https://dx.doi.org/10.4314/dujopas.v10i4b.14
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 4b December 2024*

Assessment of The Prospects of β-Sitosterol and its Derivatives as Antioxidant and Anti-Inflammation

Idorenyin Ugochi Nwaehujor*[1] and Gabriel Ademola Olatunji[2]

[1]Perishable Crop Research Department,
Nigerian Stored Products Research Institute.
P.M.B. 1489, Ilorin,
Kwara State,
Nigeria.

[2]Department of Chemistry,
Faculty of Physical Science,
University of Ilorin,
P.M.B. 1515, Ilorin,
Kwara State,
Nigeria.

Email: idorenyinugochi@gmail.com

*ORCID: https://orcid.org/0000-0001-5851-5283

Abstract
One of the most common bioactive phytosterols found in plants is β-sitosterol. It functions as inflammation reducer, oxidation inhibitor, immunosuppressive and antiarthritis. Inflammation is implicated in severe ailments, and it is a condition that has led to several dead in the world. Most of the drugs utilized in the management of inflammation have been found to restrain the performance of the immune system. β-sitosterol acetate and β-sitosterol triol were synthesized from β-sitosterol and tested for antioxidant on 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and hydrogen peroxide. Also, inflammatory inhibition was assayed with lipoxygenase, proteinase, albumin denaturation inhibition and membrane stabilization. Results of the DPPH and ABTS performance of β-sitosterol and its synthesized products were comparable while β-sitosterol acetate had higher hydrogen peroxide scavenging activity than β-sitosterol and β-sitosterol triol. The three samples showed no significant difference (P<0.05) on lipoxygenase inhibition but β-sitosterol triol had higher proteinase inhibition at 10 – 100 µg/mL. Also, at a measurement 150 µg/mL, β-sitosterol acetate recorded remarkably better performance in albumin denaturation suppressant and membrane stabilization. The synthesized products of β-Sitosterol had better antioxidant and anti-inflammatory activities than β-sitosterol. The derivatives β-sitosterol would offer enhanced therapeutic effects on inflammation and other diseases.

Keywords: Antioxidant, Derivatives, Inflammation β-Sitosterol, Synthesis

INTRODUCTION
When free radicals interact with molecular oxygen, reactive oxygen is generated leading to inflammation. Many illnesses such as rheumatoid arthritis, high blood pressure, cancers, heart diseases and inflammatory bowel diseases have been traced to inflammation which in turn

Author for Correspondence

leads to dead globally (Krishnamoorthy *et al.*, 2016). Most of the drugs utilized to remedy inflammation have negative outcome such as gastrointestinal hemorrhage, lowering of the immunity of the body and peptic ulcers (Amri *et al*., 2018). Due to safety concern in the treatment with these drugs, the search for other agents that could offer safe alternatives is considered.

β-Sitosterol is a significant metabolite in some plants. They are found in some fruits, vegetables, nuts and seeds. It is a white powder with a melting point of 136^0C. The chemical composition of β-sitosterol is similar to that of cholesterol. The compound is reported to possess various biological activities such as, pain reliever, immune regulator, antimicrobial, anticancer, inflammatory suppressant and lipid reducing property (Babu and Jayaraman, 2020). β-sitosterol being an outstanding metabolite of some plants have been commercialized because of its numerous bioactivity (Khan *et al*., 2022). Nandi *et al*. (2024), in their review reported that β-sitosterol manifested chemosensitizing properties on cancer cells, countering mechanisms such as multiplication, halting of cell cycle and invasion. It reduces the risk of coronary artery disease heart attacks and atherosclerosis (Durrani *et al.*, 2024). β-sitosterol holds potential as a safe nutraceutical against inflammation.

In spite of the fact that β-sitosterol has significant anti-tumor activity, natural phytosterols undergo auto-oxidation or enzymatic oxidation stimulated by reactive oxygen specie, light, heat, or enzymes to form phytosterol oxidation products. The effects of these compounds on human health are not ascertained. Also, β-sitosterol has poor solubility in water which limits their bioavailability and therapeutic effect (Dai and Row, 2021). It is believed that modifying the structure of the compounds would enhance drug release, solubility, targeting, and bioavailability (Xiong *et al*., 2016).

The antidepressant investigation by Yin *et al.* (2018), on β-sitosterol and its derivatives revealed that β-sitosteryl salicylate, which is one of the derivatives, showed better activity than β-sitosterol. β-Sitosterol-D-glucoside, derived from sweet potato was reported to have strong anti-cancer activity. β-Sitosterol-glucoside restrained the growth of hepatoma cells by stimulating the activity of caspase-3 and -9 through activating their pathways to induce cell apoptosis (Bao *et al.*, 2022). β-Sitosterol derivatives are found to be more potent than the main compound. There is need to assess the structural derivatives of the compound to determine their antioxidant activities and also their efficacy in combating inflammation.

MATERIALS AND METHODS

Collection of samples

The β-sitosterol (BST) used for this study was obtained from Sigma-Aldrich through Bristol Scientific Company, 14 Bristol road, Apapa, Lagos, Nigeria.

Synthesis of β-sitosterol acetate

β-Sitosterol acetate was synthesized following a procedure described by McCarthy *et al.* (2005). The product of the synthesis was confirmed using Fourier Transform Infrared (FTIR) spectroscopy and melting point. The sample was coded BSA.

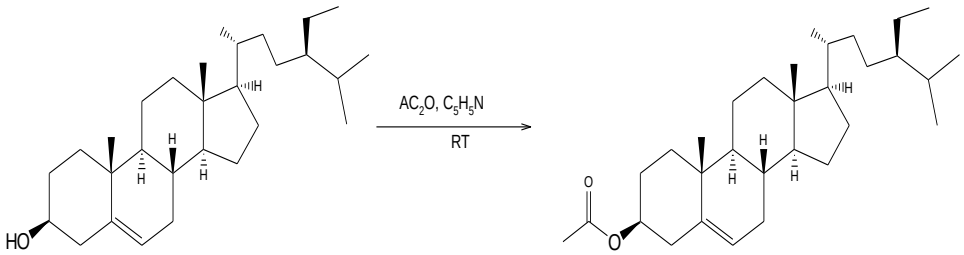

Figure 1: Equation for the preparation of β-sitosterol acetate

Synthesis of β-sitosterol triol

β-Sitosterol triol was synthesized according to the procedure of McCarthy, *et al.*, (2005). The product of the synthesis was confirmed using FTIR and melting point. The sample was coded BSTT.

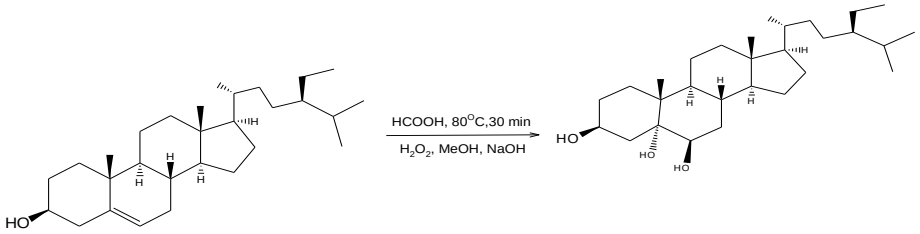

Figure 2: Equation for the preparation of β-sitosterol triol

Antioxidant assay of β-sitosterol and its derivatives

β-Sitosterol, β-sitosterol acetate and β-sitosterol triol were tested for their anti-oxidant effect using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and H_2O_2 scavenging. The experiment was carried out following the procedure of Nishaa *et al*. (2012).

Anti-inflammatory assessment of β-sitosterol and its derivatives

Anti-inflammatory studies were carried out with β-sitosterol, β-sitosterol acetate and β-sitosterol triol using lipoxygenase, proteinase, albumin denaturation inhibition and Membrane stabilization with the procedure of Leelaprakash and Das (2011).

RESULTS AND DISCUSSION

Preparation of β-sitosterol acetate (BSA)

IR (KBr) ν_{max}/cm^{-1}**:** 2951, 2863, 1726, 1467, 1368, 1261, 1039.

The product obtained was an off-white solid with a weight of 655 mg. The percentage yield was 65.5% with a melting point of 124^0C. The IR result confirmed the conversion of the hydroxyl group of β-sitosterol to acetate, depicted by the appearance of a carbonyl stretching peak at 1726 cm^{-1} (Figure 3).

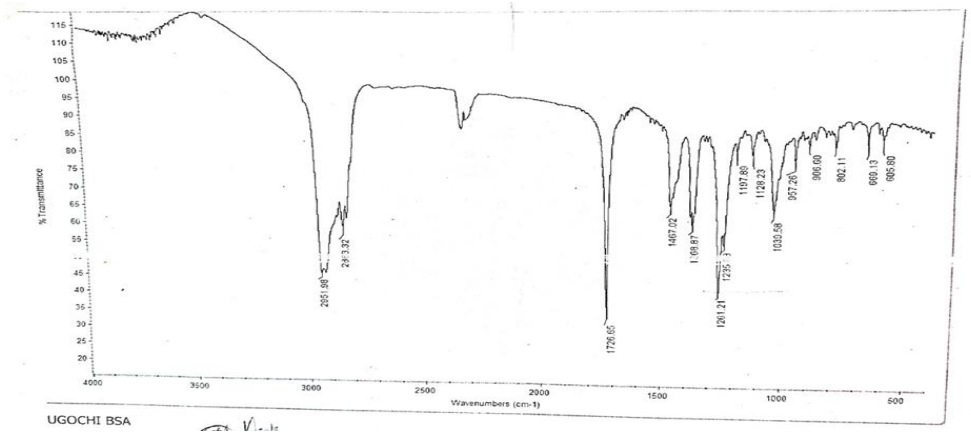

Figure 3: IR spectrum of β-sitosterol acetate

Preparation of β-sitosterol triol (BSTT)

IR (KBr) ν_{max}/cm^{-1}: 3407, 2952, 2853, 1463, 1375, 1052.

The product was a white solid, weighing 739mg (37% yield), and the melting point was 135^{0}C. The formation of multiple OH was confirmed by the appearance of the broad brand at 3407 cm^{-1} (Figure 4). The IR result was comparable with the report of McCarthy *et al*., (2005).

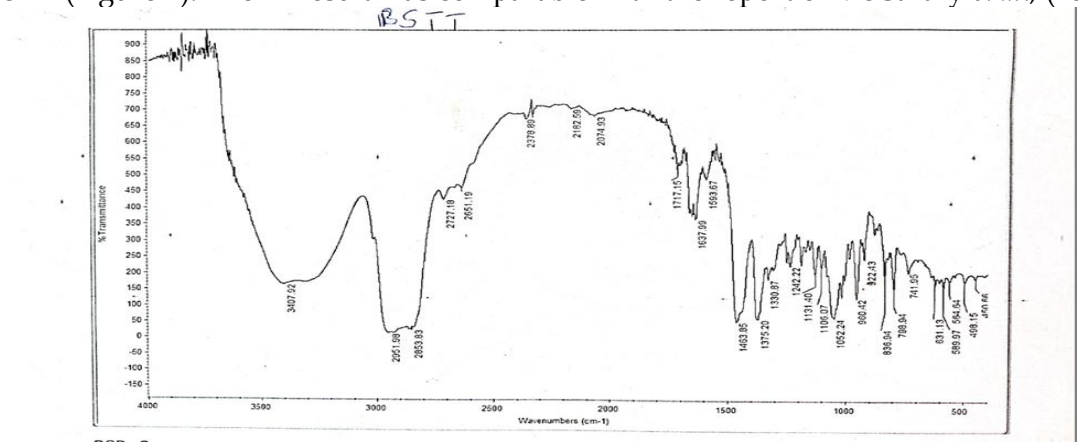

Figure 4: IR spectrum of β-sitosterol triol

Results of antioxidant assay of β-sitosterol and its derivatives.

DPPH radical scavenging

The assessment of DPPH radical inhibition showed that BSA performed better than BST and BSTT. At the concentration of 10 -15 µg/mL, no remarkable difference was noticed in the performance of all the samples and the reference standard (butylated hydroxytoluene).

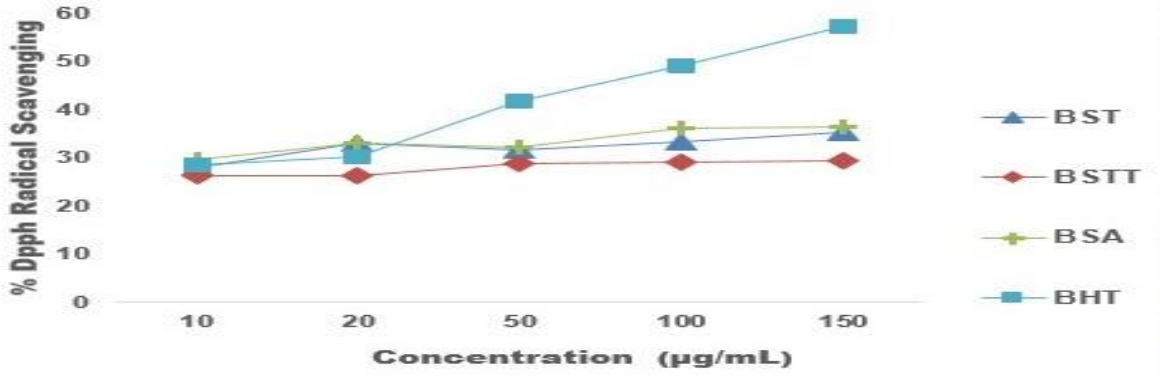

Figure 5: *In-vitro* DPPH radical scavenging of β-sitosterol and its derivatives. Each of the spot on the graph stands for mean of triplicate readings. BHT = butylated hydroxytoluene

ABTS radical scavenging

The results indicated that there was no significant difference ($p<0.05$) in the performance of β-sitosterol and its derivatives but at the dosage of 10 µg/mL, their activities were more prominent compared with the standard (BHT).

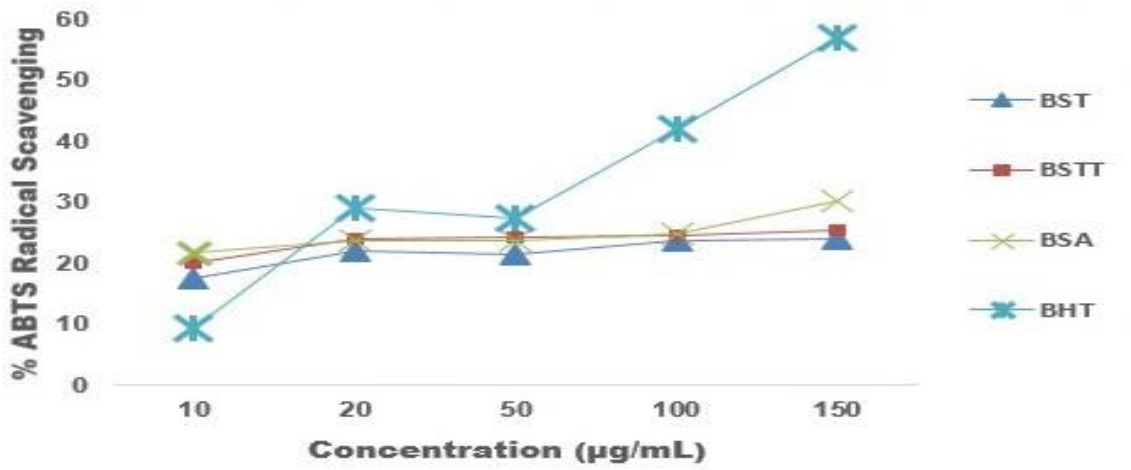

Figure 6: The *In-vitro* ABTS radical scavenging of β-sitosterol and its derivatives. Each spot on the graph stands for mean of triplicate readings.

H_2O_2 scavenging activity

The BSA had higher H_2O_2 scavenging than BST and BSTT. At concentrations 10 – 50 µg/mL, the performance of BSA were greater than the standard (BHT). BSTT had lowest activity in scavenging H_2O_2 free radicals. Hydrogen peroxide is not a free radical but it can influence several cellular processes. Although hydrogen peroxide is not toxic, it can be easily converted to toxic hydroxyl radical and hypochlorous acid (Mukhopadhyay *et al*., 2016).

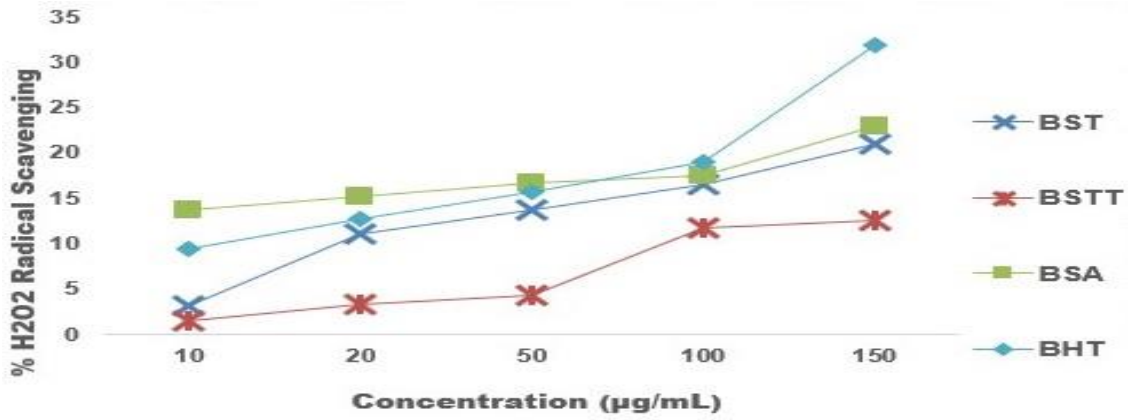

Figure 7: The *In-vitro* H_2O_2 radical scavenging of β-sitosterol and its derivatives. Each spot on the graph stands for mean of triplicate readings.

Results of anti-inflammatory effect of β-sitosterol and its derivatives

Lipoxygenase inhibition activity

The lipoxygenase inhibition assay revealed that there was no significant difference in the performance of all the samples including the standard (Indomethacin). All the samples exhibited high lipoxygenase inhibition activity (Figure 8). Lipoxygenase and their catalysis products are involved with carcinogenic operations (Wisastra and Dekker, 2014).

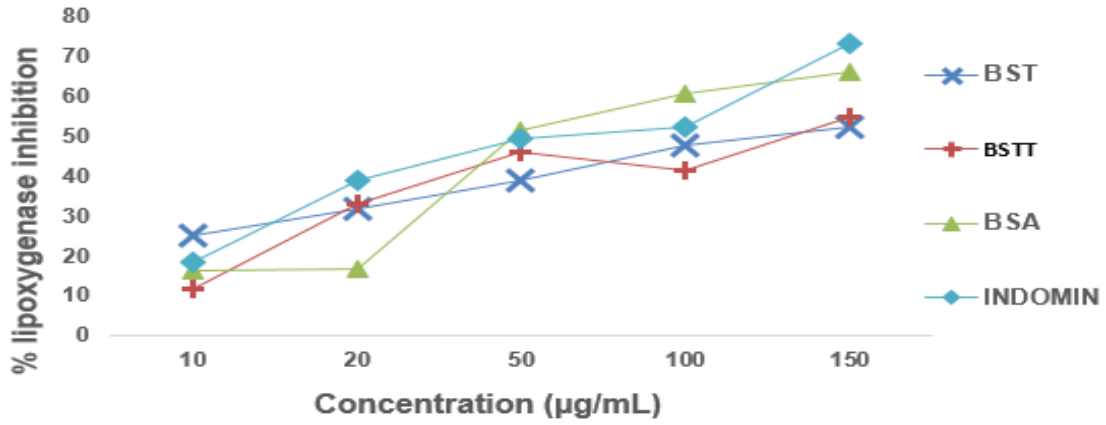

Figure 8: The *In-vitro* lipoxygenase inhibition activity of β-sitosterol and its derivatives. Each spot on the graph stands for mean of triplicate readings. Indomin = indomethacin

Proteinase Inhibition activity

The result showed that BSTT has highest proteinase inhibition at 10 – 100 µg/mL but at 150 µg/mL, the activity of BSA increased significantly. Proteinases are responsible for modulating physiological activities like growth. They also take part in different pathological situations such as hypertension, cancer, and malaria. The activities of proteinases should be controlled in order to prevent unrestrained cleavage of proteins. Proteinase inhibitors perform important function in controlling proteinase activities and also demonstrate therapeutic effects against diseases in the body (Perera *et al*., 2016).

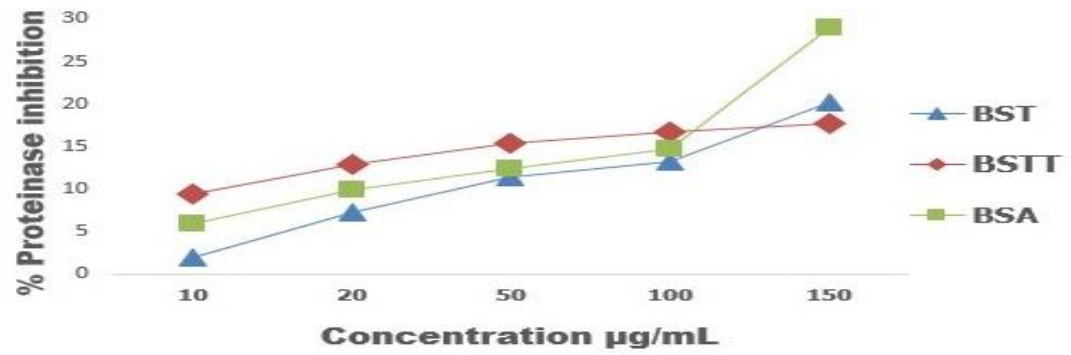

Figure 9: The *In-vitro* proteinase inhibition activity of β-sitosterol and its derivatives. Each spot on the graph stands for mean of triplicate readings.

Albumin denaturation inhibition

The activity of BSTT was moderately higher than BST and BSA at 10 – 100 µg/mL but at 150 µg/mL, BSA showed highest albumin denaturation inhibition. Similar trend was observed in proteinase inhibition. Albumin denaturation is the partial or complete modification of the structures of proteins or nucleic acids resulting in a loss of bioactivity and cell death.

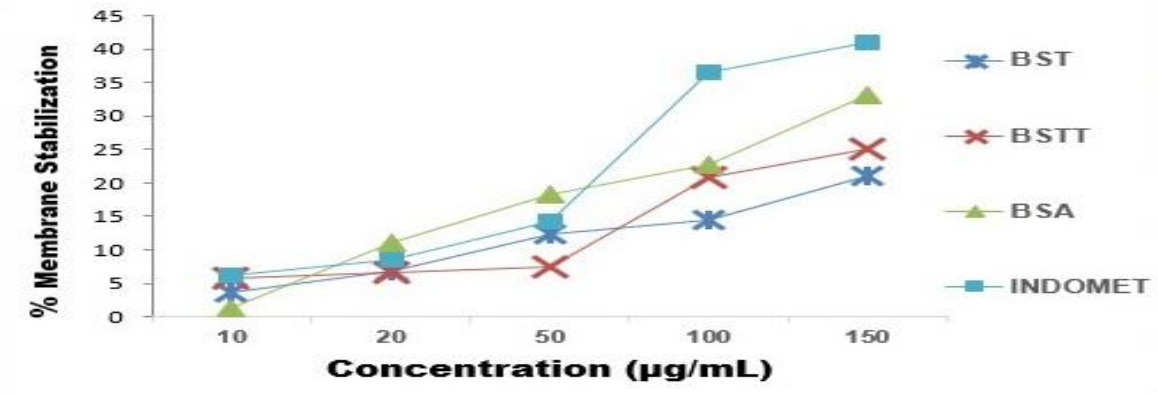

Figure 10: The *In-vitro* albumin denaturation inhibition activity of β-sitosterol and its derivatives. Each spot on the graph stands for mean of triplicate readings.

Membrane stabilization activity

The membrane stabilization assay showed that there was no remarkable contrast in the performance of the samples at 10 – 50 µg/mL. BSA was higher than BST and BSTT at 20 – 150 µg/mL. The vitality of the cells is subject to the viability of their cell membrane. When the cells are exposed to injuries, it would cause oxidation of haemoglobin and secondary damage through free radical induced lipid peroxidation. Destruction of the cell membrane results in the release of lysosomal enzymes which cause different disorders. The membrane stabilizing agents inhibit the release of these enzymes (Sumathi and Anuradha 2016).

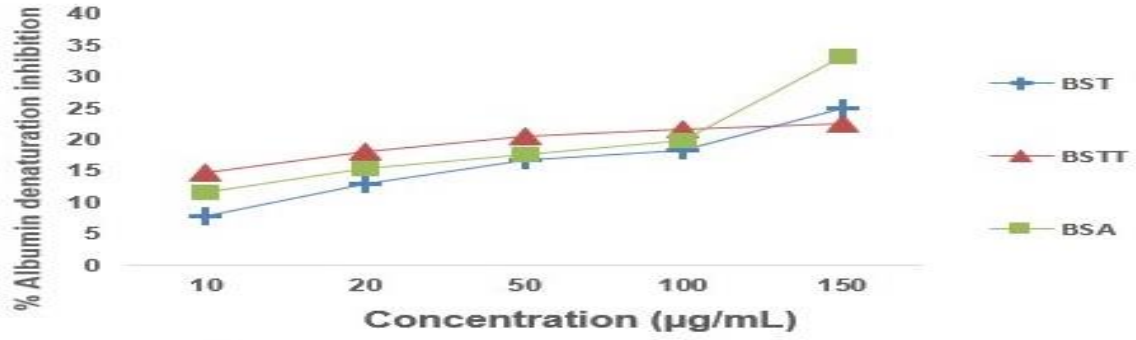

Figure 11: The *In-vitro* membrane stabilization activity of β-sitosterol and its derivatives. Each spot on the graph stands for mean of triplicate readings.

CONCLUSION

The derivatization of β-Sitosterol to β-Sitosterol acetate and β-sitosterol triol were successful and the conversion was confirmed by FTIR spectroscopy. The bioactivity studies revealed that the derivatives had stronger effect as antioxidant and also as inflammation inhibitor than β-sitosterol. Further studies are recommended to ascertain the rate and system of action of the derivatives and to ascertain their toxicological characteristics.

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