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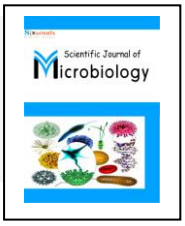

Original article

In-vitro antibacterial evaluation of ethanolic stem crude extracts of *anacardium occidentale* linn. (anacardiaceae) on *streptococcus mutans* associated with dental caries

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ABSTRACT

The preliminary phytochemistry and antibacterial activity of ethanolic stem crude extracts of *Anacardium occidentale* on *Streptococcus mutans* isolated from dental caries were determined using chemical, standard microbiological and agar disc diffusion techniques. The phytochemical analysis of the ethanolic stem crude extracts of *Anacardium occidentale* revealed the presence of alkaloids (+++), phenolics (+++), saponins (+), tannins (++), flavonoids (++), phlobatanins (+), anthraquinones (+), terpenes (++), deoxy-sugar (++) and cardiac glycosides (+). The results showed that between 65.2% to 95.6% of *S. mutans* isolated were sensitive to different concentrations of ethanolic stem crude extracts of *A. occidentale* with Activity Index (A.I) ranging from 0.38 to 1.22. Ethanolic stem crude extracts of *A. occidentale* showed the highest mean zones of Inhibition (17.9 ± 1.3mm) at 50.0 $mgml^{-1}$ and lowest inhibition diameter (6.5±1.5mm) at 12.5 $mgml^{-1}$ on *S. mutans*, thus, exhibiting concentration dependent activity. The results show that *S. mutans* with code WD09 was resistant to Streptomycin, Amoxycillin, di-methyl sulphoxide and *A. occidentale* ethanolic stem crude extracts. Therefore, there is a need to consider the use of this potent ethanolic stem crude extracts of *Anacardium occidentale* that have shown some measures of antimicrobial potency, judging by the antibacterial activity and activity index for developing synthetic drugs against dental caries caused by *Streptococcus mutans*.

1. Introduction

Over 80% of the world's inhabitants depend on herbal or traditional medicine for their primary health care (Cragg *et al*., 1999; Akinjogunla *et al*., 2011). Utilization of plants as traditional remedies still occupy a central place in developing countries especially among rural communities and many plants have shown to be highly effective for treating diverse ailments (Ali *et al*., 2001; Omojasola and Awe, 2004). Plants produce and contain a diverse range of bioactive constituents such as alkaloids, tannins flavonoids, cardiac glycoside and phenolics, consequently, purportedly provide excellent leads for new drug developments (Newman *et al*., 2000; Ali *et al*., 2007; Akinjogunla *et al*., 2009). The rediscovery of the connection between plants and health is responsible for the launching of new generations of multi-component botanical drugs, dietary supplements and plant-produced recombinant proteins (Raskin *et al*., 2002). Approximately 700 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use (Chakraborthy, 2008).

Resistance of both human and animal pathogenic microorganisms to drugs have been commonly reported in recent years from all over the world, particularly in developing countries, due to indiscriminate use of commercial antibiotics in the treatment of infectious diseases (Piddock and Wise, 1989 ; Service, 1995; Akinjogunla *et al*., 2009). Though, the resistance development by microbes cannot be stopped, appropriate action will reduce the mortality and health care costs by using antibiotic resistant inhibitors of plant origin (Ahmad and Beg, 2001). The increase in resistance of microorganisms due to multi-farious use of commercial antimicrobial drugs has encouraged scientists all over the world to search for new antimicrobial substances from various antecedents comprising medicinal plants (Akinjogunla et al., 2009).

Cashew plant, *Anacardium occidentale*, a member of genus *Anacardium* of the family *Anacardiaceae* is one of the plants which have been used for ethno-medical purposes (Bilcalho, 2001; Atata and Sani, 2003). *Anacardium occidental*e is commonly called cashew in English, 'Kashu' in Hausa, 'Okpokpo' in Ibo and 'Kaju' in Yoruba (Yusuf *et al*., 2009; Arekemase, 2011). It contains 60 to 74 genera and 400 to 600 species, made up of trees that grow up to 15m high (Morton, 1987). It is a tropical plant native to tropical America but now grown in many parts of the world. *A. occidentale* produces a pseudo-fruit called the cashew apple that is commonly consumed in the northeastern Brazil as fresh cashew apple juice or as 'cajuína' (Morton, 1987; Bilcalho, 2001; Atata and Sani, 2003). Over 50 % of annual cashew productions of 400,000 tonnes come from India and Tanzania (Opeke, 2005). The leaves stem and bark extracts of *A. occidentale* are utilized widely for the treatment of diarrhoea, dysentery and colonic pain (Bilcalho, 2001). In Brazil, it is used to treat diabetes, urinary disorders, asthma, eczema, dyspepsia and venereal diseases (Bilcalho, 2001). It has also been reported to possess anti-bacterial, anti-inflammatory and anti-ulcerogenic (Akinpelu, 2001; Adeleye and Ogunwale, 2004; Hassan *et al.,* 2004).

Dental caries are among the most important global oral health problems and *Streptococcus mutans* is the leading cause of dental caries worldwide and is considered to be the most cariogenic among the oral streptococci (Okada *et al*., 2005; Petersen *et al*., 2005). The oral streptococci acts on the available carbohydrate in the mouth, breaking it down with the subsequent production of lactic acid which eventually eats up the teeth enamel (Ophori *et al*., 2010).

The aims of this study are to determine the preliminary phyto-chemical constituents and antibacterial activities of ethanolic stem crude extracts of *Anacardium occidentale* on *Streptococcus mutans* associated with dental caries.

2. Materials and methods

Forty (40) patients diagnosed with dental caries attending Dental Clinics of the University of Uyo Teaching Hospital (UUTH), Uyo, and Winners Dental Clinic (WDC), Uyo, Akwa Ibom State, Nigeria, between June and September 2011 were studied. The infected area of the tooth was swabbed with sterile swabbed stick and transported using peptone water to Microbiology Laboratory within an hour for bacteriological analysis. The samples were streak inoculated onto Columbia agar base (Oxoid, UK) supplemented with 5% sheep blood and incubated at 37°C for 24-48 hrs. After incubation, the colonies were sub-cultured onto Petri dishes containing

nutrient agar and incubated as described above. Pure colonies were streaked on nutrient agar slopes in McCartney bottles as stock cultures and incubated at 37°C for 24hrs. The bacterial isolates were characterized using routine conventional laboratory techniques including Gram staining, motility, coagulase, catalase, oxidase , indole, urease production, citrate utilization, methyl red, Vogues Proskauer, bile solubility and carbohydrate fermentation tests such as mannitol, sucrose, glucose and lactose as described by Fawole and Oso (1988); Holt *et al.* (1994); Cheesbrough (2006). The sensitivity of the isolates to Optochin (5µg /Disk) was also determined as described by Cheesbrough (2006).

2.1. Sources of medicinal plant

The stems of *Anacardium occidentale* used in this research were obtained in Uyo, Akwa Ibom State. The plant was identified at Department of Botany and Ecological Studies and later transferred to Pharmacognosy and Natural Medicine Laboratory, Faculty of Pharmacy, University of Uyo for processing. The stems of *Anacardium occidentale* were washed under running tap water and with distilled water in order to remove extraneous matters (sand e.t.c), air-dried at room temperature for one month, pulverized in a mill and stored in polythene bag until required.

2.2. Preparation and concentration of plant extracts

The powdered *Anacardium occidentale* (2kg) were exhaustively extracted by Soxhlet Apparatus using 95% ethanol. The filtrate was evaporated using a rotary evaporator attached to a vacuum pump (Model type 3492, Corning Ltd). After complete evaporation, the extract was weighed and preserved aseptically at 4ºC. The graded concentrations (12.5 $mgml^{-1}$, 25.0 $mgml^{-1}$ and 50.0 $mgml^{-1}$) of the extracts were prepared using Dimethyl sulphoxide (DMSO) and shaken vigorously to obtain a homogenous mixture before subjecting them to antibacterial activity assays

2.3. Susceptibility of *streptococcus mutans* to the plant extracts

The ethanolic stem crude extracts of *A. occidentale* were tested for antibacterial activity on *Streptococcus mutans* obtained from dental caries by disc diffusion method (NCCLS, 2004; Junaid *et al*., 2006). Mueller – Hinton Agar was sterilized, cooled to 45 – 50^{o}C and then poured into sterilized Petri dishes. The *Streptococcus mutans* were first cultured in a nutrient broth for 18hrs before use and standardized to 0.5 McFarland Standards (10^{6} cfu / ml). The 0.5 McFarland Standard was prepared by adding 0.5 ml of barium chloride to 99.5 ml of sulphuric acid solution. Sterile filter paper discs of 6 mm diameter were impregnated with ethanolic stem crude extracts of *A. occidentale* of graded concentrations (12.5 $mgml^{-1}$, 25.0 $mgml^{-1}$ and 50.0 $mgml^{-1}$) and carefully placed onto Mueller Hinton agar plates which had previously been inoculated with *S. mutans* using sterilized forcep and incubated at 37^{o}C for 24hrs. Each ethanolic stem crude extract concentration was replicated thrice and the mean inhibition zone diameter (in millimeters) was determined in each case. The ethanolic stem crude extracts of *A. occidentale* were tested *in vitro* for purity by plating out on Petri dishes containing nutrient agar and incubated at 37^{o}C for 24 hrs. Control experiments comprising dimethyl sulphoxide, streptomycin and amoxycillin were also set up.

2.4. Phytochemical screening

The phytochemical constituents of the ethanolic stem crude extracts of *A. occidentale* were analyzed according to the methods described by Sofowora (1984); Trease and Evans (1989).

2.5. Test for saponins

(a) Half a gram (0.5g) of the filtered plant extract was put in a test tube and 2ml of distilled water was added and shaken vigorously. Formation of frothing or foam which persisted on warming was taken as preliminary evidence for the presence of saponins

(b) Half a gram (0.5g) of the filtered plant extract was mixed with few drops of 5% Na_2CO_3 (Sodium carbonate) and few drops of Fehling's solution added and boiled. The presence of a brown precipitate indicated a positive test.

2.6. Test for tannins

(a) Half a gram (0.5g) of the filtered plant extract was stirred with 5ml of distilled water and 5% Ferric chloride reagent added. A blue-black colouration indicated a positive test.

(b) Half a gram (0.5g) of the filtered plant extract was put in a test tube and 9ml of distilled water added. Decolouration observed upon addition of bromine water indicated positive test for tannin

2.7. Test for cardiac glycoside

Half a gram (0.5g) of the plant extract was dissolved in 2ml of chloroform, concentrated Sulphuric acid (H_2SO_4) was carefully added to form a lower layer. A reddish-brown colour at the interface indicated a positive test.

2.8. Test for anthraquinones

Half a gram (5g) of the plant extract was boiled with 5mls of 10% sulphuric acid (H_2SO_4) and filtered. The filtrate was cooled in ice and shaken with 2.5ml benzene, the benzene layer separated and half its own volume of 10% ammonium hydroxide (NH_4OH) was added. A pink, red or violet coloration in ammonia (lower) phase indicated a positive test.

2.9. Test for flavonoids

Few pieces of magnesium metal strip were added to 5ml of the filtrate plant extract with concentrated hydrochloric acid (5ml). The formation of orange, red, crimson or magenta was taken as a positive test.

2.10. Test for terpene

Half a gram (0.5g) of the plant extract was dissolved in 3mls of chloroform and filtered. 10 drops of acetic anhydride were added to the filtrate with 2 drops of concentrated sulphuric acid (H_2SO_4), pink colour at the interphase was taken as the positive test.

2.11. Test for deoxy-sugar

Half a gram (0.5g) of the filtered plant extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride. It was then underplayed with 1ml of concentrated sulphuric acid (H_2SO_4). Violet ring observed which settled after few minutes was an indication of a positive test.

2.12. Test for phenolics

To 2ml of the ethanolic extract, 1ml of ferric chloride solution was added. Blue or green colour indicated a positive test.

2.13. Test for resin

To 5 ml of each extract in separate test tubes, 5 ml of copper acetate solution was added and the resulting solution shaken vigorously and allowed to separate. The separation of a green coloured solution was considered positive for resins.

2.14. Test for phlobatanins

This was carried out by boiling 0.5 ml of the extract mixture with 5 ml of water and 1% HCl in a test tube for 2 min. The colour change was regarded as positive for phlobatanins.

2.15. Test for alkaloids

Half a gram (0.5g) of the plant extract was added with a few drops of picric acid reagent. A white or yellow precipitate indicated a positive test.

2.16. Determination of activity index (A.I)

Activity Index (A.I) was calculated as the mean inhibition zone of sample divided by the mean inhibition zone of the standard drug used (streptomycin / amoxycillin).

$$\text{Activity Index} = \frac{\text{Mean inhibition zone of sample}}{\text{Mean inhibition zone of the standard drug}}$$

Fig. 1: Photograph of *Anacardium occidentale*.

3. Results

The morphological and biochemical characteristics such as Gram's staining reaction, motility, coagulase, catalase, oxidase , indole, urease production, citrate utilization, methyl red, Vogues Proskauer, bile solubility, carbohydrate fermentation tests and sensitivity to Optochin of *Streptococcus mutans* isolated from patients with dental caries in Uyo are shown in Table 1. There was no bacterial growth when ethanolic stem crude extracts of *Anacardium occidentale* were plated out on Petri dishes containing nutrient agar and incubated at 37^{o}C for 24hrs. Thus, indicated and confirmed the purity of the ethanolic stem crude extracts of *Anacardium occidentale*. The results showed that ethanolic stem crude extracts of *A. occidentale* exhibited varying degrees of inhibitory effects against *Streptococcus mutans* associated with dental caries and the zones of inhibition increased with the increase in concentrations of the ethanolic stem crude extracts of *A. occidentale*, thus, exhibiting concentration dependent activity. Also, there were greater degrees of antibacterial activities of *A. occidentale* on *S. mutans* isolated from University of Uyo Teaching Hospital than *S. mutans* isolated from Winners Dental Clinic (Tables 2 and 3). The discs containing 50.0 mg/ml of ethanolic stem extracts showed the highest mean zones of Inhibition against *S. mutans*, while the discs containing 12.5mg/ml showed the lowest inhibitory zones. The results showed that di-methyl sulphoxide (DMSO) exhibited no inhibition on all the *Streptococcus mutans* isolated from both UUTH and WDC (Tables 2 and 3). Ethanolic stem crude extracts of *A. occidentale* showed the highest mean zones of Inhibition (MM) ±SD of 17.9 ± 1.3mm at 50.0 mgml^{-1} on *Streptococcus mutans* with code UU10 having activity index of 0.71 and 0.85 using streptomycin and amoxycillin, respectively (Table 2; Figs 2 and 3). The ethanolic stem crude extracts of *A. occidentale* showed the narrowest inhibition diameter (6.5±1.5mm) at 12.5 mgml^{-1} on *Streptococcus mutans* with code WD08 having the activity index of 0.45 and 0.46 using streptomycin and amoxycillin, respectively (Table 3; Figs 4 and 5). The results showed that 15/23 (65.2%), 20/23 (86.9%) and 22/23 (95.6%) of the *Streptococcus mutans* isolated from both UUTH and WDC were sensitive to 12.5 mgml^{-1}, 25.0 mgml^{-1} and 50.0 mgml^{-1} of *A. occidentale* ethanolic stem crude extracts respectively. The results showed that *Streptococcus mutans* with code WD09 was resistant to streptomycin, amoxycillin, di-methyl sulphoxide (DMSO) and different concentrations (12.5 mgml^{-1}, 25.0 mgml^{-1} and 50.0 mgml^{-1}) of *A. occidentale* ethanolic stem crude extracts. Duncan's multiple range tests showed significant difference ($P < 0.05$) among the mean diameter inhibition zones for the ethanolic stem crude extracts *of A. occidentale*. The results of the preliminary phytochemical analysis of ethanolic stem extracts of *A. occidentale* showed that resins were not detected , alkaloids and phenolics were in present in very high concentrations (+++), tannins, flavonoids, terpene and deoxy-sugar were present in moderately high concentrations (++), while saponins , cardiac glycoside , anthraquinones and phlobatanins were present in small concentration (+) (Table 4).

Table 1
Morphological and biochemical characteristics of *Streptococcus mutans* associated with dental caries

Biochemical / Antibiotic Tests	Results
Gram staining	+ cocci
Catalase	-
Citrate	-
Oxidase	-
Coagulase	-
Urease	-
Glucose	+
Lactose	-
Sucrose	-
Mannitol	+
Motility	+
Methyl Red	+
Vogues Proskauer	-
Hydrogen sulphide	+
Bile Solubility	-
Optochin	Resistant

+: Positive; - : Negative

Table 2
Antibacterial activity of *Anacardium occidentale* on *Streptococcus mutans* isolated from patients with dental caries at the University of Uyo.

Bacterial Codes	Mean zones of Inhibition (MM) ±SD					
	12.5 mgml^{-1}	25.0 mgml^{-1}	50.0 mgml^{-1}	DMSO	STR	AMY
UU01	NZ	8.9 ± 1.5^{a}	13.3 ± 2.0^{b}	NZ	17.0 ± 2.5^{b}	13.0 ± 1.5^{a}
UU03	7.8±1.0^{a}	10.6 ± 1.0^{a}	14.1 ± 0.4^{b}	NZ	17.8 ± 1.2^{b}	11.6 ± 1.0^{a}
UU04	9.0±0.2^{a}	13.2 ± 1.2^{b}	16.6 ± 0.5^{b}	NZ	23.0 ± 0.5^{c}	16.2 ± 2.5^{b}
UU05	9.5±1.5^{a}	12.7 ± 1.0^{b}	16.0 ± 2.0^{b}	NZ	18.2 ± 2.5^{b}	21.0 ± 0.5^{c}
UU07	7.5±1.0^{a}	9.3 ± 2.5^{a}	12.1 ± 1.0^{a}	NZ	15.9 ± 1.0^{a}	15.0 ± 0.5^{b}
UU08	NZ	8.8 ± 2.0^{a}	10.3 ± 1.5^{a}	NZ	NZ	NZ
UU09	8.7±1.0^{a}	13.5 ± 1.0^{b}	15.4 ± 2.0^{b}	NZ	19.0 ± 1.0^{b}	17.6 ± 1.0^{b}
UU10	9.3±1.5^{a}	14.0 ± 0.5^{b}	17.9 ± 1.3^{c}	NZ	25.0 ± 0.7^{c}	21.0 ± 1.3^{c}
UU15	7.6±0.5^{a}	10.0 ± 0.5^{a}	15.0 ± 2.3^{b}	NZ	16.3 ± 0.5^{b}	13.8 ± 2.0^{a}
UU18	10.1±1.5^{b}	10.9 ± 2.5^{a}	14.1 ± 2.0^{b}	NZ	18.5 ± 0.5^{b}	18.0 ± 1.5^{b}
UU19	NZ	NZ	9.5 ± 1.2^{a}	NZ	12.0 ± 2.0^{a}	NZ
UU22	7.0±2.0^{a}	9.4 ± 1.0^{a}	10.8 ± 1.8^{a}	NZ	16.0 ± 1.0^{b}	13.7 ± 0.5^{a}
UU23	NZ	8.0 ± 1.0^{a}	12.0 ± 1.0^{a}	NZ	18.9 ± 0.5^{b}	15.0 ± 2.2^{b}

DMSO: Dimethyl sulphoxide; STR: Streptomycin; AMY: Amoxycillin;
NZ: Absence of zone of inhibition; SD: Standard Deviation
Each inhibitory zone includes 6 mm diameter of the disc.
Each value represents the mean of three experiments and standard deviation. Means within the column followed by the same letter do not differ significantly as determined by Duncan's multiple range test ($P<0.05$) among the treatment).

4. Discussion

Streptococcus mutans is one of the most important organisms in the formation of plaque on the surfaces of teeth and has been shown to be highly cariogenic (Kornman, 1986). Increase in the resistance of microorganisms to antibiotics and the cost of production of synthetic compounds has necessitated various pharmaceutical companies to search for alternatives and medicinal plants are important for pharmacological research and drug development because they are safe, not expensive and also contain undiscovered biodynamic compounds with

unrealized potential for the use in modern drugs (Akinjogunla *et al*., 2011). Information on medicinal plants for the treatment of dental caries and reports on anti-*Streptococcus mutans* activity of *A. occidentale* is very scarce. The results on the antibacterial activities of ethanolic stem crude extracts of *A. occidentale* on *S. mutans* in this study are in agreement with Arekemase (2011). The findings in this study pointed out that the higher the concentrations of the extracts, the higher the sensitivities of *S. mutans* to the ethanolic stem crude extracts of *A. occidentale* as evidenced by the increased size of the bacterial growth inhibition zones, thus, exhibiting concentration dependent activity and these results are in conformity with Okigbo *et al*. (2009) and Jagtap *et al*. (2009). Akinjogunla *et al* (2011) also showed that the higher the concentrations of the *Vernonia amygdalina*, the larger the diameter of the bacterial growth inhibition zones. The susceptibility of *S. mutans* to ethanolic stem crude extracts of *A. occidentale* may be due to its Gram positive nature. Consequently, extracts of *A. occidentale* may interfere with the synthesis of glucan by glucosyl transferase produced by *S. mutans,* and thus, interfering in the mechanism of adherence of the organisms on the surfaces of the teeth (Marsh, 1992). The relative amount of phytochemical substances from plant extraction depends on the solubility of the phytochemical in the solvent used for extraction (Olowosulu and Ibrahim, 2006). Ethanol is generally able to dissolve multivariable types of compounds; polar and non-polar, simple and complex chemical structures (Cowan, 1999). The sensitivity of the *S. mutans* to *A. occidentale* implies that the intrinsic biosubstances in the ethanolic stem crude extracts of *A. occidentale* are naive to the drug resistance factors such as beta-lactamase expression, amino-glycoside modifying enzymes and altered ribosomal binding. Cushnie and Lamb (2005) also attributed the antibacterial efficacies of medicinal plants to the presence of some bioactive compounds. The presence of alkaloids, saponins and tannin in the ethanolic stem crude extracts of *A. occidentale* in this study agrees with the reports of Abulude *et al*. (2009). Ethanolic stem crude extracts of *A. occidentale* in this research contained phlobatanins and this is contrary to the reports of Abulude *et al*. (2009). These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins form irreversible complexes with proline rich protein, resulting in the inhibition of cell protein synthesis, flavonoids complex with extracellular-soluble proteins and bacterial cell wall proteins, while lipophilic flavonoids disrupt microbial cells membranes (Olowusulu and Ibrahim, 2006). There is a need to consider the use of this potent ethanolic stem crude extracts of *A. occidentale* that have shown some measures of antimicrobial potency, judging by the antibacterial activity and activity index for developing synthetic drugs against dental caries caused by *S. mutans*.

Table 3

Antibacterial activity of *Anacardium occidentale* on *Streptococcus mutans* isolated from patients with dental caries at the winners dental clinic.

Bacterial Codes	Mean zones of Inhibition (MM) ±SD					
	12.5 mgml^{-1}	25.0 mgml^{-1}	50.0 mgml^{-1}	DMSO	STR	AMY
WD01	NZ	NZ	8.4 ± 2.1^{a}	NZ	13.3 ± 2.0^{a}	NZ
WD02	7.0±2.3^{a}	9.8 ± 2.5^{a}	14.0 ± 1.0^{b}	NZ	17.4 ± 0.5^{b}	12.4 ± 1.5^{a}
WD03	8.8±1.0^{a}	11.0 ± 1.6^{a}	14.8 ± 0.5^{b}	NZ	17.0 ± 1.5^{b}	14.9 ± 2.0^{a}
WD04	9.0±0.5^{a}	13.6 ± 0.5^{b}	17.1 ± 2.2^{b}	NZ	22.0 ± 1.2^{c}	20.0 ± 2.0^{b}
WD06	8.4±1.2^{a}	13.0 ± 1.0^{b}	17.2 ± 1.5^{b}	NZ	19.4 ± 0.8^{b}	16.0 ± 0.5^{a}
WD08	6.5±1.5^{a}	10.9 ± 2.0^{a}	12.7 ± 0.8^{a}	NZ	14.6 ± 0.5^{a}	14.0 ± 1.5^{a}
WD09	NZ	NZ	NZ	NZ	NZ	NZ
WD12	8.2±1.1^{a}	13.4 ± 1.2^{b}	16.2 ± 1.0^{b}	NZ	24.6 ± 1.5^{c}	18.1 ± 1.2^{b}
WD13	NZ	6.8 ± 2.5^{a}	9.3 ± 2.5^{a}	NZ	13.3 ± 0.8^{a}	15.5 ± 1.0^{a}
WD15	NZ	8.0 ± 1.0^{a}	11.9 ± 0.5^{a}	NZ	15.7 ± 1.0^{a}	15.1 ± 1.2^{a}

DMSO: Dimethyl sulphoxide; STR: Streptomycin; AMY: Amoxycillin;

NZ: Absence of zone of inhibition; SD: Standard Deviation

Each inhibitory zone includes 6 mm diameter of the disc.

Each value represents the mean of three experiments and standard deviation. Means within the column followed by the same letter do not differ significantly as determined by Duncan's multiple range test ($P<0.05$) among the treatment).

Table 4
Phytochemical constituents of the ethanolic extracts of *Anacardium occidentale* Stem bark.

Plant Constituents	Occurrences
Saponins	+
Tannins	+ +
Cardiac Glycoside	+
Anthraquinones	+
Flavonoids	+ +
Terpene	+ +
Deoxy-Sugar	+ +
Alkaloids	+ + +
Phenolics	+ + +
Resin	-
Phlobatanins	+

- = Not detected
+ = Present in small concentrations
++ = Present in moderately high concentrations
+ + + = Present in very high concentrations

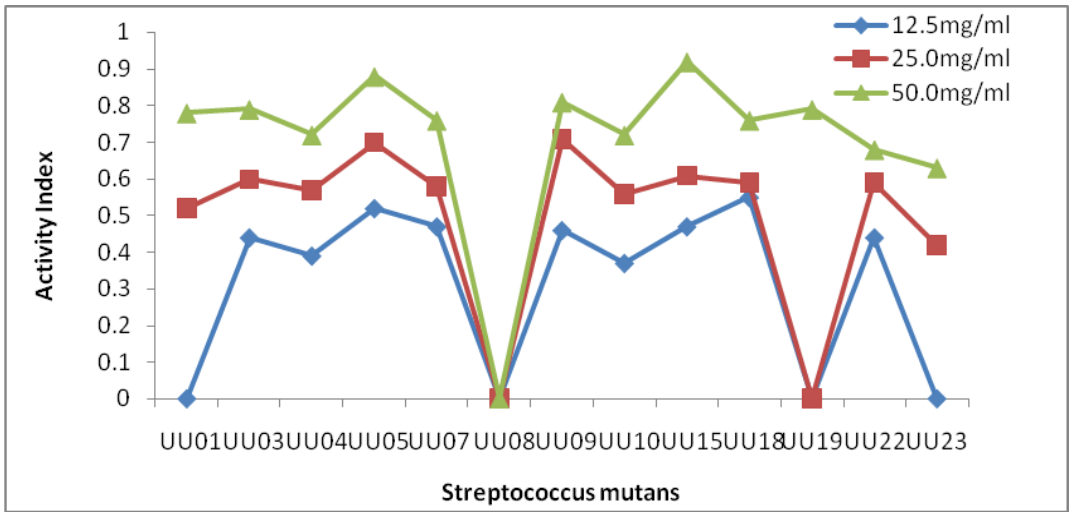

Fig. 2: Activity Index (A.I) from *Anacardium occidentale* and Streptomycin.

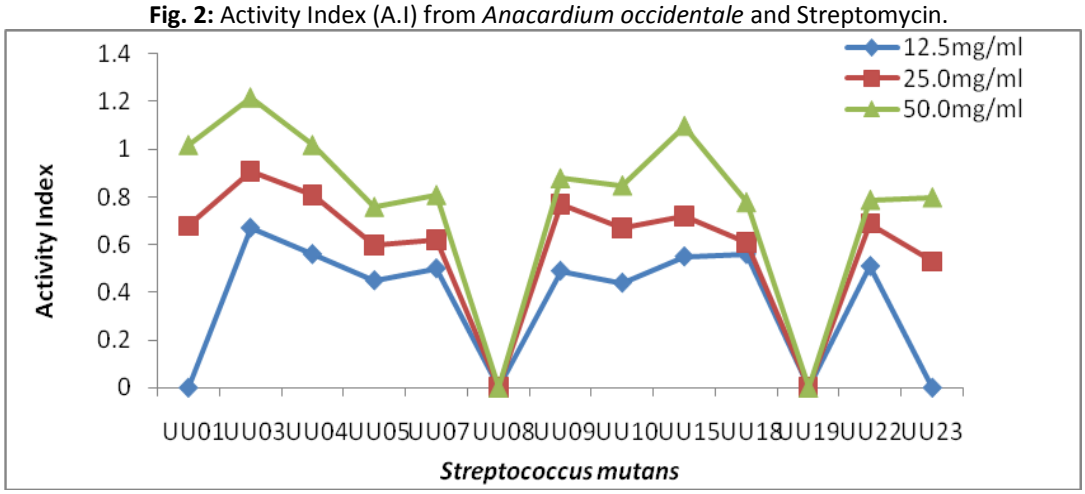

Fig. 3: Activity Index (A.I) from *Anacardium occidentale* and Amoxycillin.

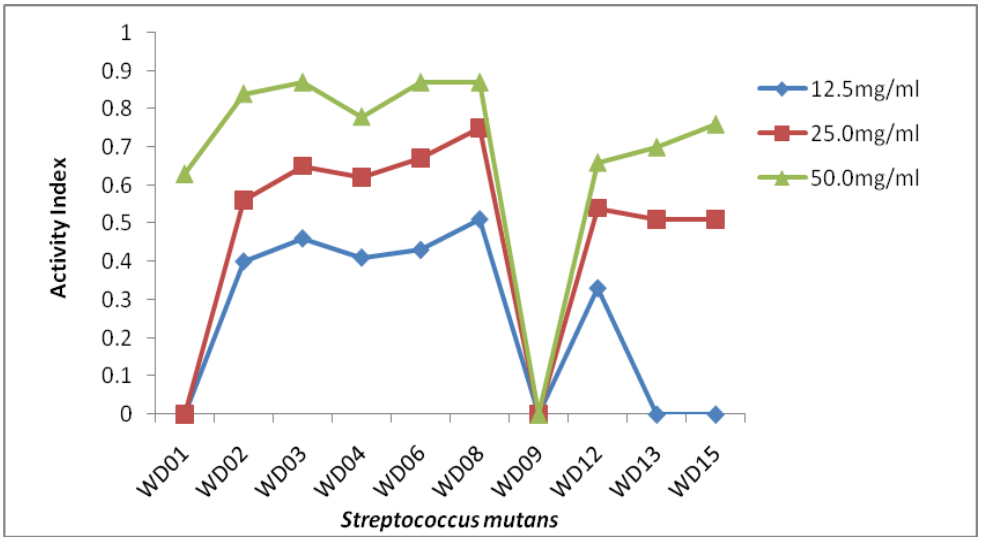

Fig. 4. Activity Index (A.I) from *Anacardium occidentale* and Streptomycin.

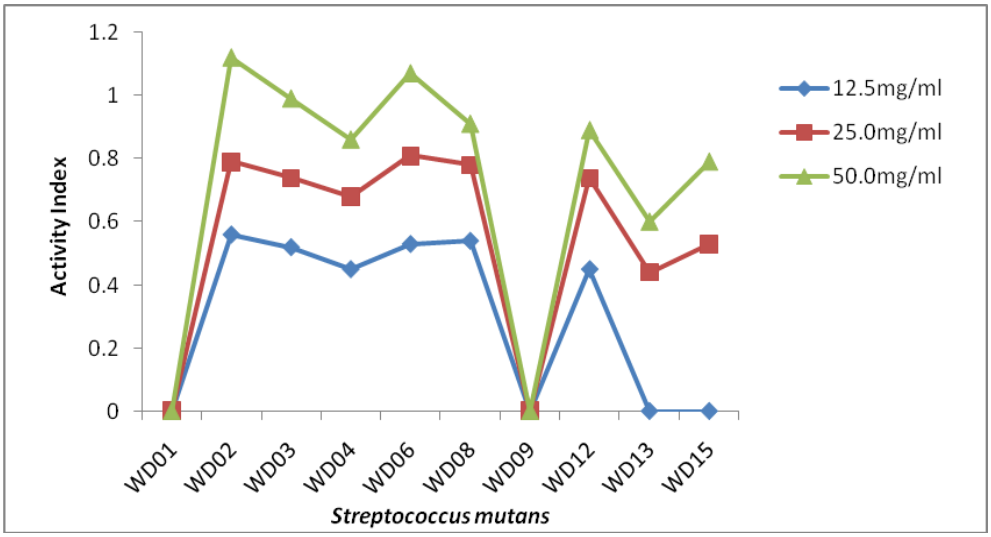

Fig. 5. Activity Index (A.I) from *Anacardium occidentale* and Amoxycillin.

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