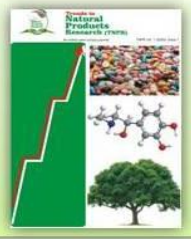
Trends in
Natural Products Research



Antibacterial activity of *Anacardium occidentale* Linn (Cashew) nut shell extract against some clinical bacterial isolates

Simeon Chukwuemeka Enemuor*, Paul Osinachi Amaje and Christian Kelechi Ezeh

Biocatalysis and Environmental Health Research Group
Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

Keywords *Anacardium occidentale*, Antibacterial activity, Cashew, extract.

Abstract: Cashew nut shell extracts were prepared using methanol and ethanol as the extracting solvents. The extracts were subject to phytochemical analysis. The antibacterial activity of the extracts on four human pathogens of clinical importance, *Escherichia coli, Salmonella typhi, Staphylococcus aureus and Bacillus subtitlis* were investigated using the agar well diffusion and tube dilution methods. The phytochemical analysis revealed the presence of terpenoids, tannins, flavonoids and phenolics. All test organisms were sensitive to the extracts, although there were differences in their zones of inhibition. In terms of sensitivity, *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* were sensitive to methanol extract at 5 mg/ml but *Bacillus substilis* was sensitive at 10 mg/ml. All test organisms were sensitive to ethanol extract at 5 mg/ml. Ethanolic extract was more potent compared to methanol extracts. Ethanol extract had the Minimum Inhibitory Concentration (MIC) of 25 mg/ml on *Escherichia coli* and *Salmonella typhi.* The results showed that ethanol extracted more antibacterial compounds than methanol from cashew nut shell. From the finding, extracts from cashew nut shell.

*Corresponding author:
simeon.enemuor@unn.edu.ng,
+234 8037789 633
DOI: 10.48245/tnpr-2734391.2023.4.104
Page No.: 38-44
Volume: 4, Issue 1, 2023
Trends in Natural Products Research

INTRODUCTION

In order to fight bacterial infections, medicine has principally relied on antibiotics which are naturally occurring or chemically synthesized. However, bacteria have developed resistance to many of the antibiotics commonly used to treat infections (Puja *et al*., 2013). Though, the resistance development by microbes is on the increase, using more effective therapies will reduce the mortality rate and health care cost. Traditional remedies utilizing plants are still common among rural communities of developing countries for managing various diseases. Medicinal plants represent a rich source of antimicrobial agents. Medicinal plants contain physiologically active components which over the years have been exploited in the traditional medical practices for the treatment of various ailments (Izah *et al*., 2018). *Anacardium occidentale* (Family *Anacardiaceae*), is a multipurpose tree of the tropics which attains a height of about 15m (Orwa *et al*., 2009). They grow on relatively dry soil in nature but in cultivation grow well in the tropical rainforest. The cashew tree produces many products and resources. According to Agedah, *et al*. (2010), the bark and leaves of *A. occidentale* are used medicinally.

India has the largest area under cashew cultivation and stands as the second largest producer of cashew in the world (Chaudhari *et al*., 2012). Today, India is the largest processor and exporter of cashew in the world (Idah *et al*., 2014; Akinhanmi *et al*., 2008). The cashew nut consists of kernel, shell and testa. It contains on average 20 to 25 % kernel (edible portion) and 5 % testa and about 65-70 % shell (Khuzkovski and Matins, 2016). The cashew nut shell contains 25-30 % dark reddish brown viscous phenolic liquid known as Cashew Nut Shell Liquid and abbreviated as CNSL (Lubi and Touchil, 2012). CNSL is amber-colored, poisonous, viscous oil obtained from the by-product shells of the cashew nut. It is obtained either by extraction in solvents or extraction by roasting (Edoga *et al.,* 2006). The in vitro activity of aqueous extracts of cashew apple peels was reported against two clinically important pathogens, *Escherichia coli* 0157:H7 and methicillin-resistant *Staphylococcus aureus* (MRSA) (Aderiye and David, 2014). The cashew nut shell liquid (CNSL) has wide commercial application, biological and medicinal properties. The biological properties of CNSL such as larvicidal, molluscicidal; antifungal and antimicrobial have been reported (Kannan *et al*., 2009). Other medicinal properties reported for CNSL are cytotoxic activity against several tumor cell lines (Leite *et al.,* 2016; Ashraf and Rathinasamy, 2018), anti-diabetic (Sahin *et al.*, 2022), anti-inflammatory and analgesic effects (Junior *et al.,* 2020). *Staphylococcus aureus* has been reported as a major cause of community and hospital acquired infections. The organism has a differential ability to spread and cause outbreaks in hospitals. Infections caused by *S. aureus* used to respond to β-lactam and related group of antibiotics. However, due to development of methicillin resistance amongst *S. aureus (*MRSA) isolates, treatment of these infections has become problematic (Lakshmana *et al*., 2011), but much have not been done on the use of CNSL extracts on the above clinically important pathogens The present investigation was carried out to evaluate the antibacterial activities of methanol and ethanol extracts of cashew nut shell against some clinical bacterial isolates.

Materials and Methods

The Cashew nuts were purchased from Ogige Market in Nsukka, Enugu state, South-east Nigeria. The nuts were bisected using a knife along the axis of the junction of the two halves of the shell. The intact kernel in the testa lining in the shell was separated from the shell. The separated shell containing the liquid of interest was then washed using distilled water and dried. The dried shell was crushed into coarse form using a manual blender.

Extraction Procedures

The coarse form of the cashew shell was used for methanol and ethanol extraction in Soxhlet extractor as described by Pandey and Tripathi (2014). Five hundred milliliters (500 ml) of methanol and ethanol were poured into the round bottom flask of Soxhlet apparatus and 50 g of crushed cashew nut shell was introduced into the thimble and fitted into the Soxhlet extractor. The set-up was heated to its boiling point and the vapour produced was subsequently condensed by water flowing in and out of the extraction set-up. This process of heating and cooling continued until a sufficient quantity of CNSL was obtained. At the end of the extraction, the thimble was removed while the remaining solvent in the extractor was discharged into the round bottom flask for a repeat of the process. Finally, the set-up was then re-assembled and a simple distillation unit was used to recover the solvent from the oil and the extracts were dried to paste.

Preparation of Stock Solution of the Cashew Nut Shell Extracts

Stock solution was prepared by weighing 2000 mg of each condensed solvent extract and dissolved in

10 ml of dimethyl sulphoxide (DMSO) giving a final concentration of 200mg/ml. The stock solution was kept in screw capped bottles for subsequent use. From this stock solution, varying concentrations of 5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml, 25 mg/ml 50 mg/ml and 100 mg/ml of the two extracts were prepared and stored in the refrigerator at 4 ^{o}C.

Phytochemical Analysis of the Extracts

The phytochemical analyses of the Cashew Nut Shell Liquid extracts were done to determine the presence of **t**he secondary metabolites (Trease and Evans, 1988)

Source of Clinical Isolates

The test microorganisms used were obtained from the Medical Diagnostic Laboratory, Department of Microbiology, University of Nigeria, Enugu State. These organisms were *Escherichia coli, Salmonella typhi, Staphylococcus aureus and Bacillus substilis.*

Standardization of the Test Organisms

Overnight Nutrient broth cultures of *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Bacillus substilis* at 37 ^{o}C were prepared. The culture was adjusted to obtain turbidity comparable to that of the turbidity of 0.5 McFarland standards. This level of turbidity is equivalent to approximately 1.5 x 10^5. Subcultures were made on nutrient agar.

Determination of Antibacterial Activity of the Extracts (Agar Diffusion method)

The antibacterial activity of methanol and ethanol extracts of CSNL against the clinical isolates was evaluated using agar well diffusion method according to the Clinical and Laboratory Standard Institute (CLIS, 2012). The antibacterial activity of the extracts was tested by inoculating nutrient plates with the test organisms. Duplicate plates were made for each test organism by flooding each plate with 18 h old culture. Thereafter, a sterile cork borer (6mm diameter) was used to make ditches on each plate. 0.1 ml of the extracts was added into each ditch and was labelled appropriately. One of the ditches had distilled water to serve as a negative control. The inoculated plates were left on the lamina flow bench for 1 h to allow the extracts to diffuse into the agar. Thereafter, the plates were incubated aerobically at 37 ^{o}C. Zones of inhibition produced after incubation were measured in millimeters.

Determination of Minimum Inhibitory concentration MIC of the extracts

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. A broth dilution method as described by Clinical and Laboratory Standard Institute (CLIS, 2012) was adopted in the determination of MIC. A portion (0.1 ml) of each prepared concentration was added to each 9 ml of nutrient broth containing 0.1 ml of standardized test organisms of bacterial cells. The tubes 37 ^{o}C were incubated at 37 ^{o}C for 24 h. The tube with the least concentration of the extracts without growth (turbidity) after incubation was taken and recorded as the Minimum Inhibitory Concentration (MIC).

RESULTS

Phytochemical Analysis

The results of the phytochemical analysis of the two extracts are shown in Table1. Ethanol and methanol extracts had terpenoids, tannins, flavonoids, and phenols with terpenoids and flavonoids abundantly present.

Table 1: Phytochemical analysis of cashew nut shell extracts

Phytochemicals	Methanol	Ethanol
Terpenoids	+++	+++
Tannins	+	+
Flavonoids	+++	+++
Phenols	++	++

Key: - = absent; + = low in abundance; ++ = moderate in abundance; +++ = high in abundance

Antibacterial Activity of Methanol and Ethanol Extracts

Antibacterial activity of methanol and ethanol extracts on the clinical isolates is represented in Figure 1 and 2. Both ethanol and methanol extract inhibited *E. coli*, *S. typhi*, *S. aureus*, and *B. substilis* at 25 mg/ml. Highest zone of inhibition was observed at 100 mg/ml for both extracts against the test organisms. Antibacterial activities increased with increase in concentration of the extracts. The extracts were more effective against *S. typhi* (methanol extract) and *E. coli* (ethanol extract). Minimum inhibitory concentration of the ethanol extract against the test organisms was 25 mg/ml while that of methanol extract was 25 mg/ml against all isolates except *E. coli* which was 100 mg/ml (Table 2).

Table 2: Minimum Inhibitory Concentration (MIC) of the extracts

Bacterial isolates	Minimum inhibitory concentration (mg/ml)	
	Methanol extract	Ethanol extract
Escherichia coli	50	25
Salmonella typhi	25	25
Staphylococcus aureus	25	25
Bacillus substilis	25	25

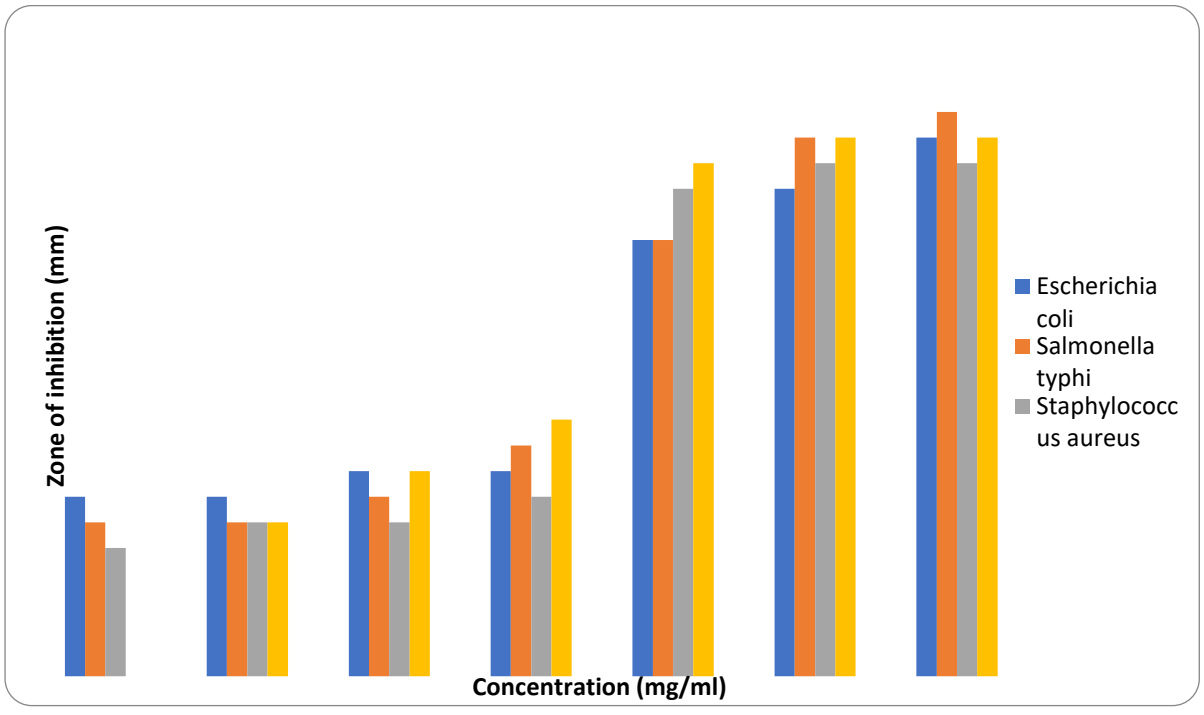


Figure 1: Antibacterial activity of methanol extract of the cashew nut shell on the clinical isolates

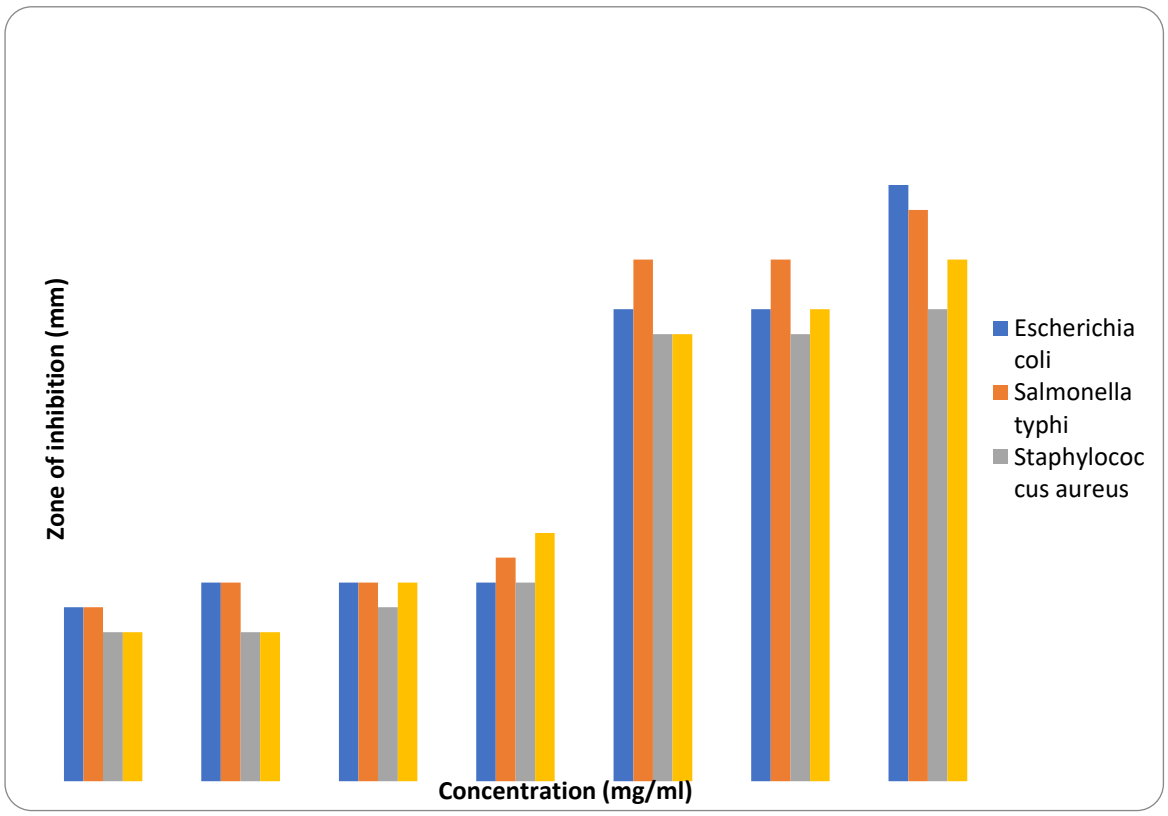


Figure 2: Antibacterial activity of ethanol extract of the cashew nut shell on the clinical isolates

DISCUSSION

Qualitative phytochemical analysis was done to determine the presence of bioactive compounds present in ethanol and methonal extract of *Aanacardium occidentale* nut shell oil. Ethanol and methanol extracts contained terpenoids, tannins, flavonoids, and phenols with terpenoids and flavonoids abundantly present. These secondary metabolites are the bioactive substances responsible for the antibacterial activities. This finding collaborates with the report of Sujatha *et al.* (2011), who carried out elementary phytochemical profiling of chloroform, ethanol and methanol extracts of *Aanacardium occidentale* nut shell oil. It also agrees with the report of Kannan *et al*. (2009) who reported the presence of triterpenoids, phenolics and volatile oils. The extracts exhibited antibacterial activity against the test organisms to various degrees. The variations in the activities could be due to the differences in the concentrations of the bioactive components in the extracts. Kannan *et al*. (2009) reported that the presence of certain compounds act synergistically/additively when mixed in several proportions. The differences in the susceptility of the test organisms could also be due to their cell composition; the highest zones of inhibition were found on the Gram negative (found to contain a thin layer of peptidoglycan and mostly lipopolysaccharides) isolates. The Gram-positive isolates with lesser zones of inhibition to the extracts suggest that thicker peptidoglycan and the teichoic acid are a bit stable to the extracts. The results show that ethanol is the best solvent for extracting cashew nut shell liquid for antibacterial purposes. Similar results were reported by Sujatha *et al*. (2011).

CONCLUSION

Preliminary phytochemical test indicated the presence of tannins, terpenoids, phenols and flavonoids in ethanol and methanol extracts of *Aanacardium occidentale* nut shell oil. Both extracts showed high antibacterial activity against *S. aureus*,

E. coli, S. typhi, and *E. coli* probably due to the phytochemicals present in the plant extracts. Further research is required for the identification and characterization of bioactive molecules present in the ethanol and methanol extracts of *Aanacardium occidentale* nut shell oil and their *in vivo* antibacterial activities against human pathogens.

CONFLICT OF INTEREST

There is no conflict of interest

References

Aderiye BI and David OM (2014). In vitro antibacterial activity of aqueous extracts of cashew (*Anacardium occidentale* L.) fruit peels using bioautography method. *European Journal of Pvledicinal Plants* 4(3): 1-8

Akinhanmi TF, Atasie VN and Akintokun PO (2008). Chemical composition and physicochemical properties of cashew nut (*Anacardium occidentale*) oil and cashew nut shell liquid. *Journal of Agricultural, Food and Environmental Sciences* 2(1): 1-10.

Ashraf SM and Rathinasamy K (2018). Antibacterial and anticancer activity of the purified cashew nut shell liquid: implications in cancer chemotherapy and wound healing. *Natural Product Research* 32(23): 2856-2860. doi: 10.1080/14786419.2017.1380022.

Chaudhari AP, Thakor NJ, Mahale DM, Haldarikar PM and Sonawane SP (2012). Physical properties of cashew nut shell liquid. International *Journal of Application or Innovation in Engineering and Management* (IJAIEM) 2(1): 1-4

Clinical and Laboratory Standards Institute (2012). Performance standards for antimicrobial susceptibility testing. Twenty Informational Supplement 29: 1–160.

Edoga MO, Fadipe L and Edoga RN (2006). Extraction of polyphenols from cashew nut shell. Leonardo Electronic. *Journal of Practices and Technologies* 9: 107-112

Idah PA, Simeon MI and Mohammed MA (2014). Extraction and characterization of cashew nut (*Anacardium occidentale*) oil and cashew shell liquid oil. *Academic Research International* 5(3)

Izah SC, Uhunmwangho EJ and Eledo BO (2018). Medicinal potentials of *Buchholzia coriacea* (wonderful kola). *Medicinal Plant Research* 8(5): 27-42.

Junior ALG, Islam MT, Nicolau LAD, de Souza LKM, Araujo TSL, de Oliveira GAL, Nogueira KM, Lopes LS, Medeiros J-VR, Mubarak MS and Melo-Cavlcante AAC (2020). Anti-inflammatory, antinociceptive, antioxidant properties of anacardic acid in experimental models. *ACS Omega* 5931): 19506-19515. doi: 10.1021/acsomega.0c01775.

Kannan VR, Sumathi CS, Balasubramanian V and Ramesh N (2009). Elementary chemical profiling and antifungal properties of cashew (*Anacardium occidentale* L.) nuts. *Botany Research International* 2(4): 253-257.

Kluczkovski AM and Martins M (2016). Cashew nuts. In: Encyclopaedia of Food and Health pp 683-686. doi: org/10-1016/B978-0-12-384947.00123-9.

Leite AS, Islam MT, Junior ALG, Sousa, JMC, de Alencar MVOB, Paz MFCJ, Rolim HML, de Medeiros MGF, Melo-Cavalcante AAC, and Lopes JAD (2016). Pharmacological properties of cashew (*Ancardium occidentale*). *African Journal of Biotechnology* 15(35): 1855-1863.

Lubi MC and Thachil ET (2012). Cashew nut shell liquid (CNSL) – a versatile monomer for polymer synthesis. Designed Monomers and Polymers 3(2): 123-153.
Doi: 10.1163/156855500300142834

Orwa C, Mutua A, Kindt R, Jamnadass R and Anthony S (2009). Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya

Pandey, A and Tripathi S (2014). Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry* 2: 115-9.

Parekh J and Chanda S (2007). *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkey Journal of Biology* 31: 53-58.

Parasa LS, Sunita T, Rao KB, Rao AH, Rao JS and Kumar LCA (2011). Acetone extract of cashew (*Anacardium occidentale,* L.) nuts shell liquid against methicillin resistant *Staphylococcus aureus* (MRSA) by minimum inhibitory concentration (MIC). *Journal of Chemical and Pharmaceutical Research* 3(5): 736-742.

Parasa LS, Tumati SR, Kumar LCA, Chigurupati SP, and Rao GS (2011). *In vitro* antimicrobial activity of cashew (*Anacardium occidentale,* L.) nuts shell liquid

against methicillin resistant *Staphylococcus aureus* (MRSA) clinical isolates. *International Journal of Pharmacy and Pharmaceutical Sciences* 3(4): 1-5

Puja S, James PM and Albert R (2013). Ten highly effective essential oils inhibit growth of methicillin resistant *Staphylococcus aureus* (mrsa) and methicillin sensitive *Staphylococcus aureus* (MSSA*). International Journal of Pharmacy and Pharmaceutical Sciences* 5(1): 1-3

Reddy SN, Rao SA, Chari, AM, Kumar, RV and Jyothy, V (2012). Synthesis and antibacterial activity of sulfonamide derivatives at C-8 alkyl chain of anacardic acid mixture isolated from a natural product cashew nut shell liquid (CNSL). *Journal of Chemical Sciences* 124(3): 1-8

Sahim C Magomedova L, Ferreira TAM, Liu J, Tiefenbach J, Alves PS, Queroz FJG, de Oliveira AS, Bhattacharyya M, Grouleff J, Nogueira PCN, Silveira ER, Moreira DC, Leite JRSA, Brand GD, Uehling D, Poda G, Krause H,Cummins CL and Romeiro LAS (2022). Phenolic lipids derived from cashew nut shell liquid to treat metabolic diseases. *Journal of Medicinal Chemistry* 65: 1961- 1978

Sujatha S, Joseph B and Antony ES (2011). Analysis of phytochemical composition and bio-activity against clinical pathogens of essential oil from *Anacardium occidentale* (L.). *International Journal of Food Safety* 13: 1-7.

Trease GE, Evans WC (1988). Pharmacognosy (13th Edition). Baillere Tindall, London, pp 386-388).

Vaou N, Stavropoulou E, Voidarou C, Tsigalou C and Bezirtzoglou E (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms* 9(10): 2041.

CITATION: Enemuor SC, Amaje PO, Ezeh CK. (2023) Antibacterial activity of *Anacardium occidentale* Linn (cashew) nut shell extracts against some clinical bacterial isolates
Trend Nat Prod Res 4(1). 38-44. DOI: 10.48245/tnpr-2734391.2023.4.104