http://dx.doi.org/10.4314/bajopas.v10i1.36

Bayero Journal of Pure and Applied Sciences, 10(1): 247 – 250
Received: November, 2016
Accepted: April, 2017
ISSN 2006 – 6996

BACTERICIDAL STUDIES OF SAPONINS FROM THE STEM-BARK OF *ADENIUM OBESUM* (FORSSK.) ROEM. AND SCHULT.

[1]Tijjani, A.*, [1]Haruna, A. and [1]Jacob, A.G.
[1]Department of Applied Chemistry, Federal University, Dutsin-Ma, Katsina, Nigeria
*Corresponding Author: tali@fudutsinma.edu.ng phone: +2348039682674

ABSTRACT
Phtyochemical screening of methanolic Stem-bark extracts of Adenium obesum indicated the presence of saponins. Consequently, the saponins were extracted using standard procedures. The saponin extract was examined for in vitro antibacterial activity using disc diffusion technique. Activity against five bacterial strains (Gram-positive and Gram-negative bacteria) was tested. The extract was active against all assayed bacteria (Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 6538, Klebsiella pneumonia ATCC 15380 and Pseudomonas aeruginosa ATCC 14028 but excepting Bacillus subtilis ATCC 6633) with minimal inhibitory concentration (MIC) values ranging from 0.5 to 5 mg/ml. This result shows that the stem-bark of Adenium obesum could produce saponins that might be utilized for the development of antibacterial drugs.
Key words: Bactericidal; Saponins; Stem-bark; Adenium obesum; Disc diffusion

INTRODUCTION

Original candidate chemical structures for many pharmaceutical compounds that promote human health originated from chemicals found in plant extracts (Patterson and Burkholder, 2003). Among these plant compounds are saponins (Avato *et al.*, 2006; Haralampidis *et al.*, 2002). Saponins are naturally occurring glycoside compounds whose chemical structures are composed of a fat-soluble nucleus (aglycone) that is commonly a triterpenoid, steroid or an alkaloid attached to one or more side chains of water-soluble sugars (glycone) through ester linkages to the aglycone nucleus at different carbon sites (Haralampidis *et al.,* 2002).

*Adenium obesum (*Apocynaceae), commonly known as 'karya' among hausa tribe of northern Nigeria, is a small deciduous succulent shrublet that can grow to shrub or small tree that belongs to plant family Apocynaceae (Codd, 1987). The plant, which belongs to the genus *Adenium,* occurs in savanna, dry bush land or woodland and wooded grassland up to 2100 m altitude, on rocky or sandy soil (Dimmitt and Hanson, 1991). It is used as poison on arrows (Watt and Breyer, 1962) and also extensively for the treatment of a variety of ailments including venereal diseases; the root or bark extract is used as a bath or lotion to treat skin diseases and to kill lice, while the latex is applied to decaying teeth and septic wounds to enhance its healing. In Somalia, the root decoction, as nasal, drops is prescribed for rhinitis while in northern Kenya, the latex is rubbed on the head to kill lice and the powdered stem is applied on camels and cattle to kill skin parasites. The bark is also chewed as an abortifacient (Neuwinger, 2000).

In recent years, multiple resistances in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. In this situation, the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections (Poole, 2001), has forced scientists into looking for new antimicrobial substances from various sources like medicinal plants (Benziane *et al.*, 2012). Saponins have been reported to possess antimicrobial activities (Crombie *et al.*, 1986; Turner, 1953; Edewor *et al.*, 2009). The phytochemical screening of the constituents of *Adenium obesum* stem-bark revealed the presence of saponin glycosides (Tijjani *et al.*, 2011). In the present study, for the first time, saponins were extracted from the stem-bark of *Adenium obesum* and then subsequently tested against some pathogenic bacterial isolates. This is important as the plant could serve as a source of promising antibacterial saponins lead compounds that can be utilized for the development of drugs that could be used to cure diseases associated with the selected microorganisms.

MATERIALS AND METHODS

Plant material Collection and Preparation

The stem-bark of *Adenium obesum* was collected from Samaru area of Zaria, Kaduna State, Nigeria. It was authenticated at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, where a specimen (voucher number 1836) was deposited. The bark was air-dried and ground into powder using a porcelain mortar and pestle. It was then sealed in a polyethene bag and stored in a dessicator prior to evaluation.

Extraction of saponins

The powdered stem-bark (1kg) of the plant was exhaustively extracted using petroleum spirit for 13 h in a Soxhlet extractor. This was to remove the lipids and other pigments available in the plant material (Tijjani *et al.,* 2011). Thereafter, methanol was used for the extraction to continue for the next 17 h.

This was to remove the saponins together with low molecular weight substances such as sugars, phenolic compounds, oligosaccharides and flavonoids that might be present (Fenwick *et al.*, 1992). The resulting solutions were concentrated *in vacuo* using rotatory evaporator at 40 ^{o}C to yield 28g methanolic extract. The presence of saponins in the methanolic extracts was detected by the characteristic frothing tests and thin layer chromatography (TLC) (spraying TLC plates with methanol : acetic acid : sulphuric acid : anisaldehyd, 85:10:5:0.1 and heating at 100° C for 5 min. On heating, saponin bands turned red and were readily visualized).

In order to obtain partially purified saponins, the methanolic extracts were loaded on to a column of RP-18 powder (Octadecyl slane bonded to silica gel particle size 15-25 YM (JT Baker, Germany). The column was washed with water to remove the sugars and oligosaccharides while further elution with 30% methanol (v/v) removed the flavonoid compounds and other phenolic compounds. Subsequent elution with 100% methanol removed the saponins (Fenwick *et al*., 1992). This yielded 18g of crude saponins.

Phytochemical Screening of Stem-bark Extracts of *Adenium Obesum*

The extracts (petroleum spirit and methanol) of Stem-bark of *Adenium Obesum* were subjected to preliminary phytochemical tests using standard techniques so as to detect the presence of saponins and other metabolites available (Trease and Evans,1984 and 1989; Sofowora,1993).

Test Bacteria

Five standard bacterial strains were selected: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumonia* ATCC 15380, *Bacillus subtilis* ATCC 6633 and *Pseudomonas aeruginosa* ATCC 14028. The bacterial strains were maintained on nutrient agar and sub-cultured every three days. An inoculum of each bacterial strain was suspended in 5 ml of Mueller Hinton broth (MHB) and incubated overnight at 37 ºC. The overnight cultures were diluted with Mueller Hinton broth and adjusted to give a concentration of bacterial cells equivalent to a McFarland 0.5 standard prior to the bacterial testing (Samie *et al.,* 2005).

Susceptibility Testing of Crude Saponins

To investigate the antibacterial activity of the crude saponins, disc diffusion method was used (Collins and Lyne, 1970; Nascimento *et al.,* 2000). The assays are based on the use of sterile discs filter paper (6 mm diameter) impregnated with 20µl of the extract solution (at a concentration of $4 \times 10^{4}\ \mu g / cm^{3}$) and allowed to dry at room temperature. A sterile disc impregnated with dimethyl sulphoxide (DMSO) was used as negative control. After incubation for 24 h at 37°C, all the bacterial plates were observed for zones of growth inhibition and the diameter of these zones was measured in millimeters using a ruler. The experiments were performed in triplicates.

Minimum Inhibitory Concentration (MIC) of Crude Saponins

A series of culture tubes (microdilution assays) were prepared all containing the same volume of medium (5 ml) inoculated with the test microorganisms. The lowest concentration of sample at which the subculture from test dilution yielded no viable organisms was recorded as minimum inhibitory concentration. Decreasing concentration of the crude saponins extract of *Adenium obesum* stem-bark (AO) was added to the tubes in a step wise dilution (two-fold serial dilutions). One tube was left without AO crude saponins extract to serve as positive control and the other without the extract and inocula to serve as negative control. The cultures were incubated at 37°C for growth of the test organism and a period of 24 hours for growth of at least 10-15 generations. The tubes were inspected visually to determine the growth of microorganisms by the presence of turbidity and the tubes in which the crude saponins extract was present in minimum concentration sufficient to inhibit the microbial growth, which remained clear was noted as MIC of the extract (Ferreira *et al.,* 2003; Nazaruk and Jakoniuk, 2005).

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the AO stem-bark revealed the presence of saponins as presented in Table 1. Saponins have been reported to possess antiviral (Apers *et al*., 2000), antifungal (Chong-Ren *et al*., 2006; Masoud, 2013), hypoglycaemic activity (Yoshikawa *et al*., 2001), anticancer (Cai *et al.,* 2002), anti-inflammatory (Camila *et al*., 2011) and antioxidant activities (Hu *et al*., 2002). The plant saponins have demonstrated significant antimicrobial activity against the microorganisms studied (Tables 2 and 3). The crude saponins were active against four out of the five organisms tested. It was only not active against *Bacillus subtilis*. Generally, the zones of inhibition ranged from 18 to 35 mm (Table 2). The highest (most active) inhibition zone was the one observed against *Staphylococcus aureus* (Gram-positive bacteria); and the least was against *Klebsiella pneumoniae* (Gram-negative bacteria). This is not surprising because on a general note, Gram-positive bacteria have less resistant cell walls than Gram-negative.

The MIC values ranged from 0.5 to 5mg/ml. The lowest (most active) was that of *Staphylococcus aureus* (0.5 mg/ml) and the highest was the one observed against *Klebsiella pneumoniae*. There was no growth seen for *Bacillus subtilis* as shown in Table 3.This result demonstrated the potentialities of AO to treat pneumonia and urinary tract infections caused by *Klebsiella pneumoniae* (Claudia *et al.,* 2014), *Escherichia coli* and *Pseudomonas aeruginosa* (Garry *et al.,* 2014; Teiji, 2014). In addition, *Pseudomonas aeruginosa* is implicated for ear infections, rashes (dermatitis) and skin lesion ecthyma gangrenosum (Fine *et al.,* 1996; Rahme *et al.,* 1995). Further, *Escherichia coli* is responsible for diarrheal diseases and meningitis (James *et al.,* 2004). *Staphylococcus aureus* is implicated with lower respiratory tract infections, cardiovascular infections, skin, wound and deep tissue infections, pneumonia, endocarditis and septicaemia diseases (Eili *et al.,* 2007; Lisa, 2013). These findings support the traditional uses of the plant stem-bark for the treatment of skin diseases and wound infections; and also suggested the ability of the saponins to cure diseases associated with the bacteria studied.

Table 1 Phytochemical Constituents of the Stem-Bark of *Adenium obesum*

Constituent	Extract	
	Petroleum Spirit	Methanol
Alkaloids	-	+
Flavonoids	-	+
Saponins	+	+
Glycosides	+	+
Anthraquinones	-	+
Tannins	+	+
Steroids	+	+
Coumarins	-	_

+/- = presence or absence of constituent tested

Table 2 Antibacterial sensitivity Profile of the crude saponins extracts of *Adenium obesum* (stem-bark*)* at Concentration of $4 \times 10^4 \ \mu g / cm^3$

Test organisms	Average Zones of Inhibition (mm)
*Escherichia coli (*ATCC 25922)	28
*Pseudomonas aeruginosa (*ATCC 14028)	-
*Bacillus subtilis (*ATCC 6633)	25
*Staphylococcus aureus (*ATCC 6538)	35
Klebsiella pneumonia (ATCC 15380)	18

- = No zone of inhibition

Table 3 Minimum inhibitory concentrations (MICs) of the crude saponins extracts of the stem-bark *Adenium obesum*

Microorganism	MIC (mg/ml)
Escherichia coli (ATCC 25922)	1
Pseudomonas aeruginosa (ATCC14028)	2.5
Bacillus subtilis (ATCC 6633)	NG
Staphylococcus aureus (ATCC 6538)	0.5
Klebsiella pneumonia (ATCC 15380)	5

NG = No Growth

CONCLUSION

Results obtained from this study demonstrated the potency of the saponins extracted from the plant to serve as a very good antibacterial agent and this could form a good basis for selection of crude saponins of AO for further phytochemical and pharmacological investigations. The antibacterial properties of the crude saponins could be exploited further for potential development of new drugs against diseases caused by the selected pathogens. The crude saponins could be fractionated and specific saponin(s) responsible for the antibacterial activity might be isolated and characterized to elucidate structure(s), which could possibly be modified for better biological activity.

REFERENCE

Apers, S., Baronikova, S., Sindambiwe, J.B., Witvrouw, M., De Clercq, E., Vanden Berghe, D., Van Marck, E., Vlietinck, A., and Pieters, L (2000): Antiviral, haemolytic and molluscicidal activities of triterpenoid saponins from Maesa lanceolata: establishment of structure-activity relationships. *Planta Medica*; 67:528–532.

Avato, P., Bucci, R., Tava, A., Vitali, C., Rosato, A., and Bialy, Z (2006): Antimicrobial activity of saponins from Medicago spp.: Structure–activity relationship. *Phytotherapy Research*; 20, 454–457.

Benziane, M., N. Kambuche Bouzidi, S. Bellahouel, B. Merah, Z. Fortas, R. Soulimani, S. Saidi and A. Derdour (2012): Antimicrobial activity of the alkaloids and saponin extracts of *Anabasis articulata. Journal of Biotechnology and Pharmaceutical Research*; 3(3) : 54-57.

Cai, J., Liu, M., Wang, Z., and Ju, Y (2002): Apoptosis induced by dioscin in Hela cells. *Biological and Pharmaceutical Bulletin*; 25: 193–196.

Camila, R. A., Bernadete, P., d., and José Paz., P (2011): A new steroidal saponin with antiinflammatory and antiulcerogenic properties from the bulbs of Allium ampeloprasum var. Porrum. *Fitoterapia* ; 82:1175–1180.

Chong-Ren Y., Ying Z., Melissa, R. J., Shabana, I., Khan, Y., and Xing-Cong, L (2006): Antifungal Activity of C-27 Steroidal Saponins. *Antimicrobial Agents And Chemotherapy*; 50(5):1710–1714

Claudia, V., Francesta, L., Maria, P.B., Gianfranco, D., and Pietro, E.V (2014): Antibiotic Resistance Related to Biofilm Formation in *Klebsiella pneumonia* ; *Pathogens*; 3:744

Codd, L.E (1987): *Adenium obesum.* The Flowering Plants of Africa*.* South African National Biodiversity Institute (SANBI), Pretoria, South Africa; 49: p1953.

Collins, C.H., and Lyne, P. M (1970): *Microbiological methods*; University Park Press, 3rd ed. Baltimore.

Dimmitt, M.A., and Hanson, C (1991): The genus Adenium in cultivation. *Diplorhynchus Welw. ex Fic. & Hiern (Apocynaceae).*Mededelingen, *Cactus and Succulent. J.*; 63: 223-225.

Edewor, T., Ibikunle, G., and Usman, L (2009): A. Phytotoxic and antimicrobial screening of Saponin isolated from ethanolic leaf extract of *Xylopia aethipioca. Science Focus*; 14(4):507 – 512.

Eili, K., David, L.S., and Ramanan, L (2007): Hospitilization and deaths caused by Methicillin-Resistant *Staphylococcus aureus*, United States, 1999-2005.*Emerging Infectious Diseases*; 13:12

Fenwick, G.R., Price, K.R., TsuKamoto, C., Okubo, K (1992): *Institute of Food Research, Norwich Laboratory*. Publication N0 03249N; pp 285-326.

Ferreira, M.J.U., Ascenso, J.R., Valdeira, L., Duarte, A., Frade, J.P., and Freitas, G (2003): Evaluation of antiviral and antimicrobial activities of triterpenes isolated from *Euphorbia segetalis*. *Nat. Prod .R e s e a r c h*; 1 7: 3 7 5 - 8 0.

Fine, M.J., Smith M.A., Carson, C.A (1996): Prognosis and outcomes of patients with community acquired pneumonia.A meta-analysis. *JAMA*; 275(2):134-141.

Garry, L. Sean, P.G., and Brendan, F.G (2014): Biomolecular mechanisms of *Pseudomonas aeruginosa* and *Escherichia coli* biofilm formation. *Pathogens*; 3:597

Haralampidis, K., Trojanowska, M., and Osbourn, A. E (2002): Biosynthesis of triterpenoid saponins in plants. *Advances in Biochemical Engineering/Biotechnology*; 75: 31–49.

Hu, J., Lee, S., O., Hendrich, S., and Murphy, P., A (2002): Quantification of the group B soya saponins by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*; 50:2587–2594.

James, B.K., James, P.N., and Harry, L.T.M (2004): Pathogenic *Escherichia coli*. *Nature Review/Microbiology*; 2: 123.

Lisa, S (2013): *Staphylococcus aureus*-Aspect of Pathogenesis and Molecular Epidemiology. *LiU-Tryck, Lin-koping*, Sweden; pp1-2

Masoud, S., Behzad, Z., Mauro, S., and Virginia, L (2013): Spirostane, furostane and cholestane saponins from Persian leek with antifungal activity. *Food Chemistry*; 14: 1512–1521

Nazaruk, J. and P. Jakoniuk (2005): Flavonoid composition and antimicrobial activity of *Crisium rivulare* (jacq) flowers*, Journal of Ethnopharmacoly*; 102: 208-12.

Nascimento, G.G. F., Locatelli., J, Freitas, P. C., and Silva, G.L (2000): Antibacterial activity of plant extracts and phytochemical on antibiotic resistant bacteria. *Brazilian. Journal of Microbiology*; 31**:** 247-256.

Neuwinger, H.D (2000): African traditional medicine: *A dictionary of plant use and applications*. *Medpharm Scientific, Stuttgart, Germany*; p 589.

Patterson, J., and Burkholder, K (2003): Application of prebiotics and probiotics in poultry production. *Poultry Science*; 82: 627–631

Poole, K (2001): Overcoming antimicrobial resistance by targeting resistance mechanisms. *Journal of. Pharmacy and Pharmacology*; 53:283–284.

Rahme, L.G., Wolfort, S.F., Shao, J., Tompkins, R.G., and Ausel, F.M (1995): Common Virulence Factor for Bacterial Pathogenicity in Plants and Animals.Science; 268 (5219): 1899-902.

Samie, A., O.b.I., C.L., Bessong, P.O. and Namrita., L (2005): Activity profiles of fourteen selected medicinal plants from Venda Communities in South Africa against fifteen clinical Bacterial species. *Afr. J. Biotech* ; 4(12):1443-1451.

Sen, S., Makkar, H. P. S., and Becker, K. A (1998): Saponins and their implication in animal nutrition. *Journal of Agricultural Food Chemistry*; 46, 131–140.

Sofowora, A (1993): *Medicinal plants and Traditional medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria .p289.

Teiji, S (2014): The molecular mechanism of acute lung injury caused by *Pseudomonas aeruginosa*: from bacterial pathogenesis to host response. *Sawa Journal of Intensive Care*; 2:10

Tijjani, A., I .G. Ndukwe and R.G.Ayo (2011): studies on antibacterial activity of *Adenium obesum* (*Apocynaceae*) STEM-BARK. *Continental J. Microbiology*; 5 (1): 12 – 17.

Trease, G.E. and C. W. Evans (1984): *Pharmacognosy*. 12th Edition .Balliere Tindall, London; p257.

Trease, G.E. and W.C. Evans (1989): *Pharmacognosy*. 13th (ed). ELBS/Bailliere Tindall, London; pp 345-773.

Turner, E.M.C (1953): The nature of the resistance of oats to the takeall fungus. *J Exp Bot.*; 11: 403-412.

Yoshikawa, M., Murakami, T., Kishi, A., Kageura, T., and Matsuda, H (2001): Medicinal flowers. III. Marigold (1):hypoglycaemic, gastric emptying inhibitory, and gastroprotective principles and new oleanane-type triterpene oligoglycosides, calendasaponins A, B, C, and D, from Egyptian Calendula officinalis. *Chemical and Pharmaceutical Bulletin*; 49: 863–870.

Watt, J.M and Breyer, G.M (1962): *The Medicinal and Poisonous Plants of Southern and Eastern Africa,* 2nd Edition, E. & S. Livingstone Ltd. Edinburgh and London; p 941.