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In vitro Antibacterial Activity of Aqueous Extracts of Cashew (*Anacardium occidentale* L.) Fruit Peels Using Bioautography Method

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Authors' contributions

This work was carried out in collaboration between the authors. Author BIA designed the study, while the protocol and the first draft of the manuscript were written by the authors.

Original Research Article

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ABSTRACT

Aims: Bark, leaves and gum of cashew (*Anacardium occidentale* L.) have been reported to be effective in curtailing the growing problems of resistance of bacterial pathogens. The *in vitro* activity of aqueous extracts of cashew apple peels was determined in this study against two clinically important pathogens.
Place and Duration of Study: The work was conducted at the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria and processed immediately. This study was carried out between June, 2009 and January, 2010.
Methodology: Bioautographic method was used to test the antibacterial activity of aqueous (cold and hot water) extracts of cashew apple peels on *Escherichia coli* O157:H7 and methicillin-resistant *Staphylococcus aureus* (MRSA). The zones of inhibition of the extracts were compared.
Results: The activity of the fifth hour hot water extract was highest with zones of inhibition of 415.48 and 346.30 sq. mm against *E. coli* O157:H7 and MRSA respectively. *E. coli* O157:H7 was more susceptible to the extract with the zone of inhibition ranging between 176.79 and 283.53 sq. mm while that of MRSA was153.94 - 346.30 sq. mm. The 5 h extract of cold water was more potent on the test organisms with 615.75 and 490.87 sq. mm diameters of inhibition on *E. coli* O157:H7 and MRSA respectively. Cold water extracts produced more active compounds (13 biologically active spots) that inhibited the growth of the test organisms than the hot water extracts, with six spots.
Conclusion: The extracts of the peels of the cashew apple against the test organisms

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are promising. However, the nature and mechanisms of action of the biologically active compounds in the extracts are still open to investigation.

Keywords: Cashew; bioauthography; Escherichia coli O157:H7; Methicillin-resistant Staphylococcus aureus; antibacterial; chromatography.

1. INTRODUCTION

Cashew, *Anacardium occidentale* L. belongs to the family *Anacardiaceae*, it originates from south and central America. It produces a pseudocarp on which the nut is attached. Apart from the nut, the primary product from the plant, other parts of the plants such as the pomace serve different purposes to man [1-5] and livestock [6-9]. Different parts and bi-products of the cashew plant e.g. the gum exudates have been reported to be very effective in inhibiting *Aspergillus flavus*, *Colletotrichum musae* and *Verticillium* sp. [10].

Furthermore, some extracts from cashew plant parts especially the apple, bark, leaves, gum and nut have been reported to inhibit the growth of medically important microorganisms [7,8,11-14]. Their high biological activities have been attributed to their high content of tannins [15,16]. Extracts from the bark of cashew tree effectively inhibited 15 different microorganisms *in vitro* [17]. The swollen peduncle of the cashew fruit, called the cashew 'apple' has high levels of ascorbic acid when compared to that of other fruits [18].

Cashew apple, the pseudo-fruit, is fibrous, juicy and very rich in polyphenols, minerals, organic acids, carbohydrates, pigments and vitamins mainly vitamin C [19-21]. Most of these chemical components of the cashew apple have been found to possess some anti-microbial and anti-mutagenic properties [22-25].

Cashew apple juice has a stringent taste with biting sensation of the tongue and throat. These attributes are believed to aid its use in the treatment of throat infections, mouth ulcers, throat infections and oral thrust [17,26]. The juice of the cashew apple has cytotoxic activity [22], antifungal, antibacterial and nematicidal activity [27,28] reported that cashew fruit can be used to manage *Helicobacter pylori*, the causative agent of acute gastritis and stomach ulcer. The antimicrobial activities of leaves, bark, nut shell liquid, apple juice and gum exudates of the tree have been investigated and documented. However, the *in vitro* activity of the 'epicarp' (peels) of cashew apple has not been reported hence the objective of this study.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

Fresh and healthy yellow variety of cashew fruits (*Anacardium occidentale*) were plucked from a single tree in a cashew plantation near the Ekiti State University, Ado-Ekiti, Nigeria and identified at the herbarium section of the Department of Plant Science of the institution. The fruits were washed under running water and made free from dust and sands. Thin layer (1 mm thick) of the endocarp of the fresh cashew apple was carefully and aseptically removed with a sterile surgical blade. The peels were weighed and divided into two portions. A part of the peels was submerged in hot water in flasks maintained at 80ºC while the other portion was put in cold water at 4ºC for 12 h. A known amount of each sample was taking

periodically at one hour interval, macerated and the resulting suspension filtered through a clean muslin cloth, followed by double filtration with Whatman No. 1 filter paper. The filtrate was brought to dryness and stored at 4ºC in sterile condition until needed. All the experiments were conducted in triplicates and repeated twice.

2.2 Thin Layer Chromatography (TLC)

A baseline was drawn on the lower edge of the 25 mm TLC plate and at the upper end (solvent front) of the plate. A spot of each of the plant extract was placed on the baseline with the use of a micro-pipette and allowed to dry. The plate was placed in the developing jar using chloroform/methanol (9:1) as eluent to separate the constituents. The developed plate was examined under the UV lamp at 530 nm and the fluoresced spots were circled with a pencil. Another set of developed plates were later exposed to iodine fumes in Vaseline-sealed desiccator for a few seconds. Again, the plates were examined under UV lamp and any new spots detected were marked. The spots were labeled and their distances from the baseline were measured. The distance between the baseline and the solvent front was measured and the $R_f$values determined. This experiment was carried out to monitor the incidence of spots and relate same to bio-active spots after bioassay.

2.3 Source and Standardization of Bacteria Inocula

Escherichia coli O157: H7 and Methicillin-Resistant *Staphylococcus aureus* (MRSA) were obtained from the Department of Microbiology Laboratory, Ekiti State University, Ado-Ekiti, Nigeria. The organisms were primarily isolated from clinical samples. The broth cultures of these organisms were standardized by the method of CLSI [29].

2.4 Thin layer chromatography bioassay

In another experiment, the developed thin-layer chromatograms were sprayed with 1 ml each of standardized bacteria suspension of *E. coli* O157: H7 and MRSA in 5ml nutrient broth and incubated at 37ºC for 24 h. This test was performed to determine effective antimicrobial spot of the fractionated extracts of cashew peel. The refractive factor (R_f) and colour reactions were observed and recorded.

2.5 Estimation of Zone of Inhibition

The zone of inhibition was estimated as the area (πr^2, where r = radius) not covered by any growth of the seeded test organisms on the developed chromatogram incubated at 37ºC for 18 h. An average of many radii of the inhibition zone was taken.

3. RESULTS AND DISCUSSION

The growing problems of resistance of bacterial pathogens are now of concern to public health and has created a critical need for the search for natural antibacterial compounds [30]. Herbal medicine practitioners in most cases make use of water (cold or hot) in extraction of active ingredients for the treatment of traditional remedies [31]. The first and twelfth hour extracts did not show any inhibitory activity against the test organisms. However, the third and sixth hour hot water extracts of the peel were more effective against *E. coli* O157:H7 than MRSA (Table 1).

Table 1. Antibacterial activity of hot water extracts of cashew apple peels against *E. coli* O157:H7 and MRSA

Extracts	*Spots	Zone of inhibition (sq. mm)	
		***E. coli* O157:H7**	**MRSA**
T_1	0	0.00	0.00
T_2	1	176.79	153.94
T_3	2	176.79	176.79
		176.79	153.94
T_4	1	201.06	176.79
T_5	1	415.48	346.30
T_6	1	283.53	226.98
T_{12}	0.00	0.00	0.00

**Spots with pronounced antimicrobial activity*

Evidence of antibacterial activity of the fractions of hot water extracts on the test organisms became obvious after 2 h, with the 4th and 6th hour extracts showing greater inhibitory activity on *E. coli* O157: H7. Bioactivity increased with time of extraction, but no inhibition was detected with the 12th h extract. Using the bioactivity of T_2 extract as the baseline, the T_5 extract exhibited about 235.01% inhibitory effect over the T_2 extract.

The activity of the hot water extract was highest at the fifth hour with 415.48 and 346.36 sq mm zones of inhibition against *E. coli* O157: H7 than MRSA respectively. Extracts at the third hour had two bioactive spots. The low susceptibility in MRSA may be due to widespread antimicrobials used against this organism and which has caused a significant increase in resistance among Gram-positive organisms in particular [32]. In spite of this, the T_5 extract showed about 224.96% inhibition on this organism when compared to that of T_2. Invariably, this shows that the hot water extract obtained after 5h was very potent on the microbes.

There was no detection of any bioactive spot from the cold water extract collected after 1h (Table 2). The second hour extract showed only one bioactive spot while two spots were subsequently detected at every stage as the extraction process continued to the 12^{th} hour. The zone of inhibition of *E. coli* O157: H7 by the cold water extracts ranged between 201.06 and 615.75 sq. mm while those of MRSA were from0 to 314.16 sq mm. Similarly, *E. coli* O157: H7 was more susceptible to the cold water extract than MRSA. The extract obtained after 5h was more potent on the test organisms (with inhibition of 615.75 and 490.87 sq mm on *E. coli* O157:H7 and MRSA respectively).

It is evident that the cold water extracts are more active on the test organisms than the hot water extracts. This might be as a result of decomposition of the active compounds by heat. High temperature significantly reduces the quantity of phytochemical like alkaloid, phenols, tannins, oxalate and saponin [33]. Furthermore, the cold water extracts showed more inhibitory spots against the microbes maybe as a result of the active biochemicals present in it. Of the two bioactive spots obtained from the cold water extract after 5h, the spot located at an R_f6.0 showed no inhibition against MRSA but its activity was poor on *E. coli* O157: H7. When the T_2 extracts (hot and cold water) are compared, the cold water extract exhibited about 177.7% and 147.44% inhibitory activity with the extract obtained after 5h.

Table 2. Antibacterial activity of cold water extracts of cashew apple peels against *E. coli* O157:H7 and MRSA

Extracts	*Spots	Zone of inhibition (sq. mm)	
		***E. coli* O157:7**	**MRSA**
T_1	0	0.00	0.00
T_2	1	314.16	226.98
T_3	2	283.53	226.98
		201.06	226.98
T_4	2	201.06	201.06
		490.87	314.16
T_5	2	615.75	490.87
		283.53	0
T_6	2	176.79	0
		283.53	153.94
T_{12}	2	254.47	176.79
		254.47	176.79

**Spots with pronounced antimicrobial activity.*

Generally, all the extracts were more effective against *E. coli* O157:H7 (a Gram negative organism) than MRSA (a Gram positive organism) this support the early report by Murali et al. [34] that confirmed *E. coli* O157: H7 to be more susceptible to the extract of lemon peels with a 3–log reduction after 1 h of exposure than MRSA with 2-log reduction. However, this is contrary to the report of Eftekhar [35] that *S. aureus* ATCC 25923 (a Gram positive bacterium) is more susceptible to extracts of *Datura*spp than *E. coli*. Shahidi-Bonjar [36] also reported that the cells of *S. aureus* were more sensitive, than those of *E. coli,* to 44 medicinal plants from Iran.

Most antimicrobial agents target the cell wall because of their affinity with the protein moiety of cellular enzymes in the cell wall [37]. The extracts of cashew peel are likely to target certain parts of the bacteria other than the cell wall as Alli et al. [38] reported that garlic acid extract interfered with DNA and RNA syntheses of both *Pseudomonas aeruginosa* and *S. aureus*. In like manner, Henie et al. [39] reported the disruption of bacterial membrane by *Psidium guajava* leaf extracts, with the methanolic extracts of guava causing a significantly higher ($p \leq 0.05$) release of RNA in *E. coli* O157: H7 compared to *S. aureus*. Medicinal plants can also cause impairment with the intracellular pH, total ATP concentration and membrane selective permeability [40]. In agreement with the results of our work, Tsuchiya et al. [41] reported that none of the 17 strains of MRSA they screened was sensitive to phytochemical compounds.

The cold water extracts produced more active compounds that inhibited the growth of the test organisms than the hot water extracts. About 13 biologically active spots were detected from the cold water extract while the hot water extract had 6 spots. From the bioautography, the extracts showed growth inhibition bands for both *E. coli* O157: H7 and MRSA. The synergic action of the phyto complex of the bands was suggested by Arias et al. [42] to have been responsible for the inhibition of the test organisms. The retention factors (R_f) of the active spots from the hot water extract ranged between 5.5 and 9.0 while the R_f values of the cold water extract ranged between 1.0 and 11.2 (Table 3). It appears that the spots located at Rfs 8.5 and 9.0 showed more bioactive spots in both aquatic extracts.

In general, the cold water extract had a better inhibitory activity than the hot water extract of cashew peels. Earlier report showed that cold water extract had a better inhibitory activity than the hot water extract of *Moringa oleifera* against an array of pathogenic bacteria [43].

Table 3. Chromatograph and retention factor (R_f) of bioactive spots from the aqueous extracts of cashew peels

Extracts (hr)	Chromatograph (numbers of spots)		Retention factors (R_f)	
	Hot water	Cold water	Hot water	Cold water
T_1	0	0	0	0
T_2	1	2	9.0	6.0; 9.0
T_3	2	2	8.2; 9.0	9.0; 11.2
T_4	1	2	7.0	7.2; 9.0
T_5	1	2	5.5	6.0; 9.0
T_6	1	2	5.6	8.0; 8.5
T_{12}	0	2	0	8.2; 8.5

4. CONCLUSION

The broad spectra of activity of the extracts of the peels of the cashew apple against *E. coli* and MRSA when determined will be promising. However, the nature and mode of action of the active antibacterial compounds in the extracts is still open to investigation.

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COMPETING INTERESTS

Authors declare that no competing interests exist.

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