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Sensitivity of Cashew (*Anacardium occidentale*) Leaf Extract against Selected Urinary Tract Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was carried out to investigate cashew extract as anti – urinary tract infection.
Methods: The leaves of cashew (*Anacardium occidentale)* were extracted using ethanol and distilled water; however the leaf extracts of cashew were screened for anti-microbial activities by the *in vitro* cup-plate method of agar diffusion technique with concentration of about 10^{-5} s cells/ml of the selected bacteria; using ethanol and distilled water as control. Simultaneously; the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the two extracts on selected bacteria were also determined.
Results: The minimum inhibitory concentration (MIC) of ethanolic and aqueous extract against the two organisms was 0.0625 g/l; namely *E. coli* and *S. aureus* except *K. pneumoniae* that occurred at 0.125 g/l. The two extracts were bactericidal at 0.25 g/l and above; below this concentration there were differentiations in the organism's reaction to the extracts; for instance, the two extracts

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at 0.125 g/l were cidal; to the other two organisms; only *K. pneumoniae* was static. However at 0.0625g/l of the two extracts; the two organisms were static but *K. pneumoniae* showed growth.
Conclusion: Hence the leaf extract of *Anacardium occidentale* dissolved in distilled water and ethanol are good potential for the development of antibacterial drugs for urinary tract pathogens like *Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus*.

Keywords: Antibacterial; Anacardium occidentale; urinary- tract; pathogen; ethanol; aqueous extract.

1. INTRODUCTION

The cashew tree (*Anacardium occidentale*) is a tropical evergreen tree that produces the cashew nut [1]. It can grow as high as 14 m (46 ft), but the dwarf cashew, growing up to 6 m (20 ft), has proved more profitable, with earlier maturity and higher yields. The species is originally native to northeastern Brazil [1]. Portuguese colonists in Brazil began exporting cashew nuts as early as the 1550s [2]. Major production of cashews occurs in Vietnam, Nigeria, India, and Cote d'voire [3]. The cashew nut, often simply called a cashew, is widely consumed. It is eaten on its own, used in recipes, or processed into cashew cheese. The shell of the cashew seed yields derivatives that can be used in many applications including lubricants, waterproofing, paints, and arms production, starting in World War II [2]. The cashew is a light reddish to yellow fruit, whose pulp can be processed into a sweet fruit drink or distilled into liquor.

Since antiquity; man uses plants to treat common infectious disease and some of the traditional medicines are still included as part of the habitual treatment of various maladies [4]. Scientific interest in medicinal plants grow rapidly in recent times due to the increase in efficiency of the new plant derived drugs and rising concerns about the side effects of modern medicine. The continuing emergence of drug resistant organisms and the increasing evolutionary adaptation of pathogenic organisms to commonly used orthodox antimicrobial agents have reduced the efficacy of antimicrobial agents currently in use. However; the search for new drugs from plants continues to grow [5]. Plants continue to be a major source of commercially consumed drugs. Even most synthetic drugs have their origin from natural plants products [6].

The cashew leaf and other plants are extensively used in traditional and Ayurvedic medicines for the treatment of various diseases such as chronic fever, rheumatism, internal worm infections, asthma, inflammations, dermatopathy, bronchitis, cough, constipation, hepathopathy, greyness of hair and baldness. Recently; many researchers have been identifying a lot of compounds used in mainstream medicine which were derived from plant sources; 80% of these compounds were used in the same or related manner as in the traditional ethnomedine especially in the countries like China and India; where orthodox and traditional medicine are practicing together. The specific objectives of the present study are to verify the antibacterial of cashew leaf extract against selected bacterial isolates and assess the antibacterial properties of cashew leaf extract; so as to identify the concentration at which the extract will inhibit or kill the bacterial isolates.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The leaves of *Anacardium occidentale* were plucked from the tree inside the Homage Estate; at Odogunyan, Ikorodu; Lagos State; at an early hour in the morning around 7 am. Pure clinical isolates of *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* were obtained from the department of Biotechnology; Federal Institute of Industrial Research, Oshodi (FIIRO). The test organisms were streaked and incubated on Nutrient Agar slants and incubated overnight at 37˚C and maintained under refrigerated condition.

2.2 Phytochemical Screening

The phytochemical analysis was carried out using the method described by Odebiyi and Sofowora [7]. The plant extracts were screened for the presence of Tannins, Saponins, Alkaloids, Oxalate and Phenol.

2.3 Extraction Procedure

The leaves were air dried at room temperature and grounded into fine powder using blender (grinding machine). Fifty grammes (50 g) of the finely ground sample was weighed into two 500 ml beakers separately; 200 ml of extracting

solvents e.g distilled water and ethanol was added to each sample respectively and kept in a dark cupboard for five days. The samples were aseptically filtered using Whatman no 4 filter paper. The resultant extracts were each concentrated using the rotary evaporator model (Buchi Rotarvapour R-114) which ensures evaporation of bulky solutions to small volume concentrates without bumping at temperature 50-60^{0}c. The resultant extracts were filtered and sterilized using Millipore filter (0.45 µ/m) and then use for the antibacterial activity.

2.4 Antibacterial Activity; Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The resultant extracts from the sample were screened for antibacterial activity and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the *in vitro* cup-plate method of agar diffusion technique [8]; by cool molten Muller Hinton agar and poured aseptically into sterile disposable Petri-dishes and allowed to set. However; a cell suspension of each test organisms was prepared to give a concentration of 10^{-5} cell of each; the plates were seeded with 1ml aliquot test organism suspensions and spread onto the agar surface with aid of hockey stick aseptically. The plates were allowed to dry for 1 hour at room temperature. Wells of 5mm diameter were dug using cork borer; each was carefully filled with 1ml aliquot of the extract of water and ethanol respectively. Same procedures were followed for the tubes containing only the diluents e.g distilled water and ethanol; which served as control and labeled accordingly .The plates were left at room temperature for 1 hour for pre-diffusion to get ahead of growth of the organism and subsequently incubated at 35˚C for 24 hours. After incubation, the plates were examined for presence or absence of zone of inhibition of growth. The degree of sensitivity was expressed as a measure of the diameter of inhibition of growth in millimeter. This screening was repeated twice and average of the two resultants of diameter of zone of inhibition was calculated and recorded.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Constituents of *Anacardium occidentale* Leaves

Table 1. List of phytochemical constituents

Active ingredients	Inference
Tannin	Present
Saponin	Present
Oxalate	Present
Phenol	Present
Alkaloids	Present

3.2 Antibacterial Screening of The cashew Leaves Extract

Table 2. Zone of inhibition in (mm) of the screening of cashew leaves ethanol extract against three selected microorganisms

Microoganism	1st Screening	2nd Screening	Average
Klebsiella pneumonia	30	32	31
Staphylococcus aureus	36	34	35
Escherichia coli	33	34	33.5

Table 3. Zone of inhibition in (mm) of the screening of cashew leaves water extract against three selected microorganisms

Microoganism	1st Screening	2ND Screening	Average
Klebsiella pneumonia	30	30	30
Staphylococcus aureus	35	33	34
Escherichia coli	32	31	31.5

3.3 Minimum Inhibitory Concentration of the Extracts

Table 4. Minimum inhibitory concentration of the ethanol extract against selected organisms

Microoganism / Zone of inhibition (mm)	Concentration (g/l)					
	2	1	0.5	0.25	0.125	0.0625
Klebsiella pneumonia	27	29	24	17	17	-
Staphylococcus aureus	32	28	25	19	19	16
Escherichia coli	29	30	31	21	21	19

Table 5. Minimum inhibitory concentration of the water extract against selected organisms

Microoganism / Zone of inhibition (mm)	Concentration(g/l)					
	2	1	0.5	0.25	0.125	0.0625
Klebsiella pneumonia	25	25	23	21	18	-
Staphylococcus aureus	30	27	23	22	20	17
Escherichia coli	31	28	30	26	22	18

3.4 Minimum Bactericidal Concentration (MBC) of the Extracts against Selected Organisms

Table 6. Minimum bactericidal concentration of the ethanol extract against selected organisms

Microorganism	2.0 g/l	1.0 g/l	0.5 g/l	0.25 g/l	0.125 g/l	0.0625 g/l
Klebsiella pneumonia	Cidal	Cidal	Cidal	Cidal	Static	-
Staphylococcus aureus	Cidal	Cidal	Cidal	Cidal	Cidal	Static
Escherichia coli	Cidal	Cidal	Cidal	Cidal	Cidal	Static

Table 7. Minimum bactericidal concentration of the water extract against selected organisms

Microorganism	2.0 g/l	1.0 g/l	0.5 g/l	0.25 g/l	0.125 g/l	0.065 g/l
Klebsiella pneumonia	Cidal	Cidal	Cidal	Cidal	Static	_
Staphylococcus aureus	Cidal	Cidal	Cidal	Cidal	Cidal	Static
Escherichia coli	Cidal	Cidal	Cidal	Cidal	Cidal	Static

Keys: Cidal- Bactericidal; Static-Bacteristatic

The leaves of the cashew plant, *Anacardium occidentale* extract contains the following phytochemical constituents as shown in the Table 1; namely tannins, saponin, steroid, oxalate, phenol and alkaloid. The antibacterial properties of *Anarcardium occidentale* are derived from the presence of a polyphenol known as Anacardic and other compounds such as Tatrols and Tannins [9]. This plant is effective in the treatment of malaria, asthma, diarrhea, dysentery, leprosy, wart and sore throat [10].

The test organisms; *Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus* were all sensitive to the extracts obtained from the leaves of the cashew plant, *Anacardium occidentale*. The difference in sensitivity could be attributed to the fact that *Staphylococcus aureus* is a gram positive organism whereas *Escherichia coli and Klebsiella pneumoniae* are gram negative organisms; however gram positive organisms lack an outer membrane in their cell walls; whereas gram negative organisms do have it. This outer membrane may be responsible for the difference in the degree of sensitivity of these organisms to the extracts of *Anacardium occidentale*. This could be due to the fact that the outer membrane may prevent a substantial amount of the extract having contact with the cell wall. However the plates (control) containing only the diluents in them do not show any zone of inhibition; rather growth of inocula full the plate concern.

Tables 2 and 3 show that Ethanol and water fraction of the extract respectively have the highest activity against *Staphylococcus aureus,* while *Escherichia coli, Klebsiella pneumoniae* were also sensitive. *Escherichia coli* was sensitive at 33.5mm with ethanol extract and 31.5 mm with the water extract, *Klebsiella pneumoniae* was sensitive at 31mm with the ethanol extract and 30mm with the water extract respectively; during the antibacterial screening.

Table 4 shows minimum inhibitory concentration **(**MIC) of ethanol extract against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* in 2.0 g/l well, it was observed that *Staphylococcus aureus* has the highest inhibitory zone of 32 mm while the lowest concentration was discovered to be *Klebsiella pneumoniae*. In 1.0 g/l well, *Escherichia coli* has the highest inhibitory zone of 30mm and *Staphylococcus aureus* has the lowest inhibitory zone of 28 mm. At 0.5, 0.25 and 0.125 g/l wells; *Esherichia coli* has the highest inhibitory zone and *Klebsiella pneumoniae* has the lowest inhibitory zone. In 0.0625 g/l well, *Escherichia coli* has the highest inhibitory zone of 19 mm, *Staphylococcus aureus* has the inhibitory zone of 16mm and *Klebsiella pneumoniae* has no inhibitory zone.

Table 5 shows MIC of water extract against *Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae* in 2.0 g/l well; it was observed that *Escherichia coli* has the highest

inhibitory zone of 31 mm while the lowest inhibitory zone was discovered to be in *Klebsiella pneumoniae*. In 1.0 g/l well, *Escherichia coli* has the highest zone of 28 mm and *Klebsiella pneumoniae* has the lowest inhibitory zone of 25 mm. At 0.5, 0.25 0.125 g/l wells, *Esherichia coli* has the highest inhibitory zone and *Klebsiella pneumoniae* has the lowest inhibitory zone. In 0.065g/l well, *Escherichia coli* has the highest inhibitory zone of 18 mm; *Staphylococcus aureus* has the inhibitory zone of 17 mm and *Klebsiella pneumoniae* has no inhibition.

Tables 6 and 7 show the minimum bactericidal concentration (MBC) of the ethanol and water extract against the three selected organisms respectively; the resulting extracts were active against the entire three selected microorganisms. The extracts killed the *Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae* at 2.0, 1.0, 0.5, 0.25, and 0.125 g/l except *Klebsiella pneumoniae* at 0.125 g/l; however the two extracts inhibited the growth of the entire three selected organisms at 0.0625 g/l.

The result agrees with the work of Vongranich et al. [11] who reported that the plant extract had a good *in vitro* antibacterial activity against *Esherichia coli and Pseudomonas aeruginosa.* This result also agrees with that of Duke [12] who reported that the bark extract of *Anacardium occidentale* exhibited antibacterial activity *in vitro* against 13 to 15 organisms tested. Based on the findings of this work, the leaf extract of *Anacardium occidentale* dissolved in distilled water and ethanol with estimated concentration have good potential for the development of antibacterial drugs. The effect of any scientific study will help to variously establish and confirm the credibility of the use of herbals as an effective source of both traditional and modern medicine [13].

4. CONCLUSION

Research on medicinal plants should be intensified at two levels; the botanical gardens and research institutes and they should be encouraged to collect and then classify the known medicinal plants to assure the authenticity of it. The fact still remains that Herbal medicine will ever remain as a priority among Africans; the growing rate of civilization among Africans will never displace this fact. Therefore Herbal medicine should be encouraged by National Agency for food, drug administration and Control (NAFDAC) in Nigeria. More research to fully establish the usefulness of this plant, *Anacardium occidentale* to humanity as a source of drugs for the alleviation of illness caused by microorganisms is hereby suggested; so as to fully harness its potentials

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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