Comparative Therapeutic Phytoconstituents of *Senecio biafrea* (Oliv. and Hiern) J. Moore and *Vernonia amygdalina* Del.

Osiyemi, O. A., Adejoh O. P., Ugbogu. A. O.*

Forestry Research Institute of Nigeria, Ibadan.

KEYWORDS

Senecio biafrea,
Vernonia amygdalina,
Phytoconstituents,
Therapeutic,
Flavonoid

ABSTRACT

The therapeutic chemical constituents' investigation of Senecio biafrae shoot and Vernonia amygdalina leaf, which are commonly used in the Nigerian ethnomedicine, were studied. Phytochemicals viz: cardiac glycosides, tannin, cyanogenic glycosides, flavonoid, alkaloid and saponin in the dried powdered plants samples were analysed quantitatively by spectrophotometric and titrimetic methods. In the results, cardiac glycoside was the most abundant in V. amygdalina (8.79 ± 0.02 mg/g [or 57.1 %]) and S. biafrae (6.07 ± 0.07 mg/g [or 42.24 %]), with the former significantly (P<0.01) higher. Flavonoid (5.79 ± 0.08 mg/g [or 28.3 %]) and tannin (0.47 ± 0.02 mg/g [or 3.1 %]) contents in V. amygdalina were higher in comparison with the flavonoid (3.55 ± 0.01 mg/g [or 24.70 %]) and tannin (0.28 ± 0.01 mg/g [or 1.95 %]) in S. biafrae. The alkaloid in S. biafrae (4.1 ± 0.04 mg/g [or 28.53 %]) was higher in comparison with that of V. amygdalina (0.1± 0.01 mg/g [or 0.6 %]). The cyanogenic glycosides (0.26 ± 0.006 mg/g [or 1.81 %]) in S. biafrae was more in comparison with that of V. amygdalina (0.12 ± 0.02 mg/g [or 0.3 %]). The saponin content of V. amygdalina (0.13 ± 0.04 mg/g [or 0.6 %]) and S. biafrae (0.11 ± 0.03 mg/g [or 0.77 %]) were relatively low with no significant difference. However, the presence of these therapeutic secondary metabolites in S. biafrae and V. amygdalina supports their uses in the herbal preparations for the treatment of some diseases like malaria, diabetes and infertility in Nigeria.

*CORRESPONDING AUTHOR

osinyemi2004@yahoo.com

INTRODUCTION

All over the world, vegetables have been used for many years in daily life to treat diseases (Krishna *et al*., 2022). More than three-quarters of the world's population depend on complementary and alternative vegetable-based medicine for health care (Edirne, 2010). The healing potential of vegetables has been attributed to their therapeutic chemical constituents known as phytochemicals. Phytochemicals like cardiac glycosides, alkaloid, flavonoid and saponin play active roles in amelioration of diseases (Edeoga, 2005). They have been found to possess antidiabetic, antioxidant, antimicrobial, cancer preventive and antimalaria activities (Ajaiyeoba *et al*., 2006 Tanimowo *et al*., 2009; Osiyemi *et al*., 2013). However, the levels of these plants chemicals may vary depending on specie and variety (Onyeka *et al*., 2007).

Senecio biafrae and *Vernonia amygdalina* are vegetables in Asteraceae family. S. biafrae grows as undercover in tree crop plantation. It is a perennial climbing herb, with stem up to 3 m long, strongly branched; branches succulent and glabrous. Leaves alternate, simple or deeply pinnately lobed, more or less succulent (Bello *et al*., 2018). Its common name is "English spinach", also called "worowo" and "Ota eke" by the Yorubas and the Igbos, respectively in Nigeria (Odugbemi, 2006). It is cultivated as vegetable on a small scale, mainly in Nigeria and Cameroon. S. biafrae is known for its therapeutic virtues, notably among the Yoruba speaking people of South-western Nigeria where its leaf extract is used to stop bleeding from cuts or injury (Adelakun *et al*., 2018) and for the treatment of diabetes or pulmonary defects (Adebayo, 2009). It is used in traditional medicine to treat many other diseases such as oedema, cough, infertility, sore eyes and rheumatic pain in Benin, Côte d'Ivoire and Cameroon (Adebooye, 2004). *V. amygdalina* is a small shrub that grows in the tropical Africa with petiolate leaf of about 6 mm diameter and elliptic shape. It is commonly called "bitter leaf" and "ewuro" by the Yorubas. The plant has being used traditionally to treat malaria and gonorrhea in Nigeria and Uganda (Shaa *et al*., 2011), diabetes, kidney disease, jaundice, ascaris and stomach discomfort (Odugbemi *et al*., 2007). Hence, this study aims to estimate and assess the phytochemical contents in *S. biafrae* and *V. amygdalina* for justification of their folk claims as medicine.

MATERIALS AND METHODS

Plant Collection and processing

Senecio biafrae shoot and *Vernonia amygdalina* leaves were collected in Ibadan, Oyo state, Nigeria. The plants were authenticated in the Forestry Herbarium Ibadan (FHI) and a voucher specimen deposited there. The plants materials were air dried and ground into powder.

Quantitative analysis of phytochemicals

Determination of alkaloids

The alkaline precipitation gravimetric method (Harborne, 1998) was utilized. In brief, five grams (5 g) of the grind plant sample was added to 200 mL of 10% acetic acid solution in ethanol. The mixture was extracted for 4 hrs. at 28ºC and later filtered. The filtrate was reduced to one quarter of its original volume by evaporation on a water bath. Concentrated ammonium hydroxide was added to the extract in drops until alkaloid was precipitated. The alkaloid precipitate was collected in a weighted filter paper, washed with 1% ammonia solution and dried in the oven at 80ºC. Alkaloid content was determined and expressed as a percentage of the weight of sample analyzed (Obadoni *et al*., 2001).

Determination of cardiac glycosides

The protocol of El-oleny *et al*., (1994) using Buljet's reagent (95 mL aqueous picric acid + 5 mL 10% aqueous NaOH) was used to evaluate cardiac glycosides in the plant sample. One gram of the pulverised plant sample was macerated in 100 mL of 70% alcohol for 2 hrs. and then filtered. Thereafter, the extract was then purified with lead acetate and Na_2HPO_4 solution before the addition of freshly prepared Buljet's reagent. The intensity of the colours produced was then measured using a spectrophotometer at 495 nm. The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples shows the absorbance which is proportional to the concentration of the glycosides.

Determination of tannin

One gram (1 g) of the fine powder sample was measured into a beaker. The sample was soaked with solvent mixture (80 mL of acetone and 20 mL of glacial acetic acid) for 5hrs. to extract tannin. The mixture was filtered and set of standard solution of tannic acid was prepared ranging from 10ppm to 50 ppm. The absorbances of the standard solutions as well as that of the filtrate were read at 500 nm on a spectrometric 20. The percentage tannin was calculated.

Determination of total flavonoid content

The total flavonoid content was done by using the methodology of Sakanaka *et al*., (2005). 1 mL of the plant extract or + (catechin standard solution (50-250 mg/mL was added to 5 mL of distilled water in a test tube. 0.3 mL of a 5% (w/v) sodium nitrite solution was also added and the mixture was left for 6 mins. Thereafter, 0.6 mL of 10% (w/v) $AlCl_3.6H_2O$ solution was added and the mixture was allowed to stand for a further 5min. before 2 mL of 1M NaOH was added. The mixture was made up to 10 mL with distilled water and mixed well. The absorbance was determined immediately at 510 nm values of triplicate analysis were expressed as mg of (±) catechin equivalents per gram of total extractable compounds.

Determination of saponin

A weight 2 g of powdered plant sample was measured into a 250 mL beaker and 100 mL of isobutylacohol (octanol) was added and left for 5 hrs. on a shaker. The mixture was then filtered using a No. 1 whatman filter paper. The filtrate is transferred to another 100 mL beaker and saturated with magnesium carbonate solution. The mixture obtained here was then filtered to obtain a clear colourless solution. This was read on a spectrophotometer at 380 nm. 0 ppm -10 ppm of standard saponin solutions were prepared from 1000 ppm saponin stock standard solution and saturated with magnesium carbonate as above which was also filtered. The absorbances of the saponin standard solutions were also read at 380 nm to obtain the gradient of plotted curve.

Determination of cyanogenic glycosides

A weighed quantity (5 g) of the powdered plant sample was dispensed into 250 mL conical flask. The sample was incubated for 16hrs. at 38ºC. After, it was extracted with 95% methanol and filtered using double layer of hardened filter paper. Distillation was done with Marharm distillation apparatus. The sample extracted was transferred into a tow-necked 500 mL flask connected with a steam generator. This was steam distilled with saturated sodium bicarbonate solution contained in a 50 mL conical flask for 60 minutes. 1 mL of starch indicator was added to 20 mL of each distillate and was titrated with 0.2N of iodine solution. The percentage hydrocyanide was calculated.

Data analysis

All tests were carried out in triplicate and the Microsoft Excel 2007 was used to compute mean and standard deviation (SD). The student t-test was used to compare the means and values of $P \leq 0.05$ were considered significant. Results were expressed as mean ± SD and relative percentage of mean.

RESULTS AND DISCUSSION

Reorientation on the choices of plant-based foods consumed has become essential for humans overall wellness. Consumption of sufficient plant-based foods like vegetables could be an important measure towards it. However, certain vegetables posses more significance quantity of therapeutic phytoconstituents than others as they boost immunity and treat diseases. Thus, daily intake of vegetables with sufficient therapeutic phytoconstituents is *sine qua non*. The therapeutic properties of vegetables are attributed to the presence of certain phytoconstituents like cardiac glycosides, tannin, cyanogenic glycosides, flavonoid, alkaloid and saponin.

Table 1: Mean weight (mg/g) of the phytochemicals in *S. biafrae* shoot and *V. amygdalina* leaf

Phytochemicals	***S. biafrae***		***V. amygdalina***	
	Mean and standard deviation (mg/g)	**Relative % of mean**	**Mean and standard deviation (mg/g)**	**Relative % of mean**
Alkaloid	$4.1 \pm 0.04^{**}$	28.53	$0.1 \pm 0.01^{**}$	0.6
Cardiac glycosides	$6.07 \pm 0.07^{**}$	42.24	$8.79 \pm 0.02^{**}$	57.1
Tannin	$0.28 \pm 0.01^{*}$	1.95	$0.47 \pm 0.02^{*}$	3.1
Saponin	0.11 ± 0.03	0.77	0.13 ± 0.04	0.8
Flavonoid	$3.55 \pm 0.01^{**}$	24.70	$5.79 \pm 0.08^{**}$	37.6
Cyanogenic glycosides	$0.26 \pm 0.006^{**}$	1.81	$0.12 \pm 0.02^{**}$	0.8
Total	14.37	100	15.4	100

$^{*}P < 0.05$, $^{**}P < 0.01$

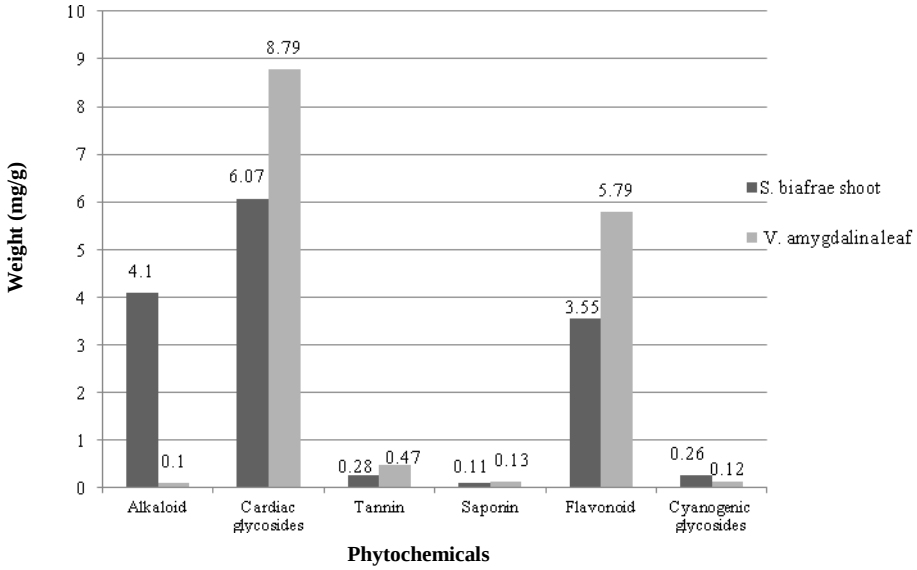

Figure 1: Comparative weights of some phytochemicals in *S. biafrae* shoot and *V. amygdalina* leaf

The results of work done on the vegetables in Figs. 2 and 3 showed that cardiac glycosides was the most abundant in *V. amygdalina* (8.79 ± 0.02 mg/g [or 57.1 %]) and *S. biafrae* (6.07 ± 0.07 mg/g [or 42.24 %]), with the former significantly ($P<0.01$) higher (Table 1). Cardiac glycosides belong to steroid-sugar hybrids, usually used for the treatment of cardiac failure (Hou *et al*., 2021) and also exhibit excellent anticancer activity (Wang *et al*., 2014). Cardiac glycosides from *Streptocaulon juventas* (Apocynaceae) inhibit HepG2 cell growth proliferation (Zhu *et al*., 2018), and the one from *Impatiens glandulifera* (Balsaminaceae) was active against A549, U373 and SKMEL-28 cancer cell lines (Cimmino *et al*., 2016).

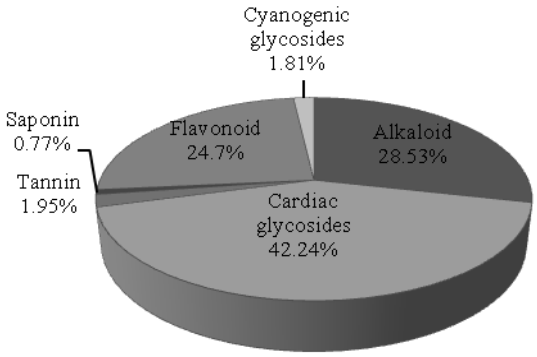

Figure 2: Relative percentages of some phytochemicals in *S. biafrae* shoot

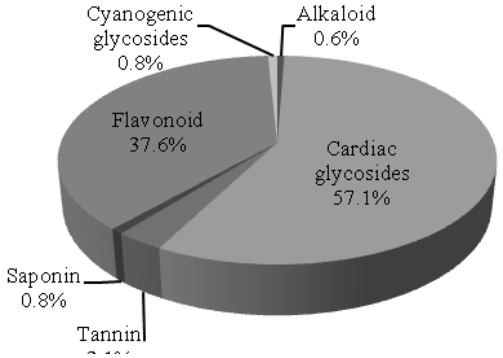

Figure 3: Relative percentages of some phytochemicals in *V. amygdalina* leaf

Flav *ɬalina* are significantly (P<0.05) higher in comparison with the flavonoid (3.55 ± 0.01 mg/g [or 24.70 %]) and tannin (0.28 ± 0.01 mg/g [or 1.95 %]) in *S. biafrae.* It has been revealed that the average intake of flavonoids by humans on a normal diet is estimated at 650 mg per day (Liu, 2013). Myriads of studies have reported the bioactivities of flavonoid compounds to include antioxidant, anti-inflammatory, antiallergic, anticancer, cardioprotective and anti-diabetic activities (Karak, 2019; Fallah *et al*., 2020). Moreover, it was shown that flavonoids were quite efficient in obesity prevention and weight control (Akhlaghi *et al*., 2018). Plant tannins are polyphenolic substances with beneficial effects for controlling chronic disorders particularly diabetes mellitus (Mohammed and Mohamed, 2019). Thus, the presence of flavonoid and tannin in these vegetables warranted their folk use especially as antioxidant and antidiabetic. *V. amygdalina* contains flavonoid, about 2 times more than that in *S. biafrae* (Fig. 1). This observation tends to indicate the more free radical scavenging effect of the former.

The alkaloid in *S. biafrae* (4.1 ± 0.04 mg/g [or 28.53 %]) was significantly (P<0.01) higher than in *V. amygdalina* (0.1± 0.01 mg/g [or 0.6 %]). Plant alkaloids have played a key role in traditional medicines as sedatives, antitussives, purgatives, and treatments for a wide variety of ailments (Gutiérrez-Grijalva *et al*., 2020). Currently, several alkaloids have served as templates for modern drugs, and there are several alkaloids used in pharmacology, such as codeine, brucine, morphine, ephedrine, and quinine (Aniszewski, 2015)

The cyanogenic glycosides (0.26 ± 0.006 mg/g [or 1.81 %]) in *S. biafrae* is significantly (P<0.01) more in comparison with that of *V. amygdalina* (0.12 ± 0.02 mg/g [or 0.3 %]). Cyanogenic glycosides are leading toxins (Vetter, 2000), their concentration is often higher in seedlings and young leaves than in mature plants (Nahrstedt, 1985). Cyanogenic glycosides have been reported to cause adverse health effects in humans, e.g., irreversible paralytic disorder, neurosensory deafness, and goiter (Chikezie *et al*., 2015). From this present study, the cyanogenic glycosides content of *S. biafrae* shoot was a little more than the toxic dose (>0.2 mg/g) hence, consumers should take caution. Moreover, a cyanogenic glycoside, amygdalin, from *V. amygdalin* has been investigated as a potential anticancer agent (Barakat *et al*., 2020). Recent results have indicated a potential neuroprotective action of a cyanogenic glycoside, prunasin 2′,3′,4′,6′-tetra-O-gallate (Tan *et al*., 2012). In addition, applications of cyanogenic glycosides from *Cardiospermum* sp. in medicine against rheumatoid arthritis have been patented (Sun *et al*., 2017).

The saponin content of *V. amygdalina* (0.13 ± 0.04 mg/g [or 0.6 %]) and *S. biafrae* (0.11 ± 0.03 mg/g [or 0.77 %]) were relatively in trace (Figs. 2 and 3) with no significant difference (Table 1). The main health effects of saponins are serum cholesterol lowering, anticoagulant, cardiovascular protection and adjuvant (Singh and Chaudhuri, 2018). Moreover, saponins have a prominent advantage as they are needed in low dose for adjuvant activity (Rajput *et al*., 2007).

CONCLUSION AND RECOMMENDATION

The presence of the phytoconstituents revealed in *S. biafrae* shoot and *V. amygdalina* leaves accounted for the various medicinal claims on these vegetables for the treatment of infections and diseases. Such information may be encouraging for researchers to carry out further advanced research on plants with potential therapeutic properties, in order to give preference to their cultivation, consumption and sustainable conservation.

REFERENCES

Adebayo, A. G. (2009). Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria. *Journal of Ethnopharmacology*, 121, 135–139.

Adebooye, O. C. (2004). *Solanecio biafrae (Oliv. and Hiern) C.Jeffrey*. PROTA, Wageningen, Netherlands.

Adelakun, S. A., Babatunde, O., Olusegun, O., and Oyebowale, O. O. (2018). Role of aqueous crude leaf extract of Senecio biafrae combined with zinc on testicular function of adult male sprague dawley rats. *Journal of Family and Reproductive Health*, 12(1), 8–17.

Ajaiyeoba, E. O., Abiodun, O. O., Falade, M. O., Ogbole, N. O., Ashidi, J. S., Happi, T. C., and Akinboye, D. O. (2006). In vitro cytotoxicity of 20 plants used in the Nigerian antimalarial ethnomedicine. *Phytomedicine*. *13*, 295-298.

Akhlaghi, M., Ghobadi, S., Hosseini, M. M., Gholami, Z., and Mohammadian, F. (2018). Flavanols are potential anti-obesity agents, a systematic review and meta-analysis of controlled clinical trials. *Nutrition, Metabolism and Cardiovascular Diseases*, 28, 675–690.

Aniszewski, T. (2015). *Alkaloids: chemistry biology ecology and applications*. Elsevier, Pacific Grove, CA.

Barakat, H. (2020). Amygdalin as a plant-based bioactive constituent. A mini review on intervention with gut microbiota anticancer mechanisms bioavailability and microencapsulation. *Proceedings*, *61*, 15.

Bello, O. A., Ayanda, O. I., Aworunse, O. S., Olukanmi, B. I., Soladoye, M. O., Esan, E. B., and Obembe, O. O. (2018). Solanecio biafrae: An underutilized nutraceutically-important African indigenous vegetable. *Pharmacognosy Reviews*, 12, 128–132**.**

Chikezie, P. C., Ibegbulem, C. O., and Mbagwu, F. N. (2015). Bioactive principles from medicinal plants. *Research Journal in Phytochemistry*, *9*, 88–115.

Cimmino, A., Mathieu, V., Evidente, M., Ferderin, M., Banuls, L. M. Y., Masi, M., Carvalho, A. D., Kiss, R., and Evidente, A. (2016). Glanduliferins A and B, two new glucosylated steroids from Impatiens glandulifera, with in vitro growth inhibitory activity in human cancer cells. *Fitoterapia*, 109, 138–145.

Edeoga, H. O., Okwu, D. E., and Hmbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, *4*, 685-688.

Edirne, T., Arica, S. G., Gucuk, S., Yildhizan, R., Kolusari, A., Adali, E., and Can, M. (2010). Use of complementary and alternative medicines by a sample of Turkish women for infertility enhancement: a descriptive study. *Journal of Alternative and Complementary Medicine*, 10, 11.

El-olemy, M. M., Al-Muhtadi, F. J., and Afifi, A. F. A. (1994). *Experimental phytochemistry: A laboratory manual*. King Saud University Press, Saudi Arabia.

Fallah, A. A., Sarmast, E., Fatehi, P., and Jafari, T. (2020). Impact of dietary anthocyanins on systemic and vascular inflammation: Systematic review and meta-analysis on randomised clinical trials. *Food and Chemical Toxicology*, 135, 110922.

Gutiérrez-Grijalva, E. P., López-Martínez, L. X., Contreras-Angulo, L. A., Elizalde-Romero, C. A., Heredia, J. B. (2020). Plant Alkaloids: Structures and Bioactive Properties. In: Swamy, M. (eds) Plant-derived Bioactives. Springer, Singapore

Harbone, J. B. (1998). *Phytochemical methods*. 1st ed. Chapman and Hall Ltd. London.

Hou, Y., Shang, C., Meng, T., and Lou, W. (2021). Anticancer potential of cardiac glycosides and steroid-azole hybrids. *Steroids, 171,* 108852. doi:10.1016/j.steroids.2021.108852

Karak, P. (2019). Biological activities of flavonoids: An overview. *International Journal of Pharmaceutical Sciences and Research*, 10(4), 1567–1574.

Krishna, H., Janakiram, T., Singh, M. K., Karuppaiah, V., Yadava, R. B., Prasad, R. N., Singh, J., and Behera, T. K. (2022). Immunomodulatory potential of vegetables vis-à-vis human health. *The Journal of Horticultural Science and Biotechnology*, 97(5), 560-579.

Liu, R. H. (2013). Dietary bioactive compounds and their health implications. *Journal of Food Science*, 78, A18–A25. https://doi.org/10.1111/1750-3841.12101

Mohammed, A., and Mohamed, E. (2019). The promising role of plant tannins as bioactive antidiabetic agents. *Current Medicinal Chemistry*, *26*, 4852-4884.

Nahrstedt, A. (1985). Cyanogenic compounds as protecting agents for organisms. *Plant Systematic and Evolution*, *150*, 35–47.

Obadoni, B. O., and Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science*, *8b*, 203-208.

Odugbemi, T. O. (2006). *Outlines and Pictures of Medicinal Plants from Nigeria*. (1st ed.) University of Lagos Press, Lagos.

Odugbemi, T. O., Akinsulire, O. R., AibinuI, E., and Fabiku, P. O. (2007). Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, southwest Nigeria. *African Journal of Traditional Complementary and Alternative Medicines*, *4*(2), 191-198.

Onyeka, E. U., and Nwambekwe, I. O. (2007). Phytochemical profile of some green leafy vegetables in south east Nigeria. *Nigerian Food Journal*, *25*(1), 67-76.

Osiyemi, O. A., Adesokan, H. K., Cadmus, S. I. B., Ajaiyeoba, E. O., and Itiola, O. A. (2013). Antitubercular activities of five medicinal plants against two strains of *Mycobacterium tuberculosis. PharmacologyOnline*, *1*, 201-205.

Rajput, Z. I., Hu, S., Xiao, C., and Arijo, A. G. (2007). Adjuvant effects of saponins on animal immune responses. *Journal of Zhejiang University Science* B, 8(3), 153–161.

Sakanaka, S. A., and Okada, Y. (2005). Preparation and antioxidant properties of leaf tea. *Food Chemistry*, *89*, 569-575.

Shaa, K. K., Oguche, S., Watila, I. M., and Ikpa, T. F. (2011). In vitro antimalarial activity of the extracts of Vernonia amygdalina commonly used in traditional medicine in Nigeria. *Science World Journal*, *6*(2), 5-9.

Singh, D., and Chaudhuri, P. K. (2018). Structural characteristics, bioavailability and cardioprotective potential of saponins. *Integrative Medicine Research*, 7(1), 33-43.

Sun, S., Qu, G., Wu, C., Zhang, H., Sun, Y., Liu, J., Yi, P., and Jing, J. (20170725). CN106974921 A: Preparation of (2R)-cardiospermin-5-phydroxybenzoate and application thereof in preparation of medicine for treating rheumatoid arthritis. *Faming Zhuanli Shenqing*, 1–4.

Tan, H. P., Wong, D. Z. H., Ling, S. K., Chuah, C. H., and Kadir, H. A. (2012). Neuroprotective activity of galloylated cyanogenic glucosides and hydrolysable tannins isolated from leaves of Phyllagathis rotundifolia. *Fitoterapia*, *83*, 223–229.

Tanimowo, W. O., Ashidi, J. S., and Osiyemi, O. A. (2009). Antihyperglycaemic and antibacteria potentials of aqueous methanolic extract of *Anthocleista djalonensis* A. Chev in Swiss albino rats and bacteria species. *Journal of Biological and Health Development*, *1*, 36-41.

Vetter, J. (2000). Plant cyanogenic glycosides. *Toxicon*, *38*, 11–36.

Wang, Y., Shao, R. G., Jiang, J. D., and Wang, Z., (2014). Research progress in anti-cancer mechanisms of cardiac glycosides. *Chinese Journal of New Drugs*, 23(6), 677–681.

Zhu, W., Su, S., Xu, Y., Xie, Z., Bai, Y., Liu, W., Abe, M., Akihisa, T., Feng, F., Zhang, J., (2018). C21 steroids from Streptocaulon juventas (Lour) Merr. induce apoptosis in HepG2. *Steroids*, 140, 167–172.