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Phytochemical Analysis *and In vitro* Antimicrobial Potential of *Colocasia esculenta* Tuber Peel Extract Against Pathogens Isolated from Water Yam *(Dioscorea alata)* Tubers

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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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ABSTRACT

The phytochemical analysis of the methanolic extract of *Colocasia esculenta* tuber peel and *In Vitro* antimicrobial sensitivity test was carried out against previously isolated and identified microorganisms from three water yam (*Dioscorea alata*) tuber varieties (*Kor, Banada* and *Azawele*) using standard methods. Phytochemical analysis of the extract revealed the presence of tannins, saponins, flavonoids, phenolics, alkaloids, steroids, and glycosides with varying contents. The

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highest phytochemical content was recorded of saponins (5.27 mg/100 g), followed by tannins (4.85 mg/100 g), alkaloids (3.46 mg/100 g), phenols (3.18 mg/100 g), flavanoids (2.50 mg/100 g), cardiac glycosides (2.16 mg/100 g), while steroids (2.01 mg/100 g) had the least content. The data obtained from the zone of inhibition (mm) was analyzed (descriptive statistics and inferential statistics to report the findings and to test hypothesis at 0.05 level of significance respectively) using statistical package for social science, SPSS Version 20. Results were reported as Mean ± SD. The statistical difference between more than 2 groups of data was evaluated using ANOVA with LSD post hoc test. Differences between means were considered significant at $p < 0.05$. The antimicrobial sensitivity test result against the fungi (*Aspergillus niger*, *Aspergillus flavus, Botryodioplodia theobromae, Penicillium marneffei,* and *Myrothecium verrucaria* as well as bacteria (*Klebsiella oxytoca, Serratia marcescens* and *Pseudomonas aeruginosa*) showed significant ($p < 0.05$) inhibitions in a dose-dependent manner when compared with standard commercial antifungal (Teraconazole) and antibacterial (Ciprofloxacin) as positive control agents at 100 mg/mL. The extract demonstrated the highest significant ($p < 0.05$) inhibition of 71.45 % and 69.10 % against *Aspergillus niger* and *Pseudomonas aeruginosa* respectively at 100 mg/mL. The lowest inhibition of 17.76 % and 17.40 % was obtained against *Myrothecium verrucaria* and *Klebsiella oxytoca* respectively at 25 mg/mL. *Colossian esculenta* tuber peel extract recorded MIC and Minimum Fungicidal Concentration (MFC) of 6.25 mg/mL against *Aspergillus niger, Aspergilus flavus, Botryodioplodia theobromae,* and *Penecillium maeneffei;* 3.13 mg/mL against *Myrothecium verrucaria, Klebsiella oxytoca, Serratia marcesces,* while *Pseudomona aeruginisa* recorded Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 3.13 mg/mL and 6.25 mg/mL respectively. The antimicrobial activities of the extract could be attributed to the high contents of saponins, tannins, phenols, and flavonoids. These research findings suggest that *Colocasia esculenta* tuber peel extract could be exploited in the control and prevention of postharvest water yam tuber rot and other tuber crops, which are the main staple foods in Nigeria and other tropical countries as it has the potentials to serve a natural antimicrobial agent. The research findings could also be of use to pharmaceutical companies in developing drugs that may help in combating drug resistance in pathogens.

Keywords: Phytochemicals; antimicrobial activity; microorganisms; In vitro inhibition.

1. INTRODUCTION

Water yam (*Dioscorea alata*) is a monocotyledonous plant that belongs to the genus *Dioscorea* and the family *Dioscoreaceae* [1,2]. It is an important food crop in West Africa and other tropical countries, including East Africa, Central Africa, the Caribbean, South America, South East Asia and India [2]. The crop is widely cultivated in Nigeria, Ghana, Ivory Coast, Togo, Gabon, Central African Republic and Western parts of the Democratic Republic of Congo [3,4]. Water yam producing states in Nigeria include: Benue, Taraba, Nasarawa and Adamawa. Others are Cross River, Oyo, Delta, Edo, Ekiti, Kaduna, Kwara, Imo, Ogun, Ondo, Osun and Plateau states [5,6].

The water yam tuber which is the thickened fleshy underground root serves as asexual reproductive organ or nutrient storage reserve for the dry months is one of the major sources of carbohydrates; minerals (phosphorus, calcium, iron, magnesium, potassium, sodium, zinc, copper and selenium); vitamins such as riboflavin, thiamine, niacin, pantothenic acid, and vitamins A, B6, C and E. It also contains large amount of water and fibre as well as small amounts of fats and proteins [5]. The water yam tuber is the most economic part of the crop and is consumed roasted, boiled, pounded or fried as well as peeled, dried and made into flour for baking and steaming for swallowing with soup. [4]. In addition, water yam has considerable social and cultural significance among the people of South-Eastern and North-Central Nigeria.

Water yam serves as a major source of food and raw materials for the production of other products like starch, ethanol, animal feeds, and other processed foods [4,5]. However, despite the nutritional, economic, and cultural values of water yam, it has been reported that over 25 % of the crop produced in the world is lost annually to diseases, pests, and nematodes especially in many African countries, including Nigeria, where yam storage is still largely by traditional methods, resulting to postharvest losses as high as 50 %

(FAO, 2023) .Nigeria lost an annual average of 10 % of her yam tubers between 1961 – 2009 [6]. The country recorded the highest yam lost in 2006 with over 3.7 million metric tons [7,8,9].

Fungi, bacteria, and nematodes are said to be the major causes of water yam tuber rot with fungi accounting for about 80 % of storage rot in West Indies and 57 – 77 % in Nigeria [10,11]. The wounding of yam tubers by rodents, nematodes, insects, and even man during weeding, harvesting and post-harvest handling makes it easy for fungi and bacteria to penetrate the tubers and cause diseases that could be transferred to the store leading to considerable quantitative loss in weight or volume and qualitative losses like reduced nutritional value, changes to taste, colour, texture or cosmetic features with the attendant adverse effects [12,13]. Substantial losses occur during prolonged storage of yam. Losses up to 10 – 20 % [12] may occur during the first 3 months and 30 – 60 % after 6 months of storage 14, 15].

Microbial rot of water yam tubers can be grouped into dry rot, soft rot, and wet or watery rot depending on the rot symptoms, invading pathogen, and the infected tissue. Dry rot is characterized by infected tissues becoming hard and dry with different colourations depending on invading pathogens [14,15]. *Fusarium species (Fusarium oxysporium, Fusarium monilforme*, and *Fusarium solani)*, *Penecillium* spp*,* and nematodes such as *Scutellonema bradys* are reported to be the causative agents of dry rot [16,17] Yam tubers showing symptoms of soft rot caused the infected tissue to become soft and sometimes ramified by the fungal mycelium that turns the tissue brown and in some cases wet with the tendency to break off due to a rapid collapse of the cell walls [17,18]. Fungi responsible for soft rot are *Armillariella mellea, Mucurcirci nelloides, Rhizoctonia solani* and *Rhizopus* spp [19]. In wet or watery rot, the external symptoms are not visible as the decay is internal and the infected tissue disintegrates into a watery mass or whitish fluid from the tissues which can easily be released on the application of slight pressure. This type of rot is characteristic of bacterial infection such as *Erwinia carotovora* [13,19].

Over the years, water yam tuber rots have been managed by conventional cultural control methods and use of synthetic chemicals like borax, captan, thiobendazole, benomyl and bleach (sodium hypochlorite) etc to inhibit the growth of pathogens during pre-harvest and postharvest stages [20,21,22]. Although, the widely use of synthetic chemicals for yam tuber rot control and prevention has been relatively effective because of the quick interventions and efficacies, there are limitations with some of the chemicals due to chemical residues in foods, toxicity, pesticide resistance in target and non-target organisms (partly due to frequent and indiscriminate applications), non-biodegradability, bioaccumulation, biomagnifications and biotransformation of the chemical residues along the food chain, thereby making them environmentally unfriendly. In addition, these chemicals are costly, not readily available, induce mutations, are often discriminated against locally and internationally, and the lack of skills in the applications of these chemicals has adversely affected the environment [23]. Other control measures involving gamma radiation [24,20,25], also have their own limitations as farmers in developing countries such as Nigeria hardly adopt these measures because of cost implications and inadequate technological knowhow among other factors.

To address these issues, there have been calls for eco-friendly natural products which have been found to possess broad spectrum antimicrobial activities against pathogens of pre-harvest and postharvest yam tubers. Biological control is generally favoured as a method of plant disease management because it does not have the disadvantages of chemicals. Bioactive substances that are found bacterial static, bactericidal and/or fungicidal *in vitro* in most cases kill the pathogens *in vivo* [21,26,27]. These plant extracts are rich in phytochemicals such as alkaloids, flavonoids, terpeniods, phenols, glycosides, tannins, phytates, saponins, steroids etc with proven antimicrobial actions [28, 29]. The extract of plants offer little or no resistance from microorganisms, inhibit partially or completely microorganisms and are environmentally friendly [30,31]. In addition, antibiotic resistance is a major concern and development of new antimicrobial agents from plants could be useful in meeting the demand for new and effective antimicrobial agents with improved safety, biocompatibility and eco-friendliness for the control and prevention of pre-harvest and postharvest tuber rot.

Cocoyam (Colocasia esculenta, L.) belongs to the Family, Araceae and is a perennial herbaceous plant that is commonly cultivated for its edible starchy roots (corms/tubers) and leaves in tropical and sub-tropical countries [32]. The leaves also serve as vegetable in most part of the world including Nigeria. Apart from the nutritional benefits of this plant, their leaves, corns/roots, tuber peels, and stem have been reported to possess medicinal properties against diverse human and animal ailments of microbial origins [33,34].

The preliminary phytochemical analysis of cocoyam leaves, stems, tubers and peels by many researchers revealed the presence of alkaloids, tannins, phenols, flavonoids, terpenoids, anthocyanins, steroids, saponins, glycosides and reducing sugars etc. in the extracts [35,25,36]. The crop is of great nutritional and economic importance to humankind. The leaves are used as vegetables and are a rich source of proteins, ascorbic acid, dietary fibre, minerals and vitamins such as calcium, phosphorus, iron, magnesium, potassium, vitamin C, thiamine, ribboflavin and niacin. The juice from the leaves is utilised to cure snakebite or scorpion stings [32,37,38]. According to Chakraborty [32], the corms have anthocyanins, cyanidin, glucosides, pelargradin, 3-glycoside, and 3-rhamnoside, while the tubers are high in starch [39,33,37]. According to Wang [40]; Tijani [41], and Azubike [29], the related anthocynin with flavonoids improve blood circulation by reducing capillary fragility, improves vision, and functions as a strong antioxidant, anti-inflammatory, and anti-cancer agent. The corms also contain calcium oxalate, an irritant, which prevent them from being eaten raw or incompletely cooked [39,33,42,43]. According to reports from Pritha et al. [12] and Nakade et al. [1], *C. esculenta* tuber is used in ethnomedicine to treat wounds, ringworm, cough, sore throats, and diabetes mellitus. It also reportedly contains antihelminthic and anticancer qualities. The presence of these photochemical confers antibacterial, antiviral, antifungal and antioxidant properties on cocoyam [44,45,46].

Considering the profound pharmaceutical and pharmacological potentials exhibited by *C. esculenta*, it is imperative to investigate the antimicrobial potentials of its tuber peels against pathogens associated with postharvest water yam tuber rot with a view to ensure food security, increase the county's export earnings, and improve the economic status of peasant farmers in Benue, Nigeria. Consequently, the tuber peels of *Colocasia esculenta was* investigated for its phytochemical qualitative and quantitative as well as antimicrobial efficacy against pathogens with postharvest water yam tuber rot with a view to providing additional information on potential natural phytochemicals needed for the management of these pathogens.

2. MATERIALS AND METHODS

2.1 Plant Material and Authentication

2.1.1 Collection of plant material

Colocasia esculenta tubers were purchased from Railway market, Makurdi, Benue State, properly labelled, packed in clean cellophane bags and transported to the Department of Botany, Benue State University, Makurdi.

2.1.2 Authentication

The plant material was taken to the Department of Botany, Benue State University Makurdi for authentication by a plant taxonomist before processing and analysis.

2.2 Preparation of Plant Extract

2.2.1 Drying and pulverization of tuber peels

The *C. esculenta* tubers were thoroughly washed with sterile water, peeled and dried in the shade for two weeks to avoid chemical decomposition. Upon drying, the peels were made into fine powder using a wooden mortar and pestle.

2.2.2 Plant extraction procedure

The method described by Dooshima et al. [16], Tiwari [47], and Srivastava [48] were employed with little modification. The sample (500 g) was packed into the thimble and placed inside the extractor. 500 mL methanol was put in the round bottom flask of the extractor and heated on a heating mantle for 8 hours. After extraction, the methanol was recovered and the extract evaporated in a beaker to a constant weight over an evaporation bath for 24 hours. The sample was then weighed and the yield calculated in percentage.

2.2.3 Storage of extract

The extract was kept in the refrigerator for further analysis.

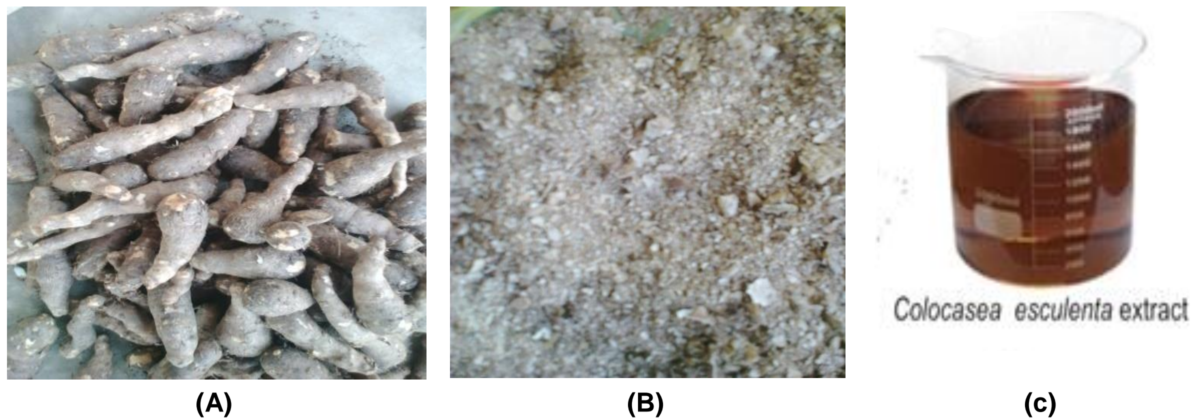

Fig. 1. *Colocasia esculenta (*A) tuber (B) tuber peels (powder), and (C) methanolic tuber peel extract

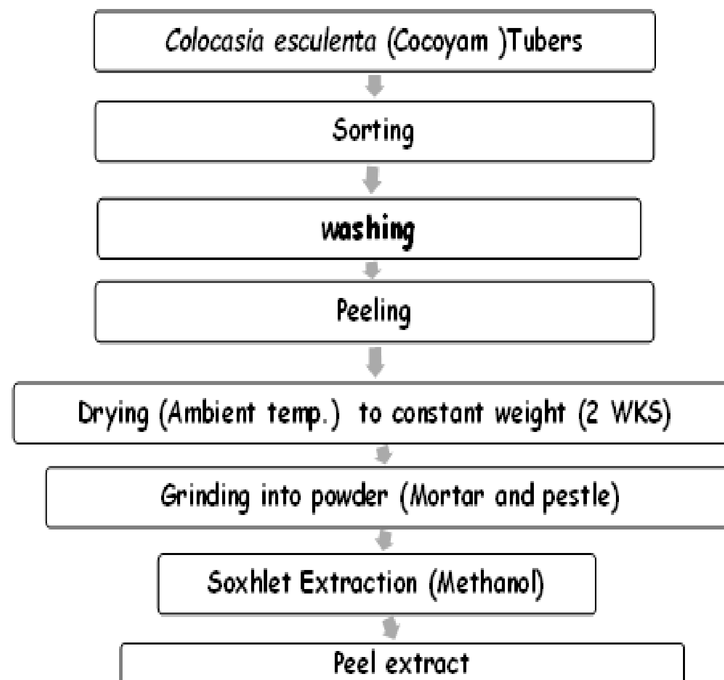

Fig. 2. Flow chart of the preparation of the cocoyam tuber peel sample

2.3 Microorganisms Handling

2.3.1 Source of microorganisms

Previously isolated and identified water yam rot pathogens comprising of five fungi: *Aspergillus niger, Aspergillus flavus, Botryodioplodia theobrome, Penecillium marneffei,* and *Myrothecium verrucaria* and three bacteria: *Klebsiella oxytoca, Serratia marcescens* and *Pseudomonas aeruginosa* of 2023 harvest year were obtained from the Laboratory, Department of Biological Sciences, Benue State University, Makurdi where they were preserved and used for the antimicrobial study.

2.3.2 Culture media preparation

The methods of [49,50,51] were used with slight modifications. The cultured microorganisms were rehydrated in sterile distilled water and inoculated onto Potato Dextrose Agar (PDA) and Nutrient Agar (NA) for fungi and bacteria respectively. Following incubation at 37 °C for 24 hours, the cultures were sub-cultured on fresh PDA and NA for fungi and bacteria respectively. Stocks for long term storage were also prepared in 20 % glycerol and kept at – 70 °C.

Throughout the experiment period, the cultures were maintained at 4 °C and sub-culturing was

done regularly to maintain fresh cultures for the experiment. Before use, the purity of each culture was also confirmed by using culture identification guides [50,51,52]. The grown colonies were then harvested and dispersed in PDA (fungi) and NA (bacteria), and the turbidity of the suspension was adjusted to an optical density of (OD_{550} nm) 0.144, which is equivalent to 1.0 x 106 cells/mL [53].

All chemicals and reagents were purchased from Agbe Sciences, Makurdi, Benue State, Nigeria. All reagents used were analytical grade and used as received without further purification. All solutions were freshly prepared using double-distilled water and kept in the dark to avoid photochemical reactions. All glassware used in the experimental procedures were sterilized in 10 % sodium hypochlorite solution, rinsed thoroughly in double-distilled water and dried before use. Aseptic condition was maintained throughout the experiments.

2.4 Phytochemical Screening

The methods described by Tiwari [47], Srivastava [48], Hortwitz [51], and James [52] were used for phytochemical analysis without modification

i. Test for tannins

Ferric Chloride Test and Spectrophotometric analysis: 4 mL of the extract was treated with 4 mL of $FeCl_3$ in a test tube. Formation of a bluish green precipitates indicated the presence of tannins. The absorbance of the test solution was measured at 700 nm using Spectrum Lab23A spectrophotometer. Standard tannic acid solution was prepared along the test solution and the absorbance obtained spectrophotometrically was used to prepare a standard curve for analysis of tannins in the test solution. Tannin contents were determined from the standard curves.

ii. Test for Saponins

Froth Test and gravimetric method: 5 mL of the extract was diluted to 20 mL with distilled water and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm^3 layer of foam indicated the presence of saponins. Thereafter, the mixture was added to 100 cm^3 of 20 % aqueous ethanol and heated with constant stirring over a water bath (55 ^oC) for about 4 hrs. After filtering the content, the aqueous ethanol extraction was heated for 4 hrs at 55 ^oC with continuous stirring. The cooled extracts were then evaporated using water bath (90 ^oC) to about 40 cm^3. The partially concentrated extract was placed in a separating funnel before adding 20 cm^3 of diethyl ether, mixed properly, and allowed to settle into layers. The aqueous layer was recovered while the ether layer was discarded before further purification using 60 cm^3 of n-butanol and 10 cm^3 of 5% sodium chloride. The sodium chloride layer was later discarded before concentrating the residues over water bath for 30 min to dryness using oven (Jenway) before determining the saponins content. The saponin content was calculated as follows:

Percentage saponin = $\frac{W2-W1}{W3}$ X 100 %

iii. Test for flavonoids

Lead Acetate Test and Colorimetric method: 1 mL of 5 % lead acetate solution was added to 1 mL of the extract solution in a test tube and the mixture was allowed to stand for five minutes. The formation of precipitate in the mixture confirmed the presence of flavonoids. The total flavonoid content was determined using the aluminium chloride colorimetric method. This method is based on the formation of a complex flavonoid–aluminium, having the absorbance maximum at 430 nm. 0.5 mL of each plant extract was mixed with 1.5 mL of methanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm on UV–visible spectrophotometer, Shimadzu UVPC-1650 (Japan). Total flavonoid contents of extract samples were expressed as mg/100 g dry weight of extract through the calibration curve with routine as standard.

iv. Test for Phenols

Ferric Chloride Test: and **Folin-ciocattean Spectrophotometer Method:** 3 drops of ferric chloride solution was added to 1 mL of the extract in a test tube. The appearance of bluish-black colour indicated the presence of phenols. Thereafter, standard gallic acid solutions of 0.5 mol/dm^3, 1 mol/dm^3, 1.5 mol/dm^3, 2.0 mol/dm^3 and 2.4 mol/dm^3 were prepared. 1 mL of the extract was treated with 1mL Folin-Citocacteau reagent, followed by the addition of 2 mL 2 % Na_2CO_3 solution. 1 mL of the standard solution was also treated with the Folin-Denis reagent

and Na_2CO_3 solution. The intensity of the resulting blue colouration was measured (absorbance) with reagent blanks at zero in a spectrophotometer.

The phenol content will be calculated as follows:

Percentage of phenol = $\frac{W2-W1}{W3}$ X 100 %

v. Test for alkaloids

**Hager`s Test and gravimetric method:** 5 mg of the extract was dissolved in 3 mL of dilute Hydrochloric acid and filtered. 2 mL of the filtrate was treated with Hager`s reagent (saturated picric acid solution) in a test tube. The formation of yellow precipitates confirmed the presence of alkaloids.

After that, the extract was treated with few drops wise addition of concentrated ammonium solution to precipitate the alkaloids. The alkaloids precipitate was removed by filtration using No. 42 filter paper, after washing with 1 % NH_4OH solution. The precipitates in the filter paper were dried at 60 ^oC and cooled in a desiccator for 3 hours.

The alkaloid content was calculated as follows:

Percentage alkaloid = $\frac{W2-W1}{W3}$ X 100 %

vi. Test for steroids

Libermann Burchard`s Test and Specrophotometric method: 2 mL of the extract was treated with 2 mL of acetic anhydride and a drop of acetic acid, heated for 5 minutes and cooled in ice followed by addition of 1 mL of concentrated tetraoxosulphate (vi) acid carefully by the sides of the test tube. An array of colours changes from violet to blue or green indicated the presence of steroids. Thereafter, equal volume of the filtrate (2 cm^3) was added to cholesterol colour reagent before taking the absorbance at 559 nm using Spectrum Lab23A spectrophotometer. The steroid content was then estimated from the standard curve.

Vii. Test for quinones

Hydrochloric Acid Test: 1 mL of the extract was treated with 3 drops of concentrated hydrochloric acid. A green colour indicated the presence of quinones.

viii. Test for glycosides

Keller- Killani Test and Titrimetirc method: 5 mL of the extract was treated with 2 mL glacial acetic acid, followed by a drop of $FeCl_3$ solution and then 1 mL of concentrated tetraoxosulphate (vi) acid. Violet green rings appearing below the brown ring in the acetic acid layer indicated a positive test for glycosides. Thereafter, 1.0 cm^3 of the sample was weighed into 200 cm^3 distilled water, allowed to autolyse for 2 hrs before complete distillation in flask containing 2.5 % sodium hydroxide and tannic acid as an antifoaming agent. The distillates were mixed with 100 cm^3 of cyanogenic glycosides, 8 cm^3 of ammonium hydroxide and 2 cm^3 of potassium iodide, before titrating the content with 0.02 M silver nitrate against a dark background to a constant turbid end point. The cyanogenic contents of the samples were then calculated.

2.5 Antimicrobial Sensitivity Test

The method as described by Dooshhima et al [16] and Espinel-Ingogtoff et al [53] were employed with slight modification. The *Colocasia esculenta* tuber peel extract was tested against five previously isolated and identified white yam pathogenic fungi: *Aspergillus niger, Aspergillus flavus, Botryodioplodia theobromae, Penecillium maeneffei,* and *Myrothecium verrucaria* as well as three bacteria: *Klebsiella oxytoca, Serratia marcenscens,* and *Pseudomonas aeruginosa*. The pure isolates were individually cultured on *Colocasa esculenta* tuber peel extract-incorporated Potato Dextrose Agar (PDA) and Nutrient Agar (NA) plates for fungi and bacteria respectively and incubated at 37 ^oC for 7 days (fungi) and 24 hours (bacteria). Triplicates samples were prepared. The controls consisted of 1 mL 100 % Teraconazole (200 mg) and 100 % of 1 mL Ciprofloxacin (500 mg) tablets for fungi and bacteria respectively. Zone of inhibition (mm) where present was recorded with a transparent plastic ruler after the incubation period and the percentage inhibition zones calculated as follows:

% Inhibition Zone (% IZ) = Average diameter of pathogen colony / Average diameter of pathogen in control x 100% [5].

The percentage inhibition was rated on the scale described by Pritha et al [40] as follows:

100 % inhibition (highly effective); 50 – 99 % inhibition (effective); 20 – 49 % inhibition (moderately effective); 0 – 19 % inhibition

(slightly effective) and ≤ 0 % inhibition (not effective).

2.6 Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the *Colossian esculenta* tuber peel extract against the pure isolates was determined using the micro-dilution method as described by Espinel-Ingroff., et al, [53]. Different concentrations of the extract were prepared with the final concentrations of 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.31 mg/mL. The positive controls were performed using Teraconazole (fungi) and Ciprofloxacin (bacteria) tablets. Triplicate wells were also prepared for each concentration of the antimicrobial agents. Isolates suspension of 10^6 cells/mL each were pipetted into each well and incubated overnight at 37°C. After this period, the turbidity of the wells were observed and recorded. The lowest concentration of the antimicrobial agents that inhibited the visible growth of the isolates was recorded as the Minimum Inhibition Concentration (MIC) value.

2.7 Determination of the Minimum Fungicidal Concentration (MFC) and Minimum Bactericidal Concentration (MBC)

The MFC and MBC of the *Colocasia esulenta* tuber peel extract were determined according to a standard procedure as described by Espinel-Ingroff et al [53] without any modification. After the overnight incubation of the isolates for MIC, 50 µL from each well which indicated no growth of all the isolates were sub-cultured onto fresh potato dextrose agar (fungi) and nutrient agar (bacteria) plates. The plates were incubated at 37 °C for 7 days (fungi) and 24 hours (bacteria) until no visible growth was observed. The MFC and MBC values were the concentrations where no growth or fewer than three colonies were obtained to give approximately 99 % to 99.5 % killing activity [54].

2.8 Statistical Analysis

The data obtained from the zone of inhibition (mm) was analyzed (descriptive statistics and inferential statistics to report the findings and to test hypothesis at 0.05 level of significance respectively) using statistical package for social science, SPSS Version 20. Results were reported as Mean ± SD. The statistical difference between more than 2 groups of data was evaluated using ANOVA with LSD post hoc test. Differences between means were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Table 1 presents the result of the phytochemical analysis of *C. esculenta* tuber peel extract. The result indicated the presence of tannins, saponins, flavonoids, phenols, alkaloids, steroids, and glycosides. Phytochemicals occur naturally in plants and form part of the plant`s defense mechanisms against pests, pathogens or diseases [55]. Plant extracts containing bioactive agents with antimicrobial properties have been found useful in treating bacterial and fungal infections [56,57,58]. The antimicrobial properties of phytochemicals in plant extracts have been linked to their quality and quantity [59,60]. Plant extracts have been exploited over the years for their nutriceutical, medicinal, and pharmacological potentials [61]. The presence of these phytochemicals in *Colossian esculent*a tuber peel extract is a confirmation of its antimicrobial potentials [62,63,64].

Table1. Phytochemical Analysis of *Colocasia esculenta* tuber peel extract

Secondary Metabolite	Test	Result	Qty(mg/100 g)
Tannins	$FeCl_3$ Test	+	4.85
Saponins	Froth test	+	5.27
Flavoniods	Lead Acetate Test	+	2.50
Phenols	Ferric Chloride Test	+	3.18
Alkaloid	Hager’s Test	+	3.46
Steroids	Libbermann Burchard’s Test	+	2.01
Quninones	Hydrochloric Acid Test	-	
Glycosides	Keller-Kallani’stest	+	2.16

Key: + = positive; - = negative

Table 2. Average zone of inhibition (cm) of the *Colocasia esculenta* tuber peel extract against the test microorganisms

	Control	100	75	50	25
Aspergillus niger	8.18± 0.01	5.84 ± 0.01^{a}	4.98± 0.69$^{a\ b}$	3.88 ± 0.01^{abc}	2.98 ± 0.01^{abcd}
Asperigllus flavus	9.71± 0.04	5.74 ± 0.05^{a}	4.85± 0.02^{ab}	3.34± 0.11^{abc}	2.68 ± 0.01^{abcd}
Botrydiplodia theobromae	10.99 ± 0.01	5.57 ± 0.01^{a}	5.07 ± 0.01^{ab}	4.46 ± 0.10^{abc}	3.18 ± 0.01^{abcd}
Penicillium marneffei	18.08 ± 0.08	9.05 ± 0.01^{a}	7.33 ± 0.01^{ab}	5.98 ± 0.01^{abc}	5.16 ± 0.01^{ab}
Myrothecium verrucaria	29.67 ± 0.01	16.04 ± 0.01^{a}	8.55 ± 0.01^{ab}	7.04 ± 0.03^{abc}	5.27 ± 0.01^{sbcd}
Klebsiella oxytoca	37.25 ± 2.20	16.17 ± 0.01^{a}	16.17 ± 0.01^{ab}	14.05 ± 0.01^{abc}	6.05 ± 0.01^{abcd}
Serratia marcescens	40.32 ± 0.04	19.74 ± 0.05^{a}	19.74 ± 0.05^{ab}	15.88 ± 3.04^{abc}	8.88 ± 0.05^{abcd}
Pseudomonas aeruginosa	38.07± 0.54	23.05 ± 0.01^{a}	23.05 ± 0.01^{ab}	15.18 ± 0.08^{abc}	9.03 ± 0.01^{abcd}

N = 5, values expressed as Mean ± SD. a = significant relative to 100 % at p <0.05, b = significant compared with 75 % at p <0.05, c = significant compared with 50 % at p <0.05, d = significant, compared with 25 % at p <0.05.

Table 3. Percentage inhibition of *C. esculenta* tuber peel extract at different concentrations (mg/mL)

	100	75	50	25
Fungi				
Aspergillus niger	71.45^{b}	61.03^{b}	47.55^{c}	36.40^{c}
Aspergillus flavus	58.95^{b}	50.00^{b}	34.13^{c}	27.61^{c}
Botrodiophodia theoromae	50.73^{b}	46.00^{c}	40.55^{c}	28.91^{c}
Penecillium marneffei	50.28^{b}	40.67^{c}	33.17^{c}	28.72^{c}
Myrothecium verrucaria	54.03^{b}	28.78^{c}	23.76^{c}	17.76^{d}
Bacteria				
Klebsiella oxytoca	54.84^{b}	46.45^{b}	40.34^{c}	17.40^{d}
Serratia marcescens	52.14^{b}	48.85^{b}	26.46^{c}	21.89^{c}
Pseudomonas aeruginosa	69.10^{b}	61.19^{b}	40.08^{b}	23.94^{c}

Key: a = 100 % inhibition (highly effective); b = 50 – 99 % inhibition (effective); c = 20 – 49 % inhibition (moderately effective); d = 0 -19 % inhibition (slightly effective); e = ≤ 0 % inhibition (not effective) [65]

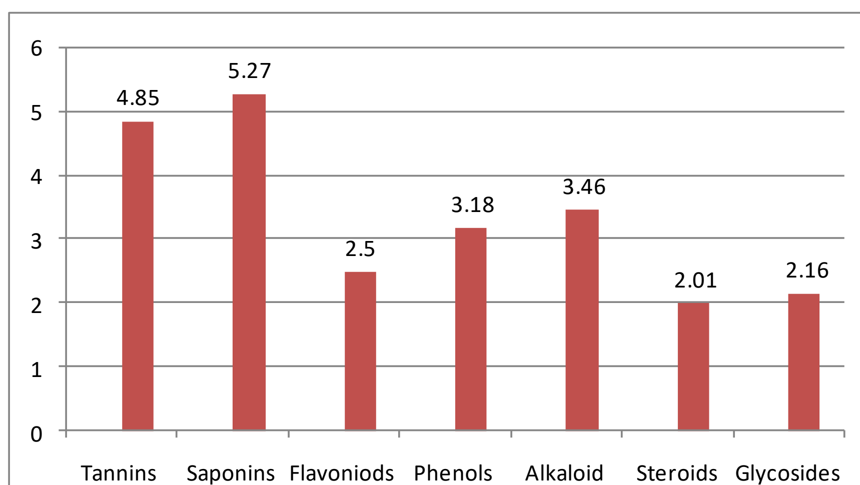

Fig. 3. Quantitative phytochemical contents of *Colocasia esculenta* tuber peel extract (mg/100 g)

Fig. 4. Antimicrobial sensitivity test plates

The antimicrobial study of the *Colocasia esculenta* tuber peel extract was carried out against five previously isolated and identified water yam pathogenic fungi: *Aspergillus niger, Aspergillus flavus, Botryodioplodia theobromae, Penicillium marneffei,* and *Myrothecium verrucaria* as well as three bacteria (*Klebsiella oxytoca, Serratia marcescens* and *Pseudomonas aeruginosa*). Generally, the results showed that the inhibitory effects of the extract increased with increasing concentration ($p < 0.05$). The result revealed significant inhibition of the extract against the test microorganisms at different concentrations, relative to that of the control (Table 2).

Table 3 showed that *Colossian esculenta* tuber peel extract inhibited effectively (71.45 %) and (61.03 %) *Aspergillus niger* at 100 mg/mL and 75mg/mL respectively, but moderately effective inhibited (47.55 %) and (36.40 %) at 50 mg/mL and 25 mg/mL respectively *Aspergillus flavus* was inhibited effectively (58.95 %) and (50.0 %) at 100 mg/mL and 75 mg/mL respectively, but moderately inhibited (34.13 %) and (27.6 %) at 50 mg/mL and 25 mg/mL respectively. *Botryodioplodia theobromae* was effectively inhibited (50.73 %) at 100 mg/mL, but moderately inhibited (46.00 %), (40.55 %) and (27.61 %) at 50 mg/mL and 25 mg/mL respectively, while at 25 mg/mL, it showed (27.61 %). The result revealed that *Penecillium marneffei* was effectively inhibited (50.28 %)*, at* 100 mg/mL; moderately inhibited (40.67 %), (33.7 %), and (28.91 %) at 75 mg/mL, 50 mg/mL and 25 mg/mL accordingly. The extract inhibited effectively *Myrothecium verrucaria* (54.03 %) at 100 mg/mL and moderately (28.78 %) and (23.76 %) at 75 mg/mL and 25 mg/mL in that order, but slightly effective (17.76 %) the microorganism at 25 mg/mL. *Klebsiella oxytoca* showed effective inhibition (54.84 %) at 100 mg/mL; moderate inhibition (46.45 %) and (40.34 %) at 75 mg/mL and 50 mg/mL respectively and slightly effective inhibition (17.40 %) at 25 mg/mL. *Serratia marcenscens* recorded effective inhibition (54.84 %) at 100 mg/mL; moderately effective inhibition (48.85 %), (26.46 %), and (2.89 %) at 75 mg/mL,

50 mg/mL, and 25 mg/mL respectively. The result also indicated that *Pseudomonas aeruginosa* was effectively inhibited (69.10 %) and (61.19 %) at 100 mg/mL, and 75 mg/mL respectively, while showing moderate inhibition (40.08 %) and (23.94 %) at 50 mg/mL and 25mg/mL accordingly.

Previous researches linked the antimicrobial activities of plant extracts to the quality and quantity of the available phytochemicals in the plant material [62,63]. Flavonoids, which are phenolic structure containing one or more carbonyl groups form complexes with extra cellular and soluble protein of bacteria cell wall, thus exhibiting antibacterial activities through these complexes [64,66]. The presence of phenolic compounds in plants has been reported to confer considerable antimicrobial properties, which is attributed to their redox potential [57]. Flavonoids are reported to be potent water soluble antioxidants with anti-inflammatory and antimicrobial properties [39]. Both alkaloids and flavonoids are documented to have antifungal properties [43,55]. The detection of flavonoids in the cocoyam peel extract could confer pharmacological properties.

Tannins bond to proteins, carbohydrates, gelatins, etc to form irreversible complexes, resulting in the inhibition of cell protein synthesis [46,49]. Tannins found in plant cells are potent inhibitors of hydrolytic enzymes, forming complexes, which interfere with growth and metabolism of microorganism in a negative manner [46]. They are able to inhibit the growth of insects and disrupt the digestive activities in ruminant animals [45,55]. The mode of antimicrobial activities of tannins include their ability to inactivate microbial adhesions, form irreversible complexes with proline-rich proteins, resulting in the inhibition of the cell protein synthesis, enzymes and cell transport disruptions, cell paralysis, and eventually, death [43,56]. Tannins-rich plants are used in the treatment of diseases like rhinnohorea and leucorrhoea and diarrhoea as well as the healing of burns and wounds [57,66]. The confirmation tannins the extract could be a potential antimicrobial agent.

Alkaloids being the largest group of secondary metabolites are the most efficient therapeutic plant metabolites, comprising basically of nitrogen bases synthesized from amino acids building blocks [29]. Pure isolated alkaloids and their synthetic derivatives are used as medicinal agents for their antibacterial, antispasmodic, hypoglycaemic and analgesic properties [42,63,64]. The presence of alkaloids in the peels could be of therapeutic and antimicrobial importance.

Steroids were found to be present in the peel extract. Steroidal compounds are of importance to the pharmaceutical industry due to their relationship with such compounds as sex hormones [31,35]. The confirmation of steroids in the extract could be of importance to the pharmaceutical industry.

Saponins are high molecular weight compounds in which sugar molecules are combined with triterpene or steroid alycone. The two major saponins, steroidal and triterpene are amorphous in nature, soluble in water and alcohol, but insoluble in non-polar solvents like benzene and n-hexane [31]. Although saponins could cause heamolysis of blood at higher concentrations, they have therapeutic potentials such as cholesterol lowering, anti-cancerous, anti-fungal and anti-bacterial activities [30,32]. The presence of saponins in the cocoyam peel extract implies that it could have considerable antimicrobial activities.

Table 4. MIC, MFC, and MBC of *Colossian esculenta* tuber peel extract

Test Organisms	Concentration (mg/mL)						
	25.0	12.5	6.25	3.13	MIC	MFC	MBC
Fungi							
Aspergilus niger	**-**	**-**	**-**	**+**	**6.25**	**6.25**	
Aspergillus flavus	-	-	-	++	6.25	6.25	
Botryodioplodia theobromae	-	-	-	+	6.25	6.25	
Penecillium marnefei	-	-	-	+	6.25	6.25	
Myrothecium verrucaria	-	-	-	-	3.13	3.13	
Bacteria							
Klebsiella oxytoca	-	-	-	-	3.13		3.13
Serratia marcescens	-	-	-	-	3.13		3.13
Pseudomonas aeruginosa	-	-	-	+	6.25		6.25

Table 4 showed that *Colossian esculenta* tuber peel extract recorded MIC and MFC of 6.25 mg/mL against *Aspergillus niger, Aspergilus flavus, Botryodioplodia theobromae,* and *Penecillium maeneffei;* 3.13 mg/mL for *Myrothecium verrucaria. Klebsiella oxytoca, Serratia marcescens,* while *Pseudomonas aeruginінosa* recorded MIC and MBC of 3.13 mg/mL and 6.25 mg/mL respectively. The low MIC, MFB and MBC could be linked to the presence and amount of the phytochemicals, especially flavonoids, phenols, saponins, and tannins which have proven antioxidant and antimicrobial activities [34,45,47].

4. CONCLUSION

The phytochemical analysis of the methanolic extract of *Colocasia esculenta* tuber peels revealed the presence of saponins, tannins, phenols, flavonoids, alkaloids, glycosides, and steroids with varying concentrations. Antimicrobial study of the extract against previously isolated and identified pathogens associated with postharvest water yam tuber rot compared favourably with standard commercial antimicrobial agents (Teraconazole and Ciprofloxacin). The result showed low MIC, MFC, and MBC against the test microorganisms which could be attributed to the quality and quantity of the phytochemicals in the extract. Based on the research findings, *Colocasia esculenta* tuber peel extract holds great potential in controlling and/or preventing postharvest water yam tuber rot and can provide an alternative to synthetic antimicrobial agents since it is less expensive, environmentally friendly, biocompatible and easy to prepare.

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 writing or editing of manuscripts.

Competing interests

Authors have declared that no competing interests exist.

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