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Chemical Composition and Antibacterial Activity of Extracts of Cymbopogon Citratus (Lemon Grass) and Phyllantus Amarus (Stone Breaker) Leaves

Akintoyese O Alabi[1], Beatrice O T Ifesan[1], Ngozi I Akosu[1] and Opeyemi l Alabi[2]

[1]Department of Food science and Technology, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Ondo State, Nigeria

[2]Department of Hospitality Management, School of Applied Science, Federal Polytechnic Auchi, Edo State

ABSTRACT

This study described the chemical composition, antioxidant and antibacterial activities of the ethanol and aqueous extracts of fresh and dried leaves of *Phyllantus amarus* (PA) (Stone breaker), *Cymbopogon citratus* (CC) (Lemon grass) and combination of stone breaker and lemon grass (PA/CC and DL/DP) using standard methods. The proximate and mineral results showed that both fresh and dried samples of *Phyllantus amarus* and *Cymbopogon citratus* were rich in crude fibre (18.53%, 14.65%), ash (16.27%, 2.29%), potassium (95.56 mg/100g, 54.04 mg/100g), calcium (58.57 mg/100g, 7.05 mg/100g), and sodium (15.23 mg/100g, 9.84 mg/100g) respectively. The ability of the extracts to scavenge 2, 2 diphenyl -2- picryl-hydrazyl (DPPH) radical showed that the fresh PA had the highest scavenging activity of 91.12 mg/g, followed (PA/CC) 90.93 mg/g and dried PA (85.61mg/g). The phytochemical composition of the extracts also revealed that they contain high content of cardiac glycoside (11.62 mg/g - 64.81 mg/g), terpenoid (14.56 mg/g - 69.66 mg/g), phenol (14.73 mg/g - 53.93 mg/g), saponin (22.61 mg/g - 30.47mg/g). Dried lemon and dried *Phyllantus amarus* (DL/DP) was found to be rich in Vitamin A (22.22 mg/g), while the lowest value of Vitamin E (0.36 mg/g) was obtained from fresh lemon grass (0.36mg/g). Antibacterial assay carried out on the two leaves extracts showed that they produced mild antibacterial activity against some foodborne pathogen (*Bacillus cereus, Shigella dysenteriae, Escherichia coli, Salmonella spp, Staphylococcus aureus, Pseudomonas spp*). with zones of inhibition of 4.3 mm -9.1mm. Further studies should be carried out to investigate the employment of the leaves in production of functional food.

***Corresponding author**

Beatrice O T Ifesan, Department of Food Science and Technology, The Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria, Tel +2348134986995; E-mail: bodewunmi2001@yahoo.co.uk

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Introduction

Several health benefits recorded in medicinal plants have increased the interest of researchers to search for alternatives to combat various chronic diseases worldwide. Some human pathogens rapidly become resistant to many of the first discovered effective antibiotics [1]. Furthermore, concerns over the safety of some chemical preservatives and negative consumers' reaction to preservatives they perceive as chemical and artificial, have prompted an increased interest in natural additives.

Phyllanthus amarus, commonly called "Jamgli amli" in Hindi; "ehin olobe" in Yoruba culture; and "ngwu" among the Igbo tribe, is a plant of the family of Euphorbiaceae with approximately 800 species spread over the Australian, American, African and Asian continent [2]. Various chronic diseases such as cancer, hepatitis and diabetes mellitus have been well treated with *P. amarus* extract in traditional medicine systems in China. *Phyllantus amarus* has been reported to possess hepatoprotective, antiviral, antimicrobial, antimutagenic and tumor suppressive properties [2].

Lemon grass is a tall plant having enormous striped leaves with an uneven edge. It is known for its smoky, sweet, herbaceous and lemony fragrance. It is broadly utilized in preparation of soups curries and teas. This herb contains calming character and widely consumed as an aromatic herb in Latin and African countries. In addition, its aerial components are widely utilized in folk medicine for the treatment of digestive disorders, diabetes, nervous disorder, inflammation and fever [3]. Several reports are available on the antioxidant and antimicrobial properties of plant extracts, herbs and spices [4-6].

Materials and Method

Sample collection

Freshly harvested leaves of *Phyllatus amarus* and *Cymbopogan citratus* were obtained from the Teaching and Research Farm of Federal University of Technology Akure, Ondo State, Nigeria. It was identified at the Department of Crop, Soil and Pest, Federal University of Technology Akure, Ondo State.

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade and water was glass distilled.

Extract preparation

The *P. amarus* and *C.citratus* leaves were air-dried for 30 days and were separately blended to powder using the laboratory blender. Method of extraction: About 20 g of powdered *Phyllantus amarus* and *Cymbopogon citratus* leaves were kept in 200 ml conical flask and 100 ml of solvent water or ethanol were individually added. The mouth of the conical flask were covered with aluminum foil and kept in a shaker for 12 h for continuous agitation for thorough mixing of powder into solvent. Then the extract was filtered by using muslin cloth followed by Whatman no 1 filter paper, the solvents from the extract were removed by using rotary vacuum evaporator at 50 OC and stored at 4 OC for further analyses [7].

Phytochemical analyses of *Phyllatus amarus* and *Cymbopogan citratus*.

Determination of tannin

About 0.2 g of finely ground sample was weighed into a 50 ml sample bottle. Ten ml litre of 70% aqueous acetone was added and properly covered. The bottle was placed in an ice bath shaker and shaken for 2 h at 30 °C .Each solution was then centrifuged and the supernatant stored in ice. About 0.2 ml of each solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannic acid solutions were prepared from a 0.5 mg/ml of the stock and the solution was made up to 1 ml with distilled water. About 0.5 ml of Folin ciocateau reagent was added to both sample and standard followed by 2.5 ml of 20% Na_2CO_3 the solution was then vortexed and allowed to incubate for 40 min at room temperature. Absorbance of solution was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared [8].

Determination of phytate composition

Phytate was determined according to the method of [9]. About 4 g of sample was soaked in 100 ml of 2% HCl for 3 h and then filter through a No 1 Whatman filter paper. Twenty five ml/litre was taken out of the filtrate and placed in inside a conical flask and 5 ml of 0.3% of ammonium thiocyanate solution was added as indicator. After which 53.5 ml of distill water was added to give it the proper acidity and this will be titrated against 0.00566 g per milliliter of standard iron (III) chloride solution that contain about 0.00195 g of iron per milliliter until a brownish yellow colouration persist for 5 min.

Alkaloid content determination

Five gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 h. This was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is then alkaloid which was dried and weighed [10].

Determination of saponin content

The saponin content of the samples was carried out using the method of Exactly 20 g of each sample was extracted with 20% aqueous ethanol by using a water bath maintained at 55 ºC, for 4 h with stirring [11]. After filtration the residue was re-extracted with 200 ml of 20 % ethanol. The combined extracts was reduced to 40 ml volume separately (water bath temperature was 90 ºC). Diethyl ether (20 ml) was used for extraction. The process was repeated three times. The ether layer was removed and 60 ml of n-butanol was added to the water layer. Butanol extract was washed with 5 % NaCl aqueous solution. After evaporation, the samples were dried in an oven to a constant weight; the saponin content was calculated as percentage of the starting material.

Determination of cardiac glycosides

The procedure described by was used 10ml the extract pipetted into a 250 ml conical flask [12]. About 50 ml chloroform was added and shaken on vortex mixer for 1 h. The mixture was filtered into 100 ml conical flask. 10 ml of pyridine and 2 ml of 29% of sodium nitroprusside were added and shaken thoroughly for 10min. About 3 ml of 20% NaOH was added to develop a brownish yellow colour. Glycosides standard (Digitoxin). A concentration which range from 0 – 50mg/ml were prepared from stock solution the abs was read at 510 nm.

Determination of terpenoid

The procedure described by was used [12]. About 0.5 g of finely ground sample was weighed into a 50 ml conical flask 20 ml of chloroform: methanol 2:1 was added the mixture was shaken thoroughly and allowed to stand for 15 min at room temp. The suspension was centrifuge at 3000rpm the supernatant was discarded and the precipitate was re-washed with 20 ml chloroform: methanol 2:1 and then re-centrifuge again the precipitate was dissolve in 40 ml of 10% SDS solution. 1ml of 0.01M ferric chloride was added and allowed to stand for 30 min before taken the absorbance at 510 nm.

Determination of Anthraquinone content

Fifty milligram of the fine powder of leaf was soaked in 50 ml of distilled water for 16 h. This suspension was heated in water bath at 70°C for 1h. After the suspension was cooled, 50 ml of 50% methanol was added to it and then filtered. The clear solution was measured by Spectrophotometer at a wavelength of 450 nm and compared with a standard solution containing 1mg/100 ml alizarin and 1mg/100 ml purpurin with the absorption-maximum 450 nm [13].

Evaluation of antioxidant properties of *Phyllatus amarus* and *Cymbopogan citratus*

Determination of 2,2 diphenyl -2- picryl-hydrazyl (DPPH) radical scavenging ability of the leaves.

Extracts (0.2 mI) were each added to 3.8 mI of 0.004% DPPH methanolic solution. After 60 min of incubation at room temperature in the dark, the absorbance was measured at 517 nm. A blank sample containing only methanol was used to zero the spectrophotometer. Ascorbic acid was used for comparison. Each experiment was performed in triplicates [14].

Determination of ABTS radical scavenging assay

Antioxidant activity of the extracts was determined using the 2, 2-azinobis- (3- ethylbenzothiazoline -6- sulfonic acid) ABTS radical scavenging assay [15]. The ABTS.+ (mother solution) was prepared by mixing equal volume of 8 mM ABTS and 3 mM potassium persulphate ($K_2S_2O_8$) (both prepared using distilled water) in a volumetric flask, which was wrapped in foil and allowed to react for a minimum of 12 h in a dark place. The working solution was prepared by mixing 5 ml of the mother solution with 145 ml phosphate buffer (pH 7.4). A range of trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-carboxylic acid) standard solution (100-1000 μM) were prepared in acidified methanol. The working solution (2.9 ml) was added to the methanolic extracts (0.1ml) or Trolox standard (0.1 ml) in a test tube and vortexed. The test tubes were allowed to stand for about 30 min after which the absorbance of the standards and samples were measured at 734 nm with a spectrophotometer.

Determination of ferric reducing antioxidant power (FRAP)
The reducing property of the extract was determined by the method of [16]. About 0.25 ml of the extract was mixed with 0.25 ml of 200 mM of Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% KFC. The mixture was incubated at 50 °C for 20 min, thereafter 0.25 ml of 10% TCA was also added and centrifuged at 2000 rpm for 10 min, 1millitre of the supernatant was mixed with 1ml of distilled water and 0.1% of $FeCl_3$ and the absorbance was measured at 700 nm.

Determination of total phenolic contents of the samples
The total phenol content of the extract was determined by the method of [17]. About 0.2 ml of the extract was mixed with 0.5 ml of 10% Folin Ciocalteau's reagent and 2 ml of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45 oC for 40 min, and the absorbance was measured at 700 nm using the spectrophotometer. Gallic acid was used as standard.

Determination of Flavonoid content of the leaves
About 10 g of each sample was extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No. 42. The filtrates were later transferred into a crucible and evaporated to dryness over a water bath. The dried extracts were weighed and the test procedure defined by was followed [18].

Determination of proximate composition of *Phyllantus amarus* and *Cymbopogon citratus* leaves
This was determined following the method described by Association of Official Analytical Chemist [19].

Determination of mineral content of *Phyllatus amarus* and *Cymbopogan citratus*
About 2 g of sample was placed in a crucible and ashed in a muffle furnace at 550oC for 5h and transferred into the desiccator to cool. The ashed samples were used by dissolving it with 1ml nitric acid and 1ml HCl and made up to 100ml. This was used to analyse for Mg, Na, Ca, K, Mn, Cu, Zn and Fe. The atomic absorption spectrophotometer was used to determine these elements 19 .The standard solution were prepared separately for each of the elements and values determined on atomic absorption spectrophotometer. The values measured were then plotted against the strength of the solution. The values of the various digest were measured from the atomic absorption spectrophotometer and the strength traced on the respective standard curve to give the corresponding values which would give the original values of the elements present in the digest. Flame photometer was used to measure the values of Na and K in all the samples, while phosphorus was determined colorimetrically [19].

Determination of vitamin content of *Phyllantus amarus* and *Cymbopogan citratus* leaves
Determination of Vitamin A
About 2 g of each sample was mixed with 30 ml of absolute alcohol and 3 ml of 5% potassium hydroxide. The mixture was boiled gently under reflux for 30 min in a stream of oxygen-free nitrogen. It was cooled rapidly by adding 30 ml of water and transferred into a separator, where it was washed with ether and the vitamin A was extracted by shaking for 1 min. It was then washed, evaporated down to about 5 ml and the remaining ether was removed in a stream of nitrogen at room temperature. The residue was then dissolved in sufficient isopropyl alcohol, the extinction was measured and the wavelength of maximum absorption was used [20].

Determination of Vitamin C
The vitamin C content of each sample was determined using the ascorbic acid as the reference compound. About 200 ml of each extract was pipetted and mixed with 300 ml of 13.3 % of TCA and 75µl of DNPH. The mixture was incubated at 37 °C for 3 h and 500 ml of H_2SO_4 was added. The absorbance was read at 520 nm [21].

Determination of Vitamin Determination of Vitamin E (Tocopherol)
About 1 g of each sample was weighed into 10 ml of absolute alcohol with 20 ml of 1 M alcoholic sulphuric acid. The unsaponifiable matter was then extracted with diethyl ether. The residue was dissolved in 10 ml absolute alcohol, the standard and the sample was transferred and 5 ml of absolute alcohol was added followed by 1 ml of concentrated nitric acid. The absorbance was measured at 470 nm against a blank containing absolute alcohol [22].

Determination of antibacterial property of *Phyllantus amarus* and *Cymbopogan citratus*
Antimicrobial activity of *P. amarus* and *C. citratus* extracts .The extracts were tested for activity against bacterial using modified agar-well diffusion method described by [23]. The ethanol, and water extracts of the leaves were individually tested against six bacterial cultures which include; *Bacillus cereus, Escherichia coli, Salmonella spp., Shigella dysenteriae, Staphylococcus aureus, Pseudomonas aeruginosa*, were provided by the Microbiology unit of Department of Food Science and Technology, Federal University of Technology, Akure. Five hour broth cultures of the test bacteria were adjusted to 108 cfu/ml and applied on the surface of Nutrient agar (Hi Media Laboratories Limited, Mumbai, India). A sterile flamed cork borer of 8 mm diameter size was used to punch four wells into each of the seeded plates and 0.5 ml of each extract was dispensed in each well. Controls were set up by filling wells with 1% of various solvents used. The plates were then incubated at 35 °C for 24 h. The experiments were performed in duplicate and the means of the diameters of the inhibition zones were calculated.

Results and Discussion
Phytochemical composition of *Phyllantus amarus* and *Cymbopogan citratus*
Table 1 shows the result of the phytochemical constituents of the aqueous and ethanol extract of *P. amarus* and *C. citratus* leaves. Tannin contents has the highest value in *raw lemon* (RL) *and raw Phyllantus amarus RL/RP* (28.33mg/g), while lowest value (1.80mg/g) was found in *dried lemon grass DL*. Tannins in *P. amarus* have astringent properties and so are remarkable in the treatment of stomach ulcers and diarrhea [24]. The oxalate value of raw lemon RL (7.60 mg/g) was lower compared to DL (11.67 mg/g), RP (16.47 mg/g), DL/DP (19.3 mg/g), DP (25.92 mg/g) and RL/RP (28.33 mg/g) respectively. The phytate content of raw *P.amarus* (RP) (8.39 *mg*/g) was higher than that of dried lemon grass DL (7.47 mg/g). However, lower value obtained for both oxalate and phytate in the leaves is considered appropriate, as high content may pose health risk to an individual [25]. Reported that the high contents of oxalate and phytate in food is known to interfere with the assimilation of nutrients, decrease the nutritive value of food and at high doses may have adverse effects on human health. It was observed that there was no significant difference in the raw and dried leaf samples as far as alkaloid content is concerned (3.81-4.73mg/g).

The presence of alkaloid in both *P. amarus* and *C. citratus* plant may be responsible for the anti-malaria property of the leaves [26,27]. The saponin content of the leaves ranged between (22.61mg/g -30.47 mg/g). The presence of saponins may be responsible for the anti-cancer activity of *C. citratus* as was reported by [28]. The most striking prospect for saponins in *P. amarus* is how they inhibited the growth of cancer cells without posing any significant risk on normal cells, as is the mode of some cancer-fighting drugs.

The cardiac glyxerine ranged from 11.62mg/g in dried *P. amarus* to 64.81mg/g in dried *C. citratus*. The cardiac glycosides found in the leaves of *P. amarus* exerts a positive effect on the heart in cardiac failures by increasing the capacity of the heart muscles to pump blood [29]. In addition the medicinal use of cardiac glycoside in *C. citratus* as treatment for congestive heart and cardiac arrhythmias was reported though its relative toxicity prevented them from being widely used [30].

The terpenoid content of leaves ranged between 14.56 (mg/g) to 69.66 (mg/g) with the highest value in (*Dry lemon*) DL and lowest value in Dry *lemon grass* and Dry *P. amarus* (DL/DP). Presence of terpenoid may be responsible for the anti-inflammatory activity of *C. citratus* as reported by [31]. Anthraquinone was found to be higher in dried *P.amarus* (16.82 mg/g) and lowest (1.48 mg/g) in dried lemon grass DL. Anthraquinone is beneficial in hampering the growth of cancer through certain activities such as inducing apoptosis and reliving bowl movements [32].

Table 1: Phytochemical Composition (mg/g) of Phyllantus amarus and Cymbopogan citratus

Samples	DL	DP	RL	RP	DL/ DP	R L / RP
Tannin	1.80 ± 0.54[b]	25.92 ± 0.25[b]	7.60 ± 0.57[a]	16.47 ± 0.37[a]	19.37 ± 0.21[b]	28.33 ± 0.70[a]
Oxalate	11.67 ± 0.06[e]	25.92 ± 0.28[b]	7.60 ± 0.57[f]	16.47 ± 0.36[d]	19.3 ± 0.12[c]	28.33 ± 0.70[a]
Phytate	7.47 ± 0.07[c]	6.66 ± 0.92[d]	7.43 ± 0.01[c]	8.39 ± 0.21[b]	10.66 ± 0.07[a]	7.36 ± 0.08[c]
Alkanoid	4.73 ± 0.21[a]	3.54 ± 0.02[a]	3.81 ± 0.07[a]	4.49 ± 1.48[a]	3.50 ± 0.07[a]	3.28 ± 0.39[a]
Saponin	30.47±0.26[a]	28.00 ± 0.28[a]	23.25 ±0.21[a]	22.93 ±0.39[a]	27.28 ± 5.89[a]	22.61 ± 0.25[a]
Cardiac glycoside	64.81 ± 8.39[c]	11.62 ± 13.63[b]	29.15 ± 2.12[d]	32.89 ± 1.05[d]	14.00 ± 0.25[a]	37.51 ± 0.38[c]
Terpenoid	69.66 ± 0.78[e]	16.70 ± 7.52[c]	41.49 ± 0.00[f]	29.28± 9.78[b]	14.56 ± 1.51[d]	42.81 ± 59.30[a]
Anthraquinone	1.48 ± 0.13[e]	16.82 ± 0.44[a]	2.39 ± 0.26[d]	5.22 ± 0.09[c]	14.39 ± 0.32[b]	4.85 ± 0.81[c]

Value are means ± standard deviation of three determinations. Values with different superscripts along the columns are significantly different ($p<0.05$)
Where; DL = Dry lemon grass leaves, RL= Raw lemon grass leaves, DL/DP = Dry lemon grass leaves /Dry *P. amarus leaves*, RP= Raw P.amarus leaves, RL/RP = Raw lemon grass leaves/ Raw *P. amarus* leaves

Antioxidant properties of *Phyllantus amarus* and *Cymbopogan citratus* extracts

The antioxidant properties of the *P. amarus* and *C. citratus* are shown in Table 2. The highest value of phenol (53-93 mg/g) found in dried *P. amarus* (DP) followed by raw *P. amarus* RP (42.62 mg/g), raw lemon and raw *P. amarus* RL/RP (42.51 mg/g), dried lemon and dried *P. amarus* DL/DP (37.28mg/g), raw lemon RL (24.88 mg/g) and dried lemon DL (14.73 mg/g) respectively. Antioxidant ability of plant phenolic compound has been attributed to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals [33]. Flavonoid content ranged from (0.40mg/g-2.24mg/g)in dried lemon grass (DL), and raw *P. amarus* leaf. Flavonoids are essential for fighting off free radicals and regulating cellular activities that promote oxidative stress in the body [34]. Raw *P. amarus* (RP) exhibited the highest DPPH scavenging activity of 91.12% followed by 90.93% in RL/RP, 85.61% from dried *P. amarus* (DP), and the lowest value of 64.45% in dried lemon grass (DL). *P. amarus* leaves are free radical inhibitors and primary antioxidants that react with free radical and the polar extracts have the highest free radical scavenging activity that can remove, prevent or slow cell damage and oxidative stress which has been found to lead to several diseases such as cancer, diabetes and stroke [35]. Antioxidant potentials of C. citratus extract have been identified and acknowledged as their abilities to reduce reactive oxygen species (ROS) [36]. The results of the FRAP shows the value ranged from 4.02 mg/m to 34.64 mg/ml with the highest value in RP and the lowest value in DL. It was observed that leaves lemon started high antioxidant activities against DPPH and ABTS, lower activity was noticed in ferric reducing properties (FRAP). *P. amarus* (RP) yielded the highest ABTS scavenging property of 82.20 mg/g and the lowest (58.27) mg/g was found in DL.

Table 2: Antioxidants Properties of Phyllantus amarus and Cymbopogan citratus

SAMPLE	FRAP	DPPH	ABTS	PHENOL FLAVONOID
DL	4.02 ±0. 00[d]	64.45 ± 1.56[e]	58.27 ± 0.30[d]	14.73 ± 1.03[e] 0.40 ± 0.09[e]
DP	21.05 ± 0.42[b]	85.61 ± 0.00[b]	81.31 ± 0.39[ab]	53.93 ±1.88[a] 1.67 ± 0.19[bc]
RL	6.86 ± 0.94[d]	70.97± 0.82[d]	75.39± 1.11[c]	24.88 ± 0.68[d] 1.41 ± 0.00[cd]
RP	34.64 ± 0.81[a]	91.12 ± 0.41[a]	82.20 ± 0.94[a]	42.62 ± 0.82[b] 2.24 ± 0.05[a]
DL/ DP	14.44 ± 0.34[c]	82.49 ± 0.26[c]	81.24 ± 0.91[ab]	37.28 ± 1.24[c] 1.17 ± 0.14[d]
RL/ RP	23.56 ± 2.82[b]	90.93 ± 10.45[a]	80.09 ± 0.99[b]	42.51 ± 1.03[b] 2.04 ± 0.33[ab]

Value are means ± standard deviation of three determinations. Values with different superscripts along the columns are significantly different ($p<0.05$)

Where; DL = Dry lemon grass leaves, RL= Raw lemon grass leaves, DL/DP = Dry lemon grass leaves/Dry *P. amarus* leaves, DP = Dry *P. amarus* leaves, RP= Raw *P. amarus* leaves, RL/ RP = Raw lemon grass leaves/ Raw *P.amarus* leaves

Proximate Composition of Phyllantus amarus and Cymbopogan citratus of the leaves extracts

The proximate composition of *P. amarus* and C. citratus leaves are represented on Table 3 .The ash content varies significantly except RL (2.70%) and DL (14.10%) that shows similar values. The ash content of *P. amarus* (2.29% - 10.63%) fell within the range of 13. 5% obtained by [37]. It was also revealed that the leaves of *P. amarus* are rich in ash, making it a good source of plant minerals required by human for normal metabolic activity of body tissues as well as the proper assimilation of vitamins [38]. However the ash content of lemon grass (2.70% - 14.10 %) is lower compare to the 20.30 % reported by [39]. Dried lemon grass exhibited the highest fat contents of 8.71% while the lowest 1.21% was recorded from raw *P. amarus* .The fat content were in the range of the value (6.07%) reported by [40]. Lipids provide excellent source of energy and enhance transport of fat soluble vitamins, insulate and protect internal tissues and contribute to vital cell processes [41]. It has been suggested that enough lipid (fat) be included in the diet to account for at least 20- 25% of the total caloric intake. The results obtained for protein showed that the value ranged from (1.29% -8.17%) % with the highest value in RL/RP and the lowest value in RL. Protein from plant sources have lower quantity, but their combination with many other sources of protein such as animal protein, legumes, may result in equivalent nutritional value [40].

The Crude fibre in RL/RP (18.53) % was the highest and RL (14.65) yielded the lowest value. *P. amarus* were low compared to the value (36.6%) reported by for raw P.amarus (RP), and 39.25 % for raw lemon grass (RL) [42,28]. The low crude fibre content of this plant may be advantageous in absorption of glucose and fat. The carbohydrate content obtained from these plants ranged from (55.17%- 66.48%) and showed that the plant has a higher source of carbohydrate.

The carbohydrate content are in line with 55.00%, reported by [39] and 60.38% [43] for C. citratus and *P. amarus* respectively. It may be an indication that the leaves are very good source of energy. Also the carbohydrate content of *P. amarus* may be one of the contributing factors for the efficacy of the leaf as an anti-diabetic agent [40].

Table 3: Proximate Composition (%) of Phyllantus amarus and Cymbopogan citratus

SAMPLE	RL	RP	RL/RP	DL	DP	DL/DR
Moisture	64.19±2.08^{a}	70.25±0.77^{b}	70.90±1.80^{b}	57.14±1.52^{a}	58.36±0.94^{a}	62.77±1.36^{a}
Ash	2.70±0.10^{c}	2.29±0.09^{f}	16.27±0.15^{a}	14.10±0.10^{c}	10.63±0.15^{d}	15.13±0.11^{b}
Fat	1.27±0.06^{c}	1.21±0.02^{c}	1.58±0.03^{b}	8.71±0.03^{a}	1.66±0.05^{a}	1.49±0.19^{b}
Protein	1.29±0.06^{e}	2.58±0.04^{d}	8.17±0.06^{a}	7.69±0.09^{b}	6.48±0.12^{c}	7.68±0.07^{b}
Crude Fibre	14.65± 0.05^{f}	17.52±0.14^{d}	18.53±0.06^{a}	15.40±0.10^{e}	18.16±0.06^{c}	18.36±0.06^{b}
Carbohydrate	55.17±0.29^{d}	63.30±0.10^{b}	64.08±1.27^{b}	60.23±0.15^{c}	66.48±0.10^{a}	66.30±0.10^{a}

Value are means ± standard deviation of three determinations. Values with different superscripts along the rows are significantly different ($p<0.05$)

Where; DL = Dry lemon grass leaves, RL= Raw lemon grass leaves, DL/DP = Dry lemon grass leaves/ Dry *P.amarus* leaves, DP = Dry *P. amarus* leaves, RP= Raw *P. amarus* leaves, RL/ RP = Raw lemon grass leaves/ Raw *P. amarus* leaves

Mineral contents (mg/100g) of Phyllantus amarus and Cymbopogan citratus leaves

These results showed in Table 4 revealed that *P. amarus* and C. citranus have appreciable quantities of minerals needed by human body for proper development. It was observed that potassium was the most predominant macro mineral; DP/DL (95.56 mg/100g), followed by RP/RL (89.67 mg/100g), DP (75.04 mg/100g), RP (69.63 mg/100g), DL (60.20 mg/100g) and RL (54.04 mg/100g) respectively. These values are significantly lower compared to the value (107.25 mg/100g) reported by [37] for *P. amarus* leaf but low compared to the value1.59mg/100g reported by [44] for C. citratus leaf. The differences in the potassium content of the leaves as compared to previous studies could be attributed to the methods of processing, geographical area of harvest and time of harvest. The presence of potassium may make them excellent sources of nutrient for boosting the immune system [45]. It has also revealed that diet rich in potassium may help to reduce blood pressure, water retention, protect against stroke and prevent osteoporosis [46].

The sodium content (11.15 mg/100g) of raw P.amarus leaf was found to be lower than the value 32.95 mg/100g reported by [37] and in other hand higher in raw C. citratus (9.84 mg/100g) as compared to 0.28 mg/100g reported by [44].The presence of sodium in the leaves might explain the nutritional bases for using the plant as indigenous tonic. Sodium shows a special role in nervous transmission and considered as an active transport of sugar and amino acids. The recommended daily intake for sodium is 2000 mg/day according to world organization [47].

The calcium content for both leaves revealed that value obtained for *P. amarus* (7.05mg/100) is higher compared to that of [48] (3.73mg/100) as well as 51.07mg/100 recorded for C. citratus compared to that of [44] 0.62mg/100. Calcium is effective for muscle activity, skeletal development and normal growth, therefore, an appreciable amount of calcium is required in the diet. The zinc content in both leaves (1.83 mg/100g-3.23mg/100g) were found to be lower than 21.73mg/100g reported by [44] for C. citratus and in the other hand higher than 0.47mg/100g reported by [37] for *P. amarus* leaf.

Zinc is an essential element in nutrition where it function as an integral part of numerous enzymes required for various metabolic processes in the body. It is said to be the second-most-abundant trace mineral in the body after iron and it is present in every cell [49]. It is also essential for the activity of over 300 enzymes that aid in many processes such as metabolism, digestion and nerve

function [50]. The recommended daily intake of zinc is found to be 8mg for women and 11 mg for adult men [47]. The second most abundant micro mineral in this study was found to be iron, ranging from (0.97mg/100g- 3.13 mg/100g) with dried lemon (DL), having the highest iron content and raw lemon grass (RL) with the lowest iron content. These values were significantly higher than 268.5mg /100g reported [44] for C. citratus and 5.78mg/100g for *P. amarus* leaf. Iron is mostly found in the red blood cells of the body and it also plays a fundamental role in treating anaemia, boosting haemoglobin, and the body's immunity [51]. Iron is also needed for physical growth, neurological development, cellular functioning, and synthesis of some hormones. The recommended daily intake of iron is 16.3–18.2 mg/day in men and 12.6–13.5 mg/day in women [47]. The iron content in both leaves is significantly lower than the recommended daily intake. The copper content recorded in this study was 0.14 mg/100g-1.13 mg/100g for both leaves. These values were within the range of value (0.13mg/100g) reported by [37] for *P. amarus* leaf, however 0.08 mg/100g was documented for C.citratus [52].

Copper plays a fundamental role in the production of red blood cells and energy production. It also aids the body in the formation of collagen and absorption of iron. The recommended daily allowance (upper limit) for adults is 10 mg/100g [47] and any intake above this could be toxic. The manganese content in this study ranged from 0.15mg/100g - 0.92 mg/100g. However the value (50mg/100g) reported for *P. amarus* was significantly higher [37] for dried *P. amarus* leaves.

Manganese is essential for bones, hormone production, nervous system function, energy metabolism and antioxidant. It is also crucial for blood clotting and connective tissue growth [53]. From the result the sodium to potassium ratio (Na:k) ratio is less than one, and according to [54] Na:k ratio should be less than one which is the recommended diets for regulating blood pressure. In addition, Na:k is also in agreement with the recommendation with WHO for reducing the risk of stroke [47]. Therefore, a diet low in sodium and high in potassium is strongly advised in other to lower blood pressure and reduce the risk of cardiovascular disease [54].

Table 4: Mineral content (mg/100g) of P.amarus and C.citratus the leaves

SAMPLE	RP	RL	RP/RL	DP	DL	DP/DL
Sodium	11.15±0.04^{b}	9.84±0.06^{c}	13.25±0.01^{c}	14.27±0.06^{b}	11.30±0.10^{c}	15.23±0.06^{c}
Potassium	69.63±0.01^{a}	54.04±0.02^{a}	89.67±0.02^{a}	75.04±0.01^{a}	60.20±0.10^{a}	95.56±0.12^{a}
Calcium	7.05±0.03^{c}	51.07±0.02^{b}	53.81±0.01^{b}	9.03±0.02^{c}	54.70±0.10^{b}	58.57±0.58^{b}
Zink	1.83±0.01^{d}	1.83±0.06^{d}	1.91±0.01^{d}	3.02±0.02^{d}	3.23±0.06^{d}	3.20±0.20^{d}
Iron	0.97±0.02^{e}	1.15±0.01^{e}	1.84±0.01^{e}	2.23±0.02^{e}	3.13±0.15^{d}	2.60±0.10^{f}
Copper	0.81±0.01^{g}	0.14±0.01^{f}	0.91±0.00^{g}	1.13±0.02^{a}	1.13±0.12^{e}	0.50±0.10^{g}
Manganes	0.26±0.02^{f}	0.15±0.01^{f}	0.41±0.00^{f}	0.92±0.01^{f}	0.63±0.06 ^{f}	0.87±0.06 ^{g}
N/K	0.16	0.18	0.15	0.19	0.18	0.16

Value are means ± standard deviation of three determinations. Values with different superscripts along the rows are significantly different ($p<0.05$)

Where; DL = Dry lemon grass leaves, RL= Raw lemon grass leaves, DL/DP = Dry lemon grass leaves/ Dry *P.amarus* leaves, DP = Dry P.amarus leaves,RP= Raw *P. amarus* leaves. RL/ RP = Raw lemon grass leaves/Raw *P.amarus* leaves

Vitamin composition of Phyllantus amarus and Cymbopogon citratus leaves

The vitamin content in Table 5 showed that vitamin A (18.71 mg/g-22.22 mg/g) was higher than vitamin C (10.14 mg/g-15.53 mg/g) and vitamin E (0.36 mg/g-1.21 mg/g). Vitamin A and E serve as antioxidants to prevent cell damage, however vitamin A is a fat-soluble vitamin that is naturally present in many foods which is essential for healthy vision and bones, cell division, reproduction and immunity. The recommended daily intake of vitamin A and E are 900mcg and 15mg respectively. Vitamin C, and vitamin E, are also the promising agents for supplementation of extrinsic factors, which could enhance the body's ability to fight the deleterious effects of oxidative stress during exercise, they are supplements containing various antioxidants.

Table 5: Vitamin composition (mg/g) of Phyllantus amarus and Cymbopogan citratus of the leave extracts

SAMPLE	VITAMIN A	VITAMIN C	VITAMIN E
DL	21.99 ± 1.73^{a}	10.14 ± 0.48^{c}	0.64 ± 0.04^{c}
DP	21.60 ± 2.59^{a}	13.57 ± 2.15^{a}	0.46 ± 0.01^{d}
RL	18.71 ± 18.16^{b}	10.72 ± 0.04^{bc}	0.36 ± 0.09^{d}
RP	21.54 ± 0.00^{a}	15.53 ± 0.47^{a}	0.82 ± 0.48^{b}
DL/DP	22.22 ± 1.73^{a}	11.97 ± 0.59^{bc}	1.21 ± 0.09^{a}
RL/RP	21.77 ± 0.00^{a}	10.28 ± 1.63^{c}	0.69 ± 0.00^{bc}

Value are means ± standard deviation of three determinations. Values with different superscripts along the rows are significantly different ($p<0.05$)

Where; DL = Dry lemon grass leaves, RL= Raw lemon grass leaves, DL/DP = Dry lemon grass leaves+ Dry *P. amarus* leaves, DP = Dry *P. amarus* leaves, RP= Raw *P. amarus* leaves, RL/ RP = Raw lemon grass leaves/ Raw *P. amarus* leaves.

Antibacterial activity of ethanol and aqueous extracts of Phyllantus amarus and Cymbopogan citratus leave extracts

Antibacterial activity of ethanol and water extracts of *P. amarus* were evaluated against some foodborne pathogens namely; *Bacillus cereus, Shigella dysenteriae*, *Escherichia coli, Salmonella spp, Staphylococcus aureu Pseudomonas aeruginosa*, as shown in Table 6. The ethanolic extracts of *P. amarus* produced mild inhibition against all the test organisms (4.5 mm-9.1 mm), inhibition zones of 4.3 mm – 7.7 mm were recorded from the antibacterial activity of ethanolic extract from *C.citratus* and the activity was similar to what was observed from mixture of *P. amarus* and *C.citratus*. All the aqueous extract could not inhibit the growth of the test organism. This study establishes one of the traditional uses of *P. amarus* against typhoid fever [55]. The result also support the findings of which revealed that the ethanolic extracts of *P. amarus* demonstrated the antimicrobial activity for all the extracts with a diameter ranges from (9.1- 4.3mm).

Table 6: Antibacterial activity of ethanol and aqueous extracts from the leaves, mm

Bacteria tested	*E. Phy*	*A Phy*	*E Phy/Lem*	*A lem*	*E lem*	*A lem/phy*
Bacillus cereus	4.5	Nil	Nil	Nil	4.3	Nil
Shigella dysenteriae	8.8	Nil	5.5	Nil	7.7	Nil
Escherichia coli	6.0	Nil	6.1	Nil	5.3	Nil
Salmonella spp	7.0	Nil	5.4	Nil	6.5	Nil
Staphylococcus aureu	9.1	Nil	7.8	Nil	5.6	Nil
Pseudomonas aeruginosa	7.2	Nil	5.6	Nil	6.2	Nil

Nil means No Inhibition
E Phy Ethanoic extracts of *P. amarus* leaf
E Phy/Lem Ethanoic extracts of lemon grass and *P. amarus* leaf
E lem Ethanoic extracts of lemon grass leaf
A Phy Aqueous extracts of *P.amarus* leaf
A lem Aqueous extracts of lemon grass leaf
A lem/phy Aqueous extracts of *P. amarus* /lemon leaves

Conclusion

The present study revealed that *C.citratus* and *P. amarus* leaves possessed several phytochemicals, and exhibited ntioxidant properties. Like other common vegetables, the leaves of *P. amarus* and *C.citratus leaves are* rich in fibre, ash, carbohydrate, minerals and vitamins. Hence, the incorporation of both leaves into human diet could be beneficial to health. In addition, ethanolic extracts of *P. amarus* and *C.citratus* demonstrated mild antibacterial activities against some pathogens. It may be concluded that findings from this work explain some of the traditional applications reported for the leaves. Therefore, there is need to carry out further research to investigate the possibility of exploring *P. amarus* and *C. ctratus* leaves as functional food agents.

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