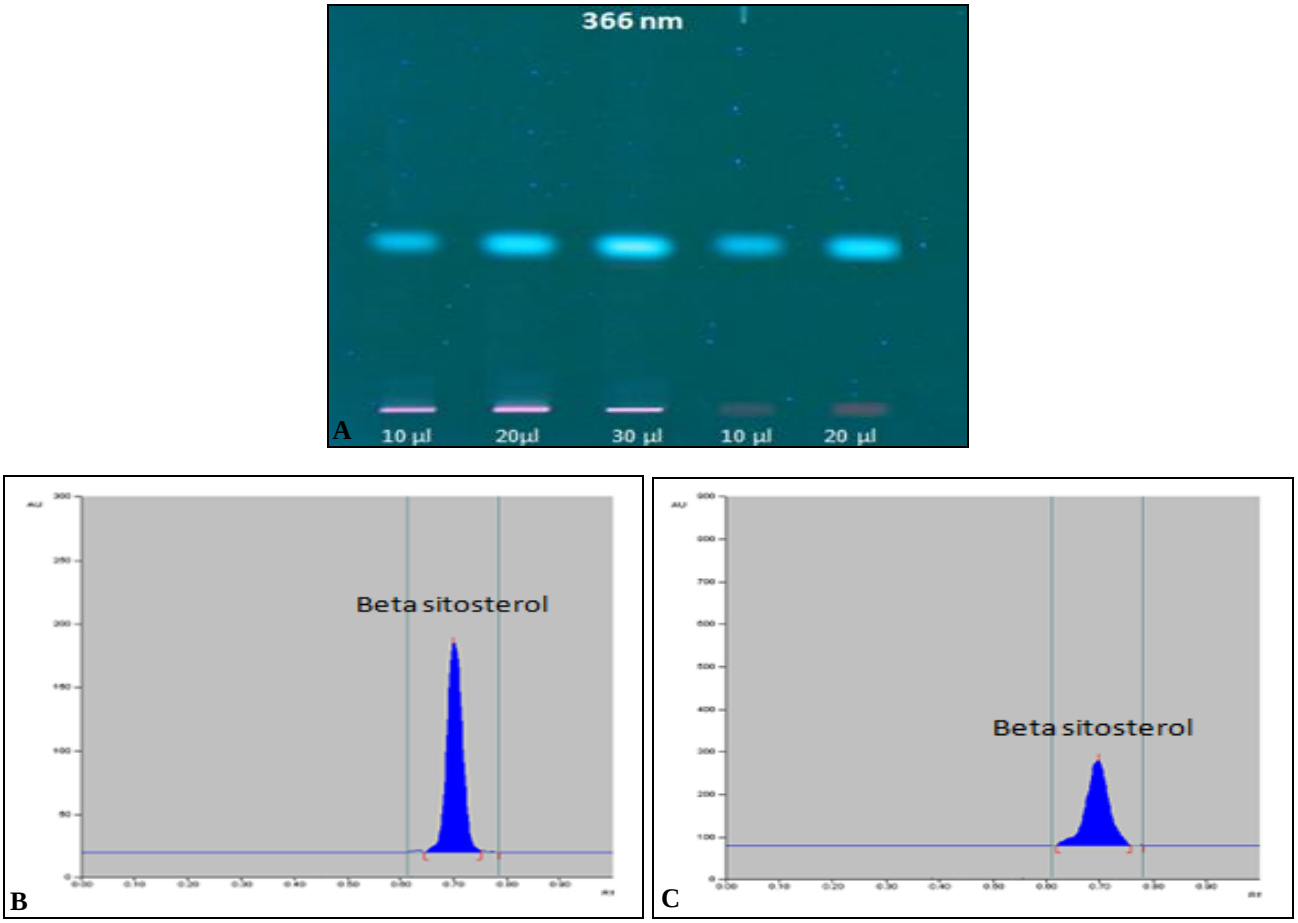

FIG. 6: HPTLC CHROMATOGRAM OF PURITY OF THE ISOLATED COMPOUND (A) STANDARD BETA-SITOSTEROL (B) ISOLATED BETA-SITOSTEROL IN ETHANOLIC EXTRACTS OF STEM OF ANDROGRAPHISECHIOIDES

The Retention time of beta-sitosterol isolated from the ethanolic extracts of stem of Andro-graphisechioides. was about 8.274 was shown by HPLC peak **Fig. 7**.

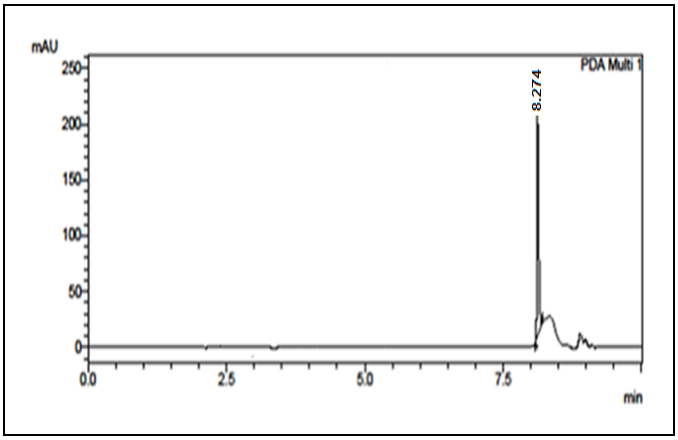

FIG. 7: HPLC SPECTRA OF PURITY OF THE ISOLATED COMPOUND BETA-SITOSTEROL

Previous studies suggested that the beta-sitosterol, are the main bioactive compound, has been reported to possess anti-inflammatory, anti-oxidant, anticancer, hepatoprotective, and hypoglycaemic activities [29]. On subjection to I.R. spectroscopic analysis, absorptions bands appeared at 3426 cm^{-1} that is characteristic of O-H stretching, 2868 cm^{-1} is due to aliphatics or C-H stretching or (CH_3), 1540 cm^{-1} due to double (C=C) stretching, 1054 cm^{-1} due to (C-O).

The absorption frequency at 738 cm^{-1} signifies cycloalkane. The out-of-plane C-H vibration of the unsaturated part was observed at 591 cm^{-1}. These absorption frequencies resemble the absorption frequencies observed for β-sitosterol as resembled data published [30, 31].

The 1H NMR spectrum (300MHz, $CDCl_3$) of compound **Fig. 7** has revealed a one proton multiplet at δ 2.26, 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 5.36 that integrated for one proton. The spectrum further revealed signals at δ 1.49 and δ 1.19 (3H each) assignable to two tertiary methyl groups at C- 18 and C-19, respectively.

The 1 HNMR spectrum showed two doublets centered at δ 0.90 (J = 6.7Hz) and δ 0.91 (J = 6.7Hz), which could be attributed to two methyl groups at C-26 and C -27 respectively. The doublet at δ 1.65 (J = 6.5Hz) was demonstrative of a methyl group at C-21. On the other hand, the triplet of three proton intensities at δ 0.86 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 close agreement with those observed for β – Sitosterol (Manoharan *et al.*, 2005 and Escudero *et al.*, 1985) [29, 30]. The 13C-NMR has shown recognizable signals of 140.76 and 129.27 ppm, assigned C5 and C6 double bonds, respectively.

The value at 24.31 ppm corresponds to the angular carbon atom (C19). Spectra show twenty-nine carbon signals, including six methyls, nine methylenes, eleven methane, and three quaternary carbons. The alkene carbons appeared at 140.76 and 129.27 ppm. In comparison, the standard data matched with the simulated data, which supports the proposed structure of this compound as β – Sitosterol [32-35].

Anti-oxidant Activity of Isolated Compound Beta-sitosterol by DPPH Method: The isolated compound beta-sitosterol contains the best anti-oxidant activity at high concentrations when compared with ascorbic acid **Fig. 8**. The compound showed 74.46% activity at 100 µl/ml at the same time ascorbic acid gave 95.03 at the same concentration **Table 1**.

The previous studies concluded that the free radical scavenging activity was showed by β-sitosterol, which implies its concentration-dependent antioxidant activity.

Low percent inhibition was observed as 11% at a concentration of 12.5 µg/ml, while high inhibition was observed at a maximum concentration of 1000 µg/ml [36].

TABLE 1: *IN-VITRO* ANTI-OXIDANT ACTIVITY OF THE BETA-SITOSTEROL USING DPPH METHOD AND COMPARISON WITH STANDARD DRUG ASCORBIC ACID

S. no	Concentration	Beta-sitosterol	Ascorbic acid
1	20 µl	36.17 ± 0.090	90.07 ± 0.014
2	40 µl	40.42 ± 0.084	91.48 ± 0.012
3	60 µl	50.35 ± 0.070	93.61 ± 0.009
4	80 µl	63.82 ± 0.051	94.32 ± 0.008
5	100 µl	74.46 ± 0.036	95.03 ± 0.007

Each value was obtained by calculating the average of four experiments, and data are presented as mean ± SEM

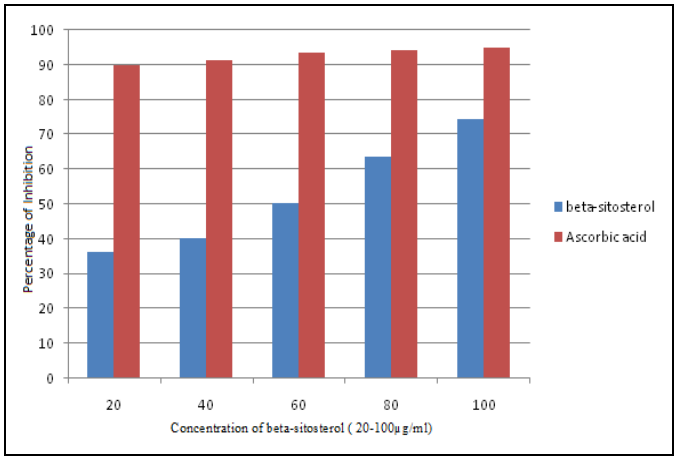

FIG. 8: GRAPHICAL REPRESENTATION OF DPPH INHIBITORY ACTIVITY OF ACARBOSE *VS.* BETA-SITOSTEROL ISOLATED FROM *ANDROGRAPHIS ECHIOIDES* STEM

***In-vitro* Alpha-Amylase Inhibitory Assay:** In this study, *in-vitro* alpha-amylase inhibitory activities of the beta-sitosterol isolated from ethanolic extract of *Andrographis echioides* stem was examined. The isolated compound 48.57 0.365 to 58.57 0.29 showed inhibitory activity at a concentration of 100 µg/ml **Table 2**.

TABLE 2: *IN-VITRO* ANTI-DIABETIC ACTIVITY OF THE BETA-SITOSTEROL USING ALPHA-AMYLASE METHOD AND COMPARISON WITH STANDARD DRUG ACARBOSE

S. no.	Concentration	Beta-sitosterol	Acarbose
1	20 µl	48.57 ± 0.365	60 ± 0.28
2	40 µl	50 ± 0.35	64 ± 0.26
3	60 µl	51.42 ± 0.34	68.57 ± 0.22
4	80 µl	54.28 ± 0.325	72.85 ± 0.19
5	100 µl	58.57 ± 0.29	77.14 ± 0.16

Each value was obtained by calculating the average of four experiments and data are presented as mean± SEM

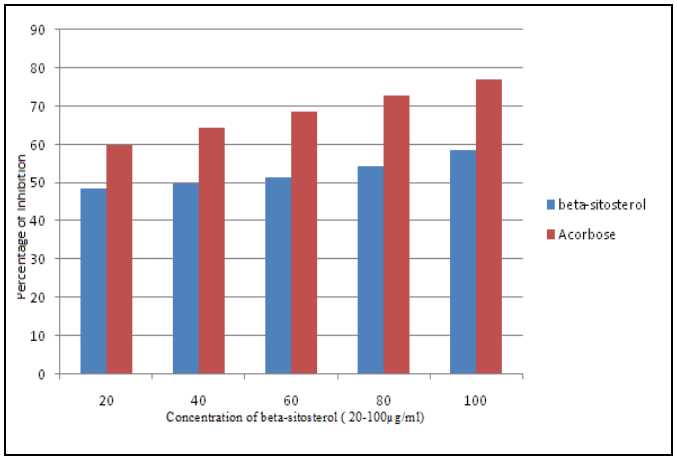

FIG. 9: GRAPHICAL REPRESENTATION OF A-AMYLASE INHIBITORY ACTIVITY OF ACARBOSE *VS.* BETA-SITOSTEROL ISOLATED FROM *ANDROGRAPHIS ECHIOIDES* STEM

Acarbose is a standard drug for α-amylase inhibitors. Acarbose at a concentration of (20-100 µg/ml) showed α-amylase inhibitory activity from 60 0.28 to 77.14 0.16% at the same concentrations 100 µg/ml. A comparison of α–amylase inhibitory activity between the standard drug is shown in **Fig. 9**. Gurupriya *et al.*, 2018 suggested that lupeol is a potential source for natural anti-diabetic and anti-oxidant compounds and could have potential use in the Management of diabetes mellitus [37].

***In-vitro* α-glucosidase Inhibitory Assay:** The result of **α** glucosidase inhibitory assay of anti-diabetic activity of beta-sitosterol isolated from ethanolic extract of *Andrographis echioides* stem are shown in **Table 3**.

TABLE 3: *IN-VITRO* ANTI-DIABETIC ACTIVITY OF THE BETA-SITOSTEROL USING ALPHA GLYCOSIDASE METHOD AND COMPARISON WITH STANDARD DRUG ACARBOSE

S. no	Concentration	Beta-sitosterol	Acarbose
1	20 µl	21.05 ± 0.0455	54.38 ± 0.026
2	40 µl	26.31 ± 0.042	61.40 ± 0.022
3	60 µl	31.57 ± 0.039	66.66 ± 0.019
4	8 0 µl	36.84 ± 0.036	71.92 ± 0.016
5	100 µl	43.85 ± 0.032	80.70 ± 0.011

Each value was obtained by calculating the average of four experiments and data are presented as mean± SEM

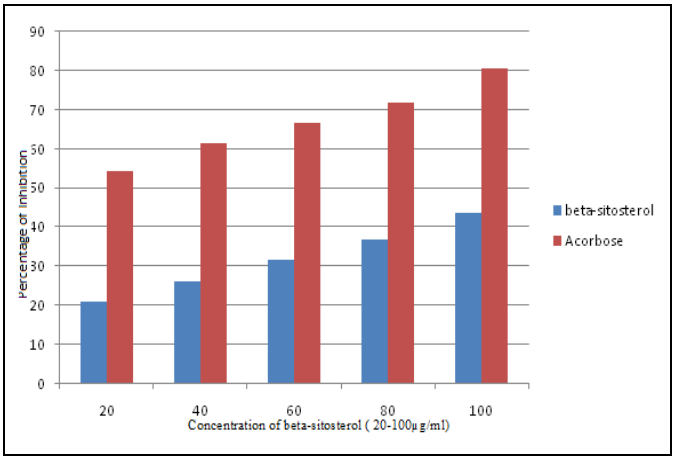

FIG. 10: GRAPHICAL REPRESENTATION OF A-GLYCOSIDASE INHIBITORY ACTIVITY OF ACARBOSE *VS.* BETA-SITOSTEROL ISOLATED FROM *ANDROGRAPHIS ECHIOIDES* STEM

The percentage inhibition various from 21.05 0.0455 to 43.85 0.032 for the high concentration to the lowest concentration. A comparison of **α** glucosidase activity between the standard and isolated compound beta-sitosterol showed in **Fig. 10**. A previous study suggested that anti-oxidant activity of the plant extract occurs due to the

presence of phenolic compounds and their redox properties, hydrogen donor capacity, and singlet oxygen quenching [38-41].

CONCLUSION: From the present study, beta-sitosterol isolated and characterized from the ethanolic extract of *Andrographis echioides* stem showed maximum inhibitory activity of anti-oxidant and anti-diabetic activities under *in-vitro* conditions. α-amylase inhibitory action decreases the digestion of carbohydrates, and α-glucosidase reduces glucose levels in blood. An isolated compound, beta-sitosterol, showed inhibitory action of free radical scavenging activity. The beta-sitosterol has inhibitory activity against α-amylase and α-glucosidase, and this therapeutic potentiality could be exploited in the management of postprandial hyperglycemia in treating type 2 diabetes mellitus.

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REFERENCES:

1. Keerthana G, Kalaivani MK and Sumathy A: *In-vitro* alpha amylase inhibitory and anti-oxidant activities of ethanolic leaf extract of *Croton bonplandianum*. Asian J Pharm Clin Res 2013; 6(4): 32-36.
2. Heise T, Nosek L and Rønn BB: "Lower within-subject variability of insulin detemir in comparison to NPH insulin and insulin glargine in people with type 1 diabetes," Diabetes 2004; 53(6): 1614-20.
3. Groop L, Forsblom C and Lehtovirta M: Characterization of the prediabetic state. Am J Hypertens 1997; 10: 172-80.
4. Grover JK, Yadav S and Vats V: Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 2002; 81: 81-00.
5. Maurya U and Srivastava S: Traditional indian herbal medicine used as antipyretic antiulcer, anti – diabetic and anticancer: A review. International Journal of Research in Pharmacy and Chemistry 2011; 1(14): 2231-81.
6. Mahesh AR, Harish K, Ranganath M.K. and Anand RD: Detail study on *Boerhaavia diffusa* plant for its medicinal importance – A Review 2012; 1(1): 28-36.
7. Patel DK, Kumar R, Laloo D and Hemalatha S: Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having anti-diabetic activity. Asian pac j trop biomed 2012; 2(5): 411-20.
8. Burke JP, Williams K, Narayan KMV, Leibson C, Haffner SM and Stem MP: A population perspective on diabetes prevention: whom should be we target for preventing weight gain? Diabetes care 2004; 26:1999-04.
9. Krolewski AS, Kosinski EJ and Warram JH: Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-depedent diabetes mellitus. Am J Cardiol 1987; 59: 750-5.
10. Chakravarthy BK, Gupta S, Gambir S.S. and Gode K.D.: Pancreatic beta cell regeneration. A novel anti-diabetic mechanism of *Pterocarpus marsupium* Roxb. J Pharmacol 1980; 12: 123-27.
11. Shen D, Juang S, Kuo P, Huang G and Chan Y: Chemical constituents from *andrographis echioides* and their antiinflammatory activity. Int J Mol Sci 2013; 14, 496-14.
12. Ramasubramania R and Jeevan R: Pharmacognostical phytochemical and anti-ulcer activity of *Andrographis echioides* (Acanthaceae) J. Pharmacogn Phytochem 2014; 3 (3): 39-49
13. Mathivanan D and Suseem SR: Phytochemical and pharmacological review of *Andrographis echiodies*. J Chem. Pharm. Res 2015; 7(7): 1167-71.
14. Nirubama K and Rubalakshmi: Bioactive Compounds in *Andrographis echioides* (L.) Nees. Leaves by GC-MS Analysis. Int J Curr Res Biosci Plant Biol 2014; 1(3): 92-7.
15. Kanchana N and Rubalakshmi: Phytochemical Screening and Antimicrobial Activity of *Andrographis echioides* (L.) Nees – An indigenous medicinal plant. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(5): 702-10.
16. Jayaprakasam D and Gunasekara B: Dihydroechioidinin, flavanone from *Andrographis echioides*. Phytochemistry 1999; 1(3): 92-7.
17. Gurupriya S, Cathrine L, Pratheema P and Ramesh J: Isolation and characterization of lupeol from methanolic extract of leaves of *Andrographis echioides*. International Journal of Current Advanced Research 2018; 7(4): 11397-02.
18. Gurupriya S and Cathrine L: *In-vitro* antimicrobial activities of (1R,3aR,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a,5a,5b,8,8,11a-hexamethyl-1-prop-1- en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9- ol isolated from the methanolic leaf extract of Andrographis echioides. International Journal of Biology, Pharmacy and Allied Sciences 2020; 9(7): 1460-71.

19. Zulfkar LQ, Beena J, Anandan R and Mohammed RU: Antibacterial activity of ethanol extracts of *Indoneesiella echioides* evaluated by the filter paper disc method. Pak J Pharm Sci 2009; 22: 123-5.
20. Deepti R, Sushila R, Permender R, Aakash D, Sheetal A and Dharmender R: HPTLC densitometric quantification of stigmasterol and lupeol from *Ficus religiosa*. Arab J Chem 2015; 8: 366-71
21. Jain PS and Bari SB: Isolation of lupeol, stigmasterol and campesterol from petroleum ether extract of woody stem of *Wrightia tinctoria*. Asian J Plant Sci 2010; 9(3): 163-7.
22. Wani MS, Gupta RC, Pradhan SK and Munshi AH: Estimation of four triterpenoids, betulin, lupeol, oleanolic acid, and betulinic acid, from bark, leaves, and roots of *Betula utilis* D. Don Using a Validated High-Performance Thin-Layer Chromatographic Method. Journal of Planar Chromatography 2018; 31 (3): 220-29.
23. Maji AK, Maity N, Banerji P and Banerjee D: Validated RP-HPLC-UV method for the determination of betulin in *Asteracantha longifolia* (L) Nees. Extract. International Journal of Phytomedicines 2013; 5: 131-15.
24. Joshi H, Saxena GK, Singh V, Arya E and Singh RP: Phytochemical investigation, isolation and characterization of betulin from Bark of *Betula utilis*. Journal of Pharmacognosy and Phytochemistry 2013; 2(1): 145-51.
25. Shuang T, Wang JX and Zheng XJ: Simple Synthesis of Allobetulin, 28-oxyallobetulin from Betulin and Betulinic Acid J Chem Soc 1998; 1: 3957-65.
26. Braca A, Sortino C and Politi M: Anti-oxidant activity of flavonoids from *Licania licaniaeflora*. J Ethnopharmacol 2002; 79: 379-81.
27. [McCue PP and Shetty K: Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in-vitro*," Asia Pacific Journal of Clinical Nutrition 2004; 13(1): 101-06.
28. Kim YM, Jeong YK, Wang MH, Lee WY and Rhee HI: Inhibitory effect of pine extract on *α*-glucosidase activity and postprandial hyperglycemia. Nutrition 2005; 21(6): 756-61.
29. Sami A, Taru M, Salme K, Jari Y.K.: Pharmacological properties of the ubiquitous natural product betulin. Eur J Pharm Sci 2006; 29:1-13.
30. Salman Ali S, Kala C and Ali Khan N: Isolation and characterization of β-sitosterol from methanolic extract of *Cordia dichotoma* linn bark. International Journal of Pharmaceutical Science and Research 2018; 9(8): 3511-14.
31. Abbas FA: Sterodial saponin from *Solanum unguiculatw* rich. Scientia Pharmazeutca 2001; 69: 219-34.
32. Rajput A.P. and Rajput TA: Isolation of Stigmasterol and β-sitosterol from chloroform extract of leaves of *Corchorus fascicularis* Lam. International Journal of Biological Chemistry 2012; 6(4): 130-35.
33. Ododo MM, Choudhury MK and Dekebo AH: Structure elucidation of β-sitosterol with antibacterial activity from the root bark of *Malva parviflora*. Springer Plus 2016; 5: 1210.
34. Jain PS and Bari SB: 'Isolation of lupeol, stigmasterol and campesterol from petroleum ether extract of woody stem-bark of *Wightia tinctoria*', Asian Journal of Plant Sciences 2010; 9(3): 163- 67.
35. [35] Panda S, Jafri M, Kar A and Meheta BK: Thyroid inhibitory, anti per oxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. Fitoterapia 2009; 80: 123-126.
36. Saeidnia S, Manayi A, Gohari AR and Abdollahi M: The story of betasitosterol-a review. Eur J Med Plants 2014; 4: 590-09.
37. Gurupriya S, Cathrine L and Ramesh J: *In-vitro* anti-diabetic and anti-oxidant activities of lupeol isolated from the methanolic extract of *Andrographis echioides* leaves. Journal of Pharmacognosy and Phytochemistry 2018; 7(4): 768-75.
38. [38]Mai TT, Thu NN, Tien PG, Van Chuyen N: Alpha-glucosidase inhibitory and anti-oxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. J Nutr Sci Vitaminol (Tokyo) 2007; 53: 267–76.
39. Pratheema P, Cathrine L and Gurupriya S: Isolation and characterization of some phytochemical compounds from the methanolic extract of *Solanum torvum* Swartz fruits. nternational Journal of Pharmaceutical Sciences and Research 2020; 11(12): 6213-21.
40. Gurupriya S, Cathrine L and Pratheema P: *In–vitro* anti-diabetic activity of 2-(3, 4-dihydroxyphenyl)-, 3, 5, 7-trihydroxy-4h-chromen-4-one isolated from the methanolic extract of *Andrographis echioides* leaves. International J of PharmaScience and Research 2019; 10(8): 3856-64.
41. Ali S, Kala C and Khan AN: Isolation and characterization of β-sitosterol from methanolic extract of *Cordia dichotoma* Lnn bark. International Journal of Pharmaceutical Science and Research 2018; 9(8): 3511-14.
42. Khanam S and Sultana R: Isolation of STB and stigmasterol as active immunomodulatory constituents from fruits of *Solanum xanthocarpum* (Solanaceae). Int J of Pharma Science and Research 2012; 3(4): 1057-60.

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