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Pharmacognostic standardisation of the leaves of *Costus afer* Ker Gawl. (Zingiberaceae) and *Palisota hirsuta* (Thunb.) K. Schum. (Commelinaceae)

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Abstract

Background Microbes' resistance to orthodox drugs and continued emergence of new infections call for search of new drugs that can mitigate such resistance and cure newly emerging infections. Despite the therapeutic importance of *Costus afer* and *Palisota hirsuta*, the detailed pharmacognostic characters of the two plants are still missing in the literature. Thus, this study evaluated the pharmacognostic characters of these plants and the antimicrobial activity of the plants' methanol extract. Macroscopic, microscopic, fluorescence analysis, phytochemical screening, heavy metal analysis, proximate, antimicrobial assay and Gas Chromatography–Mass Spectrometry analyses were carried out on both plants.

Results The microscopic evaluation showed that the epidermal layer of *Costus afer* is hypostomatic with polygonal shaped epidermal cells and cyclotoc stomata on abaxial layer while the epidermal layer of *Palisota hirsuta* leaf is amphistomatic, having polygonal shape epidermal cell, trichomes and paracytic stomata. The proximate analysis showed that both plants have a high carbohydrate content (*C. afer*: 59.13% and *P. hirsuta*: 68.28%) but a low crude fat content (*C. afer*: 1.867% and *P. hirsuta*: 0.233%). The heavy metal analysis revealed a high content of Manganese (266.80 mg/100 g) in *Palisota hirsuta*, and a high content of iron (43.94 mg/100 g) in *Costus afer*. Both plants are free of lead. The phytochemical screening showed that both plants have saponins, tannins, flavonoids, alkaloids and cardiac glycosides. Alkaloids are the highest constituents in *Costus afer* (9.1%) and *Palisota hirsuta* (7.1%). The antimicrobial assay showed both plant extracts had the highest inhibitory effect on both bacteria and fungi at 100 mg/kg. Six compounds and 23 compounds were identified by GC–MS in *C. afer* and *P. hirsuta*, respectively. Methyl stearate (40.43%) and hexadecanoic acid, methyl ester (25.62%) were the prevailing constituents identified in *C. afer* and *P. hirsuta*, respectively.

Conclusion The pharmacognostic features of *Costus afer* and *Palisota hirsuta* shown in this study can be used to design a suitable monograph which would be useful in compilation of another edition of Nigeria Herbal Pharmacopoeial. The dominant compounds in both plants can serve as chemotaxonomic markers for correct identification of the two plants.

Keywords *Costus afer*, *Palisota hirsuta*, Microscopy, Proximate analysis, Pharmacognostic, GCMS, Antimicrobial assay

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Background

The use of herbal medicine for the treatment of different infections and diseases in Africa dates back to centuries [1]. Due to renaissance in the use of herbs and herbal products in the recent time as it is believed to be safe and cost effective with little or no side effects compared to synthetic drug, World Health Organisation has set and recommended suitable pharmacopoeial standards to constitute a monograph for herbal raw materials to ensure safety, efficacy and reproducibility of herb and its products [2]. Standardisation of herbal drugs begins from the collection of herbal raw materials to its packaging and use as medicine. The authenticity, quality and purity of herbal drugs are established by reference given in pharmacopoeial [3]. Standardisation and quality assurance of medicinal plants involved the following pharmacognostic standards; correct identification, macroscopic and microscopic analysis, physicochemical analysis, biological and chemical studies, fluorescence analysis [4]. According to World Health Organisation, the macroscopic and microscopic evaluation of medicinal plants is the fundamental step in ascertaining the identity and the quality of such materials [5, 6].

Costus afer Ker-Gawl of the family Zingiberaceae, commonly known as Ginger lily, Spiral ginger and Bush cane, and it is known locally as Ireke omode (Yoruba) and Okpete (Igbo). It is commonly found in humid and thick forests and riverside [7]. It is highly valued for its anti-diabetic, anti-inflammatory and antiarthritic properties in South-east and South-West Nigeria [8, 9]. The young and tender leaves when chewed are believed to give strength to the weak and dehydrating patient [10]. Various activities of different part of the plant have been reported by several studies: anti-inflammatory [11], antioxidant [12], antidiabetic [13] and analgesic activities [14].

Palisota hirsuta (Thunb.) K. Schum. (Family Comelinaceae) locally known as jangborokun (Yoruba) and Ikpereturu (Igbo). A decoction of the leafy stems and whole plant is used in baths for oedema and for urethral discharge, respectively [15]. The roots and leaves are traditionally used to treat infertility and the root alone is used in the management of anaemia, dysentery and rheumatism [16]. The leaf extracts have been reported to have wound healing [17], anti-inflammatory [18], anti-diarrheal activity [19], anti-arthritis [20] and aphrodisiac activities [21].

These plants though in different family have some morphological similarities which have led to confusion in their correct identification as they are interchangeably collected for various medicinal purposes. Thus, this study aims at establishing some pharmacognostic characters

for the leaves of the two medicinal plants that can be used in correct identification of the plants and development of their monograph.

Methods

Plant collection and preparation

The leaves of *Palisota hirsuta* (Thunb.) K. Schum were collected from Isore farm, Ipara Remo, Ogun State, Nigeria, in April 2021, and were identified and authenticated at the Forest Herbarium Ibadan (FHI) by Mr. Adeyemo, where the voucher specimen was deposited. Also, the leaves of *Costus afer* were collected from Eleyele, Ibadan, Oyo state, Nigeria, and were identified and authenticated at the University of Ibadan Herbarium by Mr D.P.O Esimekhuai with the voucher number UIH-23090. The fresh samples of the leaves of both plants were preserved in 50% ethanol till when the anatomical analysis is fully carried out, while some of the leaves of both plants were air-dried at room temperature, pulverized and stored separately in air-tight containers for other analyses.

Macroscopic evaluation

Morphological studies were carried out by observing the plant part with the naked eyes [22]. Parameters of the plants such as size, length, width, shape, apex, margin, base, petiole, surface, colour, odour and taste were determined.

Epidermal layers preparation

The abaxial layer was carefully scraped off using a razor blade to obtain adaxial layer which is the upper leaf epidermis [22], also to get the abaxial layer of the plant; the adaxial layer was scraped off. Surface tissues obtained were further cleared using 2% v/v sodium hypochlorite for 2–3 min and rinsed severally with distilled water to remove the sodium hypochlorite. The cleared epidermal tissues were stained with Safranin O for about 2–3 min and then dehydrated in vials containing 50%, 70% and 100% ethanol successively. The sample was placed on a microslide, mounted in a drop of 25% glycerol and covered with cover slip. The slides were viewed under a light microscope (XSP-103A). Photomicrographs of the microscopic features were taken at different magnification with a camera (Infinix S5, 16MP).

Transverse section

The transverse sections of leaves and petioles of *Costus afer* and *Palisota hirsuta* were made with free hand sectioning. Briefly, scalpel was used to cut a tiny strip of the plants through the midrib region and also the petiole. The transverse sections obtained were cleared using 2% v/v sodium hypochlorite for 2–3 min. and rinsed severally with distilled water to wash off the sodium hypochlorite.

The cleared transverse sections were stained with Safranin O for about 2–3 min and then dehydrated in vials containing 50%, 70% and 100% ethanol successively. The samples were placed on microslides, mounted in 25% glycerol and viewed under a light microscope (XSP-103A). Photomicrographs of the microscopic features were taken at different magnification with a camera (Infinix S5, 16MP).

Proximate analysis

The proximate analysis of the plant samples was done according to the method of the Association of the Analytical Chemist [23]. The moisture and ash contents were determined using the weight difference method. Fiber content was estimated from the loss in weight of the crucible and its content on ignition. The carbohydrate content was determined by subtracting the sum of the percentages of moisture, ash, crude protein, and crude fiber from 100.

Phytochemical screening and fluorescence analysis

The phytochemical screening was carried out on the powdered samples of *Costus afer* and *Palisota hirsuta* following standard methods [24, 25]. Fluorescence examination of the powdered leaf samples was done using standard method. The examination was done by treating the plants powder with different reagent such as 50% HCl, 50% H₂SO₄, ferric chloride, glacial acetic acid, picric acid and distilled water. After the treatment they were examined under visible light, short ultra-violet light (254 nm) and long ultra- violet light (365 nm) [26].

Quantitative determination of the phytochemical constituents

Total phenol content was determined by the Folin-Ciocalteu method as described by [27]. Total flavonoid content was by the method described by [27]. Total Alkaloid, Saponin and terpenoid content were determined using the method described by [28]. The percentage alkaloid, saponin and terpenoid were calculated by difference.

Heavy metals analysis

Five grams of the powdered sample was weighed directly into a 250-mL Erlenmeyer flask, 25 mL of hydrochloric acid was added, and the mixture was brought to boiling on a hot plate and allowed to simmer for 5 min. The mixture was cooled and transfer to a 50 mL volumetric flask. It was made up to volume with deionised water and mixed thoroughly by shaking. The solution was filtered using No.1 Whatman filter paper. The filtrate was used for the determination of some minerals, using corresponding standards and blanks; the mineral

contents were determined using Atomic Absorption Spectrophotometer.

Preparation of plant extracts

The leaves of *Costus afer* and *Palisota hirsuta* were air-dried, pulverized into powder, weighed and stored in a dry, clean, sterile container ready for extraction. The dried pulverized plant material was extracted with methanol at room temperature for 72 h and stirred occasionally. The extract was filtered and concentrated *in vacuo* using rotary evaporator (Bibby Sterlin Ltd, London). The extract obtained was refrigerated for subsequent use.

Collection of clinical pathogens

The following bacterial clinical pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae* and the following fungi isolates, including *Candida albicans*, *Aspergillus niger*, *Penicillium notatum*, *Rhizopus stolonifer* obtained from the Department of Pharmaceutical Microbiology, University of Ibadan were used for this study.

Culture media

Bacterial test organisms were cultured using Nutrient agar while Sabouraud dextrose agar (SDA) was used to culture the fungi organisms, respectively.

Minimum inhibitory concentration (MIC) (agar dilution method)

The different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL) of each of the samples were prepared individually. Two (2) mL each of the different concentrations were added to 18 mL of the molten agar and the mixture was transferred aseptically into the Petri dish and the agar mixture was allowed to set properly for about 40 min. On the set agar plates, the different organism was streaked. The plates were incubated at 37 °C for 18–24 h and at 26–28 °C for 48 h for both the bacteria and fungi, respectively. The lowest concentration of each extract displaying no visible growth was recorded as the minimum inhibitory concentration. The concentration that inhibited bacterial/fungi growth completely (the first clear well) was taken as the MIC value. MIC values were determined at least in duplicate and repeated to confirm activity [29].

Zone of inhibition (ZI)

Each sample (0.5 g) was weighed and dissolved into 5 mL of the solvent of extraction (dimethyl sulfoxide) for proper dissolution from which 2.5 mL was taken into



Fig. 1 Habit photograph of **A** *Costus afer* and **B** *Palisota hirsuta* Habit photograph shows some macroscopic features of the plant samples which include shape of leaf apex, leaf colour, leaf arrangement and so on

another 2.5 mL of the solvent, this was taken to the 6th test tube which was the last test tube for the extract. The 7th and 8th test tubes were the negative control (dimethyl sulfoxide) and positive control (gentamycin for bacteria and Tioconazole for fungi) of the experiment.

GC–MS analysis

The methanol extracts of *Costus afer* and *Palisota hirsuta* leaves were analysed with GC (Agilent technologies 7800, USA) coupled with MS (Agilent technologies 5975, USA) to determine their phytochemical constituents. The HP5MS capillary column (30 m × 0.32 mm × 0.25 μL) was used under the following conditions: oven temperature coded from 80 °C for 2 min, then progressively increased to 240 °C for 6 min; injector temperature (250 °C), carrier gas (Helium), flow rate is 1 mL/min; the volume of the injection sample was 1μL; scan ranges from 50–500 and mode of analysis is splitless. The relative quantity of every component was computed by the comparison between its average peak area to the total area. The Identification details of the separated volatile compounds was carried out through retention indices and mass spectrometry by comparing using database of National Institute Standard and Technology (NIST), library 2008.

Results

Macroscopical evaluation

The habit photograph of *Costus afer* and *Palisota hirsuta* is shown in Fig. 1. The summary of the macroscopy and

Table 1 Macroscopy of *Costus afer* and *Palisota hirsuta*

Characters	<i>Costus afer</i>	<i>Palisota hirsuta</i>
Condition	Whole leaf	Whole leaf
Composition	Simple leaf	Simple leaf
Shape	Lanceolate	Lanceolate
Margin	Entire	Entire
Apex	Acuminate	Acuminate
Base	Truncate	Symmetrical base
Surface	Glabrous	Pubescent
Petiole	Exstipulate	Exstipulate
Leaf width	6.4 ± 0.4 cm	7.95 ± 1.13 cm
Leaf length	19.5 ± 2.04 cm	31.9 ± 4.45 cm
Petiole length	0.7 ± 0.25 cm	3.7 ± 1.09 cm

Table 2 Organoleptic properties of *Costus afer* and *Palisota hirsuta*

Characters	<i>Costus afer</i>	<i>Palisota hirsuta</i>
Colour: Adaxial	Dark green	Dark green
Colour: Abaxial	Light green	Light green
Smell	Leafy	Leafy
Taste	Bland	Bland
Surface	Glabrous	Pubescent
Touch	Soft to Papery	Papery

the organoleptic features are shown in Tables 1 and 2, respectively.

Epidermal layer

Evaluation of leaf surface constants is essential for the determination of adulteration in the leaf of medicinal plants and it is useful to differentiate closely related species [30, 31]. The epidermal cell on the adaxial surface of *Costus afer* assumes polygonal shape with straight anticlinal wall; stomata and trichomes are absent (Fig. 2A). The adaxial surface of *Palisota hirsuta* has straight walled polygonal epidermal cells,

non-glandular unicellular trichomes and cyclocytic stomata (Fig. 2B). The abaxial surface of *Costus afer* is characterised with polygonal epidermal cells with slightly wavy anticlinal wall and paracytic stomata; thus, the leaf is hypostomatic (in that they have stomata only on the abaxial surface) with no trichomes as shown in Fig. 3A. The abaxial layer of *Palisota hirsuta* has polygonal wavy anticlinal walled epidermal cells with paracytic stomata sparsely dispersed amidst the epidermal cells (Fig. 3B). The leaf of *Palisota hirsuta* is amphistomatic (both layers contain stomata) and both layers contain non-glandular unicellular trichomes

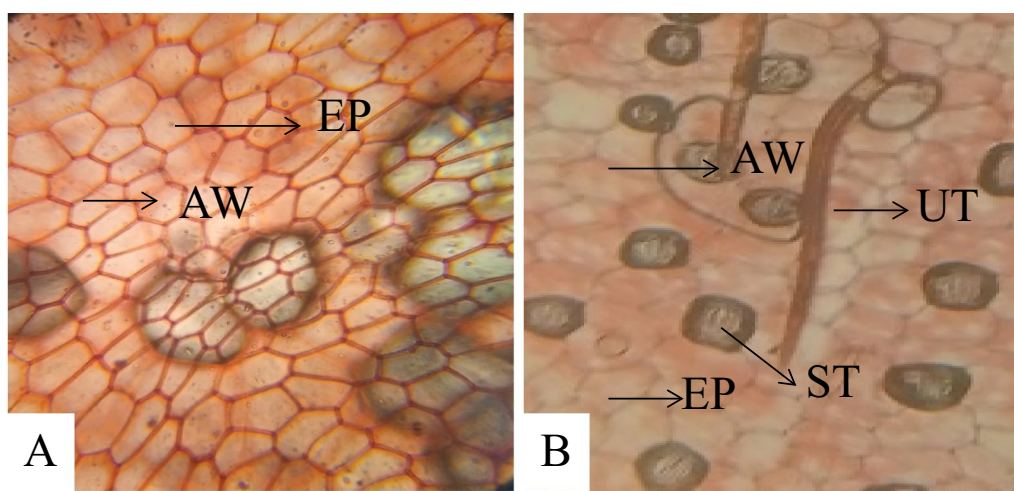


Fig. 2 Surface view of Adaxial epidermis (X400). **A** *Costus afer* **B** *Palisota hirsuta* AW: Anticlinal wall; ST: stomata; EP: Epidermal cell; UT: Unicellular trichome Adaxial epidermis also referred to as upper layer was gotten by scraping the abaxial epidermis (lower layer) of the leaf of each plant, cleared in 2% v/v Sodium hypochlorite and rinsed severally in distilled water. The cleared epidermal tissues were stained with Safranin O and then dehydrated in vials containing 50%, 70% and 100% ethanol successively. This was mounted on microslide with dilute glycerol and viewed under light microscope at $\times 400$ objective lens and photograph was taken using camera phone

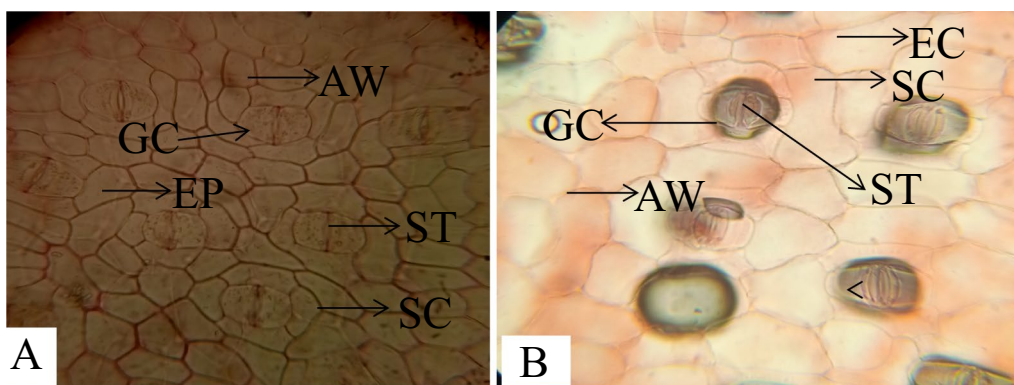


Fig. 3 Surface view of Abaxial epidermis (X400). **A** *Costus afer* **B** *Palisota hirsuta* AW: Anticlinal wall; EP: Epidermal cell; GC: Guard cell; SC: subsidiary cell; ST: stomata. Abaxial epidermis also referred to as lower layer was gotten by scraping the abaxial epidermis (upper layer) of the leaf of each plant, cleared in 2% v/v Sodium hypochlorite and rinsed severally in distilled water. The cleared epidermal tissues were stained with Safranin O and then dehydrated in vials containing 50%, 70% and 100% ethanol successively. This was mounted on microslide with dilute glycerol and viewed under light microscope at $\times 400$ objective lens

(Figs. 2B and 3B). The absence of stomata on the adaxial surface and absence of trichomes on both adaxial and abaxial surfaces of *Costus afer* can be used as diagnostic features to differentiate between its leaf and that of *Palisota hirsuta*.

Transverse section

The transverse sections of the midrib of *Costus afer* and *Palisota hirsuta* are characterised with collenchyma cells, upper and lower epidermis, cuticle (Fig. 4A and B). The mid rib of *Costus afer* has a single layered scattered upper epidermis, big circular collenchyma cells and scanty vascular bundles (Fig. 4A) while the mid rib of *Palisota hirsuta* on the other hand has double layered upper epidermis, small circular collenchyma cells and numerous

vascular bundles (Fig. 4B). The transverse sections of the petioles of the two leaves showed the presence of collenchymas, parenchyma, palisade cells, vascular bundles (Fig. 5A and B). The transverse section of the petiole of *Costus afer* showed the presence of thick cuticle, single layered upper epidermis and absence of trichomes (Fig. 5A) while *Palisota hirsuta* has thin cuticle, double layered upper epidermis and non-glandular unicellular trichomes (Fig. 5B).

Proximate analysis

The results of the proximate analysis carried out on the powdered samples of *Costus afer* and *Palisota hirsuta* are shown in Table 5. The moisture contents obtained for leaves of *Costus afer* (6.98%) and *Palisota hirsuta* (5.28%)

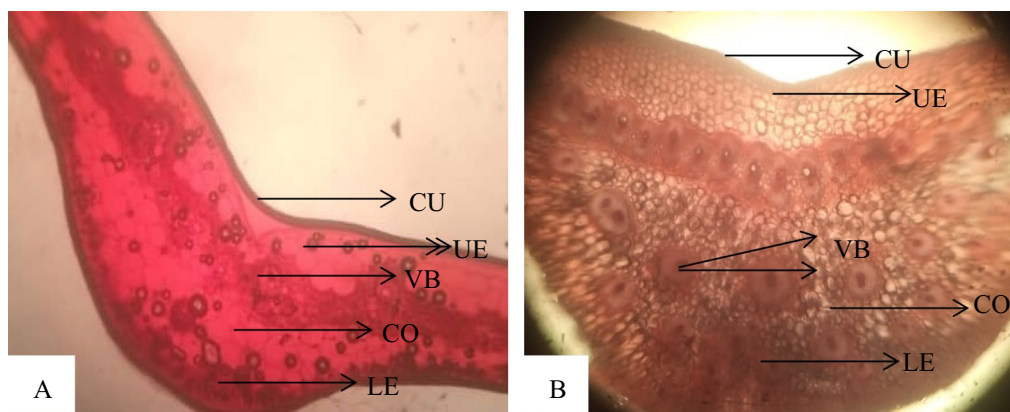


Fig. 4 Transverse sections of mid rib of leaves $\times 100$; **A** *Costus afer* **B** *Palisota hirsuta*. CO: collenchyma; CU: Cuticle; LE PP: palisade parenchyma; UE: upper epidermis, VB: vascular bundle. Briefly, scalpel was used to cut a tiny strip of the plants through the midrib region. The transverse sections obtained were cleared using 2% v/v sodium hypochlorite, washed severally in water, stained with Safranin O, dehydrated in 50%, 70% and 100% Ethanol, mounted in dilute, glycerol, viewed under microscope and, photomicrograph taken, with the aid, of phone camera

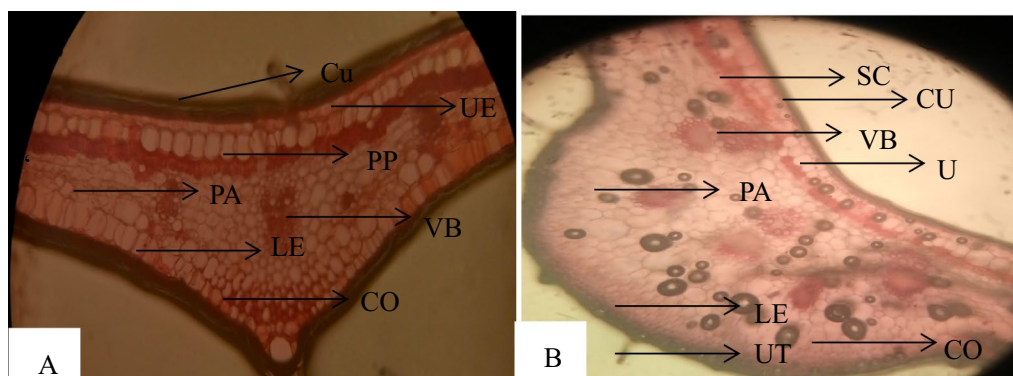


Fig. 5 Transverse sections of Petioles $\times 100$; **A** *Costus afer* **B** *Palisota hirsuta*. CO: collenchyma; CU: Cuticle; LE: lower epidermis, PA: parenchyma; PP: palisade parenchyma; UE: upper epidermis; UT: unicellular non-glandular trichome; VB: vascular bundle. Briefly, scalpel was used to cut a tiny strip of the plants through the petiole. The transverse sections obtained were cleared using 2% v/v sodium hypochlorite, washed severally in water, stained with Safranin O, dehydrated in 50%, 70% and 100% Ethanol, mounted in dilute, glycerol, viewed under microscope and, photomicrograph was taken with the aid of phone camera

Table 3 Proximate analysis of powdered leaf samples of *Costus afer* and *Palisota hirsuta*

Test	Moisture content	Crude protein	Crude fat	Crude fibre	Ash content	Carbohydrate
<i>Costus afer</i> (%)	6.98	17.14	1.87	26.53	16.55	59.13
<i>Palisota hirsuta</i> (%)	5.28	13.94	0.233	28.34	12.27	68.28

Table 4 Phytochemical screening result of *Costus afer* and *Palisota hirsuta* powdered leaf samples

Test	<i>Costus afer</i>	<i>Palisota hirsuta</i>
Saponin	++	++
Alkaloids		
• Dragendorff	++	++
• Wagner	+	+
Flavonoids	++	+
Tannins	+	+
Anthraquinones	—	—
Cardiac glycosides		
• Keller-Killiani test	+	+
• Kedde's test	+	+

Keys + = Present, ++ = Moderately Present, — = Absent

were within the recommended limits as specified by [31] which suggested that the moisture contents of medicinal plants should not exceed 14% (Table 3).

Phytochemical constituents

Both *Costus afer* and *Palisota hirsuta* tested positive for the presence of saponins, alkaloids, flavonoids, tannins, and cardiac glycosides as shown in Table 4. While both plant samples tested negative for the presence of anthraquinones. The presence of these secondary metabolites in these plants may be responsible for their various biological activities. The result gotten from the phytochemical screening of *Costus afer* supports the findings obtained by [32] and [33], while reports on from *Palisota hirsuta* was also supported by [20].

Quantitative phytochemical constituents

The results of the quantitative determination of leaf extracts of *Costus afer* and *Palisota hirsuta* are presented

Table 5 Quantitative phytochemical constituents of methanol extracts of *Costus afer* and *Palisota hirsuta*

Sample	Alkaloids (%w/w)	Flavonoid (%w/w)	Saponin (%w/w)	Terpenoid (%w/w)	Total phenol (%w/w)
<i>Costus afer</i>	9.1	3.13	0.9	1	0.77
<i>Palisota hirsuta</i>	7.5	0	1.65	0.5	0.63

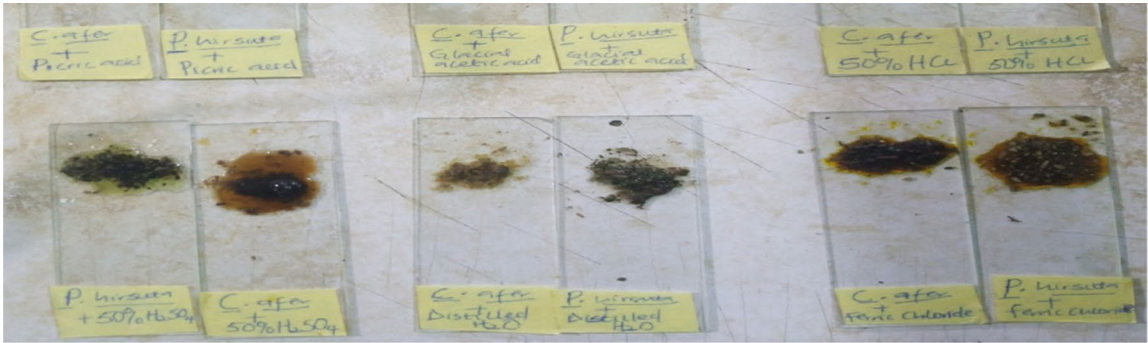
in Table 5. The methanol leaf extract of *Costus afer* has the higher percentage of alkaloid, flavonoid, terpenoid and phenol than *Palisota hirsuta* methanol leaf extract. But total *Palisota hirsuta* has the higher saponin content (1.65%) than *Costus afer* (0.9%)

Fluorescence analysis

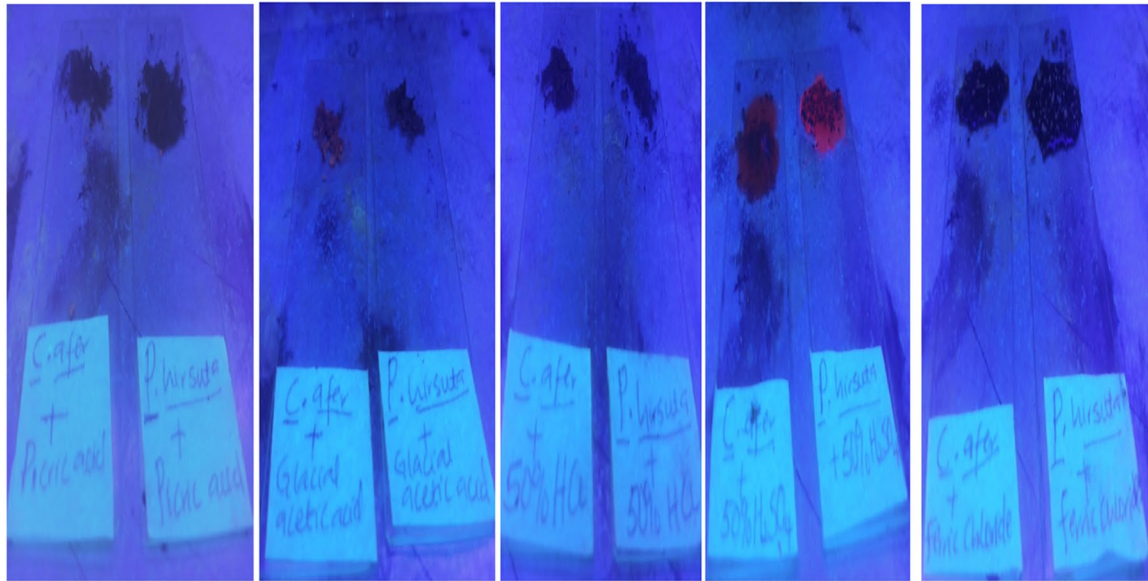
It is a pharmacognostic procedure that is used in the authentication of samples and recognizing adulterants because plant samples contain specific phytoconstituents that fluoresce under the ultraviolet light at different wavelength [31]. The fluorescence analysis is considered the simplest and one of the most essential steps in standardization with absolute scientific validation of crude drugs [6]. The various chemical reagents used react with the chemical constituents present in the samples and convert them into fluorescent derivatives, which fluoresce under UV light at different wavelength, which is distinctive for a particular drug and could be used in the detection of adulterants. Figures 6A–C show the pictorial representation of the various colour changes when viewed under daylight and ultraviolet light of 254 nm and 365 nm. Tables 5 and 6 give a summary of the different colour changes that were observed when the powdered

(See figure on next page.)

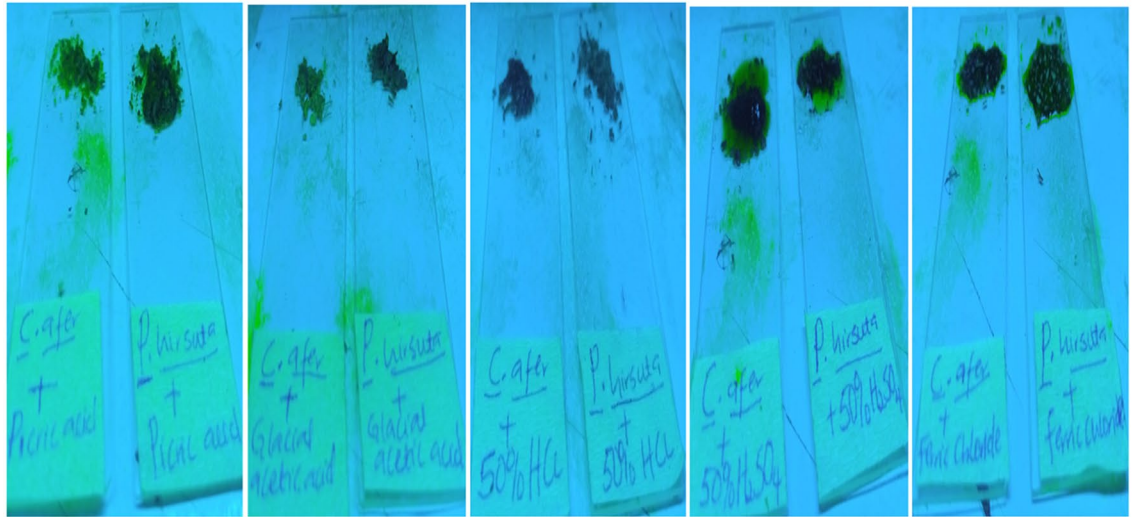
Fig. 6 **A** Fluorescence analysis of *Costus afer* and *Palisota hirsuta* at daylight. Reagents like picric acid, glacial acetic acid, 50% HCl were added to the various powdered plant samples and observed at daylight. The different colours observed were recorded. **B** Fluorescence analysis of *Costus afer* and *Palisota hirsuta* at 365 nm. Reagents like picric acid, glacial acetic acid, 50% HCl were added to the various powdered plant samples and observed under Ultraviolet rays at 365 nm. The different colours observed were recorded. **C** Fluorescence analysis of *Costus afer* and *Palisota hirsuta* at 254 nm. Reagents like picric acid, glacial acetic acid, 50% HCl were added to the various powdered plant samples and observed under Ultraviolet light at 254 nm. The different colours observed were recorded



A



B



C

Fig. 6 (See legend on previous page.)

Table 6 Fluorescence analysis of *Costus afer*

Drug + Reagent	Daylight	254 nm	365 nm
+ 50% H ₂ SO ₄	Black	Green	Yellow
+ 50% HCl	Brown	Dark green	Brown
+ Glacial acetic acid	Yellow	Light green	Deep yellow
+ Picric acid	Yellow	Light green	Black
+ Ferric chloride	Bluish black	Green	Purple
+ Distilled water	Yellow	Light green	Yellow

Table 7 Fluorescence analysis of *Palisota hirsuta*

Drug + Reagent	Daylight	254 nm	365 nm
+ 50% H ₂ SO ₄	Green	Dark green	Deep orange
+ 50% HCl	No obvious colour change	Green	Black
+ Glacial acetic acid	Light brown	Dark green	Red
+ Picric acid	Green	Deep green	Black
+ Ferric chloride	Green	Green	Purple
+ Distilled water	Green	Dark green	Purple

Table 8 Heavy metal analysis of *Costus afer* and *Palisota hirsuta*

Heavy metal	Copper	Iron	Zinc	Manganese	Lead
<i>Costus afer</i> (mg/100 g)	0.71	43.94	5.71	14.38	0.00
<i>Palisota hirsuta</i> (mg/100 g)	1.09	29.38	14.38	266.80	0.00

plant samples were treated with different reagents and viewed under daylight and ultraviolet light of 254 nm and 365 nm. The blue black colour obtained under the longer wavelength upon reaction with FeCl₃ suggests the presence of tannins according to [34]. This implied that tannin is present in the leaves of both *Costus afer* and *Palisota hirsuta*. The dark green colours observed when the sample was treated with 50% sulphuric acid suggests the presence of phytosterols [35].

Heavy metal analysis

Plants play a significant role in transferring heavy metals and trace elements from the polluted land to humans. The toxicity of medicinal plants on human health and to the ecosystem as a result of heavy metals present in them has been of great concern in the recent years. Some of the metals such as Na, K, Ca, Zn, Ni, Fe, Cu, Mn, Se, and Cr are needed in specific quantity for normal pharmacological and physiological functions of the body. A slight increase in the concentration of any of these above permitted limits becomes toxic to human [36]. The results of the heavy metal analysis are shown in Table 7. Upon analysing the heavy metal content in both plants, lead

was absent in both plants (Table 8). Copper is essential for normal neurologic function [37], but it has to be in minute quantity in food because an high intake of copper may result in hair and skin discoloration, irritation of the upper respiratory tract, and vomiting and liver damage among others [38] and the heavy metal analysis of the two plants shows that both plants have copper in minute quantity.

Minimum inhibitory concentration

The plant extract showed a significant minimum inhibitory concentration against all the bacteria and fungi organisms used for this assay at a higher concentration of 100 mg/mL and 50 mg/mL but at a lower concentration of 25 mg/mL and 12.5 mg/mL, *Costus afer* did not give a significant inhibition of the bacterial and was unable to inhibit the growth of the fungi. *Palisota hirsuta* did not inhibit the growth of all the microorganisms at a lower concentration of 12.5 mg/mL. It can be reported from this study that the minimum inhibitory concentration of both plants' extracts on all the tested microorganisms is 25 mg/mL. *Costus afer* produced a higher inhibitory effect on the both test strains bacteria and fungi at a higher concentration compared to the positive control and the inhibitory activity decreases with decrease in extract concentration (Table 9). *Palisota hirsuta* also gave a decreased inhibition down the concentration even though the inhibition produced wasn't as high as that produced by *Costus afer* and at a lower dose of 12.5 mg/mL and 6.25 mg/mL; there was no inhibitory effect on the growth of the test strains of fungi as shown in Table 10 below.

Gas chromatography-mass spectrometry

The GC/MS data and spectrum of the chemical constituents in methanol leaf extract of *C. afer* are shown in Table 11 and Fig. 7, respectively. The chromatogram showed that methanol leaf extract contained 6 compounds and they are Hexadecanoic acid, methyl ester (30.77%) class of compound fatty acid methyl esters, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (1.86%) phenylpropane Benzenepropanoic acid, 3, 5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester has been used as additive in motor cells, hydraulic fluids etc. It also possesses antioxidant and antifungal activities, 9-Octadecenoic acid fatty acid methyl esters, methyl ester (13.97%), 11-Octadecenoic acid, methyl ester (13.38%), Methyl stearate (40.43%) class of compound fatty acid methyl esters, Docosanoic acid (3.85%) while the GC/MS data and spectrum of the chemical constituents in methanol leaf extract of *P. hirsuta* are shown in Table 12 and Fig. 8, respectively. The chromatogram showed that the methanol leaf extract

Table 9 Minimum Inhibitory Concentration

Samples Concentrations	Staphylococcus aureus	Escherichia coli	Bacillus subtilis	Pseudomonas aeruginosa	Salmonella typhi	Klebsiella pneumoniae	Candida albicans	Aspergillus niger	Penicillium notatum	Rhizopus stolonifer
<i>Costus afer</i>	100 mg/mL	–	–	–	–	–	–	–	–	–
	50 mg/mL	–	–	–	–	–	–	–	–	–
	25 mg/mL	–	–	–	–	–	±	±	+	+
	12.5 mg/mL	±	–	±	±	+	+	+	+	+
<i>Palisota hirsuta</i>	100 mg/mL	–	–	–	–	–	–	–	–	–
	50 mg/mL	–	–	–	–	–	–	–	–	–
	25 mg/mL	–	–	–	+	+	+	+	+	+
	12.5 mg/mL	+	+	+	+	+	+	+	+	+

+ No inhibition of the growth of organisms
– Inhibition of the growth of organisms
± No pronounced growth of organisms

Table 10 Antimicrobial activity of methanol leaf extracts of *C. afer* and *P. hirsuta* Determined By Agar Well Diffusion Method

Samples Concentrations	Zone of Inhibition (mm)									
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhi</i>	<i>Klebsiella pneumoniae</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>	<i>Penicillium notatum</i>	<i>Rhizopus stolonifer</i>
<i>Costus afer</i>	100 mg/mL	30±0.7	28±0.7	30±0.7	30±1.4	28±0.7	20±0.7	20±0.3	18±0.7	20±0.7
	50 mg/mL	24±0.51	24±0.7	26±0.7	26±1.4	24±0.7	18±0.4	18±0.1	16±0.1	18
	25 mg/mL	21±0.7	21±0.7	22±0.7	24±1.4	21±1.1	16±0.1	16±0.3	14±0.8	16±0.6
	12.5 mg/mL	18±0.7	17±0.4	18±0.3	20±0.7	18±1.4	14±0.7	14±0.7	14±0.7	14±0.1
	6.25 mg/mL	14±0.05	13±0.4	14±0.5	14±0.8	14±1.4	12±0.7	12±0.7	12±0.7	10±0.05
<i>Palisota hirsuta</i>	100 mg/mL	24±0.7	22±0.7	22±0.8	24±0.7	22±1.4	18±0.6	16±0.7	18±0.4	16±0.7
	50 mg/mL	21±1.1	18±0.7	18±0.3	21±1.1	18±2.1	16±0.7	14±1.4	14±0.7	14±0.7
	25 mg/mL	18±1.4	14±0.7	16±0.7	18±0.7	16±1.4	14±0.7	12±1.4	12±0.7	10±0.7
	12.5 mg/mL	14±0.7	12±0.7	12±0.7	14±0.1	12±2.1	12±0.06	10±0.7	10±0.7	-
	6.25 mg/mL	10±0.3	10±0.2	10±0.7	10±0.7	10±1.4	-	-	-	-
Negative Control	Dimethyl sulfoxide	-	-	-	-	-	-	-	-	-
Positive Control	Gentamicin (10 µg/mL) / Tioconazole (70%w/v)	40	38	40	38	40	28	28	26	28

Table 11 Phytochemical Constituents of Methanol Extract of *Costus afer* leaf identified by GC–MS

Compounds names	Area %	Retention time (min)
1. Hexadecanoic acid, methyl ester	30.77	12.001
2. Benzenepropanoic acid, 3,5-bis-(dimethylethyl)-4-hydroxy-, methyl ester	1.86	12.183
3. 9-Octadecenoic acid, methyl ester	13.97	13.329
4. 11-Octadecenoic acid, methyl ester	13.38	13.376
5. Methyl stearate	40.43	13.526
6. Docosanoic acid, methyl ester	3.85	18.451

contained 23 compounds with Hexadecanoic acid, methyl ester as the highest constituent (25.62%) followed by 9-Octadecenoic acid; methyl ester; 6-Octadecenoic acid (14.67%),squalene (13.13%) and methyl stearate (10.44%). Methyl stearate, hexadecanoic, Octadecenoic acid and decanoic acids are found in both *C. afer* and *P. hirsuta* methanol leaf extracts.

Discussion

To ensure purity, safety and efficacy of herbal drugs, quality control of raw material is paramount. Correct identification, authentication and establishing pharmacognostic

Table 12 Phytochemical Constituents of Methanol Extract of *Palisota hirsuta* leaf identified by GC–MS

Compounds names	Area %	Retention time (min)
1. Hexadecane	0.29	8.95
2. Methyl tetradecanoate	1.13	10.21
3. Hexadecanoic acid, methyl ester	25.62	12.01
4. 2-Piperidinone, N-[4-bromo-n-butyl]	0.67	12.19
5. 1-Hexacosene	1.06	12.33
6. 1-Docosene	0.71	12.46
7. tert-Hexadecanethiol	0.79	12.52
8. Carbonic acid, eicosyl vinyl ester	0.30	12.57
9. Ethanol, 2-(tetradecyloxy2-Cyclohexane	0.96	13.15
10. 1-Nonadecene	0.48	13.26
11. 9,12-Octadecadienoic acid; methyl ester; (E,E)	2.35	13.30
12. 9-Octadecenoic acid; methyl ester	14.67	13.35
13. 6-Octadecenoic acid (Z)-methyl ester	12.69	13.34
14. Methyl stearate	10.44	13.54
15. Oleic Acid	1.06	13.67
16. cis-Vaccenic acid	0.37	13.73
17. 17-Pentatriacontene	6.70	14.39
18. Methyl 18-methylnonadecanoate	1.41	15.45
19. Squalene	13.13	15.76
20. Docosanoic acid	2.97	18.50
21. 1-Octadecene	1.70	22.94
22. 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	0.05	24.93
23. Octadecane,1-(ethenyloxy)	0.46	25.01

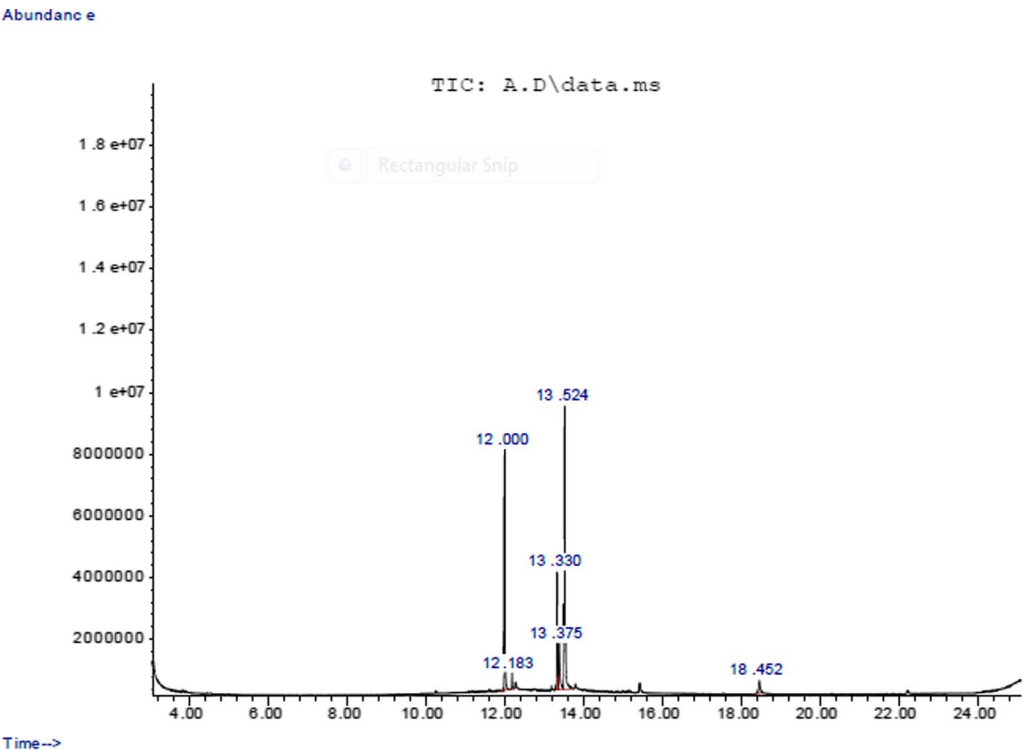


Fig. 7 GC–MS Spectra of Methanol extract of *Costus afer* leaf

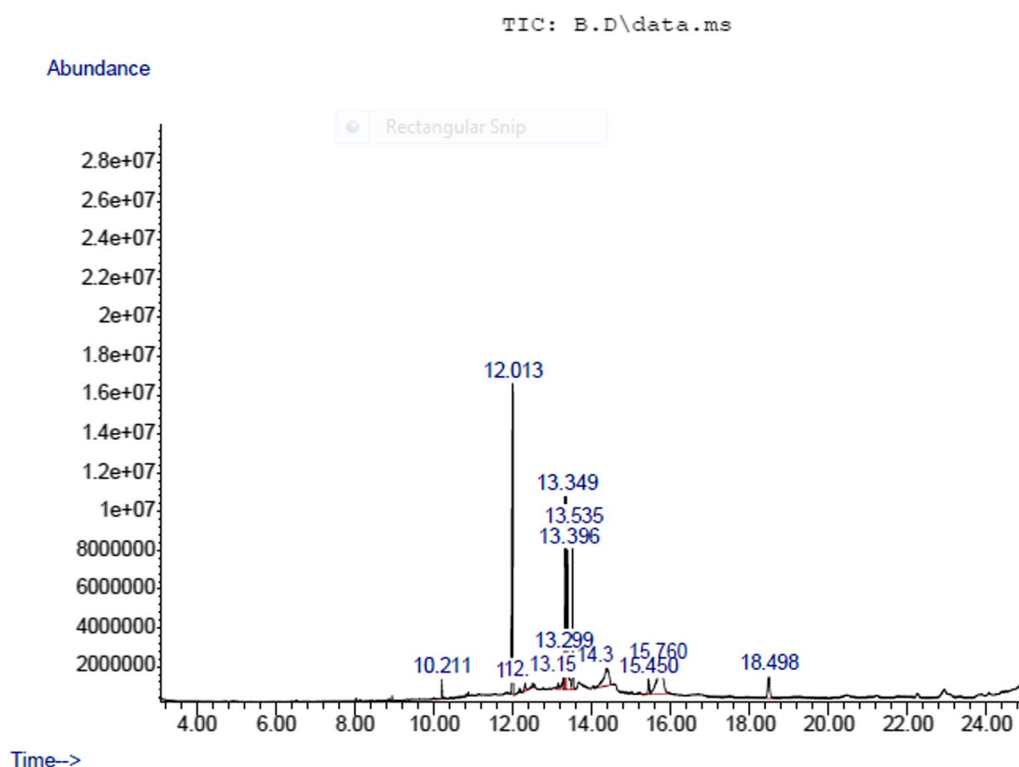


Fig. 8 GC-MS Spectra of Methanol extract of *Palisota hirsuta* leaf

standards are very significant factors for evaluation of medicinal plants. The first step towards ascertaining the identity and the degree of purity of herbal raw material according to World Health Organisation is macroscopic and microscopic evaluation and should be done before any tests are undertaken [39].

Recently, there has been rapid increase in the standardization of medicinal plants. Though modern techniques are now in existence, but still identification of medicinal plants is more reliable on pharmacognostic studies [40]. In this regard, the macroscopic and microscopic features of leaves of *Costus afer* and *Palisota hirsuta* have been studied. Macroscopic features of the leaves of the plants can serve as diagnostic characters for their correct identification. Microscopic study of the epidermal layers of the leaves revealed the absence of stomata and trichomes on the upper epidermis of *Costus afer* and presence of stomata and trichomes on the upper epidermis of *Palisota hirsuta*. Studies of physicochemical parameters can serve as an important source to judge the purity and quality of crude drugs. The ash value represents the earthy matter or inorganic components and other impurities present along with herbal drug. The phytochemical analysis revealed the presence of saponins, tannins, flavonoids, alkaloids in the leaves of the plant samples. The results

gotten from the antimicrobial susceptibility testing for *Costus afer* showed that it has both antibacterial and anti-fungi activity similar to that gotten by [41]. Also, according to [42] on the antimicrobial activity of the essential oils from the leaves of *Palisota hirsuta*, the essential oil was more active against Gram-positive bacteria and no activity at 25 mg/mL and 12.5 mg/mL against *S. typhi*, *A. niger*, and *R. stolonifer*. Also, the oil showed excellent activity at the highest concentration of 200 mg/mL against *C. albicans* when compared to the standard drug gentamycin. This can be related to the result gotten from this antimicrobial susceptibility test carried out on *Palisota hirsuta* leaf extract.

Quantitative phytochemical study showed an appreciable amount of phenols, flavonoids, terpenoids, saponins and alkaloids in both *C. afer* and *P. hirsuta*, though *C. afer* contained higher quantity of the secondary metabolites (phenols, flavonoids, terpenoids and alkaloids) than *P. hirsuta* with the exception of saponins which is higher in *P. hirsuta* methanol extract. The observed antimicrobial activity exhibited by both plant extracts could be attributed to the presence and synergism of the phytochemicals constituents they contain. The higher inhibitory effect on both bacterial and fungi observed in *C. afer* methanol extract can be attributed to the higher

percentage of phytochemicals present in it. Flavonoid, phenols and alkaloids are known to have antimicrobial, antifungal, antihelmintics, antidiarrheal and anti-inflammatory effect, and they also act as antihypertensive agent, antimalarial, antidepressant, anesthetic and amoebicide [43]. Polyphenols, such as tannins and flavonoids, have important antibacterial activity [44]. The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelope proteins [49].

Gas Chromatography-Mass analysis of *C. afer* and *P. hirsuta* revealed the presence of various groups of bioactive compounds. The chromatogram showed that *C. afer* methanol leaf extract contained 6 compounds and five of these six compounds belong to the class of compound fatty acid methyl esters (FAMES); they are Hexadecanoic acid, methyl ester (30.77%) class of compound fatty acid methyl esters, 9-Octadecenoic acid fatty acid methyl esters, methyl ester (13.97%), 11-Octadecenoic acid, methyl ester (13.38%), Methyl stearate (40.43%), Docosanoic acid (3.85%) while Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (1.86%) belong to the class of compound phenylpropane. Benzenepropanoic acid, 3, 5-bis(1,1-dimethylethyl)-4-hydroxy- methyl ester has been used as additive in motor cells, hydraulic fluids etc. It also possesses antioxidant and antifungal activities, while the GC/MS data and spectrum of the chemical constituents in methanol leaf extract of *P. hirsuta* are shown in Table 12 and Fig. 8, respectively. The chromatogram showed that the methanol leaf extract contained 23 compounds with Hexadecanoic acid, methyl ester as the highest constituent (25.62%) followed by 9-Octadecenoic acid; methyl ester; 6-Octadecenoic acid (14.67%), squalene (13.13%) and methyl stearate (10.44%). Methyl stearate, hexadecanoic, Octadecenoic acid and decanoic acids are found in both *C. afer* and *P. hirsuta* methanol leaf extracts.

All the identified compounds in both *C. afer* and *P. hirsuta* methanol leaf extracts have wide range of biological activities. Squalene has been reported to have antimicrobial, antitumor, supplements, antioxidants, anti-cancer, repellents and hypocholesterolemic activities [45], Hexadecanoic acid has been reported to have antimicrobial, antioxidant, antifungal, solvent, hypocholesterolemic, emollient, anti-inflammatory, pharmaceutical agent and 5-Alpha reductase inhibitor [46]. 9,12,15-Octadecadienoic acid has anti-inflammatory, anti-asthma, antimicrobial, antioxidant, anticancer, anti-arthritis, hepatoprotective and diuretic [47, 48]. Docosanoic acid is used as anthelmintic, hypoglycemic and antihypertoxic [49]. Hexadecanoic acid has antimicrobial, antioxidant, antifungal, solvent, hypocholesterolemic, emollient,

anti-inflammatory, pharmaceutical agent and 5-Alpha reductase inhibitor [46].

Conclusion

The present study on the leaves of *Costus afer* and *Palisota hirsuta* provide critical information on their macroscopic and microscopic characters, proximate, phytochemical, heavy metal properties antimicrobial and GC-MS analysis. The macroscopic and microscopic study revealed diagnostic features, which could be helpful in the discovery and correct identification of these plants. The prevailing compounds present in both plants can serve as chemotaxonomy marker which could be used in correct identification of the two plants, respectively. The data obtained will help in the preparation of the individual monograph for each of the plant which could be useful in the compilation of the revised edition of Nigerian Herbal Pharmacopoeia.

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Plant authentication

Diseased free leaves of *Palisota hirsuta* were collected from Isore farm, Ipara Remo, Ogun State, Nigeria in April 2021 and was identified and authenticated at the Forest Herbarium Ibadan (FHI) by Mr. Adeyemo, where the voucher specimen was deposited with voucher number FHI113303. Also, the healthy leaves of *Costus afer* were collected from Eleyele, Ibadan, Oyo State, Nigeria and was identified and authenticated at the University of Ibadan Herbarium by Mr D.P.O Esimekhua with the voucher number UIH-23090.

Author contributions

MAS originally conceived of the broad research idea and the design for the study. AOL took up the responsibility of literature search and initial stage of the manuscript preparation. She subsequently took responsibility for the statistical analysis. The three authors collectively collected the data for the study and carried out experimental studies. They all prepared conceptualisation of the key terms. OJA and MAS edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data and material are available upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare no conflicts of interest.

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