ANTIMICROBIAL AND PHYTOCHEMICAL PROPERTIES OF LEAVES, STEMS AND MALE INFLORESCENCE OF *Alchornea cordifolia* (SCHUM. & THONN.) MUEL. ARG.

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: September 29, 2017 **Accepted:** February 12, 2018

Abstract: *Alchornea cordifolia* (Schum. & Thonn.) Muel. Arg. is used by traditional medicine practitioners in Nigeria to treat stomach disorders, diarrhoea, skin infections (wound healing), ear infections, and eye defects. To evaluate these claims, 100 mg/ml concentration of aqueous and ethanolic extracts of the leaves, stem and male inflorescence of *A. cordifolia* were screened for antimicrobial properties against two bacteria (*Escherichia coli* and *Staphylococcus aureus*) and two fungi (*Aspergillus niger* and *Trichoderma viride*). The Disc Assay Method was used for antifungal screening while Agar Well Method was used for antibacterial screening. Each plant part was subjected to qualitative phytochemical screening using standard methods. The plant parts showed higher inhibitory activities against the test bacteria than the fungi. Their ethanolic extracts were more potent than their aqueous extracts. *T. viride* was the most resistant organism whereby only the ethanolic extracts showed inhibitory effect. Leaf ethanolic extract showed the highest inhibitory activity against all tested pathogens; its inhibitory activity against *E. coli* (19.01 ± 0.08 mm) was higher than the control *Tetracycline* (18.92 ± 0.02 mm). Phytochemical screening revealed that all three plant parts have alkaloids, tannins, anthraquinones, saponins, flavonoids, terpenoids, carbohydrates, glycosides, and reducing sugars. Pentose sugars were absent in all plant parts while steroids were present only in the leaves. Therefore, the ethanolic extracts of all the plant parts should be subjected to further investigation and isolation of their antimicrobial compounds.

Keywords: *Alchornea cordifolia*, antimicrobial, phytochemistry, plant extract, stomach disorders

Introduction

Ethnobotany is the study of how people of a particular culture and region use indigenous plants for food, shelter, medicine, clothing, hunting, and religious ceremonies (Acharya and Shrivastava, 2008). The indigenous traditional knowledge of medicinal plants of various ethnic communities is currently being validated scientifically since nearly 80 % of the world population depends upon traditional system of health care (Ganesan *et al*., 2004).

Similarly, in Nigeria, more than 80 % of the population use medicinal plants (Adeniyi *et al.*, 2014a). One of such medicinal plants is *Alchornea cordifolia* (Schum. & Thonn.) Muel. Arg. - a member of the family Euphorbiaceae. *A. cordifolia* is an evergreen dioecious shrub which grows up to 8 m tall (Burkill, 1997) commonly found in freshwater swamps, secondary forests and riverine forests in Nigeria (Ayodele and Yang, 2012). The leaves are green with ovate blades, cordate bases, sub-entire margins, and acuminate apices. Their leaves have parallel venations and are arranged alternately on the branches. The plant is dioecious with unisexual flowers (Burkill, 1997). Male inflorescence of the *A. cordifolia* is yellowish to green in colour and sticky; being a copious producer of pollen grains, the plant has been linked with allergy in Nigeria (Adeniyi *et al.*, 2014b; Adeonipekun *et al.*, 2016b).

These studies have revealed that the pollen of *A. cordifolia* is abundant in the air throughout the year. *A. cordifolia* has been used in several herbal remedies in Nigeria. The leaves are normally used as infusions for the treatment of respiratory problems such as sore throat, cough and bronchitis; for management of intestinal problems such as gastric ulcers, diarrhoea, amoebic dysentery and worms; and for genital-urinary problems such as venereal diseases and female sterility (Okeke *et al.*, 1999; Ebi, 2001; Ayisi and Nyadedzor, 2003; Olaleye *et al.*, 2007; Gatsing *et al.*, 2008; 2010; Ige, 2011).

The poultice of the leaves is used for the treatment of wounds. The leaves and root bark are externally applied to treat leprosy and as antidote to snake venom (Burkill, 1997). A decoction of the fruit is taken to prevent miscarriage while the sap of the fruit is used to cure eye problems and skin diseases (Ige, 2011). Other uses of the plant include: as windbreak, tea substitute, mulch, forage for small ruminants and poultry, dye processing, fish poison, fuel, and decorations (Adewunmi *et al.*, 2001; Burkill, 1997; Ige, 2011). The leaves, roots and stem bark of *A. cordifolia* have been found to contain terpenoids, steroid glycosides, flavonoids, tannins, saponins, carbohydrate, imidazopyrimidine alkaloids (alchorneine and alchornidine), and several guanidine alkaloids. The leaves also contain gallic acid and its ester, anthralinic acid, protocatechuic acid and ellagic acid (Agyare *et al.*, 2014). Banzouzi *et al.* (2002) reported the presence of alchornoic acid in the seed oils. Several authors have worked on the leaves, stems, roots, seeds, fruits, and barks of *A. cordifolia* revealing the pharmacognostic potential of the plant as well as confirming some of the herbal and traditional claims. Some of these include: antiviral activity (Ayisi and Nyadedzor, 2003); antiplasmodial activity (Banzouzi *et al.*, 2002); antimicrobial activity (Okeke *et al.*, 1999; Ebi, 2001); antibacterial activity (Gatsing *et al.*, 2008; 2010); hepato-protective activity (Olaleye *et al.*, 2006); anti-inflammatory activity (Osadebe and Okoye, 2003); antioxidant activity (Olaleye *et al.*, 2007); wound healing activity (Agyare *et al.*, 2014).

However, a part of the plant which is seldomly worked on is the male inflorescence despite its massive production. Therefore, this research is aimed at comparing the phytochemical contents and antimicrobial potential antimicrobial potential of the leaf, stem and male inflorescence of *A. cordifolia*.

Materials and Methods

Sources of plant materials and test microbes

Parts (leaves, stems and male inflorescences) of *A. cordifolia* were collected from the University of Lagos campus, Akoka, Lagos State. The plant was collected wholly and identified at the Herbarium of the Department of Botany, University of Lagos with voucher number 906. The test bacteria: *Escherichia coli* and *Staphylococcus aureus* were obtained from the Department of Microbiology, University of Lagos. The bacteria were selected because they are common

pathogens that cause diseases in man. *E. coli* is the causative agent of diarrhoea and other gastro-intestinal diseases while *S. aureus* is common as a sexually transmitted infection (STI) as well as causative agent for cough and skin allergies. The selected test fungi were *Aspergillus niger* and *Trichoderma viride* which were obtained from the Mycology Unit of the Department of Botany, University of Lagos. *A. niger* and *T. viride* have been known to cause severe allergies and invasive infections in humans. The bacteria strains were streaked on Nutrient Agar (NA) slanted while the fungi were spread on Potato Dextrose Agar (PDA). All pure microbial cultures were stored in a freezer at 4ºC.

Preparation of plant extracts

The leaves and stems were cut into pieces. All plant materials were dried in an oven at 40 ºC (Adeniyi *et al.*, 2014a; Adeonipekun *et al.*, 2014). Dried samples were ground into powder using a grinding machine. Aqueous extraction was done by soaking 50 g of each plant part in 500 ml of cold sterile distilled water in a conical flask for 48 h. The extracts were then filtered off using Whatman No 1 filter paper while the filtrates were concentrated using a rotary evaporator (Adeniyi *et al.*, 2014a; Adeonipekun *et al.*, 2014). Ethanolic extraction was done by soaking 50 g of each plant part in 500 ml of ethanol in a conical flask for 48 h. The extracts were filtered off using Whatman No 1 filter paper while the filtrates were concentrated using a rotary evaporator (Adeniyi *et al.*, 2014a; Adeonipekun *et al.*, 2014).

Antifungal activity test of plant extracts

This was done using the Agar Disc Diffusion Test Methodology by Adeniyi *et al.* (2014a) and Adeonipekun *et al.* (2014). From the serial dilution of each fungal culture, inoculums were transferred into already prepared Potato Dextrose Agar (PDA) plates and spread evenly with a sterile glass rod. Afterwards, 6 mm sterile discs from Whatman No 1 filter paper were prepared by perforating the filter paper using a paper punch. The discs were sterilized in an autoclave after wrapping with aluminum foil. The sterilized discs were then infused in each plant extract at a concentration of 100 mg/ml. Two controls were used: sterile distilled water and Fulcin. Four of the discs from each of the extracts, antibiotic and water control were aseptically placed with the aid of a sterile forceps on PDA with inoculums. Each experimental setup was replicated by repeating the above processes. All the experimental plates were incubated at 25ºC and zones of inhibition were measured after 72 h.

Antibacterial activity test of plant extracts

This was done using the Agar Well Diffusion Test Methodology by Adeniyi *et al.* (2014a) and Adeonipekun *et al.* (2014). From the serial dilution of each bacterial culture, inoculums were transferred into already prepared Nutrient Agar (NA) plates and streaked with an inoculating loop. Afterwards, 8.5 mm wells were drilled on four parts of each NA-plate using a cup-borer. Plant extracts (concentration of 100 mg/ml) and controls (*Tetracycline* and distilled water) were pipetted directly into the wells. Each experimental setup was replicated by repeating the above processes. All the experimental plates were incubated at 37 ºC and zones of inhibition were measured after 48 hours.

Phytochemical screening

Phytochemical screenings of the plant samples were carried out qualitatively using the method described by Harbone (1998) and Evans (2009). Screening involved tests for reducing sugars, alkaloids, cardiac glycosides, saponins, tannins, flavonoids, steroids, and terpenoids.

Results and Discussion

The ethanolic extract of the leaf showed the highest inhibitory activity against *Aspergillus niger* (11.17 ± 0.53 mm) while the stem aqueous extract showed no activity. Similarly, the leaf ethanolic extract showed the highest inhibitory activity against *Trichoderma viride* (4.06 ± 0.83 mm) while the aqueous extracts of all three parts showed no activity. Ethanolic extracts of the plant parts showed higher inhibition against the tested fungi than the aqueous extracts (Fig. 1). Ethanolic extract of the male inflorescence showed the highest inhibitory activity against *Staphylococcus aureus* (19.40 ± 0.85 mm) while the stem aqueous extract (11.66 ± 0.14 mm) showed the least. Against *Escherichia coli* (19.01 ± 0.08 mm), the leaf ethanolic extract showed the highest inhibitory activity while the male inflorescence aqueous extract (7.66 ± 0.14 mm) showed the least (Fig. 2).

Generally, the ethanolic extracts showed higher inhibition than the aqueous extracts of the plant parts against the tested bacteria and fungi strains while, all the plant parts tested showed higher inhibitory activity against the bacterial strains that the fungi strains. Phytochemical screening revealed that all three plant parts used tested positive for alkaloids, tannins, anthraquinones, saponins, flavonoids, terpenoids, carbohydrates, glycosides, and reducing sugars. Pentose sugars were absent in all plant parts while steroids were present only in the leaves (Table 1).

Table 1: Qualitative phytochemical screening of *A. cordifolia* parts

Test	*A. cordifolia* parts		
	Leaf	Stem	Male inflorescence
Reducing sugars	+	+	+
Alkaloids	+	+	+
Tannins	+	+	+
Cardiac glycosides	+	+	+
Saponins	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+
Steroids	+	-	-

+ = Present; - Absent

Each part of *Alchornea cordifolia* exhibited inhibitory activity against one or more microbes. All extracts except the stem aqueous extract showed inhibitory activities against *Aspergillus niger* (Fig. 1). The dangers posed by *Aspergillus niger* to humans and plants (especially stored produce) was explained by Adeniyi *et al.* (2014a) and Adeonipekun *et al.* (2014). These authors revealed the stubbornness of the fungus to sedges extracts and suggested further research on the fungus to develop more effective antibiotics. Results from the present work have however revealed that the plant is a potential source of antibiotics against *Aspergillus niger*, especially the ethanolic extract of the leaves which was the most potent (11.17 ± 0.53 mm) with no significant difference from the control, Fulcin (14.05 ± 0.75 mm).

All aqueous extracts were inactive against *Trichoderma viride* (Fig. 1). There is a paucity of data on the antimicrobial activity against *T. viride.* This is because, the fungus has biofungicide activities and mainly used as a biological control for other plant pathogenic fungi such as *Fusarium*, *Rhizoctonia* and *Pythium*. However, the fungus has also been implicated in allergies (Khan *et al.*, 2009) and invasive infections (Chouaki *et al.*, 2002) in hypersensitive and immuno-compromised humans respectively. Therefore, the mild inhibitory activity shown by the ethanolic extracts of *A. cordifolia* against the fungus could suggest a possible role of the plant in inhibiting the activities of the invasive fungus. This could also indicate that *A. cordifolia* could inhibit the biofungicide potential of the fungus. However, these claims need to be verified with further research on the inhibitory compounds contained in *A. cordifolia*. Furthermore, the high

inhibitory activities of Fulcin shows the potential of Fulcin as an effective drug against *Trichoderma*-induced allergy.

The six extracts showed inhibitory activities against *Escherichia coli* (Fig. 2), with the leaf ethanolic extract showing the highest action (19.01 ± 0.08 mm) which was greater than that of the control, Tetracycline (18.92 ± 0.02 mm). Although the difference is not significant, but it supports the claims of traditional medicine practitioners (TMPs) that an alcoholic extract of the leaf can be used to cure diarrhoea (which is mainly caused by *E. coli*). The results also supports the works of Okeke *et al.* (1999), Ebi (2001), and Gatsing *et al.* (2008, 2010) who have recorded the antibacterial property of *A. cordifolia* leaves and the entire plant. Thus, the leaf ethanolic extract of *A. cordifolia* is a potential source of active antibiotic which should be extracted, purified and pharmacologically evaluated.

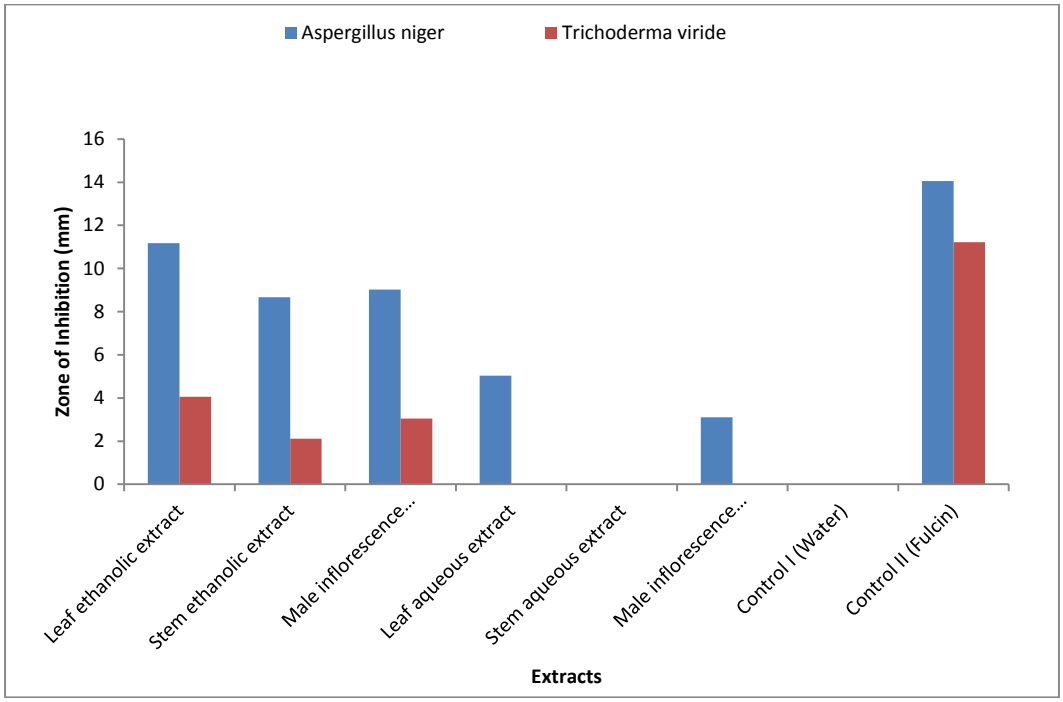

Fig. 1: Antifungal property of *A. cordifolia* extracts

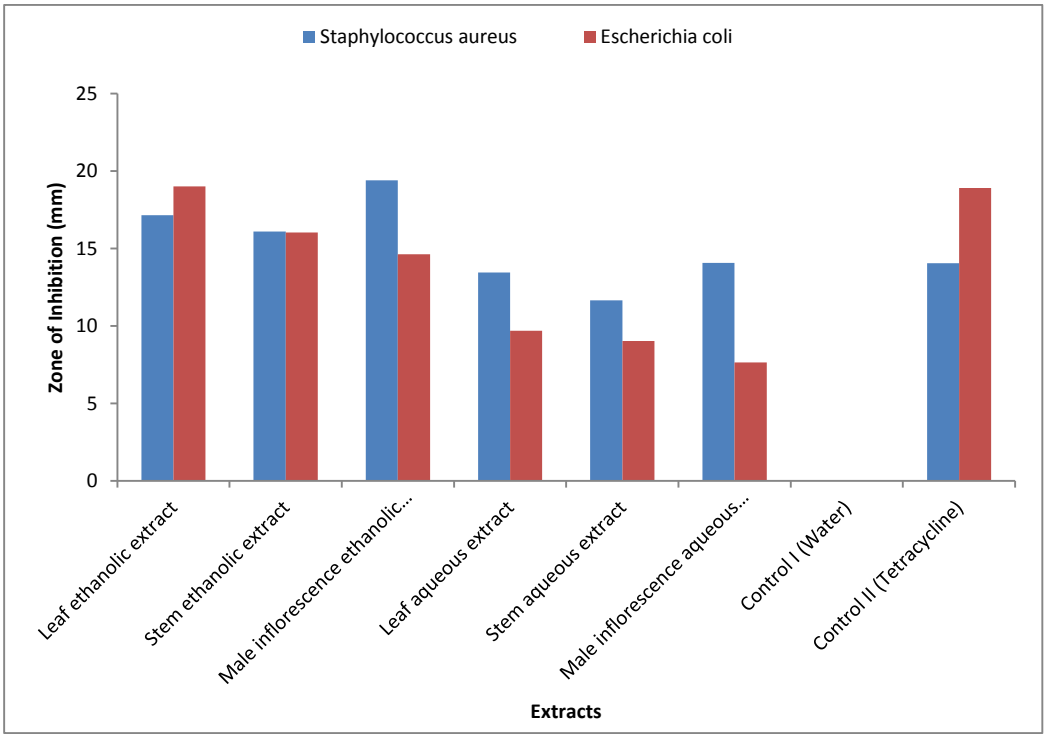

Fig. 2: Antibacterial property of *A. cordifolia* extracts

Also, the six extracts showed inhibitory activities against *Staphylococcus aureus* (Fig. 2) with the male inflorescence ethanolic extract having the highest inhibition (19.40 ± 0.85 mm) which is lower than that of the control, Tetracycline (21.38 ± 0.43 mm). This also supports the claims of TMPs that the plant can be used to cure skin infections and genital diseases (which are mainly caused by *S. aureus*). A source of this inhibitory activity could be the pollen grains present on the inflorescence. Adeonipekun *et al.* (2016a) has remarked that the chemical contents of some pollen grains confer on them some degree of antimicrobial potential. Therefore, the male inflorescence ethanolic extract of *A. cordifolia* is here suggested as a potential source of active antibiotic. Further studies are required on the antimicrobial potential of the pollen grains only to ascertain this claim.

Traditional medicine practitioners make use of water primarily as a solvent, but studies have shown that alcohol extracts of plants are much better and powerful, this may be due to the better solubility of the active components in organic solvent (De Boer *et al.*, 2005; Adeniyi *et al.*, 2014a; Adeonipekun *et al.*, 2014). In this study, ethanolic extracts show higher antimicrobial activities than the aqueous extracts. This implies that ethanol extracted more active phyto-constituents as compared to water (Nwachukwu and Uzeato, 2010; Peni *et al.*, 2010; Adeniyi *et al.* 2014a; Adeonipekun *et al.*, 2014). The fact that the aqueous extract did not inhibit as much as the ethanolic extract does not mean that the plant parts tested did not contain the sought phytochemical. Rather, the extraction solvent is a strong determinant of its recovery even when present.

In the studied plant parts, the high number of phytochemicals present could account for their use in curing several ailments in traditional medicine. The presence of flavonoids, glycosides and tannins may account for the use of *A. cordifolia* in treating stomach disorders (diarrhoea and dysentery), gastro-intestinal infections, tumor growth, and wound healing as suggested by Agbor *et al.* (2004). Similarly, as suggested by Vogel (2005) the presence of anthraquinones in plants (such as *A. cordifolia*) may account for the use of such plants as anti-inflammatory drugs and laxatives. Furthermore, Yukihiro *et al.* (2002), Adeniyi *et al.* (2014a) and Adeonipekun *et al.* (2014) all suggested that the combination of reducing sugars present in plants constitute a building block for the production of phytoalexins and this may be responsible for high antimicrobial activity. This work further supports their claims, as all three plant parts had reducing sugars and exhibited strong antimicrobial activity. The presence of alkaloids in the three plant parts may be responsible for protecting the plant against pest and diseases (Aniszewski, 2007). This attribute has led to dominance of the plant in freshwater environments and its seemingly allelopathic effect on other plants in that environment. The phytochemical results indicating the presence of steroids only in the leaves may account for the highest inhibitory activities of the leave extracts.

Conclusion

The leaves, stem and male inflorescence of *Alchornea cordifolia* are here confirmed as possessing antimicrobial properties. Highest inhibitory activities against the tested pathogens: *S. aureus*, *E. coli*, *A. niger* and *T. viride* were recorded for the leaves compared to the stem and inflorescence possibly due to the presence of steroids. The plant parts were more active against bacteria than fungi while their ethanolic extracts were more potent than their aqueous extracts. Therefore, the ethanolic extracts of the plant parts should be subjected to further investigation and isolation of their antimicrobial compounds. The outstanding inhibitory activities of the leaves ethanolic extract against *E. coli* which is even higher than the standard antibiotic showed that the plant contains compounds that can be used as antimicrobial agents in drug development.

Acknowledgments

The Departments of Botany and Microbiology in the University of Lagos are appreciated for the supply of tested microbes and use of facilities.

Conflict of Interest

The authors declare there is no conflict of interest associated with the research.

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