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

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

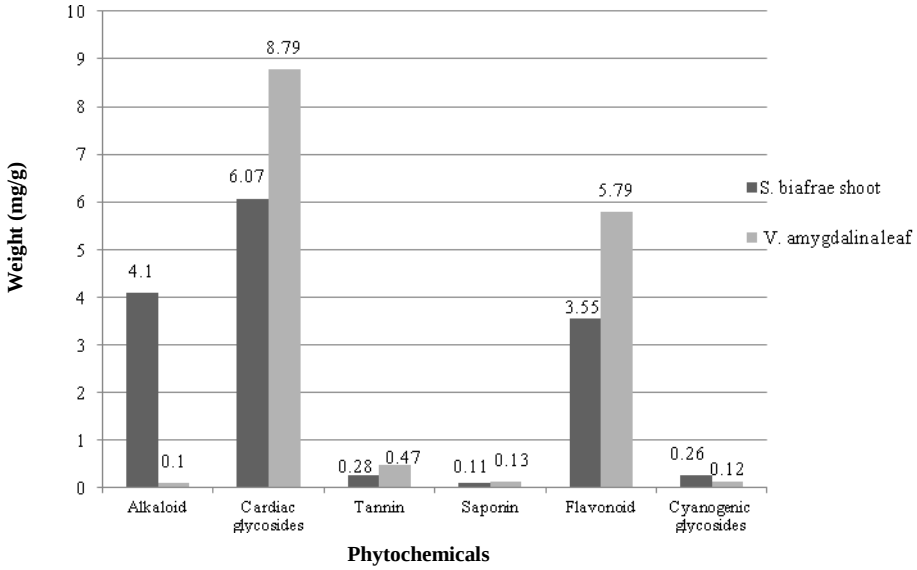


Figure 1: Comparative weights of some phytochemicals in *S. biafrae* shoot and *V. amygdalina* leaf

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).

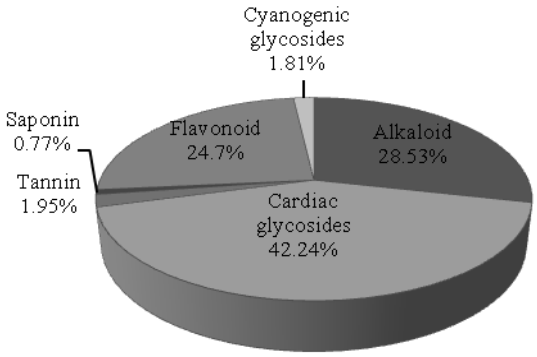


Figure 2: Relative percentages of some phytochemicals in *S. biafrae* shoot

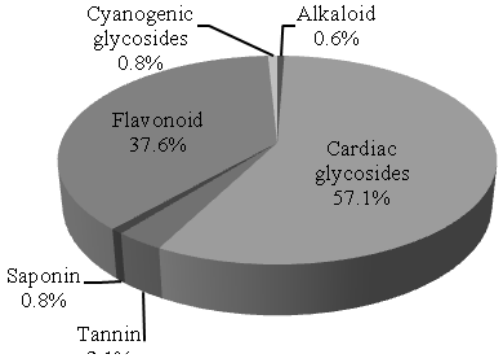


Figure 3: Relative percentages of some phytochemicals in *V. amygdalina* leaf

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.

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