Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1885-1892 | ISSN:2251-6727

# Characterization of Phytoestrogen 3 (0) -2, 4, 6-Trimethoxyphenyl Androstane Carboxyaldehyde from Piper Guineense Leaf

**Irenus Chinonye Iwu[1*] , Veronica Ifeoma Ukachukwu[2] Augustina Adanma UKAOMA[3] , Leonard Chukwuemeka Anyika[4] Ifeoma Chinwendu Obiagwu[5], Maryann Chioma Igbomezie[6] , Miracle Oluchukwu Ezekoye[7] , Ali Bilar [8], Basil Ngozichukwu Anukam[9], Michael Babalola Oluboyo [10], Ali Agaka Aremu[11] Uchenna Lyda Onu[12]CH Chikadibia Edward [(13)]**

*Corresponding author Irenus Chinonye IWU

Department of chemistry federal university of technology owerri Nigeria

[1,2,,5,6,7 8,9,10, 11,12 ,.13] Department of Chemistry Federal University of Technology Owerri Nigeria

[3] Department of Biological Sciences, Federal University of Technology Owerri Nigeria

[4] Department of Chemistry Madonna University Elele River State Nigeria

***(Received: 16 June 2025 Revised: 20 July 2025 Accepted: 04 August 2025)***

**KEYWORDS**

bioactive constituents, piper guineense, infra-red ,spectrometer, characterization

**ABSTRACT:**

Introduction

Piper guineense is a plant commonly used as a spice in Eastern Nigeria; there have been confirmed and unconfirmed reports of its numerous applications in medicine. The parts of the plant are said to be of immense benefit to the health and wellbeing and our research was aimed at unveiling the bioactive compounds that were responsible for its numerous applications.

Objectives.

The research objectives were to isolate bioactive compounds from the dried and pulverized leaf of Piper guineense using convectional laboratory techniques

Method

Sample was collected, identified, room dried and extracted. The Compound was isolated from column chromatography using chloroform and methanol mix which gave yellow oil. Thin layer chromatography (TLC) carried out on the compound showed one spot upon iodine vapour development with retardation factor (Rf = 0.40). An infra-Red analysis of the sample was carried out using FTIR (Fourier transform infra-red) Spectrophotometer BX-model Perkin Eimer. The Proton nuclear magnetic resonance spectrum ( IH) and carbon-13 Nuclear Magnetic Resonance) spectrum (13C)NMR were obtained on a Bruker Avance 400 FT NMR Spectrometer using tetra methyl silane as internal standard. LC – ESIMS spectrum was determined in the positive ion mode on a PE. Biosystem APl 156 single quadruple instrument. .

Result

Analysis of the results gave the compound 3[0] -2', 4', 6'- trimethoxypheyl, Androstan carboxaldehyde with molecular formula C¬¬¬29 H40 O5, m/z 468.

Conclusion

The isolated compound is a phytoestrogen and , could be used in the treatment of infertility in women and low sperm count in men. The compound could act as a fertility booster to men.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1885-1892 | ISSN:2251-6727

## 1. Introduction

*Piper guineense* Schum. & Thonn. (Piperaceae) is a herbaceous perennial plant grown in Nigeria, usually the southern part. Its common known as West African Black pepper.[1] . The plant is said to be rich in phytochemicals [2,3] . It has good flavor and could be useful as spices , it is also useful for preparing soups for post-partum women. The leaves contain basic phytochemicals and some considerable amount of anti-nutrients which have medicinal values [4]. Similarly the fruit has been shown to contain calcium, copper, iron, sodium, potassium, manganese, magnesium, selenium and zinc. [5] .The leaves and stems of *P. guineense* are rich in protein, lipid, fiber, carbohydrate, ascorbic acid, beta-carotene .The seed contains calcium and magnesium [6,2] Previous researches have shown that the leaves contains amino-acid, vitamins and minerals such as calcium, copper, iron, magnesium, potassium, phosphorus, sodium and zinc in lower amounts comparatively to daily intakes [7]) .

Similarly, research has shown the significant effect of using the single extract of *Abelmoschus esculentus* or *Piper guineense* and also the combination of both extracts as feed of albino rats. Their findings revealed glandular erosion, mucosal erosion, inflammation, ulceration, degeneration of mucosal lining cells and epithelium, degeneration of chief and parietal cells . [8].They concluded that the usage of the two extract as single and combine doses posed a degree of pathological condition on the gastric mucosa. Similarly ,other researchers have examined the values of phytochemicals (dry and wet weight) obtained from three different market sources found that there was no significant difference among heavy metals detected in Piper guineense across the three markets. They concluded that all the metals in the P. guineense were below world health organizations' maximum permissible limit. Their findings indicated that P. guineense collected from the markets were safe for consumption and good for medicinal use [9].

There are hormone like compoundds obtainable from plants, such as brassinosteroids, which are hormones that have a multidimensional activity and are engaged in plant growth, development, and its response to environmental stresses[10,11] Interestingly, however, mammalian steroid hormones are also part of the metabolic profile in plants [12] .In plants (for example, the Fabaceae family) phytoestrogens (genistein, daidzein) are present. Due to their similar structure to estrogens, they can interact with human estrogen receptors and have many physiological activities in humans [13] . The effect of feeding rats with Piper guineense leaves revealed that there was increased agility, faster consumption and enhanced coordination in the rats [14] . Studies on the volatile compounds in the leaves and seeds of Piper guineense plant from South East Nigeria has been carried out and a total of thirty-three volatile constituents in the leaves and seeds of Piper guineense was identified [15] . The major compounds identified includes, acids, esters, alcohols, and hydrocarbons. It is claimed that when P. guineense is added to food meant for pregnant and nursing mothers as a medicinal spice and among the post-partum women, it assists in the contraction of the uterus. [16] .Though these and more researches have been carried out on the plant, new compounds are been identified from the plant, our research there is aimed at identifying compound yet unknown of medicinal importance from the leaf of the plant.

## 2. Objectives

Research objectives includes collection, drying and preservation of samples, extraction of the constituents of the sample using ethanol and concentrating the extract to obtain a semi solid crude extract,. Re-extracting the crude extract using chloroform and subjection the chloroform extract partitioning between chloroform and water. Re-concentrating the organic phase and subjecting it to column chromatography and later to thin layer chromatography .The pure isolated compound are then subjected to spectral analysis which includes Infra-red spectroscopy, proton and carbon 13 Nuclear mass spectroscopy and mass spectroscopy for structural elucidation

## 3. Methods

### Sample collection and preparation

Leaves of Piper *guineense* (uziza) was collected from Owerri environs Imo State of Nigeria. They were identified by Prof. Mbagwu in Department of Plant Science and Biotech, School of Agriculture And Agricultural Technology, Federal University of Science and Technology, Owerri. The leaves of piper guineense

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1885-1892 | ISSN:2251-6727

were washed and air dried at room temperature , pulverized. and stored in air tight container [17]

**Ethanol extraction procedures**

500g of the sample was percolated in 1L of ethanol for 48 hours and filtered. The filtrate was concentrated with rotary evaporator. The concentrated crude extract was partitioned between chloroform and water mixture and separated, .the water phase was discarded while the chloroform phase was retained.

**Column Chromatographic separation**

The bioactive compound was isolated from the extract [18]. The extract was separated into different components in a chromatographic column using silica gel (stationary phase). Slurry of finely powdered silica gel dispersed in diethyl ether was packed into the glass column to a height of about 12m and loaded with 10ml of the extracts dissolved in 100% diethyl ether. Using different concentrations of diethyl/chloroform and chloroform/methanol mixtures, different fractions were collected at intervals of 30ml each

**Qualitative thin-layer chromatographic test**

Thin-layer chromatographic analysis was carried out to identify pure samples using TLC plates (coated with silica gel) . The eluents obtained from column chromatography were then placed on the TLC plates with a microcapillary tube. The plates were placed in a glass tank containing the mobile phase (diethyl ether,chloroform and methanol measured to same volume and allowed to run until the mobile phase reached the highest point on the plate. The TLC plates were removed, dried in air and developed in iodine tank. The Retardation factor value was obtain from the distance moves by solute and distance moves by solvent.

**Infra-red spectroscopic analysis**

Infra-Red analysis of the selected pure chromatographic fraction was carried out at the applied informatics and communication African Centre of Excellence (CAPIC-ACE), Covenant University Ota Nigeria using FTIR Spectrophotometer BX-model manufactured by Perkin Eimer.

**$^{1}$H-NMR and $^{13}$C-NMR analysis**

The $^{1}$H and $^{13}$C NMR spectra were obtained on a Bruker Avance 400 FT NMR Spectrometer using tetra methyl silane as internal standard. Chemical shifts are expressed in ð values.[19]

**Mass spectroscopy** LC – ESIMS spectra was determined in the positive ion mode on a PE. Biosystem APl 156 single quadruple instrument. HRESIMS (positive ion mode) spectrum was recorded on a thermo Finniga MAT 95 x L

## 4. Results

**Result of the infra-red spectral analysis of compound**

**Table 1 Infrared (IR) analysis of compound**

| IR Absorption ($cm^{-1}$) | Bond type | Functional group |
|---|---|---|
| 667.193 | C=C | Aromatic |
| 864.742 | C=C | Aromatic |
| 1092.110 | C-O | Ether |
| 1162.930 | C-O | Ether |
| 1367.933 | C-O | Ether |
| 1461.116 | C=C | Aromatic |
| 1599.030 | C=C | Aromatic |
| 1740.667 | C=O | Carbonyl (aldehyde) |

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1885-1892 | ISSN:2251-6727

| | | |
|---|---|---|
| 2855.141 | C-H | Aliphatic |
| 2922.233 | C-H | Aliphatic |
| 3380.695 | O-H | Alcohol |

The infra-red (IR) spectrum depicted the presence of out of plane aromatic and aromatic bending at 667.19cm$^{-1}$ and 864.74cm$^{-1}$. Table (1). There are absorptions at 1092.11cm$^{-1}$, 1162.93cm$^{-1}$, 1259.84cm$^{-1}$ and 1367.93cm$^{-1}$ showing presence of ethers. Aromatic bonds were seen at 1461.12cm$^{-1}$ and 1599.03cm$^{-1}$. Carbonyl (C=0) bonds were observed at 1740.67cm$^{-1}$ indicating an aldehydic functional group. Aliphatic bonds were seen at 2855.14cm$^{-1}$ and 2922.23cm$^{-1}$. Absorption showing the presence of hydroxyl group was seen at 3380.69cm$^{-1}$.

**Result of proton and carbon 13 NMR**

**Table 2 $^{1}$HNMR and $^{13}$CNMR analysis of compound**

| **Position** | **$^{13}$C Chemical Shift ( ð)** | **Types of carbon** | **$^{1}$H chemical shift (ð)** | **Type of proton** |
|---|---|---|---|---|
| 1 | 20.860 | **C**$H_2$ | 1.2604 | **CH$_2$** |
| 2 | 21.500 | **C**$H_2$ | 1.2842 | **CH$_2$** |
| 3 | 77.985 | **C**H | 1.6200 | **CH** |
| 4 | 22.300 | **C**$H_2$ | 1.2900 | **CH$_2$** |
| 5 | 31.050 | **C**H | 1.6210 | **CH** |
| 6 | 24.686 | **C**$H_2$ | 1.3790 | **CH$_2$** |
| 7 | 25.971 | **C**$H_2$ | 1.3800 | **CH$_2$** |
| 8 | 35.720 | **C**H | 1.6800 | **CH** |
| 9 | 43.290 | **C**H | 1.7100 | **CH** |
| 10 | 48.210 | **C** | --- | ---- |
| 11 | 27.252 | **C**$H_2$ | 1.4000 | **CH$_2$** |
| 12 | 28.541 | **C**$H_2$ | 1.4100 | **CH$_2$** |
| 13 | 49.015 | **C** | ------ | ---- |
| 14 | 44.738 | **C**H | 1.7190 | **CH** |
| 15 | 28.850 | **C**$H_2$ | 1.4210 | **CH$_2$** |
| 16 | 29.839 | **C**$H_2$ | 1.4400 | **CH$_2$** |
| 17 | 48.000 | **C**H | 1.7310 | **CH** |
| 18 | 203.595 | **C=O** | ---- | ----- |
| 19 | 9.216 | **C**$H_3$ | 0.8802 | **CH$_3$** |

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1885-1892 | ISSN:2251-6727

| | | | | |
|---|---|---|---|---|
| 20 | 10.520 | **C**$H_3$ | 0.8910 | C**H₃** |
| 1’ | 130.000 | **=C** | ---- | ---- |
| 2’ | 130.589 | **=C** | ---- | ---- |
| 3’ | 130.610 | **=C**H | 7.8478 | C**H** |
| 4’ | 131.200 | **=C** | --- | ---- |
| 5’ | 131.700 | **=C**H | 7.9500 | C**H** |
| 6’ | 132.110 | **=C** | --- | ---- |
| 7’ | 50.434 | **OC**$H_3$ | 3.3100 | OC**H₃** |
| 8’ | 51.840 | **OC**$H_3$ | 3.3816 | OC**H₃** |
| 9’ | 52.600 | **OC**$H_3$ | 3.3920 | OC**H₃** |

**Proton nuclear magnetic resonance ($^1$HNMR) of compound**

From the analysis of $^1$HNMR spectrum, table (2), the presence of methyl protons were seen at ð 0.8802 and ð 0.8910. Methylene protons showed their peaks at ð 1.2604, ð 1.2842, ð 1.2900, ð 1.3790, ð 1.3800, ð 1.4000, ð 1.4100, ð 1.4210, ð 1.4400. Absorptions of methine protons were observed at ð 1.6210, ð 1.6800, ð 1.7100, ð 1.790, and v1.7310. These were labeled H-5, H-8, H-9, H-14 and H-17. Aromatic protons were seen at ð 7.8478 and ð 7.9500 labeled H-3’ and H-5’. The spectrum depicted the presence of methoxy protons whose chemical shifts were seen at ð 3.3100, ð 3.3816 and ð 3.3920

**Carbon nuclear magnetic resonance ($^{13}$CNMR) of compound**

$^{13}$CNMR spectrum showed the presence of methyl carbons at ð 9.2160 and ð 10.520, labeled C-19 and C-20 respectively. Methylene carbons showed their peaks at ð 20.860, ð 21.500, ð 22.300, ð 24.686, ð 25.971, ð 27.252, ð 28.541, ð 28.850 and ð 29.839. These were labeled C-, C-2, C-4, C-6, C-7, C-11, C-12, C-15 and C-16 respectively. Methine carbons were seen at ð 31.050, ð 35.720, ð 43.390, ð 44.738, ð 44.738, ð 46.161, ð 47.589 and ð 48.000 labeled C-5, C-8,C-9,C-14, C-3’, C-5’and C-17 respectively. Quaternary carbons showed their absorptions at ð 48.210, ð 49.015, ð 110.150, ð 110.620 and ð 120.000 labeled C-10, C-13, C-2’, C-4’ and C-6’ respectively. Absorptions due to the presence of methoxy carbons were seen at ð 50.434, ð 51.860, and ð 52.600 labeled C-7’, C-8’, and C-9’ respectively. The carbonyl carbons (C=O) absorption was indicated at ð 203.595 confirming presence of an aldehyde. Aromatic carbons were absorbed at ð 130.000, ð 130.589, ð 130.610, ð 131.200, ð 131.700 and ð 132.110 labeled C-1’, C-2’, C-3’, C-4’, C-5’ and C-6’ respectively

**Mass spectroscopy**

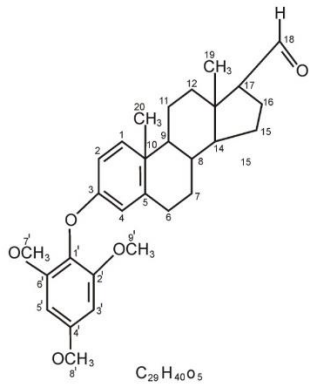
The result obtained from the mass spectroscopic analysis revealed that the molecular ion peak was obtained at m/z 468 which corresponds to a molecular mass of 468 for the compound

The Compound was isolated using chloroform and methanol mix which gave yellow oil. Thin layer chromatography (TLC) carried on the compound showed one spot on iodine vapour (Rf = 0.40). From the analysis of infra-red (IR), proton nuclear magnetic resonance ($^1$HNMR) and carbon-13 nuclear magnetic resonance ($^{13}$ CNMR), and mass spectra, compound was characterized and the compound proposed as 3[0] -2’, 4’, 6’- trimethoxyphenyl, Androstan carboxaldehyde with molecular formula $C_{29}$ $H_{40}$ $O_5$, mz = 468.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1885-1892 | ISSN:2251-6727




3 [0] - 2', 4', 6' - trimethoxyphenyl, Androstan Carboxaldehyde

FIG 1 Structure of compound

## 5. DISCUSSION

Androstane is a steroid that has been reported to be useful in the treatment of asthma, inflammatory pruritic dermatoses and breast cancer in postmenopausal women. It has been reported that androgen (4-androstene-3,17-dione (androstenedione) was present in the leaves of wheat and that its content decreased fourfold after the plants were exposed to cold . Androstenedione was also found in the leaves of Nicotiana tabacum and Inula helenium .[20,21] Androsterone is used for weight loss, to reduce sexual problems and to improve athletic performance. Thus, it helps to solve the problem of erectile dysfunction and fatigue. Androstenedione is a natural steroidal hormone produced in male and female gonads and in adrenal glandsand it helps to increase testosterone levels [22]. The isolated compound is a derivative of androstane and could be the reason piper guineense is used in the treatment of coughs and bronchitis .[23] The presence of this compound may be the reason, the plant is used to treat intestinal diseases and rheumatism[24] .This compound in *Piper guineense* confirms the claim that piper guineense is suitable for treatment of infertility in women and low sperm count in men[25,26] .Phytoestrogens including flavonoids are commonly found in fruits and other parts of various medicinal plants , [27].Phytoestrogens apart from their antioxidant and anti-inflammatory properties may have many other therapeutic uses including being considered as anti-cancer drugs , regulators of cholesterol metabolism, hepatoprotective and cardioprotective compounds

## Conclusion

Our findings lend credence to the trado- medical application of the leaf of the plant in treatment of certain fertility related ailments due to the high phytoestrogens in the plant leaf and seed,. It could be useful in the treatment of infertility in women and low sperm count in men. The plant could act as a fertility booster to men. It is therefore recommended that men with fertility issues could consider including the leaf and seed of this plant in their daily meal

## Acknowledgements

The authors wish to thank Prof Olayinka .O. Ajani of Department of Chemistry Covenant University Ota Nigeria for his numerous assistance in carrying out the IR and NMR analysis at the applied informatics and communication African Centre of Excellence (CAPIC-ACE), Covenant University Ota Nigeria.

## Conflict of interest

Authors have no competing interest to declare that are relevant to the content of this article

## REFERENCES

1. Ojiako F. O., Echerobia C .O., Okudoh S. U., 2018 Insecticidal Activity of Piper guineense Schumacher extract on some field insect pests of Cucumber (Cucumissativus Linnaeus) in Imo State, Nigeria. Futo Journal Series (FUTOJNLS) 4 (1) 45- 66.

2 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 Piperguineense: A Review. 25 (6) 927- 937.

DOI: https://dx.doi.org/10.4314/jasem.v25i6.6

3 Nwachukwu C.U., Umeh C.N., Kalu I.G., 2010 Identifi-cation and traditional uses of some common medici-nal plants in Ezinihitte Mbaise L.G.A., of Imo State, Nigeria. Report and Opinion 2 (6) 1 - 8.

4 Asaolu S.S., Adefemi O.S., Oyakilome I.G., Ajibulu K. E., Asaolu M. F., 2012 Proximate and mineral

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1885-1892 | ISSN:2251-6727

composition of Nigerian leafy vegetables. Journal of Food Research 1 (3) 2014 – 2018

5 Iloh E. O., Onyema-iloh O.B., Agbafor K.N., Omogo S. E., 2015 Preliminary Photochemical and Antibacterial Screening of the Leaf Extracts of Gongronema Latifolium (Utazi) *Quest Journals Journal of Research 2 (10) 06-09 ISSN(Online) : 2394-076X ISSN (Print):2394-0751* www.questjournals.org

6 Ojinnaka M.C., Ubbor S.C., Okudu H.O., Uga U., 2016 Volatile compound analysis of the leaves and seeds of *Piper guineense* using gas chromatography- mass spectrometry (GC-MS) African Journal of Food Science 10 (11) 327- 332, DOI: 10.5897/AJFS2016.1472

7 Ndulaka J. C., Ekaiko M. U., Ogbonna C. R., Asiegbu E. I., Achinewhu S.C., Ogbonna C.C., Hart A.D ., 1995 Chemical composition of indigenous wild herbs, spices, fruits, nuts and leafy vegetables used as foods. Plant Foods Hum. Nutr. 48 :341-348.

8. Sadura I., Janeczko A. (20180 Physiological and molecular mechanisms of brassinosteroid-induced tolerance to high and low temperature in plants. Biol. Plant. 62 601–616. [CrossRef]

9 Nolan T.M., Vukasinovic N., Liu D.R., Russinova E., Yin Y.H., 2020 Brassinosteroids: Multidimensional regulators of plant growth, development, and stress responses. Plant Cell 32, 295–318. [CrossRef] [PubMed]

10 Gorzkiewicz J., Bartosz G., Sadowska-Bartosz I., 2021 The potential effects of phytoestrogens: The role in neuroprotection. Molecules, 6, 2954. [CrossRef] [PubMed]

11. Achinewhu S.C.,Ogbonna C.C., Hart A.D.,1995 Chemical composition of indigenous wild herbs, spices, fruits, nuts and leafy vegetables used as food. 48 (4) 341-3440.

12. Slavin J.L., Lloyd B. 2012 Health Benefits of Fruits and Vegetables. Adv. Nutr. 3 506–516

13 Asokan S.M., Kuo W.W., Kuo C.H., Day C.H., Shen C.Y., Chung L.C.,2017 Potential Phytoestrogen Alternatives Exert Cardio-Protective Mechanisms via estrogen Receptors. Biomedicine (Taipei) 7 (2) 11. doi:10.1051/bmdcn/2017070204

14 Wasswa J., Omorodion T. N., Avwioro G.O ., Asimiyu O.S. , 2017 Histological Effect of *Piper Guineense* (UZIZA) Leaves on the Liver of Wistar Rats International Journal of Research and Review 4 (3) 36-41

15 Badawy M.T., Sobeh M., Xiao J., Farag M.A.., 2021 Androstenedion (a natural steroid and a drug supplement); A comprehensive Review of its consumption, metabolism, Health Effects and Toxicity with Sex Difference" molecules 26 (20) 6210.

16. Ojinnaka M.C., Ubbor S.C., Okudu H.O., Uga U. (2016) Volatile compound analysis of the leaves and seeds of *Piper guineense* using gas chromatography-mass spectrometry (GC-MS) African Journal of Food Science 10 (11) 327-332, doi. 10.5897/AJFS2016.1472

17. Iwu I.C., Onu U.L., Ukaoma A.A., Oze R.N. 2018 Phytochemical, Antimicrobial and GC/MS Analysis of the Root of Stachytarpheta *Cayennensis (L .Vahl) Grown in Eastern Nigeria. International Research Journal of Natural Sciences* 6 (2) 1-14.

18. Uyanwa M.N., Okenwa U., Iwu I., Nwoke L., Echeme J.O. 2024 Isolation and Characterization of Glucocorticoid Steriod from the Leaf of *Rauvolfia vomitoria* Int. Res. J. Pure Appl. Chem., 25 (3 )76-89, 2024; Article no.IRJPAC.115202

19 Iwu I.C., Echeme J.O., Uchegbu R.U., 2014 Isolation and characterization of 4 – 15.Cholestene – 3 – yl Benzoate from the stem Bark of pentacle thramacrophylla (P.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1885-1892 | ISSN:2251-6727

Benth), International Journal of Emerging knowledge IJEK 11 (10) 185 – 192.

20 Janeczko A. 2021 Estrogens and Androgens in Plants: The Last 20 Years of Studies. Plants ,10 2783. https://doi.org/10.3390/ plants10122783

21 .Madu A..N., Iwu I.C., Edeh E.C., Joseph E.E. , 2023 The Extraction and GC- MS Characterisation Of Leaf Extract Of Piper Guineense (Uziza Leaf) African- British Journals. 6 (12) 71-83 DOI 10.52589/AJBMR- KHLVVGEG.

22. Janeczko A., Oklest'kova J., Siwek A., Dziurka M., Pociecha E., Kocurek M., Novak O., (2013)Endogenous progesterone and its cellular binding sites in wheat exposed to drought stress. J. Steroid Biochem. Mol. 138 384–394. [CrossRef] [PubMed]

23. Owolabi M.S., Lawal O.A., Ogunwande I.A., Hauser R.M., Setzer W.N.., 2013 Aroma chemical composition of *Piperguineense* Schumach. & Thonn.From Lagos, Nigeria: a new chemotype American Journal of Essential Oils and Natural Products 1 (1): 37-44 Journal DOI: 10.46654/ij.24889849 Article DOI: 10.46654/ij.2488

24.Tarkowská D.(2019) Plants are capable of synthesizing animal steroid hormones. Molecules , 24 2585. [CrossRef] [PubMed]

25 Simerský R., Novak O., Morris D..A., Pouzar V., Strnad M.,2019 Identification and quantification of several mammalian steroid hormones in plants by UPLC- MS/MS. J. Plant Growth Regul. 28 125–136. [CrossRef]

26 Khaleel T.F., Dillman R., Gretch D., 2003 Estradiol distribution during the development and expression of reproductive structures in Populus tremuloides Michx. Sex. Plant Reprod. 16 , 35–42. [CrossRef]

27.Milanesi L., Monje P., Boland R., 2001 Presence of estrogens and estrogen receptor-like proteins in Solanum glaucophyllum. Biochem. Biophys. Res. Commun. 289 ( 1175–1179. [CrossRef]