International Journal of Pathogen Research

1(3): 1-8, 2018; Article no.IJPR.44703

Antifungal Efficacy of Saponin Extracted from *Phyllanthus niruri*

V. A. Ajibade[1*], O. A. Ajenifuja[1], F. T. Akinruli[1], F. A. Ajayi[1] and O. Famurewa[2]

[1]Department of Science Technology, Federal Polytechnic, P.M.B. 5351, Ado-Ekiti, Ekiti State, Nigeria.
[2]King's University, Odeomu, Osun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author VAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VAA and OAA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Nagwa Mostafa El-Sayed, Professor, Department of Medical Microbiology& Parasitology, Research Institute of Ophthalmology, Giza, Egypt.
Reviewers:
(1) Emmanuel Ifeanyi Obeagu, University Health Services, Michael Okpara University of Agriculture, Umudike, Nigeria.
(2) Mohamed M. Deabes, National Research Centre, Egypt.
(3) Ochieng O. Anthony, Sumait University, Tanzania.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/27208

Original Research Article

Received 15 August 2018
Accepted 02 November 2018
Published 14 November 2018

ABSTRACT

The *in-vitro* and *in-vivo* antifungal activities of saponin extracted from *Phyllanthus niruri* were investigated. The proximate composition of the plant determined using the methods of the Association of Official Analytical Chemists showed that the moisture, dry matter, crude protein, crude fibre, total ash and fat were available in 1.34 g, 98.66 g, 12.33 g 18.45 g, 7.36 g and 2.54 g/100 g respectively. The elemental analysis determined using the Flame- Photometric method reveals the presence of major, minor and trace elements in appreciable quantities. Nine elements; Zn Mn, Ca, Mg, Na, K, Fe and P were present. The susceptibility of three *Trichophyton* species determined by the radial- mycelial methods showed that *T. mentagrophytes* was more susceptible to the saponin. These favourable effects point to the potential of the saponin as a remedy against mycotic infections caused by *T. mentagrophytes*. The fact that this extract exerted an inhibitory effect on these epidermal fungi indicates that they can potentially be further developed into

**Corresponding author: Email: ajibvijay@yahoo.com;*

antifungal clinically used agents.

Keywords: In vitro; in vivo; antifungal; saponins; Phyllanthus niruri.

1. INTRODUCTION

For the past 60 years, antifungal chemotherapy has been the mainstay of medical intervention against infectious diseases caused by fungal pathogens. The continuous decline of therapeutic effectiveness as a result of extensive use of antifungal chemotherapy has been long predicted and seems inescapable [1]. Many surveillance studies have over the last decade (1997-2007) drawn attention to this phenomenon [2]. At the same time, the once- abundant supply of new and improved antimicrobial compounds have reduced, as drug development becomes increasingly challenging and pharmaceutical companies invest in more lucrative markets [3]. It is therefore critical to realise that the effectiveness of antimicrobial agents, widely accepted as a common good, cannot be taken for granted and that such substances are increasingly attaining the status of nonrenewable resources.

Studies have revealed that traditional plant medicine from various parts of the world can provide a rich source of antiviral and antibiotic activities [4]. These types of study can be justified in the context of phytochemical leading to the pharmaceutical development and bioactive extracts which can also be considered as "ethical phytomedicines", if appropriate phytochemical standardisation and toxicology investigations are undertaken [5].

Many medicinal plants of Africa especially Nigeria have been investigated for their chemical components and some of the isolated compounds have been shown to possess interesting biological activity. Amongst these plants is *Phyllanthus niruri* L., (*Syn. P. fraternus* Webster), Euphorbiaceae, which is a common kharif (rainy season) weed found in both cultivated fields and wastelands [6]. It carries different nomenclature in different parts of the world. However in Nigeria it is called *Asasa* or *Arunjeran* in Yoruba, *Majiryar Kurumi* in Hausa, *Asivi* or *Igbehen* in Edo, *Egu eza* in Ibo and *Oyomo-ke-iso-aman-ke-edem* in Efiki [7]. Although considered a problematic weed for farmers, it is a valuable medicine for herbalist [8] and holds a reputable position in both Ayurvedic and Unani systems of medicine. Recently it has attracted the attention of researchers, because of its hepatoprotective properties. Although no effective specific therapy is available for viral hepatitis, *P. niruri* has shown clinical efficacy in the treatment of viral Hepatitis B [9].

Saponins are naturally occurring surface-active glycosides that are not only produced by plants. They derive their name from their ability to form stable, soap-like foams in aqueous solutions. This easily observable character has attracted human interest from ancient times [10]. Saponins have high toxicity against fungi [11]. Fungicidal activity against *Trichoderma viride* was previously used as an identification method for saponins. Kalopanax-saponin A isolated from *Kalopanax pinctus* exhibited strong and specific antifungal activity against *Candida albicans* and *Cryptococcus neoformans* [12]. The monodesmosidic spirostanol saponins from *Nelumbo mucifera* destroy certain food-deteriorating yeasts, film-forming yeasts, and dermatophyte yeast and fungi [13]. The major mechanism suggested for the antifungal activity of saponins is their interaction with membrane sterols.

Despite successes achieved in controlling infectious fungal diseases, efforts to defend against the wide range of microbes that threaten human health continued to be challenged by emerging and re-emerging of infectious pathogens and possible use of a variety of virulent agents as biological weapons [14]. A defensive strategy based solely on developing new vaccines and antifungal drugs, each specific for only one or a few agents, is unlikely to be successful in dealing with potential microbial threats and these are exceedingly expensive. An alternative approach attempts to exploit plants. Therefore the aim and objective of this study is to determine the proximate and mineral composition of *P. niruri*, and to extract crude saponin from the whole plant of *P. niruri* and determine the *in-vitro* antifungal potency and efficacy on strains of *Trichophyton* spp.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The matured and fresh leaves of the plant, *Phyllanthus niruri* were collected from farmlands in Ado-Ekiti during the raining season between

the months of May and October (2004 – 2006). The plant material was air-dried at room temperature (27°C ± 1°C), was ground into the powdered form using milling machine (Retsch GmbH 5657 HAAH) and stored in air-tight plastic container. Identification and authentication of the plant were performed in the Department of Plant Science, University of Ado-Ekiti, Nigeria where a voucher specimen was deposited.

2.2 Extract Saponins

The method described by Martson et al. [15] was employed. The dried and powdered plant material (500 g) was defatted in a Soxhlet with petroleum ether at between 40°C and 60°C for 16 h. The residue was added to 100 ml of absolute methanol and left overnight under reflux at 70°C. It was filtered with Whatman No. 2 mm filter paper and the filtrate evaporated to dryness with a rotatory evaporator. The yield was dissolved in 100 mL of distilled water, extracted in a separating funnel with 1-butanol three times and dried by evaporating. Finally, the extract was dissolved in 25 mL of absolute methanol and the saponins compound was precipitated by adding 75 mL of diethyl ether.

2.3 Proximate Analysis of the Plants

2.3.1 Estimation of crude fiber

The crude fiber is the non-digestive dried residue remaining after digestion of sample with 1.25% H_2SO_4 and 1.25% NaOH solution under specific conditions [16]. The method of Park et al. [17] was employed in this estimation.

Ground plant material (2 g) was defatted with petroleum ether at 35 – 38°C and boiled with 200 mL of sulphuric acid for 30 mins. The extract was filtered through muslin and washed with boiling water until washings were no longer acidic. The filtrate was boiled in 200 mL of sodium hydroxide solution for 30 mins and filtered again through a muslin cloth. It was washed with 25 mL of boiling 1.25% H_2SO_4, three 50 mL portions of water and transferred to a preweighed ashing dish (W_1) dried for 2 h at 130 ± 2°C, cooled in a desiccator and weighed (W_2). The residue was ignited for 30 min at 600 ± 150°C and cooled in a desiccator and weighed (W_3). The observations were calculated thus:

$$\%\ \text{Crude fiber in sample} = \frac{\text{Loss in weight on ignition}(W_2 - W_1) - (W_3 - W_1)}{\text{Weight of sample}} \times 100$$

2.3.2 Estimation of fats

The method described my Martson et al. [15] was employed to estimate the fat content. The dried powdered plant materials (50 g) were placed in a thimble of a Soxhlet extraction apparatus. Extraction was done with petroleum ether (150 drops/min) for 6h without interruption. The extract was allowed to cool and the petroleum ether was evaporated in a water bath until no odour of ether remains. It was cooled at room temperature. The residue was weighed, heated until constant weight was recorded. The fat content was calculated as follows;

$$\%\ \text{Fat w/N} = \frac{\text{Weight loss by sample (g)}(Extracted\ fat)}{\text{Weight of sample (g)}} \times 100$$

2.3.3 Total ash determination

The total ash content of the sample was determined using the method described by AOAC [16]. The dried powdered plant material (10 g) was ashed in a furnace at 500°C for 3 h. It was allowed to cool in a desiccator, weighed and the new value taken. The percentage ash was then calculated as follows:

$$\%\ \text{Ash} = \frac{\text{Weight of dish after ignition} - \text{Weight without sample}}{\text{weight of sample}} \times 100$$

2.3.4 Estimation of moisture and total solids

The over-drying method described in the official method of Analysis [16] was employed. The dried powdered plant material (190 g) was dried in an oven at 100°C for 1 h. The sample was cooled in a desiccator, weighed and the weight loss determined. Drying was continued until a constant weight was obtained. The percentage moisture was then calculated as follows:

$$\%\ Moisture\ content\ \frac{Final\ sample\ weight}{Initial\ sample\ weight} \times 100$$

Total solid (%) = 100 – % moisture content

2.3.5 Estimation of protein

The Kjeldahl digestion method described in the official method of Analysis [16] was used. The dried powdered plant material (2 g) was put inside a Kjeldahl digestion flask. One tablet of selenium catalyst was added followed by the

addition of 25 mL of concentrated H_2SO_4. The mixture was digested, heated gently for 5 – 10 minutes and then vigorously for 45 mins. It was allowed to cool, diluted with distilled water and transferred into the semi-macro apparatus for distillation. Distillation was carried out for 15 minutes with all the pinch corks closed and the contents titrated with 0.02 HCL. The crude protein was calculated as shown below.

$$Total\ protein\ content = \frac{0.0014 \times volume\ of\ acid\ \times 250}{5 \times 100} = A$$

$$\therefore \frac{\mathrm{A}}{\mathrm{W} \times 6.25};\ where\ \mathrm{W} = \text{weight of sample digested}$$

2.3.6 Estimation of carbohydrate

The carbohydrate content was estimated by subtracting the moisture, protein, fat, crude fibre and ash contents from 100.

2.4 Determination of Mineral Contents

The atomic absorption spectrophotometer (AAS) method [16] was used to determined Na^+, K^+, Ca^{2+}, Fe^{2+}, Mg^{2+}, Cu^{2+}, Zn^+, and P. Standard stocks of the metals to be estimated were prepared according to standard procedures [16]. The dried powdered sample of the plant (2.0 g) was pretreated by ashing a furnace at 550°C until the sample was completely ashed. It was put in a desiccator to cool. To the pretreated sample in a Kjeldahl flask, 20 mL of 1.0 nitric acid (dilute) was added, gently boiled for 10 minutes and allowed to cool at room temperature. The digested samples were filtered with a filter paper (125 mm) into 100 mL of the graduated flask. The Kjeldahl flasks and filter paper were washed three times each with 10 mL of distilled water. The 100 ml flasks were made up to mark with distilled water (solution A). Blank solutions were similarly prepared but without the sample (Solution B). The standard stocks of the metals were prepared into solutions and were measured with the correct filters to standardize the spectrophotometer. The solutions for the metals were subsequently measured.

2.5 Isolation and Identification of *Trichophyton* species

Scrapping from the face and scalp of the head of two infected children in Aba-Erifun, Ado-Ekiti were collected and inoculated onto different plates of Sabouraud dextrose agar (Oxoid). (Fig. 1 had an infection on the face, while Fig. 2 had the infection almost all over the scalp involving the hair around the infected area). They were then incubated at room temperature (25°C ± 1°C) for seven days.

The two samples were identified by their mycelia characteristics, spore formation and appearance on agar plates as described by Larone (1995). The plates were placed on a microscopic stage and examined by using 100 x and 400 x magnification objectives to observe the spore-bearing structures. Inoculating needle was used to collect growth and teased on a slide stained with Loffler's methylene blue.

2.6 Determination of Antifungal Potency and Efficacy of Crude Saponins

2.6.1 Antifungal activities

Two different methods described by Fernandez et al. [18] were used to determine the antifungal activities of the saponin extract.

(i) Seed agar plate bioassay technique

The saponin extract used at various concentrations of 0.3 – 2 mL were introduced ascetically into different pre-sterilized Petri-dishes. Sterilised Sabouraud dextrose agar (15 mL) was added to each of the Petri-dishes containing the extracts. They were swirled carefully to ensure proper mixing and allowed to set. The fungi plates were then bored using cork borer (7.0 mm). Small inoculum (agar plug) of each test fungi was then placed on the seeded agar and incubated at room temperature (28°C ± 1°C). Control plates were made without the addition of extract for comparison of growth after four days.

(ii) Preparation of Spore suspension for TLC bioassay

The test fungi were cultured on Sabouraud dextrose agar plates at room temperature (28°C ±1°C) for 5 days. The fungal spores were harvested by flooding plates of the fungus with sterile distilled water and rubbing the culture with a sterile glass rod. The spore suspensions were filtered through two layers of sterile muslin to remove the mycelia fragment. Centrifugation of the filtrates was carried out for 15 mins at 3,500 rpm. The supernatant was discarded and the spores washed in two changes of distilled water.

This was taken into 2% Sabouraud broth and the spore concentration was determined by means of a Hemacytometer. The concentration was adjusted to 5.0 x 10^5 spore/mL by dilution with distilled water.

2.6 Statistical Analysis

The data were expressed as mean ±S.D., which for biochemical and physiological parameters were analyzed statistically using one way ANOVA followed by Dunnet-+-test using the SPSS Statistical software for comparison with the control group and saponin treated group. $p < 0.05$ was considered as significant while $p < 0.01$ and $p < 0.001$ were considered as insignificant.

3. RESULTS AND DISCUSSION

3.1 Results

The proximate compositions of the leaves of the plant are shown in Table 1. Moisture content of the whole plant was 1.34 g/100 g. The dry matter value was 98.66 g/100 g. The crude protein value was 12.33 g/100 g while that of crude fiber, total ash and fats were 18.45 g/100 g, 7.36 g/100 g and 2.54 g/100 g respectively. These reported values are average of replicates.

Elemental analysis of the whole plant reveal the presence of major, minor and trace elements in appreciable quantities (Table 2) Nine elements i.e., Zn, Mn, Ca, Mg, Na, K, Fe and P were found to be present in the plant extract while Zn and K were in low percentage. These reported values are average of duplicates.

Table 3 shows the susceptibility of each of the species of *Trichophyton* determined by their radial growth at different concentrations after 28 hr, 56 hr and 84 hrs respectively. It was observed that among the fungi tested; *T. mentagrophytes* was more susceptible to the extract.

The results of the treatment of infected scalp and skin are shown in Fig. 1 (A and B) and Fig. 2 (A and B). The infected portions were cleared after administration of the saponin extract for four weeks.

Table 1. Proximate composition (g/100 g) of *P. niruri*

Parameter	Mean	± SD
Moisture	1.34	0.06
Dry matter	38.66	3.93
Crude Protein	12.33	3.26
Total ash	7.16	0.52
Carbohydrate	29.52	2.62
Cellulose (fiber)	8.45	9.94
Fats	2.54	0.70

Means are for replicate determinations
±SD = Standard deviation

Table 2. Elemental analysis (mg/kg) of *P. niruri*

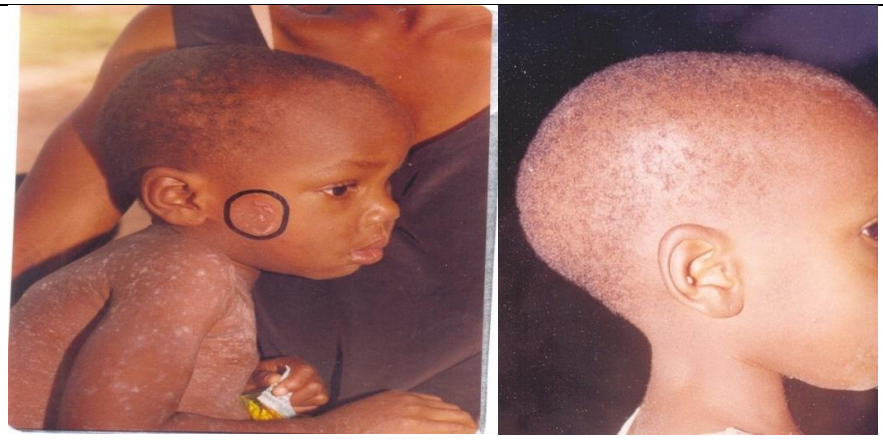
Parameter	Mean	+ SD
Zn	10	0.07
Mn	Nd	-
Ca	170	17.35
Mg	270	26.16
Na	120	29.46
K	50	20.29
Fe	100	1.04
Cr	Nd	-
P	1550	-

Means are for duplicate determination
± SD = Standard deviation
nd = not detected

Table 3. Antifungal activities of crude saponins by radial mycelia growth determination

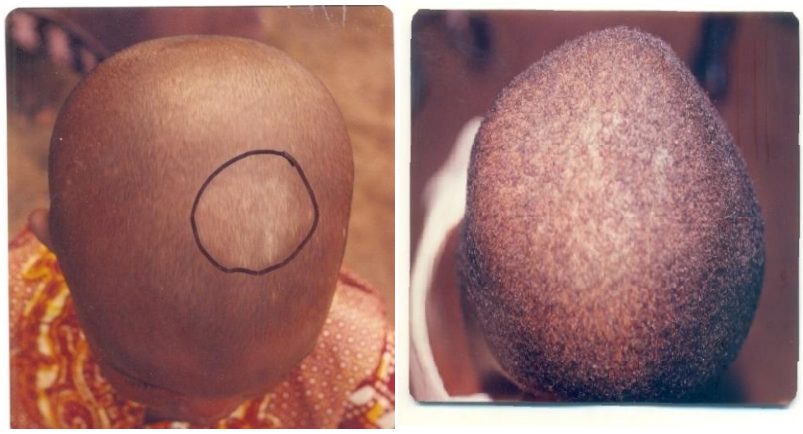
Organisms	Radial mycelia growth (mm)				
	Concentration (g/ml)				
	Hours	0.1	0.2	0.3	Control
Trichophyton rubrum	28	7.0	7.0	7.0	15.0
	56	7.2	7.2	7.2	13.0
	84	7.2	7.3	7.2	25.0
Trichophyton mentagrophytes	28	7.0	7.0	7.0	20.0
	56	7.0	7.0	7.0	19.0
	84	7.0	7.0	7.0	30.0
Trichophyton megninii	28	7.8	7.6	7.7	23.0

	56	8.0	7.7	7.9	20.0
	84	9.8	9.0	8.0	32.0



A B

Fig. 1. (A) Before treatment (B) After treatment with crude saponin extract



A B

Fig. 2. (A) Before treatment (B) After treatment with crude saponin extract

3.2 Discussion

The results obtained in the proximate analysis of the plant showed that the dry matter of the plant is as high as 98.66 g/100 g. This has always been the findings of most researchers and it is complemented by the reports of Oyekanmi and Osho, [19]. The carbohydrate and crude protein composition of a plant is of importance because of their nutritive values. Both exist as 57.98 g/100 g and 12.33 g/100 g respectively in the plant. The value of carbohydrate is high because most plants store glucose as starch which is a source of energy. The elemental analysis revealed the presence of phosphorus, magnesium, calcium and sodium in appreciable quantities. This precludes that the plant could be a good source of nutrient for body building and a booster to the immune system. Phosphorus has been reported to be good for bones and teeth formation. It contributes to energy production by participating in the breakdown of carbohydrates, protein and fats. It is needed for growth, maintenance and repair of tissues and cells, and for the production of DNA and RNA. Phosphorus is also needed to balance, and metabolise vitamins and minerals such as vitamin D, calcium, iodine, magnesium and zinc. Magnesium is an essential mineral involved in various metabolic reactions [20]. It is necessary for major biological processes, including the production of cellular energy and the synthesis of nucleic acids and proteins. It is also important for the electrical stability of cells and maintenance of membrane integrity and

plays a key role in many physiological functions especially protein synthesis of chlorophyll [11].

In this work, the saponin of interest was present in 1.86 mg/100 g concentration. This is however substantial, a reflection of the findings of Nwanjo, [21]. With the presence of phytic acids and cyanides at substantial concentration, one expects signs of toxicity. It is, however, important to note that in all the published works over the last twenty (20) years. There has not been any report of toxicity or side effects made in any human or animal [20,21]. This could be because these chemicals are not always available in a pure form. This finding substantiates the result of this work.

The activity of the saponin on *Trichophyton* spp. isolated from the skin and head of infected children showed that the extract is more active against *T. rubrum* and *T. mentagrophytes* with little activity against *T. megninii*. The skin and head treatment of the patients over one week also proved the efficacy of the extracts. Antifungal activities of saponin have been reported by Ibrahim et al. [22]. The major mechanism suggested for the antifungal activity of saponins was their interaction with membrane strolls. It was observed that those saponins having a branched-chain trisaccharide moiety without any oxygen-containing groups at C2 and C12 exhibited anti-yeast activity while saponins with 2P-hydroxyl or 12keto groups showed very weak or no activity [23]. A saponin with a disaccharide moiety exhibited relatively low activity and the aglycones or bidesmodic furostanol saponin showed no activity [11].

4. CONCLUSION

Saponins that are extracted from plants showed the same therapeutic effect even though they have varying structures because of several types that exist. As regarding the observation recorded in this study, the therapeutic functions regarding the effects of saponin in the integumentary system (skin) was able to be established.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO (World Health Organization). The anatomical therapeutic chemical classification system with defined daily doses (AT/DDD). Oslo: The Organization; 2005.
2. Mackenzie FM, Bruce J, Van Looveren M, Cornaglia G, Gould IM, Goosens H. Antimicrobial susceptibility testing in European hospitals: Reports from the ARPAC study. Clin. Microbiol Infect. 2006;12:1185-1195.
3. Moel GS, Jones RN, Biedenbach DJ, Shlwell MG, Fritsche TR. Contemporary causes of skin and soft tissues infections in North America, Latin America, and Europe: Report from the SENTRY Antimicrobial Surveillance Program (1998-2004). Diagn Microbiol Infect Dis. 2007;57:7-13.
4. Patel Jay Ram, Tripathi Priyanka, Sharma Vikas, Chauhan Nagendra Singh, Dixit Vinod Kumar. *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology: A review. Journal of Ethnopharmacology. 2011;138(2):286–313.
5. Berhow MA, Kong SB, Vermillion EE, Duval SM. Complete quantification of group A and B soyasaponins in soybeans. Journal of Agricultural and Food Chemistry. 2006;54(6):2035-2044.
6. Rain SA. Chanca piedra (*Phyllanthus niruri*) plant and leaf. Scientific References; 2002.
7. Irvine FR. West African Botany (3rd edition) Oxford University Press, Amen House, London E.C.4; 1986.
8. Qudhia P, Tripathi RS. Prospects of cultivation of medicinal plants in Chattisgarh, India. In: Recent progress in medicinal plants, crop improvement, production technology, trade and commerce. Sci Tech publ, USA. 2002;5:211-236.
9. Paranjpe P. Indian medicinal plants: Forgotten healers. Chaukhamba Sanskrit Pratisthan, Delhi. 2001;48-49.

10. Yoshikawa M, Murakami T, Kishi A, Kageura T, Matsuda H. Medicinal flowers 111, marigold (1): Hypoglycemic gastric emptying inhibitory, and gastro protective principles and new oleanane-type triterpene oligoglycosides, calendasaponins A, B, C and D, from Egyptian *Calendala officinalis*. Chemical and Pharmaceutical Bulletin. 2001;49:863-870.
11. Giribabu N, Rao PV, Kumar KP, Muniandy S, Rekha SS, Salleh N. Aqueous extract of *Phyllanthus niruri* leaves displays *in vitro* antioxidant activity and prevents the elevation of oxidative stress in the kidney of streptozotocin-induced diabetic male rats. Evid Based Complement Alternat Med. 2014;20(14):815-834.
12. Kim DW, Bang KH, Rhee YH, Lee ET, Park HJ. Growth inhibitory activities of kalopanax saponins A and 1 against human pathogenic fungi. Archives of Pharmacol Research. 1998;21:688-691.
13. Rai S, Wahile A, Mukherjeek PK, Saha BP, Mukheek A. Antioxidant activity of *Nelumbo mucifera* (sacred lotus) seeds. Journal of Ethnopharmacol. 2006;104:322-327.
14. Douglas WM, Brian DG, William BB, Shukal B, Paul OG, Paul H, Segarra-Newnham. Population mobility, globalization, and antimicrobial drug reststance. Emerg. Infect. Dis. 2009;15(11):1727-1732.
15. Martson A, Wolfender JL, Hostettmann K. Analysis and isolation of saponins from plant material. In saponins in food, feedstuffs and medicinal plants. Annual Proceedings of the Phytochemical Society. Clarendon Press, London. 2000;120-138.
16. AOAC. Official methods of analysis 15th ed. Association of Official Analytical Chemist, Washington D.C. 1990;777-784.
17. Park HJ, Kwon SH, Lee JH, Lee KH, Miyamoto K, Lee KT. Kalopanx saponin A is a basic saponin structure for the anti-tumor activity of hedragnin mono-desmosides. Planta Medica. 2001;67:118-121.
18. Fernandez R, Takahashi F, Liu Z, Steward R, Stein D, Stanley ER. The Drosophila shark tyrosine kinase is required for embryonic dorsal closure. Genes Dev. 2000;14(5):604-614.
19. Oyekanmi BA, Osho IB. Antimicrobial, phytochemical and pharmacological properties of *Phyllanthus niruri* Linn. FASEB Journal of Pharmacology. 2016; 30(1):cxv.
20. Nishiura J, Campus A, Boim M, Schor N. Effect of *Phyllanthus niruri* on urinary calcium levels in calcium stone forming patients. Journal of Clinical and Laboratory Investigation of Urolothies and Related Areas. 2005;32(15):362-366.
21. Nwanjo HU. Studies on the effect of aqueous extract of *Phyllanthus niruri* leaf on plasma glucose level and some hepatospecific marker in diabetic Wistar rats. The Internet Journal of Laboratory Medicine. 2007;2(2):55-62.
22. Ibrahim D, Hong LS, Kuppan N. Antimicrobial activity of crude methanolic extract from *Phyllanthus niruri*. Nat Prod Commun. 2013;8(4):493-496.
23. Miyakoshi M, Tamura Y, Masuda H, Mizutani K, Tanaka O, Ikeda T, Ohtani K, Kasai R, Yamasaki K. Antiyeast steroidal saponins from *Yucca schidigera* (Mohave yucca), a new anti-food deteriorating agent. Journal of Natural Products. 2000;63:332-338.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/27208