Umar et al (2023)
Vol 11(2): 6284
https://doi.org/10.20884/1.api.2023.11.2.6284

Acta Pharmaciae Indonesia:
Acta Pharm Indo
E-ISSN 2621-4520

RESEARCH ARTICLE Open Access

Characterization and tyrosinase activity of a mixture of beta-sitosterol and stigmasterol from *Bauhinia rufescens* Lam.

Abdulganiyu Umar[1], Musa Maikudi Idris[2], Umar Ali[3], Aminu Muhammad[2*], Hasnah Mohd Sirat[4]

ABSTRACT

Background: Phytosterols are steroid compounds present in plants that are similar to cholesterol in structure and functions. In this work, stem bark of *Bauhinia rufescens* was extracted with petroleum ether.

Objective: This work aims to determine anti-tyrosinase activities of a mixture of β-sitosterol and stigmasterol from the stem bark *B, rufescens* for the treatment of dermatological disorders.

Methods: This work is presenting isolation, characterization and anti-tyrosinase activities of a mixture of β-sitosterol and stigmasterol from the stem bark *B. rufescens*. The extract was subjected to chromatographic techniques to afford a phytosterol identified as a mixture of β-sitosterol and stigmasterol based on GC-MS, IR, ^{1}HNMR and ^{13}CNMR data.

Results: The phytosterol isolated showed inhibition of tyrosinase activity at 57.1%±0.03 as compared to kojic acid with 85.0% ± 0.01 (sample concentrations of 0.1 mg/mL).

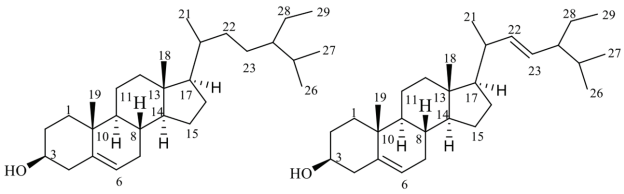
Conclusion: The finding of this study revealed that, the mixture of β-sitosterol and stigmasterol has potential to be developed as tyrosinase inhibitors for the treatment of dermatological disorders.

Keywords: *B. rufescens*, tyrosinase, phytosterol, spectroscopy

Introduction

Phytosterols, structurally similar to cholesterol with variations in carbon side chains and the presence or absence of a double bond, are steroid compounds found in plants. These plant cholesterols, similar in structure and functions to cholesterol [1], are secondary metabolites considered health-promoting constituents of natural foods. Stigmasterol and sitosterol, two widely distributed phytosterols in plants, animals, and fungi, have been reported for their potent antioxidant, hypoglycemic, and thyroid-inhibiting properties [2].

The genus *Bauhinia* (Fabaceae) comprises around 300 species known as 'cow's paw' or 'cow's hoof' due to their leaf shape. Our investigation into the stem bark of *Bauhunia guianensis* led to the isolation of β-sitosterol and stigmasterol [3]. In a previous study, we reported the tyrosinase inhibitory effect of the leaves and stem bark extract of *B. rufescens*, with the stem bark extracts showing higher inhibitions [4]. This current work presents a novel finding of isolation, characterization, and anti-tyrosinase activities of a mixture of β-sitosterol and stigmasterol (Figure 1) from the stem bark of *B. rufescens*.



β-sitosterol **stigmasterol**

Figure 1. Structure of beta-sitosterol and stigmasterol

[1]Department of Chemistry, Faculty of Science, Yusuf Maitama Sule University, Kano State, Nigeria

[2]Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, Bayero University Kano, Kano State, Nigeria

[3]Department of Chemistry, Faculty of Natural and Applied Sciences, Kafin Hausa, Jigawa State, Nigeria

[4]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia.

*Corresponding author: Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, Bayero University Kano, Kano State, Nigeria. Email: amuhammad.chm@buk.edu.ng

Methods

General

Melting points were determined using a Leica Gallen III Kofler micro melting point apparatus. UV spectra were measured with a Shimadzu UV 1601PC spectrophotometer, and IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer. The 1H- and 13C-NMR spectra were recorded on Bruker Avance 400 MHz spectrometer. Chemical shifts were recorded in parts per million (δ) in deuterated chloroform. Column chromatography was carried out on silica gel 70-230 mesh (Merck). Silica gel 60 F254 pre-coated aluminum plates (0.2 mm, Merck) were used for TLC analysis; detection was performed by spraying with 5 % H2SO4 in methanol and 1 % vanillin in methanol, followed by heating at 120 °C for 5 min. The GC analysis was performed using Hewlett Packard HP 6890 series II A gas chromatograph equipped with an HP-5 column (30 m long, 0.25 µm thickness, and 0.25 mm inner diameter). The GC-MS chromatograms were recorded using a Hewlett Packard Model 5890A gas chromatography and a Hewlett Packard Model 5989A mass spectrometer.

Plant material

The stem bark of *B. rufescens* was collected at Kiru, Kano State, Nigeria, in August 2011. A voucher specimen (Acc. 99) was deposited in the herbarium of the Department of Biological Sciences, Bayero University, Kano, Nigeria.

Extraction and isolation

The dried stem bark of *B. rufecsens* (800 g) was ground and extracted with petroleum ether (4.0 L) in a soxhlet extractor each for 18 h. The sample was concentrated using a rotary evaporator to give sticky brown substances of petroleum ether (5.82 g, 0.73 %).

The petroleum ether extract, BRSP (5.0 g), was fractionated over a silica gel column (180 g silica, column size 60.0 cm x 5.0 cm) using petroleum ether - diethyl ether - chloroform - ethyl acetate - methanol step gradient to afford 230 fractions. They were pooled into 32 fractions, BRSP1-BRSP32 based on thin layer chromatography profile.

Fraction BRSP17 (495.5 mg) was chromatographed over silica gel (20 g silica, column size 30.0 cm x 2.5 cm) and eluted with petroleum ether, diethyl ether, and ethyl acetate with increasing polarity to give 65 fractions (BRSP17-1 to BRSP17-65). BRSP17-33 to 37 were combined and purified by crystallization using *n*-hexane to yield a mixture of *β*-sitosterol and stigmasterol (24.3 mg, 0.49%) as a white solid.

Characterization

R_f = 0.38 (petroleum ether-diethyl ether, 3:2); m.p 149-154°C, literature [5] 138°C for *β*-sitosterol and 162°C for stigmasterol; IR (KBr) ν_{max} cm^{-1}: 3433, 3029, 2937, 1640, 1062; ^{1}H NMR ($CDCl_3$): δ 5.36 (2H, d, *J* = 5.2 Hz, H-6, 6′), 5.15 (1H, dd, *J* = 15.2, 8.6 Hz, H-22′), 5.03 (1H, dd, *J* = 15.2, 8.6 Hz, H-23′), 3.53 (2H, m, H-3, 3′),0.96 (3H, d, *J* = 7.6 Hz, H-26′), 0.94 (6H, d, *J* = 6.8 Hz, H-21, 21′), 0.87 (6H, s, H-18,18′), 0.81 (6H, t, *J* = 7.2 Hz, H-29, 29′), 0.71 (3H, d, *J* = 7.2 Hz, H-26); ^{13}C NMR ($CDCl_3$): δ 37.2 (C-1, 1′), 31.6 (C- 2, 2′), 71.8 (C-3, 3′), 42.2 (C-4), 42.3 (C-4′), 140.7 (C-5, 5′), 121.6 (C-6, 6′), 31.9 (C-7, 7′), 31.9 (C-8, 8′), 50.1 (C-9, 9′), 36.5 (C-10, 10′), 21.0 (C-11, 11′), 39.6 (C-12′), 39.7 (C-12), 42.3 (C-13, 13′), 56.7 (C-14), 56.8 (C-14′), 24.2 (C-15), 24.3 (C-15′), 28.2 (C-16), 28.8 (C-16′), 55.9 (C-17′), 56.0 (C-17), 12.0 (C-18, 18′), 19.3 (C-19, 19′), 36.1 (C-20), 40.4 (C-20′),18.7 (C-21), 21.0 (C-21′), 33.9 (C-22), 138.2 (C-22′), 26.1 (C-23), 129.2 (C-23′), 45.8 (C-24), 51.2 (C-24′), 29.1 (C-25), 31.8 (C-25′), 19.7 (C-26), 21.1 (C-26′), 19.0 (C-27), 18.9 (C-27′), 23.0 (C-28), 25.3 (C-28′), 12.2 (C-29, 29′); EIMS *m/z* 414 [M^+, $C_{29}H_{50}O$] *β*-sitosterol and 412 [M^+, $C_{29}H_{48}O$] stigmasterol.

Tyrosinase inhibitory assay

Tyrosinase inhibitory activity was determined using the method described by [6], with slight modifications. Each sample (0.1 mg/mL, 40 µL) was mixed with sodium phosphate buffer (100 mM, 80 µL, pH 6.8) and then L-DOPA solution (2.5 mM, 40 µL) and mushroom tyrosinase enzyme (EC1.14.18.1; 100 units/mL, 40 µL) were added into a 96-well plate. The test mixture (200 µL) was mixed well and incubated at 37°C for 10 min. DMSO instead of the sample was used as a negative control, and kojic acid (0.1 mg/mL) was employed as positive control. The absorbance level was obtained with a multiplate reader at 515 nm with reference to 655 nm, and the percentage inhibition of tyrosinase activity was calculated by the following formula:

$$\text{Inhibition (\%)} = \left(\frac{\text{Absorbance (blank)-Absorbance (tyrosinase+sample)}}{\text{Absorbance (blank)}}\right) \text{x } 100$$

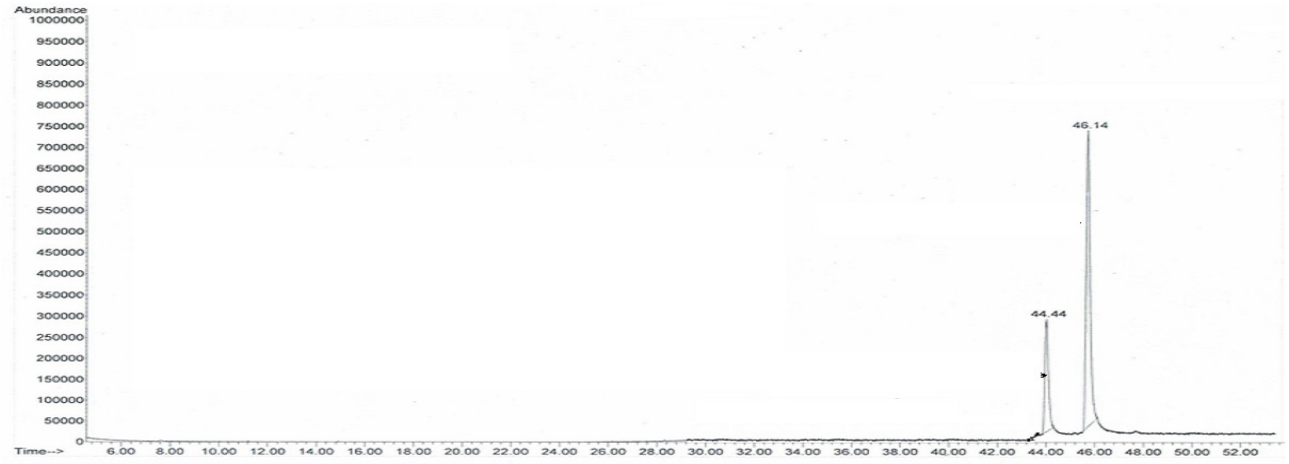


Figure 2. Gas chromatogram of the phytosterols

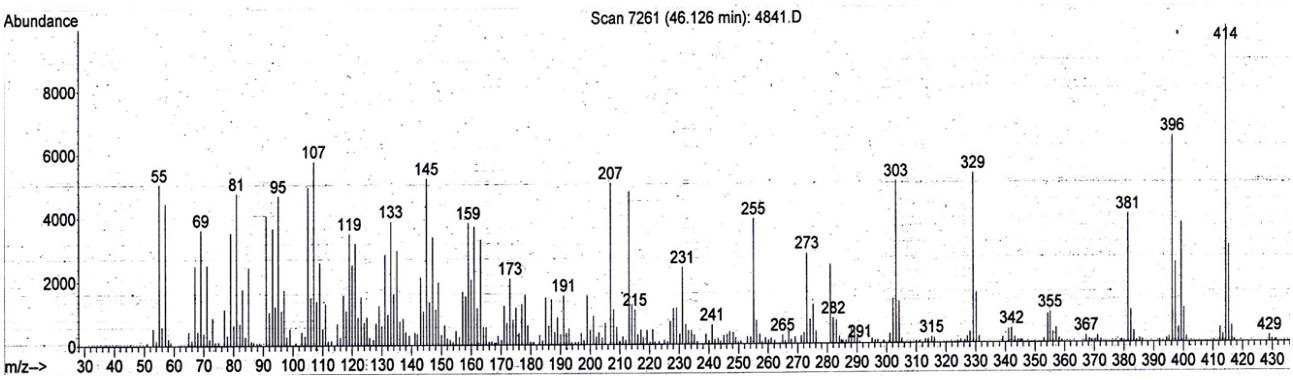


Figure 3. Mass spectrum of the β-sitosterol

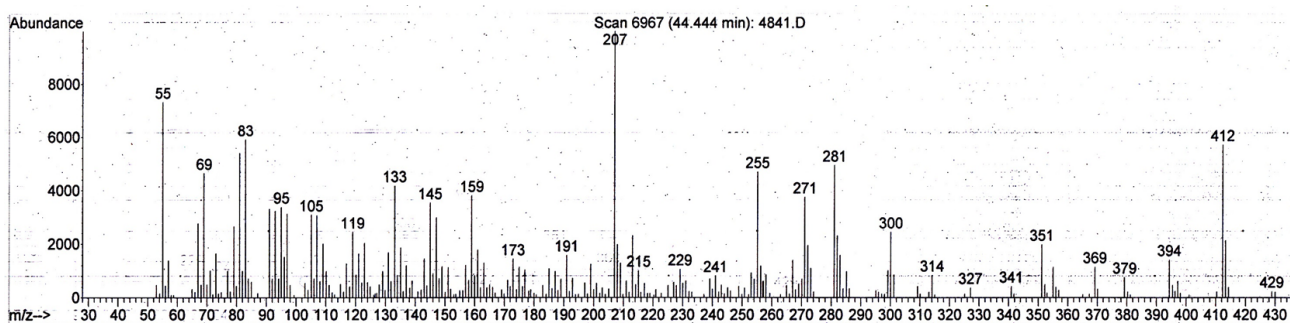


Figure 4. Mass spectrum of the stigmasterol

Results

Purification of BRSP17 (495.5 mg) using silica gel CC, eluted with petroleum ether, diethyl ether and ethyl acetate gradient yielded a white solid substance (24.3 mg, 0.49%) melted at 149-154°C [7], 144-146°C. From the GC-MS analysis, the gas chromatogram of the white solid obtained (Figure 2) revealed two peaks with retention time 46.13 and 44.44 minutes corresponding to molecular ion peak at *m/z* 414 ($C_{29}H_{50}O$) and 412 ($C_{29}H_{48}O$) respectively (Figures 3 and 4).

The IR spectrum (Figure 5) of the mixture showed absorption peaks at 3433 cm^{-1} for OH stretching, 2937 cm^{-1} for CH stretching and 1062 cm^{-1} for C-O stretching. The ^{1}H NMR spectrum (Figure 6) showed the presence of oxymethine protons at δ 3.53 (H-3, H-3′) and olefinic protons at δ 5.36 (H-6, H-6′). In addition, pair of double of doublet at δ 5.15 (*J* = 15.2, 8.6 Hz) and 5.03 (*J* = 15.2, 8.6 Hz) assignable to H-22′ and H-23′ were also observed for the characteristic steroid nucleus.

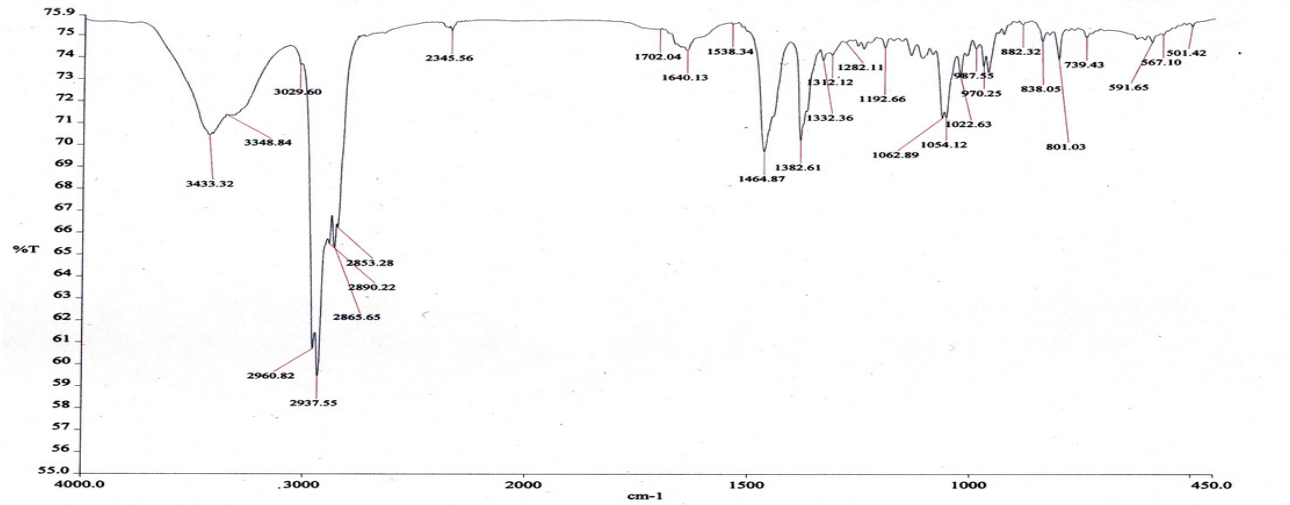


Figure 5. IR spectrum of the phytosterol

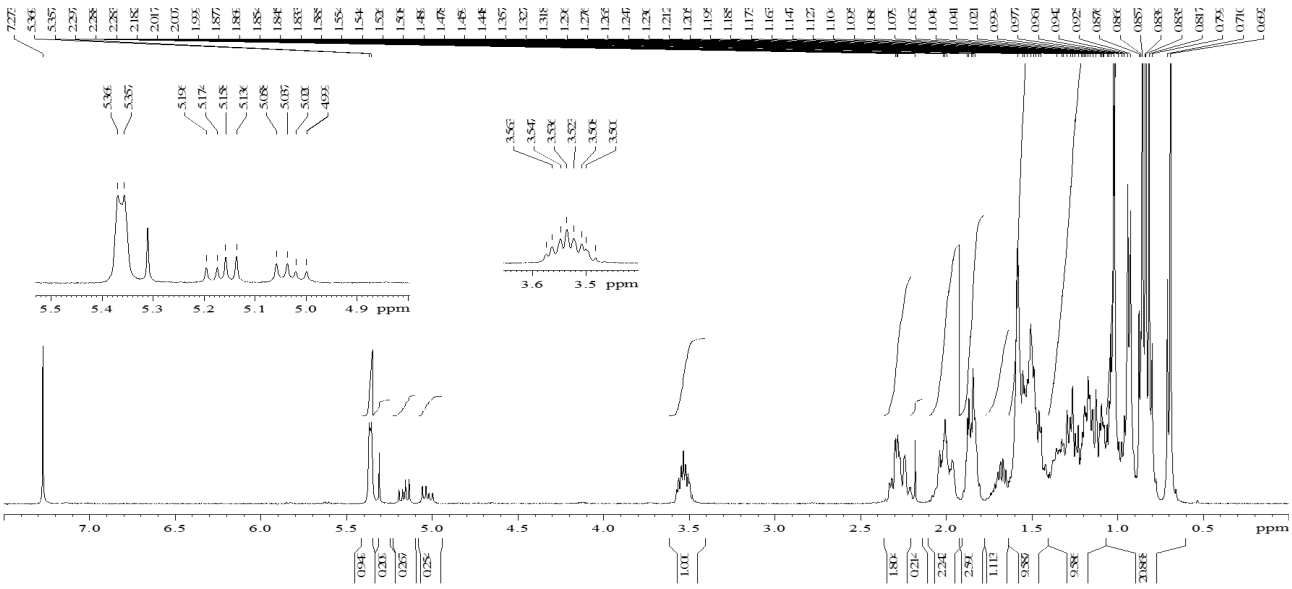


Figure 6. [1]HNMR spectrum of the phytosterol

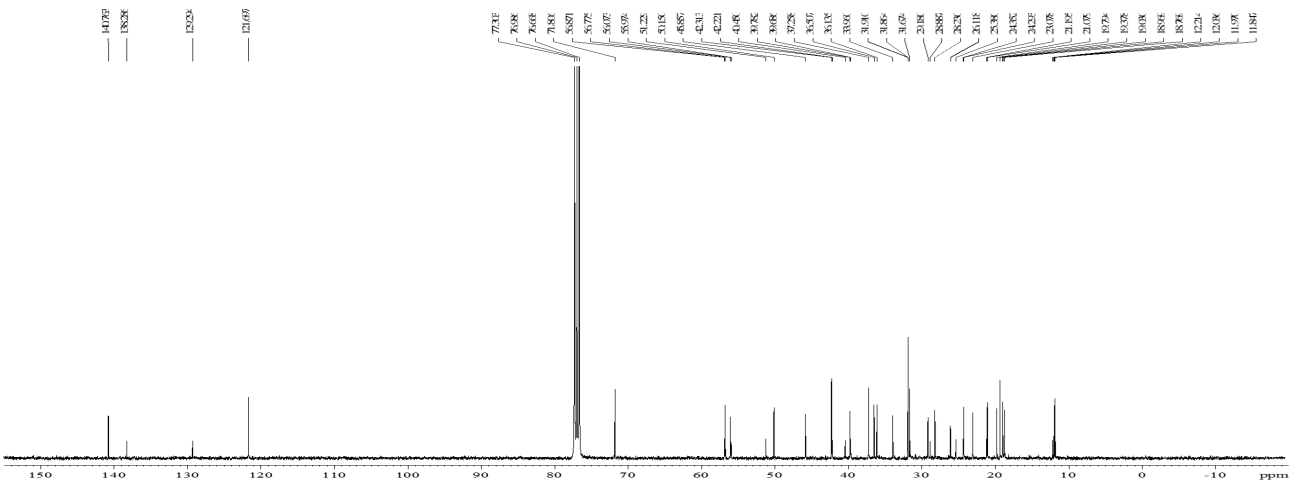


Figure 7. [13]CNMR spectrum of the phytosterols

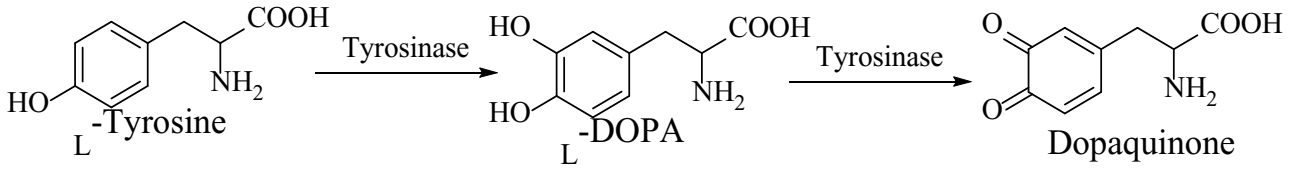


Figure 8. Oxidation of tyrosine

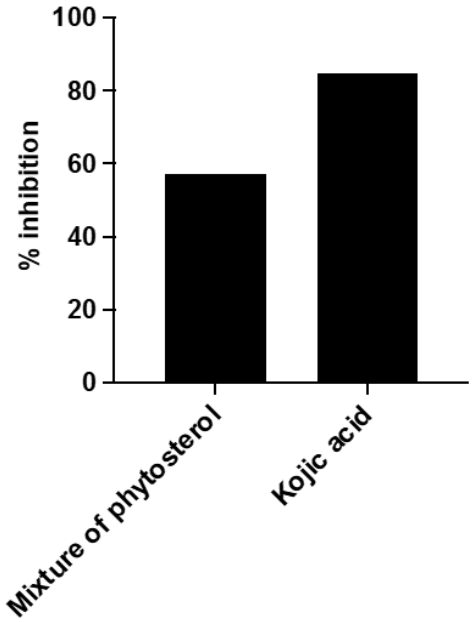


Figure 9. Tyrosinase inhibitory activity of the samples at 0.1 mg/mL

The ^{13}C NMR data (Figure 7) revealed the presence of 4 olefinic carbon atoms at δ 140.7 (C-5, C-5′), 121.6 (C-6, C-6′), 138.2 (C-22′) and 129.2 (C-23′), oxymethine carbon at δ 71.8 (C-3, C-3′) and other signals assignable to methyl, methylene and methine of aliphatic system. The physical properties and spectral data of the mixture were in accordance to the reported data of sterols [7-9]. Therefore, the mixture was identified as *β*-sitosterol and stigmasterol.

Discussions

Tyrosinase involves in melanin biosynthesis that catalyses the oxidation process of tyrosine to dihydroxyphenyl alanine (DOPA) and to a melanin precursor, DOPAquinone (Figure 8). Melanin in human skin is a major defense mechanism against the ultraviolet light of the sun. The production of abnormal pigmentation, such as melasma, freckles, age spots, liver spots and other forms of melanin hyperpigmentation are dermatological disorders associated with the melanin biosynthesis. Therefore, regulating melanin biosynthesis through the inhibition of tyrosinase enzyme will control hyperpigmentation disorders [10, 11].

Tyrosinase inhibitory activity of the isolated sterols from *B. rufescens* using L-DOPA as substrate was expressed as mean of percent inhibition, mean±S.E.M of three independent experiments, $P<0.05$ compared with kojic acid used as reference standard indicated percent inhibitions of 57.1%±0.03 and 85.0%±0.01 at the concentration of 0.1 mg/mL, respectively (Figure 9). The inhibiting activity of some triterpenes, phenolics [12], curcumin derivatives [13], and flavonols [6] for tyrosinase were previously reported, and we reported here a siginificant antityrosinase activity exhibited by a mixture of sterols from *B. rufescens*.

Conclusion

In this study, mixture of phytosterol, the stigmasterol and *β*-sitosterol was successfully isolated and characterized using chromatographic techniques and spectroscopic data. The isolated phytosterol exhibited a significant inhibitory activity against tyrosinase enzyme. The results of this study provided a potent anti-tyrosinase agent that could be developed in the treatment of dermatological disorders associated with the melanin abnormal pigmentation.

Acknowledgment

Authors acknowledge Faculty of Science Universiti Teknologi Malaysia for providing facilities.

Conflict of Interest

The authors declare no conflict of interest.

Author contributions

AU and AM conceptualized the study design, AU, MMI investigated the data, AU, UA, HMS wrote original draft, AU, MMI, UA, AM reviewed and edited the final

version, AM supervised all experiment. All authors have read the final manuscript.

Received: 29 June 2022
Revised: 12 August 2022
Accepted: 6 September 2022
Published online: 31 December 2023

References

1. Scolaro B, de Andrade LFS, Castro IA (2020) Cardiovascular Disease Prevention: The Earlier the Better? A Review of Plant Sterol Metabolism and Implications of Childhood Supplementation. Int J Mol Sci 21(128):1-19. https://doi.org/10.3390/ijms21010128
2. Pierre LL, Moses MN (2015) Isolation and Characterisation of Stigmasterol and β-Sitosterol from Odontonema Strictum (Acanthaceae). J Innov Pharm Biol Sci 2(1):88-95.
3. Viana EP, Santa-Rosa RS, Almeida SSMS, Santos LS (1999) Constituents of the stem bark of Bauhinia guianensis. Fitoterapia 70(1):111-112. https://doi.org/10.1016/S0367-326X(98)00032-X
4. Muhammad A, Sirat HM (2013) Antimicrobial, antityrosinase and brine shrimp lethality test of Bauhinia rufescens Lam (Fabaceae). J Coast Life Med 1(2):123-128.
5. Oja V, Chen X, Hajaligol MR, Chan WG (2008) Sublimation Thermodynamic Parameters for Cholesterol, Ergosterol, β-Sitosterol, and Stigmasterol. J Chem Eng Data 54(3):730-734. https://doi.org/10.1021/je800395m
6. Kubo I, Kinst-Hori I, Chaudhuri SK, Kubo Y, Sánchez Y, Ogura T (2000) Flavonols from Heterotheca inuloides: Tyrosinase Inhibitory Activity and Structural Criteria. Bioorg Med Chem 8(7):1749-1755. https://doi.org/10.1016/S0968-0896(00)00102-4
7. Kamboj A, Saluja AK (2011) Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of Ageratum conyzoides (Asteraceae). Int J Pharm Pharm Sci 3(1):94-96.
8. Okoro IS, Tor-Anyiin TA, Igoli JO, Noundou XS, Krause RWM (2017) Isolation and Characterisation of Stigmasterol and β-Sitosterol from Anthocleista djalonensis A. Chev. Asian J Chem Sci 3(4):1-5. https://doi.org/10.9734/AJOCS/2017/37147
9. Gololo SS, Shai LJ, Sethoga L, Agyei N, Bassey KE, Mogale MA (2016) Isolation of a mixture of Phytosterol compounds from the n-Hexane extract of Jatropha lagarinthoides (Sond) collected from Zebediela sub-region in Limpopo province, South Africa. J Chem Pharm Sci 9(4):3084-3087.
10. Kim CS, Noh SG, Park Y, Kang D, Chun P, Chung HY, Jung HJ, Moon HRA (2018) Potent Tyrosinase Inhibitor, (E)-3-(2,4-Dihydroxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, with Anti-Melanogenesis Properties in α-MSH and IBMX-Induced B16F10 Melanoma Cells. Molecules 23(2725):1-14. https://doi.org/10.3390/molecules23102725
11. Muhammad A (2014) Chemical Constituents and Bioactivity Studies of Bauhinia rufescens Lam. (Fabaceae) and Ficus platyphylla Del. (Moraceae). PhD Thesis, Universiti Teknologi Malaysia. p. 121.
12. Muhammad A, Sirat HM (2013) Potent Microbial and Tyrosinase Inhibitors from Stem Bark of Bauhinia rufescens (Fabaceae). Nat Prod Commun 8(10):1435-1437. https://doi.org/10.1177/1934578X1300801025
13. Jiang Y, Du Z, Xue G, Chen Q, Lu Y, Zheng X, Conney AH, Zheng K (2013) Synthesis and Biological Evaluation of Unsymmetrical Curcumin Analogues as Tyrosinase Inhibitors. Molecules 18:3948. https://doi.org/10.3390/molecules18043948