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Research Article

PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF DIFFERENT FORMS OF CRUDE STEM BARK EXTRACT OF *UAPACA STAUDTII*

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ABSTRACT
Uapaca species are part of ethno medicinal plants used by Africans in treating various diseases especially the infectious diseases and there are claims that *Uapaca staudtii* is one the species mainly used in our local communities. The study was carried out to determine the phytochemical and antibacterial properties of different extract forms of *Uapaca staudtii* stembark extract using standard phytochemical and microbiological techniques. The extract and fractions were screened for phytochemical constituents and antibacterial activity against *Escherichia coli, Staphylococcus aureus, Proteus* sp., *Streptococcus* sp. and *Klebsiella pneumoniae* respectively using the agar-disc diffusion method. The results showed that *U. staudtii* stembark extracts contained carbohydrates, alkaloids, glycosides, flavonoids, cardiac glycosides, saponins polyphenols and anthraquinones. The antibacterial sensitivity test showed *Escherichia coli* was sensitive to the ethanol form of extract with the Z.I range of 13.5±0.5mm -16±1.0mm. No zone of Inhibition (NZ) was observed for *Staphylococcus aureus* at the lowest concentration of 25 mg/ml. The chloroform extract showed no zone of Inhibition (NZ) for *Staphylococcus aureus* at 25 mg/ml and 50mg/ml and 75mg/ml respectively. *At t*he least concentration of 25 mg/ml, *Escherichia coli, Klebsiella pneumonia* and *Streptococcus sp exhibited* resistance to *U. staudtii* chloroform extract as NZ were recorded. The n-butanol extract form on the isolates revealed that *Escherichia coli and Proteus spp expressed resistance to* the n-butanol extract form at concentration of 25%, 50%,75% but moderately sensitive at 100% with Z.I of 12±0.2mm and13.6±0.1mm respectively. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanol extract as low as 12.5mg/ml for some test isolates show that *Uapaca staudtii* has potential to be used for production of potent antibiotics and therefore justifies the use of *Uapaca* species in traditional medicine to treat some infectious diseases.

Keywords: *Phytochemical constituents, Antibacterial properties, Uapaca staudtii, Treatments*

INTRODUCTION
In the recent years, research on medicinal plants has attracted a lot of attentions globally; large body of evidence has accumulated to demonstrate the promising potential of medicinal plant used in various traditional, complementary and alternate system of treatment of human diseases (Sher, 2009). Medicinal plants have been used for centuries to remedy human diseases because they contain substances of therapeutic values recently, some higher plant products have attracted the attention of microbiologist to search for some phytochemicals for their exploitation as antimicrobials, such products would be biodegradable and safe to human health (Kumar *et al*., 2008; Wang *et al.*, 2010).In developing countries, notably in West African, new drugs are not often affordable. Thus, up to 80% of the population uses medicinal plants as remedies. Extracts from the various plant parts (leaves, stem bark and roots) of various higher plants are used in herbal medicine production. Plant extracts are given singly or as concoctions for various ailments (Okoro *et al.*, 2010). Up to 80% of the population uses traditional medicine in primary healthcare in Africa. Although, many African plants are used in traditional medicine as antimicrobial agent but it was discovered that only few have been documented. However, in spite of

vast improved health and longevity in some countries such as United State and Europe, millions of their people are turning back to traditional herbal medicine in order to prevent or treat many illnesses and to circumvent resistance of many human pathogens to conventional antibiotics (Levy, 1998).

Uapaca staudtii is a small to medium-sized tree under phyllanthaceae family and belongs to the order malpighiales. The plant is distributed in tropical Africa and Madagascar. Other members of the famil are known to include *Uapaca pilosa, Uapaca togoensis, Uapaca guinessis, Uapaca bail,* and *Uapaca paludosa,* Uapaca species are part of ethno medicinal plants used by Africans in treating various diseases (Levin, 1986*). Uapaca heudelotti* is medicinally useful for treating skin infections, female sterility and haemorrhoids (Dalziel, 2000). *Uapaca togoensis* is also used for treating female infertility and the wood of *Uapaca staudtii* is termite-proof, it is used for making furniture, railway sleepers and barrel staves (Dalziel, 1937; Burkiil, 1985). *Uapaca paludosa* and *Uapaca vanhouttei* have also been reported for making charcoal and used as firewood (Dalziel, 2000). In view of many claims on the uses of Uapaca species in the treatments of some ailments, this study was carried out to determine the phytochemical and antibacterial properties of *Uapaca staudtii* stembark extract with the aim of highlighting its potentials.

MATERIALS AND METHODS

Collection of plant materials

The plant was collected at "Itak Ikot Akap" in Ikono Local Government Area, identified and authenticated using the sample voucher in the herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo. The plant material was dried under shade and pulverized in a clean mortar. The resulting powder was weighed and stored in a dry, clean plastic container with screw cap for subsequent processing.

Preparation of different forms of crude extracts

The powdered plant material (1Kg) was macerated in 70% aqueous methanol for 72 h filtered and concentrated on water bath (45 – 50˚C) to obtain a black solid residue (100 g) tagged as crude ethanol extract. The crude ethanol extract (20 g) was dissolved in distilled water and partitioned with ethanol, chloroform and n-butanol to obtain ethanol, chloroform and n-butanol fractions respectively. The fractions obtained were rid of solvent using water bath (45 - 50˚C) and subsequently screened for phytochemical constituents.

Preliminary phytochemical screening

The different extract forms were subjected to phytochemical screening to detect the bioactive constituents using methods of Harborne (1984), Trease and Evans (1994) and Sofowora(1994).

Collection of bacterial test isolates

Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Kkebsiella sp., and *Proteus* sp. were obtained from University of Uyo Medical centre Laboratory. Confirmation by re-characterization and identification were done according Holt *et al*.., (1996) and (Cheesbrough, 2006). The bacteria were sustained on Nutrient agar (Oxoid, USA) slant at 4^0C prior to use.

Evaluation of antibacterial activities of the plant's extracts

The antibacterial activities of the different extracts were determined using the agar-disc diffusion method as previously described by12 with some modifications. Sterile Whatmann No 1 filter paper discs of 6mm in diameter were perforated soaked in equal volumes of varying concentrations of extracts of 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml) and left for 2 hours undisturbed. The discs were dried. The antibacterial activities tests were carried out using Kirby- Bauer method (Cheesbrough, 2006). *Uapaca staudtii* stem bark extracts in ethanol, chloroform, and n-butanol were tested against test isolates.

Eighteen (18) hours cultures of each of the five human pathogenic bacteria in Nutrient Broth (Oxoid) were used for the *in vitro* antibacterial assay as standard inoculum. Using spread-plate method, each Mueller-Hinton agar (Difco Laboratories, Detroit, Mich) plate was then seeded with 0.1 ml standard inoculum of the bacteria; the inoculum was spread evenly over the surface of the media by the use of sterile glass spreader. A sterile forcep was used to pick the perforated Whatmann No 1 filter paper already soaked in different concentrations of the extract and dried were impregnated on the seeded plates, the standard reference drugs (Gentamycin and Streptomycin) were also impregnated on the plates The cultures were done in three replicates. The plates were dried at 37°C for 30 mins. The inoculated plates were then incubated at 37°C for 24 hours after which each plate was observed for the zones of inhibition of growth. The zones were measured with a transparent ruler and the result recorded in millimeters (mm) after which the mean and standard deviation was also determined.

Measurement and interpretation of zones inhibition

The diameters of the zone of inhibition (Z.I) of the growth were measured in milliliter (mm) by the use of scale ruler No growth or clear zones of inhibition around the discs indicated the susceptibility of the organism to the extracts while absence of such zones showed lack of inhibitory effect of extracts on the test organism. The values recorded were the means of three measurements of zones of inhibitions on the triplicate cultures and their standard deviation. The Z.I values recorded were compared with that of Streptomycin and Gentamycin values who served as control and sensitivity profile was established. The Z.I values > 15mm =Sensitive, 12mm-15mm = moderately sensitive and < 12mm = Resistant.

Determination of minimum inhibitory concentration (MIC) of the extracts on each isolates

The MIC of each extract was determined against the test organisms using broth dilution method. The bacterial isolates were prepared by making a direct suspension of the inoculum in broth medium (nutrient broth). Two-fold serial dilution of each extract was performed to obtain the concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. The initial concentration was obtained by dissolving 1 g of the extracts in 10 ml of the distilled water. Having obtained the different concentrations of the extract, 0.1 ml of the standard inoculums of the test microorganisms in the nutrient broth was then inoculated into the different concentrations of the extract. The test tubes were then incubated at 37°C for 24 hours after which the test tubes were checked for turbidity (growth). The lowest concentration of the extract which showed no turbidity was recorded as the minimum inhibition concentration (MIC).

Determination of minimum bactericidal concentration (MBC) of the extracts on the isolates

The MBC of the extract was carried out to determine whether the test microorganisms were killed or only their growth was inhibited. For this purpose, Mueller-Hinton agar was prepared according to manufacturer's instruction. The plates were covered and the media were allowed to cool and solidify. The tubes that had lowest concentration of the extract which showed no turbidity were recorded as the minimum inhibition concentration (MIC) were then sub-cultured onto the prepared Mueller-Hinton agar plates and the plates were then incubated at 37°C for 24 hours after which the plates was observed for colony growth.The extract concentration without any visible colony growth was recorded as the MBC of the extract form.

RESULTS AND DISCUSSION

Phytochemical screening was performed on the plant stem bark extracts. It was seen that the extracts contains bioactive components such as carbohydrates, flavonoids, alkaloids, steroids, terpenoids, polyphenols, digitalis glycosides, cardiac glycosides, steroidal nucleus, saponins and anthraquinones in varied concentrations as presented on Table 1.

Table 1: Phytochemical constituents of different forms of stembark extracts of *U. Staudti*

Bio-active Constituents	Occurrences in different extract forms		
	Ethanol extract	**Chloroform extract**	**N-butanol extract**
Alkaloids	++	+	+
Steroids	++	+	+
Flavonoids	++	--	-
Terpenoids	+	+	+
Polyphenols	+	+	+
Digitalis glycosides	+	+	+
Steroidal nucleus	+	+	+
Saponins	+	+	+
Carbohydrates	++	+	+
Tannins	+	+	-
Anthraquinone	+	+	+

Keys: *++ = Abundantly Present, + = moderately present, - = Not detected*

The antibacterial sensitivity test was carried out on the isolates using the ethanol extract at concentration of 25mg/ml, 50 mg/ml,75 mg/ml and 100 mg/ml showed *Escherichia coli* was sensitive to the ethanol form of extract with the Z.I range of 13.5±0.5mm -16±1.0mm. No zone of Inhibition (NZ) was observed for *Staphylococcus aureus* at the lowest concentration of 25 mg/ml, *Proteus* spp and *Klebsiella pneumonia* exhibited moderately and high sensitivity to the extract with Z.I range between 11.7±0.2mm -15±0.6 mm but *Streptococcus* sp resistance with Z.I ranged 9.2 ± 0.2 mm -11.2±0.1mm. Streptomycin and Gentamycin is used as the standard reference drug, their ZI ranged from 15.2±0.3mm - 22±0.4mm. None of the ethanol extract forms produced a ZI as large as those of Streptomycin and Gentamycin. The ethanol stem bark extract of *U. staudtii* is not as active as Streptomycin and Gentamycin but exihited some inhibitory effect on some test isolates (Table 2).

Table 2: Antibacterial activities of ethanol extract stembark extracts of *U. staudtii*

Bacterial isolates	Concentration of the extract (mg/ml) and zones of inhibition (mm)					
	25	50	75	100	Strep	Gen
E. coli	13.5±0.5	15.7±0.5	15.1±0.5	16±1.0	22.1±1.0	18.8±0.5
S. aureus	NZ	10.7±0.2	11.2±0.2	12.1±0.3	15.2±0.3	20±1.0
Proteus sp	12.4±0.5	15±0.6	15±0.5	15.5±0.3	16.2±0.3	20±0.5
K. puemonia	11.7±0.2	13.9±0.1	14.3±0.2	12.5±0.55	18.2±0.4	22±0.4
Strept. spp	9.2±0.2	11.2±0.1	10.2±0.4	10±0.3	18.4±0.6	16.1±0.1

Keys: *NZ: No zone of inhibition; each value represents the measurements of three replicate cultures and standard deviation.*

Antibacterial activity of the chloroform extract was carried out. It is seen that the chloroform extract showed no zone of Inhibition (NZ) for *Staphylococcus aureus* at 25 mg/ml and 50mg/ml and 75mg/ml respectively. At the least concentration of 25 mg/ml, *Escherichia coli, Klebsiella pneumonia* and *Streptococcus sp exhibited* resistance to *U. staudtii* chloroform extract as NZ were recorded for them. For *Proteus* sp., ZI ranges from 12.1±0.6mm -13.2±12 mm. Streptomycin and Gentamycin had ZI ranged from 16.0±0.3 mm-26.4±0.5mm (Table 3).

Table 3: Antibacterial Activities of Chloroform of *U. staudtii*

Bacterial isolates	Concentration of the extract (mg/ml) and zones of inhibition (mm)					
	25	50	75	100	Strept	Gen
E. coli	NZ	15.1±0.2	15.3±0.4	16±0.6	24..1±1.0	21.2±0.6
S. aureus	NZ	NZ	NZ	13.±0.5	16.0±0.3	23.0±1.0
Proteus sp	12.1±0.6	13.2±12	13±0.6	13.±1.2	18.0±0.5	20.5±0.5
K. puemonia	NZ	NZ	12±0.1	12.1±0.6	23.1±1.2	26.4±0.5
Strept. spp	NZ	NZ	11±0.3	12.4±1.3	18.2±0.5	22.0±1.0

Keys: *NZ: No zone of inhibition; each value represents the measurements of three replicate cultures and standard deviation*

Antibacterial activity of the n-butanol extract form *on the isolates revealed that Escherichia coli and Proteus spp expressed resistance to* the n-butanol extract form at concentration of 25%, 50%,75% but moderately sensitive at 100% with Z.I of 12±0.2mm and13.6±0.1mm respectively. *Staphylococcus aureus, K. pneumonia* and *Streptococcus* spp showed total resistance at all concentration of the extract form with little Z.I or N.Z. However, Streptomycin and Gentamycin standard drug used as control were seen to have wider Z.I for the test isolates that ranged from 15.0 ± 0.3 mm to 23.1 ± 1.2 mm and 18.8 ± 0.5 mm to 26.6 ± 0.5mm (Table 4).

Table 4: Antibacterial Activities of n-Butanol of *U. staudtii*

Bacterial Isolates	Concentration of the extracts (mg/ml) and zone of Inhibition (mm)					
	25	50	75	100	Streptomycin	Gentamycin
Escherichia coli	9.9±0.2	11.3±0.2	11.5±0.2	12.0±0.2	22.1±1.0	18.8±0.5
Staphylococcus aureus	NZ	NZ	NZ	8.4±0.2	15.0±0.3	20.0±1.0
Proteus spp	NZ	8.7±0.2	9.0±0.2	13.6±0.1	16.2±0.5	20.5±0.5
Klebsiella pneumonia	8.7±0.1	10.1±0.1	10.3±0.1	10.8±0.2	23.1±1.2	26.4±0.5
Streptococcus spp	NZ	7.7±0.1	10.2±0.1	12±0.2	18.2±0.5	22.0±1.0

Keys: *NZ: No zone of inhibition; each value represents the measurements of three replicate cultures and standard deviation.*

The Minimum Inhibitory Concentration (MIC) of the various extracts form carried out on the test isolates showed the ethanol extract had the least MIC of 12.5 mg/m for *Escherichia coli* and *Proteus* spp.,25 mg/ml for *Klebsiella pneumonia*, 50mg/ml for *S. aureus* but the highest MIC of 100 mg/ml from chloroform and n-butanol extract form were recorded for *Klebsiella pneumoniae* , *Streptococuss* sp respectively. The n-butanol extract also was observed with the least MIC of 12.5mg/ml mg/ml .for *Escherichia coli* the least MIC of 12.5mg/ml mg/ml (Table 5).

Table 5: Minimum Inhibitory Concentration (MIC) of the Extract

Isolates	**Concentrations of the extract (mg/ml)**						
	100	**50**	**25**	**12.5**	**6.25**	**MIC**	**Extract form**
E. coli	-	-	-	-	+	**12.5**	E.E
	-	-	+	+	+	**50**	C.E
	-	-	-	-	+	**12.5**	N,B.E
S. aureus	-	-	+	+	+	**50**	E.E
	-	+	+	+	+	**100**	C.E
	-	+	+	+	+	**100**	N,B.E
Proteus spp	-	-	-	-	+	**12.5**	E.E
	-	-	-	+	+	**25**	C.E
	-	-	+	+	+	**50**	N,B.E
K .pneumonia	-	-	-	+	+	**25**	E.E
	-	+	+	+	+	**100**	C.E
	-	+	+	+	+	**100**	N,B.E
Streptococcus sp	-	+	+	+	+	**100**	E.E
	-	+	+	+	+	**100**	C.E
	-	-	+	+	+	**50**	N,B.E

KEYS : + = Growth - = No Growth, E.E = Ethanolic Extract C.E = Chloroform Extract, N.B.E = N-Butanol Extract, C.E = Chloroform Extract, N.B.E = N-Butanol Extract

The Minimum Bactericidal Concentration of the extracts was also carried out on the bacterial isolates. For ethanol extract, it was observed that *S. aureus* and *Streptococcuss* sp. had the highest MBC of 100 mg/ml but the least MBC of 12.5mg/ml was recorded for *E. coli* of and *Proteus* sp from ethanol extract form. For the chloroform extract and n-butanol extract, MBC of 50 mg/ml and the highest MBC of 100 mg/ml were recorded for *S. aureus*, *Proteus* sp. *K. pneumoniae* and *Streptococuss* sp respectively **(Table 6).**

Table 6: Minimum Bactericidal Concentration (MBC) of the Extract

Isolates	Concentrations of the extract (mg/ml)						
	100	**50**	**25**	**12.5**	**6.25**	**MBC**	**Extract form**
E. coli	-	-	-	-	+	**12.5**	E.E
	-	-	+	+	+	**50**	C.E
	-	-	+	+	+	**50**	N,B.E
S. aureus	-	+	+	+	+	**100**	E.E
	-	+	+	+	+	**100**	C.E
	-	+	+	+	+	**100**	N,B.E
Proteus spp	-	-	-	-	+	**12.5**	E.E
	-	-	+	+	+	**50**	C.E
	-	+	+	+	+	**100**	N,B.E
K .pneumonia	-	-	+	+	+	**50**	E.E
	-	+	+	+	+	**100**	C.E
	-	+	+	+	+	**100**	N,B.E
Streptococcus sp	-	+	+	+	+	**100**	E.E
	-	+	+	+	+	**100**	C.E
	-	+	+	+	+	**100**	N,B.E

KEYS : + = Growth - = No Growth, E.E = Ethanolic Extract C.E = Chloroform Extract, N.B.E = N-Butanol Extract, C.E = Chloroform Extract, N.B.E = N-Butanol Extract

Stem bark extracts of *Uapaca staudtii* contains bioactive components such as carbohydrates, flavonoids, alkaloids, steroids, terpenoids, polyphenols, digitalis glycosides, cardiac glycosides, steroidal nucleus and saponins. These metabolites have been reported to have potentials for antimicrobial activity (Cowan, 1999; Draughon, 2004). Some researchers have discovered that antibacterial properties are usually associated with these bioactive constituents ((Nweze et al., 2004; Ameh, 2010; Udoh *et al.*, 2017). These substances have their different functions and are known to work using different mechanisms. Alkaloids present in plant are known to function as anesthetic, spasmolytic and anti-cholinergic agent (Iroabuchi, 2008). Alkaloids have also reported by many researchers to possess clinical importance potentials such as anticancer, antibacterial, antiasthmatic activities Saponins are basically act as emulsifying agents and have antibacterial, anti-inflammatory and anti-exudative properties (Hussain, and Deeric 1991; Batisa *et*

al., ; 2004; Burile *et al*., 2009) Tannins are known to inhibit the synthesis of cell proteins of bacteria by forming complexes that are irreversible with proline rich protein in bacteria. Tannis has been reported to be responsible for the haemostatic activity where they arrest bleeding from damaged or injured vessels by precipitating protein to from vascular plugs (Chung *et al*., 1998; Okwu and Iroabuchi, 2004). Flavoniods which is also found in *U. staudtii in* a very high concentration especially in ethanolic extracts and it is reported to function well in some biological aspects such as the protection against allergy, platelet aggregation, microbes invasion, ulcer, hepatoxin, viruses and tumors (Batisa *et al*., 2004; Burile *et al.*, 2009). In particular the flavonoids were reported to be responsible for ethno medicinal plants (Singh and Bhat, 2003). Cardiac glycosides is useful in heart pumping (Godfraind, 1984). Nascimento *et al.,* (2000) reported that flavonoid works well in human system as it reduces the risk of estrogen-induced cancer by interfering with the activities of the enzymes that produce estrogen. Moreover, some researchers observed and reported that flavonoids are also found to form complexes with the extracellular soluble proteins leading c to disruption of microbial cell membranes (Boham, 1974; Tsuchiya, *et al.,* 1996). The results of antibacterial activities of the extracts on the some isolates in this study portray direct variations of antibacterial activities of these extracts forms based on the nature solvents used for extraction and the concentration of the extracts. This agrees with Udoh *et al*., (2012), who reported that the ability of the bioactive constituents to come out from the complex structure depends largely on the potency of the solvents used for the extraction. In this study, it was noted that the test organisms were susceptible to ethanol extract form more than any other form. Phytochemically, some bioactive substances were abundantly present in ethanol fraction than any other form. Moreover, the wider Z.I, least MIC and MBC were obtained from ethanol fraction. Hence, the results indicated that ethanol is a better extraction solvent for extraction of stembark of *U. staudtii* active constituents. This agrees with the reports of Obi and Onuoha (2000) and Ogueke *et al*; (2006) who stated that ethanol is a better extraction solvent for the extraction of most plants bioactive substances of medicinal importance. No wonder the tradition medicine practitioners always recommend the use of ethanol (local gin) for the maceration and extraction of plants for local treatments.

The present study of antibacterial evaluation of the stembark of *U. staudtii*, it was observed that in all the extract forms tested on the isolates, the zones of inhibition (Z.I) increased with increasing concentration. Hence, if concentration of the stembark of *U. staudtii* is increased with appropriate solvent, the antibacterial potency may increase as well. The extracts of *Uapaca staudtii* has high potential to be used as an antibacterial agent. It showed varying degree of activities against all the tested bacteria (both Gram positive and Gram negative) with some inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, *Proteus* sp, *Klebsiella pneumoniae* and *Streptococuss* species as demonstrated with MIC and MBC and this gives further evidence that the plant could be exploited for more therapeutic use. According to Pieme *et al*., (2008), plant's extract found with some antimicrobial properties can serve as promissory extract that can open the possibility of finding new clinically effective antibacterial compounds. Thus, this assessment can form a primary platform and baseline research for further phytochemical and pharmacological studies. These findings therefore provide an insight into the usage of this plant in treatment of bacterial infections.Attempt should also be made to isolate and characterize the pure organic compound constituting the active secondary metabolites found in the plants so as to estimate its chemotherapeutic value, which may lead to its spontaneous use as a potent antimicrobial drug.

CONFLICTS OF INTEREST: We state that the work has no potential conflicts of interest

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