

## ISOLATION, CHARACTERIZATION OF B-SITOSTEROL FROM THE HERBAL EXTRACTS AND EVALUATION OF IN VITRO ANTI-OXIDANT AND ANTI-DIABETIC ACTIVITIES

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**ABSTRACT**

Plants naturally produce phytochemicals, physiologically active chemical compounds that serve as a natural defence mechanism and have considerable potential for treating a wide range of ailments. *Cyperus rotundus* and *Tinospora cordifolia* are the potent traditional herbs used in Ayurveda medicine, especially for diabetes. The current study aimed to isolate and characterize the bioactive compound  $\beta$ -sitosterol from the combined ethanolic extracts of the rhizome of *Cyperus rotundus* (*C. rotundus*) and the stem of *Tinospora cordifolia* (*T. cordifolia*). Furthermore, the *in vitro* antioxidant and anti-diabetic activities of isolated compounds are tested by DPPH assay, alpha amylase inhibition assay, alpha glucosidase inhibition assay and glucose uptake activity in 3T3L1 cells. The combined ethanolic extract of both medicinal plants was initially subjected to column and preparative thin layer chromatography for the isolation of bioactive compounds, followed by their characterization by spectral methods like UV, FT-IR, <sup>1</sup>H and <sup>13</sup>C NMR. The structure of the isolated compound is identified as  $\beta$ -sitosterol and further confirmed by structural elucidation. Similarly, the isolated compound  $\beta$ -sitosterol exhibited higher antioxidant scavenging activity of 64.54±1.38  $\mu$ g/ml against DPPH at a concentration 100 $\mu$ g/ml. The study also confirmed that  $\beta$ -sitosterol showed good inhibitory activities towards alpha-amylase (62.21±1.25  $\mu$ g/ml) and alpha-glucosidase (65.48±1.47  $\mu$ g/ml).  $\beta$ -sitosterol showed significantly increased glucose uptake (28% at 100 $\mu$ g/ml). Based on the conclusion that the organic compound  $\beta$ -sitosterol exhibited good antioxidant and anti-diabetic efficacy, it could have been used in the treatment of free radical disease and can be utilized in the management of diabetes mellitus.

**Keywords:** *Cyperus rotundus*, *Tinospora cordifolia*, Isolation, Characterization, Anti-oxidant activity, Anti-diabetic activity

**INTRODUCTION**

Plants are a significant source of potentially beneficial bioactive components for the creation of novel chemotherapeutic drugs. Many plants' biological and pharmacological characteristics are yet unknown. Scientists are investigating the prospect of using pharmacologically active chemicals from medicinal plants all over the world. 80% of individuals use herbal medicines because of their great efficacy and lack of negative side effects. In comparison to traditional therapies; modern synthetic pharmaceuticals are more expensive. Herbal plants are the only source that consistently provides the active ingredients used to create pharmaceuticals for modern therapies (Rakesh K Joshi et al., 2015, Dayana Jeyaleela et al., 2019). Globally, researchers in microbiology, biochemistry, botany, pharmacology and the chemistry of natural products are interested in studying medicinal plants to find more and more bioactive components and potential lead molecules that might be used to treat a wide range of ailments. (Abdel Nasser Singab et al., 2014). In the current scenario, scientists and researchers are turning to herbals for natural products and chemicals, which have led to a rise in the demand for herbal remedies and other alternative treatments. (Bailey et al., 1989, Elavarasi et al., 2014). Hyperglycemia and improper insulin secretion or action is a complicated metabolic condition of diabetes mellitus. 5–10% of people with DM have type 1 diabetes, formerly known as juvenile diabetes, occurs due to the damages in the insulin-producing  $\beta$  cells in the pancreas. (Mahabir et al., 1997, Dayana Jeyaleela et al., 2017). Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance, a reduction in hepatic glucose production and impaired glucose uptake by body cells. Type 2 diabetes affects 90% of elderly people; however, it steadily rises in youth due to obesity and inactivity. Large-scale consequences like heart disease, renal disease and stroke may result from it if left untreated. Patients with diabetes mellitus experience great exhaustion, weight loss, thirst, hunger and blurred eyesight as symptoms (Ripsin et al., 2009, Chung-Hung et al., 2012). Alpha-amylase and alpha-glucosidase inhibition are key therapeutic objectives for the treatment of diabetes mellitus (DM). Both enzymes play an essential role in carbohydrate digestion and regulate postprandial glucose levels in individuals with diabetes. Long-chain carbohydrate digestion by alpha amylase is complex, and the conversion of starch and disaccharides to glucose by alpha glucosidase is also complex. Oral antidiabetic

drugs suddenly decrease blood sugar levels and produce side effects such as nausea, abdominal pain, diarrhoea, and weight gain. Herbal medicines are in great demand because of their efficacy, safety, and better compatibility with human beings without any side effects (Grover et al., 2002). *Cyperus rotundus* also known as motha, purple nutsedge or nutgrass, Korai kizhangu in Tamil language belongs to the Cyperaceae family which is widely spread in the regions of Southern Europe, Africa, Central Europe and then Southern Asia (Gupta et al., 1971). The extract of *C. rotundus* is mostly used in traditional folk medicine for the treatment of inflammatory diseases, stomach ailments, bowel disorders, wounds, boils and blisters, dysentery, diarrhoea, fever, malaria, skin disease, loss of memory, insect bites and food poisoning. (Dhillon et al., 1993, Yu J et al., 2004, Sivapalan et al., 2013). According to research, *C. rotundus* extracts have biological properties that are anti-diabetic, anti-microbial, anti-inflammatory, anti-cytotoxic, anti-mutagenic, anti-pyretic and anti-diarrheal. (Sharma et al., 2011, Sivapalan et al., 2012) Likewise, *Tinospora cordifolia*, of Menispermaceae origin, is distributed in the tropical and subtropical regions of India. *Tinospora cordifolia*, also known as guduchi and "Shindilakodi," in Tamil (Singh et al., 1970, Kirti Sinha et al., 2004, Patel MB et al., 2012). The extract of *Tinospora cordifolia* reported many medicinal evaluations in which it is more commonly used in fever, diabetes, vaginal discharge, diuretic, dyspepsia, stomachic, urethral discharge, allays thirst and enriches the blood and cures jaundice (Vaibhav et al., 2010, Bhawya et al., 2010). Also, the pharmaceutical activities of anti-stress, anti-periodic, anti-arthritis, anti-malarial, anti-spasmodic, anti-allergic, anti-neoplastic and anti-leprotic drugs were reported in the previous studies. (Upadhaya et al., 2010, Spandana et al., 2013). Due to the medicinal properties of both plants, *Cyperus rotundus* and *Tinospora cordifolia* were chosen to explore the synergistic effects on free radical scavenging activities and diabetic and as well as to identify the potential bioactive compound that may be the source of the combined extract's therapeutic effects. The pharmacological effects such as antioxidant and anti-diabetic activities of combined ethanolic extracts of the plants *Cyperus rotundus* and *Tinospora cordifolia* were previously studied and compared with the individual extracts. The combined extract exhibits the highest potential compared with the individual ethanolic extract of *Cyperus rotundus* and *Tinospora cordifolia*. Based on the previous results, it was aimed to isolate the bioactive compound which is responsible for the pharmacological effects.

## MATERIALS AND METHODS

### Collection and authentication of plant materials

The rhizome part of *C. rotundus* and stem part of *T. cordifolia* were collected in January 2019 from Lalgudi area, Tiruchirappalli district, Tamil Nadu, India. The collected plant samples were authenticated (voucher specimens 2958, 2959) and deposited at the Rapinat Herbarium, St. Joseph's college (Autonomous), Tiruchirappalli, Tamil Nadu India

### Preparation of ethanol extracts

The collected plant materials were washed with distilled water to remove dirt and impurities and then dried under shade. The dried plant samples were ground using a mechanical grinder and sieved. The dry powder of the rhizomes of *C. rotundus* and the stem of *T. cordifolia* were equally mixed in a ratio of 1:1. 400g of each plant material are mixed together and denoted as 'CT Combination'. The CT combination powder was extracted with ethanol by the soxhlet extraction method. The final extract was filtered and concentrated by evaporation. The final crude extract is stored at 4°C in the refrigerator for further analysis (Pradeep Singh *et al.*, 2015).

### Identification of Bioactive Compounds in the CT Combination by GC-MS Analysis

The formulation of ethanolic extract of *C. rotundus* and *T. cordifolia* were subjected to identify bioactive compound by GC-MS. This analysis was performed on a "Thermo GC-Trace Ultra ver; 5.0" gas chromatography attached with a "Thermo MS DSQ II" for mass evaluation. Components were detected on a "ZB 5-MS Capillary Standard Non-Polar Column" with 600 mm length with a 0.25- $\mu$ m film thickness. During the experiment, temperature was elevated from 70 to 260 °C. The flow-rate of carrier gas, helium, was 10 mL/min and the injection volume of the samples was 1  $\mu$ l. The identification of the chemical components was performed on the basis of comparison of their respective retention time and mass spectra with those attained from authentic sample. Analysis of the mass spectrum, the compound was identified using National Institute of Standards and Technology (NIST) database.

### Phytochemical screening

#### Liebermann-Burchard Test

2mg of combined ethanolic extract of *C. rotundus* and *T. cordifolia* was dissolved in chloroform. Then acetic anhydride and concentrated sulphuric acid was poured from the tube's side. Sterol was present because of a brief colour change from red to blue to green.

### Identification of compound in the CT combination by HPTLC

#### Sample preparation

2g of the crude extract of the CT was dissolved in 10 ml of ethanol and filtered through Whatmann filter paper No. 41. The filtrate was subjected to HPTLC analysis for the identification and the quantification of  $\beta$ -sitosterol (Ridhi Joshi *et al.*, 2020).

#### Standard solution preparation

The stock samples were made by weighing 10 mg of  $\beta$ -sitosterol standard and carefully diluted with 10 ml of ethanol to obtain standard stock concentration solutions.

### HPTLC Analysis of standard and CT combined Extract

1  $\mu$ l to 6  $\mu$ l of standard solutions of  $\beta$ -sitosterol and 1  $\mu$ l to 6  $\mu$ l of the CT combined extract were uniformly loaded on the percolated silica gel 60 F<sub>254</sub> HPTLC plate (preparative) under a semiautomatic spotter under a nitrogen stream. Toluene: Ethyl acetate: Formic acid (5:4:1) was the mobile phase. The HPTLC plate and sample applicator is used in the analysis. A temperature of 25°C was used for the chromatographic circumstances, which also comprised the ascending separation technique, a trough chamber for plate growth and a migration distance of 10 cm. The sample and the standard-loaded plates were dried in hot air and then scanned densitometrically at UV light and a short wavelength of 254nm using TLC Scanner. The calibration curves of the sample and the standard were studied by plotting the peak area versus concentration of the sample.

### Isolation of $\beta$ -Sitosterol by Column and TLC

#### Column and Preparative Thin-layer chromatography

The ethanolic extract of the CT combination was subjected to TLC and column chromatography to separate the bioactive compound  $\beta$ -sitosterol, followed by the methods of Arjun *et al.*, (2010) and Rand *et al.*, (2018). The column chromatography was packed with activated silica gel and the concentrated extract was loaded on top of the column. Then, the column was first eluted with n-hexane (a low-polar solvent), then different ratios of ethyl acetate and n-hexane were poured into the column (1:9 to 9:1), and finally the un-eluted compound was separated with the methanol solvent. At the end, several fractions were obtained. The fractions with isolated compounds were further separated by TLC. 5  $\mu$ l of the isolated compound was applied to silica gel plates, Merck (Germany), 20 x 20 cm, 0.25 mm in thickness. Plates were developed using the solvent system n-hexane: ethyl acetate (70:30 v/v). The compound was detected on TLC plates by spraying with Liebermann-Burchard reagent and heating at 100°C for 10 minutes.

#### Purification of isolated components from HPLC

The analytical HPLC system was equipped with a diode array detector, a 20  $\mu$ l of sample injector and a 200 x 4.6 mm C18 column. The mobile phase consisting of methanol and water (75:25 v/v), was filtered through a membrane filter and pumped into the column at a flow rate of 1.0 ml/min. The column temperature was 30°C. 40  $\mu$ l of the isolated compound was injected into the column and detected at 346 nm.

### Characterization and structural elucidation study of isolated compound

Different kinds of spectroscopic methods were used to analyse the structure of the isolated compound CT-1, which were UV, FTIR, <sup>1</sup>H NMR, and <sup>13</sup>C NMR. All these methods were used to explain the structure of the CT-1 isolated compound. The UV-visible spectrum of the compound CT-1 was screened with methanol using the Shimadzu 160A UV-visible spectrophotometer. The FT-IR spectra of CT-1 were recorded with a resolution of 4 cm<sup>-1</sup> within the wave number range of 400 to 4000 cm<sup>-1</sup> using the KBr pellet method. <sup>1</sup>H and <sup>13</sup>C NMR spectra were acquired on Bruker WP 200 SY and AM 200 SY instruments (<sup>1</sup>H, 200.13 MHz; <sup>13</sup>C, 50.32 MHz) using TMS (tetra methyl silane) as an internal standard in CDCl<sub>3</sub> solvent (deuterated chloroform) (Muhammad Aurang Zeb *et al.*, (2017), Pierre *et al.*, (2015), Habib *et al.*, (2007).

### In Vitro Antioxidant Activity

#### DPPH assay method

The antioxidant activity of the isolated compound CT-1, i.e.,  $\beta$ -sitosterol, was examined by the low-cost and effective DPPH method. A 0.05 mM concentration of DPPH reagent was prepared with 100 ml of ethanol. The different concentrations of 20, 40, 60, 80 and 100  $\mu$ g/ml of sample and the standard ascorbic acid were prepared. Around 3 ml of freshly prepared DPPH reagent was added to the sample and the standard test tubes were left separately for 30 minutes. The absorbance of each sample and the standard was measured spectrophotometrically at 517nm. The radical scavenging activity of the standard and the isolated compound was observed and the percentage of inhibition of DPPH was calculated by following the method of Arif Hussain Bhat *et al.*, (2019).

**Percent (%) of inhibition of DPPH = (A-B)/A x 100.**

A - absorbance values of blank  
B - absorbance values of sample

#### In vitro antidiabetic activity

##### Alpha-Amylase Inhibition Assay

The inhibition of alpha-amylase enzyme was used to assess the anti-diabetic effect of the isolated compound CT-1. The isolated compound CT-1 was prepared in different concentrations of 20, 40, 60, 80 and 100  $\mu$ g/ml. 250  $\mu$ l of different concentrations of the isolated compound were taken in a test tube; 200  $\mu$ l of 0.02 M sodium phosphate buffer and 1 ml of alpha amylase solution (0.5 g/ml) were added at a pH of 6.9. The reaction mixture was pre-incubated at room temperature for 10 minutes. After that, 400  $\mu$ l of 1% starch solution in 0.02M sodium phosphate buffer was added at regular time intervals and then further incubated at 25°C for 10 minutes followed by 1 ml of dinitrosalicylic acid, which was added and incubated in a boiling water bath for 5 minutes, then cooled at room temperature. Finally, the reaction mixture was further diluted by adding 10 ml of double distilled water, and the absorbance of the test and standard sample solutions was measured at 540 nm by a spectrophotometer Manigandan *et al.*, (2021). The alpha-amylase inhibitory potential was calculated in terms percentage inhibition.

**%Inhibition = [(Abs control - Abs test) / Abs control] x 100**

**Alpha-glucosidase inhibition assay**

Alpha-glucosidase inhibition was used to assess the anti-diabetic effect of the isolated drug CT-1 by the following method of Saraf *et al.*, (2015). 20mM concentration of the reagent buffer p-nitro phenyl glucopyranoside (p-NPG) was prepared and the pH of the reaction was maintained at 6.9. To do that, 50 µl of alpha-glucosidase was mixed with 60 µl of the different concentrations of 20, 40, 60, 80 and 100 µg/ml of the isolated compound β-sitosterol. Then it was left for 10 min for a complete reaction, and then 50 µl of 3.0 mM (pNPG) substrate was dissolved in 20 mM phosphate buffer (pH 6.9). The reaction mixture was incubated at 37°C for 20 min and stopped by adding 160 µl of 0.1 M sodium carbonate. The yellow-colored p-nitro phenol was measured at 405 nm.

The alpha-glucosidase inhibitory potential was calculated by percentage inhibition.

$$\% \text{Inhibition} = [(\text{Abs control} - \text{Abs test}) / \text{Abs control}] \times 100$$

**Cell culture**

3T3-L1 (Mouse fibroblast cell) cell line was purchased from NCCS, Pune and were cultured in liquid medium (RPMI) supplemented with high glucose, 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin, and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO<sub>2</sub> at 37°C.

**Materials Required**

RPMI medium, Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and D-Glucose and HEPES were from Sigma, (USA), 1X PBS was from Himedia, (India). The tissue culture plate and wash beaker were from Tarson (India).

**Glucose uptake activity**

The effect of extracts on glucose uptake assay was measured using 3T3-L1 cells according to the methods described by Murad Alsawalha *et al.*, (2019) with slight modifications. The cell cultures with 70-80% confluency in 40mm petri plates were allowed to differentiate by maintaining in RPMI with 2% FBS for 4-6 days. The differentiated cells were serum-starved overnight and at the time of the experiment, cells were washed with HEPES buffered Krebs Ringer Phosphate solution (KRP buffer) once. Then cells were incubated with KRP buffer with 0.1% BSA for 30min at 37 °C. The differentiated 3T3-L1 preadipocytes were incubated with 100µg/ml of metformin and 10µl of β-sitosterol at various concentrations. 50mM concentration of glucose solution was added simultaneously to each well and incubated at 37°C for 30 min. After incubation, the uptake of the glucose was terminated by aspiration of solutions from wells and washing thrice with ice-cold KRP buffer solution. Cells were lysed with 0.1M NaOH solution and an aliquot of cell lysates was used to measure the cell-associated glucose. The glucose levels in cell lysates were measured using glucose oxidase method by measuring the OD at 520nm using a micro plate reader, (Thermo fisher Scientific,

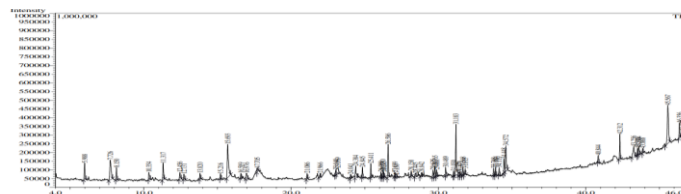
USA). Three independent experimental results were taken to determine the percentage enhancement of glucose uptake over controls.

**RESULTS AND DISCUSSION**

Medicinal plants offer a naturally occurring supply of drugs for the treatment of illness as well as prospects for the discovery of novel medications. Identifying the various biological activities of plants would therefore benefit from an examination of their elements. The presence of such phytoconstituents in medicinal plants can be determined through a variety of techniques. Chromatography and spectroscopic techniques are the most standard tools used for this purpose. Gas chromatography plays a significant role in the determination of phytoconstituents. However, spectroscopic approaches (UV-VIS, FT-IR) are simple, accurate, and can be used as standard methods for detecting phytoconstituents.

**Identification of Bioactive compounds in the CT combined Extract by GC-MS Analysis**

The GC-MS chromatogram of ethanolic extract of *C. rotundus* and *T.cordifolia* were determined by their retention time (RT), peak area (%), height (%) and concentration (%) are represented in **Table 1** and **Figure 1**. According to the GC-MS analysis, 54 chemical components were found in the combined plant extract. Among the identified bioactive components, β-sitosterol is majorly present in the combined extract with an area percentage of 12.51 and the retention time value of the compound is 45.567. This compound has antioxidant and anti-candidal activity. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy has antioxidant, antimicrobial, anticancer, anti-inflammatory, and cytotoxic activities Xiangying Yu *et al.*, (2013). 1, 2, 3-Benzenetriol [Pyrogallol] has antimicrobial, anti-inflammatory, antioxidant, analgesic, insecticide, anticancer, cytotoxic, antiseptic, antibacterial, antidermatitic, fungicide and pesticide properties Narayana V. V. S *et al.*, (2014). Stigmasterol is an abundant phytosterol compound that has anticancer, antimutagenic, hyperglycemic, antioxidant and anti-inflammatory properties Zeb MA., *et al.*, (2017). N-hexadecanoic acids have antioxidant, pesticide, flavour, 5-alpha reductase inhibitor, antifibrinolytic, hemolytic, lubricant, nematocidal and antiallopecic Kishor Mazumder *et al.*, (2020).



**Figure 1** GC-MS analysis of ethanolic extract of combination of *C. rotundus* and *T.cordifolia*

**Table 1** Retention Time, Compounds, Molecular Formula, Molecular Weight, area%, and activity of combination of ethanolic extract of *C. rotundus* and *T.cordifolia*

Peak#	R.Time	Area	Area%	Molecular formula	Molecular Weight (g/mol)	Name
1	5.988	343643	2.56	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub>	126.11	Thymine
2	7.726	742899	5.53	C <sub>11</sub> H <sub>14</sub> O	194.22	2-Butanone, 4,4-hydroxy-3-methyl phenyl-
3	8.150	227513	1.69	C <sub>5</sub> H <sub>6</sub> O <sub>4</sub>	144.12	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-
4	10.354	190919	1.42	C <sub>6</sub> H <sub>13</sub> Br	165.07	Hexane, 2-bromo-
5	11.317	344207	2.56	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub> S	148.23	2-(1-Hydroxyethyl)-2-methyl-1,3-oxathiolan
6	12.456	176353	1.31	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144.12	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6 methyl
7	12.731	136514	1.02	C <sub>12</sub> H <sub>28</sub> OSi	216	2-Methyl-1-di(tert-butyl)silyloxypropane
8	13.820	128360	0.96	C <sub>9</sub> H <sub>10</sub> O	150.17	2-methoxy-4-vinylphenol
9	15.216	100460	0.75	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	164.2	Eugenol
10	15.693	1098168	8.17	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11	1,2,3-benzenetriol
11	16.589	115734	0.86	C <sub>16</sub> H <sub>18</sub> F <sub>3</sub> NO <sub>4</sub>	345.31	3-Methoxy-4-[3-oxo-3-(pyrrolidin-1-yl)propoxy benzaldehyde
12	16.976	236725	1.76	C <sub>8</sub> H <sub>15</sub> N	125.21	3-azabicyclo[3.2.2]nonane
13	17.705	80553	0.60	C <sub>10</sub> H <sub>18</sub> O <sub>3</sub>	154	(E)-2,5,5-trimethylhepta-3,6-dien-2-ol
14	21.086	103564	0.77	C <sub>15</sub> H <sub>24</sub> O	220.35	(1R,2R,4S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.0 <sup>2,7</sup> ]decan-4-ol
15	21.966	81279	0.60	C <sub>15</sub> H <sub>22</sub> O <sub>2</sub>	234.33	Tricyclo[5.1.0.0(2,4)]oct-5-ene-5-propanoic 3,3,8,8-tetramethyl-
16	23.040	94363	0.70	C <sub>13</sub> H <sub>17</sub> NO <sub>2</sub>	219.28	N-(5-hydroxy-7-oxabicyclo[4.1.0] hept-2-yl
17	23.169	360889	2.69	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.4	Hexadecanoic acid
18	24.041	91372	0.68	C <sub>15</sub> H <sub>24</sub>	204.35	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1- (1-methylethyl)-, (1S-cis)-
19	24.384	314765	2.34	C <sub>15</sub> H <sub>26</sub> O	222.37	Alpha.-cadinol
20	24.845	190869	1.42	C <sub>15</sub> H <sub>22</sub> O	218.33	8-Isopropyl-1,5-dimethyltricyclo[4.4.0.0 <sup>2,7</sup> ] dec-4-en-3-one
21	25.411	248431	1.85	C <sub>15</sub> H <sub>22</sub> O	218.33	Cyperotundone -(3ar,4R,7R)-1,4,9,9-Tetramethyl-3,4,5,6,7,8 hexahydro-2H-3a,7-methanoazulen-2-one
22	26.099	78950	0.59	C <sub>15</sub> H <sub>24</sub>	204.36	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-tri methyl-4-vinyl-

Table 1 Continue

23	26.230	161872	1.20	C <sub>10</sub> H <sub>12</sub> O	180.20	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxy phenol
24	26.295	79363	0.59	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	238.36	1-((1S,3ar,4R,7S,7as)-4-Hydroxy-7-isopropyl-4-methyloctahydro-1H-inden-1-yl)ethanone.
25	26.586	596139	4.44	C <sub>15</sub> H <sub>22</sub> O	218.33	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl
26	27.039	84877	0.63	C <sub>14</sub> H <sub>22</sub> O	206	2-Isopropyl-tricyclo[4.3.1.1(2,5)]undec-3-en-10-ol
27	27.150	99403	0.74	C <sub>27</sub> H <sub>46</sub> O <sub>3</sub>	418.65	3.alpha.,7.beta.-dihydroxy-5.beta.,6.beta.-epoxycholestane
28	28.150	99996	0.74	C <sub>33</sub> H <sub>56</sub>	452.8	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane
29	28.445	99869	0.74	C <sub>14</sub> H <sub>22</sub> O	208.34	4-(5,5-dimethylspiro[2.5]oct-4-yl)-3-buten-2-one
30	28.842	91849	0.68	C <sub>14</sub> H <sub>22</sub> O	204.15	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol
31	29.635	151036	1.12	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	238.36	(3S,3as,6R,7R,9as)-1,1,7-Trimethyldecahydro-3a,7-methanocyclopenta[8]annulene-3,6-diol
32	29.765	239021	1.78	C <sub>15</sub> H <sub>24</sub> O <sub>2</sub>	236.34	Corymbolone
33	29.881	127392	0.95	C <sub>15</sub> H <sub>24</sub> O	220.35	(-)-Spathulenol
34	30.489	185436	1.38	C <sub>17</sub> H <sub>34</sub> O	270.45	Hexadecanoic acid, methyl ester
35	31.030	85207	0.63	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278.34	Di-sec-butyl phthalate
36	31.183	1082076	8.05	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.40	N-Hexadecanoic acid
37	31.375	91673	0.68	C <sub>19</sub> H <sub>23</sub> NO <sub>6</sub>	361.39	Cyclooctanepentanoic acid, 1-nitro-.beta.,2-dioxo-, phenylmethyl ester
38	31.473	106431	0.79	C <sub>12</sub> H <sub>20</sub> O	180.29	Ethanone, 1-(4,5-diethyl-2-methyl-1-cyclopenten-1-yl)
39	31.668	155847	1.16	C <sub>17</sub> H <sub>24</sub> O <sub>9</sub>	372.36	Trans-Sinapyl alcohol
40	31.815	131476	0.98	C <sub>18</sub> H <sub>28</sub> O <sub>3</sub>	292.41	Benzenepropanoic acid, 3,5-bis(1,1-dimethyl-4-hydroxy-, methyl ester
41	33.748	107560	0.80	C <sub>19</sub> H <sub>34</sub> O	294.47	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
42	33.884	131516	0.98	C <sub>19</sub> H <sub>36</sub> O	296.48	9-Octadecenoic acid (Z)-, methyl ester
43	34.119	146411	1.09	C <sub>18</sub> H <sub>24</sub> O	256.38	(+)-Bakuchiol, acetate
44	34.448	270193	2.01	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.44	9,12-Octadecadienoic acid (Z,Z)-
45	34.572	581517	4.33	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.47	Oleic Acid
46	40.844	130882	0.97	C <sub>19</sub> H <sub>38</sub> O	330.50	HEXADECANOIC ACID, 2-HYDROXY-1-1-(hydroxymethyl)ethyl ester
47	42.312	423240	3.15	C <sub>15</sub> H <sub>26</sub>	206.36	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl
48	43.256	419486	3.12	C <sub>29</sub> H <sub>48</sub> O	412.69	Stigmasterol
49	43.518	167797	1.25	C <sub>22</sub> H <sub>40</sub> O	336.55	Butyl 9,12-octadecadienoate
50	43.604	200578	1.49	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	356.53	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
51	43.868	80635	0.60	C <sub>19</sub> H <sub>22</sub> O <sub>4</sub>	314	2-(3-FURYL)-6A,10B-DIMETHYL-1,4A,5,6,6A,10,10A,10B-OCTAHYDRO-2H-BENZO[F]ISOCROMENE-4,7-DIONE
52	45.567	1277363	12.51	C <sub>29</sub> H <sub>50</sub> O	414.71	Beta.-Sitosterol
53	46.356	266070	1.98	C <sub>21</sub> H <sub>38</sub> O <sub>3</sub>	338.52	9-Octadecenoic acid (Z)-, oxiranylmethyl ester
54	46.577	79152	0.59	C <sub>30</sub> H <sub>48</sub> O	424.00	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one

13437893 100.00

### Separation and Isolation of $\beta$ -Sitosterol by Chromatographic Methods:

The combined ethanolic extract of *C. rotundus* and *T. cordifolia* was subjected to preparative TLC chromatography. Through trial and error, the solvent system was identified. The silica gel plates were reactivated by heating them in an oven set to 100°C for 15 minutes, followed by 10 minutes of cooling. The mobile phase for phyosterols was n-hexane: ethyl acetate (70:30 v/v). The fractions with isolated chemicals (CT-1) were added to the baseline of TLC plates and subsequently further separated by TLC. The isolated compound CT-1 was compared with the standard  $\beta$ -sitosterol and detected on TLC plates by spraying with Libermann-Burchard reagent and heating at 100°C for 10 minutes. The  $R_f$  value of the isolated compound was found to be 0.50, which indicated the steroid group. Then, the isolated compound CT-1 was subjected to column chromatography and the column was first eluted with n-hexane, increasing the amount of ethyl acetate in n-hexane and finally with methanol, yielding a number of fractions. The fraction collected in the ethyl acetate-methanol (40:60) ratio shows positive results for terpenoids. Also, it showed a similar  $R_f$  value to the standard  $\beta$ -sitosterol and it was further taken for the purification process.

### HPTLC Analysis of the $\beta$ -sitosterol rich fraction (CT-1)

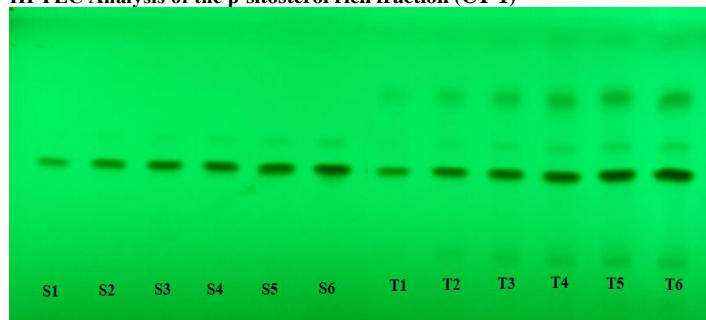


Figure 2 HPTLC chromatogram of 1 µl to 5µl of standard  $\beta$ -sitosterol and 1 µl to 5µl of isolated compound CT-1

HPTLC fingerprint patterns have been evaluated for the isolation of compounds from the ethanolic extracts of *C. rotundus* and *T. cordifolia*. The isolated compound was quantitated accurately using silica gel F<sub>254</sub> HPTLC pre-coated plates with the mobile phases toluene, ethyl acetate, and formic acid (5:4:1). The chambers are saturated with mobile phase for 60 min. The development time of the compound was 15 minutes. After development, the plate was air-dried for 5 minutes. To attain the greatest resolution and peak, the chromatographic conditions had previously been tuned. The peak area versus concentration (ng/spot) for each spot was plotted to create the calibration curve. The maximum  $R_f$  values of both the standard and the isolated compound CT-1 are 0.50 in figure 2. The isolated compound is  $\beta$ -sitosterol and correlation curves for the isolated compound  $\beta$ -sitosterol in combined plant extracts were regression via height  $y = 626.017 + 16.922x$  and  $r = 0.92985$   $sdv = 2.80$  and regression via area  $y = 8624.057 + 2202.518 X$  and  $r = 0.99996$ ,  $sdv = 0.31$  in figures 3 and 4.

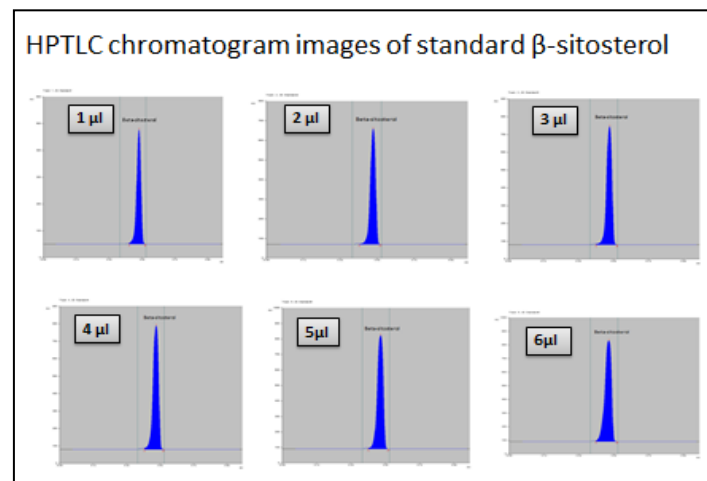


Figure 3 HPTLC chromatogram images of standard  $\beta$ -sitosterol

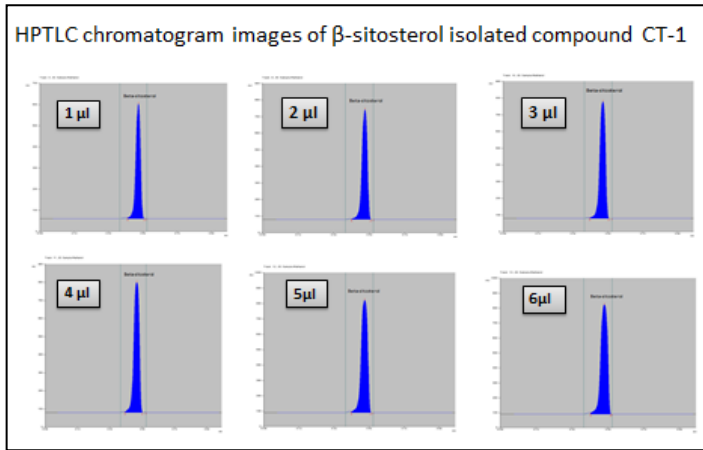


Figure 4 HPTLC chromatogram images of  $\beta$ -sitosterol isolated compound CT-1

**Characterization of isolated component of  $\beta$ -sitosterol:**

**UV visible of spectroscopy**

A spectrophotometer is helpful in the study of photon spectroscopy within the UV-VIS range. The absorption in the visible range is directly influenced by the colour of the compound used. The isolated compound CT-1  $\beta$ -sitosterol is a white powder with a MW of 414.7 g/mol and a melting point of 136°C, which corresponds to the molecular formula  $C_{29}H_{50}O$ . The UV  $\lambda_{max}$  value of compound  $\beta$ - sitosterol was 216 nm in Fig. 5.

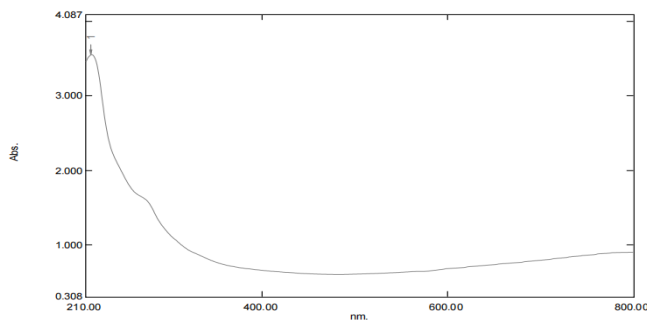


Figure 5 UV spectra for  $\beta$  sitosterol isolated compound CT-1

**FTIR spectra of isolated compound  $\beta$ -sitosterol**

For characterizing and identifying chemicals or functional groups (chemical bonds) present in the combination of plant extracts, the FT-IR has proven to be an invaluable method. It is a quick, non-destructive method that requires little sample preparation. As a result of the bands seen in the infrared spectrum, it enables the qualitative identification of chemical molecules.

**IR:** (KBr)  $\nu_{max}$ : The IR absorption spectrum showed absorption peaks at 3433.72  $cm^{-1}$  (O-H stretching), 2937.46  $cm^{-1}$  and 2868.48  $cm^{-1}$  (aliphatic C-H stretching), 1642.92  $cm^{-1}$  (C=C absorption peak), and other absorption peaks includes 1463  $cm^{-1}$  (CH<sub>2</sub>), 1379.54  $cm^{-1}$  (OH def), 1058.75  $cm^{-1}$  (cycloalkane) and 881.50  $cm^{-1}$  in Fig. 6.

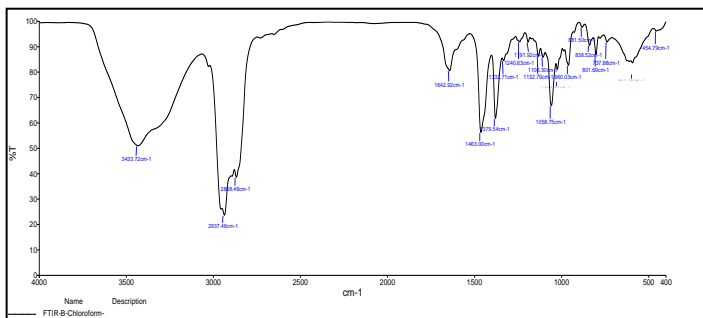


Figure 6 FTIR spectra for  $\beta$  sitosterol isolated compound CT-1

**Proton NMR of isolated compound  $\beta$ -sitosterol**

<sup>1</sup>H NMR ( $CDCl_3$ , 300MHz) has given signals at  $\delta$  3.506 (m, 1H of C3), 5.337 (t, 1H of C6), 0.661 (dd, 2H of C23), 0.679 (dd, 2H of C22), 0.787 (d, 1H of C24), 0.800 (dd, 2H of C28), 0.811(d, 1H of C25), 0.827 (d, 1H of C20), 2.263&2.268 (dd, 2H of C7), 4.695 (dd, OH of C3), 0.898 (d, 3H of C26&C27, J=6.6), 0.823 (d, 3H of C29, J= 6.4 H2), 0.789 (d, 3H of C19, J= 6.4), 1.670 (s, 3H of C18) and

0.990 (s, 3H of C21). The previous studies suggested that the <sup>1</sup>H NMR spectrum of the isolated compound  $\beta$ -sitosterol showed the presence of six methyl signals that appeared as two methyl signals at  $\delta$  0.78 (C19) and 1.67 (C18). Three methyl doublets at  $\delta$  0.99 (C21), 0.82(C29) and 0.89(C26 & C27) and a methyl triplet at  $\delta$  81 correspond to the methyl protons. The <sup>1</sup>H NMR spectrum of this substance also showed one olefinic proton at  $\delta$  5.33 in Fig.7. Its <sup>1</sup>H NMR spectrum showed a proton corresponding to the hydroxyl group which appeared as a multiplet at  $\delta$  4.695ppm (OH of C3).

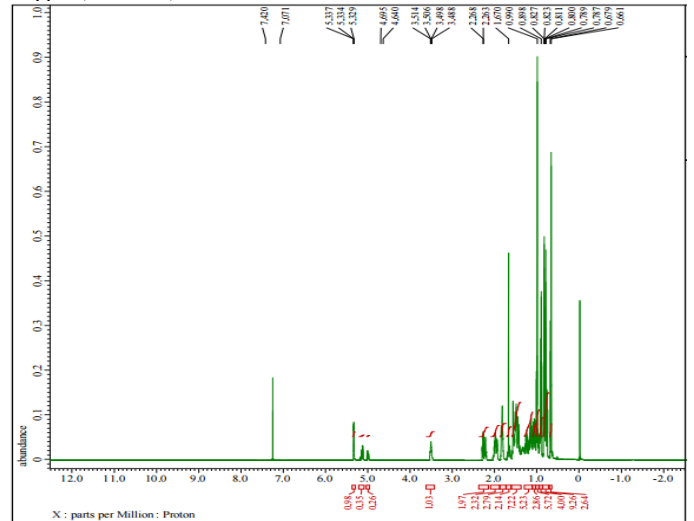


Figure 7 <sup>1</sup>H NMR spectra for  $\beta$  sitosterol isolated compound CT-1  
<sup>13</sup>C NMR of isolated compound  $\beta$ -sitosterol

<sup>13</sup>C NMR ( $CDCl_3$ , 100MHz) has given signal at 37.333(C-1), 31.971(C-2), 71.899(C-3), 42.398(C-4), 140.827(C-5), 121.802(C-6), 31.971(C-7), 31.722(C-8), 50.202(C-9), 36.586(C-10), 24.888 (C-11), 39.881(C-12), 42.369(C-13), 56.847(C-14), 24.388(C-15), 28.333(C-16), 56.847(C-17), 12.065(C-18), 19.486(C-19), 39.851(C-20), 21.161(C-21), 138.404(C-22), 129.337(C-23), 50.202(C-24), 36.586(C-25), 18.863(C-26), 19.192(C-27), 24.388(C-28), 11.941(C-29) in Fig. 8. The <sup>13</sup>C NMR spectrum also showed twenty-nine carbon signals made up of six methyl, eleven methylene, nine methane and three quaternary carbon signals. The spectra showed signals of 140.827 ppm and 121.802 ppm, which are alkene double bonds. The values of 19.192 ppm and 11.941 ppm correspond to methyl carbon atoms. The signal at 71.899 ppm is the  $\beta$ -hydroxyl group attached to the carbon.

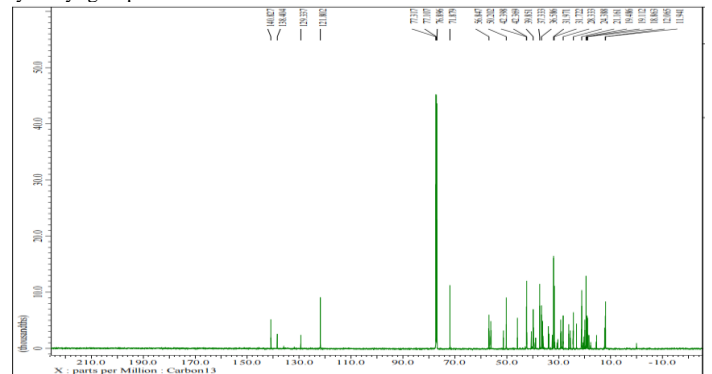


Figure 8 <sup>13</sup>C NMR spectra for  $\beta$  sitosterol isolated compound CT-1

**Purification of  $\beta$  sitosterol isolated compound by HPLC**

The isolated compound CT-1 from the ethanolic extract of sample was purified by HPLC method. The retention time of  $\beta$ -sitosterol was about 3.045 and was shown by HPLC peak in Fig. 9.

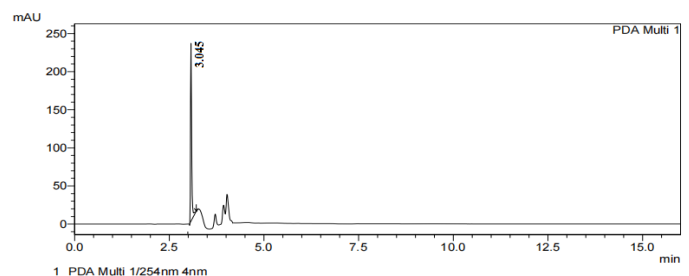


Figure 9 HPLC SPECTRA FOR ISOLATED  $\beta$ - SITOSTEROL

### Antioxidant activity of isolated compound $\beta$ -sitosterol from plant extracts by DPPH method

The antioxidant activity of the isolated compound  $\beta$ -sitosterol showed 64.54 % of inhibitory activity at a concentration of 100 $\mu$ g/ml while ascorbic acid exhibited 82.72% at a concentration of 100 $\mu$ g/ml. The IC<sub>50</sub> value for the isolated compound  $\beta$ -sitosterol is 44.33 $\mu$ g/ml (Table 2).

**Table 2** *In vitro* antioxidant activity of the compound  $\beta$ -sitosterol using DPPH method

S. No	Concentrations	Scavenging Effect (%)	
		Sample	Ascorbic acid
1	20 ( $\mu$ g/ml)	43.63 $\pm$ 0.92	48.18 $\pm$ 1.40
2	40 ( $\mu$ g/ml)	47.27 $\pm$ 1.42	60 $\pm$ 1.42
3	60 ( $\mu$ g/ml)	55.45 $\pm$ 1.55	70.9 $\pm$ 1.38
4	80 ( $\mu$ g/ml)	60.90 $\pm$ 1.49	79.09 $\pm$ 1.47
5	100 ( $\mu$ g/ml)	64.54 $\pm$ 1.38	82.72 $\pm$ 1.33
<b>IC<sub>50</sub> Value</b>		<b>44.33</b>	<b>18.81</b>

### *In Vitro* alpha amylase and alpha-glucosidase inhibitory assay

In this study, the *in vitro* alpha amylase inhibitory activities of the isolated compounds  $\beta$ -sitosterol from both plant extracts (ratio 1:1) were examined. The isolated compound showed the maximum inhibitory activity 62.21 $\pm$ 1.25 at a concentration of 100 $\mu$ g/ml (Table 3). Acarbose, a standard drug for alpha amylase inhibitors, showed inhibitory activity of 81.51 $\pm$ 1.25 at a concentration of 100 $\mu$ g/ml. The IC<sub>50</sub> value for the isolated compound  $\beta$ -sitosterol was indicated as 45 $\mu$ g/ml. This indicates that the isolated compound  $\beta$ -sitosterol is a very potent alpha amylase inhibitor in the management of diabetes mellitus.

The results of antidiabetic activity using an alpha-glucosidase inhibitory assay of the isolated compound  $\beta$ -sitosterol are shown in Table 3. The isolated compound  $\beta$ -sitosterol showed inhibitory activity of 65.48 $\pm$ 1.47 at a concentration of 100 $\mu$ g/ml. Acarbose showed an inhibitory activity of 80.53 $\pm$  1.37 at the same concentration of 100 $\mu$ g/ml. The IC<sub>50</sub> value for the isolated compound  $\beta$ -sitosterol was indicated as 61.11 $\mu$ g/ml. A comparison of alpha-amylase inhibitory activity between the standard drugs has been depicted.

**Table 3** *In vitro* antidiabetic activity of the  $\beta$ -sitosterol and standard drug acarbose against alpha-amylase and alpha-glucosidase

S. No	Concentrations	Alpha amylase (%)		Alpha-glucosidase (%)	
		Sample	Acarbose	Sample	Acarbose
1	20 ( $\mu$ g/ml)	45.37 $\pm$ 1.24	50.42 $\pm$ 1.42	31.85 $\pm$ 1.20	52.32 $\pm$ 1.33
2	40 ( $\mu$ g/ml)	59.73 $\pm$ 1.47	61.34 $\pm$ 1.47	41.59 $\pm$ 1.35	61.94 $\pm$ 1.42
3	60 ( $\mu$ g/ml)	57.94 $\pm$ 1.37	67.22 $\pm$ 1.37	56.44 $\pm$ 1.24	69.91 $\pm$ 1.50
4	80 ( $\mu$ g/ml)	69.30 $\pm$ 1.35	73.1 $\pm$ 1.25	72.4 $\pm$ 1.42	78.10 $\pm$ 1.47
5	100 ( $\mu$ g/ml)	73.21 $\pm$ 1.25	81.51 $\pm$ 1.34	75.48 $\pm$ 1.47	80.53 $\pm$ 1.37
<b>IC<sub>50</sub> Value</b>		<b>25.94</b>	<b>14.82</b>	<b>46.02</b>	<b>10.55</b>

### Glucose uptake activity

*In vitro* glucose uptake activity of  $\beta$ -sitosterol in 3T3L1 cells was analysed at different concentrations (50, 100, 150, 200 and 250  $\mu$ g/ml) as shown in Table 4. The glucose uptake activity of isolated compound  $\beta$ -sitosterol was compared to that of the standard antidiabetic drug metformin (100  $\mu$ g/ml). The glucose uptake was significantly increased in the metformin-treated cell lines (52%) over the untreated control group. However,  $\beta$ -sitosterol showed significantly increased glucose uptake compared to the standard drug metformin (28% at 100 $\mu$ g/ml) in a concentration-dependent manner.

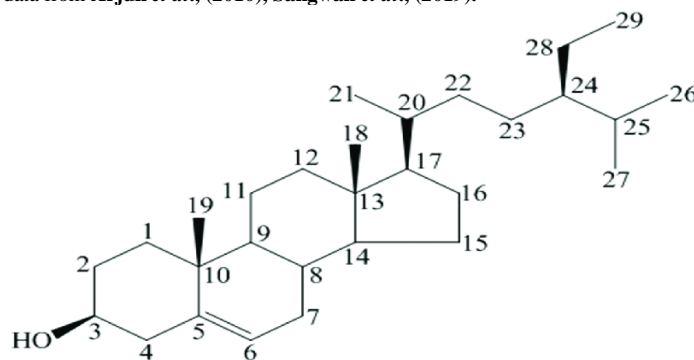
**Table 4** Percentage of Glucose uptake

S. No	concentration ( $\mu$ g/ml)	Mean Value (%)
1	Control	100
2	Metformin (100 $\mu$ g/ml)	52.54945
3	10 $\mu$ g/ml)	18.36327
4	50 $\mu$ g/ml)	25.99837
5	100 $\mu$ g/ml)	28.23226
6	250 $\mu$ g/ml)	30.97423
7	500 $\mu$ g/ml)	32.86984

### DISCUSSION

Phytosterols, which are similar types of plant-based cholesterol, are essential in the therapeutics sector since they are more advantageous, less expensive, more accessible and without negative effects.  $\beta$ -sitosterol is one of the phytosterol compounds and has been shown to possess various pharmacological activities in many diverse applications. The current study aimed to isolate and characterize  $\beta$  sitosterol from the ethanolic extracts of *C. rotundus* and *T. cordifolia* and evaluate antidiabetic activity under *in vitro* condition. The combined ethanol extract of *C. rotundus* and *T. cordifolia* was subjected to column chromatography by using different mobile phase solutions. The CT-1 (white crystalline) compound was isolated. The isolated compound was identified by phytochemical analysis (Lieberman-Burchard test). The results of the phytochemical analysis indicated that the isolated compound may be a steroid or terpene. On HPTLC, the R<sub>f</sub> values of both the standard and the isolated compound CT-1 are 0.50. Previous studies by Rajeshwari Prabha Lahare *et al.*, (2019) reported that the R<sub>f</sub> value of  $\beta$ -sitosterol in the leaves, stem and roots of *Andrographis paniculata* was visualised by the HPTLC method.

The isolated compound's structure was proposed to be  $\beta$ -sitosterol based on the melting point and other relevant information (UV, FTIR, <sup>1</sup>H NMR, and <sup>13</sup>C NMR).  $\beta$ -sitosterol is a white crystalline substance, and these absorption frequencies coincide with the absorption frequencies observed for  $\beta$  -sitosterol, according to data from Arjun *et al.*, (2010), Sangwan *et al.*, (2019).



**Figure 10** Structure of the isolated  $\beta$ - sitosterol

The HPTLC Calibration curve and linearity of isolated compound CT-1 was analysed. The CT-1 regression equation and correlation curves values of plant extract was 0.28 and 0.31. Shahnaz sultana *et al.*, 2017 reported that the methanol extract of *Cyperus rotundus* rhizomes were isolated and identified two steroidal esters such as lupenyl 3 $\beta$ -O-arabin pyranosyl 2'-oleate and  $\beta$ -sitosterol-3 $\beta$ -O-glucoside. Devprakash *et al.*, 2011, Rand *et al.*, 2018 showed that the phytoconstituents such as  $\beta$ -sitosterol, berberine, tinosporine, heptacosanol, palmatine and columbin has been reported from the stem part of *T. cordifolia*. The DPPH free radical scavenging activity assay is an essential for determining the antioxidant activity of plant extracts due to its comparatively quick analytical time. To prevent the detrimental impact of free radicals on several diseases, including cancer, diabetes and oxidative stress disorders, eliminating free radicals is essential. The results revealed that the isolated compound  $\beta$ -sitosterol had a significant free radical scavenging activity compared to the standard ascorbic acid. An earlier study reported that the presence of phenolic compounds and their redox characteristics, hydrogen donor capacity and singlet oxygen quenching are responsible for the plant extract's anti-oxidant action. Gupta *et al.*, 2011, Arif Hussain Bhat *et al.*, 2019, demonstrated that the  $\beta$ -sitosterol compound isolated from rhizomes of *Arisaema utile* showed DPPH free radical scavenging activities. The metabolic disorder known as diabetes mellitus is characterized by hyperglycemia and decreased insulin secretion or activity. The main contributing factors to diabetes mellitus are increased glycogenolysis and gluconeogenesis, as well as decreased tissue glucose uptake. Oral diabetes medications cause weight gain, nausea, abdominal pain and diarrhoea in addition to a fast drop in blood sugar levels. Alpha-amylase and alpha-glucosidase inhibition are the key therapeutic goals for the treatment of diabetes mellitus (DM). In this study, the *in vitro* alpha amylase inhibitory activity of the isolated compound  $\beta$ -sitosterol was examined. The IC<sub>50</sub> value for the isolated compound  $\beta$ -sitosterol was denoted as 45 $\mu$ g/ml. The isolated compound showed the maximum inhibitory activity and indicates a potent alpha-amylase inhibitor in the management of diabetes mellitus. Manigandan and Subramaniyan, 2021 suggested that the phytochemicals compound such as scirpusin A, Scirpusin B isolated from the rhizomes of *C. rotundus* showed antidiabetic inhibitory effect against both alpha amylase and alpha glucosidase activities under *in vitro* conditions. The alpha glucosidase-inhibited activity of the isolated compound  $\beta$ -sitosterol was determined and showed inhibitory activity at a concentration of 100 $\mu$ g/ml compared to the standard acarbose. The previous study Sunil Kumar *et al.*, 2013 showed that the  $\beta$ -sitosterol compound isolated from the leaves of *Dillenia indica* and potential source for natural antidiabetic.

Cell lines are an important *in vitro* model system for providing diagnoses and identifying the effect of  $\beta$ -sitosterol. The 3T3-L1 cell line was selected for the current study because it exhibits relevant key junction pathways for glucose

homeostasis and lipid accumulation. One of the important target mechanisms to achieve glucose homeostasis is the assay of glucose uptake activity in the 3T3-L1 adipocyte cell line through the translocation of GLUT4 on the plasma membrane. This assay is thought to be an effective target for the development of effective medications for diabetes and obesity. In the current research, the glucose uptake activity of the isolated compound  $\beta$ -sitosterol from *C. rotundus* and *T. cordifolia* was determined *in vitro*. Hence,  $\beta$ -sitosterol significantly enhanced glucose uptake in 3T3-L1 adipocytes comparable to the standard antidiabetic drug metformin. Metformin is a biguanide that improves the sensitivity of insulin by activating the AMP-activated protein kinase pathway (Lachin et al., 2021).

## CONCLUSION

Plants contain phytosterols, which are essential bioactive substances. Among the various kinds,  $\beta$ -Sitosterol is one of the most renowned substances, with a structure resembling that of cholesterol. The main function of an isolated organic compound is to maintain the membrane's fluidity. The present study demonstrates that  $\beta$ -sitosterol was isolated and characterised from a combined ethanolic extract of the rhizome part of *Cyperus rotundus* and the stem part of *Tinospora cordifolia*. As an isolated compound, it exhibited inhibitory activity against alpha amylase and alpha glucosidase and enhanced glucose uptake in 3T3-L1 adipocytes. The results indicated that an isolated compound is considered a potential drug and has promising antioxidant and antidiabetic effects. The therapeutic potential of  $\beta$ -Sitosterol could be utilized as a novel drug for the treatment of type 2 diabetes mellitus by regulating postprandial hyperglycemia.

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**Authors contributions:** All authors have contributed equally.

**Conflicts of interests:** Nil

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