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Synthesis, Characterization and Antifungal Activitivies of Copper Complex of the Aqueous Extract of CYMBOPOGON CITRATUS (Lemon Grass)

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Abstract
Copper complex was synthesized from copper chloride and lemon grass extract in the ratio of 1:5 (metal to ligand) and characterized. The physicochemical parameters ranging from pH, conductivity, melting boiling, boiling point and solubility of the complex in water, ethanol, diethyl ether, methanol, and acetone were studied. Whilecharacterization of the synthesized complex was done using: FT-IR, UV-VIS, XRD and XRF spectroscopic methods. The complex was soluble in water and insoluble in organic solvents, with a basic pH of 8.87 and conductance of 12.35 mho cm^2 mol^{-1} making the complex a non-electrolyte. The melting and boiling point of the complex was 165.8 ^{0}Cand 188.8^{0}Crespectively. It was a black complex. The lemon grass extract was also screened for its secondary metabolites constituents and the result revealed it contained: alkaloid, tannins, flavonoids, carbohydrates and steroids. The FT-IR spectrum reveals bands at 3398(O-H), 2924 (C-H),1635cm^{-1} (C=O), and 1404cm^{-1} (C-C) which was shifted other frequencies in the complex with a band 468cm^{-1}v(M-O) indicating complex formation. The XRD data revealed complex is crystalline and XRF showed elemental composition of both extract and complex. Both the extract and complex showed antifungal activities against test organisms.

Keywords: ***Synthesis, characterization, antifungal, Complex, lemongrass***

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I. Introduction

Natural resources including plants and their extract have been used in folk medicine for the treatment of many diseases (Vishu*et al*., 2013). Medicinal plants are plants which are believed or tested to have components which are capable to heal diseases, wounds or other ailment affecting humans. Hence plants have been used in traditional medicine science pre-historic (El-seed *et al*., 2013) and the knowledge passed from generation to generation.Plant synthesizes hundreds of chemical compounds for function against insects, fungi, diseases and herbivorous mammals (WHO, 2003).

Sona*et al.* (2012) reported that medicinal plants and their components possess a range of beneficial preventive properties showing promising effect on different health problems such as coughs, cold, throat irritations, stomach ache, indigestion as well as gastro-internal diseases. Also they have positive protecting activities such as spalmplytic, sedative, antiviral, anti-inflammatory, anti-septic, hepatoprotective, anti-hyperglycemic and immunostimulating.Medicinal plants are widely used in non-industrialized societies because the cheaper and readily available than modern medicine and in rural areas, local tradition medicine is the only source of care for people and in the developed countries, alternative medicine including use of dietary supplements is marketed using the claims of traditional medicine.

Transition metals exhibit different oxidation states and can interact with a few negatively charged molecules. These properties of transition metals led to the development of metal-based drugs with promising pharmacological application and unique therapeutic opportunities. The advances in inorganic chemistry provide better opportunities to use metal complexes as therapeutic agents. The use of transition metal complexes as therapeutic compounds has become more and more pronounced. These complexes offer a great diversity in their action such as; anti-inflammatory, anti-infective and anti-diabetic compounds. Considerable efforts are made for the development of transition metal complexes as drugs. Beside several limitations and side effects, transition metal complexes are still the most widely used chemotherapeutic agents and make a large contribution to medicinal therapeutics. The therapeutic properties of metals when bound to organic compounds mainly plants which are rich in phytochemicals with great therapeutic properties, is proven to possess better potential activities

in the areas of biological, clinical, analytical, catalytical, microbial, insecticidal, antibiotic, growth factors, food additives, tumor inhibitor, cell division. This could be attributed to either the unused coordination sites in the metal and ligand system or due to the selective oxidation state of the complex ions in the co-ordination sphere, (Cotton and Wilkinson 1996).

II. Materials And Methods

The sample *cymbopogoncitratus* (Lemon grass) was collected from a farm in Jengre, Bassa LGA of Plateau State and transported in a polythene bag to chemistry department Laboratory University of Jos, where it was handpicked to removed other plant that are harvested with the sample and wash with Tap water thoroughly and then distilled water to removed dirt. The sample was then chopped into smaller parts and air dried under shade at room temperature. It was then ground into fine powder usingpestle and mortar, the fine powder stored in plastic containers for future use.250g of the ground dried sample was weighed and poured into a glass container in which distilled water was added. The mixture was kept for five days with manual agitation each day for adequate extraction, after which it was filtered with a lint free cloth and filter paper to obtain a pure and clear extract.The filtrate was then concentrated to complete dryness, the weight of the dry concentrate measured and kept in a sample bottles for further used.

Synthesis of copper Complex

The metal salt of copper chloride was dissolved in water and plant extract dissolved in sodium hydroxide and mixed in a 1:5 metal to ligand ratio. The salt solution was added drop wise to the extract to avoid precipitation. The solution was heated for 30 minutes at control temperature 60^0C on a heating mantel, it was then allowed to dry to obtain the complex which was packed and packaged in sample bottles for characterization. Same was done for the copper complex

Characterization of complexes

The synthesized complex and the extract were characterized employing the used of different analytical procedures: physical properties (color, boiling and melting points, pH, Conductance), elemental analysis, UV-Vis, FTIR, XRD, XRF and percentage yield. the instrumental analysis where carried out at NATIONAL RESEARCH INSTITUTE FOR CHEMICAL TECHNOLOGY, ZARIA and analysis for their physical properties were carried out at the Post Graduate Laboratory, Chemistry Department and the antimicrobial assay conducted at Microbiology Department of University of Jos, Jos Nigeria.

III. Results and Discussion

Table 1:physicochemical parameters of Copper complex

Physicochemical parameters	Copper complex
Water(25^0C)	Very soluble
Methanol(25^0C)	Slightly soluble
Ethanol(25^0C)	Sparingly soluble
Diethyl Ether(25^0C)	Insoluble
Acetone(25^0C)	Sparingly soluble
pH(25^0C)	8.87
Conductivity	12.35 mho cm^2 mol^{-1}
Melting point	165.8^0C
Boiling point	188.8^0C
Color	Black

The physical properties of the synthesized copper complex was studies and the result reveals the complex is only soluble in water and slightly soluble in methanol, ethanol acetone and insoluble in diethyl ether, the pH of the complex is 8.87 which indicated the complex was form in a basic condition, it is a non-electrolyte with conductivity of 12.35 mho cm^2mol^- . the melting and boiling points of the complex are 165.8^0C and188.8^0C which is lower than that of the pure copper metal .

Table 2: Phytochemical screening of Extact and copper complex.

Class of phytochemicals	Lemon Grass Extract	Copper Complex
Alkaloids	+++	_
Saponins	_	_
Tannins	+++	++

Flavonoids	+++	+++
Carbohydrates	+	_
Steroids	+	+
Terpenes	_	_
Anthraquinones	_	_
Cardiac glycosides	_	_

Key: +++ = Highly present, ++ = Moderately Present, + = Present, - = Absent

The result of the aqueous extract of lemon grass revealed the presents of alkaloid, tannins, Flavonoids, carbohydrates and steroids which is in agreement wth the constituents gotten by(Abbas etal., 2019;Amer and Baida, 2018). These secondary metabolites have been found to possess pharmacological activities which make it very suitable and effective for used of plants in traditional medicine for treatment of some diseases cause by microorganisms. Medicinal value and property differs for all the phytochemicals, as it is reported Saponins, Terpernoids, Tannins and steroids have anti-inflammatory effects (Liu, 2003), Glycosides and tannins have hypoglycemic effects(Udeozo Etal,2015), Flavonoid has antioxidant properties which enhance effective functioning of the immune system. Tannins also can serve as antidote to poisoning and helps healing of fresh injury and also posse's antiseptic properties which serves to expel parasites. Saponins effects hydrolysis of red blood cells which has made it been used to treat a number of cardiovascular disorder and ease the process of digestion (Ashutosh, 2010).

Table 3:FT-IR Absorption Wavelength and Functional Group Assignment

Assigned Functional Group	**Extract cm^{-1}**	**Copper complex cm^{-1}**
O-H stretching of Alcohol	3398	3404
C-H Stretch Alkanes	2924	2926
C=N nitriles	2370	2366
C=O carbonyl group	1635	1631
C-C stretch in rings aromatic	1404	1570
C-H Wag(-CH_2X)	1271	1413
C-N Stretch Aliphatic Amines	1124	1116
N-H stretch of amines	1041	1028
C-H "oop"	773	879
v(M-O)		615

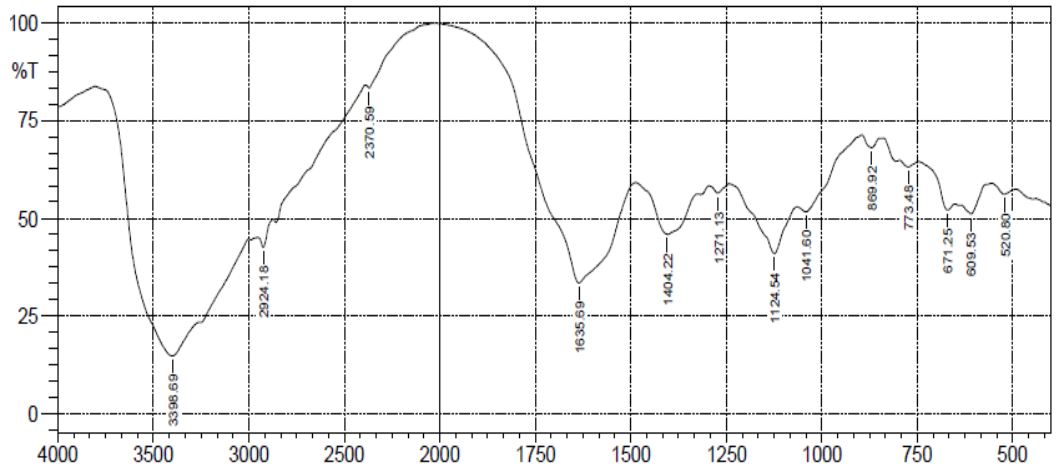

Figure 1: FT-IR Spectrum Lemon Grass Extract

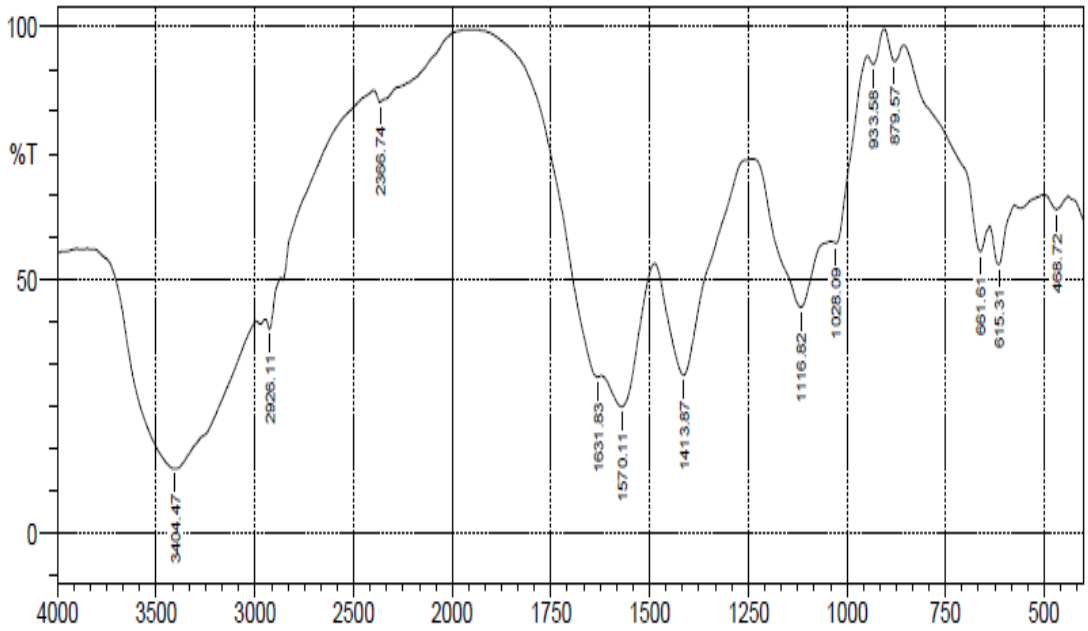

Figure 2: FT-IR Spectrum Copper complex

FT-IR analysis was done using a Shimadzu Fourier transform Infrared Spectrophotometer. Samples were weight-in at 0.01 g and homogenized with 0.01 g KBr anhydrous by mortar agate. The FT-IR of the extract and complex were compared which provided information on site of coordination of the ligand and the metal(Rathakrishnan et al., 2014). The IR spectra are given in fig 1 and 2 above. The interpretation of the IR is based on the most important band of absorption because of the complex nature of the extract with many functional group, the complex showed absorption in the region above 3200cm^{-1} for the ligand and metal complex associated with the hydroxyl group (O-H), 2924 assigned to (C-H) the band at 1635cm^{-1} is assigned to (C=O), and 1404cm^{-1}(C-C), all these bands are present in the spectrum of the complex but shifted to lower frequencies, with a new band at 615cm^{-1} v(M-O) which established the coordination of the ligand to metal ion(Sani and Dailami., 2015)

Table 3: UV-VIS Analysis

Sample	Wavelength(nm)	Band Assignment
$CuCl_2$	285.00-368.20	
Cu-Lemon grass complex	205.00-368.20	$n\rightarrow\pi^x$, L→M(LMCT)

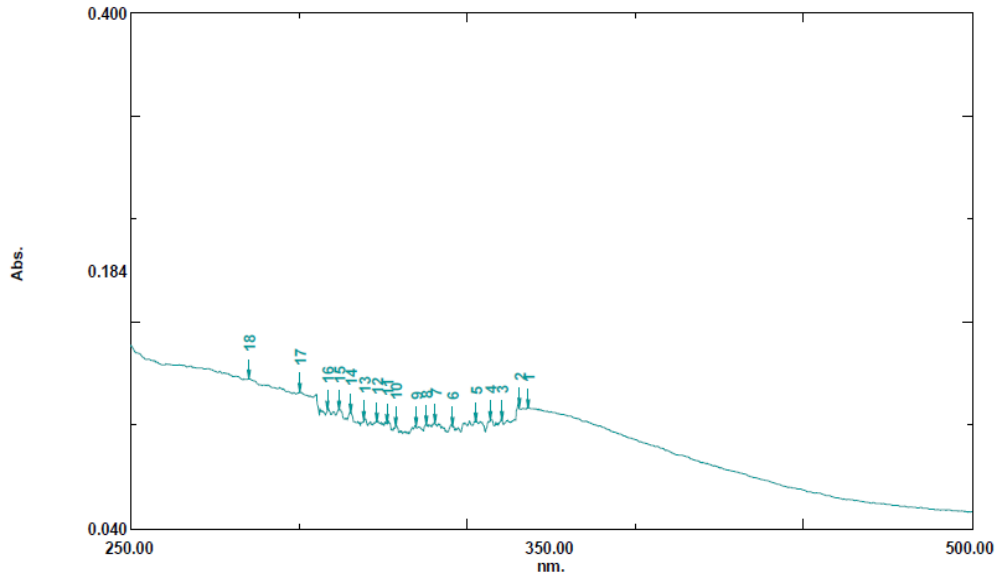

Figure 3a UV.vis spectrum of copper chloride.

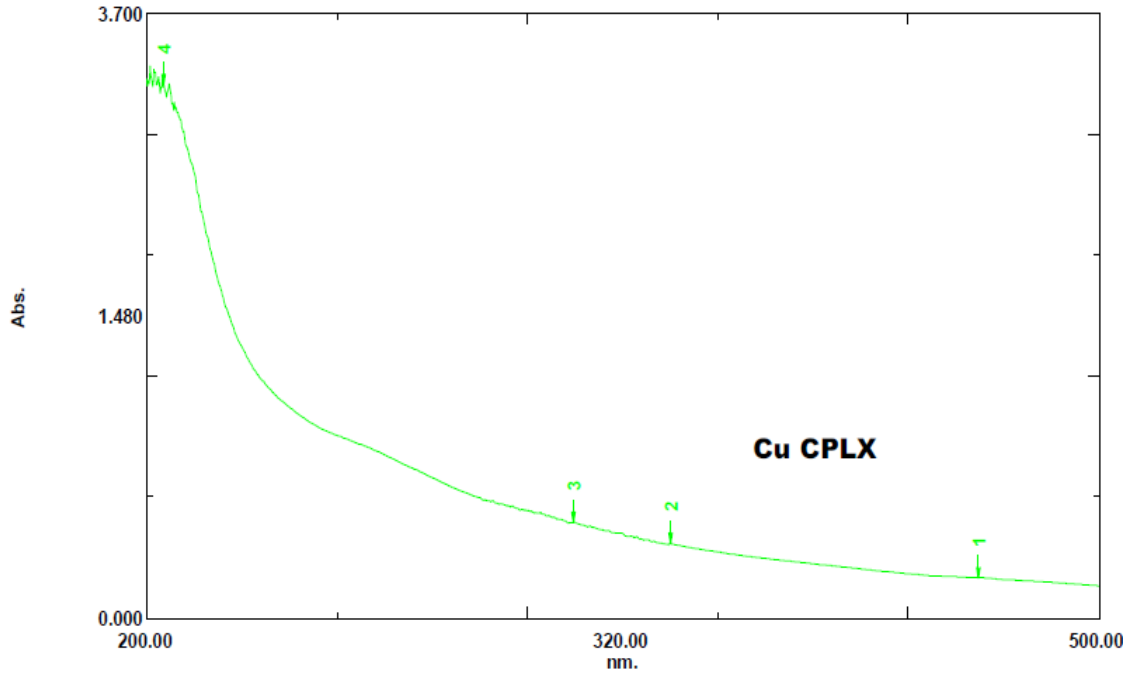

Figure 3b. UV-vis spectrum of copper(ii) complex

The U-V-visible spectra of the copper chloride and complex was recorded in DMSO and results presented as above. The copper salt has showed absorptions at 285.00nm and 368.20nm as minimum and maximum wavelength and the complex 206.00nm and 462.00nm which indicated an increase in the maximum wavelength of the complex to a higher wavelength. The increase in the absorbance in the spectra of the complex is attributed to the complexation of the ligand to the metal ion which confirmed the coordination of the ligand to the metallic ion. The absorption band at 485.00nm and 462.00nm were assigned to Ligand Metal charge transfer.

Table 4:XRF analysis of extract and complex

Elements %	Mg	Al	B	P	K	Cu	Ca	Fe	Zn
Extract	1.0651	2.5319	1.8862	1.1217	34.5662	0.0000	6.2774	0.1429	0.0733
Cu-complex	0.1290	1.4311	1.4311	0.5140	0.2841	9.0486	0.2828	0.1238	0.5902

The result for elemental analysis of the extract is presented in table 4 above and fig below. Plants apart from being good source of organic constituents are been reported to contain also inorganic components bio-accumulated from their habitat. From the result it indicated that the elements present were at tolerable limited and the increased in the content of the copper in the copper complex compared to that of the extract could be attributed to the complex formation. The result also validate that antifungal effect of the extract is because of its rich phytochemicals and not of toxic elements accumulated by the plant and hence the complex.

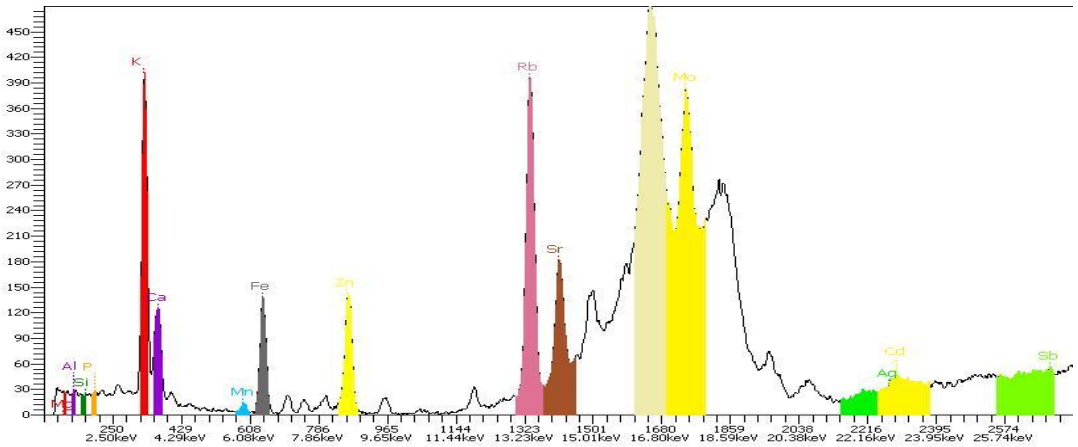

Figure 4: XRF spectrum of Lemon Grass Extract

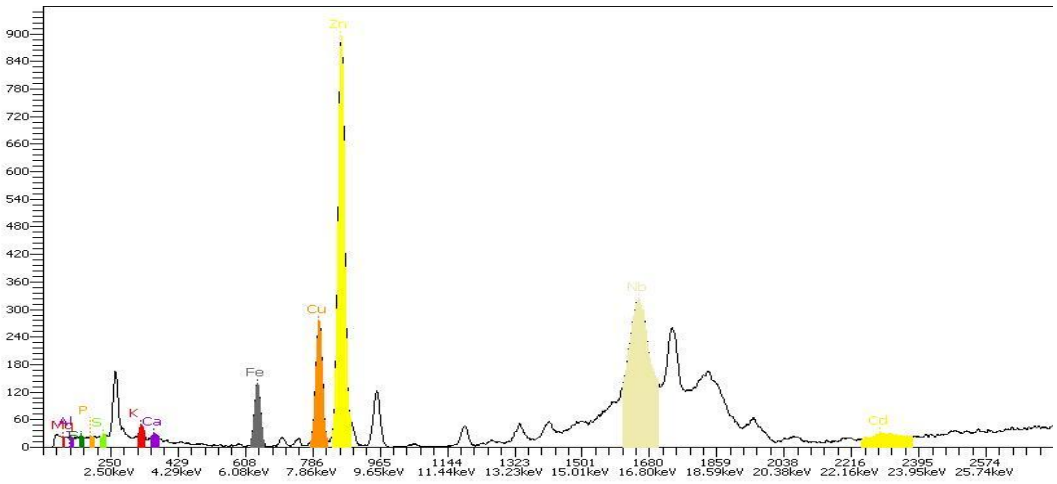

Figure 5: XRF Spectrum of Copper Complex

XRD ANALYSIS OF COPPER COMPLEX

The XRD of Copper complex of lemon grass was made with the help of X-ray diffractometer with Cu as the anode material. K-alpha [Å] =1.54060 and the generator setting 30mA, 40kV. Powdered XRD pattern of the metal complex show sharp crystalline peaks indicating the complex is crystalline in nature.

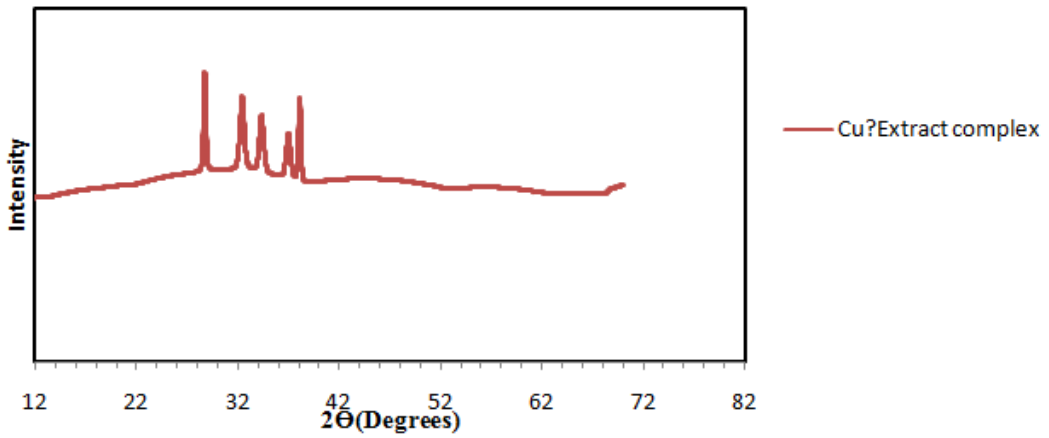

Figure 6:XRD Spectrum for Copper Complexes

ANTIMICROBIAL ASSAY

The antimicrobial activities of lemon grass extract and its copper complex was studied for its efficacy and the result presented below.

Table5: Zone of Inhibition of Plant Extracts on Different Plant Extract and Fungi

Plant Extract	**Mg/mL**	**Microorganism (Fungi)**	**Zone of Inhibition (mm/Mg)**					
			200	**100**	**50**	**25**	**12.5**	**Nystatin**
A. Lemon Grass Extract		*Aspergilusflavus*	20	18	13	9	0	26
		Aspergilusniger	21	18	14	7	0	24
		Fusarimspp	19	16	12	9	0	24
		Penicilliumspp	20	15	11	6	0	25
B. Copper Complex		*Aspergilusflavus*	26	24	18	14	8	24
		Aspergilusniger	27	22	18	15	7	25
		Fusarimspp	27	23	17	14	7	24
		Penicilliumspp	26	22	15	9	5	25

Key C= Positive Control, NYSTATIN =Standard Antifungal Drug

Table 6: Minimum Inhibitory Concentrations (MIC)

Plant Extract	**Microorganism (Fungi)**	**Zone of Inhibition (mm/Mg)**					
		200	**100**	**50**	**25**	**12.5**	**MIC**
A. Lemon Grass Extract	*Aspergilusflavus*	-	-	+	+	+	100
	Aspergilusniger	-	-	+	+	+	100
	Fusarimspp	-	-	+	+	+	100
	Penicilliumspp	-	-	+	+	+	100
B. Copper Complex	*Aspergilusflavus*	-	-	-	+	+	50
	Aspergilusniger	-	-	-	+	+	50
	Fusarimspp	-	-	-	+	+	50
	Penicilliumspp	-	-	-	+	+	50

Key: MIC= Minimum Inhibitory, - = No Turbidity, + = There is Turbidity

Table 7: Fungicidal Concentration of Plant Extract, Zinc and Copper Complexes of Fungi

Plant Extract	Microorganism (Fungi)	Zone of Inhibition (mm/Mg)					
		200	100	50	25	12.5	MFC
A. Lemon Grass Extract	*Aspergilusflavus*	-	+	+	+	+	200
	Aspergilusniger	-	+	+	+	+	200
	Fusarimspp	+	+	+	+	+	-
	Penicilliumspp	+	+	+	+	+	-
B. Copper Complex	*Aspergilusflavus*	-	-	+	+	+	100
	Aspergilusniger	-	-	+	+	+	100
	Fusarimspp	-	+	+	+	+	200
	Penicilliumspp	-	+	+	+	+	200

Key= No Growth, + = there is growth on the plate with microorganism, MBC = this is the final concentration that kills the microorganisms.

The result indicated that the extract and the complex have inhibitory activity on the tested fungi: *Aspergillusflavus, Aspergillusniger, Fusarium SPP and Penicllium SPP* at almost all concentration based on the observation of zone of inhibition. At high concentration (200mg/ml) the copper complex exhibited the highest activity which produced almost the same zone of inhibition with the control drug Nystatin. At the lowest concentration (12.5mg/ml) the plant extract exhibit no activity against the four fungi as indicated from the observation of zone of inhibition. The result obtained for the MIC of plant extract against all fungi was found to be 100mg/ml and the MIC for copper complexe was found to be 50mh/ml. minimum inhibitory concentration is very important and necessary in evaluating the antimicrobial activity as a guide towards predicting the efficacy of a promising product. The Plant extracts has MFC for *Aspergillusflavus* and *Aspergillusniger* at 200mg/ml and fungistatic for *Fusarium SPP* and *Penicillium SPP* while the copper complex has fungicidal activity for *Aspergillusflavus* and *Aspergillusniger*at 100mg/ml and for *Fusarium SPP* and *Penicillium SPP* at 200mg/ml. The results showed that the copper complex has better fungicidal activity compared to plant extract and zinc complex.

IV. Conclusion

The extraction of lemon grass and synthesis of its copper complex was successful with the following results obtained: The complex was found to be soluble in water with a pH 8.87 of which is basic and a conductivity of12.35 mho cm^2 mol^{-1} making it a non electrolyte. The phytochemical screening of the extract revealed the extract has flavonoids, alkaloids, tannins and steroids. The complex was found to be crystalline in nature with inhibitory activity on the tested organism at 200mg/ml.

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