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Phytochemical and antimicrobial Screening of methanolic Extract of leaf and bark of three varieties of Terminalia catappa lin

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The phytochemical constituents of the leaf and bark of the varieties of Terminalia catappa Linn (red, pink, and white) was analyzed using gas chromatography/mass spectrometry method. A total of 80 constituents were identified in the six methanolic extracts. Cis-oleic acid was identified with highest percentage, 40.85% in the leaf Terminalia cattapa red variety followed by erucic acid, 31.76% in the leaf of T.cattapa pink variety, 25.82% in the leaf of T.cattapa white variety and methyl(16E)-16-octadecanoate, 21.84% in the leaf of T.cattapa white variety. Methyl tridecanoate and methyl heneicosanoate are present in all the six crude extracts. All the six extracts were found to be active against Escherichia coli, Klebsiella pneumonia, Proteus aeruginosa, Streptococcus agalactiae, Staphylococcus aureus, Salmolenella typimurium and Proteus mirabilis at a concentration of 1000, 500 and 250µgml-1 except staphylococcus aureus at 250µgmol-1 in the leaf of T.catappa pink variety. This study, therefore, serves to justify the use of the leaf and bark of this plant in herbal medical practice.

Keywords: Terminalia catappa, phytochemical constituent, methanolic extract

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INTRODUCTION

Terminalia belongs to the family Combretaceae and is a pantropical genus comprising 150 species [1]. *Terminalia catappa* (tropical almond) is deciduous moderate-sized tree (10-35m) tall, with pagoda-like habit, particularly when the tree is young. Stem often buttressed at the base, diameter up to 1.5m., bark is dark grey-brown and the fruit is an ovoid or ellipsoid drupe, slightly flattened, with a prominent keel, usually glabrous, green to yellow and red at maturity but other varieties remain as green or yellow at maturity. The fruit looks like a stone surrounded by a 3-6 mm thick layer of juicy flesh. Fruits vary greatly in shapes, sizes, and colour. The quality of the fruits differs considerably, the flesh being edible and sweet to bitter [1]. *T.catappa* is native to southeast Asia but is now widely planted throughout the tropics, including west and east Africa [2]. In Nigeria, there are majorly three varieties of this plant, the classification based on the colour of the fruit when the mesocarp is removed. These are red, pink and white varieties, which are common in the southwest part of the Country.

Tropical almond is a multipurpose tree. The bark and leaves and sometimes roots and green fruits are locally used for tanning leather and provide a black dye used for dyeing cotton and as ink. The juice of its fresh leaves is used in the preparation of medicinal lotion for leprosy and scabies and it is taken internally for stomach ache and headache [3]. The fruit and the seed within the fruit are edible when fully ripe. The seed contains a pale odourless oil, similar to almond oil. The oil is employed medicinally as a substitute for true almond oil to relieve abdominal inflammation. The timber is of good quality and is used for house and boat building because it is susceptible to termites [2,4 , 5].

[6] identified various phytoconstituents from the fruits, seeds, and barks of *T.catappa*, the bark contains glycoside, cardiac tannins, volatile oils, saponin, steroid, glycosides and phenols. Recently [7] isolated punicalagin (Polyphenol), its derivative and other several compounds in the leaves of *T.catappa*. the leaves contain 1-degalloyl-eugeniin, 2,3-(4,4,5,5,6,6-hexahydroxy-diphenoyl) – glucose, chebulagic acid, gentistic acid corilagin, geraniin, granatin B, kaempferol, punicalagin, punicalin, quercetin, tercatain, tercatain, tergallagin, terflavin A and terflavin B. [8] also identified quercetin in the leaf of *T.catappa*. The phytoconstituents like flavonoids, carotenoids, and phenolic compounds may be responsible for the traditional use of this plant.

Numerous pharmacological investigations have confirmed this plant's ability to exhibit anti-microbial, anti-inflammatory, antidiabetic, anti-oxidant, hepatoprotective, antisickling and anticancer activities, all of which support its traditional uses. Ethanolic leaf extract of *T.catappa* exhibit anti-inflammatory effect on 12-O-tetradecanoyl phorbol-13-acetate (TPA) – induced ear edema in both acute and chronic animal models. [9, 10] found that the extract from the tender leaves of this plant has anti-inflammatory as well as analgesic activities.

The various polyphenolic compound, triterpenoids, and other chemical compounds found in the plant may be responsible for the anti-inflammatory activities. The aqueous and cold extract of fresh and tender leaves of *T.catappa* has the capacity to decrease the high blood glucose level and lipids in alloxan-induced animal models, while the fallen dry leaf decoction has also been reported to have hypocholesterolemic effects in rats [11]. [12] found that treatment with aqueous extract of *T.catappa* exhibited antihepatotoxic activity against carbon tetrachloride (CCl_4) – induced toxicity in rat, while [13] found out that

T.catappa extract possesses antioxidant activity in a dose-dependent manner by DPPA assay, nitric oxide assay, reducing power assay and H_2O_2 assay. Also [14] reported that phenol present in almond leaf is partly responsible for the observed scavenging activity.

The hepatoprotective activity on the leaf extract on *Terminalia catappa* has been reported by [15, 16, 17, 18]. [19] reported that *T.catappa* extract provides a powerful chemopreventive substance against cancer. Anti-sickling activities of the extract of *T. Catappa* was reported by [20], two recipes used by traditional healers in Ibadan, southwest Nigeria were investigated and it was reported that the inclusion of reddish-brown, freshly fallen leaves of *Terminalia catappa* in recipe 2 is responsible for its higher antisickling activity than recipe 1.

[21] reported that immersing *T.catappa* leaf extract above 375 ppm in water improves the water quality and is beneficial to enhance survival of ornamental fish (Betta sp). The choloroform, as well as methanolic extracts, show good antimicrobial activity against Gram-positive and Gram-negative bacteria. The aqueous and methanolic extracts of the leaves of *T.catappa* show different degrees of activity against *Pseudomonas* a*eruginosa, Staphylococcus aureus, Proteus mirabilis, Bacillus subtilis, Samonella typhimurium,* and other fifteen microorganisms, the methanolic extract is significantly more efficient than aqueous extract [22].

To the best of our knowledge, from the literature available, there has been no research on the Gc-Ms analysis of the methanolic extracts of the leaf and bark of the different species of *T.catappa* available in Nigeria. The research is aimed at a comparative study on the Gc-ms analysis of the methanolic extracts of the different cultivars of *T.catappa* linn available in Nigeria.

MATERIALS AND METHODS

Plant Collection and Identification

The leaves and bark of three varieties of *Terminalia catappa* lin were harvested from different parts of Oyo town, Southwest Nigeria in November 2018 and were identified at the herbarium of the Department of Biology, Emmanuel Alayande College of Education, Oyo, Oyo state.

Extraction of the Crude Extract

Fresh matured leaves and bark of three varieties of *Terminalia catappa* linn were harvested and air-dried separately for two weeks. The air-dried samples were pulverized separately, 50g of each were soaked in 250ml of 95% methanol for 48 hours. The residues were separated from the extracts by filtering through filter paper (Whatman No 1), the residues were further extracted with fresh solvent. The extracts recovered from both the extractions were evaporated using rotary evaporator and stored at 4^0C until used as a crude extract.

Gas Chromatography/mass spectrometry (Gc/ms) Analysis

The analysis of the leaf and bark crude extract of three varieties of *Terminalia catappa* was performed using multidimensional gas chromatography-mass spectrometer (shimadzu, japan) equipped with double capillary columns that have different characteristics (non-polar and polar)

Antimicrobial screening

The antibacterial activity of the crude extracts was measured against gram-negative bacteria (*Escherichia coli, Klebsiella pneumonia, Salmonella typhymurium,* and *Proteus mirabilis*) and gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus agalactiae* using a well diffusion method

according to the National Committee for clinical laboratory standard [23]. Petri plated containing approximately 25-30ml of nutrient agar medium were swabbed using cotton applicator with 24hours sub-cultured bacteria strains which were prepared in dilution to match the turbidity intensity of the MacFarland standard.

Wells (6mm diameter) were punched in the agar and filled 10Ml of the extract of different concentrations (1000, 500 and 250μgml^{-1}). The plates were incubated at 37^{0}C for 24 hours. the antibacterial activities were assessed by measuring the inhibition zone diameter (mm) around the well.

Table 1: GC-MS analysis of the methanolic extract of the leaf and bark of three varieties of *T. catappa* lin

S/N	RT	COMPOUND	TCB RED	TCB PINK	TCB WHITE	TCL RED	TCL PINK	TCL WHITE
1	7.2022	Decenal, (E)				1.61		
2	7.830	3 – Methyl tridecane				0.24		
3	8.273	2,4 – Decadienal (E,E)				0.42		
4	9.586	1 – Undecene			1.04			
5	9.770	2,4 Dilsopropenyl-1-methyl-1-vinyl cyclohexane.	0.09		0.19			
6	9.967	3a,7-methano-3aH-cyclopenta cyclo octane – 1,4,5,6,7,8,9,9a-octahydro-1,1,7- trimethyl-[3aR-(3a.alpha.)7.alpha 9a.beta)]	0.69	0.96	1.03			
7	10.211	Aromadendrene	0.63					
8	10.432	1,5-Heptadiene, 6 – methyl – 2 – (4 – methyl – 3 – cyclohexen -1-yl) – (5) – (-)			0.14			
9	10.660	Ocimene	0.26		0.18			
10	11.002	Seychellene	0.27		1.18			
11	11.006	gamma- Elemene		0.44				
12	11.484	3,5 – Xylenol	0.54					
13	11.490	4 – (1 – Methylethylidene) – 1,2 – divinyl cyclohexane.		0.71				
14	12.320	(+) – Nerolidol						0.66
15	12.321	9,12,15 – Octadecatrienal			0.74			
16	12.336	2 – Tridecen – 1 – ol, (E)				0.33		
17	12.643	(5E) – 5 –Decenylacetate	0.60					
18	12.644	3 – Decyne			0.75			
19	12.648	Limonene Oxide		0.67				
20	13.009	Longipine epoxide					1.28	
21	13.016	alpha – Bisabolol	1.42	1.01				
22	13.018	1beta – cadin – 4 – en – 10 – ol			0.42			
23	13.021	Methy (5E, 8E) -5,8 – Octadecadienoate		1.00				
24	13.192	tau – Muurolol	1.08		0.53			
25	13.298	2(E) -2, 6 – Dimethyl -2, 7 – Octadiene – 1, 6 – diol.	2.59					

26	13.300	Isothujol			1.43			
27	13.303	Limonediepoxide		2.08				
28	13.425	Bergamotol, Z – alpha-trans					0.72	
29	13.575	2 – Nonen – 1 – ol				0.64		
30	14.106	3Hydroxy-2-methyl-2-nitrocyclohexyl acetate					0.54	
31	15.044	Octadecenal				0.22		
32	15.317	3-Allyl-2,6,6-trimethylbicycto [3.1.1] heptanes				0.48		
33	15.325	(5E) -2,3,5,8-Tetramethyl-1,5,9 Decatriene	0.66				2.62	0.43
34	15.322	9-methy-5-methylene-8-decen-2-one		1.17				
35	16.061	Myristic acid		0.91				
36	16.480	1-octadecyne				0.75		
37	17.069	Methyl(16E)-16-octadecenoate			21.84			
38	17.075	Methyltridecanoate	15.26	19.90	17.06	4.64	9.73	12.10
39	18.266	Nonadecanoic acid	1.33					
40	18.273	Pentadecanoic acid			2.09		7.83	7.12
41	18.276	[1, 1-Bicyclopropyl]-2-octanoic acid, 2-hexyl, methylester.						
42	18.295	Margaric acid				9.97		
43	18.715	2,6,10,14-Hexadecatetraen-1-ol, -3,7,11,15-tetra methyl acetate(E,E,E)	0.84					
44	19.073	Methyl(10E)- ol – octadecenoate	1.18		9.82			
45	20.181	Methylpetroselinate		16.36				
46	20.216	Dodecylmethacrylate	29.78					
47	21.093	Erucic acid		12.24	14.17		31.76	25.82
48	21.117	Cis-oleic acid	8.27			40.85		16.93
49	9.110	9,11-octadecadienoic acid methyl ester.	5.93					
50	21.339	Methyllinolelaidate		5.57		1.55	2.25	2.75
51	21.341	(11E, 132) -1,11,13 – Octadecatriene			5.55			
52	21.351	Linoleic acid Chloride						10.36
53	21.380	Nonadecanoic acid				10.78		
54	21.390	Arachic acid					7.56	
55	21.610	1,12-Tridecadiene		11.85				
56	22.075	1,4 Heptadiene – 3,3,6 – trimethyl	1.23					
57	22.079	(4E) -3,3,6 – Trimethyl-1,4 – heptadiene		1.35				
58	22.081	1,11-Dodecadiene					1.31	
59	22.254	1-Propoxyheptane		1.10				
60	22.434	4,11,11-Trimethyl-8-methylene bicyclo [7.2.0] undec-4-ene					0.91	
61	22.620	5,6 –Dimethylundecane				4.36		

62	22.628	1 Fluorodecane		2.03	3.31	1.17	2.27	
63	22.645	Nonanoylchloride			2.74		2.16	
64	22.654	Methynervoate	2.75					2.65
65	22.930	Methylheneicosanoate	5.23	5.23	9.70	4.47	2.85	6.81
66	23.791	Brassidic acid		1.29				
67	23.985	Z – 13 –Octadecenal				1.87		
68	23.989	9-Tetradecen-1-ol, acetate	2.24					
69	23.991	(6Z) – 6, 17-Octadecadienyacetate		3.10				
70	23.992	E-9-Tetradecenylacetate			3.80			
71	23.993	9-Octadecenal					10.84	2.93
72	24.308	2-Dodecenal, (E)				5.30		
73	24.439	10 – Undecenal	6.86	5.76	10.29	0.52		8.24
74	24.653	Glyceryl-1,3-distearate		2.31				
75	24.656	Laurylacetate						2.67
76	24.876	Behenic acid methylester	5.37				1.02	
77	25.318	Eicosane	3.28					
78	26.840	Methylheptacosanoate		1.14				
79	26.842	Methyl-14-methyl pentadecanoate			1.58			
80	27.512	Hexadecane	0.971					

Note: TCL = Terminalia catappa Leaf

TCB = Terminalia Catappa Bark

Table II: Zones of inhibition (mm) showing the antimicrobial potentials of the methanolic extract of the leaf and bark of three varieties of *Terminalia catappa* lin.

Plant sample	Concμgml^{-1}	E. Coli	K. pneumoniae	P. aeruginosa	S. agalactiae	S. aureus	S. typhimurium	P. mirabilis
TCB (red)	1000	28	14	16	11	12	12	10
	500	12	11	11	11	11	12	10
	250	12	11	10	11	11	12	10
TCB (Pink)	1000	14	14	11	11	11	14	10
	500	14	14	11	11	11	14	10
	250	14	11	11	11	11	11	10
TCB (White)	1000	12	11	10	12	11	14	11
	500	12	11	10	12	11	12	08
	250	12	11	10	12	11	10	08
TCL (RED)	1000	14	16	24	10	15	12	14
	500	14	16	20	10	13	12	10
	250	14	15	20	10	13	12	10
TCL (Pink)	1000	11	15	16	13	12	10	10
	500	11	15	16	13	12	10	10
	250	11	15	12	11	-	10	10
TCL (White)	1000	15	17	21	13	12	13	12
	500	15	16	15	10	12	12	12

	250	15	16	14	10	10	12	12
Synthetic antibiotic								
nitrofuranton	200*µ*g	25	20	18	11	28	15	15
Ofloxacin	5*µ*g	20	20	18	-	20	22	33
Gentamicin	10*µ*g	24	15	22	-	-	18	16
					-	-		

Keynote: resistant = (-), not sensitive (< 8mm), sensitive (9-14mm) very sensitive, (15-19mm) and ultra sensitive (> 20mm)

The Gc-Ms analysis of the crude methanolic extract of three varieties of the leaf and bark of *Terminalia catappa* linn revealed the presence of eighty constituents as shown in table 1, cis-oleic acid was identified as the constituent with the highest percentage 40.85% in *Terminalia catappa* leaf extract(red variety) erucic acid has the second-highest percentage 31.76% in *Terminalia catappa* leaf extract (pink variety), followed by Dodecylmethacrylate 29.78% in *Terminalia catappa* bark extract (red variety) and erucic acid 25.82% in *Terminalia catappa* leaf extract (white variety). Methyl tridecanoate and methyl heneicosanoate are present in all the six extracts and are present in appreciable percentages. All the six extracts were active against all the tested micro-organism (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, S. agalactiae, Staphylococcus aureus, Salmonella typhimurium* and *Proteus mirabilis*) at all concentrations (1000*µ*gm^{-1}, 500*µ*gml^{-1} and 250*µ*ml^{-1}). As shown in Table II.

Terminalia catappa leaf extract (Pink variety) is not active at 250*µ*gm^{-1} against *Staphylococcus aureus*. The high antimicrobial activity of all the extract is due to the high percentage of phenolic compounds and fatty acids.

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