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Characterization of Tumeric (*Curcumin Longa L.*), Neem Leaves (*Azardiractin Indica*), and Lemon Grass (*Cymbopogan Citratus*) as Natural Preservatives

Nyom P. N[1,2*], Itodo A. U[1], Wuana R. A.[1]

[1]*Department of Chemistry, Faculty of Physical Sciences, Joseph Sarwuan Tarka University, P.M.B 2373, Makurdi, 970101, Nigeria*
[2]*Department of chemistry, Centre for Food Technology and Research (CEFTER), Benue State University, Makurdi, Nigeria.*
** Email address: nyomp@yahoocom*
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ABSTRACT

Tumeric, neem leaves, and lemon grass were Soxhlet-extracted using n-hexane and methanol as extracting solvents. Maximum yields in methanol extracts were observed for both Tumeric, Neem leaves, and Lemon grass, with values of 36.30 ± 0.98, 42.82 ± 0.09, and 45.3 ± 0.16, respectively. The highest yield percentage was obtained from lemon grass. The result of the antimicrobial activity of the extracts showed that the tumeric extract exerts its highest inhibition zone on the *mucor* specie, 17.00±1.41 mm, following closely were saccharomyces, 16.00±0.00, *proteus*, 14.00 ± 1.41 mm while the least zone was on *bacillus* species, 8.00 ± 0.00 mm. Lemon grass on the other hand was most effective on *klebsiella*, 15.00 ± 1.41 mm followed by *staphylococcus* and *proteus* species, 14.50 ± 2.12 mm. On the other hand, Lemon grass extract demonstrated no antimicrobial activity against *Aspergillus* species, showing 0.00 mm. The trend is similar to the Neem leave extract, which is not likewise reactive against the *Fusarium* spp. Highly Statistical difference in the zone of inhibition of the three extracts on microbes, $p< 0.05$. Such extracts' chemical characterizations were performed using GC-MS, and consequently, many compounds with their antimicrobial, antifungal, and antioxidant activities could be identified. The main bioactive compounds that represent tumeric extract are tumerone and curlone. Linoleic acid is the major compound present in neem leaves extract. Compared to the tumeric and neem leaves, lemon grass extract revealed the highest number of compounds. The identified compounds are primarily hydrocarbons, fatty acids, alcohols, esters, and phenols, among others. It has been reported that turmeric, neem leaves, and lemon grass exhibit significant antimicrobial activities in terms of growth inhibition of pathogens and spoilage organisms accordingly.

Key words: Neem leaves, Tumeric, Lemon grass, Antimicrobial and Antioxidant

Introduction

In developing countries, the preservation of food with the use of herbs is still very popular and essential [1]. Such organic forms have always appealed to mankind. Synthetic food preservation techniques have been developed for a long time [2] and combine hydrothermal methods of preservation and handling, such as dry, cold and heat treatments. However, these typical methods of conservation have their drawbacks with respect to many factors such as cost, flavor, safety and efficiency.

The development and application of preservatives based on conventional herbs, spices, and their oils for the functional roles they play in protecting foods and people against microbes, pathogens, and food-borne infections could provide a possibility to reduce food security concerns [3]. Plant extracts are among the most effective organic resources for preventing the advancements of oxidative damage and preserving foods. Therefore, many other new fresh herbs extracts may serve as a good food preservative agent having antibacterial and antioxidant activities hence allowing the reduction or replacement of the use of synthetic preservatives in order to maintain food and quality. The study of natural products as a food preservative agent has grown significantly from both the researchers and individual points of view [4].

Various plant extracts or plant products are known to exhibit broad-spectrum antimicrobial activities. They can, therefore, be regarded as bio-preservatives without being harmful to human health [5]. Thus, herbal extracts have great potential to be used on fruits and most foods generally to give an extended shelf-life [6]. They are nontoxic and harmless. They are easy to apply and do not lose their potency at normal storage temperatures [7].

Use of herbal extracts has opened new avenue for control of spoilage. The extracts of garlic and ginger at 10 % concentration were found to be inhibitory for most of the bacterial and fungal isolates, [8]. *Tulsi* leaf extracts containing polyamine biosynthesis inhibitor blocked ornithine decarboxylase pathway that could be exploited to control fruit rots. Spraying of tomato fruits with 10 %, garlic and ginger extract retarded spoilage. Thus, significantly lower PLW % was recorded in the tomato fruits sprayed with 10% garlic extract followed by spray of 10% ginger extract. In this

line, fresh-cut tomato with improved shelf life using natural antimicrobials like herbal extracts maintained/increased the contents of lycopene, ascorbic acid, and total phenolic compound [10].

Curcumin longa L., Azadirachta indica, and Cymbopogon citratus are plants that have been used as food and medicine [11]. *Curcuma longa* L. contains a group of complex secondary metabolites known as curcuminoids, which consist of curcumin, demethoxycurcumin, and bis-demethoxycurcumin.

This is the most important *curcumin* extract for its biological activities, but it is rapidly degraded and low bioavailable because of its poor solubility. Medically, *curcumin* has been used as an anti-inflammatory, anti-arthritic, immune stimulant, and wound medicament [12]. It can also inhibit platelet aggregation. It is also a strong antioxidant, acting to quench reactive oxygen species.

Curcumin has better efficiency in reducing the toxic effects caused by various chemicals [13]. For this reason, the food is usually preserved against spoilage by adding *Curcuma longa* L. The insects' spoilage of preserved food is controlled by the neem tree, scientifically known as *Azadirachta indica*. Despite being used medicinally by the local populace, the isolated components of the tree possess unique properties [14]. These can be obtained from the roots, flowers, leaves, fruits, and stem barks of *A. indica*. Neem leaves were reported to have anti-bacterial, anti-cancerous, and anti-diabetic activities [15].

The leaf extracts can prevent the growth of other microorganisms. Aside from these, it is also found out that some *arithrinoids* or tetranortriterpenes, tablets, or fractionated purities, glycosides, polysaccharides, proteins, and amino acids, and limonoids, toxic effects of which are also used for controlling farm pests. The lemon grass, *Cymbopogon citratus*, which manifests bactericidal effects and can kill or reduce several kinds of bacteria including *Escherichia* coli, and *Streptococcus* mutants which may cause tooth decay, gum diseases, and bad breath [16].

Perennial lemongrass powder is also under control in the packaging of beef and feed and sheep meat due to grey mould. The flavour of its essential oil has been suggested to be good. These methods, though in need of development, are urgent; applying advanced detection techniques ensures quality and safety of the nanoemulsions in production.

The role of natural plant products has also been referred to in storage rot of mangoes, where the fruits immersed in the plant extracts showed less incidence of the disease [17]. *Azardiractin*, an active principle in neem oil, develops pectin molecules by removing the chances of removal of methyl group from the α-galactouronic acid residue of pectin. Thus, it helps in lowering the breakdown of pectin molecules during storage [18].

It was a study on the extraction of turmeric, neem leaves, and lemon grass and involved various aspects of their extracts for chemical and biological characterization to ascertain its potency in food preservation. This paper, however, looks at their efficacy as natural preservatives.

Materials and methods

Sample collection and pre-treatment

The Neem leaves and Lemon grass plant materials were air dried, then grounded into powdered form for extraction. The turmeric rhizome was first washed to clean, then peeled and dried, followed by grinding into powdered form for extraction.

Extraction of the plant materials

The dried and powdered samples of the Neem leaves, Lemon grass, and Turmeric, 300.0 g each were separately extracted using soxhlet apparatus with methanol and n-hexane as the extracting solvents in each case. The extract was evaporated to dryness under reduced pressure at 90 °C by a rotary vacuum evaporator to obtain the crude extracts, which were placed in dark bottles and stored in the refrigerator at 4 °C until use [19].

The yield estimation was calculated as follows;

$$\text{Yield (\%)} = \frac{weight\ of\ recovered\ extract}{weight\ of\ dry\ \ powder!}\ x\ 100$$

Phytochemical Quantification/Characterization.

The phytochemical quantification of the crude extracts was done using GC-MS to identify and quantify the bioactive components present in the extracts, [20].

Microbial Analysis/Characterization of the Extracts.

This was by the method of agar well diffusion. Broth media were measured and dissolved in an appropriate volume of distilled water, in line with the manufacturer's specification, and were sterilized by autoclaving. The pour plate technique, which is a method used for the isolation and enumeration of microorganisms in a sample by mixing the liquid sample with molten agar and allowing it to pour into a petri dish, was employed.

Approximately 1.0 cm^3 of standardized inoculum was introduced into the medium in a sterile container to ensure that the test organisms were well distributed, transferred into sterile petri dishes, and allowed to gel. All the plates received an equal volume of the media. Antibacterial activities of the plant extract were determined according to the standard agar-well diffusion method.

The wells on the agar medium were bored using a cork borer of 0.6 cm, and into the wells was dispensed 0.1 mL of the solution of the extract. The plates were incubated at 37 °C for bacterial activity. After 24 hours, the plates were observed for clear zones of inhibition. This, therefore, implied that

any clear zone of inhibition seen was because of the action of the extract. One control serving to test the activity of the solvent used to dissolve the extract was used to ensure that activity was not due to the action of the solvent on the test organisms. Plates were read by measuring any clear zones-an area without growth-of inhibition around the wells containing the extract.

Measurement was done from well edge to the end of clear zone of inhibition using millimeter measuring rules. The MIC and MBC of the crude extracts were done by a standard method where all the tubes showing no growth were plated out in their appropriate agar and incubated accordingly to observe those which would recover to form colonies. Those that did not recover to grow were regarded as bactericidal or fungicidal.

Results and discussion

Percentage yield of the extract

The results of the percentage yield of Neem leaves, Tumeric and Lemon grass extract using hexane and methanol as the extracting solvents are presented in Figure 1.

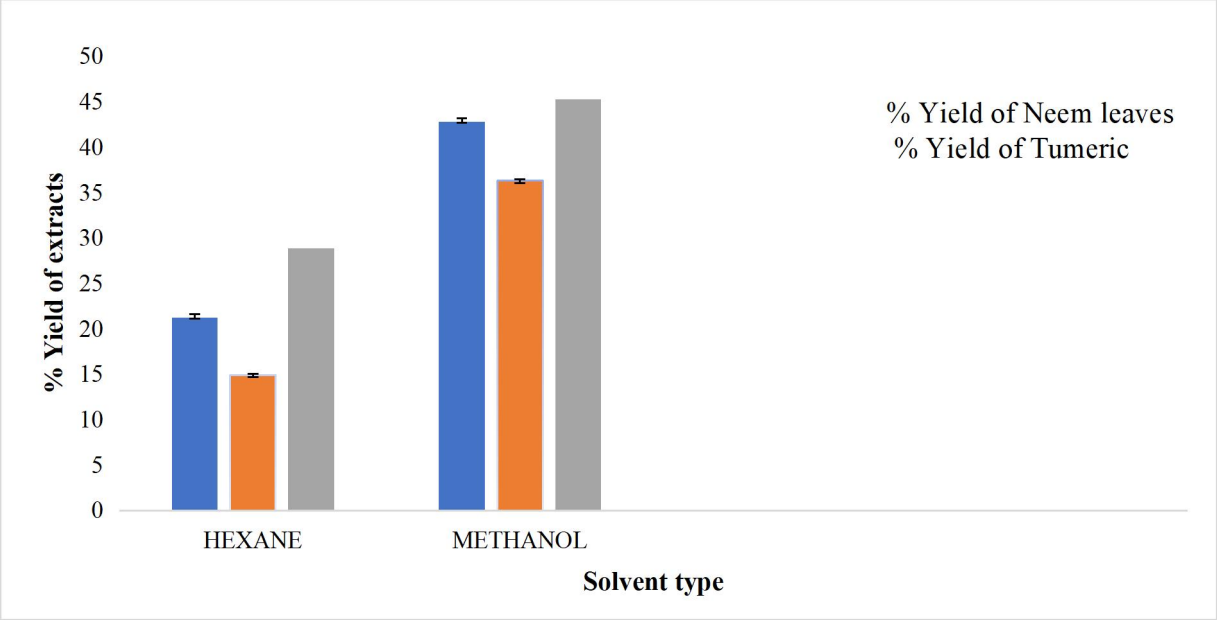

Figure 1: **% Yield of Neem Leaves, Tumeric, and Lemon Grass extract using Hexane and Methanol as Solvents**

Antimicrobial analysis

Table 1. Shows result of the antimicrobial analysis of Neem leaves, Tumeric and Lemon grass.

Table 1: Antimicrobial susceptibility test – Zones of inhibition (mm).

Microbial specie	Tumeric Extract	Lemon g. Extract	Neem leaves Extract
Staphylococcus	13.00 ± 1.4^{c}	14.50 ± 2.29^{b}	10.50 ± 2.12^{a}
Bacillus	8.00 ± 00^{d}	11.50 ± 0.71^{bc}	11.00 ± 1.41^{a}
Klebsiella	12.00 ± 1.4^{c}	15.00 ± 1.41^{a}	14.50 ± 2.12^{a}
Pseudomonas	$7.50. \pm 0.71^{bc}$	10.00 ± 0.00	13.00 ± 1.41^{a}
Proteus	14.00 ± 1.41^{bc}	14.50 ± 2.12^{ab}	12.00 ± 0.00^{a}
Saccharomyces	16.00 ± 0.00^{ab}	12.00 ± 1.41^{abc}	12.00 ± 0.00^{a}
Mucor	17.00 ± 1.41^{a}	10.00 ± 1.41^{c}	4.00 ± 5.66^{b}
Aspergilles	12.50 ± 0.71^{c}	0.00 ± 0.00^{d}	00.00 ± 0.00^{b}

Fusarium	13.50 ± 0.71^{c}	11.00 ± 1.41^{c}	00.00 ± 0.00^{b}
P-value	**0.00**	**0.00**	**0.00**

Values are mean ± standard deviation of triplicate determinations. Means within the sample column bearing different superscripts are significantly different ($p \leq 0.05$)

Phytochemical Characterization of the extracts

Tables 2, 3 and 4 and figures 2, 3 and 4 present results of the GC-MS for *Curcumin Longa L* (Tumeric), *A. Indica extract* (Neem) leaves and *Cymbopogan Citratus* (Lemon grass) respectively

Table 2: GC-MS Result for *Curcumin Longa L*. Extract (Tumeric)

Peak No	Retention Time (s)	Name of Compound	Molecular Formulae	Molecular Weight g/mol	Peak Area
1	15.753	Ar-tumerone	$C_{15}H_{20}O$	216.319	37.32
2	16.446	2-Methyl – 6 - (4-Methylenecyclohex -2- en-1-yl) hept -2-en-4-one	-	-	2.52
3	16.502	Curione	-	-	2.02
4	26.418	8 – Hexadecenal, 14-methyl	$C_{17}H_{32}O$	252.4	0.50
5	37.237	Oleic Acid	$C_{18}H_{34}O_2$	282.461	0.44
6	37.505	7 pentadecyne	$C_{15}H_{28}$	208.38	0.21
7	37.676	9- oxabicyclo [6.1.0] nonane	$C_8H_{14}O$	126.196	0.26
8	37.740	9- ocatadecenoic acid	$C_{18}H_{34}O_2$	282.461	0.21
9	37.882	6- octadecenoic acid	$C_{18}H_{34}O_2$	282.461	0.39

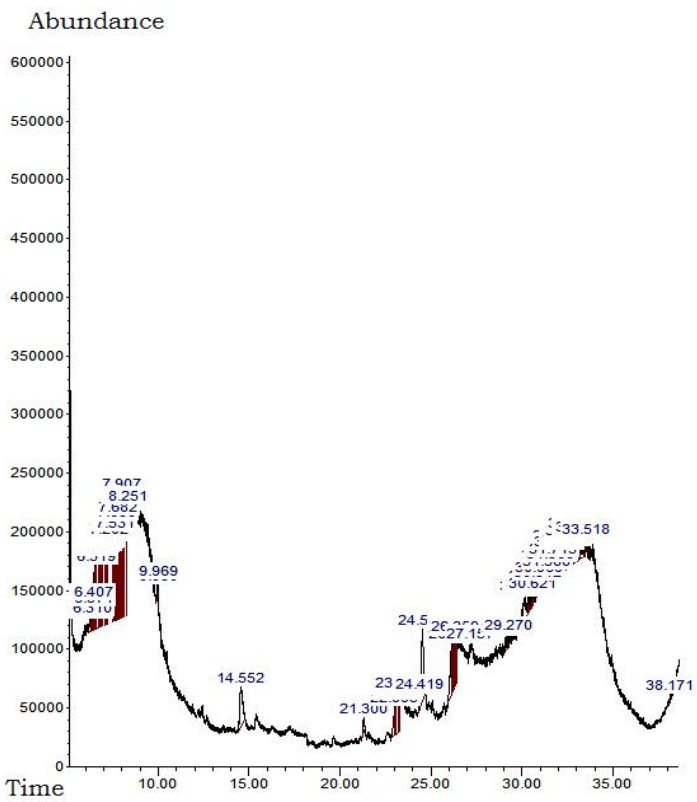

Figure 2: GC-MS Chromatogram for *Curcumin Longa L*. extract. (Tumeric)

Table 3: GC-MS Result for *A. Indica extract* (Neem Leaves)

Peak No.	Retention Time (s)	Name of Compound	Molecular Formulae	Molecular Weight g/mol	Peak Area
1	19.679	2 Underanone	$C_{11}H_{22}O$	178.29	0.58
2	21.389	Methyl ester	$C_{19}H_{58}O_2$	298.3	1.86
3	23.297	n- Hexadecanoic acid	$C_{16}H_{32}O$	256.22	1.01
4	24.570	6,9,12 octadectrien –i-ol	$C_{18}H_{32}O$	264.4	2.12
5	33.967	Oleic acid	$C_{18}H_{34}O_2$	252.5	1.73
6	36.484	13 octadecenal	$C_{18}H_{34}O$	266.47	1.77
7	36.615	3, 8, nonadien – 2-one	$C_9H_{14}O$	138.21	2.56

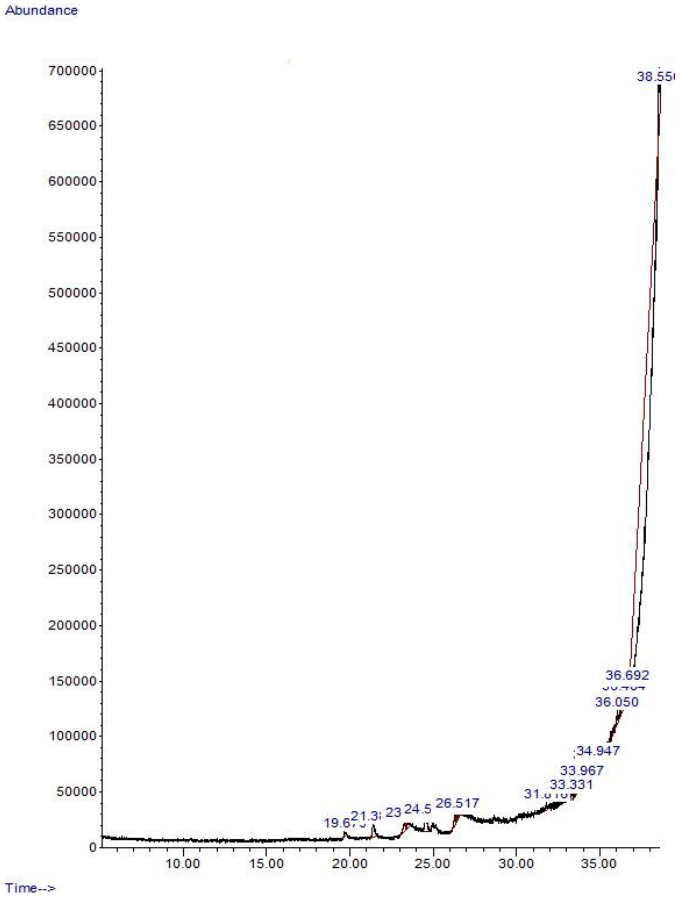

Figure 3: GC-MS chromatogram for *Azadirachta indica* (Neem leaves) extract

Table 4: GC-MS Results for *cymbopogan citratus* extract (lemon grass)

Peak No	Retention Time (s)	Name of compound	Molecular formulae	Molecular weight (g/mol)	Peak area %
1.	6.374	1-methoxy-3-hydroxymethylocatane	$C_9H_{18}O_3$	174.24	0.25
2.	6.732	2,6-Ocatediene	C_8H_{14}	110.20	1.29

3.	6.799	2-methlyl-z,z-13-octadecadienol	$C_{19}H_{36}O$	280.5	1.51
4.	7.587	5-octadecene	$C_{18}H_{36}$	252.5	2.32
5.	7.638	9-octadecadecenal	$C_{18}H_{34}O$	266.5	1.55
6.	7.682	6-tridecene	$C_{13}H_{26}$	182.35	1.55
7.	14.552	Selin-6-en-4 alpha-ol	$C_{15}H_{20}O$	222.37	3.51
8.	21.300	Hexadecanoic acid , methyl ester	$C_{17}H_{20}O$	270.45	0.63
9.	22.993	n-Hexadecanoic acid	$C_{16}H_{32}O$	256.42	1.32
10.	29.270	Cycloeicosane	$C_{20}H_{40}$	280.5(17)	0.07
11.	30.621	Cyclopropaneoctanal,	$C_{11}H_{20}O$	168.28	0.25
12.	31.540	E-9-Hexadecenal	$C_{16}H_{30}O$	238.41	0.27
13.	31.672	Oxacyclotetradecane-2.	$C_{13}H_{22}O_3$	226.31	0.95
14.	32.019	z-8-methyl-9- tetradecenoic acid	$C_{15}H_{28}O_2$	240.38	0.66
15.	33.218	Palmitoleic acid	$C_{16}H_{30}O_2$	254.41	1.24
16.	32.461	7,11-Hexadecadienal	$C_{16}H_{28}O$	236.39	0.38
17.	33.151	13-octadecenal	$C_{18}H_{34}O$	266.5	0.38

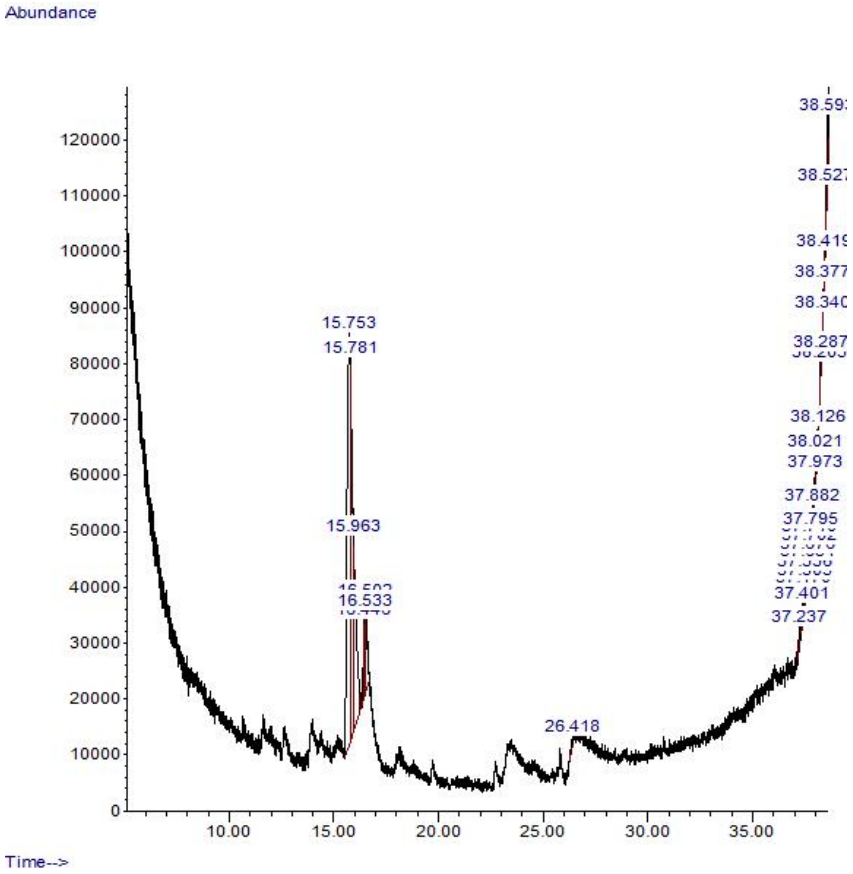

Figure 4: GC-MS chromatogram for *cymbopogan citrates* extract (lemon grass)

The overall yields for the three plants were in methanol extracts, with values of 36.30 ± 0.98, 42.82 ± 0.09 and 45.3 ± 0.16 respectively, while hexane extracts gave the minimum yield with values of 14.9 ± 0.14, 21.82±0.35 and 28.85 ± 0.15 as shown in figure 1. Methanol was found to give maximum yield in all three plant extracts. This can be attributed to the higher solubility of compounds in methanol than other solvents; also, methanol is a polar

solvent. The results obtained agreed with that of Amanpreet, (2016) and Barchan et al., (2014), who reported that percentage yields of two cultivars of turmeric extracts and three men the sp.

It can be noticed from the above figure that the polarity of the solvents increased, the yield of extracts prepared using the solvents: hexane, dichloromethane, methanol, and water. A similar study carried out by Garba et al. 2020, showed that methanol gave the highest percentage yield of plant extract-Commiphora Africana, 47.50% amongst other solvents.

From the result, it was observed that lemon grass showed better percentage yield of 45.3 ± 0.16 compared to neem leaves and turmeric.

This effect is most probably due to a summation of polyphenol adsorption to the bacterial membranes with accompanying membrane disruption and leakage of the cellular contents, besides generation of hydrogen peroxides from the polyphenols.

From the antimicrobial analysis used to determine the susceptibility or otherwise of microbes against the extracts of Tumeric, Lemon grass, and Neem leaves, as presented in Table 4.1, it could be seen that the highest inhibition zone expressed by the Tumeric extract was on *mucor* species which is 17.00 ± 1.41 mm, next is saccharomyces with 16.00 ± 0.00, *proteus*, 14.00 ± 1.41 mm, while the least zone was on *bacillus* species which is 8.00 ± 0.00 mm.

On the contrary, Lemon grass was most effective on *klebsiella* with a zone of inhibition at 15.00 ± 1.41 mm, followed by *staphylococcus* and *proteus* species at 14.50 ± 2.12 mm. Conversely, Lemon grass extract does not show any antimicrobial activity against *Aspergillus* species with 0.00 mm. The same trend is found in Neem leave extract, which is also non-reactive to *fusarium* spp. There is a significant difference in the zone of inhibition of the three extracts on microbes at $p< 0.05$.

These results would, therefore, imply that tumeric extract exhibited antimicrobial activities against both the fungi and bacteria isolated, lemon grass against all with the exception of *Aspergillus* spp. while neem leaves reacted against all with the exception of *Aspergillus* spp. and fusarium spp. This explains the ability of these extracts to extend the shelf life of the fruits by preventing such microbes from acting on the mango fruits hence their preservation.

Similar results were reported by, where the antimicrobial activity of tumeric extracts was tested against *E. coli, S aureus, S. typhi,* and *C. albicans* by disc diffusion method. This is reported to be due to the presence of some essential oils, which are responsible for the antimicrobial activity of turmeric. Fatima, et al., 2022, equally reported that lemon grass extract tested for antimicrobial activities against *bacillus cereus, E. coli, Klebsiella, staphylococcus aureus,* and *Candida albicans* were all positive.

GC-MS analysis was done to identify the chemical constituents of the plant extracts. The chromatogram of the methanolic extract of tumeric, neem leaves and lemon grass are presented in Figures 2, 3 and 4 respectively. The mass spectra are also depicted in Tables 2, 3 and 4 respectively. The spectrum of the compounds was compared with the database spectrum of known compounds stored in the GC-MS library. The result indicated that most of the compounds detected have antimicrobial, antifungal, and antioxidant activities. It has been observed from the literature that these compounds act as preservatives by being antimicrobial in action.

The major bioactive compounds in tumeric extract are tumerone and curlone. Other compounds include 6 and 9-octadecenoic acid, 7-pentadecyne, oleic acid, etc. Tumerone is an aromatic compound, which is also found to be a natural antibiotic, anti-tumor, and immune-activating agent; it's a membrane stabilizer and inhibitor surfactant.

Neem leaves extract shows the presence of compounds like 2-udecanone, 6,10-dimethyl oxirane, methyl ester ($C_{19}H_{38}O_2$), n-Hexadecanoic acid (synonym; palmitic acid, ($C_{16}H_{32}O$), oleic acid, nonadecane, 6,9,12-octadecatrien-1-ol (synonym linoleic acid, ($C_{18}H_{34}O$), etc. among which linoleic acid is the most abundant compound present. Literature study reveals that most of the predominant compounds are biologically active molecules used as natural antioxidant and antimicrobial agents. Methyl ester is a typical food preservative. Hexadecanoic acid is an inhibitor and a plant metabolite. Linoleic acid has been known for some activities like its antimicrobial, antifungal, anti-inflammatory, among others, Pandey et al., 2018.

It was observed that the number of compounds in lemon grass extract is the highest compared to tumeric and neem leaves. The classes of compounds identified were mostly hydrocarbons, fatty acids, alcohols, esters, and phenols. Some of the compounds in the lemon grass extract include Hexadecanoic acids, palmitoleic acid, selin - 6 - en - 4 alpha - ol, 5-octadecene, 6-tridecen, methyl ester,2,6-octadiene, octadecenoic acid, eicosane aldehyde amongst others. These compounds are responsible for C. citratus being an effective anti-microbial and antioxidant natural bioproduct widely applied to food protection, replacing the use of synthetic compounds (Fatima, et al., 2022). The essential oil of lemongrass protects the sensory property of food and inhibits the activity of microbes preventing deterioration, hence, preserving quality and prolonging the shelf-life of food products.

Similarly, this preservative effect of LEO can be attributed to its active constituent citral (an aldehyde) forming the major portion of LEO,, Mukarran et al., 2021. Antioxidative activity of lemon grass is because of the synergistic effects of its active principles. A study conducted concerning the utilization of lemon grass ethanolic extract on the storage of cooked and shredded chicken breast showed that the water activity in the product did not show any alteration by adding lemon grass extract, Keiling, et al., 2021.

Conclusion

Therefore, based on this study, recommendations can be made for turmeric, neem leaves, and lemon grass as natural food preservatives. Besides, the use of natural preservatives has the additional benefits of increasing health safety in food products, thereby reducing synthetic additives and chemicals in general, which can also lead to a healthier and cleaner food industry.

The prospects of using turmeric, neem leaves, and lemon grass as natural preservatives that can be applied to improve the quality and safety of food products have been very encouraging.

Further research into the fields for their optimal concentration and methods of application in different food products is required. Besides, regulatory approval and consumer acceptance are important variables that would need consideration before natural preservatives can be widely used within the food industry.

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