

ANTIMICROBIAL ACTIVITY OF SAPONINS FROM *PARINARI CURATELLIFOLIA* (PLANCH. EX BENTH) ROOT BARK EXTRACT

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ABSTRACT

Parinari curatellifolia is used in traditional medicine for treatment of various diseases and microbial infections. The present study was to determine antimicrobial potentials of saponins from the root bark extract of the plant against some selected pathogenic fungi and bacteria. The saponins were extracted using standard protocol and were subjected to qualitative phytochemical screening. The antibacterial activity of the saponins was evaluated against clinical isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* using the cup plate method. The saponins demonstrated strong antibacterial activity on the test organisms with diameter of zone of inhibition ranging between 8.0 -21.0 mm. The saponins produced concentration-dependent responses on each test bacteria. *Staphylococcus aureus* was most susceptible having the highest diameter of zone inhibition of 21.0 mm at 30 mg/mL. MIC and MBC of 3.75 mg/mL was obtained on *S. aureus*. This indicated that the saponin had bactericidal effect on *S. aureus*. The antifungal activity of the saponin was also evaluated using the agar diffusion cup plate method against clinical isolates of *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Aspergillus niger* with typed isolates of and *Candida albicans* (ATCC 102311). The saponin showed diameter of zone of inhibition ranging from 12-18 mm at 140 mg/mL on *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Aspergillus niger* but no activity was observed on *Candida albicans*. The result showed that *Aspergillus niger* was the least susceptible to the saponin extract among the fungal isolates. This study revealed the antimicrobial potential of the saponin from *Parinari curatellifolia* root bark extract.

Key words: Antibacterial, antifungal, *Parinari curatellifolia*, saponins

INTRODUCTION

Saponins are naturally occurring non-volatile surface active glycosides. They are mainly produced by plants [1]. They are a large group of secondary plant constituents which are highly polar in nature and are readily soluble in water but insoluble in non-polar solvents. They produce intense froth when shaken in aqueous solution but chloroform, acetone and diethyl ether inhibit their frothing property [2]. On hydrolysis with dilute acid, they produce aglycone and glycone moieties. Saponins can be steroidal or terpenoidal in nature [2]. Saponins cause haemolysis of red blood cells. On this basis, they are used as fish poisons (sapotoxins). The physiological role of saponins in plants is not yet fully understood [1]. Many Saponins are known to be antimicrobial, to inhibit mould and to protect plants from insect attack. Saponins may be considered a part of plants' defence systems and as such have been included in a large group of protective molecules found in plants named 'phytoanticipins'. The first term describes those saponins, such as avenacosides in oat that are activated by the plant's enzymes in response to tissue damage or pathogen attack. Phytoprotectants is another term used to describe those saponins that have a general antimicrobial or anti-insect activity [3]. The biological activities of saponins include: analgesic anti-inflammatory, antidiarrhoeal, antioxidant, anticarcinogenic, antiulcerogenic and antimicrobial [4-6].

Parinari curatellifolia is used in traditional medicine for treatment of several diseases. The hot infusion of the bark is used for treatment of pneumonia. The crushed or pulped leaves are used in dressing fractures or dislocations, wounds, sores and cuts. After being stripped, the twigs can be used as chewing sticks. Teeth are washed with the hot infusion of the root and stem barks to treat toothache [7-9]. The seeds are used in the treatment of diabetes [10]. Traditionally the bark is used for washing clothes, vaginal douches, itchy scalp, dandruff and cough [11]. African traditional medicine practitioners used the various parts of the plant for treatment of cancer, diabetics, malaria fever, epilepsy, toothache, stomach aches, dysentery, venereal diseases, fungal and bacterial infections [12-14]. *Parinari curatellifolia* is used for several economic purposes which include its use as food. The fruit has a pleasant, sweet, tasting yellow flesh which is highly valued for its carbohydrate (88.2 %) content and high content of vitamin C [9]. The fruits are eaten fresh, cooked as porridge or made into beer. The delicious syrup prepared from it provides the basis of a refreshing, non-alcoholic drink. The fruit can also be dried and used as a reserve

food; the nuts are eaten or mixed with vegetables [2]. The seeds contain about 40 % oil and the kernels contain 70% oil. The seeds are pounded and used for soup making. The edible oil is used for cooking, paints, varnishes, soap, printing and engraving inks [9].

P. curatellifolia root bark and leaves contains K, Na, Ca, Al, Cu, Ni, Zn, Mn, Co, Cr, Pb and Cd in very low concentrations [15]. The pharmacognostic evaluation of the stem bark of *P. curatellifolia* revealed the presence of cell wall materials (cellulose, lignin and mucilage) with ergarstic cell contents (tannins, starch grains, calcium oxalate crystals, oils and proteins). The physical constants which include: moisture content, total ash, acid insoluble ash, water and alcohol soluble extractive values have been reported by [16]. The preliminary evidence for the presence of anthraquinones, terpenoids, saponins, flavonoids, cardiac glycosides and tannins as secondary metabolites in *Parinari curatellifolia* stem bark, root bark and leaves have been reported [17 &18]. Terpenoids and steroids have been isolated from *P. curatellifolia* with fascinating biological activities mostly anticancer [19-24]. Extracts of *P. curatellifolia* have demonstrated antimicrobial activity [16 & 21], antiplasmodial activity [20], hypoglycemic [10] and antioxidant activity [25].

Antibiotic resistance has been reported world wide as many bacterial and fungal species have defied treatment with antibiotics [26]. Plant chemicals especially the phenolics have been reported to have tremendous antimicrobial activities [27 & 28]. Reports on antimicrobial activities of saponins have not been well documented [2]. This study was designed to extract saponins from the root bark of the plant and to evaluate its antimicrobial activity.

EXPERIMENTAL

Collection and identification of plant sample

The plant was identified previously as described in Halilu *et al.* [16]. The root bark of *P. curatellifolia* was collected from Zaria, Kaduna State, Nigeria, in September, 2011. It was pulverized and stored in an air tight plastic container.

Extraction of Saponins

The method described by Woo *et al.* [29] was followed with slight modification. The powdered root bark (50 g) was extracted by maceration using 300 mL of n-hexane for 24 h and then

filtered. The marc was washed (rinsed) with 300 mL of hexane until the resulting liquid extract was clear. The marc was allowed to dry and then extracted by maceration with hydro-alcoholic solution of Methanol/water (70:30) for 48 h, filtered and subsequently washed with 200 mL of the hydro-alcoholic solution. The resulting solution was concentrated using rotary evaporator. The solid extract obtained was dissolved in 50 mL distilled water and then transferred into a separating funnel and then partitioned using 200 mL of diethyl ether. The aqueous portion was partitioned with 400 mL of n-butanol which was concentrated in a hot air oven at 60 °C to obtain the saponins.

Phytochemical analysis of Saponins

Frothing test

The saponins (2 g) were dissolved in 8 mL of distilled water in a test tube and then shaken vigorously for 2 min. The formation of persistent froth which lasted for more than 15 min indicated the presence of saponins.

Haemolysis test

The solution of 1.8 % sodium chloride solution (2 mL) was placed in two separate test tubes, A (control) and B (test). This was followed by addition of 2 mL of distilled water to each of the test-tubes. The aqueous solution of the saponins (1mL) was added to test-tube B. This was followed by addition of 2 drops of blood to each test-tube. The test tubes were covered with aluminium foil and then inverted gently for the content to mix. This was observed for 2-3 min. Haemolysis in test tube B and none in test-tube A confirmed the presence of saponins as described by Brain and Turner [30].

Chloroform test

The aqueous solution of saponin (2 mL) was shaken vigorously in a test tube for 5 min when persistent froth was formed. Chloroform (0.5 mL) was added to the solution containing the froth. The inhibition of frothing indicated the presence of saponins.

Fehling's test

An equal volume (1 mL) of Fehling's solution A and B were added to the 3 mL of the saponins in a test tube and then boiled over water bath. The formation of a brick red precipitate indicated the presence of reducing sugar and confirmed the presence of the glycone moiety.

Salkowski's test (Test for aglycone)

The solution of saponins (2 mL) was transferred into separating funnel and then extracted with 4 mL of chloroform. Few drops of concentrated sulphuric acid were added to 2 mL of the chloroform portion and observed. The formation of brown ring at the interface indicated the presence of steroids/triterpenoids.

Liebermann – Burchard's test (Test for aglycone)

The chloroform portion of the saponin (2 mL) was treated with 5 drops of acetic anhydride and then followed by addition of 0.5 mL of concentrated sulphuric acid by the wall of the test tube. The formation of brown ring at the interface without greenish colour in the upper layer indicated the presence of triterpenoids.

Antibacterial screening of Saponins

Test Organisms

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Aspergillus niger* and typed isolate of *Candida albicans* (ATCC 102311) were obtained from the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

Standard antifungal and antibacterial

The stock solution of fluconazole was prepared by dissolving 0.6 mg in 10 mL of 10% DMSO and then diluted to the required concentration with sabouraud dextrose liquid medium. Ofloxacin disk (10 mg) was used as standard antibiotic.

Preparation of fungal and bacterial Isolates

The solid cultures (18 h) of the fungal and bacterial isolates were sub-cultured into sterile sabouraud dextrose/ nutrient agar liquid medium respectively. These were standardized according to Clinical Laboratory Standards Institute [31] to produce inoculum size of 10^6 cfu/mL

for *Candida albicans* and 10^5 cfu/mL for *Trichophyton mentagrophytes* and *Trichophyton rubrum*, while inoculum size of 10^5 cfu/mL was produced for the bacteria.

Susceptibility test

The cup plate method was used to test the susceptibility of the saponins. The molten agar was allowed to cool, then transferred into Petri dishes and then allowed to solidify. The plates were flooded with the test bacteria (10^5 cfu/mL). Wells measuring 6 mm in diameter were bored on the inoculated plates using a sterile cork borer. The wells were filled with 0.1mL of saponins containing 15 mg/mL, 20 mg/mL, 25 mg/mL and 30 mg/mL. Distilled water (0.1 mL) was used as negative control and ofloxacin disk (10 mg) was used as positive control. The plates were allowed to stand for a pre-diffusion time of 2 h and then incubated for 24 h at 37 °C. The diameters of zones of inhibition were measured to the nearest millimetre using a metric rule. The experiments were carried out in five replicates. The plates for fungal strains were flooded (1.0 mL) of organisms and the excess drained aseptically. The plates were allowed to dry at 30 °C temperature in a sterilized incubator. Using the agar diffusion cup plate method, a sterile cork borer (6 mm) was used to bore holes in the agar plates. The bottoms of the wells (holes) were sealed with the appropriate molten sabouraud dextrose agar. The saponins (140 mg/mL), 10% DMSO (negative control) and 128 µg/mL of fluconazole were dispensed into the wells using micropipette. These were allowed to diffuse into the agar at room temperature for 1 h before incubation at 30 °C for 3 days. The diameter of zones of inhibition of the test organisms were measured to the nearest millimetre, using a calibrated metric rule. The experiment was carried in five replicates.

Determination of Minimum Inhibitory Concentration (MIC)

Two fold serial dilutions were used to determine the MIC of the saponins. The first test tube containing 2 mL of 30 mg/mL of the saponins was serially diluted to give 10 different concentrations. The culture of *S. aureus* (0.5 mL) containing 10^5 cfu/mL was added to the test tubes and was incubated at 37°C for 24 h. The MIC *Aspergillus niger* was determined using the broth micro dilution method as described by Pereira *et al.* [32] was used for the determination of the MIC of *Aspergillus niger* using a 96 micro well plate. The nutrient broth (100 µL) was transferred into each of the 96 micro well. The crude saponin (50 µL) was added to row A1 to

A10 of the 96 micro well to have a total volume of 150 μL . A 50 μL of the mixture was serially diluted to row H. Row A11 down to H11 were used as blank while row A12 down to H12 as positive control where fluconazole was used. A 100 μL of the standardized fungal suspension (2×10^6 spores per mL) was added to each micro well. The initial absorbance at 600 nm was measured after 30 min using a (96 well microplate reader). The microplate was covered and incubated at 27 °C and the absorbance at 600 nm was measured after 48 h and 72 h of incubation. The correction absorbance was determined by subtracting the absorbance after 30 min from the absorbance after 48 h and 72 h. The MIC is the lowest concentration to the control. The data obtained were transferred to Microsoft Excel programme and the percentage inhibition of each well was determined using the formula mode. The percentage inhibition (PI) was calculated thus:

$$\text{PI} = \frac{\text{Ac} - \text{At}}{\text{Ac}} \times 100$$

Note: PI > 90 % is the MIC and this is termed as MIC 90; Where Ac = Absorbance control and At = Absorbance test

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined from the concentrations just before and after the MIC. The nutrient agar was prepared and plated with *S. aureus*. The plates were incubated at 37 °C for 24 h. The minimum fungicidal concentration was determined by transferring 20 μL of the content of the MIC well and two wells after the MIC well into a nutrient agar and incubated aerobically at 37 °C. The plate was observed for growth after 72 h. The plate with the least concentration in which no growth was observed is the minimum fungicidal concentration.

Data analysis

The data was subjected to student T-test for test of significance using Microsoft Excel programme 2007.

RESULTS AND DISCUSSIONS

The mass of the saponins obtained was 9 g and the percentage yield was 18 % from 50 g of the powdered root bark. The result of the qualitative phytochemical analysis of the glycone and aglycone moieties associated to saponins are presented in Table 1. The analysis of the saponins

revealed a triterpenoidal type of saponins. This deduction was based on the fact that it gave positive reaction of triterpenoids using Liebermann-Burchard's test [17].

Table 1: Qualitative phytochemical analysis of Saponins

Saponins tests	Observation	Inference
a) Frothing test	Persistent Froth	+
b) Chloroform test	Inhibition of froth	+
c) Fehling's test	Brick red precipitate	+(Reducing sugar)
d) Salkowaski's test	Reddish brown ring	+(Triterpenoids)
e) Liebermann-Burchard's test	Reddish brown ring	+(Triterpenoids)
f) Haemolysis test	Haemolysis	+

Key: + = Positive Result

The saponins inhibited the growth of both Gram positive and Gram negative organisms, with diameter of zone of inhibition ranging between 8.0 to 21.0 mm at 15 mg/mL, 20 mg/mL, 25 mg/mL and 30 mg/mL on the test organisms. The activity demonstrated was concentration dependent (Table 2). From the result (Table 1), the saponins demonstrated broad-spectrum activity on both Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *P. aeruginosa*). The results in Table 2 also showed that *S. aureus* is most susceptible producing zone of inhibition of 21.0 mm at 30 mg/mL. This observation may be due absence of the lipopolysaccharide and protein (outer membrane) in the cell wall of Gram-positive bacteria [33]. The antibacterial activity demonstrated by the standard antibiotic (ofloxacin 10 mg/mL) was slightly higher than that of the saponins (Table 2). This observation was expected as ofloxacin is a pure compound. On comparing the activity of the saponins with ofloxacin, there was significant ($p < 0.0001$) increase in the activity of the ofloxacin on *S. aureus* and *B. subtilis*, *E. coli* and *P. aeruginosa* than saponins.

Table 2: Antibacterial activity of Saponins

Concentration mg/mL	Diameter of Zone of inhibition (mm)			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
15	10.0±0.12*	8.0±0.11*	14.0±0.10*	12.0±0.12*
20	14.0±0.10*	12.0±0.12*	18.0±0.11*	14.0±0.13*
25	18.0±0.13*	15.0±0.11*	18.0±0.12*	17.0±0.11*
30	21.0±0.11*	19.0±0.13*	20.0±0.10*	20.0±0.12*
Ofloxacin disk (10 mg/mL)	25.0±0.12	30.0±0.11	26.0±0.10	31.0±0.10

Halilu, M.E., Ifeanyi, G.I., Salvia, T.M., Ungokore H.Y., Dibal, M.Y. And Mathias, S.N:
Antimicrobial Activity of Saponins from *Parinari Curatellifolia* (Planch. Ex Benth) Root Bark
Extract

Distilled water	0	0	0	0
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Values are mean inhibition zone (mm) \pm S.D of five replicates; Diameter of cork borer = 6 mm
*There is significant difference between the saponin and ofloxacin at $p < 0.0001$

The MIC and MBC of the saponins on *S. aureus* was 3.75 mg/mL (Table 3). This result indicated that the saponin is bactericidal on *S. aureus*.

Table 3: MIC and MBC of Saponin fraction on *Staphylococcus aureus*

Test organism	MIC (mg/mL)	MBC (mg/mL)
<i>S. aureus</i>	3.75	3.75

The saponins demonstrated strong antifungal activity on *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Aspergillus niger* at concentration of 140 mg/mL with diameter of zone of inhibition ranging between 12.7 to 18.3 mm (Table 4). The saponins could not inhibit the growth of *Candida albicans* (Table 4). This may be due to variation in of the cell wall composition of the fungal species [34]. This observation may also be attributed to the pattern of the reaction of the fungal strains to the saponins. This may be related to the mode of interaction of the saponins with the chitin of the fungal cell wall [35]. On comparing the activity of the saponins with fluconazole, there was significant increase ($p < 0.0001$) in the activity of the fluconazole on *Trichophyton mentagrophytes* and *Aspergillus niger* than saponin and ($p < 0.001$) for *Trichophyton rubrum*.

Table 4: Susceptibility of the test fungi to the saponin fraction

Organism/Conc.(140 mg/mL)	Diameter of Zone of Inhibition (mm)		
	Saponins	10% DMSO	Fluconazole (128 μ g/mL)
<i>Trichophyton mentagrophytes</i>	18.3 \pm 0.47**	0	26.67 \pm 0.58
<i>Trichophyton rubrum</i>	16.0 \pm 0.00*	0	23.00 \pm 0.58
<i>Aspergillus niger</i>	12.7 \pm 0.47**	0	14.67 \pm 0.00
<i>Candida albican</i>	0	0	24.33 \pm 0.58

Values are mean inhibition zone (mm) \pm S.D of five replicates; Diameter of cork borer = 6 mm
*Significant difference between the saponin and fluconazole at $p < 0.0001$;
**Significant difference between the saponin and fluconazole at $p < 0.001$

The saponins showed an MIC₉₀ and MFC₉₀ of 195 µg/mL on *Aspergillus niger* (Table 5). This result demonstrated that the saponin was fungicidal on *Aspergillus niger*.

Table 5: MIC and MFC of Saponin fraction on *Aspergillus niger*

Test organism	MIC ₉₀ (µg /mL)	MFC ₉₀ (µg /mL)
<i>Aspergillus niger</i>	195	195

CONCLUSION

The key discovery that has emerged from this research is that the saponins from the root bark of *P. curatellifolia* inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Aspergillus niger*. Another major finding is that the saponins produced bactericidal effect on *Staphylococcus aureus* and fungicidal effect on *Aspergillus niger*. The saponins did not inhibit the growth of *Candida albicans*. The saponin from *P. curatellifolia* is a potential source of antibiotic as it has emerged from the result of this research.

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REFERENCES

1. George, F., Zohar, K., Harinder, P., Makkar, S. & Klaus, B. (2002). A Review: The biological action of saponins in animal systems, *British Journal of Nutrition* Vol. 88, Pp. 587–60
2. Negi, J.S., Negi, P.S., Pant, G.J., Rawat, M.S.M. & Negi, S.K. (2013). A review: Naturally occurring Saponins: Chemistry and Biology. *Journal of poisonous and medicinal plant research*, 1 (1), 1-6.
3. Halimatu, H. S., Sule, M. I., Musa, M. A., Emmanuel, A. A., Ibrahim, Hajara I., Hassan, A. S. & Yaro, A. H. (2010). Analgesic and anti-inflammatory activities of the saponins extract of *Carissa edulis* root in rodents, *International Journal of Biological and Chemical Sciences*,

- 4(4), 1310-1317.
4. Morrissey, J.P. & Osbourn, A.E. (1999). Fungal resistance to plant Antibiotics as a Mechanism of pathogenesis. *Microbiological and Molecular Biological Reviews*, 63,708–724.
 5. Hassan, H. S., Sule, M. I., Musa, M. A., Emmanuel, A. A., Ibrahim, Hajara I., Hassan, A. S. & Yaro, A. H. (2010). Analgesic and anti-inflammatory activities of the saponins extract of *Carissa edulis* root in rodents, *International Journal of Biological and Chemical Sciences*. 4(4), 1310-1317
 6. Abubakar, K., Abubakar, M. R., Ugwah-Oguejiofor, J. C., Muhammad, A. A., Usman, M. & Mshelia, H. E. (2015). Antidiarrhoeal activity of the saponin and flavonoid fractions of *Anarcadium occidentale* leaves in albino rats, *Advancement in Medicinal Plant Research*, 3(1), 23-28
 7. Sidi, S., Dominic, G., Charlotte, R. & Moctar, S. (2006). Seed Leaflet No.110, *Parinari curatellifolia* Planch Ex. Benth. Millenium seed bank project, KEW. Phytotrade Africa, Forest and landscape, Denmark. www.SL.kvl.dk
 8. Hines, D.A. & Eckman, K. (1993). *Indigenous Multipurpose Tree for Tanzania, Uses and Economic benefits to the people*. Cultural Survival, Canada and Development Services Foundation. pp 1- of Tanzania 50.
 9. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Simons, A. (2009). *Parinari curatellifolia* Agroforestree Database: a tree reference and selection guide version 4.0 Pp 1-5.
 10. Ogbonnia, S.O., Sunday, O.O., Emmanuel, N. A., Veronica, N. E. & Olawale, O. P. (2009). Evaluation of acute toxicity in mice and subchronic toxicity of hydroethanolic extract of *Parinari curatellifolia* (Chrysobalanaceae) seeds in rats. *African Journal of Biotechnology*, 8 (9), 1800-1806.
 11. Qasem, J.R. & Abublan, H.A. (1996). Fungicidal activity of some weed extracts against different plant pathogenic fungi. *Journal of Phytopathology*, 44 (3), 157-161.
 12. Uys, A.C., Malan, S.F., VanDyck, S. & VanZyl, R.L. (2002). Antimalarial compounds from *Parinari capensis*. *Bioorg Medic Chem Lett* 12: 2167.
 13. Arnold, H.J. & Gulumian, M.J. (1984). Pharmcopoeia of traditional medicine in venda. *Journal of Ethnopharmacology*, 12, 35-74.
 14. Ssegawa, P. & Kasenene, J.M. (2007). Medicinal plant diversity and uses in the

- Sango bay area, Southern Uganda. *Journal of Ethnopharmacology*, 113: 521-540.
15. Halilu, M.E., Abah, J.O., Almustapha, N.L. & Achor, M. (2010). “Phytochemical Screening And Mineral Element Analysis of The Root Bark of *Parinari macrophylla* Sabine and its Effect on Microorganisms”. *Continental Journal of Biological Sciences*, Vol.3, 46-50.
16. Halilu, M. E., Agunu, A., Ibrahim, H. & Abdurahman, E. M. (2008a). Pharmacognostic evaluation of the stem bark of *Parinari curatellifolia* Planch. Ex. Benth (chrysobalanaceae), *Nigerian Journal of Pharmaceutical Sciences*, 7 (1), 79-85.
17. Halilu, M.E., Akpulu I.K., Agunu, A., Ahmed, A. & Abdurahman, E.M. (2008b). Phytochemical and Antibacterial Evaluation of *Parinari curatellifolia* Planch Ex Benth (Chrysobalanaceae)”, *Nigerian Journal of Basic and Applied Sciences*, 16 (2), 272-276.
18. Lee, I.S., Shamon, L.A., Chai, H.B., Chagwedera, T.E., Besterman, J.M., Farnsworth, N.R., Cordell, G.A., Pezzuto, J.M. & Kinghorn, A.D. (1996). Cell-cycle Specific cytotoxicity mediated by rearranged ent-kaurene diterpenoids from *P. curatellifolia*. *Chemical and Biological Interaction*, 1 (3), 193-204.
19. Rundle, N.T., Xu, L., Andersen, R.J. & Roberge, M. (2001). G2 DNA damage checkpoint inhibition and Antimitotic activity of 13-hydroxy-15-oxozoapatlin. *Journal of Biochemistry*, 51, 48231- 48236.
20. Kraft, C., Jenett-Siems, K., Siems, K., Jakupovic, J., Mavis, S., Bienzle, U. & Eich. E. (2003). *In-Vitro* antiplasmodial Evaluation of Medicinal Plants from Zimbabwe. *Phytotherapy Research*, 2: 123- 128.
21. Chimponda, T. & Mukanganyama, S. (2010). Antimycobacterial activities of selected medicinal plants from Zimbabwe against *Mycobacterium aurum* and *Corynebacterium glutamicum*. *Tropical Biomedicine*, 27 (3): 595–610.
22. Simeon, O., Asa, A., Kennedy, I.A. & Mriose, V.I. (2012). Anti-snake venom activity of flavonoids from the root bark of *Parinari curatellifolia* in mice. *International journal of pharmaceutical research*, 4, 55-58.
23. Stanley, M., Simbosho, C.D. & Leseilane, M. (2012). Anti-Proliferative effect of plant extract from Zimbabwean medicinal plant against human leukemia cell lines. *The African journal of plant science and biotechnology*, 14-20.
24. Halilu, M.E., October, N., Balogun, M., Agunu, A., Abubakar, A & Abubakar, M.S. (2013a).

- Isolation and Characterization of Steroids from Petroleum Ether Extract of Stem Bark of *Parinari curatellifolia* Planch ex. Benth (Chrysobalanaceae). *Journal of natural Science Research*, 3 (6), 53-61.
25. Halilu, M.E., October, N., Balogun, M., Musa, K.Y. & Abubakar, M.S. (2013b). Isolation and Characterization of Triterpenes from Petroleum Ether and Ethyl acetate Extract of Stem Bark of *Parinari curatellifolia* Planch ex. Benth (Chrysobalanaceae). *Journal of Chemistry and Materials Research*, 3(9), 100 – 107
26. Halilu, M.E., October, N., Balogun, M., Namrita Lall & Abubakar, M.S. (2013c). Studies of *In vitro* Antioxidant and Cytotoxic Activities of Extracts and Isolated Compounds from *Parinari curatellifolia* (Chrysobalanaceae), *Journal of natural Science Research*, 3 (11), 149 - 154
27. Arne, R., Torsten, B., Santiago, E., Peter, K. and Ekhard, M. (2008). Susceptible, Intermediate, and Resistant- The intensity of Antibiotic Action, Medicine, *Deutsches Arztlatt International*, 105 (39), 657-62
28. Silvia Helena Taleb-Contini, Marcos José Salvador¹, Evandro Watanabe², Izabel Yoko Ito², Dionéia Camilo Rodrigues de Oliveira (2003). Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species, *Brazilian Journal of Pharmaceutical Sciences*, 39 (4), 403-408
29. Woo, W. S., Shin, K. H. & Kang, S.S. (1980). Chemistry and Pharmacology of Flavone C-Glycoside from *Ziziphus* seeds. *Korean Journal of Pharmacognosy*, 11(3-4), 141-148.
30. A. Doss, H. Mohammed Mubarack and R. Dhanabalan (2009). Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. *Indian Journal of Science and Technology*, 2 (2), 41-43.
31. Brain, K.R. & Turner, T.D. (1975). The practical Evaluation of Phytopharmaceuticals. Pp. 90-92
32. Annette W. F. (2012). Antifungal Susceptibility Testing: Clinical Laboratory and standards Institute (CLSI) Methods, Health Science Center , San Antonio , TX , USA, Pp 65-74
33. Pereira, F. O., Wanderley, P. A., Viana, F. A. C., Lima, R. B., Sousa, F. B., Lima, O. (2011). Growth Inhibition and morphological alterations of *Trichophyton rubrum* induced by essential oil from *Cymbopogon winterianus* (Jowitt Ex. Bor). *Brazilian Journal of*

Microbiology, 42, 233-242

34. Carter, S. J. (1972). Corper and Gunn's Tutorial Pharmacy 6th Ed. Pitman Medical Printed in Northern Ireland at the University Press, Belfast. pp. 72-373.
35. Babatunde, I.A. & Olusola, A.O. (2015). Antifungal agents that target fungal cell wall components (A review). *Agricultural and Biological Sciences Journal*, 1(5), 206-216.