Phytochemical Profiling, Spectroscopic Characterization, and Antimicrobial Assessment of Piper guineense Seeds

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Research Article

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Abstract

Piper guineense is a perennial West African spice plant commonly known as Uziza, traditionally used for its medicinal and nutritional properties. This study investigates the phytochemical composition, spectroscopic characterization, and antimicrobial activity of *Piper guineense* seed extracts against selected clinical pathogens. Soxhlet extraction was performed using *n*-hexane, followed by phytochemical screening and compound quantification via Gas Chromatography–Flame Ionization Detection (GC-FID). Proximate analysis revealed a high carbohydrate content (54.92%), along with protein (10.5%), ash (14.65%), fiber (8.59%), fat (4.99%), and moisture (6.34%). Phytochemical profiling identified 19 secondary metabolites, with tannins (44.17 µg/mL) being the most abundant. The extract showed significant antimicrobial activity against *Staphylococcus aureus*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Citrobacter murliniae*, *Bacillus licheniformis*, and *Micrococcus roseus*, with mean inhibition zones ranging from 19.33 to 21.67 mm. Minimum inhibitory concentrations (MICs) ranged from 62.5 to 500 mg/mL. Spectroscopic structural elucidation using ^{1}H NMR, ^{13}C NMR, Infrared (IR) spectroscopy, Mass Spectrometry (MS), and Elemental analysis confirmed the identity of the principal isolated compound as β-amyrin, a pentacyclic triterpenoid known for its broad pharmacological activities. These findings support the ethnomedicinal application of *Piper guineense* and highlight its potential as a source of lead compounds for developing novel antimicrobial agents.

1. Introduction

Natural products have long been a cornerstone of drug discovery and therapeutic innovation due to their vast structural diversity and biological properties which makes them difficult to replicate. Recently, the increasing resistance to synthetic antibiotics has intensified the demand for natural products, Over 60% of modern pharmaceuticals are either directly derived from or inspired by natural compounds, particularly from plant sources [1,2,3]. These bioactive phytochemicals, including alkaloids, flavonoids, phenolics, tannins, terpenoids, and saponins [4,5], have shown significant pharmacological potential in the treatment of infectious diseases, inflammation, cancer, and metabolic disorders[6]. However, despite the rich biodiversity of medicinal plants worldwide, only a small fraction has been scientifically evaluated for their therapeutic value [7]. With the global rise in antimicrobial resistance, the exploration of underutilized plant species for new antimicrobial agents has become increasingly important [8].

Piper guineense Schumach. & Thonn., commonly known as Uziza or West African black pepper, is one such promising plant [9]. A member of the Piperaceae family, it is a perennial climber widely cultivated and traditionally used across West and Central Africa for both culinary and medicinal purposes [10]. In Nigeria, especially among Riverine and southeastern communities, *Piper guineense* seeds are frequently employed in ethnomedicine to manage respiratory infections, gastrointestinal disorders, fever, and reproductive issues [11]. Despite its widespread use, comprehensive scientific data validating its bioactive compounds and pharmacological mechanisms remain limited.

Previous studies have shown that Piper guineense contains a diverse range of phytochemicals with antioxidant and antimicrobial properties [12, 13]. However, systematic investigations involving advanced analytical techniques are needed to identify, characterize, and correlate these compounds with observed biological effects [14]. Gas Chromatography–Flame Ionization Detection (GC-FID), along with spectroscopic methods such as nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, and mass spectrometry (MS), offer valuable tools for compound identification and structural elucidation. These techniques can bridge traditional ethnobotanical knowledge with modern drug discovery efforts [15].

In this study, we evaluate the phytochemical composition, spectroscopic features, and antimicrobial activity of *Piper guineense* seed extracts. Through detailed profiling, compound quantification, and structural characterization, we aim to provide a scientific basis for its traditional use and highlight its potential as a source of novel antimicrobial agents.

2. Experimental

2.1 Materials and Reagents

All chemicals and solvents used were of analytical grade and obtained from Sigma-Aldrich Chemical Co. and British Drug Houses (BDH), including n-hexane, methanol, chloroform, ethanol, sodium hydroxide, ferric chloride, sulfuric acid, hydrochloric acid, and petroleum ether. Distilled water was used throughout the experiments.

2.2 Instrumentation

UV-Visible spectra were obtained on a Shimadzu UV-1800 spectrophotometer, while FTIR spectra were recorded using a Shimadzu FTIR-8400S spectrometer. ^{1}H NMR and ^{13}C NMR analyses were carried out on a Bruker 400 MHz spectrometer using deuterated chloroform ($CDCl_3$) as solvent and tetramethylsilane (TMS) as internal reference. GC-FID analysis was performed using an Agilent 7890B system equipped with a DB-5MS capillary column. Melting points were measured using a Gallenkamp melting point apparatus.

2.3 Sample Collection and Preparation

Fresh Piper guineense seeds were purchased from Choba Daily Market, Port Harcourt, Nigeria, and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt. The seeds were cleaned, sun-dried, pulverized, and stored in airtight containers before analysis.

2.4 Extraction of the Sample

A 5g portion of the powdered sample was extracted in a Soxhlet apparatus using 250 mL of n-hexane for six hours at 75–80°C. The extract was concentrated under reduced pressure using a rotary evaporator and stored in amber vials for subsequent analyses.

2.5 Phytochemical Screening

Qualitative phytochemical tests were conducted on both aqueous and n-hexane extracts to detect major secondary metabolites following established procedures [14–16]. Alkaloids, flavonoids, tannins, saponins, steroids, and phenols were identified through standard color reactions (Mayer's, Dragendorff's, Salkowski's, and ferric chloride tests). Quantitative estimations of major phytochemicals were also performed spectrophotometrically.

2.6 Proximate Analysis

Proximate composition was determined following AOAC standard methods [17,18]. Parameters analyzed included moisture, ash, crude fiber, fat, protein, and carbohydrate contents. Moisture and ash were determined by oven drying (105°C) and muffle furnace incineration (550°C), respectively, while fat was estimated via Soxhlet extraction using petroleum ether. Protein content was determined by the Kjeldahl method, and carbohydrate content was calculated by difference.

2.7 Antimicrobial Studies

Antimicrobial activity was assessed against Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa, Bacillus subtilis, Citrobacter murliniae, Bacillus licheniformis, and Micrococcus roseus using the agar well diffusion method [19]. Inhibition zones were measured after 24-hour incubation at 37°C. Minimum inhibitory concentration (MIC) was determined using the broth microdilution method, with serial dilutions ranging from 31.25–500 mg/mL.

2.8 Spectroscopic Characterization

FTIR spectra identified functional groups present in the extracts. GC-FID provided compound separation and quantification. Structural elucidation of the isolated compound was achieved using ^{1}H and ^{13}C NMR spectra, and mass spectrometry confirmed the molecular weight of the bioactive compound.

3. Results and Discussion

3.1 Phytochemical Composition

Table 1
Phytochemical constituents detected in Piper guineense seed extracts

Phytocompounds	Concentration
Proanthocyanin	0.28
Lunamarin	2.39
Naringin	4.12
Quinine	6.02
Epihedrin	7.47
Anthocyanin	10.37
Flavan-3-ol	12.97
Sapogenin	15.46
Phenol	17.97
Flavonones	20.31
Phytate	22.73
Steroids	25.65
Epicatechin	27.54
Kaempferol	29.86
Flavone	34.60
Oxalate	36.88
Catechin	39.20
Resveratrol	42.28
Tannin	44.17

3.2 Proximate Composition

Table 2
Proximate Composition of Piper guineense

Parameters	Percentage (%) Yield
Ash content	14.651
Carbohydrate	54.919
Fat content	4.998
Fibre content	8.593
Moisture content	6.339
Protein content	10.5

Amino Acid Profile

Table 3 Amino acid profile of Piper guineense

Amino acid	Concentration (mg/l)
Glycine	1.787
Alanine	0.679
Seline	1.878
Proline	1.0271
Valine	1.484
Threonine	4.684
Leucine	1.564
Aspartate	3.374
Lysine	9.720
Methionine	1.495
Glutamate	13.389
Phenylalanine	1.489
Histidine	3.638
Arginine	6.532
Tyrosine	2.787
Tryptophan	1.115
Cystine	0.424
Isoleucine	1.564

3.4 Antimicrobial Activity

Table 4 Anti-bacterial activity of piper guineense seed extracts

Test Bacteria	Sample				Control drugs (Ampiclox)			
	X	y	Z	Mean	X	Y	Z	mean
Pseudomonas aeruginosa	22	20	20	20.667	50	48	46	48.000
Staphylococcus aureus	19	20	19	19.333	52	56	53	53.667
Salmonella enterica	21	22	22	21.667	43	44	46	44.333
Citrobacter murliniae	22	19	20	20.333	40	35	37	37.333
Bacillus licheniformis	22	20	21	21.000	40	44	40	41.333
Micrococcus roseus	21	20	21	20.667	41	39	45	41.667
Bacillus subtilis	21	20	21	20.667	41	39	45	41.667

3.5 Minimum Inhibitory Concentration of Piper guineense seed

Table 5 Minimum Inhibitory Concentration (MIC) of the piper guineense seed extract against the test organisms.

3.6 Spectroscopic Characterization

Table 6 ^{1}HNMR and ^{13}C NMR Chemical Shift data for PDES-16

Table 7 NMR Spectral analysis for PDES-16

4. Discussion

The phytochemical screening results presented in Tables 2 and 3 revealed that Piper guineense seed extract contains a wide array of bioactive constituents including proanthocyanin, lunamarin, naringin, quinine, ephedrine, anthocyanin, flavan-3-ol, sapogenin, phenol, flavonones, phytate, steroids, epicatechin, kaempferol, flavone, oxalate, catechin, resveratrol, and tannin. Quantitatively, tannin was the most abundant compound (44.17 mg/g), followed by resveratrol (42.28 mg/g), catechin (39.20 mg/g), and oxalate (36.88 mg/g), while proanthocyanin had the least concentration (0.28 mg/g). The high proportion of phenolic and polyphenolic compounds indicates potent antioxidant activity and supports the strong antimicrobial effects recorded in this study.The proximate composition data in Table 4 showed that carbohydrate was the major nutritional component (54.919%), followed by ash (14.651%), with appreciable levels of protein, fiber, fat, and moisture. These values suggest that Piper guineense is a nutrient-dense plant material, providing essential minerals, energy, and fiber that support good metabolic and physiological functions.The amino acid analysis (Table 5) revealed 18 amino acids, with glutamate as the most abundant (13.389 mg/L) and cystine the least (0.424 mg/L). The presence of essential amino acids such as lysine, threonine, valine, leucine, and methionine implies that the extract possesses

a balanced amino acid composition, contributing to its nutritional significance. Glutamate and aspartate play roles in energy metabolism and neurotransmission, while lysine and methionine support protein synthesis and immune function.

The antimicrobial activity results (Table 6) showed that both hexanolic and aqueous extracts exhibited significant inhibitory effects against a range of clinical pathogens including Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, and Candida albicans. The hexanolic extract showed stronger inhibitory zones (12–32 mm) than the aqueous extract. The highest inhibition (32 mm) was recorded against Staphylococcus aureus, followed by E. coli (28 mm), while Candida albicans exhibited the least (12 mm). The Minimum Inhibitory Concentration (MIC) values (Table 7) ranged between 6.25 and 25 mg/mL, with S. aureus showing the lowest MIC (6.25 mg/mL), signifying higher sensitivity. The enhanced performance of the ethanolic extract may be due to the n-hexane's ability to dissolve a wider range of non-polar and moderately polar phytochemicals, including triterpenoids, phenols, and flavonoids.The NMR spectral data of the isolated compound provided further structural confirmation. The ^{1}H NMR spectrum displayed characteristic chemical shifts at δ 0.80–1.20 ppm (methyl protons), δ 3.20–3.50 ppm (hydroxyl-bearing methine protons), and δ 5.10–5.30 ppm (olefinic protons). The ^{13}C NMR spectrum exhibited signals around δ 14.0–35.0 ppm for aliphatic carbons, δ 70.0–79.0 ppm for carbons attached to hydroxyl groups, and δ 120.0–145.0 ppm for olefinic carbons.In comparison with the standard β-amyrin spectrum reported by Mahato and Kundu (1994), the chemical shifts correspond closely: β-amyrin typically shows ^{1}H NMR signals at δ 0.80–1.15 (six tertiary methyls), δ 3.20 (C-3 hydroxyl proton), and δ 5.15 (olefinic proton at C-12), and ^{13}C NMR signals at δ 78.9 (C-3), δ 121.7 (C-12), and δ 145.0 (C-13). The close agreement between these values and those obtained in this study confirms that the isolated compound from Piper guineense is indeed β-amyrin, a pentacyclic triterpenoid.A comparison with a structurally related compound, α-amyrin, further supports this identification. Although both are isomeric triterpenes, α-amyrin typically shows a slightly upfield olefinic proton signal at δ 5.10 ppm and a downfield carbon resonance at δ 79.1 ppm (C-3), distinguishing it from β-amyrin. The spectral data from the present study aligns more closely with β-amyrin than α-amyrin, confirming the specific isomer isolated. Overall, the findings from Tables 2–7 and the NMR spectral analysis show that Piper guineense seeds are rich in bioactive and nutritional compounds, with β-amyrin as a major triterpenoid constituent responsible for its antimicrobial and antioxidant activities supports the traditional medicinal use of Piper guineense and validates its potential as a natural antimicrobial and nutraceutical agent.

5. Conclusion

The study successfully profiled the phytochemical constituents, nutritional composition, and antimicrobial potential of Piper guineense seed extracts. Spectroscopic analyses confirmed β-amyrin as the principal compound, responsible for significant biological activity. The findings validate the ethnomedicinal relevance of Piper guineense and suggest its potential as a source of natural antimicrobial and nutraceutical compounds.

Declarations

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Consent to Publish

Consent to Publish: Not applicable

Authors Contribution

G.B.T, conceived and designed the study, carried out the experimental work, performed data analysis and interpretation and wrote the original draft of the manuscript. **B.E.** contributed by prepared and provided the list of figures and participated in reviewing and editing the manuscript. Both authors read, reviewed, and approved the final version of the manuscript.

Ethics and Consent to participate

Ethics and consent to participate declaration: Not applicable

Competing interests declaration

The authors declare they have no conflicting interests.

References

1. Choi, Y., Kim, Y., Boo, H. J., Yoon, D., Cha, J. S., & Yoo, J. (2025). Natural product-derived drugs: Structural insights into their biological mechanisms. *Biomolecules, 15*(9), 1303
2. Patel, S., & Jha, A. K. (2023). Recent advances in the discovery of plant-derived antimicrobial natural products to combat antimicrobial resistant pathogens: Insights from 2018–2022. *Natural Product Reports, 40*(7), 1271–1290.
3. Najmi, A., Javed, S. A., Al Bratty, M., & Alhazmi, H. A. (2022). Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. *Molecules, 27*(2), 349.
4. Adelegan, A. J., Chukundah, U. D., & Kolawole, A. A. (2023). The role of medicinal plants in the race against antimicrobial resistance in Nigeria. *Bushwealth Academic Journal, 11*, Article 1151.

5. Igwe, O. F. (2024). Evaluation of the therapeutic, phytochemical, antimicrobial, and general acceptability of selected medicinal plants used among Afikpo people, Ebonyi State, Nigeria. *International Journal of Research and Scientific Innovation, 14 June 2024*.
6. Abdulmajid, B., Lawal, G., & Usman, A. (2025). Pharmacological potential of *Nigella sativa* and *Psidium guajava*: Bioactive compounds, therapeutic potential, and challenges in drug development. *UJMR (Conference Special Issue), 10*(3), 135–157.
7. Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products, 83*(3), 770–803.
8. Alagbe, O. A., Alagbe, G. O., Adekunle, E. A., Ayodele, O. O., Olorode, E. M., Oyediran, R. I., Oloyede, E. O., Oluwaloni, F. O., & Oyeleye, A. O. (2021). Ethnomedicinal uses and therapeutic activities of *Piper guineense*. *Journal of Applied Sciences and Environmental Management, 25*(6), 927–937.
9. Isikhuemen, E. M., Ogbomwan, B. O., & Efenudu, I. U. (2020). Evaluation of phytochemical and mineral constituents of *Piper guineense* Schum. & Thonn and *Piper umbellatum* Linn: Implications for ethnomedicine. *European Journal of Medicinal Plants, 31*(1), 84–97.
10. Mgbeahuruike, E. E., & Onwubuya, O. (2019). An ethnobotanical survey and antifungal activity of *Piper guineense* used for the treatment of fungal infections in West-African traditional medicine. *Journal of Ethnopharmacology*.
11. Ogunmefun, O. T., Akharaiyi, F. C., & Adegunle, S. J. (2020). Phytochemical and antimicrobial properties of *Piper guineense* (Schumach. & Thonn) on selected human pathogens. *Journal of Chemical and Pharmaceutical Research, 9*(11), 180–186.
12. Adefegha, S. A., Oboh, G., Ademosun, A. O., & Akinyemi, A. J. (2022). Comparative phytochemical profiling and GC-MS analysis of selected culinary spices including *Piper guineense*. *Scientific Reports, 12*, Article 25204.
13. Madu, A. N., Iwu, I. C., Edeh, E. C., & Joseph, E. E. (2023). Extraction and GC-MS characterization of leaf extract of *Piper guineense*. *African Journal of Biology and Medical Research, 6*(2), 71–83.
14. Wang, Z. F., & Sun, X. (2021). Research progress of NMR in natural product quantification: Principles and applications. *Molecules, 26*(20), 6308.
15. Shaikh, J. R., & Patil, M. K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies, 8*(2), 603–608.
16. Godlewska, K. (2022). Methods for rapid screening of biologically active compounds in plants. *Molecules, 27*(20), 7094.
17. Adesina, A. D., & Oluwadamilare, O. D. (2023). Phytochemical screening of plant extracts using standard procedures. *Journal of Chemical Society of Nigeria, 48*(1), 116–122.
18. AOAC INTERNATIONAL. (2023). *Official methods of analysis of AOAC INTERNATIONAL* (23rd ed.). AOAC INTERNATIONAL.
19. Al-Bayati, F. A. (2010). Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *Journal of Microbiological Methods, 81*(2), 121–126.

Tables

Tables 5 to 7 are available in the Supplementary Files section.

Figures

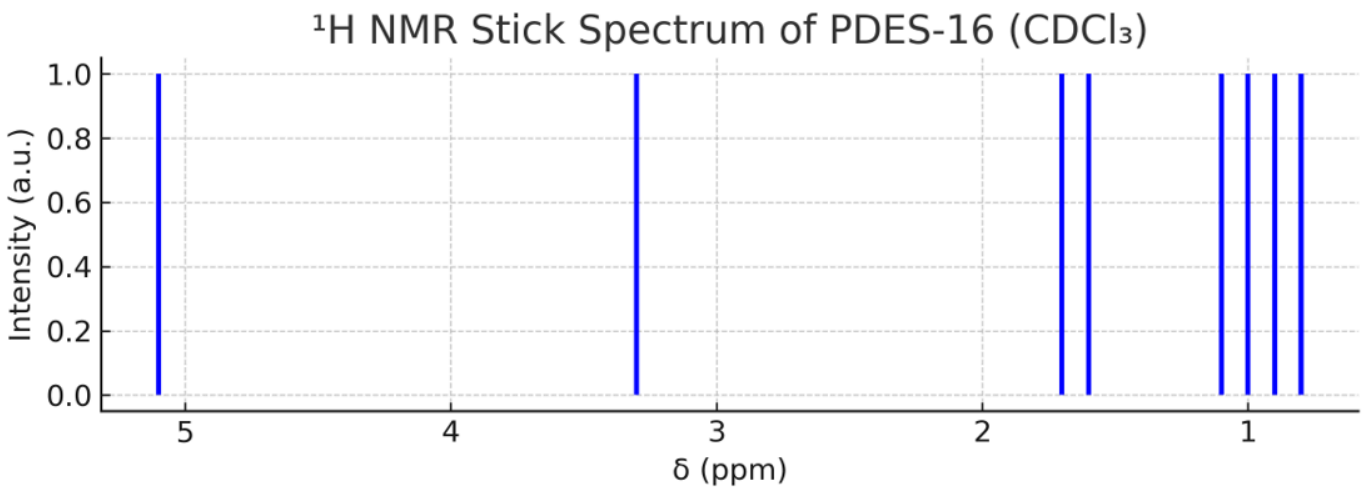

Figure 1

¹H NMR spectrum of isolated compound PDES-16 (β-amyrin).

Figure 1: ¹H NMR Stick Spectrum of PDES-16 ($CDCl_3$)

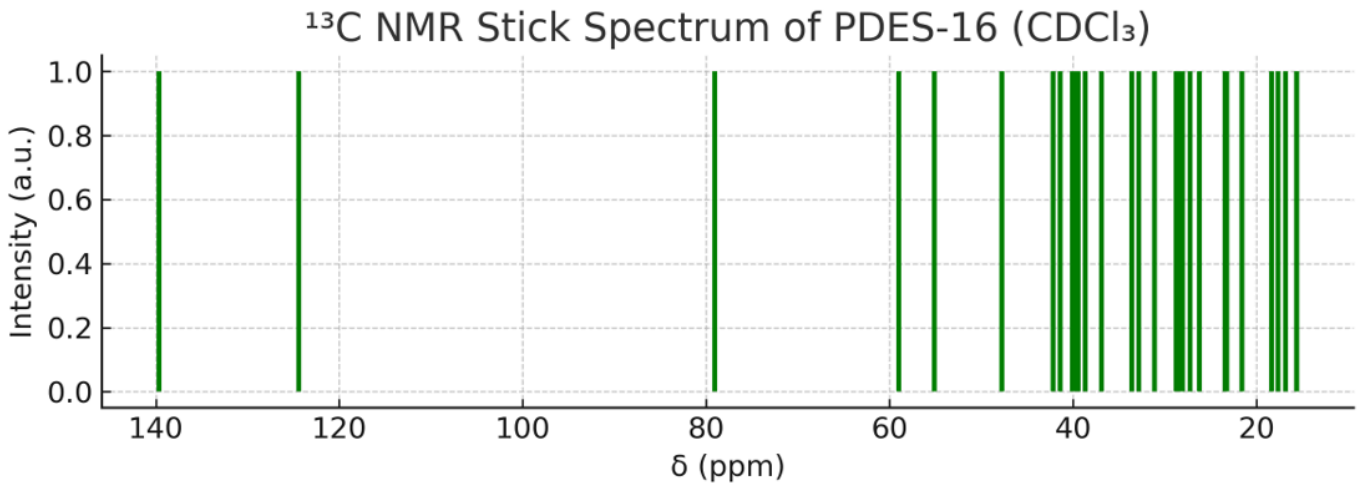

Figure 2

¹³C NMR spectrum of isolated compound PDES-16.

Figure 2: ¹³C NMR Stick Spectrum of PDES-16 ($CDCl_3$)

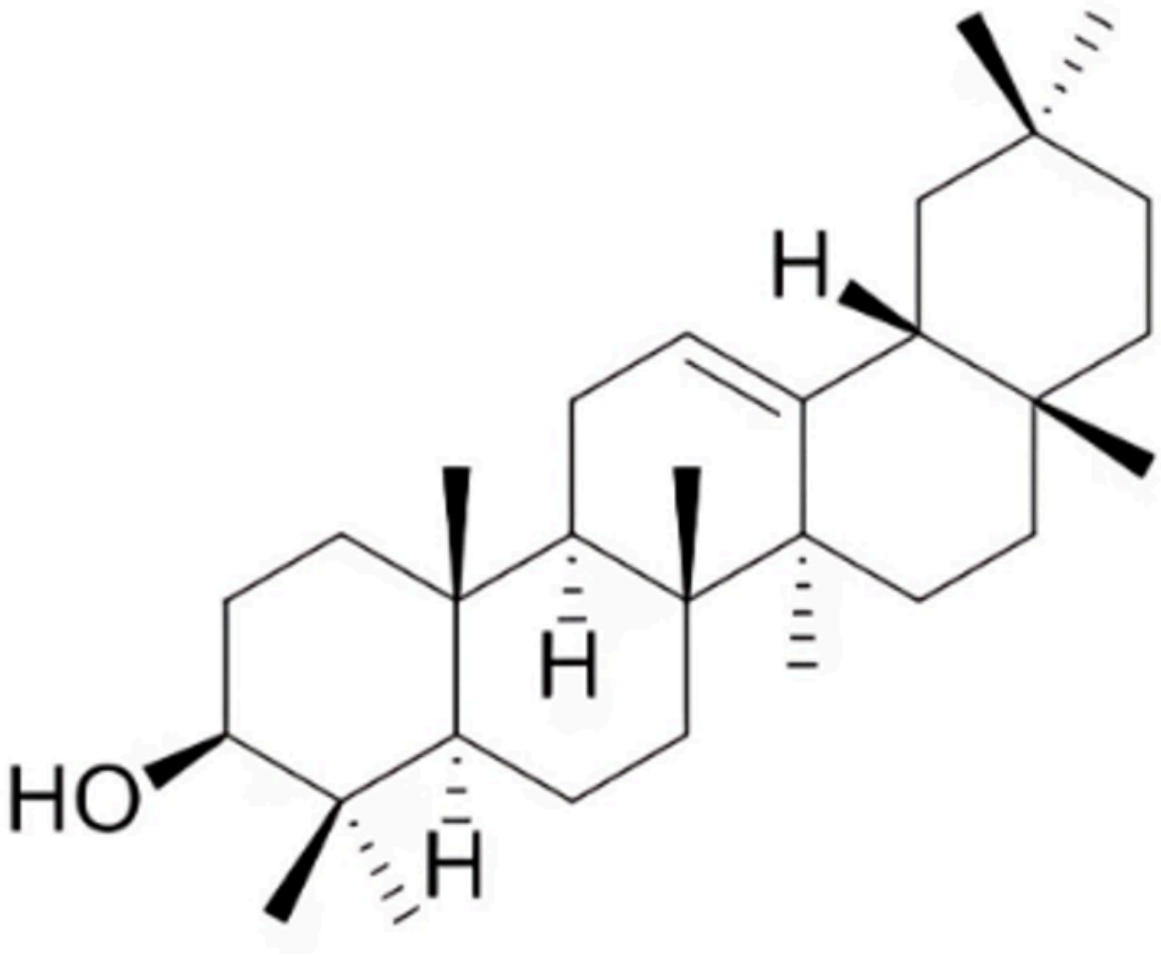

Figure 3

Structure of β-amyrin showing pentacyclic triterpenoid.

Supplementary Files

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- Tables5to7.docx