The Efficacy of Extracts of the Leaf and Bark of Cashew Plant (*Anacardium occidentale)* on Bacterial Isolates Isolated from Banana (*Musa paradisica*)

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Abstract: This study was undertaken with the aim to investigate the potency of cashew leaf and bark extracts on microorganism associated with banana spoilage. Banana (*Musa paradisica*) was subjected to spoilage in the laboratory for 7days. Bacteria isolated from the sample include *Staphylococcus aureus*, *Proteus vulgaris* and *Corynebacterium* spp. The efficacies of the extracts were tested against the isolated microorganisms at various concentrations for 8 weeks. The results showed that the ethanolic extracts of both leaf and bark of *Anacardium occidentale* at the last concentration (10%) showed the highest antimicrobial activity. At 8.5% concentration, the activity of the aqueous leaf extract decreased with an increase in microbial growth and later decreased at 7.0% concentration. At the highest concentration of the ethanolic bark and leaf extract (10%), there was no count of *Corynebacterium* spp. and *Staphylococcus aureus* respectively. The aqueous extract showed a bit of effectiveness at the 7.0% of the extract by reducing the numbers of growth of the microorganism, but at the 8.5% concentration there was an increase in the microbial growth. The phytochemicals present in the *Anacardium occidentale* leaf and barks include saponins, tannins, flavonoids, phenol, glycosides, terpenoid and alkaloids. The study shows that the ethanol extract was more effective than aqueous extract.

Keywords: Banana, Cashew, Extraction, Concentration, Phytochemical, Susceptibility.

INTRODUCTION

Ever since the discovery of microorganisms as the principal cause of diseases, man has employed various agents to control the spread of these disease-causing agents. In general terms, these materials have been collectively referred to as antimicrobial agents (Saga and Yamaguchi, 2009).

Of all antimicrobial agents, plant materials have had the widest and most common use over the course of history. The field of phytomedicine has been wholly dedicated to the research and development of plant based antimicrobial agent. Indeed according to World Health Organization (2003), about 80% of Africans have recoursed to traditional medicine, which involves the use of plants' active principles, to treat most diseases. Plant derived substances have recently become of great interest owing to their versatile application (Baris *et al*., 2006).

It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant-derived components were discovered after following up on ethno-medicinal use of the plants (Ncube *et al*., 2008). The medicinal value of these plants is due to some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, porphyrins, flavonoids and phenolic compounds (Hill, 1991).

Anacardium occidentale (Family: Anacardiaceae) is a native plant of tropical America, naturalized and cultivated throughout India especially near the coastal area. *Anacardium occidentale* is used medicinally wherever it is found growing. All parts of the plant like leaves, false fruit and bark have been traditionally used to relieve variety of ailments. It is a small tree with a short, thick, crocked trunk and is commonly called cashew in English, 'Kashu' in Hausa, 'Okpokpo' in Ibo and "Kaju" in Yoruba. The leaves of *Anacardium occidentale* is simple, alternate, criaceus, gabrous, obovate, rounded at ends, with short petiole, pale green or reddish when young, and dark green when mature (Orwa *et al*. 2009).

Cashew apples and cashew nuts are excellent sources of nutrients. The leaves, barks, roots and stems are traditionally used for the treatment of numerous diseases such as, allergy, cough, stomach ache, diarrhea, skin infections etc. (Chabi *et al*., 2013).

*Anacardium occidental*e tree has been used medicinally worldwide. It contains vitamins and unsaturated fatty acids (Oleic acid 73.3% and Linoleic acid 7.67%). All the parts of the plant (apple, bark, leaves, gum, nut and roots) have different ethno medicinal uses. Bananas (*Musa paradisica*) are perennial and herbaceous plants belonging to the family Musaceae. *Musa* is one of the three genera in the family. In popular culture, banana usually refers to soft, sweet dessert *Musa* cultivars. Banana is a major staple crop of considerable importance in the developing world. *Musa* fruits play major roles in the nutrition and well being of people of the tropical and sub-tropical regions of the world.

They are good sources of income to the farmers in their respective growing regions (Bridge, 2000; Akinyemi *et al.*, 2010) and animal alike (Ramiakajato *et al*., 2001).

Banana fruit can be contaminated by microorganisms through skin penetration, natural opening or mechanical damage. Microbial spoilage of fruits may be due to bacteria or fungi causing the fruits to be undesirable, reducing the market value and may also cause some side effects such as gastroenteritis, when consumed (Prescott *et al*., 2002). Post-harvest diseases can cause serious losses of fruits both in terms of quantity and quality. Fruits with microorganisms have no market value. Some of the bacteria associated with the spoilage of banana include; *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Corynebacterium* spp., *Lactobacillus* spp., *Bacillus thuringensis, Klebsiella pneumonia, Pseudomonas aeruginosa, Streptococcus* spp.

The aim of this study was therefore to isolate bacteria associated with the spoilage of banana and carry out laboratory studies on the efficacy of leaf and bark extracts of *Anacardium occidentale* using water and ethanol as extracting solvent.

MATERIALS AND METHODS

Collection of Plant Materials

The plant materials used were the leaves and bark of *Anacardium occidentale.* Fresh leaves and bark of cashew were collected from University of Ilorin permanent site road, Ilorin, Kwara State. The identification was carried out at the herbarium of the Department of Plant Biology, University of Ilorin with voucher number: UILH/001/970.

Preparation of Leaves and Bark Extract

Aqueous extraction (Cold Water)

Twelve grams of the blended sample was measured into a conical flask and 100 ml of sterile distilled water was added, covered with a cork, mixed together properly and left in the shaker at 100 revolution per minute for 24 hours. The sample was filtered and squeezed through four layers of muslin cloth. The filtrate was then centrifuged at 2,000 revolutions per minute (r.p.m.) for 5 minutes after which it was decanted. The pellet was discarded and the supernatant was sterilized by using the membrane filtration unit with type HC filters. The filtrate obtained was stored in sterile McCartney bottles and kept in the refrigerator at 4^{o}C and later used for antimicrobial tests.

Ethanol extraction

Twelve grams of the blended sample was measured into a conical flask and 100 ml of 95% ethanol was added; covered with a cork, mixed together and left on the shaker at 100 r.p.m. for 24 hours after which the extract was filtered and squeezed through four layers of muslin cloth. The filtrate was then centrifuged at 2,000 r.p.m. for 5 minutes after which it was decanted. The pellet was discarded and the supernatant was sterilized by using the membrane filtration unit with type HC filters. The filtrate obtained was stored in sterile McCartney bottles and kept in th nbe refrigerator at 4°C and later used for antimicrobial tests.

Isolation of Test Microorganisms From Banana (*Musa paradisica)*

Bacteria

The banana (*Musa paradisica)* was subjected to spoilage by subjecting it to a humid condition in a micro humidity chamber and spoilage occurred within 1 week. The banana was surface-sterilized with 70% alcohol to kill the unwanted microorganisms before placing in the sterile container. The spoilt areas were cut out and ground using sterilized mortar and pestle (swabbed with 70% ethanol) and serial dilutions were made up to 10^{-6}.

The method used was Pour plate in which 0.1ml of the 10^{-3} and 10^{-4} dilutions were withdrawn using a sterile syringe and aseptically put into petri dish. For the isolation of bacteria, about 20ml of molten Nutrient Agar was poured onto the petri dish containing 0.1 ml of the dilutions. The plate was swirled properly and allowed to solidify before incubating at 30^0C for 24hours. It was then examined for bacterial growth. Several sub-culturing were carried out to obtain pure cultures of the isolates, they were then put in McCartney bottles to make agar slant. The bottles were then incubated at 30^0C for bacteria growth. They were then kept at refrigeration temperature of 4^0C to avoid contamination.

Preparation of Extracts for Efficacy/Antimicrobial Test

This was done by method described by Das *et al*. (2010).

Qualitative Phytochemical Screening Test for the Extract

The crude extracts of cashew powders was subjected to phytochemical tests using standard procedures to identify the constituents present as described by Herbune (1973), Odebiyi and Sofowora (1978) and Tiwari *et al.* (2011). The methods were deployed in order to detect the presence of plant constituents such as alkaloids, saponins, tannins, phenols, glycosides and flavonoids in the plant's extract.

RESULTS AND DISCUSSION

The following bacterial isolates were associated with the spoilage of banana sample they were; *Proteus vulgaris*, *Corynebacterium* spp. and *Staphylococcus aureus*.

A total of three bacterial isolates were identified and this negates the findings of Mokbel *et al,* (2005) who observed that there were no microorganisms in banana and this they attributed to the antimicrobial properties of the unripe peel of banana fruit. The presence of these organisms in the fruits was due to various predisposing factors. The organisms present might be as a result of contamination from the atmosphere, storage methods, pH, and moisture content.

The presence of organisms might also be due to nutritional composition of banana and they have the opportunity to grow and establish themselves during fermentation stage of spoilt banana. Some of these organisms when ingested are very harmful and can be dangerous to human health (Schardi *et al*., 2006).

The antimicrobial activity of the aqueous leaf extract on the bacterial isolates against *Proteus vulgaris*, *Corynebacterium* spp. and *Staphylococcus aureus* for week 1 was less effective due to the low concentration of the extract as shown in Figure 1. As the concentration increased from week 2 to week 6 as shown in Figures 2-6, the bacterial counts reduced. At week 7, there was a slight increase in the bacterial counts for the test organisms. The antimicrobial activity of the aqueous bark extract was found to reduce bacterial counts from week 1 to week 5. There was a slight increase in the bacterial counts on the 6^{th} and 7^{th} week and later a decrease on the 8^{th} week.

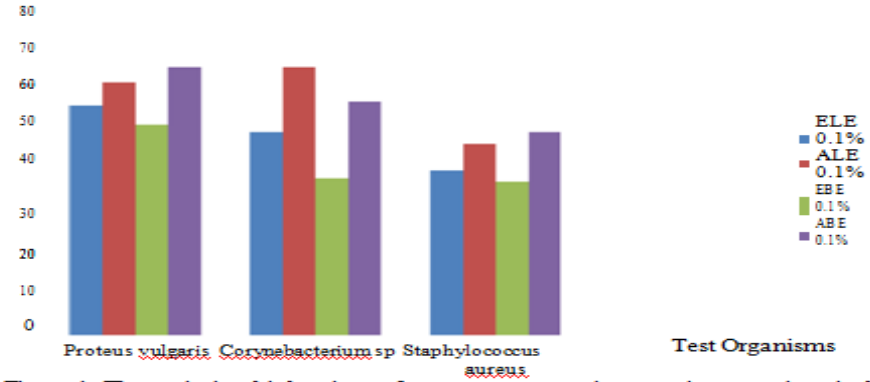

Figure 1: The antimicrobial actions of extract concentration on microorganisms isolated from banana for the first week

KEY
ELE = Ethanolic leaf extract
ALE = Aqueous leaf extract
EBE = Ethanolic bark extract
ABE = Aqueous bark extract

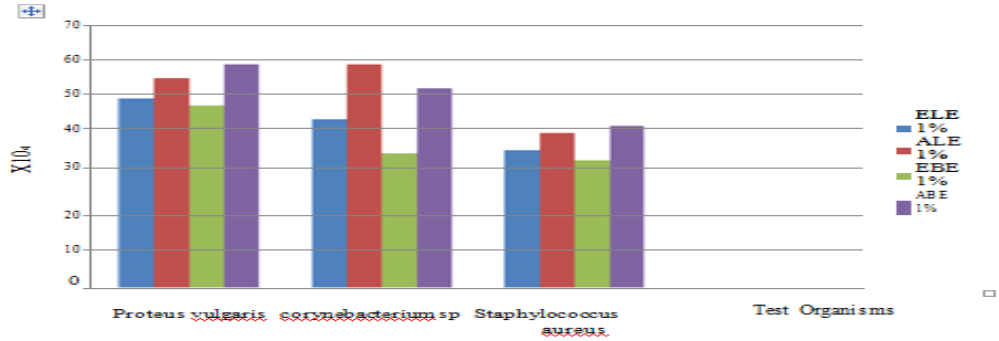

Figure 2: The antimicrobial actions of extract concentration on microorganisms isolated from banana for the second week

KEY:
ELE = Ethanolic leaf extract
ALE = Aqueous leaf extract
EBE = Ethanolic bark extract
ABE = Aqueous bark extract

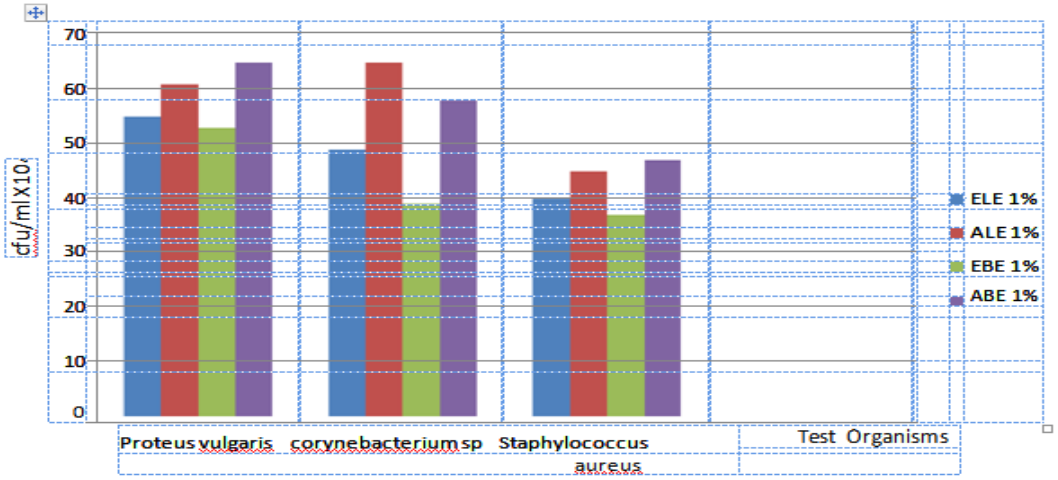

Figure 3: The antimicrobial actions of extract concentration on microorganisms isolated from banana for the third week

KEY:

ELE = Ethanolic leaf extract ALE = Aqueous leaf extract, EBE = Ethanolic bark extract ABE = Aqueous bark extract

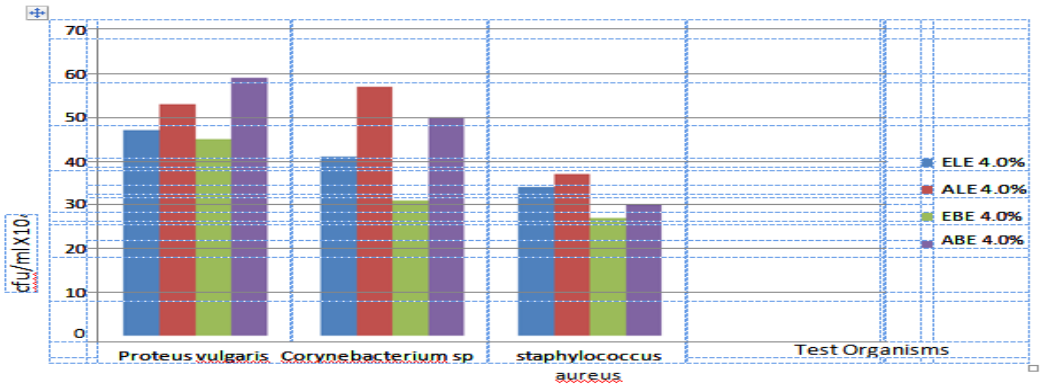

Figure 4: The antimicrobial actions of extract concentration on microorganisms isolated from banana for the fourth week
KEY: ELE = Ethanolic leaf extract ALE = Aqueous leaf extract, EBE = Ethanolic bark, extract ABE = Aqueous bark extract

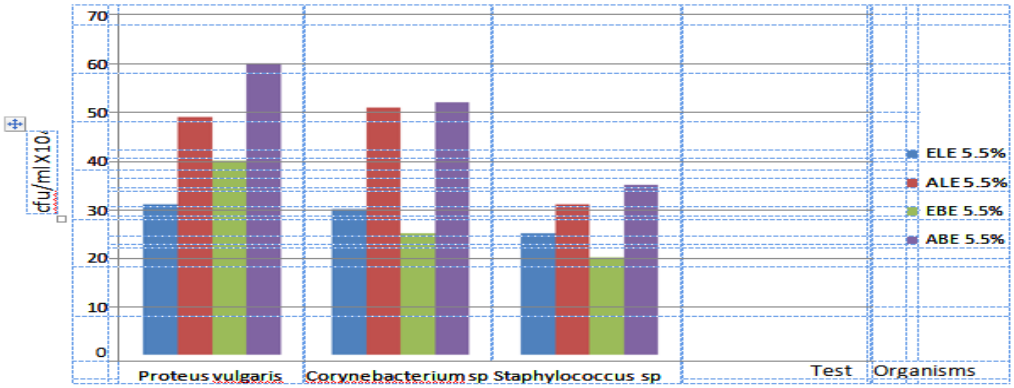

Figure 5: The antimicrobial actions of extract concentration on microorganisms isolated from banana for the fifth week
KEY: ELE = Ethanolic leaf extract ALE = Aqueous leaf extract, EBE = Ethanolic bark extract, ABE = Aqueous bark extract

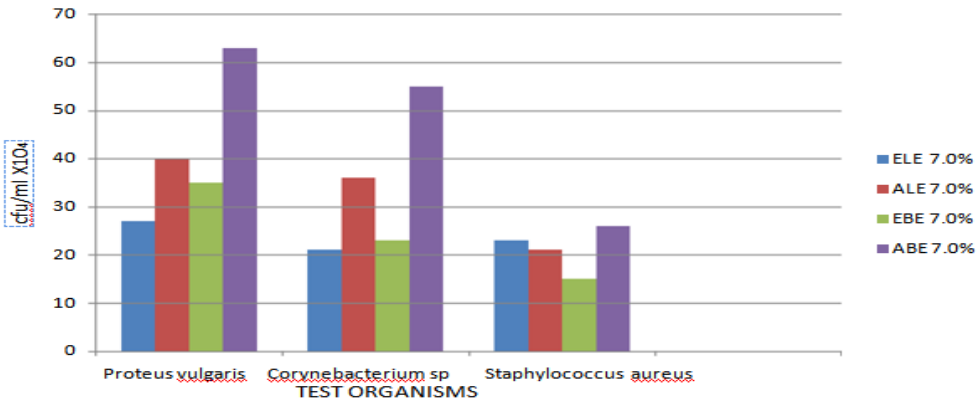

Figure 6: The antimicrobial actions of different extract concentration on microorganisms isolated from banana for the sixth week
KEY: ELE = Ethanolic leaf extract ALE = Aqueous leaf extract, EBE = Ethanolic bark extract, ABE = Aqueous bark extract

The antimicrobial activity of the ethanolic leaf extract showed a reduction of bacterial counts from the 1st to 8th week on the test organisms. The antimicrobial activity of the ethanolic bark extract showed a reduction of bacterial counts from the 1st to 8th week on the test organisms. This showed that the effectiveness of the ethanolic extract was higher than that of the aqueous extract. The antimicrobial activity on microbial counts is presented in Figures 1-8.

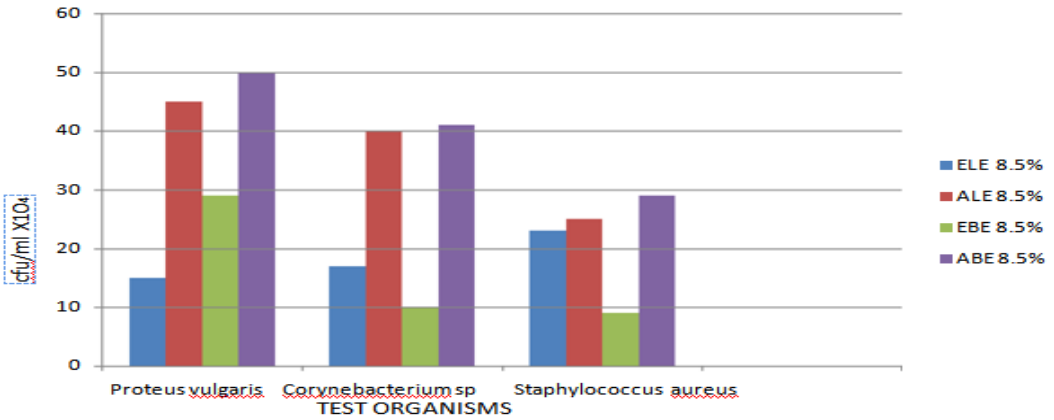

Figure 7: The antimicrobial actions of extract concentration on microorganisms isolated from banana for the seventh week
KEY:ELE = Ethanolic leaf extract ALE = Aqueous leaf extract, EBE = Ethanolic bark extract ABE = Aqueous bark extract

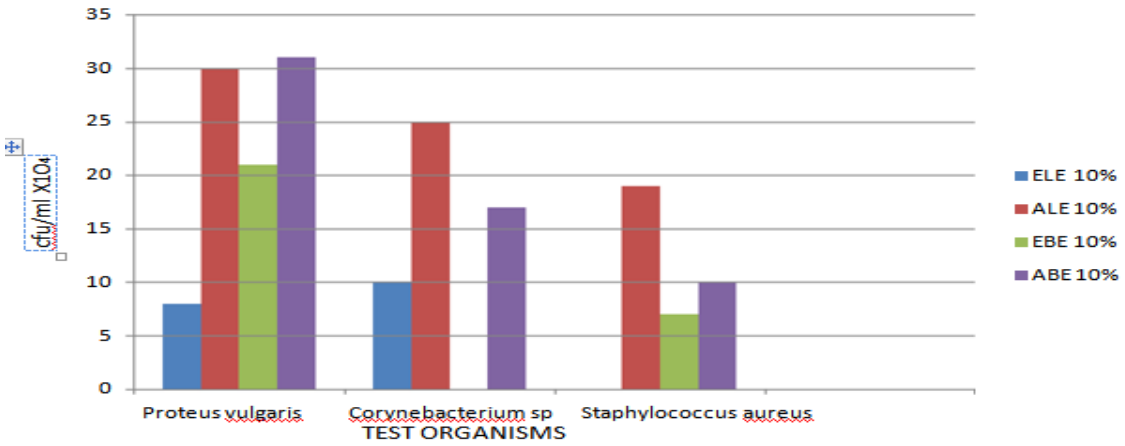

Figure 8: The antimicrobial actions of extract concentration on microorganisms isolated from banana for the eighth week
KEY: ELE = Ethanolic leaf extract ALE = Aqueous leaf extract EBE = Ethanolic bark extract ABE = Aqueous bark extract

From the 1st to 8th week, the ethanolic bark extract was found to be highly effective against the test organisms from 0.1-10% concentration by reducing the microbial counts drastically. At 10% concentration, there was no count of *Corynebacterium* spp. due to the effectiveness of ethanolic bark extract which supports the findings made by Mahadevappa *et al*., 2011 that cashew bark was effective against some respiratory diseases caused by *Corynebacterium* spp. such as asthma, bronchitis.

From the 1st to 4th week, the aqueous bark extract was observed to be effective from 0.1 to 4.0% concentrations against the test organisms (*Staphylococcus aureus, Proteus vulgaris, and Corynebacterium* spp.). There was an increase in the bacterial counts from 5.5 to 8.5 % concentrations and at 10% concentration there was a slight decrease which might be due to the increase in the activity of the extract as its concentration increased.

The leaf and bark of *Anacardium occidentale* has been found to be effective against *Staphylococcus aureus* supporting the findings of Aderiye *et al.* (2015) that the leaf and bark of cashew can cure some of the diseases caused by *Staphylococcus aureus* such as urinary disorder and skin infection.

The phytochemical screening showed the presence of saponins, flavonoids, alkaloids, tannins, phenol, and terpenoid in both the leaf and bark extracts of *Anacardium occidentale* as presented in Table 1.

TABLE 1: Phytochemicals present in *Anacardium occidentale* extract

Phytochemical	Ethanolic leaf extract	Ethanolic bark extract	Aqueous leaf extract	Aqueous bark extract
Flavonoid	++	++	++	++
Glycosides	+	+	+	-
Alkaloids	+	++	+	++
Saponins	++	++	++	+
Tannins	+	+	+	+
Phenols	+	+	+	+
– Terpenoids	+	+	+	+

KEY:
-, =Absent
+, = moderately present
++, =Heavily present

Preliminary Phytochemical analysis of *Anacardium occidentale* extracts showed an array of rich secondary metabolites such as tannins, terpenoid, alkaloids, flavonoids, phenols, steroids, glycosides. The aqueous extract was found to be less effective in dissolving the phytochemicals than the ethanolic solvent; hence, there was an absence of glycosides in the aqueous bark extract. The absence of some phytochemicals might be due to differences in the polarity of the solvents, as the types of solvent used determined the kind of biologically active compounds that can be extracted from plant (Tiwari *et al.,* 2011). The differences in the phytochemicals observed in this study might be attributed to different cultivars and geographical locations from where the plant was harvested and different extraction procedure employed.

The results obtained are in accordance with the work of Omojate (2014) who reported that the phytochemical analysis of *Anacardium occidentale* showed the presence of some phytochemicals such as flavonoids, alkaloids, phenols, glycosides, tannins, saponins.

CONCLUSION

The antibacterial study of the cashew leaf and bark extracts gave its effectiveness against some spoilage microorganisms which were isolated from banana. This result also showed that the ethanol extract was more effective than aqueous extract. Thus, the use of this plant might justify the treatment of certain diseases like dysentery, diarrhea, skin diseases and urinary tract infections by traditional healers.

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