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Antioxidant Activity, Total Phenolic Contents and Functional Group Identification of Leaf Extracts among Lemongrass (*Cymbopogon citratus*) Accessions

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Abstract
Lemongrass leaves are widely used for tea and the treatment of malaria. In the present study, Soxhlet extraction was carried out with aqueous ethanol (v/v). Fresh and dried leaves of selected ten lemongrasses (Cymbopogon citratus) accessions from different geographical regions in Nigeria were examined for total phenolic contents, and antioxidant activities. Aqueous methanol extraction was carried out and further partitioned into hexane, ethyl acetate and butanol to obtain fractions according to their polarities and Fourier Transform Infrared Spectroscopy (FTIR) was carried out to identify the functional groups that may be present. Among the ten accessions, the leaf extracts at five different concentrations exhibited increasing antioxidant activities using DPPH (2,2-diphenyl- 1-picrylhydrazyl) radical scavenging test, stronger activities for dried leaves (71.15 ± 0.14 - 89.79 ± 0.16μg/ml) than fresh leaves (71.65 ± 0.45 -81.94 ± 0.84 μg/ml) at 100 μg/ml of sample extract. The total phenolic contents of dried leaf extracts, revealed higher amounts in all lines ranging from 19.57±0.57 to 43.17±0.67mg gallic acid equivalent /100 g DW when compared with fresh leaf extracts, where the values ranged from 9.68 ± 2.20 to 28.5 ± 3.90 mg gallic acid equivalent /100 g fresh weight except for two lines which showed greater total phenolic contents than in the dried leaves. High total phenolic content may help contribute to the overall high antioxidant activity of the plant. FTIR identified the presence of major active functional groups including alcohol, ester, amide, alkanes, alkenes, carboxylic acid, ketones and aldehyde in four partitioning solvents (n-hexane, ethyl acetate, butanol, and methanol) leaf extracts of lemongrass samples.
Keywords: *Cymbopogon citratus,* Antioxidant activity, Phenolic content, Morphology, FTIR

Introduction
Lemongrass is a perennial grass plant that is distributed worldwide particularly in tropical and subtropical countries (1). It could grow up to 6 inches high with bulb-like stems of smooth linearly sheathed leaves with a narrow base and acute apex. Lemongrass has been traditionally used to remediate a plethora of medical conditions. This is due to the broad spectrum of secondary metabolites that it produces. It has been used to treat fever, cough, elephantiasis flu, leprosy, malaria and digestive problems among many other illnesses (2, 3). Studies on different lemongrass extracts have also shown other important therapeutic potentials such as anti-cancer, anti-hypertensive, and anti-mutagenicity (4). Lemongrass contains nutrients such as fats, proteins, fiber and minerals, and also several important bioactive compounds which may be grouped into alkaloids, terpenoids, flavonoids, phenols, saponins, and tannins (5).

In recent times, interest has increased considerably in discovering naturally occurring antioxidants in medicinal plants (6). Antioxidant potentials of lemongrass extract have been identified and acknowledged their abilities to reduce reactive oxygen species (ROSs). Mechanisms such as inhibition of lipoperoxidation and decolorization of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) have been used to determine antioxidant activity in medicinal plants (2, 7). Phenolic compounds are dietary constituents widely existing in plants and have been considered to have the high antioxidant capacity and free radical scavenging capacity (8). They have attracted more and more attention for the prevention and reduction of many oxidative stress-related diseases, including cancer (9, 10).

Functional groups have been reported to influence the absorption, distribution, metabolic extraction, and toxicity of bioactive molecules and they vary in type, number, and position of functional groups, resulting in variations in chemical properties which can influence the solubility of these compounds in different solutions (11, 12). Consequently, the use of Fourier Transform Infrared (FTIR) spectroscopy in functional group analysis plays an essential role in understanding the overall physicochemical properties of the extract. In addition, identification of the

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functional group helps to evaluate their structure-activity relationships. A study (13) reported that FTIR spectral analysis of lemongrass showed the presence of phytochemicals carrying hydrogen bonded –OH functional group which is a fundamental part of most of the phenolic phytochemicals such as flavonoids and tannins. FTIR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures (14).
In the present study, aqueous ethanol extracts of fresh and dried leaves of selected ten lemongrasses (*Cymbopogon citratus*) accessions from different geographical regions in Nigeria were examined for total phenolic contents and antioxidant activities. Their functional groups were also determined.

Materials and Methods

Plant materials

Stalks of ten genotypes of lemongrass (*C. citratus)* were obtained from 9 locations in Nigeria (Abia, Badagry, Kano, Kwara, Lagos, Maiduguri, Niger, Ogbomosho, Ondo, and Osun) for this study. They are listed in Table 1. The stalks were planted in garden pots in the University of Lagos Botanical and Zoological Garden for a period of seven months.

Preparation of Extracts from fresh and dried leaves

To five (5) g of pulverized lemon grass samples were extracted with aqueous ethanol (v/v) in a Soxhlet apparatus. Next, the extracts were concentrated to dryness in a rotary evaporator under reduced pressure. The dried residues were re-dissolved in 100 mL of 80% methanol. Triplicates extractions were made for each sample. The extracts were used for total phenolic and antioxidant assay. Dry matter (DM) refers to the initial ground dry sample after correction of the residual water.

Total Phenolic Contents by the Folin-Ciocalteu method

The total phenolic content of the extract solutions was determined by using the Folin-Ciocalteu assay (15). An aliquot (1 mL) of extracts or standard solution of Gallic acid (100µg/mL) was mixed with 9 ml of distilled water. 1 ml of Folin-Ciocalteu reagent was added, after 5 minutes, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The mixture was incubated for 90 minutes in the dark at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Visible spectrophotometer using a mixture of water and reagents as a blank. The total phenolics were expressed as mg of gallic acid equivalents per gram of dry matter (mg GAE/g). Triplicates of sample extract solutions were analyzed.

2, 2-diphenyl- 1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of the extract, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated from a method (16,17). An aliquot of 0.5 mL of extract at different concentrations (20, 40, 60, 80, 100µg/ ml) was added to freshly prepared 2.0 mL of reagent solution (0.004 g of DPPH in 100 mL methanol). The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of the test mixture (due to quenching of DPPH free radicals) was read at 517 nm with an UV-Visible spectrophotometer.
DPPH solution at 1mM in methanol was used as the blank. All determinations were carried out in triplicates of sample extract solutions. The results were expressed as µg/mL of gallic acid equivalents per gram of dry /fresh extract mg gallic acid equivalent /100 g. Gallic acid calibration curves were obtained from five concentrations. Triplicates of sample extract solutions were analyzed. The scavenging effect was calculated using the expression:

$$\left[\frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}}\right]*100$$

Infra-red spectrometry for functional groups determination

Lemongrass leaves were harvested, weighed, dried for 4 days and then reweighed to determine the moisture content in each sample. The lemongrasses were ground using a kitchen blender and then soaked in a mixture of water/methanol (50%) in a conical flask for 3 days. The extract was removed and stored in the refrigerator then the fresh mixture of the solvent was added to the residue and then left for another 1 day. This method is solvent extraction of the dried plant materials. The second extract was removed and added to the first one. For the partitioning of extracts, rotary evaporation was used for gentle removal of solvents from samples - according to the descriptions of Craig *et al*. (18) and four partitioning organic solvents; n-hexane, ethyl acetate, butanol, and methanol were used. The dried aqueous methanol extract obtained after evaporation was weighed, then dissolved in little water (about 10 ml), this was partitioned first using 15 mls at a time and repeated three times until hexane looked plain. This was followed by ethyl acetate, the same way as hexane then butanol and methanol. The four fractions obtained were hexane (containing non-polar fraction), ethyl acetate (slightly polar fraction), butanol (polar fraction) and methanol from the aqueous/methanol extract. Infra-red spectrometry of the different fractions was used

to determine the various functional groups present in the l components present in 5 samples of *C. citratus.* The dried paste of the extract was placed on the sample chamber of FTIR spectrophotometer (Alpha Bruker, Laser Class 1, Platinum ATR Model) and the spectra were recorded on the FTIR spectrophotometer.

Statistical Analyses

Results were expressed as mean (n =3) Pearson correlation test was carried on DPPH radical scavenging activity with phenolic content. Significant differences at a 95% confidence interval were assessed through the analysis of ANOVA.

Results and Discussion

Phenolic compounds as an essential category of phytochemicals are food constituents widely existing in plants and have been considered to have high antioxidant capacity and free radical scavenging capacity (8). In this present study, the total phenolic content (TPC) of the lemongrass varied largely among the fresh extracts of lemongrass, ranging from (9.68) mg GAE/g to (64.52) mg GAE/g (Figure 1). The dried lemongrass leaves extract, however, showed higher total phenol content, generally above 20mg GAE/g except for LGN03 with TPC of (19.57) mg GAE/g (Figure 1). Among the accessions, dried lemongrass leaves extract; LGO01, LGD02, LGK04, LGS05, LGL06, LGK08, and LGA10 showed the highest total phenolic contents (above > 30 mg GAE/g of DW) and fresh leaves extract LGN03 and LGM09 had total phenolic contents above 30 mg GAE/g. Among the accession, only LGO01 fresh lemongrass extract had phenolic content below 10 mg GAE/g (Figure 1). The total average of the phenolic content evaluated for all accession prepared from dried leaves 31.47 mg GAE/g is relatively higher compared to the average of total phenolic content of fresh leaves 23.44 mg GAE/g, this is comparable to the phenolic content (30.74 mg GAE/g) of dried leaves of lemongrass reported by (19). This may be as a result of concentration increasing as the sample dries, since less water is present.

In this study, it was observed that lemongrass accessions from northern Nigeria (Niger LGN03, Kano LGK04, and Maiduguri LGM09) showed higher phenolic contents in both fresh and dried lemongrass leaf extracts, this high concentration could be attributed to the environmental condition of the region (20). Several factors may affect the content of phenolic compounds found in extracts, such as the way of preparation (plant processing, concentration, time and temperature of infusion), the plant (species, part used, stage of development), growth environment, genotypes, stage of growth during harvest the method of extraction. The high phenolic content from LGN03, LGK04 and LGM09 indicates they may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity (21, 22).

Table 1. Morphological characteristics of the 10 *C. citratus* accessions used in the study

Morphology	Collection sites	Leaf colour	Sheath colour	Plant height (cm)	No of tillers
LGO01	OYO (Ogbomoso)	Light green	Green	93	0
LGD02	Ondo	Light green	light green	68	5
LGN03	Niger	Light green	light green	110	6
LGK04	Kano	Light green	light green	107	4
LGS05	Osun	Light green	Green	85	4
LGL06	Lagos (Badagry)	Light green	Green	126	3
LGL07	Lagos (Lagos)	Light green	Light green	98	0
LGK08	Kwara	Light green	Green	112	3
LGM09	Maiduguri	Light green	Sea green	107	7
LGA10	Abia	Light green	Water green	103	4

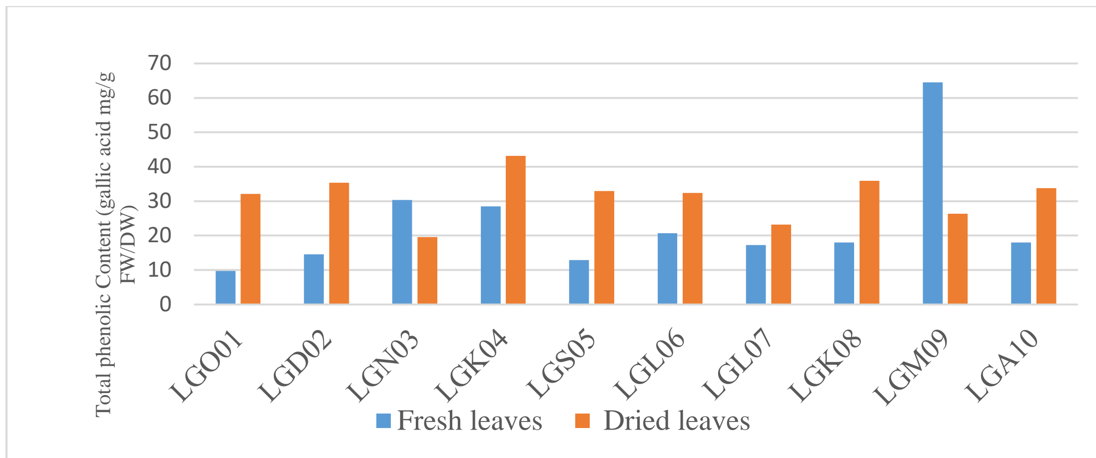

Figure 1. Total phenolic content of fresh and dried leaves of ten accessions of lemongrass. FW (fresh weight), DW (dry weight).

Generally, the 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) percentage scavenging increased as the concentration of lemongrass extract increased. The percentage inhibition of fresh leaf extracts of 10 lemongrass (*C. citratus*) accessions at different concentration is shown in Figure 2A. It was observed from the fresh lemongrass that LGA10 had the highest percentage inhibition while LGO01 had the lowest percentage of inhibition at 20µg/ml. The highest and the lowest percentage inhibition of fresh lemongrass extracts at 40µg/ml was observed in LGM09 and LGO01 accessions respectively, while the highest and the lowest percentage inhibition of fresh lemongrass at 60µg/ml was observed in LGK04 and LGL07 respectively. LGD02 had the highest percentage Inhibition at 80µg/ml while LGL07 had the highest percentage, while at 80µg/ml and 100µg/ml LGA10 had the highest percentage. Natural antioxidants in the plants may be due to the presence of phenolic compounds (23).

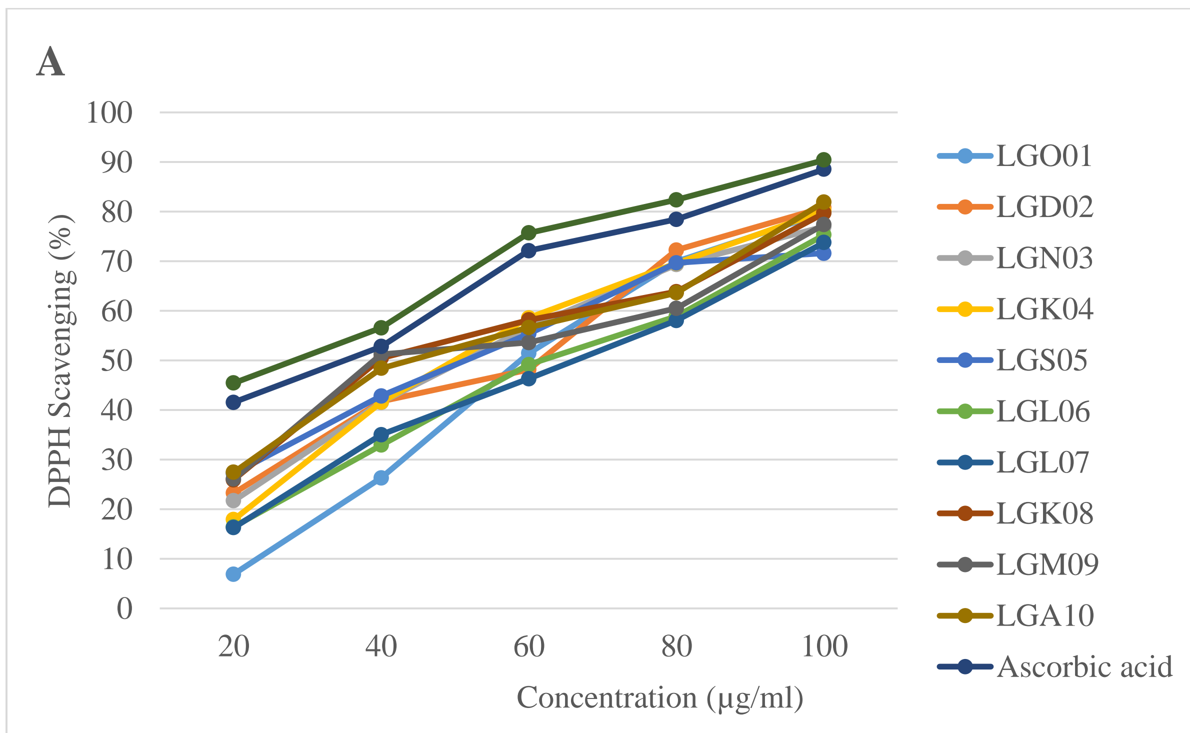

Figure 2A. Antioxidant activities of the aqueous ethanol extract of leaves of ten lemongrass at various concentrations. Each value represents a mean (n = 3): (A) DPPH radical scavenging activity of fresh leaves extracts. Gallic acid and ascorbic acid used as standards

The percentage inhibition of dried lemongrass leaf extracts at the different concentration indicated that LGA10 had the highest percentage inhibition while LGO01 had the lowest percentage inhibition at 20µg/ml (Figure 2B). The highest and the lowest percentage inhibition of dried lemongrass at 40µg/ml was observed to be (LGO01 and LGD02 respectively), the lowest and highest percentage inhibition of dried lemongrass at 60µg/ml was observed to be (LGD02 and LGN03 respectively), LGM09 had the highest percentage of inhibition at 80µg/ml while LGD02 had the highest percentage of inhibition at 80µg/ml and at 100µg/ml, LGL07 had the highest percentage distribution while LGK08 had the lowest percentage distribution. The antioxidant activity in the lemongrass is due to the presence of bioactive compounds, such as phenols, flavonoids, tannins, as well as compounds that have other groups, such as sulphide and alkaloids (24).

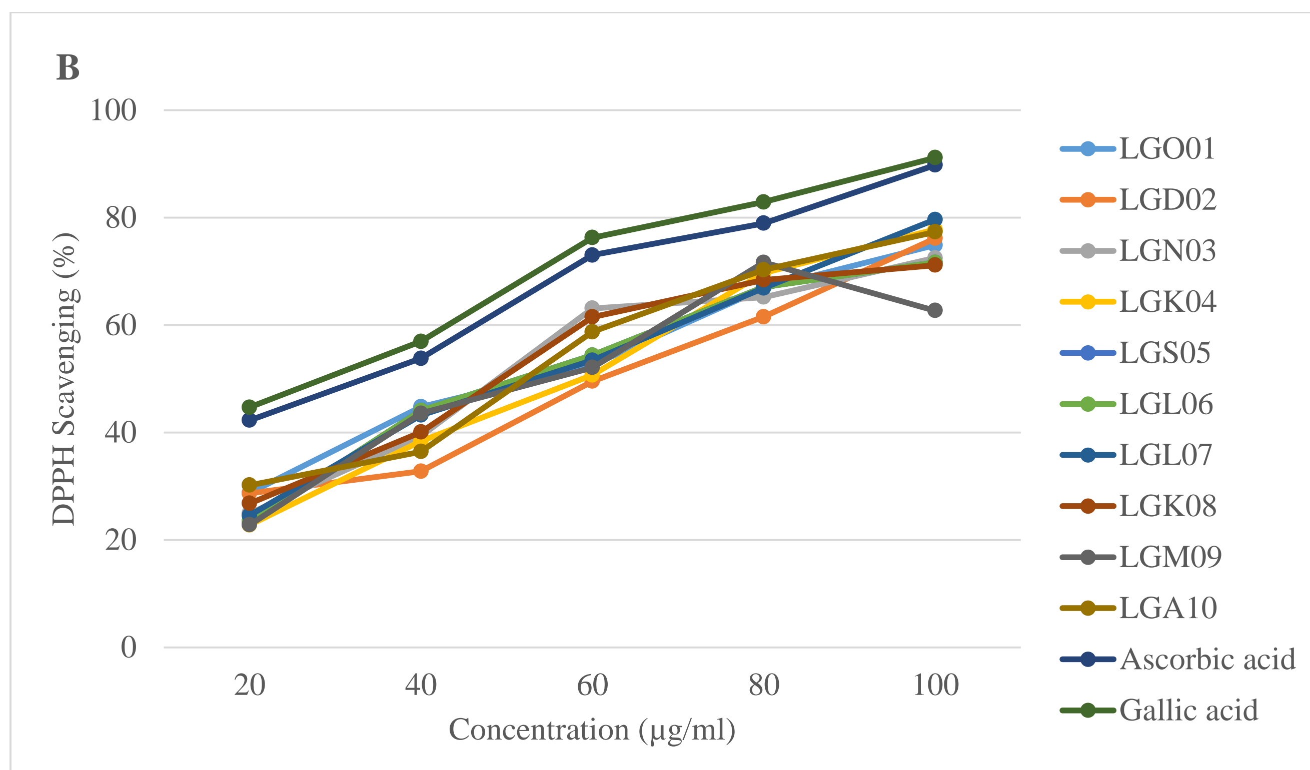

Figure 2B. Antioxidant activities of the aqueous ethanol extract of leaves of ten lemongrass at various concentrations. Each value represents a mean (n = 3): (B) DPPH radical scavenging activity of dried leaves extracts. Gallic acid and ascorbic acid used as standards

Functional group identification plays a vital role in understanding the overall physicochemical properties of an extract and to evaluate their structure-activity relationships (11). The infrared spectroscopy was used to detect the functional groups of biomolecules present in the lemongrass accessions. The results were recorded on an FTIR spectrometer between the range 3500-500 cm^{-1}. FTIR spectral analysis data of four extracts (butanol, ethyl acetate, methanol, and n-hexane) revealed the occurrence of multiple functional groups in five lemongrass accessions (LGN03, LGL07, LGK08, LGM09, and LGA10). Spectral data of most of the extracts confirmed the presence of bioactive functional groups such as –OH, N-H, –CHO, C=O, C=C, C-H, and C-O.

All four extracts exhibited the presence of broad peaks for hydrogen bonded –OH stretching in functional group region. It should be of note here that the methanol extract is the crude from which hexane, ethyl acetate, and butanol fractions were partitioned. The methanol extract will contain most of the functional groups present in the other

fractions. Important IR absorption frequencies are tabulated in Table 2. The FTIR spectra of n-hexane extract for LGK08 is shown in Figure 3(A), and ethyl acetate extract for LGM09 shown in Figure 3 (B). FTIR spectra of methanol extract for LGA10 and LGK08 are shown in Figure 3 (C and D). FTIR spectra of methanolic extract shows there was broad peaks at 3327.32cm^{-1} (LGN03), 3330.77 cm^{-1} (LGL07), 3289.59 cm^{-1} (LGM09) and 3329.92cm^{-1} (LGA10) (Table 2). The FTIR spectra methanolic extract also shows sharp peaks at 1632.08cm^{-1} (LGN03), 1631.63cm^{-1} (LGL07), 1630.18 cm^{-1} (LGK08), 1635.45cm^{-1} (LGM09) and 1632.08 (LGA10), which correspond to the presence of hydroxyl (OH), alkanes (C-H), alkenes (C=C) and amine (N-H) functional groups. The ethyl-acetate extract spectra (Figure 3C) also confirms the presence of bioactive compounds of H-bonded O–H stretching; broad N–H stretching in LGN03, LGK08 and LGM09 from the broad peak 3338.4cm^{-1}, 3371.92cm^{-1} and 3375.17cm^{-1} respectively which indicates the probable phytochemicals that have –NH bonds which can be present in such secondary metabolites as alkaloids, 2983.75 cm^{-1} that may have –OH bonds which are present in flavonoids, polyphenols and tannins. The FTIR spectral analysis of ethyl acetate extract reported by (25) is similar to this study's findings which found probable phytochemicals to be alkaloids, flavonoids, polyphenols, carboxylic acid and tannins in *Mammea suriga*.

The FTIR spectra of n-hexane extract (Table 2) identified major sharp peaks values observed at 2983.75 cm^{-1} (LGN03), 2852.97 cm^{-1} (LGL07), 2872.48 cm-^{1} / 1717.59 cm^{-1} (LGM09), 2920.05 cm^{-1} / 1741.23 cm^{-1} (LGA10) which can be attributed to the existence of alkanes (C-H), esters (C=O) functional groups. Also, from the FTIR spectra of ethyl-acetate extract showed the presence of functional groups like carboxylic acid O–H, this may be linked with fatty acids which may have both antimicrobial and antioxidant properties, aldehyde/ketone C=O, alkane C-H, and amine C–N. The peak at 2925.35 cm^{-1} of LGN03, 2983.75 cm^{-1} of LGL07, 2852.98 cm^{-1} of LGK08, 2873 cm^{-1} of LGM09 and 2984.44 cm^{-1} of LGA10 is due to the asymmetric stretching of C-H groups of aromatic compounds (Table 2). The characterization of n-hexane extract of the lemongrass accessions reveals the presence of C=O, C-O, C=C band stretching, suggests that phytochemical components may be aromatic or aliphatic (26, 27).

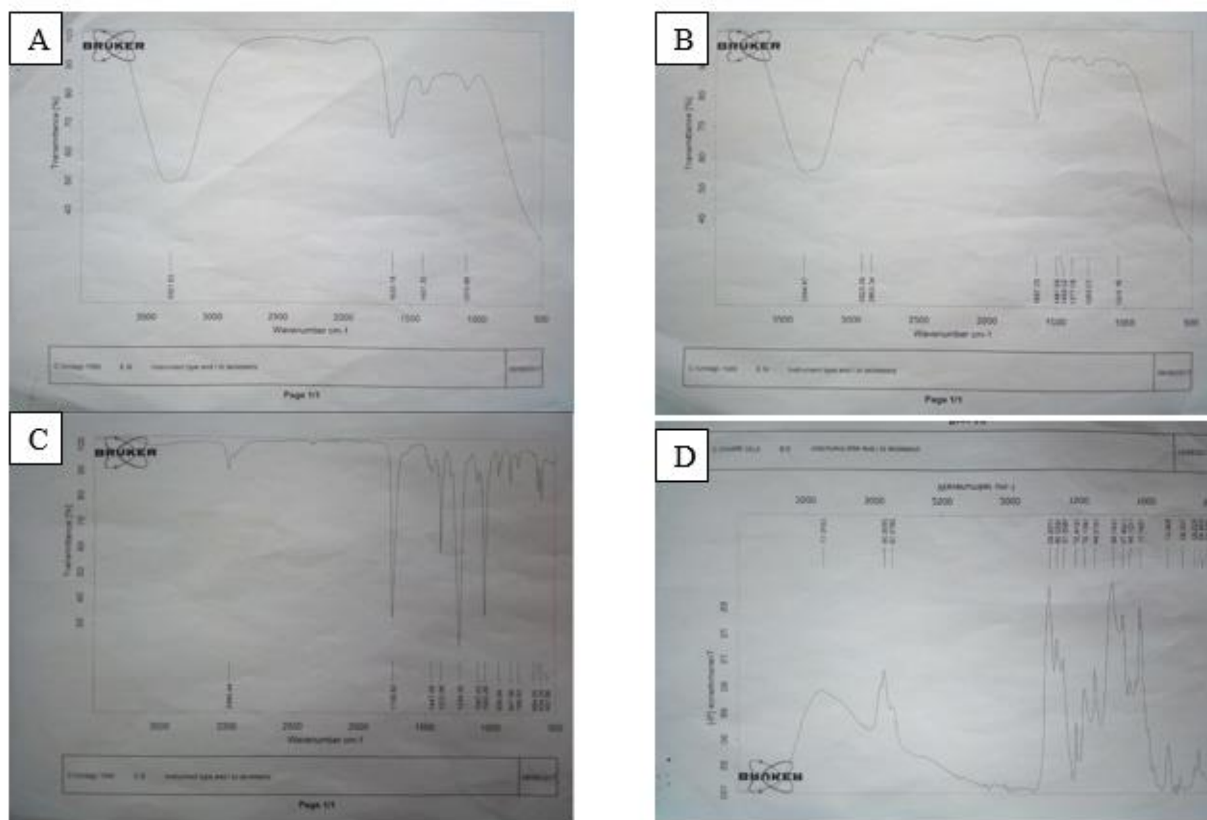

Figure 3: FT-IR spectrum of methanol extract for LGK08 (A), ethyl-acetate extract for LGM09 (B), ethyl-acetate extract for LGA10 (C) and n-Hexane extract for LGK08 (D).

Table 2. Major bands observed in the FTIR spectra of various extracts of lemongrass

Accessions	Extract	IR*vmax* (cm-[1]) *(vibration mode)*	Extract	
LGN03	**But**	1708.37 (C=O Acid); 2873.37 (C-H Alkane); 3339.67 (O-H Alcohol)	**Met**	1632.08 (C=C Alkene); 3327.32 (N-H Amine)
	Ethyl	1513.7 (C-O Ether); 1603.46 (N-H Amide);1650.74 (C=C Alkene) 1706.59 (C=O Acid); 2925.35 (C-H Alkane);3338.4 (N-H Amine)	**n- Hex**	1646.44 (C=C Alkene); 1717.71 (C=O Acid);2852.97 (C-H Alkane) 3370.79 (N-H Amine); 2983.75 (C-H Alkane)
LGL07	**But**	1653.09 (C=C Alkene); 2873.19 (C-H Alkane); 3328.55 (N-H Amine)	**Met**	1631.63 (C=C Alkene); 3330.77 (N-H Amine)
	Ethyl	1736.69 (C=O Ester); 2983.75 (C-H Alkane)	**n- Hex**	1710.92 (C=O Acid); 1739 (C=O Ester);2852.68 (C-H Alkane)
LGK08	**But**	1461.25 (Aromatic compounds); 2873-25 (C-H Alkane);329.82 (O-H Alcohol)	**Met**	1632.08 (C=C Alkene);3327.32 (N-H Amine)1401.3 (C-H Alkane)
	Ethyl	2852-98 (C-H Alkane); 1651.69 (C=C Alkene); 15131.67 (C-O Ether) ;1603.96 (N-H Amide); 3371.92 (N-H Amine)	**n-Hex**	1491.68 (C-H Alkane); 1637.25 (C=C Alkene); 344.47 (N-H Amine)
LGM09	**But**	3329.8 (N-H Amine); 1461.33 (C-H Alkane)	**Met**	1631.63 (C=C Alkene); 1401.14 (C-H Alkane);3289.59 (O-H Alcohol)
	Ethyl	2873 (C-H Alkane); 3375.17 (N-H Amine); 1705.43 (Carboxylic acid)		1075.69 (-OH Carboxylic acid-)
			n-Hex	2872.48 (C-H Alkane); 1717.59 (C-O Esters)
LGA10	**But**	1657.2 (C-H Alkenes); 2873.46 (C-H Alkanes); 2932.27 (C-H Alkanes)	**Met**	1632.08 (C=C Alkenes); 3329.92 (O-H Hydrogen-bonded alcohols, phenols)
	Ethyl	1447.05 (C-H Alkane); 1736.82 (C=H Aldehydes, ketones, carboxylic acid, esters) 2984.44 (C-H Alkanes)	**n-Hex**	1461.3 (C-H Alkanes); 1711.2 (C=O Aldehydes, Ketones, Carboxylic acid, Esters) 1741.23 (C=O Aldehydes, Ketones, Carboxylic acid, Esters); 2851.24 (C-H Alkanes) 2920.05 (C-H Alkanes)

But- Butanol, Ethyl- Ethylacetate, Met- Methanol, N-Hex - n-Hexane

In this study, FTIR analysis showed spectra of all extract indicating the presence of functional groups like hydroxyl group OH, carboxylic acid O–H, aldehyde/ketone C=O, alkane C-H, amine C–N which also were present in *Mammea suriga* (25) and in garlic cloves (28). In this study also, FTIR analysis showed a strong presence of hydroxyl group (OH) in all accessions (Table 2), (OH) stretching vibrations indicates phenolics which are a group of compounds containing hydroxyl functional groups (-OH) attached to an aromatic hydrocarbon. Phenolic compounds from natural resources displayed antifungal activity (29) and antioxidant activity. The number of hydroxyl groups found in the phenols is related to their relative toxicity towards microorganisms and also able to remove ROS with evidence that increased hydroxylation is directly proportion to toxicity or antioxidant activity (30). Also, carboxylic acids (-OH) were found in all accessions and it has been linked with many antimicrobial and antifungal activities which are found to exist in various plant metabolite molecular structures such as ursolic acid, which had been reported as a strong antibacterial agent (31).
Significant differences were not observed among the ten lemongrass accessions with regards to their total phenolic contents and antioxidant activities ($p > 0.05$). They also possessed almost similar functional groups in the four solvents used for partitioning. The use of the plant in the management of the various ailments can be attributed to the presence of the secondary metabolites which are mainly phenolics with very good antimicrobial and antioxidant properties. Most of the diseases can be implicated from ROS and mopping up these will give a relief or reduce the ailments.

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