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Antimicrobial Activity of the Aqueous and Ethyl Acetate Sub-Fractions of *Alchornea cordifolia* Leaf

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Research Article

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ABSTRACT

Aims: To investigate the antimicrobial activity of ethyl acetate and residual aqueous fractions of the methanol extract of *Alchornea cordifolia* leaf against *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 12600, *Escherichia coli* ATCC 11775 and *Candida albicans* ATCC 18804 in comparison to standard antibiotics.
Study design: Extraction of *Alchornea cordifolia* leaf, partitioning of the extract, susceptibility tests (Zones of inhibition) and Minimum Inhibitory and Bactericidal Concentrations determination.
Place and Duration of Study: Department of Medicinal Plant Research and Department of Pharmaceutical Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Idu – Abuja, Nigeria and Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria, July and October.
Methodology: The leaves of *Alchornea cordifolia* (Schum. & Thonn.) Muell. Arg. were collected, dried at room temperature and extracted with methanol using a soxhlet extractor. The methanol extract was partitioned between ethyl acetate and distilled water to obtain an ethyl acetate sub-fraction (EAF) and an aqueous residual fraction (AF). Agar well diffusion and agar dilution methods according to Clinical Laboratory Standards Institute (CLSI) were used to test the antimicrobial activity of the ethyl acetate and aqueous fractions of *Alchornea cordifolia* against the above mentioned microbial species.
Results: Both fractions; ethyl acetate and residual aqueous fractions of the methanol

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extract showed antimicrobial activity against the standard organisms viz: *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 12600, *Escherichia coli* ATCC 11775 and *Candida albicans* ATCC 18804. The highest activity was observed for the ethyl acetate fraction against *Staphylococcus aureus* ATCC 12600 with zone of inhibition of 27 mm, Minimum Inhibitory Concentration (M.I.C) of 1.25 mg/ml and Minimum Bactericidal Concentration (M. B. C) of 2.5mg/ml.

Conclusion: *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 12600, *Escherichia coli* ATCC 11775 and *Candida albicans* ATCC 18804 were susceptible to the ethyl acetate sub-fraction and residual aqueous fractions of the methanol extract of *Alchornea cordifolia* leaf.

Keywords: Antimicrobial; Alchornea cordifolia; ethyl acetate fraction; aqueous fraction.

1. INTRODUCTION

One of the main sources of medicines consisted of drug preparations from plants and vegetables until the early twentieth century. Natural products, as crude plant extracts have been use for thousands of years and many of these ancient formulations have been recorded in ancient literatures (Hamta and Robert, 2004). World Health Organization (1996) notes that despite the immense technological advancement witnessed from the early part of the twentieth century in modern medicine, about 75% of the populations of Africa still rely on traditional healing practices and medicinal plants for their daily health care needs (cited in Patel *et al.,* 2011, p. 42). The floral biodiversity of Africa provides the African traditional medical practitioner with an impressive "natural pharmacy' from which plants are selected as remedies or as ingredients to prepare herbal medicines for various human diseases and disorders (John, 2004). In recent years, some of the widely used African medicinal plants have been selected for investigation of their chemical constituents and biological activity by researchers and scientists working in the field of natural products research.

Alchornea cordifolia (Schum. & Thonn.) Muell. Arg. which is of the family Euphorbiaceae, is geographically distributed in secondary forest usually near water, moist or marshy places. It is called *Agyama* in Ghana, *Susu bolonta* in Sierra Leone, *Casamance bugong* in Senegal, *Tschiya* in Togo, *Bondji* in Cameroon, *Ewe ipa, Ubobo* and *Bambami* in Nigeria. The plant grows to a considerable height but is always of a shrubby or scrambling habit.

Alchornea cordifolia is an important crude drug in indigenous system of medicine in the coastal regions of West Africa. A decoction of the leaves is taken as a remedy for veneral diseases and urethral discharges in the Cameroon Mountain and in Senegal (Dalziel, 1956; Le Grand, 1989). In Nigeria, the decoction of the plant leaf is used against gonorrhoea (Ogungbamila and Samuelsson, 1990). The infusion of the leaf of *A. cordifolia* is taken orally for urinary tract infection in Zaire (Muanza *et al*., 1994). The decoction of the leaf is used for conjunctivitis in Senegal (Le Grand, 1989). For ringworm, the juice of the leaves and fruit is rubbed on the skin (Okeke *et al*., 1999). The plant is used for treating infected wound in Zaire (Muanza *et al*, 1994). The infusion of the dried leaf of *A. cordifolia* is used for diarrhoea in Zaire (Kambu *et al*, 1990; Muanza *et al*, 1994). The fresh chewed leaf juice of the plant is used for diarrhoea in Sierra Leone (Macfoy and Sama, 1990).

Secondary metabolites which constitute an important source of pharmaceutical drugs have been reported to be present in *Alchornea cordifolia* leaf: alkaloids such as alchornine; tannins and polyphenols (GHP, 1992); tannins, phenolic acids such as gallic acid, ellagic acid, protocatechuic acid (Ogungbamila and Samuelsson, 1990; Lamikanra *et al*., 1990; Banzouzi *et al*., 2002); flavonoids: quercetin, hypericin and guaijaverin (Lamikanra *et al*., 1990; Ogungbamila and Samuelsson, 1990; Ajali, 2000); an alkaloid: triisopentenylguanidine (Lamikanra *et al*., 1990); inulin, tannins, alchornine, saponins, flavonoids, glycosides and phenol, anthraquinones, sterols and cardiac glycosides (Abdullahi *et al.,* 2003; Adeshina *et al.,* 2007; Adeleye *et al.,* 2008; Amos-Tautua *et al.,* 2011).

Secondary metabolites have been observed to possess antimicrobial properties. Tannin compounds inhibit the microbial growth by causing the bacterial colonies to disintegrate which probably results from their interference with the bacterial cell wall (Erasto *et al.,* 2004). Shivananda *et al*., (2007) and Manjunatha *et al*., (2007) reported that tannins hasten the healing of wounds, inflamed mucous membrane and arrest bleeding. Tannins are pore-closing substances; tannins and flavonoids possess ability to increase colonic water and electrolyte reabsorption, therefore plant containing tannins and flavonoids are used in the treatment of diarrhoea and dysentery (Akinpelu and Onakoya, 2006; Agbor *et al*., 2004; Palombo, 2006) (cited in Adeshina *et al.,* 2010, p. 653). El-Mahmood, (2009) also reported that glycosides and flavonoids are known to protect against gastrointestinal infections.

Saponins are surface active agents, which interfere with or alter the permeability of the cell wall thus facilitating the entry of toxic materials or leakages of vital components from the cell. Phenol is generally a protoplasmic poison and is toxic to all types of cells. Precipitation of protein occurs with high concentration of phenol, while at low concentrations it denatures proteins without coagulating them. It is free to penetrate the tissues because of its denaturing activity.

Adeshina *et al*., (2010) showed that ethyl acetate extract of *Alchornea cordifolia* leaves possesses antimicrobial activity against the clinical and type isolates of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans.* However, we investigated the antimicrobial activity of an ethyl acetate sub-fraction of a methanol extract.

This study aims at investigating the antimicrobial properties of the sub-fractions of a methanol extract of *Alchornea cordifolia* leaf.

2. MATERIALS AND METHODS

2.1 Collection, Identification and Preparation of Plant Leaf

Alchornea cordifolia leaves were collected from Abuja, Nigeria. They were authenticated in the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria where a voucher with specimen number 4334 was kept for future reference. The leaves were air-dried at room temperature and then reduced to powder using mortar and pestle.

2.2 Preparation of the Extract and its Derived Fractions

Using the Soxhlet extractor 300 gm of the powdered leaves was extracted with 450 ml of methanol at room temperature until all the extractable components were exhausted. The

methanol extract was concentrated and kept in a dessicator. Twenty-five gram (25 g) of the methanol extract was partitioned between ethyl acetate and distilled water resulting in an ethyl acetate fraction (EAF) (yield 32.1%) and a residual aqueous fraction (RAF) (yield 67.2%). The ethyl acetate sub- fraction was evaporated under reduced pressure at 40°C, yielding dry residue, considered as the fraction, while the residual aqueous fraction was freeze-dried. The fractions were stored in a dessicator until required.

2.3 Antimicrobial Activity

2.3.1 Purification of organisms

Pseudomonas aeruginosa ATCC 10145, *Staphylococcus aureus* ATCC 12600, *Escherichia coli* ATCC 11775 and *Candida albicans* ATCC 18804 were collected from the Department of Pharmaceutical Microbiology, University of Benin, Benin City, Nigeria. The organisms were confirmed by sub-culturing into Nutrient broth and Sabouraud Dextrose liquid media and incubated at 37^{o}C for 18 hours and 25^{o}C for 5 days respectively.

They were further streaked on the Nutrient agar and Sabouraud Dextrose agar and incubated at 37^{o}C for 18 hours and 25^{o}C for 5 days respectively. Biochemical tests were used to confirm the organisms. The organisms were kept on agar slants at 4^{o}C until needed.

2.3.2 Preparation of inoculums

Eighteen-hour broth culture of the test organism was suspended into sterile nutrient broth. It was standardized according to Clinical Laboratory Standards Institute (CLSI, 2002) by gradually adding normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0×10^{6} cfu/ml.

2.3.3 Susceptibility testing

The washed overnight broth cultures were diluted appropriately using sterile normal saline to 0.5 McFarland scales (0.5 McFarland is about 10^{6} cfu/ml). The molten sterile nutrient agar (20 ml) was poured into sterile Petri dish and allowed to set. The sterile nutrient agar plates were flooded with 1.0 ml of the standardized test organism and the excess was drained off and dried at 30^{o}C for 1 hr. A sterile cork borer (No. 4) was used to bore equidistant cups into the agar plate. One drop of the molten agar was used to seal the bottom of the bored hole, so that the extract will not sip beneath the agar. One hundred microlitres of the fractions of different concentrations (1.25 – 20.0 mg/ml) was added to fill the bored holes. Negative control was prepared by putting 0.1 ml of pure solvent in one of bored holes and aqueous solution of 2 µg/ml of Gentamicin (for Gram positive bacteria) and 4 µg/ml of Gentamicin (for Gram negative bacteria) (Sweetman, 2005) in another bored hole which served as positive control. One hour pre-diffusion time was allowed, after which the plates were incubated at 37°C for 18 h. The zones of inhibition were then measured in millimetre. The above method was carried out in triplicates and the mean of the triplicate results were taken. Sabouraud's dextrose broth and agar were used for *C. albicans* and incubated at 25^{o}C for 5 days. Cotrimoxazole was used as positive control for *C. albicans*.

2.3.4 Minimum Inhibitory Concentration (M.I.C.) and Minimum Bactericidal/Fungicidal Concentration (M. B. C/M. F. C)

Graded concentrations of the fractions ranging from 1.25 – 20.0 mg/ml were used. These concentrations in sterile melted Mueller-Hinton agar plates were prepared using double dilution method. The solidified leaf extract-agar admixture plates were inoculated with 2.0 µl of standardized 18 h culture test organism. The inocula were allowed to diffuse into the test agar plates for 30 min. The test agar plates were then incubated at 37°C for 18 h and the lowest concentration of the extract in the test agar plates that showed no growth was considered as the M.I.C. of the extract against the test organism.

The M. B. C./M. F. C. was carried out by inoculating the concentration of the extract/fraction in the test agar plates showing no visible growth into sterile nutrient broth test-tubes containing inactivating agents 3% v/v Tween 80. These test-tubes were then incubated at 37°C for 24 h after which they were examined for presence or absence of growth.

Same experiment was carried out for *C. albicans* using Sabouraud's dextrose agar and liquid medium and incubated at 25°C for 5 days.

2.4 Statistical Analysis

Results were expressed as mean ± standard deviation. The data was analysed using Student's t-test. $P<0.05$ was considered significant and $P>0.05$ not significant.

3. RESULTS AND DISCUSSION

3.1 Susceptibility of the Ethyl Acetate and Residual Aqueous Fractions

All the test organisms were susceptible to the ethyl acetate and residual aqueous fractions with *P. aeruginosa* being the most susceptible to the aqueous fraction and *S. aureus* the most susceptible to the ethyl acetate fraction (Tables 1 - 2). There is significant difference between the zones of inhibition showed by ethyl acetate fraction against *S. aureus* at all concentrations used, *C. albicans* at 20 mg/ml and that showed by aqueous fraction against the same organisms at $P<0.05$ while there is no significant difference between the zones of inhibition showed by ethyl acetate fraction against the other organisms and that showed by aqueous fraction against the organisms at $P>0.05$ (Tables 1 – 2).

The ethyl acetate and aqueous fractions of methanol extract of *Alchornea cordifolia* showed antimicrobial activity against Gram positive bacteria, Gram negative bacteria and the yeast *Candida albicans, a*lthough, the ethyl acetate fraction was more active against Gram positive bacteria - *S. aureus* and the yeast *Candida albicans* (Table 1). This suggests that these fractions have broad spectrum of antimicrobial activity. Gram negative bacteria have been known to show resistance to antimicrobial agents due to the composition of their cell membrane (Wiley, 2008). The ethyl acetate fraction displayed significant antimicrobial activity against such recalcitrant pathogenic bacteria like *S. aureus* that are increasingly becoming more difficult to treat due to the development of resistance to known antibiotics including the newer ones. This result agrees with the finding of Adeshina *et al.,* (2010) who reported that ethyl acetate extract showed antimicrobial activity against clinical and type *S. aureus* isolates. These pathogens are known to cause majority of community and hospital acquired infections and are capable of elaborating several virulence factors including the

formation of biofilms on colonized surfaces (Indrayan *et al*., 2002; De and James, 2002). *Candida albicans* also responded to ethyl acetate fraction which indicates that the fraction possesses to some extent anti-candida agent. Adeshina *et al.,* (2010) reported that type *C. albicans* was susceptible to ethyl acetate extract.

The standard antibiotics Gentamicin and Cotrimoxazole used showed larger zones of inhibition against *P. aeruginosa, E. coli,* and *C. albicans* (Tables 1 and 2). This may be attributed to the fact that conventional antibiotics are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, while herbal medicinal products prepared from plant and animal origins are subject to contamination and deterioration most of the time (El-Mahmood and Ameh, 2007). However, the ethyl acetate fraction showed larger zone of inhibition against *S. aureus* than that showed by Gentamicin against the same organism (Table 1).

The RAF showed more antimicrobial activity against *P. aeruginosa* than the ethyl acetate fraction (Table 2). The active ingredient which possesses the antimicrobial activity against *P. aeruginosa* might have been present in the aqueous fraction. The solvent system plays a significant role in the solubility of the active principles in the plant and influences the antibacterial activities.

3.2 Antimicrobial Activity of Ethyl Acetate and Residual Aqueous Fractions of *A. cordifolia*

Figures 1 and 2 showed that the ethyl acetate fraction had more antimicrobial activity against all the organisms except *P. aeruginosa* than the aqueous fraction while the aqueous fraction was more active against *P. aeruginosa* than ethyl acetate fraction.

Generally, ethyl acetate fraction (non-polar solvent) of the methanol extract of *A. cordifolia* leaf was relatively more active than the aqueous fraction (polar solvent) (Figs.1 and 2). This result is contrary to the results of Ajaiyeoba, (2002) and Udobi and Onaolapo (2009) who reported that aqueous fractions (polar solvent) showed more antimicrobial activity than hexane (a non-polar solvent), and ethanol and methanol (polar solvents) were more active than chloroform (a non-polar solvent) respectively.

The low M.I.C values of the ethyl acetate fraction against *S. aureus* and *C .albicans* confirm the high activity of the fraction at low concentrations. High activity of antimicrobial agent at low concentration is very essential for chemotherapeutic purposes because of their toxicity to the patient's system (Adeshina *et al*., 2010).

It was generally observed that the ethyl acetate extract showed higher antimicrobial activity against *P. aeruginosa* ATCC 10145*, S. aureus* ATCC 12600*, E. coli* ATCC 11775 and *C. albicans* ATCC 18804 as reported by Adeshina *et al*., (2010) than the ethyl acetate fraction of methanol extract of *Alchornea cordifolia*.

The ethyl acetate fraction had the lowest M.I.C. value of 1.25 mg/ml and 2.5 mg/ml against *Staphylococcus aureus* and *Candida albicans* respectively while the highest values of 5.0 mg/ml and 10.0 mg/ml were observed against the gram negative bacteria; *E. coli* and *P. aeruginosa*. The M. I. C. values of aqueous fraction against the organisms were higher; 5.0 mg/ml – 20.0 mg/ml.

Table 1. Susceptibility of the test organisms to ethyl acetate fraction of *Alchornea cordifolia*

Test organisms	Zones of Inhibition (mm)						
	20mg/ml	10mg/ml	5.0mg/ml	2.5mg/ml	1.25mg/ml	GTM*	CMZ**
P. aeruginosa ATCC 10145	17 ± 0.1	15 ± 0.2	12 ± 0.1	11± 0.1	NI***	30 ±0.0	NA****
S. aureus ATCC 12600	27 ± 0.2	24 ± 0.4	21 ± 0.1	18 ± 0.4	15 ± 0.3	25 ±0.2	NA****
E. coli ATCC 11775	19 ± 0.2	17 ± 0.1	14 ± 0.3	11 ± 0.1	NI***	23± 0.0	NA****
C. albicans ATCC 18804	21 ± 0.1	17 ± 0.3	15 ± 0.1	NI***	NI***	NA****	27± 0.2

*The results are expressed as mean ± standard deviation, *Gentamicin, **Cotrimoxazole, ***No Inhibition, ****Not Applicable.*

Table 2. Susceptibility of the test organisms to residual aqueous fraction of *Alchornea cordifolia*

Test organisms	Zones of Inhibition (mm)						
	20mg/ml	10mg/ml	5.0mg/ml	2.5mg/ml	1.25mg/ml	GTM*	CMZ**
P. aeruginosa ATCC 10145	18 ± 0.5	16 ± 0.1	13 ± 0.1	11 ± 0.2	NI***	30 ± 0.0	NA****
S. aureus ATCC 12600	13 ± 0.1	11 ± 0.1	NI***	NI***	NI***	25 ± 0.2	NA****
E. coli ATCC 11775	17 ± 0.2	15 ± 0.2	13 ± 0.1	11 ± 0.1	NI***	23 ± 0.0	NA****
C. albicans ATCC 18804	15 ± 0.1	12 ± 0.3	11 ± 0.5	NI***	NI***	NA****	27±0.2

*The results are expressed as mean ± standard deviation, *Gentamicin, **Cotrimoxazole, ***No Inhibition, ****Not Applicable.*

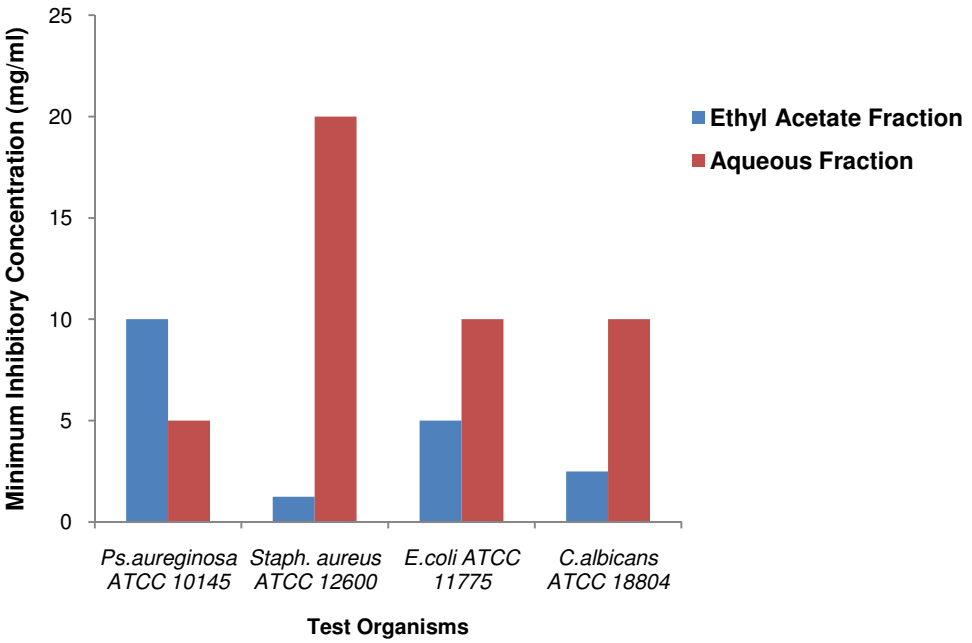

Figure 1. Comparative minimum inhibitory concentrations of ethyl acetate fraction and residual aqueous fraction of *A. cordifolia* leaf.

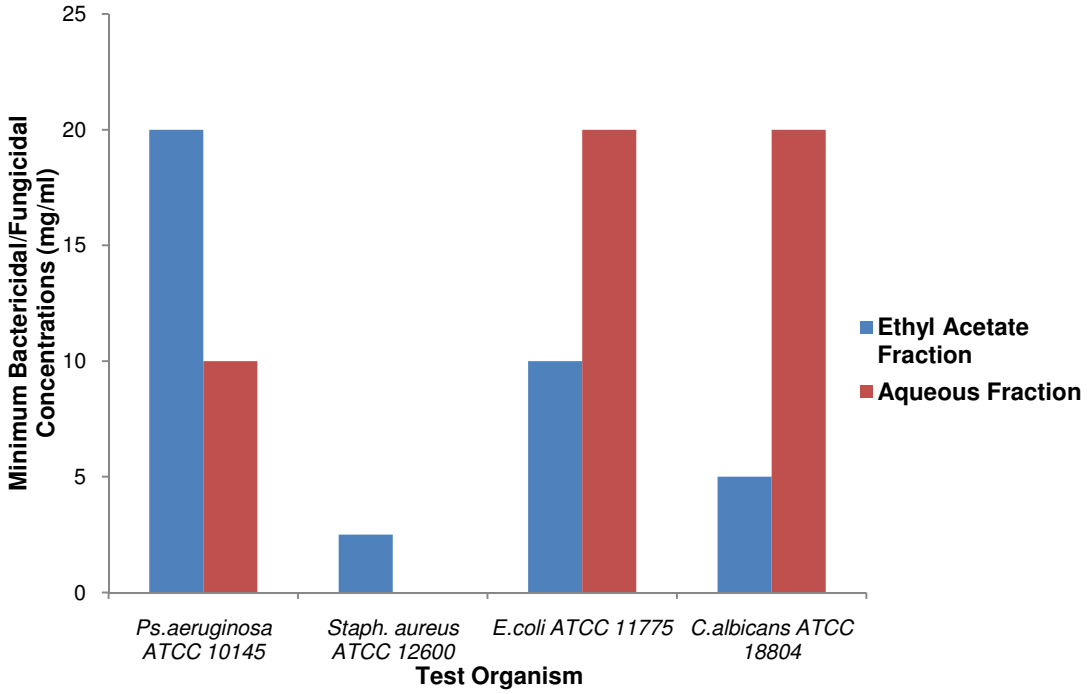

Figure 2. Comparative minimum bactericidal/fungicidal concentrations of ethyl acetate fraction and residual aqueous fraction of A*. cordifolia* leaf

The ethyl acetate fraction showed bactericidal action against *Staphylococcus aureus* and *Candida albicans* at 2.5 mg/ml and 5.0 mg/ml while the M. B. C. values of 10 mg/ml – 20 mg/ml were showed by aqueous fraction against all the organisms.

4. CONCLUSION

Ethyl acetate and residual aqueous fractions from the methanol extract of the *Alchornea cordifolia* leaf possess antimicrobial activity against the test Gram positive and negative bacteria species and the yeast-*Candida albicans.* The ethyl acetate sub-fraction displayed a potential antimicrobial activity that can be explored as remedy for human microbial infections which could justify the claimed ethno-medicinal uses of *Alchornea cordifolia* leaf.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Abdullahi, M., Mohammad, G., Abdukadir, N.U. (2003). Medicinal and economic plants of Nupe Land. ed1, Jube-Evans Books and Publications, Bida, Nigeria. pp. 106-107.

Adeleye, A., Ezekiel, A.O., Smith, S., Odusola, O., Sobande, J. (2008). Antibacterial activity of extracts of *Alchornea cordifolia* (Schum and Thonn) Mull.Arg.*, Boerhavia diffusa* (L) and *Bridellia micranthal* (Hoscht) Baill. used in traditional medicine in Nigeria on *Helicobacter pylori* and four diarrhoeagenic bacterial pathogens. Afr. J. Biotechnol, 7, 3761-3764.

Adeshina, G.O., Onaolapo, J.A., Ehinmidu, J.O., Odama, L.E., Kunle, O.F. (2007). Phytochemical and toxicologic activity of the leaf extracts *Alchornea cordifolia* (Schum and Thonn) Muell. Arg. (Euphorbiaceae). Nigerian J. Pharm. Res, 6, 19 - 24.

Adeshina, G.O., Onaolapo, J.A., Ehinmidu J.O., Odama L.E. (2010). Phytochemical and antimicrobial studies of the ethyl acetate extract of *Alchornea cordifolia* leaf found in Abuja, Nigeria. J. Med. Plants Res, 4, 649-658.

Agbor, C.A., Leopold, T., Jeanne, N.Y. (2004). The antidiarrhoeal activity of *Alchornea cordifolia* leaf extract. Phytother. Res, 18, 873-876.

Ajaiyeoba, E.O. (2002). Phytochemical and antibacterial properties of *Parkia biglobosa* and *Parkia bicolor* leaf extracts. Afr. J. Biomed. Res., 5, 125 -128.

Ajali, U. (2000). Antibacterial activity of *Alchornea cordifolia* stem bark. Fitoterapia, 71, 436-438.

Akinpelu, D.A., Onakoya, T.M. (2006). Antimicrobial activities of medicinal plants used in folklore remedies in south-western Nigeria. Afr. J. Biotechnol, 5, 1078-1081.

Amos-Tautua, B.M.W., Angaye, S.S., Jonathan, G. (2011). Phytochemical screening and antimicrobial activity of the methanol and chloroform extracts of *Alchornea cordifolia*. J. Emerging Trends Engineering Appl. Sci, 2, 445-447.

Banzouzi, J.T., Prado, R., Menan, H., Valentin, A., Roumestan,C., Mallie, M., Pelissier, Y., Blache, Y. (2002). In vitro antiplasmodial activity of extracts of *Alchornea cordifolia* and identification of an active constituent: ellagic acid. J. Ethnopharmacol, 81, 399-401.

Clinical and Laboratory Standard Institute. (2002). Performance standard for antimicrobial disc susceptibility testing. Twelfth International Supplement. Approved standard M100-S12. National Committee for Clinical Laboratory Standards, Wayne, Pa.Dalziel, J.M. (1956). The useful plants of West Tropical Africa, 3rd edition, Crown Agents for Oversea Government and Administration, Millbank, London.

De, N., James, N.A. (2002). Antibacterial spectrum of extracts of Ocimum gratissium L. (Basil) and Xylopia aetiopica A. Rich. (Dunah). Niger. J. Basic Appl. Sci, 11,165- 175.

El-Mahmood, A.M. (2009). Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. J. Med. Plants Res, 3, 498-505.

El-Mahmood, A., Ameh, J. M. (2007). *In-vitro* antibacterial activity of *Parkia biglobosa* (Jacq) root, bark extract against some microorganisms associated with Urinary tract infections. Afr. J. Biotechnol, 6, 195-200.

Erasto, P., Moleta, G.B., Majinda, R.R. (2004). Antimicrobial and antioxidant flavonoids from the root wood of *Bolusanthus speciosus*. Phytochemistry, 65, 875 - 880.

Ghana Herbal Pharmacopoiea (1992). Alchornea. ed 1, The advent Press, Accra pp. 7-8.

Hamta, M., Robert, S.J. (2004). An analysis of cytotoxic botanical formulations used in traditional medicine of ancient Persia as abortifacients. J. Nat. Prod, 67, 1204.

John, A.O. (2004). Evaluation of the analgesic, anti-inflammatory and antidiabetic properties of Sclerolarya birea (A. Rich) Hochst. Stembark aqueous extract in Mice and Rats. Phytother. Res, 18, 601.

Indrayan A. K., Sharma, A., Gideria, B. S., Gupta, C.P. (2002). Antimicrobial activity of dye from *Caesalpina sappan* (Patang/Braziluvoral). Indian J. Microbiol, 42, 359-360.

Kambu, K., Tona, L., Kaba, S., Cimanga, K., Mukala, N. (1990). Antispasmodic activity of extracts proceeding of plant, antidiarrhoeic traditional preparations used in Kinshasa, Zaire. Ann. Pharm. FR. 48, 200-208.

Lamikanra, A., Ogundaini, A.O., Ogungbamila, F.O. (1990). Antibacterial constituents of *Alchornea cordifolia* leaves. Phytother. Res., 4, 198-200.

Le Grand, A. (1989). Anti-infectious phytotherapy of the Tree-Savannah, Senegal (West Africa) III; A review of the phytochemical substances and anti- microbial activity of 43 species. J. Ethnopharmacol, 25, 315-338.

Macfoy, C.A., Sama, A.M. (1990). Medicinal plants in Pujehun District of Sierra Leone. J. Ethnopharmacol, 30, 610-632.

Manjunatha, B.K., Krishna, V., Vidya, S.M., Mankani, K.L., Manohara, Y.N. (2007). Wound healing activity of *Lycopodium serratum*. Indian J. Pharm. Sci, 69, 283-287.

Muanza, D.N., Kim, B.W., Euter, K.L., Williams L. (1994). Antibacterial and antifungal activities of nine medicinal plants from Zaire. Int. J. Pharmacog, 32, 337-345.

Ogungbamila, F.O., Samuelsson, G. (1990). Smooth muscle relaxing flavonoids from *Alchornea cordifolia*. Acta. Pharm. Nordica, 2, 421- 422.

Okeke, I.N., Ogundaini, A.O., Ogungbamila, F.O., Lamikanra, A. (1999). Antimicrobial spectrum of *Alchornea cordifolia* leaf extract. Phytother. Res, 13, 67 – 69.

Palombo, E.A. (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: mode of action and effects on intestinal function. Phytother. Res, 20, 717-724.

Patel, K., Kumar, N., Sharma N. K. (2011). Pharmacognostic & Phytochemical Evaluation of *Bryophyllum Pinnatum* Leaves. J. Adv. Sci. Res, 2, 42-49.

Shivanand, B., Sivachandra-Raju, S., Chalapathi-Rao, A.N. (2007). Wound healing activity of *Matricaria recutita* L. extract. J. Wound Healing, 16, 298-302.

Sweetman, S. (2005). Sweetman S (Ed), Martindale: The complete drug reference. Pharmaceutical Press. Electronic version.

Udobi, C.E., Onaolapo, J.A. (2009). Phytochemical analysis and antibacterial evaluation of the leaf stem bark and root of the African locust bean (*Parkia biglobosa*). J. Med. Plants Res, 3, 338-344.

Wiley, J.M., Sherwood, L.M., Woolverton, C.J. (2008). Presscott, Harley and Klein's Microbiology. 7th ed. McGraw-Hill Companies, Inc.

World Health Organisation. (1996). WHO Guideline for the Assessment of herbal Medicines, WHO expert committee on specification for pharmaceutical preparation.Technical Report series No 863. Geneva.