Phytochemical and Antibacterial Studies of *Cordia Africana Lam*

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Abstract
Cordia Africana antibacterial activity was determined using stem bark extract, which was tested against four bacterial isolates: Staphylococcus Aureus, Bacillus Subtilis, Escherichia Coli and Pseudomonas Aeruginosa using the agar well diffusion method. Ethyl acetate extract shows highest activity with 8.0 mm, zone of inhibition.TLC was carried out and the column chromatography profile yielded 97 fractions, which were pooled to six combined sub-fractions (A-F). The pure isolate A shows antibacterial activity on all the bacterial with highest zone of inhibition of 22.0 mm on Escherichia Coli. The spectroscopic studies revealed that isolate A consist of three compounds i.e. isobutyloctadecylester, butyl undecyl ester, methyl-12-oxo-9-dodecanoate

1.0 Introduction

The discovery of plant as therapeutic agents started in ancient times and has continued to thrive even in modern days. Documentation of plants with medicinal values dates back as far as 78AD (Alice, 1996). Research has proved that developing countries rely mainly on medicinal plants for treatment of their prevailing ailments especially in areas where hospitals are not readily accessible (Lambo, 1979). In Japan, China and Great Britain, the use of herbal remedies is prevalent due to side effect associated with the use of synthetic drugs and the rising cost of effective drugs (Chin *et al.,* 2006, Rainer and Douglas, 2006).

The bioactive antimicrobial constituents in plants are alkaloids, tannins, flavonoids, saponins, phenolic compounds and others (Edeogal*et al.,* 2005). If bioactive compounds are carefully isolated, purified and identified, they can combat diseases and infections of human and animals when properly utilized. Knowledge of the chemical constituent of any plant is desirable not only for discovery of therapeutic agents, but also because such information is useful for discovery of new resources from such chemical substance (Sathish *et al.,* 2013).

1.1 Statement of the Problem

Medicinal plants are considered as a rich source of ingredients which can be used in drug development either pharmacopoeial, nonpharmacopoeial or synthetic drugs. Harwig and Scott (1971). Concern over rising health challenges regarding antibiotic resistant by different bacterial strains world over, necessitated the need and quest for alternative medicine that can fight these bacterial (El-Hamidi, 1970). Cordia Africanais a popular medicinal plant used in Africa and in Hausa traditional medicine for management of various ailments including treatment of diseases related to bacterial infections.

1.4 Justification

Cordia Africana have been reported to be effective in management of bacterial infections. This research has the potential to be able to provide an alternative source of antimicrobial agent.

1.5 Aim and Objectives

The aim of this research is to evaluate the Phytochemical and antibacterial properties of the stem bark extracts of the Cordia Africana lam*plant.*

The objectives are: -

a) To qualitatively identify some phytochemicals, present in *Cordia Africana* lam.
b) To isolate compound from the extract using column chromatography.
c) To characterize the compound isolated using GC- MS and FTIR.
d) To determine the antibacterial activities of the various extract of *Cordia Africana*.

1.6 Scope and Delimitation

The scope of this research is limited to extraction, phytochemical analysis, isolations and screening of its anti-bacterial activities on some selected bacterial strains.However, this research would not evaluate the anti-fungal, anti-viral activity and **acute toxicity study** *of the extract.*

2.0 Literature Review

The stem bark and leaf extracts of *Cordia Africana*have been evaluated for their antibacterial, anti-inflammatory and cytotoxic activities Sathish *et al.* (2013). Fred (2016) investigated the antimicrobial activity of *Cordia* plant extracts against bacterial strains causing food poisoning diseases, spoilage of food; food poisoning is caused by pathogens. Chin *et al.* (2006) investigated the review of existing literature on ethnobotanical knowledge of *Cordia Africana*to assist in the proper utilization, management and conservation of the species.Edeogal*et al.* (2005) describes evaluation of some phytochemical tests for Gumbail (*Cordia Africana*) and its uses in termite management.

In are related development, El Mahmood(2009) evaluate the extraction and physico - chemical characterization of Cordia Africana seed oil, used for skin disease, wound, diarrhea, and ascaris infection in human.Rainer and Douglas. (2006) investigated the anti-diarrheal activity of methanolic extract and ethnobotanical study in Agew-Awi and Amhara peoples in northwest Ethiopia.Tijjani*et al.* (2016) investigated anti-nociceptive activities of the ethanolic bark of *Cordia Africana (Boraginaceae),*

2.2 Phytochemical

Phytochemical are chemical compounds produced by plants that are of pharmacological importance to human health (Trease, *et al*., 1989). Examples of such compounds are tannins, saponins, flavonoids, steroid, anthraquinones and alkaloids.

2.0 Materials and Methods

2.1 Materials

The list of reagents and apparatus used in the research are indicated in Table 2.1 and 2.2

Table 2.1: List of reagents

Reagent	Chemical formula	Purity	Grade	Manufacturer
Sulphuric acid	H_2SO_4	98.0	A.R	British Drug House
Sodium chloride	NaCl	99.5	A.R	LOBA chemie
Dichloromethane	CH_2Cl_2	99.0	A.R	LOBA chemie
Sodium sulphate	Na_2SO_4	99.0	A.R	British Drug House

Table 2.2: List of Apparatus

Apparatus	Model	Manufacturer
Analytical balance	AW320	Shimadzu Japan
FT-IR Spectroscopy	8400S	Shimadzu., Japan.
GC-MS	QP2010SE	Shimadzu., Japan.

2.4 Chemicals

The chemicals used for this research work were of laboratory and analytical grade reagents.

2.5 Extraction of Plant Sample

The extraction was carried out according to method described by El-Mohmood (2009). Serial exhaustive extraction was carried out by maceration using solvents of increasing polarity with the aid of separating funnel.

2.7 Phytochemical Screening of the Crude Extracts and Fractions.

The Ethyl acetate extracts showed the highest bacterial activity and was subjected to qualitative and phytochemical screening in other to identify the various classes of phytochemicals using the methods described by Martinez and Valencia (1999), Sofowora (1993) and Harborne (1984).

2.8 Antibacterial Studies.

2.8.2 Test for Antibacterial Activities

The antibacterial activity of *C. Africana*stem bark extract was carried out against the four bacteria using the agar well dilution method (El-mohmood, 2009). Each extract was dissolved in 10 cm^3 of dimethyl sulphuxide (DMSO) to obtain a concentration of 10 mg/cm^3. The sterilized medium (nutrient agar) (20 ml) was poured into a sterile Petri dish, covered and allowed to cool and solidify. The medium was inoculated with 0.1 cm^3 of the standardized bacteria culture (1.5 x 10^8 CFU/ml) and allow to dry at 39^0C for 30 minutes. A standard cork borer (6mm diameter) was used to make a well at the centre of each inoculated plate and filled with earlier prepared 25, 30, 35 and 40mg/ml of extract. The same procedure was used to prepare a ciprofloxacin standard (positive control).

2.9 Thin Layer Chromatography

Thin layer chromatography was carried out for the ethyl acetate fraction of stem bark, which showed potent antibacterial activity according to the method described by (Chin *et al.,* 2006). Pre-coated TLC plate (silica gel F_{254}) was used.

2.12 Column Chromatography

The ethyl acetate extract was separated using gravity column chromatography wet packed with silica gel. The extracts were pooled to six, A; F.

Antibacterial studies of isolate A.

Isolate A was subjected to antibacterial screening following the previous method.

2.13 Spectroscopic Studies

a. **FTIR characterization of active fraction A was recorded by a shimadzu 8400 (shimadzu, Kyoto, Japan) FTIR spectrometer using KBr pellet.**
b. **The GC-MS Analysis of A was also carried out.**

3.0 Results

3.1.1 Extraction

The yield of the extract in various solvent is presented in Table 3.1. The methanol had the highest percentage yield.

Table 3.1: Yield of Extracts

Extract	Yield (%)
n- Hexane	2.36
Chloroform	0.81
Ethyl acetate	0.29
Methanol	4.56

3.1.2 Phytochemical Screening

The result of the phytochemical screening is presented in table 3.2.

Table 3.2: Preliminary Phytochemical Screening of *Cordia Africana* extracts

Test/ Phytochemical	n- Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloid				
Meyer test	-	-	-	-
Wagner test	-	-	-	+
Dragendorff test	-	-	-	+
Hager test	-	-	-	+
Carbohydrate				
Molish's test				
Fehling test				
Saponins				
Frothing test	-	-	-	+
Flavonoids				
Shinodas test	-	-	+	+
Ferric chloride	-	-	+	-

Key: - = absent, + = present, and ++= appreciable amount.

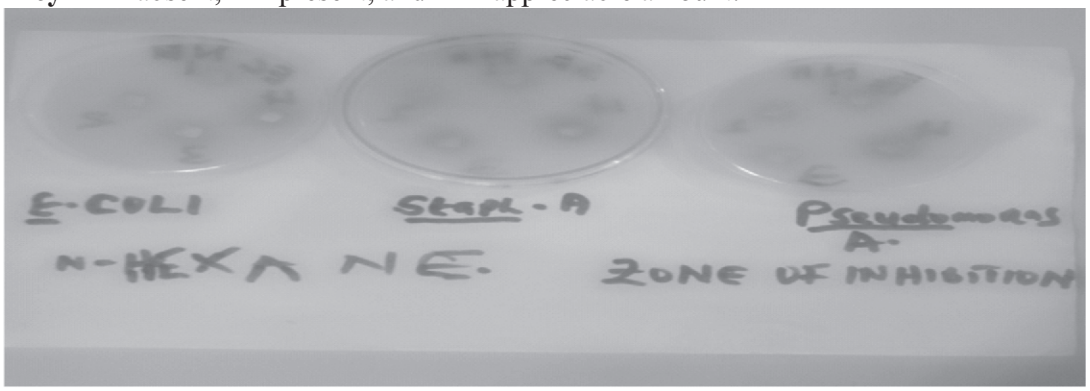

Plate II: Anti-bacterial activity of the extracts on microorganism

3.3 THIN LAYER CHROMATOGRAPHY

TheTLC Plate of the ethyl acetate showed six spots. The R_F value of each spot is presented in Table 3.5 and Plate 3.2

Table 3.5: Table of R_F values of TLC viewed under UV- light at 254nm

Pooled Eluate	R_F value
A	0.56
B	0.03
C	0.41
D	0.45
E	0.04
F	0.62

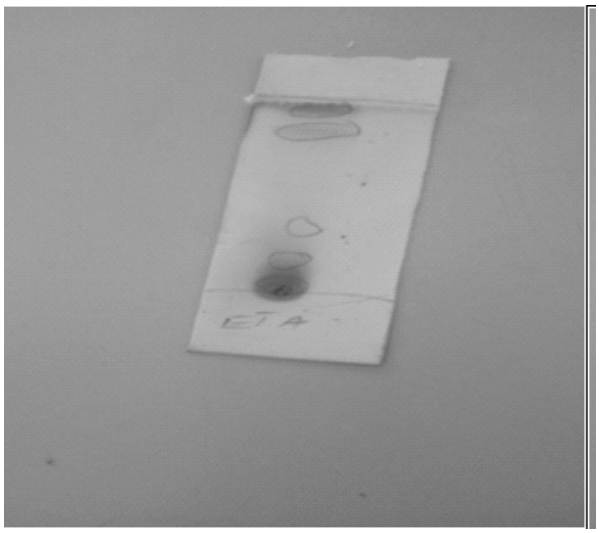

Plate III: TLC profile of Ethyl acetate

3.4 COLUMN CHROMATOGRAPHY

The column chromatography produced an isolate A which is pale green in colour and is soluble in chloroform and ethyl acetate.

3.5 ANTIBACTERIAL ACTIVITY OF ISOLATE (A)

The results are presented in Table 3.6. the isolate shows strong antibacterial activity on *E. coli* and *B. subtilis.*

Table 3.6: Zone of inhibition recorded against pure sample on microbes

Microorganism	zone of inhibition in millimeter (mm)
Staphylococcus aureus	5.0
Escherichia coli	22.0
Bacillus subtilis	16.0
Pseudomonas aeruginosa	7.0

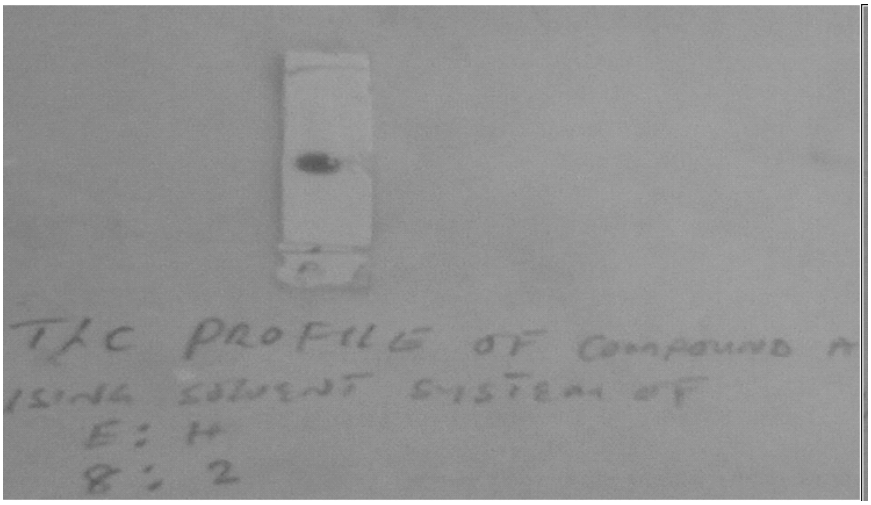

Plate IV: TLC profile of isolate A

3.6 SPECTROSCOPIC STUDIES ON ISOLATE A

3.6.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The results are presented in Table 3.7 and Appendix 1. The spectrum showed frequencies corresponding to the functional group in the isolate.

Table 3.7: FTIR Result of Isolate (A)

Frequency (cm-1) Literature	frequency (cm-1) Sample	intensity	assignment	functional group
675 – 900	837.13	63.93	C – H Aromatic	ester Aromatic
650 – 1000	945.15(w)	60.76	C – H bending	1,2,4 – tri substituted
	968.30(w)	61.57	C – H bending	1,2-disubstituted
1000 – 1300	1168.90(w)	59.86	C – O stretching	ester, carboxylic acid
1000 – 1750	1743.71(m)	53.98	C = O stretching	carboxylic acid and its derivative

Key: Where (m) – medium peak, s – strong peak, w – weak peak

3.6.2: GC-MS

The GC-MS revealed the presence of some compounds.

The results showed the molecular ion peak of various compounds as presented in Table 3.8,

Table 3.8: GC-MS Analysis Result

Peak No	Retention time	Compound name	Molecular formula	Base peak	Molecula ion peak
1	16.793	isobutyloctadecylester	$C_{30}H_{50}O_4$	149	419
2	17.127	butyl undecyl ester	$C_{23}H_{36}O_4$	149	321
3	20.417	methyl – 12- oxo-9-dodecanoate	$C_{13}H_{22}O_3$	55	226

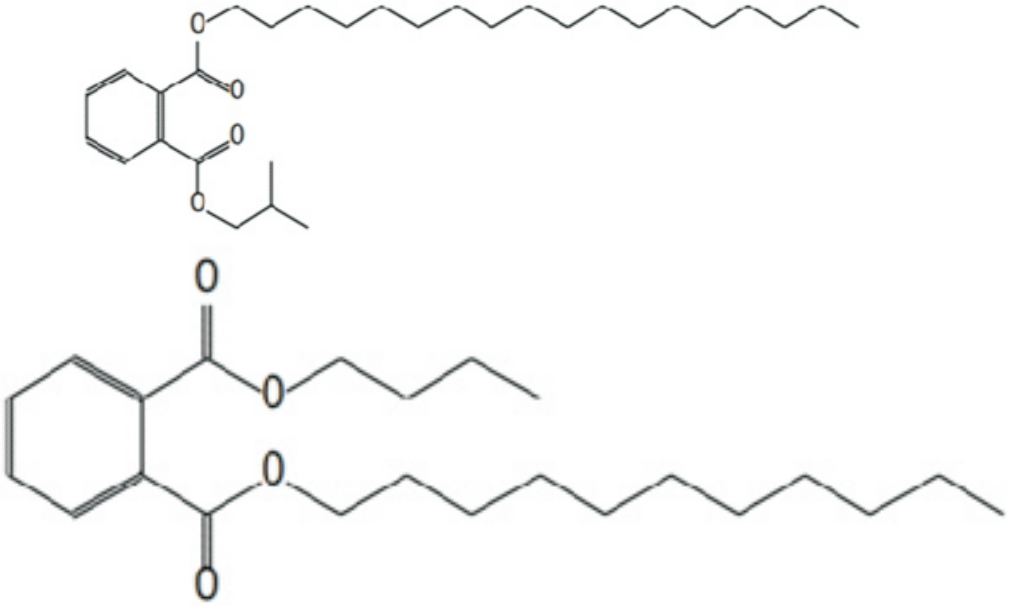

Figure 3.2: structure of butyl undecyl ester

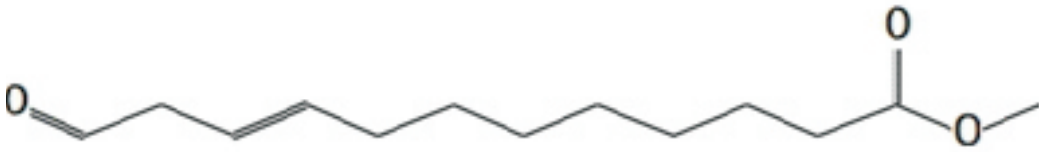

Figure 3.3: Methyl–12-oxo-9-dodecanoate

3.7 Discussion

The plant material was subjected to serial exhaustive extraction by maceration using solvents of increasing polarity, n- Hexane, Chloroform, Ethyl acetate and Methanol. The percentage yield of the extract was calculated, with Methanol having the highest yield of 4.56% as shown in Table 3.1 while Ethyl acetate with percentage yield of 0.29% shows the highest anti-bacterial activity when all the extract was subjected to anti-bacterial screening. Table 3.2 shows the various phytochemicals present. extracts were tested against two Gram-positive (*S. aureus* and *B. subtilis*) and two Gram-negative (*E. coli*and *P. aeruginosa*) bacteria. The FTIR spectrum of Isolate A as shown in Table 3.5 indicates the presence of aliphatic C-H stretching vibration at 2924 cm^{-1}, C = O stretch at 1743.71 and 1168.90 cm^{-1} respectively and C = C stretch of aromatics and aliphatic at 1446.66 and 1168.90 cm^{-1} respectively. The presence of C= C double bond between 1600 and 1475 is mainly unique to compounds with aromatic ring. The presence of C- O stretching vibration at 1168.90 further indicates the presence of aromatic ester.

The GC-MS results as shown in Table 3.8 indicate the presence of three compounds. These compounds include isobutylundecylester, butyl undecyl ester and methyl–12-oxo-9-dodecanoate. The result is in agreement with our FTIR result that shows the functional group of esters in the spectrum. These compounds are in agreement of some previous work on the plant *Cordia*. (Adeleke *et al*., 2015). However, the GC-MS revealed that the isolate could no longer be said to be a pure compound but rather a mixture of three compounds.

4.0Conclusion

Phytochemical potentials of the stem bark of *Cordia Africana* has been evaluated in this research and it revealed the presence of phytochemicals, such as, Alkaloids, Carbohydrate, Saponins, Flavonoids, Tannins, and Triterphenoids. The fraction gave the following compounds on GC-MS chromatogram; Isobutyloctadecylester, Butyl undecyl ester and Methyl-12-oxo-9-dodecanoate.

Further researches need to be conducted on this plant in terms of: -Isolation of the active compound from the chromatographic fraction, and its characterization and toxicity test for possible use as an alternative drug.

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