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Systemic evaluation of antibacterial activity of *Anacardium occidentale*

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Abstract

Background: Evaluation of antibacterial activity of different parts of two varieties of *Anacardium occidentale* L. tree {red fruited variety (RFV) and yellow fruited variety (YFV)}, namely, the fruit, leaf, stem bark and root extracts on human pathogens (*Staphylococcus aureus*, *Eschericjia coli*, *Pseudomonas aeroginosa*, *Salmonella typhi*, *Proteus mirabilis* and *Klebsella spp*) was carried out *in vitro*. **Materials and Methods:** Phytochemical screening and preparation of the extracts was by standard methods. Antibacterial activity was measured by the agar diffusion methods, which ascertained the diameter of inhibition halos around wells after 24 h incubation at 37°C. **Results:** Alkaloids, saponins, flavonoids, tannins, HCN, phenols and anthocyanin were present in leaf and stem bark extracts of the RFV and YFV. HCN was absent in the fruit and root of both varieties. Aqueous fruit extract of the RFV did not exhibit antibacterial effect on *P. aeroginosa*, *S. typhi*, *P. mirabilis* and *Klebsella spp*. Likewise, ethanolic fruit extract of the RFV did not inhibit bacterial activity of *S. typhi* and *P. mirabilis*, whereas *S. aureus*, *E. coli*, *P. aeroginosa* and *Klebsella spp* were inhibited by ethanolic red fruit extract in the following corresponding order: *E. coli* = 65.11% > *P. aeroginosa* = 64.30% > *S. aureus* = 53.01% > *Klebsella spp* = 46.76%. The zone of inhibition (ZOI) of aqueous leaf extract of the RFV was between 10.50 ± 0.05 mm and 14.50 ± 0.01 mm halos. Generally, antibacterial activities of aqueous and ethanolic stem bark, leaf and root extracts of the RFV and YFV were identical. **Conclusion:** The results offered precise reference information on comparative antibacterial activities of various parts of *A. occidentale* L. tree for possible optimum exploitation and usage.

Keywords: *Anacardium occidentale*, Antibacterial activity, Human pathogens, Phytochemicals, Zone of inhibition.

Introduction

Infectious diseases caused by pathogens such as bacteria, fungi and viruses are major challenges to public health, in that microbial activity promotes morbidity and mortality of the human host. *Staphylococcus aureus* is the causative organism of skin infections, pneumonia, food poisoning, toxic shock syndrome and bacteriemia. *Eschericjia coli* are of several types and haboured in the digestive tracts of human and animals. Although most *E. coli* are harmless microbes, some can cause enterohemorrhage, urinary tract infections and anemia. *Pseudomonas aeroginosa* infection is complicated and recently, reports showed the wide spread of resistant strains to antibiotics[1,2]. *Salmonella typhi* and *Proteus mirabilis* belong to the family of *Enterobacteriaceae*; *S. typhi* is the causative organism of typhoid fever. *P. mirabilis* causes 90% of *Proteus* infections and

can be considered a community-acquired infection, especially in persons with underlying diseases or compromised immune systems. Patients with recurrent infection have increased frequency of diseases caused by *Klebsella spp* and other microbes. For instance, *Klebsiella pneumonia* accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias and soft tissue infections.[3]

The cashew (*Anacardium occidentale* L.) tree grows up to 10 m or above in height and has large stripe, oval shaped leaves, fragrant red or yellow bulbous fruit when ripe, called the cashew apple. Bioactive principles derivable from cashew nut kernels have been previously reported by Trox J *et al*.[4] Evidence of worldwide ethnomedicinal practices showed that extracts of different parts of *A. occidentale* L tree have therapeutic potentials for the treatment of malaria, bronchitis, dyspepsia, eczema, psoriasis, syphilis, urinary insufficiency and nasal congestion.[5] In addition, Chikezie[6], mentioned other multipurpose medicinal values of *A. occidentale* L tree.

The use of herbs and medicinal plants for the alleviation of pathologic conditions is a universal phenomenon. Medicinal plants contain substances known to modern and ancient civilization for their therapeutic potentials. Plants, because of their biodiversity, are unlimited veritable sources of a variety of bioactive principles with medicinal and therapeutic values. Notwithstanding the advent and advances in synthetic organic chemistry at the dawn of the 19th century, modern pharmaceutics continue to source therapeutic principles from plant materials. More so, 80% of Africans as well as 60% of the world population rely on plant-derived medicines.[7,8]

There are increasing incidences of infectious agents becoming resistant to orthodox antibacterial drugs[1,2,9,10] and therefore, it has become exigent to develop alternative antibacterial drugs in order to mitigate these and other related health care challenges. The increasing quest for antibacterial agents has shifted to plant materials and studies have shown that phytomedicine is reliable, sustainable and a rewarding prospect in this regard.[11-17] In the present study, evaluation of antibacterial activity of different parts of two varieties of *Anacardium occidentale* L. tree {red fruited variety (RFV) and yellow fruited variety (YFV)}, namely, the fruit, leaf, stem bark and root extracts on human pathogens (*Staphylococcus aureus, Eschericjia coli, Pseudomonas aeroginosa, Salmonella typhi, Proteus mirabilis* and *Klebsella spp*) was carried *in vitro*.

Materials and Methods

Collection and preparation of plant extract

Different plant parts, namely, fruit, leaf, stem bark and root were harvested, between the months of February and March, 2012, from two varieties of *A. occidentale* L. trees {red fruited variety (RFV) and yellow fruited variety (YFV)} growing in the wild along Uturu/Okigwe Express Road, Okigwe, Imo State, Nigeria. The specimens were authenticated by Dr. F.N. Mbagwu at the Herbarium of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. Voucher specimens were deposited at the Herbarium for reference purposes. The separate plant parts were washed under a continuous flow of tap water to remove dust/debris and air-dried at room temperature for 5 h. The specimens were cut into bits and further dried at 60°C in an oven (WTC BINDER, 7200 Tuttlingen, Germany) for 3 days [18] till sufficiently devoid of moisture and subsequent ground in Thomas-Willey milling machine. Preparation of separate ethanolic and aqueous extracts of the various plant parts was, according to the methods of Ojiako *et al.*[19] Percentage yield (%Y) was calculated based on the ratio of weight of extract to that of the specimen (%; *w/w*). Stock solutions of the various botanical extracts were prepared by reconstituting the separate extracts in 20 mL of 10% dimethylsulfoxide (DMSO, Merck). The solutions were allowed to stand in a thermostatically controlled water bath at 37°C for 30 min with thorough shaking. The final concentration of the extracts = 400 mg% in DMSO *w/v*; were prepared and used for sensitivity test.

Phytochemical screening

Phytochemical screening was carried out in the presence of alkaloids, saponins, flavonoids, tannins, HCN, phenols and anthocyanin using standard methods.[20].

Pathogenic microorganisms

Clinical isolates of known human pathogens (*S. aureus* AX456, *E. coli* CG673, *P. aeroginosa* HJ731, *S. typhi* MV675, *P. mirabilis* BR095 and *Klebsella spp* FA412) were obtained from National Root Crop Research Institute, Umudike, Abia State, Nigeria. The pathogens were examined for purity by streaking in nutrient agar plates.

Stock cultures were prepared in Bijioux bottles at controlled temperature of 37°C. The microorganisms were finally maintained in nutrient agar at refrigerated temperature of 4°C until the tests were carried out.

Antibacterial test

The agar diffusion test according to the methods of Oliveira *et al.*,[17] was used to ascertain the antibacterial activity of the various plant extracts. A 10 µL of each cultured microorganism in 1×10^{-3} UFC/mL was plated in Muller-Hinton agar (MHA, Sigma), which was perforated yielding 1 central well surrounded by 10 wells. In the central well, which served for the positive control, 30 µL of ciprofloxacin in DMSO = 16.6 µg/mL was added, whereas 30 µL of each plant extract was introduced in the surrounding wells. The 10^{th} well contained 10 µL of DMSO, which served as negative control. Antibacterial activity was ascertained by a measure of the diameter of inhibition halos around the wells after 24 h incubation at 37°C, which indicated absence of bacterial growth.

Results

A cursory look at Table 1 showed that ethanolic extracts of the various specimens gave greater %Y than the corresponding aqueous extracts. The %Ys of aqueous and ethanolic extracts of both varieties were within the range of: RFV_{AQ} = 2.65—5.89%; RFV_{ETH} = 3.04—6.93%; YFV_{AQ} = 2.55—5.66%; YFV_{ETH} = 3.12—6.64%. Specifically, in relation to the two extracting solvents, aqueous fruit extract of the YFV and ethanolic fruit extract of the RFV gave the lowest %Ys. The highest %Ys of the two extracts were obtained from aqueous and ethanolic stem bark extracts of the RFV.

Phytochemical screening of the samples showed the presence of alkaloids, saponins, flavonoids, tannins, HCN, phenols and anthocyanin in the leaf and stem bark extracts of the RFV and YFV. HCN was absent in fruit and root of both varieties. Furthermore, the phytochemical profile revealed that the RFV and YFV were identified (Table 2).

Aqueous fruit extracts of the RFV did not exhibit antibacterial effects on *P. aeroginosa, S. typhi, P. mirabilis* and *Klebsella spp*, which were contrary to *S. aureus* and *E. coli*, representing 47.08% and 47.98% inhibition respectively (Table 3). Likewise, ethanolic fruit extract of the RFV did not inhibit bacterial activity of *S. typhi* and *P. mirabilis*, whereas *S. aureus, E. coli, P. aeroginosa* and *Klebsella spp* were inhibited by ethanolic red fruit extract in the following corresponding order: *E. coli* = 65.11% > *P. aeroginosa* = 64.30% > *S. aureus* = 53.01% > *Klebsella spp* = 46.76%. Table 3 showed that both aqueous and ethanolic leaf, stem bark and root extract of the RFV exhibited antibacterial effects on the six experimental human pathogens (SEHP). The zone of inhibition (ZOI) of aqueous leaf extract of the RFV was between 10.50 ± 0.05 mm and 14.50 ± 0.01 mm halos. ZOI of ethanolic leaf extract of the RFV was within the range of 12.00 ± 0.02—14.53 ± 0.01 mm halos. A cursory look at Table 3 showed that ethanolic stem bark extract of the RFV showed greater capacity to inhibit bacterial growth of the SEHP than the corresponding aqueous extract. Specifically, the lowest ZOI was observed when aqueous stem bark extracts of the RFV was incubated with *S. aureus*. Ethanolic root extract of the RFV exhibited comparative greater capacity to inhibit the SEHP than the corresponding aqueous extract.

Table 4 showed the absence of halos in culture plates of *P. aeroginosa, S. typhi, P. mirabilis* and *Klebsella spp* incubated with aqueous fruit extracts of the YFV. However, antibacterial activity of an aqueous fruit extract of the YFV incubated with *S. aureus* and *E. coli* represented 44.20% and 47.98% inhibition compared to the positive control (Table 5) respectively. Tables 3 and 4 showed that aqueous and ethanolic leaf extracts of the RFV and YFV as well as ethanolic stem bark extracts of the RFV and YFV exhibited relatively greater potency in inhibiting bacterial activity of *P. aeroginosa* compared to the standard antibacterial drug-ciprofloxacin (positive control). Likewise, antibacterial activity of aqueous and ethanolic leaf extracts of the RFV and YFV on *S. typhi* were higher than that of the standard antibacterial drug. For instance, antibacterial activity of ethanolic leaf extract of the YFV was higher than the standard antibacterial drug by 12.45%.

Table 1: Percentage yields of aqueous and ethanolic extracts of *A. occidentale* L.

	%Y; *w/w* ratio							
	Fruit		Leaf		Stem Bark		Root	
	RFV	YFV	RFV	YFV	RFV	YFV	RFV	YFV
AQ	2.65	2.55	3.98	3.55	5.89	5.45	5.45	5.66
ETH	3.04	3.12	4.22	4.01	6.93	6.45	6.41	6.64

AQ: aqueous extract; ETH: ethanolic extract.

Table 2: Phytochemicals of red and yellow fruited varieties of *A. occidentale* L.

Sample	**Phytochemicals**						
RFV	Alkaloids	Saponins	Flavonoids	Tannins	HCN	Phenols	Anthocyanin
Fruit	+	+	+	+	-	+	+
Leaf	+	+	+	+	+	+	+
Stem Bark	+	+	+	+	+	+	+
Root	+	+	+	+	-	+	+
YFV							
Fruit	+	+	+	+	-	+	+
Leaf	+	+	+	+	+	+	+
Stem Bark	+	+	+	+	+	+	+
Root	+	+	+	+	-	+	+

+: present; -: absent.

Table 3: Halo measurement of antibacterial activity of extracts of red fruited variety of *A. occidentale* L.

	Inhibition Zone Diameter (mm) (*X*) ± SEM								
		Fruit		**Leaf**		**Stem Bark**		**Root**	
Pathogens	DMSO	AQ	ETH	AQ	ETH	AQ	ETH	AQ	ETH
SA	0.00±0.00	7.99±0.03	8.89±0.04	10.50±0.05	12.09±0.05	9.50±0.01	12.00±0.00	9.00±0.07	11.00±0.00
EC	0.00±0.00	7.00±0.05	9.50±0.02	12.50±0.01	14.07±0.06	11.50±0.05	14.49±0.00	10.50±0.05	12.50±0.05
PA	0.00±0.00	0.00±0.00	8.50±0.02	14.00±0.12	14.53±0.01	12.00±0.00	13.50±0.00	10.50±0.03	11.00±0.05
ST	0.00±0.00	0.00±0.00	0.00±0.00	14.50±0.01	14.50±0.01	12.00±0.00	12.00±0.01	9.50±0.05	11.50±0.01
PM	0.00±0.00	0.00±0.00	0.00±0.00	11.50±0.01	12.00±0.02	11.00±0.00	10.00±0.11	10.00±0.06	12.33±0.32
KS	0.00±0.00	0.00±0.00	8.00±0.08	12.50±0.32	13.50±0.02	14.00±0.00	14.06±0.02	10.00±0.05	12.50±0.01

Number of determinations (*n* = 3); SA: *S. aureus,* EC: *E. coli,* PA: *P. aeroginosa,* ST: *S. typhi,* PM: *P. mirabilis,* KS: *Klebsella* spp; AQ: aqueous extract; ETH: ethanolic extract; DMSO (Dimethylsulphoxide) = negative control.

Table 4: Halo measurements of antibacterial activity of extracts of yellow fruited variety of *A. occidentale* L.

	Inhibition Zone Diameter (mm) (*X*) ± SEM								
		Fruit		Leaf		Stem Bark		Root	
Pathogens	DMSO	AQ	ETH	AQ	ETH	AQ	ETH	AQ	ETH
SA	0.00±0.00	7.50±0.02	7.19±0.15	10.50±0.00	12.50±0.02	9.00±0.06	12.00±0.00	8.50±0.05	11.03±0.03
EC	0.00±0.00	7.00±0.05	9.03±0.03	12.00±0.00	13.50±0.02	11.00±0.28	14.00±0.05	10.00±0.07	11.87±0.15
PA	0.00±0.00	0.00±0.00	8.50±0.01	14.50±0.01	14.00±0.05	11.50±0.03	14.00±0.00	9.00±0.01	9.50±0.01
ST	0.00±0.00	0.00±0.00	12.03±0.03	14.00±0.05	14.00±0.02	11.00±0.12	11.50±0.01	9.00±0.05	11.00±0.02
PM	0.00±0.00	0.00±0.00	7.00±0.11	11.00±0.04	10.50±1.05	12.00±0.04	12.00±0.00	9.00±0.03	11.03±0.30
KS	0.00±0.00	0.00±0.00	8.00±0.02	13.00±0.06	12.50±0.02	14.50±0.03	14.00±0.01	9.50±0.02	13.00±0.05

Number of determinations (*n* = 3); SA: *S. aureus,* EC: *E. coli,* PA: *P. aeroginosa,* ST: *S. typhi,* PM: *P. mirabilis,* KS: *Klebsella* spp, AQ; aqueous extract; ETH: ethanolic extract; DMSO (Dimethylsulphoxide) = negative control.

Table 5: Halo measurements of positive controls.

Pathogens	Inhibition Zone Diameter (mm) (*X*) ± SEM
SA	16.97 ± 0.04
EC	14.59 ± 0.03
PA	13.22 ± 0.05
ST	12.45 ± 0.11
PM	15.09 ± 0.15
KS	17.11 ± 0.21

Number of determinations (*n* = 3); SA: *S. aureus,* EC: *E. coli,* PA: *P. aeroginosa,* ST: *S. typhi,* PM: *P. mirabilis,* KS: *Klebsella* spp.

On the contrary, ethanolic fruit extract of the YFV inhibited the SEHP; ZOI ranged between 7.00 ± 0.11 mm halo and 12.03 ± 0.30 mm halos. In the same characteristic manner as the RFV, both aqueous and ethanolic leaf, stem bark and root extracts of the YFV exhibited antibacterial effects on the SEHP. An overview of Tables 3 and 4 showed that the capacity of aqueous and ethanolic leaf, stem bark and root extracts of the RFV and YFV to inhibit bacterial growth of the SEHP were identical, except for

ethanolic fruit extract of the RFV, which showed no inhibitory effect on *S. typhi,* and *P. mirabilis.*

Discussion

The present study showed the various capacities of the different parts of *A. occidentale* L. tree, especially the leaf, stem bark and root to inhibit microbial activity, which conformed to those reported by previous researchers elsewhere.[13, 14, 17, 21] Notable plant secondary metabolites that have been adduced to be responsible for antibacterial activity of botanic extracts are the phenols, flavonoids, saponins and tannins.[22-25] More so, several other reports have also shown that the production of a large variety of antibacterial molecules generally referred to as phytoalexins (MW < 500) in plants, especially in wild types, are responsible for their capability to exterminate invasive noxious microorganisms, as well as mitigate abiotic stressors and traumas.[26, 27] Furthermore, natural products such as terpenoids, glycosteroids, flavonoids and polyphenols are antibacterials and have been reported to be contained within the structural space of phytoalexins.[28, 29] As mentioned by Jørgensen *et al.*[30], inhibition of phytoalexins biosynthesis by cyanide, prompted by the presence of cyanogenic glycosides in *Hevea braziliensis* (rubber tree), increased its sensitivity to attack by *Microcyclis ulei*, which confirmed the evidence in support of the protective function of phytoalexins.

The present study showed there were variations in the capacities of extracts of the various plant parts to inhibit bacterial growth of the SEHP *in vitro*. Furthermore, the absence of antibacterial activity, particularly the fruit extracts of the RFV on *S. typhi* and *P. mirabilis* amongst the other SEHP, was an indication that the antibacterial phytochemicals are not evenly distributed and their absolute cellular concentrations may vary within or even absent in some plant organs that constitute the plant system. That leaf, stem bark and root extracts of *A. occidentale* L. exhibited relatively greater potency than the fruit extracts to inhibit the activity of the SEHP, which was an affirmation of the above assertions. Similarly, studies by Karima *et al.*[31], corroborate the present observations of disparities in levels of activity of antibacterial phytochemicals in plant systems. They noted that root extracts of *Carthamus caeruleus L.* were more active than the leaf extracts against certain strains of gram-negative and gram-positive bacteria as well as pathogenic fungus. However, previous studies have provided evidence that bioactivity of phytochemicals in botanic extracts is additive, in that the combinatorial actions of phytochemicals are responsible for their therapeutic benefits or toxicologic outcomes in animals.[32, 33] It is also important to note that negative interactions (interferences) do occur in botanic extracts, in which the interplay and interactions of composite phytochemicals cause attenuation or outright inhibition of maximum biologic activity of supposedly pharmacologic active plants.[33, 34] Thus, mixture of phytochemicals in botanic extracts may be more or less biologically active than individual bioactive components.

The capability of ethanolic and water, fruits extracts of the RFV and YFV to inhibit the SHEP was in consequence of the nature of the extracting solvents. Studies have shown that solubility of antibacterial principles in polar or non-polar solvent is critical in order to exert biologic activity *in vitro.*[35, 36] Accordingly, the present findings showed that ethanolic solvent extracts exhibited greater antibacterial activity than the corresponding aqueous extracts, which conformed to previous assessments by several authors.[13, 37-40] Thus, the results presented here suggest that by virtue of the %Y index in connection to the two extracting solvents, there were greater tendencies that higher concentrations of antibacterial elements were extracted into ethanol than the corresponding aqueous environment as exemplified in Table 1. In corroboration with the present findings, Bashir *et al.*[41], had previously noted that organic plant extracts exhibited greater antibacterial activity than the corresponding aqueous suspension. However, comparative inspections of outcomes of the present investigations revealed that the antibacterial potential of the RFV and YFV of *A. occidentale* L. were of equivalent status. On the contrary, the research reports by Ababutain[24], mentioned that disparity in antibacterial activity of the same plant species may manifest when variation in climatic factors and soil composition exist in locations where the plants are cultivated in conjunction with the stage of vegetative cycle of the plants. Also, Cock[42] had reported that genetic variation within a plant species could cause dissimilarity in their phytochemical composition, and consequently, affect their corresponding pharmacological properties.

Conclusion

The present preliminary *in vitro* findings offered precise reference information on comparable levels of antibacterial activities of various parts of *A. occidentale* L. tree for possible optimum exploitation and usage. However, it's essential to extend the frontiers of the present scope of

study to basic and clinical research endeavours in order to achieve the quest for sourcing antibacterial therapeutics from *A. occidentale* L. tree.

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