Original Research Article

***In vitro* activity guided Isolation and Characterization of Stigmasterol from *Plectranthus esculentus.* N.E.Br.**

UNDER PEER REVIEW

ABSTRACT

Aim: *The study was aimed at the in-vitro antibacterial activity evaluation and isolation of phytocompound(s) from Plectranthus esculentus crude extract.*

Place and Duration: *This research work was carried out at Gombe State University in the Department of Chemistry for a period of one and half year which involved sample collection, preparation, purification and analysis of isolate.*

Methodology: *The coarsely powdered plant tuber was subjected to maceration extraction method with methanol. The filtrate obtained was concentrated on a rotary evaporator under reduced pressure. The crude extract was re-dissolved in water: methanol (9:1) and partitioned with n-hexane, ethyl acetate and n-butanol respectively. The crude fractions were tested against a panel of microbes namely Helicobacter pylori, Shigella flexneri, and Salmonella typhi, using macro-dilution technique. The most active and sufficient n-hexane fraction was purified on open column chromatography.*

Results*: From a sample of 3.1 kg, about 172 g of crude extract was obtained. Fractionation of the crude extract yielded 18.7g, 13.6 g and 26.4 g for n-hexane, ethylacetate and n-butanol respectively. All the crude fractions displayed moderate to relatively significant activity against the tested microbes. The n-hexane and ethyl acetate fractions were identified as the most active fractions with MICs ranging from 62.5 - 150 µg/mL on all tested microbes where Salmonella* *typhi and Helicobacter pylori were the most susceptible with MIC of 62.50 μg/mL. The n-butanol fraction was relatively weak with MIC ranging from 125 - 250 μg/mL. Gradient elution of the n-hexane fraction led to the isolation of a phytocompound coded C5 with* R_f *value of 0.56 in n-hexane: ethyl acetate (6:4) and melting point of 136-138°C. Characterization of C5 using spectroscopic data from FT-IR, GC-MS,* ^1H *NMR and in comparison with literature suggests C5 as stigmasterol.*

Conclusion: *The study had shown that Plectranthus esculentus contains bioactive principles and may serve as a potential source of antibacterial agents.*

Key words*: Plectranthus esculentus, antibacterial agent, column chromatography, characterization, stigmasterol.*

UNDER PEER REVIEW

1.0 Introduction

Natural products of plant origin had been used by man for centuries as a source of medicines for healthcare [1]. It is believed that more than eighty (80%) percent of the World population depend on medicinal plants for cultural reasons, affordability and the renewed interest in natural products out of safety concern for synthetic drugs [2, 3]. These plants contain bioactive principles such as alkaloids, flavonoids, glycosides, saponins, terpenoids, polyphenols, polyketides and tannins with potential pharmacological properties such as antimicrobial, anticancer, antioxidant, anti-inflammation, antinociceptive and anthelmintic to mention just a few [4, 5].

The adoption of interdisciplinary research and technological advances has facilitated the successful isolation and characterization of single chemical entities (SCEs) with defined pharmacological activities at tolerable doses from complex bio-matrices [6, 7]. Many medicines and drugs contain active ingredients that originated from plant sources. These include vinblastine and vincristine from *Catharanthus roseus* used in the treatment of cancer [8, 9], artemisinin from *Artemisia annua* used in the treatment of malaria and morphine from *Papaver somniferum* used as a pain reliever [10].

Plectranthus esculentus is a dicotyledonous plant from the family Lamiaceae commonly known as living-stone potato or "rizga" (kaffir) in Hausa can be found in the Nigerian middle belt region, Southern Africa and Zimbabwe [11, 12]. It is used ethnomedicine to treat stomachache, cancer, backache, worms and skin disorders [13].

The FT-IR analysis of *Plectranthus esculentus* tuberous root acetone extract revealed the presence of organic chromophores such as organic hydroxyl (-OH), amines, carbonyl, alkyl, and aromatic functional groups while the HPLC analysis revealed the presence of kaempferol and vanillic acids. These results confirms the presence of medicinally important phytochemical substances in *Plectranthus esculentus* extracts. Most of these phytochemicals have demonstrated pharmacological properties such as antifungal, antidiabetic anticancer and antimicrobial activities [13, 14]. Balogun *et al*. [15] reported the isolation and characterization of β-sitosterol and oleanolic acids from *Plectranthus esculentus* leaf extract while 19-dehydroursolic acid, yarumic acid and β-sitosterol were obtained from its tuberous root extract. Despite the widespread use of *Plectranthus esculentus* as food and phytomedicine, reports on isolation and characterization of its phytocompounds are quite scarce. Hence, we report for the first time the isolation and characterization of stigmasterol from *Plectranthus esculentus* root tuber methanol crude extract based on available literature.

UNDER PEER REVIEW

2. MATERIAS AND METHODS

2.1. Sample collection and identification

The plant *Plectranthus esculentus* (tubers) was collected from Zawan Du Jos South LGA of Plateau State Nigeria in December, 2021. The plant was identified by Dr. Zainab Abubakar Department of Botany Gombe State University, Nigeria.

2.2 Preparation and Extraction plant material

The tubers were sliced; air-dried and coarsely powdered using a motorized miller. The powdered *Plectranthus esculentus* tubers (3.1 kg) was extracted with 10 L of methanol at room temperature using maceration method with occasional shaking for seven (7) days. The extract was filtered using Whatman No. 1 filter paper and then concentrated under reduced pressure with the aid of a rotary evaporator (BÜCHI R-100, Switzerland) at 40^{o}C. The crude extract obtained was dried to a constant weight in a desiccator and kept in a cool and dry container until required for use.

2.3 Antibacterial activity

The antibacterial activity of *Plectranthus esculentus* crude extract fractions was evaluated using the broth dilution method reported by Kwaji *et al*. [16] with slight modifications. The bacterial strains employed were *Helicobacter pylori, Salmonella typhi* and *Shigella flexneri.* Nine test tubes containing two-fold serial dilutions of extracts or commercial antibiotic (Gentamicin) were made from the stock concentration to obtain final concentrations in the range of 500 µg/mL to 3.9 µg/mL. The ninth test tube (No. 9) served as negative control (broth + bacterium inoculum). Each test tube got culture medium (2 mL) + plant extract or Gentamicin (1.8 mL) + 0.2 mL standardized bacterial inoculums (1 x 10^{6} cfu/mL). The test tubes were covered with sterile aluminium foil and incubated at 37^{o}C for 24 hours. Test tubes were observed for turbidity or growth after 18-24 hrs period. The lowest concentration that showed no turbidity

UNDER PEER REVIEW

or growth in the test-tube was recorded as the minimum inhibitory concentration (MIC). Tests were carried out in triplicate.

2.4 Isolation of compounds from *Plectranthus esculentus* crude extract

Plectranthus esculentus n-hexane crude extract fraction (10 g) initially dissolved in methanol sufficient to form solution was adsorbed to 20 g of activated silica gel 60 **(**70-230 mesh**)** and then allowed to dry. Column packing was effected using the wet slurry method. Gradient elution was performed with hexane/ethyl acetate and ethyl acetate/methanol at 5% increase in volume (100:00), (95:05), (90:10), (85:15), (80:20), (75:25), etc. Eluents of column fractions were collected in batches of 100 mL. Each column fraction was collected in a separate conical flask and numbered serially. All column fractions were concentrated and combined based on their thin layer chromatography (TLC) profile. Final purification of compound(s) was done using recrystallization procedure for compounds that showed one spot while fractions with two or more spots and of sufficient quantity are subjected to further purification on a smaller column [16].

3.0 Results and Discussions

The crude extract of *Plectranthus esculentus* displayed significant to moderate activity against the tested bacterial isolates (Table 1). According to Teke et al. [17], MIC values of 24.4 to 78.2 µg/mL are reckoned as significant (i.e. MIC < 100 µg/mL), while 100 < MIC = 625 µg/mL are moderate or inactive for MIC > 625 µg/mL for both bacterial and fungal pathogens. Based on this classification and also relative to the gentamicin standard, the *Plectranthus esculentus* crude extract fractions displayed significant activity against *H. pylori* and *Salmonella typhi* and hence could be used to control infections associated with these organisms. The hexane and ethyl acetate fractions were identified as the most active fractions with MICs ranging from 62.5 - 125 µg/mL on all the microbes. This may partly justify the use of *Plectranthus esculentus* in ethnomedicine [19].

UNDER PEER REVIEW

Table 1: Showing the minimum inhibitory concentration (MIC) of *Plectranthus esculentus* crude methanol extract fractions and the gentamicin antibiotic as control..

Bacteria	n-Hexane MIC (µg/mL)	Ethyl acetate MIC (µg/mL)	n-Butanol MIC (µg/mL)	Gentamicin MIC (µg/mL)
H. pylori	62.5	62.5	125	31.25
Shigella flexneri	125	125	250	31.25
Salmonella typhi	62.5	62.5	125	15.5

3.1 Characterization of *Plectranthus esculentus* Isolate (C5).

The FT-IR spectrum of C5 (Fig.1) revealed the presence several functional groups as presented in Table 2. These absorption frequencies align with those of sterol based on literature reports [18, 19].

UNDER PEER REVIEW

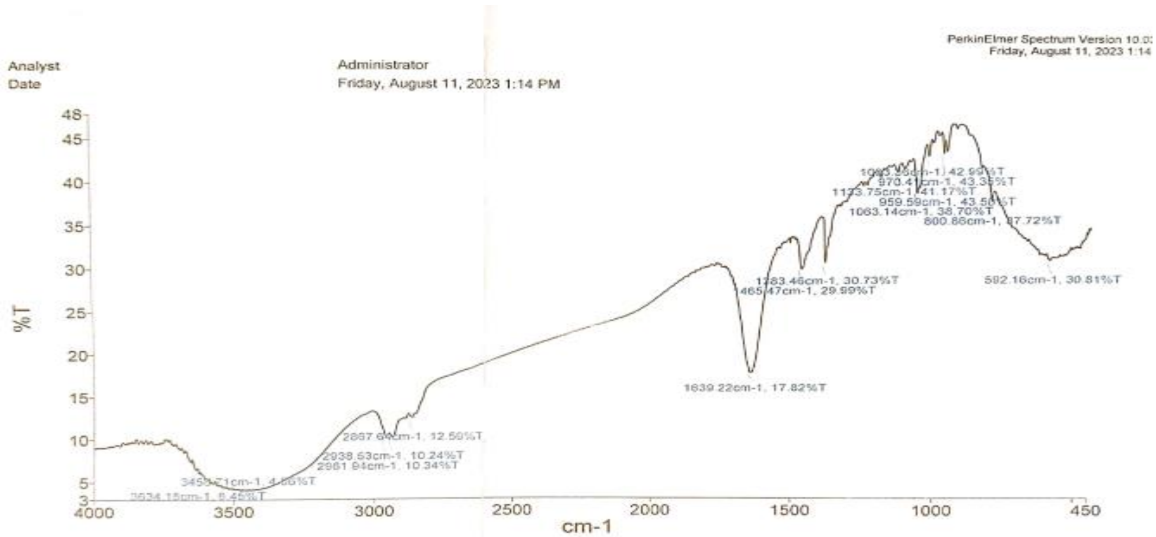

Fig. 1: FT-IR spectrum of C5

Table 2 shows the peaks or the functional groups frequencies of vibration

Table 2: FT-IR Spectrum frequencies of C5

SN	Frequency (cm^{-1})	Type of Vibration
1	3458	O-H stretching
2	2938	C-H stretching.(CH_3)
4	2867	C-H stretching (CH_2)
5	1639	C=C stretching
6	1465	CH_3 bending
7	1383	CH_2 bending
8	1133	CH_2 bending
9	800	CH bending

UNDER PEER REVIEW

Inspection of the ^{1}H NMR spectrum of C5 (Fig.2) revealed the presence of a typical steroidal nucleus with three basic regions showing signals at 0.62 ppm – 2.2 ppm representing methyl, methylene, and methine protons. Overlapping the signal at 3.5 ppm are three olefinic resonances at 5.34, 5.14 and 5.01 ppm indicating the presence of both an endocyclic and exocyclic double bonds. A proton on an oxygenated carbon appeared at 3.51 ppm. The peak due to OH is shown at 4.99 ppm. These assignments are consistent with the results obtained from FT-IR spectrum of C5 (Table 2)..

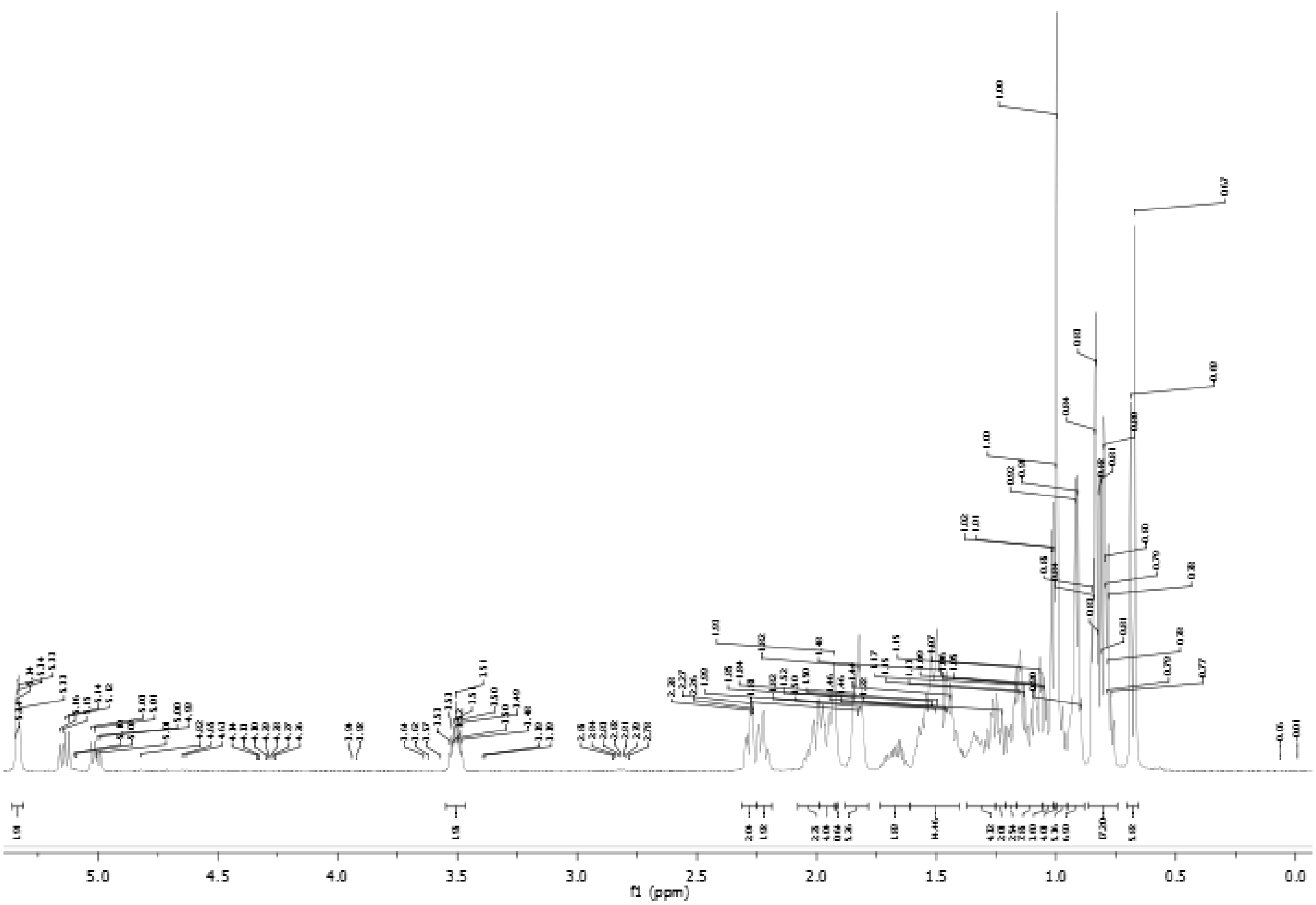

Fig. 2: 1H NMR Spectrum of C5

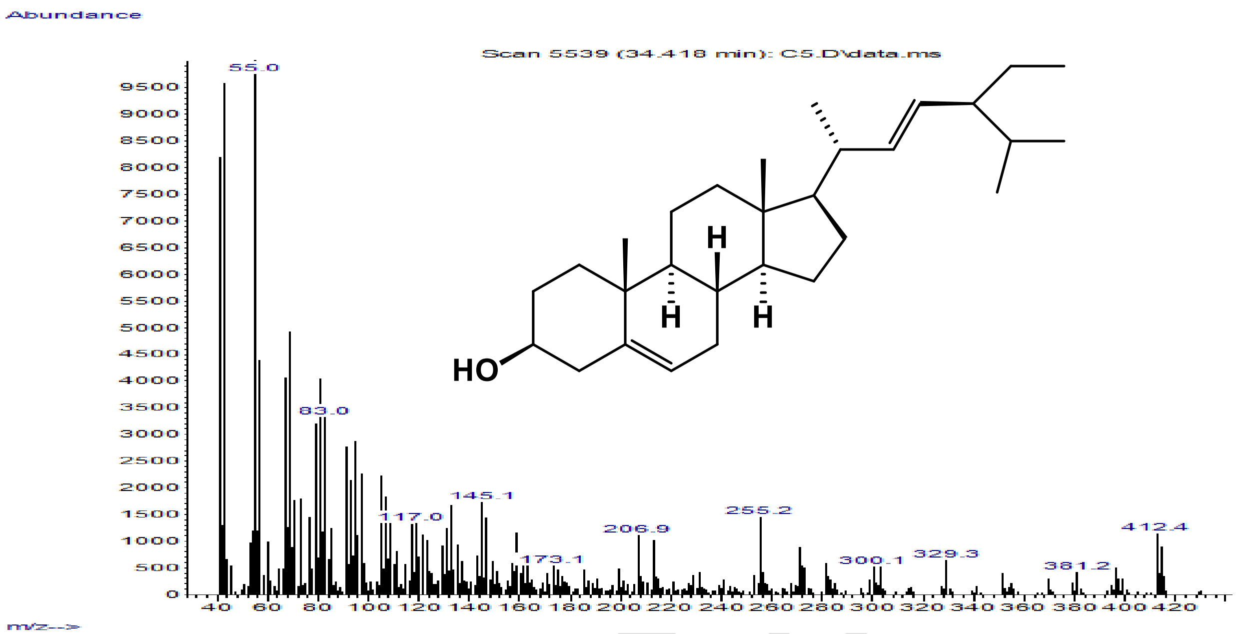

Fig. 3: GC-MS spectrum of C5

The GC-MS analysis indicated that the isolated compound C5 is Stigmasterol as suggested by GC-MS library (Fig.3) with 99% hit. The fragmentation pattern is in agreement with literature [18]. The mass spectrum of the isolated compound showed a parent molecular ion [M+] peak at m/z 412 amu (Fig.3). The base peak m/z is 55(100). The percentage fragment of other peaks relative to the base peak are m/z - 412(12), 381(6), 351(6), 329(10), 300(7), 271(12), 255(16), 206(12), 145.1(20), 83(40) with the molecular formula $C_{29}H_{48}O$ (Fig. 3).

The activity guided isolation and characterization of *Plectranthus esculentus* root tuber crude extract led to the identification of stigmasterol (Fig. 4). The root tubers of *Plectranthus esculentus* can be considered as a natural reservoir for stigmasterol in addition to other plant sources. The pharmacological activities of stigmasterol such as antimicrobial, antidiabetic, anticancer, anti-inflammatory, antioxidant, anti-osteoarthritis and neuroprotective are well documented [20, 21]. The study therefore justifies the use of *Plectranthus esculentus* in ethnomedicine.

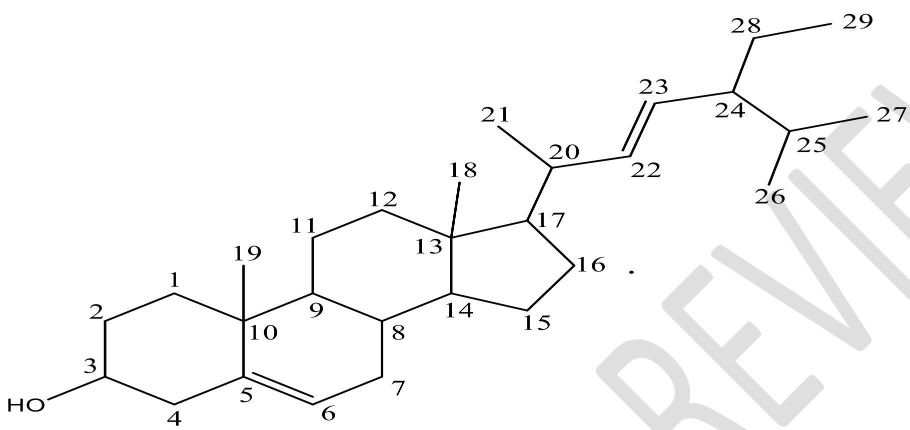

Fig. 4: Chemical Structure of Stigmasterol.

Disclaimer: Artificial Intelligence (AI) Tools.

No AI generated texts or images were used in drafting or editing of the manuscript.

REFERENCES

1). Ahmad, S. R. and Karmakar, S. The Role of Medicinal Plants in Drug Discovery across the World. Indian Journal of Pure and Applied Biosciences. 2023; 11(2): 30-41 http://doi.org/10.187.82/2582-2845.8995.

2). Miraldi, E. and Baini, G. Medicinal Plants and Health in Human History; From Empirical Use to Modern Phytotherapy. Journal of Siena Academy of Sciences. 2018; 10:7-12 DOI: 10.4081/jsas.2018.8529.

UNDER PEER REVIEW

3). Rasul, M. G. Extraction, Isolation and Characterization of Natural Products from Medicinal Plants. International Journal of of Basic Sciences and Applied Computing. 2018; 2(6): 1-6.

4). Dias DA., Urban S., Roessner U. A Historical Overview of Natural products in Drug Discovery. Metabolites 2012; 2: 303-336. Doi: 10.3390/metabo2020303

5). Mahre MB, Umaru B, Ojo NA, Saidu AS, Yahi D, Ibrahim RM., Mshelbwala PP Acute Toxicity, Phytochemistry and Antidiarrheal Effects of *Celtis integrifolia* Lam. Aqueous Leaf Extract in Wistar Albino Rats. British Journal of Pharmaceutical Research. 2016; 14(5):1-7.

6). Najmi, A., Javed, SA., AL Bratty, M. and Alhazmi, HA. Modern Approaches in the discovery and Development of Plant-Based Natural Products and their Analogues as Potential Therapeutic Agents. *Molecules*, 2022; 27: 349-364. https://doi.org/10.3390/molecules2702049.

7). Queiroz, E. F., Guillarme, D. and Wolfender, J. Advanced high Resolution Chromatographic Strategies for efficient Isolation of Natural Prooduct from Complex Biological Matrices; From metabolite Profiling to Pure Chemical Entities. Phytochem. Rev. 2024; https://doi.org/10.1007/s1101-024-09928-w.

8). Hisiger, S. and Jolicoeur, M. Analysis of *Catharanthus roseus* Alkaloids by HPLC. Phytochem. Rev. 2007; 6: 207-234 Doi: 10.1007/s1101.006-9036-y.

9). Dhyani P., Quispe C., Sharma E., Bhahukhandi A., Sati P., Attri DC. et al. Anticancer Potential of Alkaloids; a key emphasis to Colchicine, Vinblastine, Vincristine,

UNDER PEER REVIEW

Vindesine, Vinorelbine and Vincamine. BMC Cancer Cell International. 2022; 22:206-225. https://doi.org/s12935-022-02624-9.

10). Wani KI, Choudhary S., Zehra A., Naeem M., Weathers P. and Aftab T. Enhancing Artemisinin content in and delivery from *Artemisia annua:* a review of alternative, classical, and transgenic approaches, Planta. 2021; 254: 29-43. https://doi.org/10.1007/s00425-021-03676-3.

11). Schippers, R.R. African indigenous vegetables: An overview of the cultivated species. University of Greenwich, Natural Resources Institute: London, UK. 2000; pp. 81- 83.

12). Mwanja, Y., Goler, E. and Gugu, F. Flowering and seed-setting studies in Livingstone potato (*Plectranthus esculentus* NE BR.) in Jos-Plateau, Nigeria. International Journal of Plant Breeding and Genetics. 2015; 9: 275-279.

13). Eleazu, C.O., Eleazu, K.C., Ironkwe, A., Iroaganachi M.A. Effect of Livingstone potato (*Plectranthus esculentus* N.E.Br) on diabetes and its complications in Streptozotocin induced diabetes in rats. Diabetes & Metabolism Journal. 2014; 38:366-374.

14). Tapera M., Gitore SA., Shumba T., Male NP., Kaseke S. Phytochemical profile of *Plectranthus esculentus* N.E.Br obtained from Zimbabwe authenticates its medicinal uses International Journal of Pharmacognosy and Phytochemical Research, 2019; 11(1); 27-36

UNDER PEER REVIEW

15). Balogun, OK., Alemika T., Ojerinde O. Phytochemical, Antimicrobial and Antioxidant Screening of Leaf and Tuber Extracts of Three Varieties of *Plectranthus esculentus* Grown by Berom People of Nigeria. International Journal of Pharmacognosy and Phytochemical Research, 2019, 11. 27-36.

16). Kwaji, A., Adamu, H. M. and Chindo I. Y. Isolation, characterization and biological properties of betulin from *Entada africana* Gull. and Perr. (Mimosaceae). Journal of Applied and Advanced Research, 2018; 2(3): 28-31.

17). Teke, N. G., Lunga, P. K., Wabo, K. H., Kuiate, J., Vilarem, G., Giacinti, G. et al. Antimicrobial and antioxidant properties of methanol extract fractions and compounds from the stem bark of *Entada abyssinica* Stend ex A. Satabie. BMC Complementary and Alternative Medicine. 2011; 11 (57): 1-8.

18). Yohana, C., Kwaji, A and Atiko, R. Antibacterial Activity, Antioxidant Potential and Stigmasterol Isolation from *Laggera aurita* Linn (Asteraceae). International Journal of Biochemistry Research & Review. 2021; 30(9): 24-39.

19). Jiang, K., Gachumi, G., Poudel, A., Shurmer, B., Bashi, Z., El-Aneed, A. The establishment of tandem mass spectrometric fingerprints of phytosterols and tocopherols and the development of targeted profiling strategies in vegetable oils. American Society for Mass Spectrometry. 2019; 30 (1): 1700-1712.

20). Zhang X., Wang J., Zhu L., Wang X., Meng F., Xia L., et al. Advances in Stigmasterol on its Anti-cancer effect and Mechanism of Action. Frontiers in Oncology, doi: 10.3389/fonc.2022.1101289 .

UNDER PEER REVIEW

21). Backrim S., Benkhaira N., Bourais I., Benali T., Lee L-H., ElOmri N., et al. Health benefits and Pharmacological Properties of Stigmasterol. Antioxidants 2022; 11, 1912. https://doi.org/10.3390/antiox11101912.

UNDER PEER REVIEW