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# ISOLATION OF β-SITOSTEROL FROM CASSIA FISTULA FLOWER EXTRACT AND ITS CHARACTERIZATION: A NOVEL METHOD

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**ABSTRACT**

This paper explores the Novel method of isolation of β-Sitosterol from *Cassia Fistula* flower extracts. The compound was obtained by purifying the chloroform extract. Colum chromatography techniques with various compositions of eluents have been employed for purification. The purified compound was then characterized by various spectrophotometric techniques. The chloroform extract of *Cassia Fistula* flower showed moderate glucoamylase and α-amylase inhibition as well as DPPH radical scavenging activity.

**Keywords:** Novel Method, B-Sitosterol, Column Chromatography Technique, Glucoamylase, A-Amylase Inhibition, DPPH Radical Scavenging Activity.

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## INTRODUCTION

The term "Phytochemicals" refers to plant-based chemicals and was coined in 1994. Phytosterols are a subgroup of steroids and are considered a very important class of bio-organic molecules. Phytosterols are plant compounds that have similarities to cholesterol. The National Institute of Health states that there are more than 200 distinct phytosterols, with the greatest amounts naturally occurring in vegetable oils, beans, and nuts.[1] Phytosterols are so valued for their benefits that they are being added to foods as fortifying. Phytosterols are best known for their scientifically proven ability to reduce cholesterol levels.[2] A phytosterol is a plant compound that closely resembles cholesterol. Phytosterols compete with cholesterol for absorption in the digestive tract. Although they inhibit the absorption of dietary cholesterol, they are poorly absorbed, resulting in an overall decrease in cholesterol levels. Reducing cholesterol levels can result in decreased chances of heart disease, stroke, and heart attack.[3,4] Phytosterol is crucial for the physiology of eukaryotic organisms.[5] β-Sitosterol is a phytosterol that possesses a chemical structure resembling cholesterol. Sitosterols are white, waxy powders with a distinct smell. They are soluble & hydrophobic in the alcohol. The IUPAC name of the compound is 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimehyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H cyclopenta[a]phenanthren-3-ol. The plant sources of β-Sitosterol include *Hymenocrather calcinus, Salvia hypoleuca, Salvia macrosiphon, Achilleatalagonica, Lomatopodium staurophyllum, Ferulago subvelutina* and *Genum irancium.*[6] β-Sitosterol has been discovered to impact various stages of tumor development, including inhibiting the creation, promotion, and induction of cancerous cells, as well as inhibiting the invasion and metastasis of tumor cells. Supplementation of β-Sitosterol reduced levels of circulating 17β-estradiol (E2) and inhibited E2-induced MCF-7 tumor growth in ovariectomized athymic nude mice. This indicates that a high dietary intake of phytosterol may be advantageous for women with breast cancer.[7] The impact of cocoa extract containing β-Sitosterol on 2 human prostate cancer cell lines (nonmonastic & metastatic) and one normal cell line has been assessed.[8] β-Sitosterol has been reported to possess anthelminthic properties.[9] Adding β-Sitosterol to cell membranes can prevent oxidative stress and lipid peroxidation caused by Glucose oxidase. This compound has shown promising effects in neurodegenerative disorders such as Alzheimer's disease.[10] Angiogenesis is a significant process involved


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in the wound-healing properties of Aloe Vera gel. The extracts have shown angiogenic activity on the CAM (Chorioallantoic Membrane) of chick embryos. β-Sitosterol, identified as the primary compound in this gel, demonstrated potent angiogenic effects in the CAM assay.[11] *Cassia Fistula* which is also known as the Golden shower tree is one of the most precious ornamental plants and is grown in numerous areas of India in the summer season. It is also used in Ayurvedic medicines. The literature review suggested that very little research has been carried out on extracting the contents from the *Cassia fistula* flower. The main objective of this paper is to design a novel method to isolate the β-Sitosterol from the *Cassia fistula* flower.

## EXPERIMENTAL

### Material and Methods

The chemicals have been utilized as of analytical grade i.e. Petroleum ether (BVR-1), Chloroform (BVR-2), Ethyl acetate (BVR-3), Ethanol (BVR-4), acetate buffer (pH 4.5), starch solution, DNS (3,5-dinitrosalicylic acid), Methanol, phosphate buffer solution (pH 7.0), 1,4-Dioxane, DPPH(2,2-Diphenyl-1-picrylhydrazyl), Hexane, Silica Gel-H., silica gel (mesh 60-120) slurry. The compounds' IR spectra were analyzed using a Shimadzu IR Affinity FT-IR spectrophotometer with KBr discs, and the results are reported in $cm^{-1}$. The compounds' 1H NMR spectra have been obtained using a Bruker Avance II 400MHz NMR spectrophotometer with DMSO as an internal standard, and the values are reported in δppm. The compounds were analyzed using an Elemental Vario El III, Carlo Erba 1108 Elemental analyzer to determine their elemental composition. The synthesized compounds' structures were verified through spectral and elemental analysis.

### General Procedure

### Preparation of *Cassia fistula* Flower Extract and its Phytochemical Study

***Cassia fistula*** flower was collected from the university campus and was air dried. Air-dried flower weighed (350g) and was then subjected to extraction with many solvents in the increasing order of their polarity like Petroleum ether (BVR-1), Chloroform (BVR-2), Ethyl acetate (BVR-3) and Ethanol (BVR-4). Each solvent was replaced one by one after soaking the plant material for 48 hrs. All extracts obtained were further subjected to phytochemical screening to study the phyto-constituents that exist in the extracts. An in-vitro research has been carried out to examine the impact of extracts on glucoamylase. The reaction mixture consisted of 0.5mL total volume, comprising 0.1mL of extract ranging from 20-100µg, 0.3mL of 100mM acetate buffer (pH 4.5), and 0.1mL of glucoamylase (1.3µg). The mixture was then incubated at 37ºC for 30 minutes. Next, a starch solution of 0.5mL (5mg/mL in 100 mM acetate buffer pH 4.5) was added and incubated at the temp of 37ºC for the time of 30 minutes. The reaction was stopped by placing the test tubes in a boiling water bath for the time of 1-2mins. and then cooling them under running tap water. The test tubes were placed in a boiling water bath for 15 minutes after adding 2mL of DNS. The test tubes were then cooled and mixed with 7mL of double distilled water. The spectrophotometer measured the absorbance at 530nm to estimate the amount of liberated glucose. The experiment was conducted three times. A unit activity (U) is the ratio of milligrams of glucose released to milligrams of protein over a minute. 20-100µg/mL solutions of all the extracts have been made in methanol, along with corresponding controls. To study the effect of the extracts on α-amylase, *in vitro,* 0.5mL of the reaction mixture consisting of 0.1mL of extract ranging from 20-100µg/mL, 0.3mL of 20mM PBS (pH 7.0) and 0.1mL of α-amylase (0.4µg) have been then incubated at the temp of $37^0$C for the time of 30mins. Next, 0.5mL of starch solution (5mg/mL in 20mM phosphate buffer at pH 7.0) was added and incubated at 37°C for 30 minutes. The reaction was stopped by placing the test tubes in a boiling water bath for the time of 1-2mins. and then cooling them under running tap water. 2mL of DNS (3,5-dinitrosalicylic acid) reagent was added to the test tubes, which were then placed in a boiling water bath for the time 15 mins. Then, the test tubes were cooled as well as diluted with 7mL of distilled water. The spectrophotometer measured the absorbance at 530 nm to estimate the amount of liberated glucose. 20-100µg/mL solutions of all the extracts were prepared using double distilled water. Concentrated solutions ranging from 20 to 100µg/mL of all the extracts were prepared in 1,4-Dioxane, along with a corresponding control sample. The extracts were further examined for their impact on DPPH radical scavenging activity. 1mg/mL solutions of the extracts were prepared using Methanol. A 0.2mM solution

of DPPH in methanol had been formed. Then, 1mL of this solution had been mixed with 1mL of the extract solution in methanol at several concentrations (100, 200, 300, 400, 500µg/mL). Thirty minutes later, the absorbance has been measured at 517nm. A blank has been synthesized without adding the extract. Ascorbic acid and BHT have been utilized as the standards. The BVR-2 extract showed moderate glucoamylase and α-amylase inhibition as well as DPPH radical scavenging activity. Therefore, we decided to purify the Chloroform extract.

**Purification of Extract & Isolation of the Compound**

Chloroform (BVR-2) extract (47.94g) has been dissolved in the minimum amount of mixture of chloroform and methanol to prepare silica gel (mesh 60-120) slurry. After evaporation of the solvent, the sample was loaded on a column packed with silica gel. The column was washed with varying concentrations of Hexane (100%) and mixtures of Hexane & Ethyl Acetate (90:10, 80:20, and 50:50). The eluents were collected at regular intervals and monitored using TLC (Silica Gel-H) with a Hexane solvent system: Ethyl acetate (7:3) and iodine vapor as the detecting agent. The fraction was eluted with Hexane: Ethyl Acetate (90:10) showed a single spot-on TLC with the $R_f$ value at 0.4 afforded a white amorphous solid (82mg).

**Detection Method**

The compound obtained after purification was further subjected for the structure elucidation by, EI-MS, $^{13}$C-NMR, $^{1}$H-NMR, DEPT-135, IR, and Elemental Analysis.

## RESULTS AND DISCUSSION

*Cassia fistula* flower has been subjected to extraction with various solvents in polarity increasing order. The yield obtained from each solvent extract is shown in Table-1.

Table-1: Percentage Yield of Extracts

| S. No. | Extracts | Amount Obtained (In gram) | % Extraction |
|---|---|---|---|
| 1 | Petroleum ether (BVR-1) | 35.49 | 10.14 |
| 2 | Chloroform (BVR-2) | 47.94 | 13.42 |
| 3 | Ethyl Acetate (BVR-3) | 89.76 | 25.64 |
| 4 | Ethanol (BVR-4) | 97.97 | 27.99 |
| | Total | 271.16 | 77.19 |

All extracts obtained were further subjected to phytochemical screening to study the phyto-constituents that exist in the extracts. The data is shown in Table-2.

Table-2: Phytochemical Screening of Extracts

| Phytochemicals | BVR-1 | BVR-2 | BVR-3 | BVR-4 |
|---|---|---|---|---|
| Alkaloids | - | + | + | + |
| Carbohydrates | - | - | + | + |
| Flavonoids | - | - | + | + |
| Triterpenoids | + | + | - | - |
| Saponins | - | - | + | + |
| Steroids | + | + | - | - |

Table-3: Effect of the Extracts on Glucoamylase, *in Vitro*

| Concentration (µg/mL) | % Inhibition | | | | |
|---|---|---|---|---|---|
| | BVR-1 | BVR-2 | BVR-3 | BVR-4 | Acarbose |
| 20 | -5.63 | 11.27 | 9.86 | 0.56 | 23.85 |
| 40 | -0.22 | 21.41 | 16.90 | 6.20 | 34.77 |
| 60 | 6.76 | 31.83 | 26.76 | 13.52 | 43.67 |
| 80 | 9.58 | 37.46 | 35.77 | 23.09 | 55.17 |
| 100 | 9.86 | 43.38 | 39.15 | 25.35 | 58.90 |
| $IC_{50}$ | 521.43 | 115.26 | 127.69 | 197.22 | 74.85 |

From Table-3, we observed that glucose inhibition is concentration-dependent. $IC_{50}$ values of BVR-1, BVR-2, BVR-3, and BVR-4 are 521.43, 115.26, 127.69 and 197.22 µg/mL respectively. As we compared the $IC_{50}$ value of Acarbose (74.85 µg/mL) with the $IC_{50}$ value of all the extracts, it was observed that the BVR-2 extract showed the lowest $IC_{50}$ value (115.26 µg/mL). Further, the % inhibition of BVR-1, BVR-2, BVR-3, and BVR-4 at 100µg/mL was 9.83, 43.38, 39.15 and 25.35%. The results obtained for the Effect of the extracts on α-amylase, *in vitro* than with acarbose which is used as the positive control and tabulated as shown in Table-4.

Table-4: Effect of the Extracts on α-amylase, *in vitro*

| Concentration (µg/mL) | % Inhibition | | | | |
|---|---|---|---|---|---|
| | BVR-1 | BVR-2 | BVR-3 | BVR-4 | Acarbose |
| 20 | -1.60 | 12.78 | 10.22 | 6.39 | 21.72 |
| 40 | -0.96 | 19.17 | 15.33 | 8.95 | 28.75 |
| 60 | 4.15 | 20.13 | 20.13 | 12.14 | 37.38 |
| 80 | 3.83 | 31.95 | 28.11 | 18.53 | 46.32 |
| 100 | 3.51 | 38.34 | 34.50 | 28.75 | 58.46 |
| $IC_{50}$ value | 1424.50 | 130.41 | 144.92 | 173.91 | 85.18 |

From the above table, we observed that, as glucose inhibition is concentration-dependent. $IC_{50}$ values of BVR-1, BVR-2, BVR-3, and BVR-4 are 1424.50, 130.41, 144.92, and 173.91µg/mL, respectively. As we compared the $IC_{50}$ value of Acarbose (85.18µg/mL) with the $IC_{50}$ value of all the extracts, it was observed that the BVR-2 extract showed the lowest $IC_{50}$ value (130.41 µg/mL). Further, the % inhibition of BVR-1, BVR-2, BVR-3, and BVR-4 at 100µg/mL was 3.51, 38.34, 34.50, 28.75%. The results attained for extracts' Antioxidant activity by the DPPH radical scavenging method were compared with Ascorbic acid and BHT and tabulated as shown in Table-5. Reducing the reaction mixture absorbance signifies increased free radical scavenging activity.

Table-5: Antioxidant Activity of the Extracts by the DPPH Radical Scavenging Method

| Concentration (µg/mL) | % Inhibition | | | | | |
|---|---|---|---|---|---|---|
| | BVR-1 | BVR-2 | BVR-3 | BVR-4 | AA | BHT |
| 200 | 4.39 | 63.75 | 81.63 | 91.30 | 96.83 | 94.73 |
| 400 | 11.81 | 69.81 | 81.76 | 92.07 | 96.77 | 95.00 |
| 600 | 20.50 | 74.19 | 81.50 | 92.14 | 96.65 | 94.61 |
| 800 | 34.10 | 77.51 | 82.21 | 91.88 | 96.55 | 94.65 |
| 1000 | 35.97 | 80.44 | 83.37 | 93.04 | 96.46 | 94.73 |

From Table-5, was observed that BVR-3 as well as BVR-4 extracts exhibit excellent DPPH radical scavenging effect than to the standards of ascorbic acid and BHT. The decreasing order of the radical scavenging effect is BVR-4>BVR-3>BVR-2>BVR-1. The BVR-2 extract showed moderate glucoamylase and α-amylase inhibition as well as DPPH radical scavenging activity. Therefore, we decided to purify the Chloroform extract.

**Spectral Characterization of Novel Compound**

| | |
|---|---|
| Molecular Formula | $C_{29}H_{50}O$ |
| Melting Point | 137-138ºC |
| Elemental Analysis | C= 83.86%, H= 12.25%, O =3.89%. |

**Mass Spectrum (m/z) (% relative intensity): EI-MS(m/z):** 414($M^+$), 396, 381, 354, 329, 273, 213, 173, 159, 145, 107, 95, 81.

**FT-IR:** 3456 $cm^{-1}$, 2916.01 $cm^{-1}$, 2848.54 $cm^{-1}$, 1703.91 $cm^{-1}$, 1463.15 $cm^{-1}$, 1376.89 $cm^{-1}$,1260.72 $cm^{-1}$, 1206.53 $cm^{-1}$, 1097 cm-1, 1018.83 $cm^{-1}$.

**$^{1}$HNMR ($CDCl_3$, 300MHz, δ in ppm):** 3.387(1H, m, H-3), 5.270 (1H, d, J=4.5Hz, H-6), 1.184 (3H, s, H-18), 0.936 (3H, d, J=6.6Hz, H-21), 0.691 (3H, s, H-19), 0.840 (1H, d, J=6.6Hz, H-26), 0.775 (3H, d,J =6.9Hz, H-27)

**$^{13}$C NMR ($CDCl_3$ ,75MHz, δ in ppm) :** 36.726 (C-1), 28.997 (C-2), 71.287 (C-3), 45.098 (C-4), 139.767 (C-5), 120.661 (C-6), 33.076 (C-7), 35.438 (C-8), 49.391 (C-9), 36.082 (C-10), 19.981 (C-11), 38.872 (C-12), 41.663 (C-13), 55.831 (C-14), 23.201 (C-15), 27.280 (C-16), 55.402 (C-17), 11.395 (C-18), 18.479 (C-19), 35.008 (C-20), 19.337 (C-21), 31.144 (C-22), 25.133 (C-23), 26.207 (C-24), 28.139 (C-25), 13.327 (C-26), 18.049 (C-27), 22.128 (C-28), 11.180(C-29).

**Specific Rotation**

The compound showed $\lambda_{max}$=248 nm and the specific rotation of the compound is $[\alpha]_D$=38. The Cotton effect curve of the compound given in Fig.-1 signifies (+) rotation i.e. rotation to the right which confirms that the compound is (24R) Stigmast-5-en-3-ol.

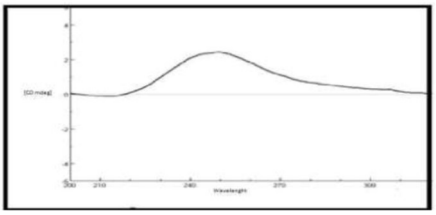

Fig.-1: Specific Rotation of Compound

The obtained component IV was a white amorphous solid which gave a positive Liebermann-Buchard's test and Salkowski test for Steroids.

**Spectral Analysis of the Compound**

**Mass Spectra**

The mass spectrum displayed a prominent peak at m/z 248 and a smaller peak at m/z 207, which are characteristic fragments of the Retro-Diels-Alder (RDA) reaction related to a C12-C13 double bond. The presence of α- and β-amyrine series with a –COOH group was verified by the m/z 203 [248-COOH] peak, along with other fragments at m/z 438 $[M\text{-}H_2O]^+$, 410 $[M\text{-}COOH]^+$, 189 $[C_{14}H_{21}]^+$, 133, 119, and 55.

**IR Spectrum**

The IR spectrum depicted a distinctive absorption peak for the hydroxyl group at 3419.47 $cm^{-1}$. The absorption peak at 1687.92$cm^{-1}$ suggests the existence of a carboxyl group. The absorption band at 1030.81$cm^{-1}$ and 996.19$cm^{-1}$ indicate the –C-OH and another peak at 2923.75$cm^{-1}$ and 2825.55$cm^{-1}$ indicate the presence of aliphatic C-H stretching.

**$^{1}$H-NMR**

The $^{1}$H –NMR spectrum of the compound revealed that the compound has a steroidal nucleus at 0.69 to 2.2 ppm indicating overlapping of methyl, methylene, and methine protons. A multiplet at δ 3.387 ppm represents an oxymethine proton (H-3). A single unsaturated proton gave a doublet at 5.270 ppm(H-6)(J=4.5 Hz).

**$^{13}$C- NMR**

The $^{13}$C NMR spectrum of the compound revealed a total of twenty-nine carbon signals, out of which six were methyl signals, eleven were methylene signals, nine were methine and three were quaternary carbon signals. The region between δ11.180 and δ55.831 represents the overlapping of methyl, methylene, and methane carbon atoms, a signal at δ71.281 represents the oxymethine signal at position 3 and finally the unsaturated carbon signals at δ120.661 and δ 139.767 represents the two carbon atoms in olefinic system. The prominent peaks at higher field of δ11.395, δ18.479, δ19.337, δ13.327, δ18.049, δ11.180 were attributed to methyl carbons of C-18, C-19, C-21, C-26, C-27 and C-29. The peaks at δ36.726, δ28.997, δ45.098, δ33.076, δ19.981, δ38.872, δ23.201, δ27.280, δ31.144, δ25.133, δ22.128 represents methylene

carbon of C-1, C-2, C-4, C-7, C-11, C-12, C-15, C-16, C-22, C-23 and C-28. The peaks at δ71.287, δ120.661, δ35.438, δ49.391, δ55.831, δ55.402, δ35.008, δ26.207 and δ28.139 were attributed to methine carbon of C-3, C-6, C-8, C-9, C-14, C-17, C-20, C-24 and C-25.The signals at δ139.767, δ36.082, and δ41.663 represent quaternary carbons at C-5, C-10, and C-13.

**DEPT-135 Spectrum**

In DEPT-135 Spectral data there were 26 carbon signals out of which six were methyl signals, eleven were methylene signals and nine were methine signals. The data showed nine methylene (-$CH_2$- negative) groups with signals at δ36.726, δ28.997, δ45.098, δ33.076, δ19.981, δ38.872, δ23.201, δ27.280, δ31.144, δ25.133, δ22.128 represents methylene carbon of C-1, C-2, C-4, C-7, C-11, C-12, C-15, C-16, C-22, C-23 and C-28. On the basis of the above spectral and chemical evidence, the isolated compound was identified as β-Sitosterol Fig.-2, Fig.-3.

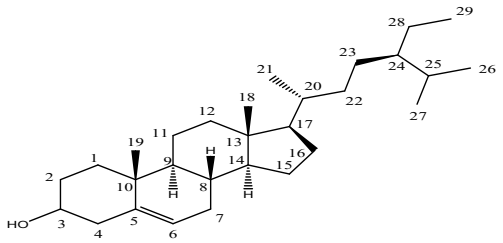

Fig.-2: β-Sitosterol

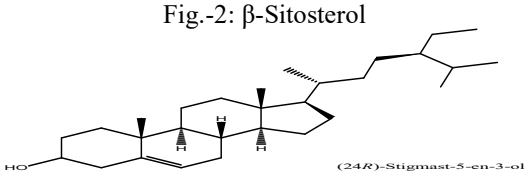


Fig.-3: (24R)-Stigmast-5-en-3-ol

The compound β-Sitosterol isolated from the Chloroform extract of *Cassia fistula* flower was further evaluated for its effect on carbohydrate metabolizing enzymes, glucoamylase, and α-amylase as well as for its DPPH radical scavenging effect.

**Effect of β-Sitosterol on Glucoamylase, *in vitro***

The solution of β-Sitosterol was prepared in various concentrations ranging from 20-100 µg/mL using Methanol as per the procedure mentioned. The results obtained were compared with Acarbose which is used as the positive control (Table-6).

Table-6: Effect of β-Sitosterol on Glucoamylase

| | Concentration (µg/mL) | % Inhibition Acarbose | Concentration (µg/mL) | % Inhibition β-Sitosterol |
|---|---|---|---|---|
| 1 | 20 | 33.15 | 2 | 35.5 |
| 2 | 40 | 38.20 | 4 | 42.2 |
| 3 | 60 | 43.82 | 6 | 56.8 |
| 4 | 80 | 53.65 | 8 | 64.8 |
| 5 | 100 | 66.01 | 10 | 79.7 |
| | $IC_{50}$ value | 67.46 | $IC_{50}$ value | 5.8 |

From Table-6, it was observed that the percentage inhibition is directly proportional to the concentration of β-Sitosterol. Comparative studies with acarbose show 56.8% and 66.01% inhibition respectively at 10 µg/mL concentration for β-Sitosterol and 100 µg/mL for acarbose. The $IC_{50}$ values of β-Sitosterol and acarbose have been observed to be 5.8 and 67.46µg/mL correspondingly.

**Effect of β-Sitosterol on α-amylase, *in vitro***

The solution of β-Sitosterol was prepared in various concentrations ranging from 20-100 µg/mL using Methanol as per the procedure mentioned. The results obtained were compared with Acarbose which is used as the positive control (Table-7).

Table-7: Effect of β-Sitosterol on α-amylase

| S.No. | Concentration (µg/mL) | % Inhibition Acarbose | Concentration (µg/mL) | % Inhibition β-Sitosterol |
|---|---|---|---|---|
| 1 | 20 | 21.47 | 2 | 38.2 |
| 2 | 40 | 33.65 | 4 | 41.5 |
| 3 | 60 | 44.55 | 6 | 46.2 |
| 4 | 80 | 51.60 | 8 | 51 |
| 5 | 100 | 63.78 | 10 | 58.3 |
| | $IC_{50}$ value | 73.62 | | 7.19 |

From Table 7, it was observed that the percentage inhibition is directly proportional to the concentration of β-Sitosterol. Comparative studies with acarbose show 58.3% and 63.78% inhibition respectively at 10µg/mL and 100µg/mL concentration. The $IC_{50}$ value of β-Sitosterol and acarbose was found to be 7.19µg/mL and 73.62µg/mL correspondingly.

**Antioxidant Activity of β-Sitosterol by DPPH Radical Scavenging Method**

The result obtained for the Antioxidant activity of β-Sitosterol by the DPPH radical scavenging method is shown in Table-8.

Table-8: Effect of β-Sitosterol on Radical Scavenging Activity by DPPH Method

| S.No. | Concentration (µg/mL) | Ascorbic acid | BHT | β-Sitosterol |
|---|---|---|---|---|
| 1 | 20 | 95.24 | 65.23 | 43.90 |
| 2 | 40 | 96.66 | 75.78 | 62.19 |
| 3 | 60 | 96.91 | 83.46 | 75.68 |
| 4 | 80 | 97.20 | 89.65 | 86.75 |
| 5 | 100 | 97.11 | 94.20 | 92.14 |

From Table-8, it is concluded that β-Sitosterol possesses good radical scavenging activity by the DPPH approach. At 100 µg/mL, β-Sitosterol exhibits a 92.14 % of inhibition, and at 20 µg/mL, it exhibits 43.90% of inhibition. Whereas the percent inhibition of Acarbose at 100 and 20 µg/mL is 97.11 and 95.24%, respectively. % inhibition of BHT at 100 and 20 µg/mL is 94.20 and 65.23%.

## CONCLUSION

The present study paves the way for a novel method of the synthesis of β-Sitosterol which shows moderate inhibition activities against glucoamylase and α-amylase and good radical scavenging activity by the DPPH method. The molecular mechanisms responsible for these biological activities is a part of further research.

## CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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## REFERENCES

1. H.I. Katcher, A.M. Hill, J.L. Lanford, J.S. Yoo, P.M. Kris-Etherton., *Endocrinology and Metabolism Clinics of North America*, **38(1)**,45(2009), https://doi.org/10.1016/j.ecl.2008.11.010

2. A. Poli, F. Marangoni, A. Corsini, E. Manzato, W. Marrocco , D. Martini, G. Medea, F. Visioli, *Nutrients,***13(8)**,2810(2021), https://doi.org/10.3390%2Fnu13082810
3. R. E. Jr. Ostlund., *Annual Review Nutrition,* **22**, 533(2002), https://doi.org/10.1146/annurev.nutr.22.020702.075220
4. J.W. Anderson, L. D. Allgood, A. Lawrence, L.A. Altringer, G.R. Jerdack, D.A. Hengehold, J.G. Morel., *American Journal of Clinical Nutrition,***71(2)**, 472(2000), https://doi.org/10.1093/ajcn/71.2.472
5. Y. Du, X. Fu, Y. Chu, P. Wu, Y .Liu, L. Ma, H. Tian, B. Zhu, *International Journal of Molecular Sciences,* **23(4)**, 2332(2022), https://doi.org/10.3390%2Fijms23042332
6. Soodabeh Saeidnia, Azadeh Manayi, Ahmad R.Gohari, *European Journal of Medicinal Plants*, **4(5)**,590(2014), https://doi.org/10.9734/EJMP/2014/7764
7. Y.H Ju, L.M Clausen, K.F Allred, A.L Almada, W.G Helferich, *Journal of Nutrition,* **134**, 1145(2004), https://doi.org/10.1093/jn/134.5.1145
8. C. Jourdain, G. Tenca, A. Deguercy, P. Troplin, D. Poelman.. *European Journal of Cancer Prevention*,**15**, 353(2006), https://doi.org/10.1097/00008469-200608000-00009
9. I.M. Villasenor, J. Angelada, A.P. Canlas, D. Echegoyen, *Phytotherapy Research*, **16**, 417(2002), https://doi.org/10.1002/ptr.910
10. C. Shi, F. Wu, X. Zhu, J. Xu, *Biochimica et Biophysica Acta*, **1830**, 2538(2013), https://doi.org/10.1016/j.bbagen.2012.12.012
11. E.J .Moon Y.M.Lee, O.H. Lee M.J.Lee, S.K. Lee, M.H. Chung, et al., *Angiogenesis*, **3**, 117(1999), https://doi.org/10.1023/A:1009058232389

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