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In vitro Bioactivity Guided Isolation and Partial Characterization of Stigmasterol from *Plectranthus esculentus.* N.E.Br. Crude Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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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 extracted with methanol. The filtrate obtained was concentrated on a rotavapor. 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 using column chromatography.

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Results: The crude extract (≈172 g) was obtained from 3.1 kg of the stembark sample. 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 strong activity against the microbes. The n-hexane and ethyl acetate fractions were the most active fractions with MICs ranging from 62.5 - 150 µg/mL where Salmonella typhi and Helicobacter pylori were the most susceptible with MIC of 62.50 µg/mL. The n-butanol fraction was weaker with MIC ranging from 125 - 250 µg/mL. Gradient elution of the n-hexane fraction yielded 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, ^{1}H NMR and compared with literature identified C5 as stigmasterol.
Conclusion: The study had shown that *Plectranthus esculentus* contains bioactive principles and may serve as a potential source of antibacterial agents.

Keywords: Plectranthus esculentus; antibacterial agent; column chromatography; characterization; stigmasterol; phytocompound.

1. 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 depends on medicinal plants for cultural reasons, affordability and the renewed interest in natural products out of safety concerns 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 confirm 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.

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 and Voucher No.GSUH484 was allocated.

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 the maceration method with occasional shaking for seven (7) days. Methanol is quite useful as a solvent because it facilitates the extraction of both polar and non-polar organic constituents and can be easily removed from the crude extract due to its low molecular weight; also methanol containing water below 20% acts as preservative and does not allow the growth of mold during extended extraction periods [16]. 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 as reported by Kwaji et al. [17] 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 solution 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 inoculum (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 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. Finally, the isolate C5 which showed a single spot was washed and recrystallized from methanol. This involves the use of suitable solvent in which the isolate is insoluble while the impurities are soluble at room temperature [17].

3. RESULTS AND DISCUSSION

The crude extract of *Plectranthus esculentus* displayed significant to moderate activity against the tested bacterial isolates (Table 1). According to Teke et al. [18] and Demgne et al. [19], 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 [20].

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

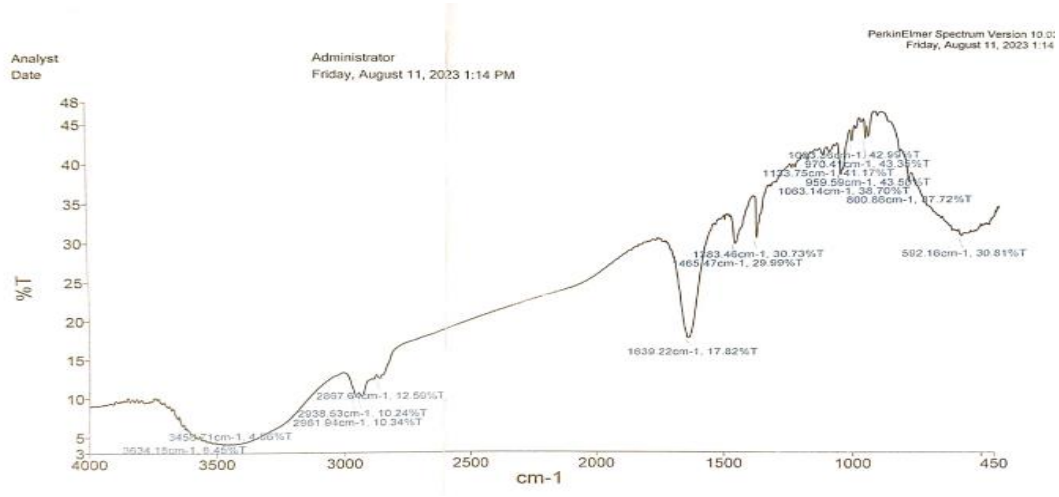

Fig. 1. FT-IR spectrum of C5

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

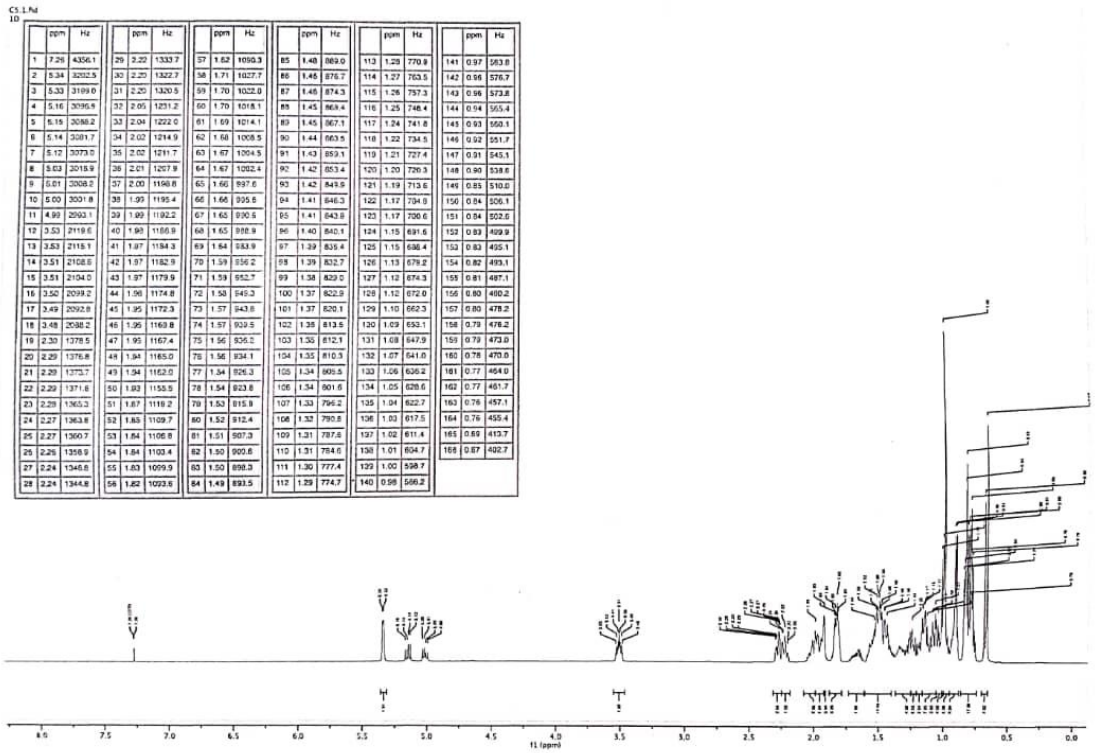

Fig. 2. 1H NMR Spectrum of C5

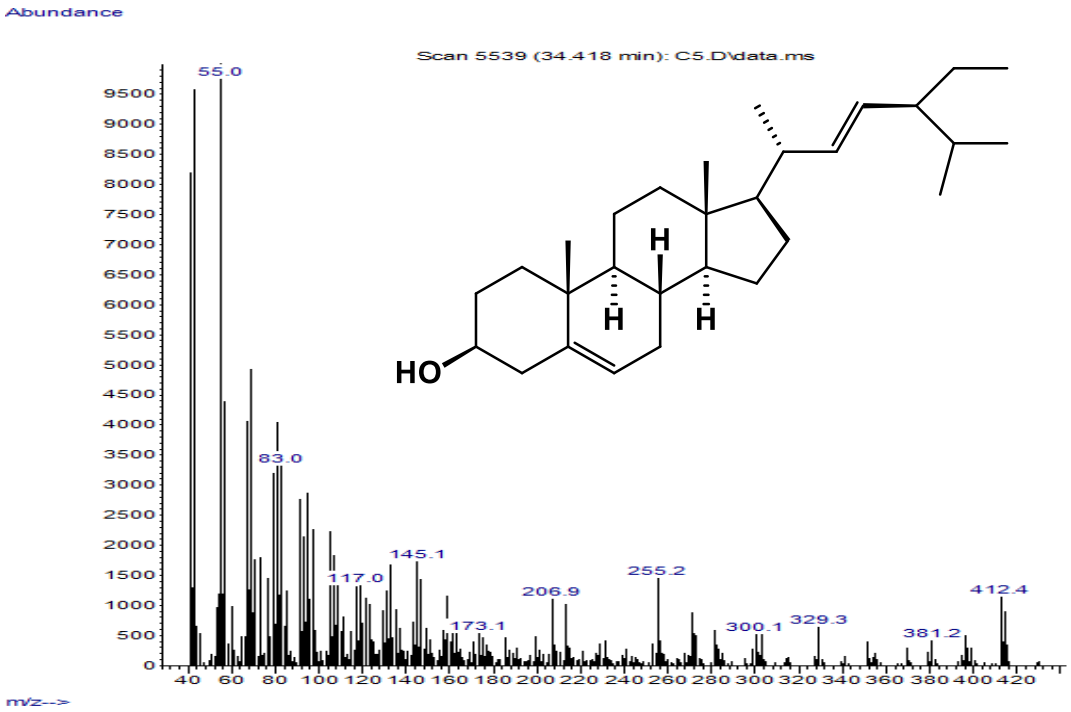

Fig. 3. GC-MS spectrum of C5

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 [21].

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).

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 [18a]. 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. Stigmasterol isolated from *Psychotria sycophylla* and tested against *Enterobacter aerogenes* EA27 yielded an MIC value of 64 µg/mL. The antibacterial activity was attributed to the presence of hydroxyl (-OH) at position 3 on the chemical structure of stigmasterol (Fig. 4) which in solution provides an acidic environment that is both inhibitory and bactericidal to pathogens depending on the concentration [20]. It is important to note that glycosylation of the stigmasterol hydroxyl group led to loss of antimicrobial activity [22]. The pharmacological activities of stigmasterol such as antimicrobial, antidiabetic, anticancer, anti-inflammatory, antioxidant, anti-osteoarthritis and neuroprotective activities are well documented [23, 24].

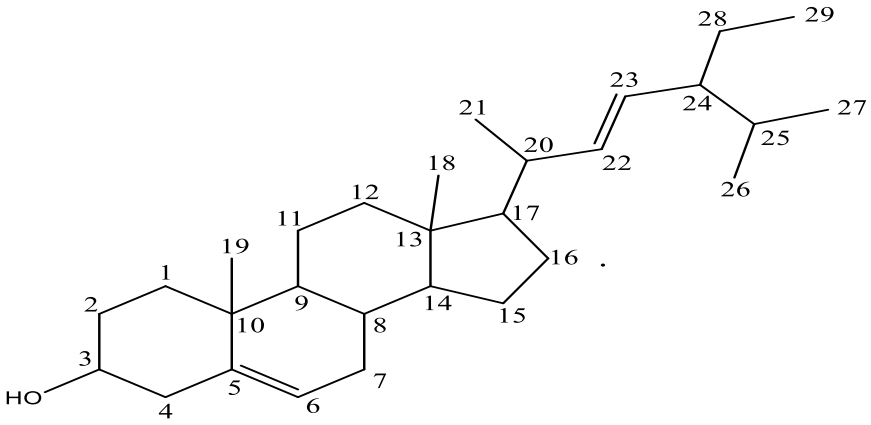

Fig. 4. Chemical Structure of Stigmasterol

4. CONCLUSION

The evaluation of the antibacterial activity of *Plectranthus esculentus* revealed that the n-hexane and ethylacetate fractions exhibited strong activity against *Salmonella typhi* and *Helicobacter pylori* with MICs of 62.50 µg/mL. The observed strong antibacterial activities could be as a result of the ability of the nonpolar constituents of n-hexane and ethylacetate fractions to diffuse faster across the bacterial lipophilic cell membrane than the predominantly polar constituents of n-butanol fraction. The n-hexane fraction which was of higher amount than either the ethylacetate and the n-butanol fraction was purified on column chromatography to obtain stigmasterol. The isolation and characterization of stigmasterol demonstrates the presence of bioactive compounds in the root tubers of *Plectranthus esculentus* and validates its use in ethnomedicine. It is recommended that the ethylacetate which demonstrated similar activity to that of the n-hexane fraction should also be investigated for the presence of bioactive compounds.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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