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Research Article

Antimicrobial Analysis and Structural Elucidation of Active Compounds of *Dialium Indum* Leaves Extract (Valvet Tarmarind)

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Abstract

Antimicrobial analysis and structural elucidation were carried out on the purified leaves of *Dialium indum*. The Harbone method was used for the extraction. The extracts were separated using a combination of column chromatography and thin layer chromatography, which gave rise to the isolation of two fractions, these fractions were further purified using recrystallization. The melting point of each pure fraction was determined. The purified extracts were subjected to structural elucidation using various spectroscopic techniques which includes; FTIR, UV, H^1 NMR, C^{13} NMR, DEPT 135^0, COSY, TOCSY, HMBC and HSQC . The spectral analysis suggested the presence of Stigmasterol, and Lauric acid. The antimicrobial analysis (anti fungal and anti bacterial analysis) using the punched agar diffusion method was carried out on the isolated fractions comparatively with a standard drug Funbact-A cream (a broad spectrum antibiotic). A total of thirteen test organisms were used for this analysis amongst which were ten bacteria test organism and three fungi test organisms. The results from the average diameter zones of inhibition, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and fungicidal concentrations (MFC) showed that all the fractions were all active on the entire test organism with zones of inhibition ranging from 14mm-36mm comparatively. None of these fractions showed similar antimicrobial effect as the standard drug Funbact-A cream but individually could serve as anti microbial to diseases caused by these test organisms from their MIC, MBC and MFC.

Keywords: *Dialium indum*, antimicrobial analysis, Structural elucidation, NMR, FTIR, UV-Spectrum and Funbact-A cream

1. Introduction

Every community in Nigeria has peculiar herbs and plants which are used in some ways for the treatment of systems and disease [1]. Out of hundreds of plants species that are recognized as having medicinal values, four out of every five are collected from the wild forest while most of them are from the floras of developing countries [2] nearly 50 percent of drugs used in medicine are of plant origin [3].

Compounds which exert various physiological effects of therapeutic value are collectively known as drugs [4]. Herbal medicine sometimes referred to as herbalism or Botanical medicine is the use of herbs for their therapeutic or medicinal value [2].

It has been shown that different parts of the tree (*Dialium indum*) have been used in folkloric medicine for the treatment of different diseases such as cancer, headache and pains (bark), fever, prenatal pains and oedema (leaves) and diarrhea (fruits) [5].

Evaluation of the analgesic activity of the methanolic stem Bark extract of *Dialium Guineensse* (wild) specie of the Dialium genus. Showed that the stem bark have analgesic properties [6]. The stem bark and the leaves of *Dialium indum* have been employed in the treatment of guinea worm infections [7].

Therefore, this work is aimed at giving in-depth screening into properties of *Dialium indum* that made it useful in the curing of ailments caused by the test organisms used in this assay since not much is known about its antimicrobial and class of active principle.

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2. Materials and Methods

2.1 Plant Collection, Identification and Preparation

The leaves of *Dialium indum* used in this study were collected from Nnodo Amike-Aba Abakaliki Ebonyi state. It was identified and authenticated as *Dialium indum* by Prof. S.S.C Onyekwelu of the department of Applied Biology Ebonyi State University. The Fresh leaves samples were dried under sunlight; pulverized and stored in a Glass jar for subsequent analysis.

2.2 Extraction and Fractionation of *Dialium indum* into different classes

500g of the pulverized leaves were soaked in 2000ml and 500ml of methanol/water mixture in a ratio of 4:1 for about 72hour. The mixture was filtered and the filtrate heated on a water bath to one-tenth (1/10) of the volume at temperature of about 40˚C. The filtrate was then acidified with 2ml of 2M H_2SO_4 and then extracted with chloroform. The mixtures were separated using separating funnel. The chloroform extract was heated to dryness and re-dissolved with chloroform giving the chloroform extract [3]. This extract was thereafter purified using column chromatography and flash chromatography the process was monitored using preparative thin layer chromatography. The isolated fractions were further recrystalized three times using methanol.

2.3 Anti-bacterial Assay

The sensitivity of the fractions were compared to that of a standard drug (Funbact-A cream) against the selected test organisms (*Bacillus typhi, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi, Staph albus, Staphylococcus aureus, Streptococcus muteus, Aspergillus flavus, Aspergillus niger* and *Candida albican*) was carried out using the punched agar diffusion method [8]. The MIC and MBC were determined using the serial dilution method while MFC was determined using the Punched Agar diffusion method [8].

2.4 Structural Elucidation

Plausible structures and molecular formulae were proposed for the two isolated fractions of leaves of *Dialium indum* leaf using a combination of spectroscopic techniques such as: FTIR, UV-visible, 1H, 13C, Dept-135, COSY, TOCSY, HSQC and HMBC NMR analysis.

3. Results and Discussions

The results of the organoleptic examination of the leaves of *D. indum* are shown in the table below

Table 1: Results of the Organoleptic Examinations of *D. indum*

Parameter	Fresh Leave	Dried Leave
Colour	Green	Brown
Odour	Odourless	Pungent
Taste	+	+

+ = sample had undefined taste

The presence of a taste in a plant tissue indicates that the said plant possesses medicinal values [9].

Table 2: TLC Results of Dialium indum Crude extracts

Parameter	R_f Value	Solvent System
D. indium Leaves fraction 1	0.222	Ethanol: chloroform (3:2)
D. indium Leaves fraction 2	0.456	Ethanol: chloroform (3:2)

The TLC results showed two spot which was later resolved using a solvent system of petroleum ether and chloroform which gave two distinct colours under iodine vapour with R_f values as shown in Table 3.

Table 3: TLC results of *Dialium indum* pure extracts

Parameter	R_f Value	Solvent System
D. indum Leaves fraction 1	0.488	Petroleum Ether: Chloroform (4:1)
D. indum Leaves fraction 2	0.987	Petroleum Ether: Chloroform (4:1)

The resulting fractions were recrystallized three times using methanol; and this afforded two white crystalline solids.

Table 4: Melting point Results of *Dialium indum* pure extracts

Parameter	Melting Range (^{O}C)
D. indum Leaves fraction 1	165^{o}C-167^{o}C
D. indum Leaves fraction 2	44^{o}C-46^{o}C

The melting points were uncorrected for all the extracts. The melting temperature range of any chemical analysis is an indication of the extent of purity, hence can be used to estimate whether a substance is pure or contaminated. The melting point of a pure unknown compound is sharp and ranges from 5^{o}C or less from the pure known compound [10].

Table 5: Results of Average Diameter zone of inhibition for *D. indum* leaves fraction 1, *D. indum* leaves fraction 2 and Funbact-A

Extract Solvent	Vol. Used In cm^3	Average diameter (mm) of zones of inhibition on test organism									
		E. coli 10418	*S. aureus* NCTC 6571	*Bacillus Species* L.C.I	*Proteus Vulgaris* L.C.I	*Salmonella Typhi* L.C.I	*Pseudomonas Aeroginosa* L.C.I	*Klebsiella pneumonia* L.C.I	*Staph Albus* L.I.C	*Strept Muteus* L.C.I	*Enterobacter Aerogenes* L.C.I
D. indum Leaf fraction1	0.05	18	20	14	36	16	22	18	26	17	34
D. indum Leaf fraction 2	0.05	16	18	14	32	14	18	16	24	14	32
Funbact A cream	0.05	24	32	18	36	22	28	24	34	28	38

L.C.I - Local clinical isolate
NCTC - National collection of type culture
Ditch diameter = mm

The results of the antimicrobial activities of *D. indum* leaves fraction 1 shown in Table 5 indicated that they inhibited the growth of all the test organisms (both gram-positive and gram-negative). The average diameter zones of inhibition were maximum at 36mm and minimum at 14mm against the test organisms. The antimicrobial activities properties of *Dialium indum* has been reported [5-7].

Table 6: Results of MIC and MBC for Funbact-A, *D. indum* leaves fraction 1, and *D. indum* leaves fraction 2

Extract Solvent	*E. coli* NCTC 10418	*S.aureus* NCTC 6571	*Bacillus Specie* L.C.I	*Proteus Vulgaris* L.C.I	*Salmonella typhi* L.C.I	*Pseudomonas aeroginosa* L.C.I	*Klebsiela Pneumonia* L.C.I	*Staph. Albus* L.C.I	*Enterobacter Aerogenes* L.C.I	*Strept Mutans* L.C.I
Funbact-A										
MIC	1:32	1:64	1:16	1:64	1:32	1:32	1:32	1:64	1:64	1:32
MBC	1:16	1:32	1:8	1:32	1:16	1:16	1:16	1:32	1:32	1:16
Leaves fraction 1										
MIC	1:16	1:16	1:4	1:64	1:8	1:16	1:16	1:32	1:64	1:16
MBC	1:8	1:8	1:2	1:32	1:4	1:8	1:8	1:16	1:32	1:8
Leaves fraction 2										
MIC	1:8	1:16	1:4	1:64	1:4	1:16	1:8	1:32	1:64	1:4
MBC	1:4	1:8	1:2	1:32	1:2	1:8	1:4	1:16	1:32	1:2

From Table 6, it could be seen that the MIC values of *D. indum* leaves fraction 1 and *D. indum* leaves fraction 2 was minimum at 1.5625mg/ml and maximum at 25mg/ml while MBC values was minimum at 3.125mg/ml and maximum at 50mg/ml. these results indicated that these fractions were active and inhibited the growth of microorganisms even at a very low concentrations. This has informed the traditional use of the plant *D. indum* in the treatment of diseases caused by these bacterial organisms.

Table 7: Results of Average Diameter Zones of inhibition for *D. indum* Leaves fraction 1, *D. indum* Leaves fraction 2 and Funbact-A

S/N	Extract Solvent	Volume used in cm^3	Average Diameter (mm) of zones of inhibition on test organism		
			Aspergillus flavus. L.C.I	*Aspergillus niger*. L.C.I	*Candida albicans* L.C.I
1	*D. indum* Leaf fraction 1	0.05	14	14	16
2	*D. indum* Leaf fraction 2	0.05	14	14	16
5	Funbact-A Cream+5ml distilled water	0.05	36	36	38

L.C.I - Local clinical isolate
NCTC - National collection of type culture
Ditch diameter r = 6mm

The results of the antifungal activities of *Dialium indum* leaves fraction 1 and *Dialium indum* leaves 2 showed that they had a moderate to susceptible antimicrobial characteristics as seen from Table 7. The average diameter of zones of inhibition ranged from 14mm-16mm. *Candida albicans* was the most susceptible.

Table 8: Results of MIC and MFC for *D. indum* leaves fraction 1, *D. indum* leaves fraction 2, Funbact-A Cream

Extract Solvent	*Aspergillus flavus* L.C.I	*Aspergillus niger* L.C.I	*Candida albicans* L.C.I
D. *indum 1*			
MIC	1:4	1:4	1:8
MFC	1:2	1:2	1:4
D. indum 2			
MIC	1:4	1:4	1:8
MFC	1:2	1:2	1:4
Funbact-A Cream			
MIC	1:64	1:64	1:64
MFC	1:32	1:32	1:32

According to the report by Ibeh *et al*., 2003, that an inhibitory zone diameter of 10mm or less indicates that the organism was resistant. An inhibitory zone diameter of 11-15mm shows intermediate effect while 16mm and above indicated that the organism was susceptible to the compounds [11]. Hence all the bacterial organisms were susceptible to *D. indum* fractions and *D. indum* had an intermediate effect on the fungi organisms.

The MIC and MFC results of *Dialium indum* leaves fraction 1 and *Dialium indum* leaves fraction 2 was evaluated as seen from Table 8, it indicated that the MIC values was minimum at 12.5mg/ml and maximum at 25mg/ml while the MFC values was minimum at 25mg/ml and maximum at 50mg/ml. this indicated that these fractions can serve as antimicrobial agents event at such a low concentration. These results have further confirmed the ethnomedical use the Plant *Dialium indum* for the treatment of ailments caused by these micro organisms.

From the results obtained from the antimicrobial analysis, it was confirmed that *D. indum* had an excellent antimicrobial effect on the test organisms comparable to the standard drug Funbact-A cream, and as such its therapeutic benefits can be harnessed for the treatment of ailment caused by the organisms used in this assay

3.1 Structural Elucidations of *D. Indum* Leaves Fractions

Table 9: FTIR spectrum results of *Dialium indum* leaves fraction 1

Wave band (cm^{-1})	Description
1814.00	C=C stretch
1893.96	
2054.32	C-H Stretch
2134.22	
2214.28	
2294.52	
2374.73	
2454.94	
2857.94	C-H of alkanes and aromatics
2938.73	
3019.55	
3100.40	
3181.29	
3262.26	
3343.38	O-H of alcohols and esters
3424.37	
3505.33	
3586.53	
3667.59	
3748.64	

Table 10: Results of UV-Visible of *Dialium Indum* Leaves Fraction 1

λmax (nm)	Chromophore Description
326.50	C=C of cyclic bond ($\boldsymbol{n} \rightarrow \boldsymbol{\pi} *$)
319.00	
299.00	C-OH (n→ $\boldsymbol{\pi}$*)
270.50	
249.00	C=C of alkanes ($\boldsymbol{n} \rightarrow \boldsymbol{\pi}$*)
242.50	
236.00	C-O of alkanes and alcohol ($\boldsymbol{\pi} \rightarrow \boldsymbol{\pi}$*)
227.00	
221.00	
216.00	
210.50	

Note: All NMR analysis was carried out in Deuterated chloroform ($CDCl_3$)

Table 11: Summary of the H^1 and C^{13} NMR of *Dialium Indum* Leaves Fraction 1

Experimental Position	Literature (Pierre *et al*, 2015 and Chaturvedula and Prakash, 2012)			
	^{1}H	^{13}C	1H	13C
1	-	37.25	-	37.6
2	-	31.89	-	32.1
3	3.51 (tdd, 1H)	71.79	3.51(tdd, 1H)	72.1
4	-	42.29	-	42.4
5	-	140.75	-	141.1
6	5.34 (t, 1H)	121.71	5.31(t, 1H)	121.8
7	-	31.89	-	31.8
8	-	31.64	-	31.8
9	-	50.14	-	50.2
10	-	36.51	-	36.6
11	-	21.23	-	21.5
12	-	39.68	-	39.9
13	-	42.21	-	42.4
14	-	56.86	-	56.8
15	-	24.37	-	24.4
16	-	28.93	-	29.3
17	-	55.94	-	56.2
18	1.03 (s, 3H)	12.05	1.03(s, 3H)	12.2
19	0.70 (s, 3H)	18.99	0.71(s, 3H)	18.9
20	-	40.51	-	40.6
21	1.01 (d, 3H)	21.10	0.91(d, 3H)	21.7
22	3.55 (m, 1H)	138.33	4.98 (m, 1H)	138.7
23	5.14 (m, 1H)	129.26	5.14 (m, 1H)	129.6
24	-	51.24	-	51.13
25	-	25.42	-	29.6
26	0.82 (d, 3H)	21.07	0.82(d, 3H)	20.2
27	0.80 (d, 3H)	19.40	0.82 (d, 3H)	19.8
28	-	25.42	-	25.4
29	0.83 (t, 3H)	12.07	0.83(t, 3H)	12.1

The combination of the FTIR, UV-visible, 1H-NMR, 13C-NMR, DEPT-135^o -NMR, COSY, TOCSY, HMBC, and HSQC spectral was matched with previous literature on Stigmasterol [12-13] and the structure was proposed as shown in figure 1a.

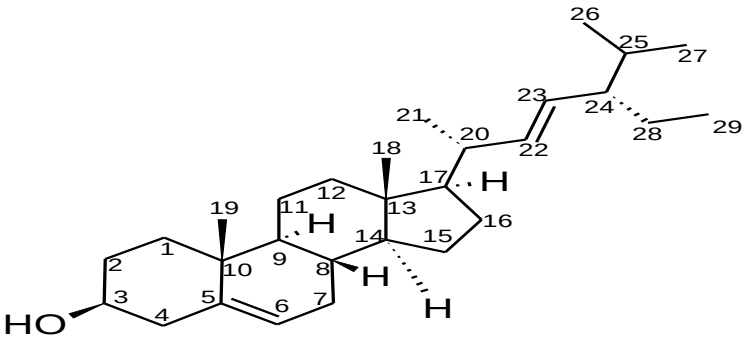

Figure 1(a): Structure of Stigmasterol

Table 12: Result of FTIR of *Dialium indum* leaves fraction 2

Wave band (cm^{-1})	DESCRIPTION
893.45	O-H bends of carboxylic acid
1053.57	C-O deformation bonds for ketones aldehydes, acids and esters
1161.37	C-O stretch vibrations of acids, esters and aldehydes
1327.75	
1396.58	
1471.80	C-H vibration for alkanes
1550.13	
1629.67	C=O stretch of carbonyls, ketones, acids and esters

Table 13: Results of UV-Visible of *Dialium indum* leaves fraction 2

λ max (nm)	**Chromophore Description**
447.50	C=O of acids and esters ($\boldsymbol{n} \rightarrow \boldsymbol{\pi}*$)
330.50	
305.00	
270.50	C=O of acids and esters ($\boldsymbol{\pi} \rightarrow \boldsymbol{\pi}^*$)
249.50	
242.50	
235.50	RCOOH (n→ $\boldsymbol{\pi}^*$)
226.50	
221.50	
216.00	
210.00	

Table 14: ^{1}H and C^{13} NMR Results of *Dialium indum* leaves fraction 2

Experimental Position	Literature (Pouchert and Behnke, 1993 Wahidulla *et al.*, 1998, and Abe *et al.*, 2009)			
	^{1}H	^{13}C	^{1}H	^{13}C
1	-	180.18	-	180.23
2	2.35 (t, 2H)	34.07	2.35 (t, 2H)	34.08
3	1.63 (m, 2H)	24.69	1.63 (m, 2H)	24.68
4	1.26 (m, 16H)	29.35	1.27 (m, 16H)	29.33
5	1.28 (m, 16H)	29.45	1.28 (m, 16H)	29.43
6	1.28 (m, 16H)	29.61	1.28 (m, 16H)	29.59
7	1.29 (m, 16H)	20.51	1.29 (m, 16H)	21.70
8	1.32 (m, 16H)	29.26	1.30 (m, 16H)	29.24
9	1.31 (m, 16H)	29.07	1.30 (m, 16H)	29.06
10	1.30 (m, 16H)	31.93	1.30 (m, 16H)	31.91
11	1.30 (m, 16H)	22.71	1.30 (m, 16H)	22.69
12	0.88 (t, 3H)	14.13	0.88 (t, 3H)	14.10

The combination of the FTIR, UV-visible, 1H-NMR, 13C-NMR, DEPT-135°, COSY, TOCSY, HMBC, and HSQC spectral was matched with previous literature on Lauric acid [14-16] the structure was proposed as Lauric acid.

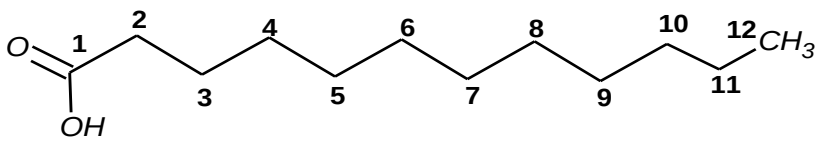

Figure 1(b): Structure of Lauric acid

4. Conclusion

The antimicrobial (antibacterial and antifungal) analyses showed that the *Dialium indum* leaves fraction 1 and *Dialium indum* leaves fraction 2 had broad spectrum antimicrobial properties. From the average diameter of Zones of inhibition the order of susceptibility of the microorganisms for *Dialium indum* leaves fraction 1 was *Proteus vulgaris>Enterobacter aerogenes>Staph albus>Pseudomonas aeroginosa>S. aureus>Klebsiella pneumona/E.coli>Salmonella typhi>Strept Muteus>Bacillus specie* whereas *Dialium indum* leaves fraction 2 was in the order *Proteus vulgaris/Enterobacter aerogenes>Staph albus>Pseudomonas aeroginosa/S. aureus>Klebsiella pneumona/E.coli>Salmonella typhi/Strept Muteus/Bacillus specie*. The structural elucidations was done using IR, UV, 1D and 2D NMR spectroscopy and indicated that Stigmasterol and Lauric acid were amongst compounds responsible for the antimicrobial properties of *D. indum*, the physical properties and antimicrobial properties of these isolated compounds Stigmasterol and Lauric acid correlated with those reported in literature [12-16].

References

[1] Filgona, J., Dunah, C.S., and Wakshama, P.S. An invitro study of the Antimicrobial Actrivity of the root extract of *Calotropis procena* and *Moringa oleifera*. Ife Journal of science, 2005; 7(1): 43-44.

[2] Ajiwe, V.I.E., Dimonyejiaku, N., Ajiwe, A.C., Chinweuba, A.J., and Chendo N.M., Preliminary study on the pharmaceutical constituents of *Emilia sonchifolia* leaf. *Anachem Journal*, 2008; 2(2): 302-309.

[3] Harbone, J.B., Phytochemical Methods- A guide to modern Techniques of plant analysis, 3rd edition, Chapman and Hall, London, 1998; pp.38-90.

[4] Finar, I.L. Stereochemistry and the chemistry of natural products, vol. 1, 5th edn., Pearson Education limited. New York, 1975; pp 273-293.

[5] Arbonnier, M. Trees, shrubs and lianas of West African dry zones French Agricultural Research Centre for International development and Museum of National History Naturalis publishers, Ltd. Atze Jan, Wageningen, the Netherland 2002; p573.

[6] Ezeja, M.I., Omeh, Y.S., Ezeigbo, I.I., Ekechukwu, A.J. Evaluation of the analgesic activity of the methanolic stem bark extract of *Dialium guineense* (Wild). *Annals of Medical and Health Science Research*, 2011; 1(1): 55-62.

[7] Adekunle, M.F. "Economic Valuation of Forest Plants used in Traditional Treatment of Guinea Worm Infections in Ogun State Nigeria". PhD thesis, Dept of forestry and wildlife management, University of Agriculture, Abeokuta Nigeria. (Unpublished) 2005; 1-199.

[8] Bryant, M.C., (1972). Antibiotics and their laboratory control, 2nd edition, Butterworth, London, 1972; 85.

[9] Onyema, C.T., and Ajiwe, V.I.E., Phytochemical and antimicrobial analysis of leaves of *Cola gigantea*, *International Journal of Applied Science and Technology*, 2014; 4(2): 121-122.

[10] Fessenden, R.J., Fessenden, J.S and Feist, P.F. (2000). Organic Laboratory Techniques, 3rd edn., Brooks Coles; California

[11] Ibeh, I.N and Uraih, N. Practical Microbiology, Vol.1, Ambik Press Ltd, 2003; pp 82-93.

[12] Pierre, L.L., and Munkombwe N.M. Isolation and Characterisation of Stigmasterol and β –Sitosterol from *Odontonema Strictum* (Acanthaceae). *Journal of Innovations in Pharmaceuticals and Biological Sciences*, 2015; 2(1): 88-95

[13] Chaturvedula, P.S.V., and Prakash, I. Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of *Rubus suavissimus*. *International Current Pharmaceutical Journal*, 2012; 1(9): 239-242

[14] Pouchert, C.J and Behnke, J. The Aldrich library of 13C and 1H FT NMR Spectra; Aldrich Chemical Company, Inc., Milwaukee, WI, USA. 1993; 1: 753-785.

[15] Abe, M., Yoshihiko, I., Asahi, S., Satomi, O., Hiroshi, N., and Shizuo Y. Isolation and *Pharmacological* Characterization of Fatty Acids from Saw Palmetto Extract. *Japan Society for Analytical Chemistry*, 2009; 25: 553-557.

[16] Wahidulla, S., D'souza, L., and Govenker, M. Lipid constituents of the red alga *Acantophora Spicifera*. National Institute of Oceanography, Dona Paula, Goa 403004, India *Phytochemistry* 1998; 8(7): 1203-12.