Synergy of methanolic leave and stem-back extract of *Anacardium occidentale* L. (cashew) against some enteric and superficial bacteria pathogens

**Abstract**

The synergy of methanolic leave and stem-back of *Anacardium occidentale* (Cashew) against some enteric (*Escherichia coli*) and superficial (*Staphylococcus aureus*) bacteria pathogens was investigated. The stem-bark and leaves of *Anacardium occidentale* were dried, extracted using methanol and evaluated for sensitivity using agar well diffusion method. The zone of inhibition for leaves, stem-bark and synergy (leaves+stem-bark) was 9.67mm, 12.00mm and 12.33 mm respectively for *Escherichia coli*; and 11.33mm, 13.67mm and 14.00mm respectively for *Staphylococcus aureus*. The synergy extract had superior effects for both test organisms. Hence, there is the need for research to focus on the isolation and purification of the bioactive ingredients of the plants that enable them to have antibacterial activities.

**Keywords:** *anacardium occidentale*, antibacterial, bacteria pathogens, methanol, medicinal plants

**Introduction**

Several groups of microorganisms have been implicated with gastrointestinal disorder. Among these bacteria is *Escherichia coli* which have been severally studied. Kigigha & Zige, Kigigha et al., reported that *Escherichia coli* and *Staphylococcus aureus* as indicators for enteric and superficial disease causing agents respectively. Typically, the occurrence of enteric pathogens in ingestible items such as food and water is among the main cause of diarrhea and dysentery especially in developing nations. These organisms have been widely reported in several food items including gari, suya, ground nut, palm oil, slice fruit (paw-paw, water melon, pineapple), meat pie, smoke fish, potable water sources. Instances of antibiotics resistance is a major issues in the field of pharmaceutical sciences. Among the common organisms that confers resistance to antibiotics is *Staphylococcus aureus*. According to Nwinyi et al., infections associated with methicillin resistant *Staphylococcus aureus* and vancomycin resistant *Staphylococcus aureus* tend to cause high morbidity and mortality, and also increase treatment cost.

Probably due to the challenges of drug resistance and emerging and re-emerging microbial infections, the search for new antibiotics has increased. Globally, plant resources is used for the treatment of several diseases especially by individuals residing in rural areas in many developing countries. Medicinal plants have been widely described as plants whose one or more parts have therapeutic properties against diseases including bacterial diseases. The medicinal potentials of plants is associated with their secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. they contain. Scientific validations have been made with some of the claims of traditional medicine practitioners, but many others claims need to still be studied. Also there is the need for isolation and purification of exact chemical constituent that make plant confers pharmacological properties.

*Anacardium occidentale* L., which belong to the Anacardiaceae family is one of the plant species that are used by traditional medicine practitioners for the treatment of different diseases. The plants can grow up to 15m in height in the tropics and tropical rain forest. Traditionally, the parts of *Anacardium occidentale* are used for preparing several medicine in different regions of the world. Agedah et al., reported that *Anacardium occidentale* is used for the treatment of diarrhoea, dysentery, stomach ulcer in Ghana, Nigeria, Togo; eye and ear infections in Africa and Brazil; wound healing, malaria and prevention of bleeding in Africa; antidiabetic in Ghana, Haiti, Kenya, Mexico, Nigeria; and treatment of fever, poisoning, warts, toothache in Haiti, Turkey. The authors further reported that the plant also has antibacterial, antiseptic, Anti-diabetic, anti-inflammatory activities, and it also promotes blood coagulation. Furthermore, different parts of *Anacardium occidentale* including bark, stem, and leaf extracts, have antimicrobial, anti-diabetic, and anthelmintic potentials.

Among the antimicrobial potentials of *Anacardium occidentale* using different solvents for extraction, information about the synergy of stem-bark and leaves methanolic extracts against some microbes that causes gastrointestinal and superficial infections is scanty in literature. Hence, this study aimed at assessing the synergistic efficacy of methanolic stem-bark and leaves extracts of *Anacardium occidentale* against *Escherichia coli* and *Staphylococcus aureus*.

**Materials and methods**

**Samples preparations and extraction**

Fresh stem-bark and leaves *Anacardium occidentale* used for the study were washed with distilled water. The water was allowed to drain off. The samples were shade dried and then chop into small pieces with knife. The samples were then blended independently using Binatone blender. 40g of the powdered samples were soaked in 100ml of methanol for 48 hours, while mixture of stem-bark and leaves were soaked in 100ml of methanol for 48 hours. The samples were filtered, washed with methanol and then dried in a fume hood. The dried plant was then powdered using a mortar and pestle.
leaves (1:1 ratio) was soaked in methanol for 48 hours as well. After 48 hours, they were filtered with muslin cloth, and the resultant filtrate was re-filtered using Whatman filter paper. The solvent were allowed to evaporate in a water bath.

**Isolates characterization**

*Staphylococcus aureus* and *Escherichia coli* used for the study were obtained from Medical Microbiology units, Federal Medical Centre, Yenagoa, Bayelsa state, Nigeria. The characteristics of the bacteria isolates were determined following the scheme provided by Cheesbrough.

**Antimicrobial screening of the extract**

The zone of inhibition was determined using Agar well diffusion techniques that was previously described by Lino & Deogracious cited in Doherty et al., with slight modification by Agu & Thomas, Kigigha et al., Epidi et al., Izah et al., Izah & Aseibai. Nutrient agar was used as the agar medium; the medium prepared according to manufacturers’ instruction was dispensed onto sterile petri dish and which was allowed to solidify. Approximately 0.3ml of 24 hours incubated isolate in peptone water was spread over the surface of the agar. A total of 3 wells of 6mm in diameter were made in each agar plate and 0.3ml of the extracts was dispensed into the agar wells. The medium were incubated at room temperature for 24 hours and the resultant zones of inhibition were measured.

**Statistical analysis**

The mean were calculated and the resultant values were used to plot the chart using Microsoft excel.

**Results and discussion**

The zone of inhibition of methanolic leaves and stem-bark of *Anacardium occidentale* against superficial (*Staphylococcus aureus*) and enteric (*Escherichia coli*) bacteria agents is presented in Figure 1. The zone of inhibition for leaves, stem-bark and synergy (leaves+stem-bark) was 9.67mm, 12.00mm and 12.33mm respectively for *Escherichia coli*; and 11.33mm, 13.67mm and 14.00 mm respectively for *Staphylococcus aureus*. The result showed that the leaves confer superior effect against *Escherichia coli*, while the stem-bark has apparent higher effect against *Staphylococcus aureus*. The synergy extract had superior effects for both test organisms. The parts of *Anacardium occidentale* have been reported to have antimicrobial potentials against several types of microbes. The antimicrobial potentials of *Anacardium occidentale* could be due to the presence of several phytochemical and bioactive constituents. Oviasogbe et al., reported that ethanolic stem stick of *Anacardium occidentale* contain alkaloids, steroids, saponins, tannins, flavonoids, carbohydrate, while the aqueous extract of small part of the plant contain glycosides. Ojezele and Agunbiade reported that aqueous (hot and cold water) and methanol extracts of leaf, bark and root of cashew contain tannin, total polyphenol, oxalate, saponin and alkaid. Agedah et al. reported that antimicrobial potentials of *Anacardium occidentale* could be due to the presence of anacardic acid–a polyphenol and other compounds such as trols and tannins.

The findings of this study had some similarity with the work of other authors. Wonghirundecha and Sumpavapol reported that hot water extracts of cashew apple peels have inhibitory effectiveness against *Escherichia coli* O157:H7 and methicillin resistant *Staphylococcus aureus*. Typically, slight variation occurred among both type of organisms as well as the extracts. The variation in sensitivity could be due to the gram reaction of both organisms and thus *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative). Agedah et al. reported that gram positive bacteria lack an outer membrane in their cell walls which gram negative bacteria do possess. The authors further reported that outer membrane (which could hinder substantial amount of the extract from having contact with the cell wall) could be responsible in variation in the level of sensitivity of the extract. In additions, authors have variously reported that sensitivity of plant extract could be determined by the nutrition, physiology, metabolism and biochemistry of the isolates, age of the plant, extraction solvent, plant parts and concentration of the extracts.

**Figure 1** Zone of inhibition of methanolic leaves and stem-bark extract of *Anacardium occidentale* against some superficial and enteric bacteria pathogens.

**Conclusion**

This evaluated the synergistic potency of methanolic stem-bark and leaves of *Anacardium occidentale* against *Escherichia coli* and *Staphylococcus aureus*. The showed that methanolic stem bark and leaves extract of *Anacardium occidentale* confers sensitivity against *Escherichia coli* and *Staphylococcus aureus*. In addition, the synergy resulted has slight higher effects compared to the individual extracts.

**Acknowledgments**

None.

**Conflict of interest**

The author declares that they have no competing interests.

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