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Research Article

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Phytochemical and antibacterial activity of stem bark extract of *Cordia africana* Lam.

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ABSTRACT
Traditional African medicine treats microbiological infections with *Cordia africana*. The maceration method was used to extract powder samples in stages using hexane, chloroform, ethyl acetate and methanol. The presence of carbohydrates, anthraquinones, phenols, proteins, amino acids, saponins, tannins, flavonoids, alkaloids and triterpenoids/steroids were tested in the extracts by conventional phytochemical screening. The study provides evidence-based support for the use of *Cordia africana* by healers in the treatment of microbial diseases and has the potential to be used in medicine.
Keywords: Phytochemical, microorganisms and *Cordia africana*.

INTRODUCTION

The use of plants as healing agents was first discovered in ancient times and is still popular today. The literature on plants with medicinal properties dates back to AD 78 (Alice, 1996). Research has shown that underdeveloped countries, especially in areas where clinics are not readily available, depend mainly on herbal medicines to treat their common ailments (Lambo, 1979). Herbal remedies are commonly used in Japan, China, and the U.K due to the side effects of synthetic drugs and the increased cost of effective drugs (Chin et al., 2006, Rainer et al., 2006, Douglas, 2006). Alkaloids, tannins, flavonoids, saponins, phenolic compounds and others are some of the bioactive antimicrobial components found in plants (Edeogal et al., 2005). When used correctly, bioactive chemicals can treat infections and diseases in humans and animals if carefully extracted, purified and identified. Knowing the chemical composition of any plant is desirable as it can lead to the discovery of new resources from these chemical constituents, as well as the development of therapeutic methods for curing diseases (Sathish et al., 2013). Traditional applications of medicinal plants have gradually developed, resulting in increasingly sophisticated and modern medicines. The type, quality, presentation and idea of the pharmaceutical composition have undergone several adjustments, improvements and newer discoveries (Sathish et al., 2013). The need to reduce the adverse effects frequently associated with the use of synthetic antibiotics and the development of harmful resistant bacteria has increased the search for new effective compounds from plant sources. (Fred, 2006). A genus of flowering plants called Cordia is found in the subfamily Cordioideae of the family Boraginaceae. There are about 2,700 species in the family Boraginaceae, found in tropical, subtropical and warm climates around the world (Gohschling et al., 2003). There are six subfamilies and about 130 genera. Evergreen shrubs and trees of the genus Cordia of the subfamily Cordioideae. There are more than 300 species of Cordia worldwide (Thirupathi et al., 2008). Allilliba, also known as *Cordia africana* lam, is the name of a deciduous tree that can grow up to 30 meters tall and has large, dark green leaves (Bekele - Tesemma et al., 1993). It is a large shade tree that spans about 10 meters. "Gumbail" is the local name in Sudan (ElBein, 1996). In tropical Africa, it is also widely distributed. The tree grows only in the Damazin, Darfor and Kordofan regions of Sudan (Drummond, 1981). Traditional medicine in northern Nigeria uses *Cordia africana* bark powder to relieve pain and inflammation associated with haemorrhoids (personal communication). In East Africa, it is used to treat schistosomiasis, jaundice, and skin problems, and to heal open wounds in general. *Cordia africana* Lam, grown in Egypt, has been the subject of biological and phytochemical investigations, according to Begum et al., (2002). The phytochemical screening results revealed the presence of several components. Using GCMS to analyze the chemical composition of flower essential oils, the percentage of oxidized chemicals in the essential oil was determined (84.16%). When examining the lipoidal content of the leaves, pentanediol was found to be the most abundant hydrocarbon (53.95%). Investigating the carbohydrate

content of *Cordia africana* Lam, the fruit was examined and mannitol was found (27.60%). The fruits' nutritional value was assessed. Fruits are abundant in vitamins, minerals, and total protein. The evaluation of a few phytochemical tests for Gumbail (*Cordia africana*) and its use in termite management is provided by Edeogal et al. (2005). To control termites, the active components from leaves are separated for phytochemical evaluation. This study aimed to identify and identify several phytochemicals found in *Cordia africana* sheep carcass extracts.

MATERIALS AND METHODS

Sample collection and preparation

The stem bark of the plant was collected at Gidan Bubu, a village about 15 kilometres from kwalkwalawa in Wamakko LGA of Sokoto State, Nigeria. The plant was identified and authenticated by Mal Abdulazeez Salihu of the Herbarium section of the botany unit in the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto with voucher number (UDUH/ANS/0323). The stem bark was air-dried under shade for seven days. This was grounded with the aid of a manual crusher, a Victoria steel hand crusher, to obtain a coarse powder, which was then stored in an air-tight bottle until when required. The chemicals used for this research work were laboratory and analytical-grade reagents. The extraction was performed according to the procedure described by El Mohmood (2009). By placing 300 g of stem bark in a separating funnel with increasing polarity of the solvent, complete extraction was achieved in batches. 1200 Cm^3 n-hexane was applied to the bark of the stem and left for 24 h. Extracts were collected and concentrated using an open space. After drying the residue in the previous step, it was extracted with 1074 cm3 of chloroform as described earlier. The resulting extract was concentrated, and the remainder was further extracted using 1370cm^3 ethyl acetate after being allowed to dry. The extracted material was then concentrated and 1200 cm^3 of methanol was used to remove the remaining material. Formula 1 was used to determine the yield % of the extracts, and the extracts were then separated and stored in sealed containers for future use. Using the agar well dilution method, the antibacterial activity of *C. africana* bark extract was tested against all four microorganisms (El-mohmood, 2009).

Plate 1. *Cordia africana* in its natural habitat

To achieve a concentration of 10 mg/cm^3, each extract was dissolved in 10 cm^3 of dimethylsulfoxide (DMSO). A sterile petri dish is filled with sterilized medium (nutritional agar) (20 ml), covered with a lid, and allowed to cool and solidify. Add 0.1 cm^3 of standard bacterial culture (1.5 x 108 CFU/ml) to the medium, and then allow drying at 390°C for 30 min. Each culture plate has a hollow centre well filled with extracts prepared previously at concentrations of 25, 30, 35 and 40 mg/ml using a standard cork borer (6 mm diameter). The control of ciprofloxacin was performed by the same method (positive control).

$$\%\ \boldsymbol{yield} = \frac{\boldsymbol{weight\ of\ extract\ obtained}}{\boldsymbol{weight\ of\ sample}} \times \frac{\mathbf{100}}{\mathbf{1}} \quad \ldots\ldots (1)$$

RESULTS AND DISCUSSION

The results of the experiment carried out are given in the Tables below.

Phytochemical Screening

The result of the phytochemical screening is supplied in table 1 below. The methanol extract incorporates all the phytochemicals screened and is then followed by way of ethyl acetate which no longer incorporates alkaloids. The n-Hexane extract incorporates only steroids and Triterpenoids. The chloroform extract contains steroids/Triterphenoids and carbohydrates. Anthraquinones is absent in all of the extracts.

Table 1. 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	-	-	+		-
NaOH	-	-	+		+
Tannins					
Ferric chloride	-	-	+	+	
Lead- sub acetate	-	-	+		+
Phenols					
Ferric chloride	-	-	+	-	
Protein and amino acid					
Xanthoproteic test	-	-	-		+
Triterphenoids/ steroids					
Salkowski test	++	++	++		++
Leiberrmann burchard test	++	++	++		++
Anthraquinones	-	-	-	-	

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

Antimicrobial Screening

The effects of the antimicrobial research are provided in table 2 and Plate 2. the n- Hexane inhibited all of the microorganisms except *B. subtilis*. The chloroform extract did not inhibited any of the microbes. The ethyl acetate extract is more on *S. aureus* with the region of inhibition of 8.0mm. The methanol extract inhibited all except boom of *E. coli* best.

Table 2. Zone of inhibition in millimetres (mm)

Micro Organism	mg/ml	n- Hexane	Chloroform	Ethyl acetate	Methanol
Staphylococcus aureus	25	2.0	-	2.0	-
	30	2.0	-	2.1	-
	35	4.0	-	3.0	-
	40	5.0	-	8.0	-
Escherichia coli	25	1.0	-	-	1.5
	30	2.0	-	-	2.0
	35	2.1	-	-	3.0
	40	3.0	-	-	3.5
Bacillus subtilis	25	-	-	-	-
	30	-	-	-	-
	35	-	-	-	-
	40	-	-	-	-
Pseudomonas aeruginosa	25	2.0	-	-	-
	30	2.1	-	-	-
	35	3.0	-	-	-
	40	5.0	-	-	-

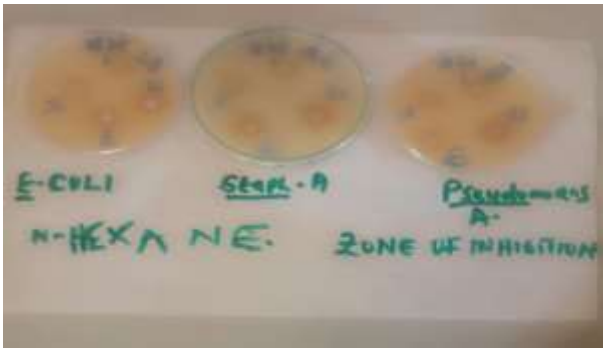

Plate 2. Anti-bacterial activity of the extracts on microorganisms

Thin Layer Chromatography

Thin-layer chromatography was done using silica gel (GF254 nm) and many mobile phases were tested. The best separation was observed with mobile phase (n-Hexane: Ethyl acetate) (99: 1). The TLC Plate of the ethyl acetate showed six spots. The R_F value of each spot is presented in Table 4 and Plate 3.

Table 3. Standard antibiotic on test microorganism zone of inhibition of ciprofloxacin (control)

Microorganism	Zone of inhibition in millimetres (mm)
Staphylococcus aureus	10.0
Escherichia coli	12.0
Bacillus subtilis	1.0
Pseudomonas aeruginosa	14.0

Table 4. 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

Table 5. Showing n-Hexane: Ethyl acetate solvent profile during column chromatography

n-Hexane:		Ethyl acetate
99	:	1
94	:	6
93	:	7
92	:	8
92	:	8
90	:	10
89	:	11
88	:	12

Antimicrobial Activity of Isolate (A)

The results are offered in table 6. The isolate shows strong antimicrobial activity on E. coli and B. subtilis.

Table 6. Zone of inhibition recorded against pure sample on microbes

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

Fourier Transform Infrared Spectroscopy (FTIR)

The results are presented in Table 7. The spectrum showed frequencies corresponding to the functional group in the isolate.

Table 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
3100 – 3000	3055.35	57.41	= C – H stretching	Alkene
1600 – 1475	1446.66	62.07	– C = C	Aromatic
3075 – 3095	3055.35	57.41	– C = C	Alkene substitution
2850 – 3000	2924.18 (s)	45.51	CH_3,CH_2,CH	Alkanes

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

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 8,

Table 8. GC-MS Analysis Result

Peak No	Retention time	Compound name	Molecular Base formula	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

Fig 1: Isobutyloctadecylester`

Fig 2: butylundecylester`

The plant material was subjected to serial exhaustive extraction by maceration using solvents of increasing polarity, n- Hexane, Chloroform, Ethyl acetate and Methanol. Table 1 shows the various phytochemicals present. However, the result shows the absence of anthraquinone. extracts were tested against two Gram-positive (*S. aureus* and *B. subtilis*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacteria. Diameters of zones of inhibition for different fractions against standard organisms are shown in Table 2. Ethyl acetate fraction showed activity ranged between 2.0 -8.0 mm, n-Hexane shows activity between 2.0 -5.0 mm while methanol shows activity between 1.5 -3.5 mm. The highest zone of inhibition by the standard ciprofloxacin was against *P. aeruginosa* with diameter of 14.0 mm. the chloroform extract does not show any activity on any of the bacterial. This is in agreement with similar work on *Cordia africana* (Emtinan *et al*., 2015). Thin layer chromatography was done using of silica gel (GF254 nm) and many mobile phases were tested. The best separation was observed with mobile phase (n- Hexane: Ethyl acetate) (99: 1). The fraction showed the presences of six florescence spots with different RF values when inspected under UV lamps (254 nm). The Plate after being sprayed with Para anisaldehyde spray reagent gave six spots, RF values of separated spots ranged between 0.03 and 0.62 and the colours were grey, violet, pale violet, green and blue. Colours of separated spots indicate that a fraction may contain triterpens, sterols, sugars and phenols. Table 5 shows results of column chromatography. A total of 97 fractions obtained and pooled together based on their thin layer chromatography (TLC) profile, gave six combined sub-fractions (A–F) as shown in the Table. Sub-fraction A, with an R_F of 0.56 (80:20 H: E), appeared to be a pure compound, whereas sub-fractions B–F were mixtures of various compounds as revealed on their TLC plates. Since our interest is on obtaining a pure isolate,

Sub fraction A that shows a single spot was further subjected to anti-bacterial activity using the same stock culture of a clinical isolate of two-gram positives, *Staphylococcus aureus* and *Bacillus subtilis* and two-gram negatives, *Escherichia coli* and *Pseudomonas aeruginosa* with the same procedure. An improved zone of inhibition was noticed in all four bacterial except *Staphylococcus aureus*. *Escherichia coli* showing the highest zone of inhibition of 22.0 mm, greater than that recorded for ciprofloxacin. Another point of interest is that *Bacillus subtilis*, that shows no activity with ethyl acetate extract and very low activity with ciprofloxacin, show improved activity on Isolate A. However, *Staphylococcus aureus* with the highest activity of 8.0 mm on ethyl acetate shows a lesser activity of 5.0 mm. Thus, it is plausible that the disparity in the antimicrobial activity observed in this study shows that Isolate A recorded a higher antibacterial activity as against the initial extract obtained from the various solvents. It is also observed that there is a general increase in the zone of inhibition with an increase in the solvent concentration. The FTIR spectrum of Isolate A as shown in Table 7 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 aliphatics 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 an 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 8 indicate the presence of three compounds. These compounds include isobutyl undecyl ester, butyl undecyl ester and methyl–12-oxo-9- dodecanoic. The result is in agreement with our FTIR result which shows the functional group of ester in the spectrum. These compounds are in agreement with 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.

CONCLUSION

Phytochemical potentials of the stem bark of *Cordia africana* have been evaluated in this research and it revealed the presence of phytochemicals, such as Alkaloids, Carbohydrate, Saponins, Flavonoids, Tannins, and Triterpenoids. Result obtained at various concentration of the extract s, indicated that the Ethylacetate extracts possesses the highest antibacterial activity on tested bacteria, and one of its column chromatographic fraction showed high activity against *E. coli.* The TLC profile was used to determine the column chromatography of the fraction in which 97 eluents obtained were pooled together to obtain six sub-fractions (A-F), where fraction A Appear as a pure isolate. Isolate A shows a stronger antibacterial activity than the initial extract as revealed in table 6. However, upon FTIR and GC-MS analysis, the isolate through the GC-MS chromatogram revealed the following compounds; Isobutyloctadecylester, Butyl undecyl ester and Methyl-12-oxo-9-dodecanoate.

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