Nigerian Journal of Pharmaceutical and Applied Science Research, 6(2):19-23, September 2017

Evaluation of the phytochemical constituents and antioxidant activity of the stem of *Senecio biafrae* (Asteraceae)

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ABSTRACT

The use of herbal products is of global importance because of their low side effects, accessibility and affordability when compared with conventional medicine. The objective of this study was to investigate the phytochemical content and *in-vitro* antioxidants activities of the methanol extract of *Senecio biafrae* stem. The methanol extract was obtained by cold maceration method. Phytochemical investigation was carried out using standard procedures. *In-vitro* anti-oxidant activity was estimated by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP) assay. The qualitative analysis revealed the presence of alkaloids, reducing sugars, cardiac glycosides, steroids, flavonoids and saponins while tannins and anthraquinones were absent in the extracts. The DPPH assay showed percentage inhibition ranging from 40.08±0.26 to 47.27±0.02% for *Seneco biafrae* stem extract, 96.64±0.07 to 98.76±0.01% for ascorbic acid and 54.62±0.06 to 58.99±0.06% for alpha tocopherol, with IC_{50} 112.58 µg/ml, 5.66 µg/ml and 18.51 µg/ml respectively. The FRAP assay of the extract was 107 µM Fe^{2+}/g dry extract while the positive control (ascorbic acid) had a value of 272.5 µMFe^{2+}/g dry material. This study shows that the methanol extract of *Senecio biafrae* stem possesses various phytochemical constituents, has antioxidant potential and could be very useful in the prevention and treatment of various diseases and ailments.

Key words: Phytochemicals, *Senecio biafrae,* Antioxidant, 2, 2-diphenyl-1-picryl hydrazyl (DPPH)

INTRODUCTION

Senecio biafrae Olive & Hiern belongs to the family Asteraceae. It occurs naturally in the forest zone most especially in Cocoa plantation. *Senecio biafrae* (which is called "worowo" among the Yorubas in Nigeria) leaf extract is used to stop bleeding from cuts or injury and treatment of eye sores (Adebooye, 2001). Fresh succulent leaves of *Senecio biafrae* (*S. biafrae*) are used as a leafy vegetable in Sierra Leone, Ghana, Benin, Nigeria, Cameroon and Gabon. They are especially popular in southwest Nigeria. The high edible mucilaginous fibre, leaves and stem are used to treat indigestion or as laxative (Fowomola and Akindahunsi, 2005). In the Western region of Cameroon, *S. biafrae* is principally used for fertility improvement and the treatment of some reproductive tract ailments that can impair fertility of women (Telefo *et al*., 2011). Literature also shows that *S. biafrae* contains many secondary metabolites of different polarities such as dihydroisocoumarins, terpenoids, sesquiterpenes, amino acids etc, which can play many therapeutic roles (Dairo and Adanlawo, 2007). Study on *S. biafrae* leaves and stems extracts reveals that the levels of ovarian cholesterol and protein as well as uterine protein were doubled principally in animals (immature female rats) treated with the ethyl acetate fraction, no matter what dose was administered (Landry *et al*., 2015). The proximate analysis results of *S. biafrae* leaves revealed the presence of protein (14.26±2.01%) and fibre (15.78±0.13%), the observed minerals content carried out using atomic absorption spectrophotometer were sodium, iron, potassium, aluminium, calcium, zinc, selenium, magnesium and colbalt (Ajiboye *et al.,* 2013a). The leaves also contained essential fatty acid (Linoleic, linolenic and arachidonic acids), indispensable amino acid (threonine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan and histidine) and vitamins such as vitamin E, C, K, A and B complex (Ajiboye *et al*.,2013b). The qualitative phytochemical constituents of the leaves of *S. biafrae* consumed in Ekiti State, Nigeria showed the presence of alkaloids, tannins, phlobatanins, phenols, saponins, terpenes, steroids, flavonoids, glycosides and chalcones (Ajiboye *et al*., 2013b). The effect of aqueous leaf extract of *Senecio biafrae* on hyperglyceic and haematological parameters of alloxan-iduced diabetic in rat, suggest that the extract have both hypoglycaemic and antianaemic properties (Ajiboye and Ojo, 2014).

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Antioxidants prevent oxidative damage by inhibiting the action of free radicals such as hydroxyl (OH-), superoxide (O-), nitric oxide (NO-), nitrogen dioxide (NO-2), peroxyl (ROO-) and non-free radical like hydrogen peroxide and singlet oxygen (Hamzah *et al.*, 2013). This had been implicated in the aetiology of these pathological conditions related to cardiovascular diseases, diabetes, inflammatory diseases, cancer, ageing process and perhaps dementia (Aruoma, 2003; Amin *et al.*, 2004; Knekt *et al.*, 2002). The previous study of free radical scavenging capacity based on 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay for *Senecio biafrae* root extracts gave percentage inhibition: 14.05±0.78% to 50.88±1.08% for aqueous extract, 17.72±1.29% to 42.15±1.30% for ethanol extract, 10.43±1.71% to 26.23±0.94% for acetone extract and 16.09±0.74% to 39.47±0.68% for petroleum ether extract and the standard drug, ascorbic acid (87.21±0.82%-99.46±2.54% in the concentration of 0.05-1 mg/ml) (Okoro *et al.*, 2014). *S. biafrae* leaves extract showed percentage inhibition of 64.15% based on DPPH assay at 1mg/ml (Azeez *et al*., 2015). The reducing power of aqueous, ethanol, acetone and petroleum ether extract of *Senecio Biafrae* root extract were 40, 28 70 and 72 mg/100 mg dry weight respectively (Okoro *et al*., 2014). Hence, the present study focuses on the evaluation of the phytochemical constituents and the antioxidant activity of the methanol stem extract of *Senecio biafrae*.

MATERIALS AND METHODS

DPPH (2, 2-diphenyl-1-picryl hydrazyl), Vitamin C, Vitamin E and TPTZ (2,4,6-tripyridyl-s-triazine) were obtained from Sigma Aldrich, USA. All other reagents and chemicals were of analytical grade and were freshly prepared. The double beam UV-Visible Spectrometer (PG Instruments Ltd, T80 +, S/N 151885-01-0094) was used to measure absorbance of the samples.

Collection of Plant Material

Senecio biafrae vegetables were purchased from Aramoko market in Ekiti state, Nigeria in August, 2014. The plant was authenticated by Mr Oyebanji of the Herbarium unit, Department of Botany, University of Lagos, Nigeria and a voucher specimen (a reference sample) was deposited with the voucher number LUH 6155. The stems were separated, dried at 45^0C and milled into uniform dry powder. Powdered stem materials were then weighed and kept in air-tight containers until further usage.

Preparation of Plant Extract

The powdered plant sample (80 g) was extracted in 1400 ml of methanol for 72 hours using cold extraction (maceration) method. The solution was filtered with a Whatman paper and concentrated by recovering the solvent at 40 °C in a rotary evaporator (Rotavapor Buchi model R-124). The concentrated solution was then completely dried in a ventilated oven at 45 °C. It was stored in the refrigerator prior to use.

Phytochemical Screening

Phytochemical screenings were carried out on the extract using standard procedures to identify the chemical constituents as described by Tease and Evans (2005).

Antioxidant screening

Two methods were employed for the determination of the in-vitro antioxidant activities of the methanol extract of *Seneco biafrae* stem.

DPPH Radical Scavenging Activity

The ability of the plant extract to scavenge 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radicals was assessed by the standard method (Hirano *et al*., 2001). The stock solution of the extract was prepared in methanol to achieve the concentration of 100 µg/ml. Dilutions were made to obtain concentrations of 20, 40, 60 and 80 µg/ml. Diluted solutions (2 ml each) were mixed with 0.5ml of 1mM DPPH methanol solution in test tubes. The mixture was shaken and allowed to stand for 15 min at room temperature and the reduction of the DPPH free radical was measured by reading the absorbance at 517nm using UV-Visible Spectrophotometer. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as control. Ascorbic acid and alpha-tocopherol were used as standards. The experiments were carried out in triplicate. Percentage inhibition was calculated using equation.

DPPH Scavenging effect (%) = $(A_0 – A_1 / A_0) \times 100$

Where A_0 = Absorbance of blank sample

A_1 = Absorbance of test sample or standard.

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Ferric Reducing Antioxidant Power (FRAP)

A modified method of Benzie and Strain (1996) was adopted for the ferric reducing antioxidant power (FRAP) assay. It depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ at low pH. Fe (III)-TPTZ has an intense blue colour which can be read at 593nm. Freshly prepared FRAP solution containing 25ml of 300 mM acetate buffer, 2.5ml of 10 mM 2,4,6-tripyridyl-5-triazine (TPTZ) in 40 mM HCl and 2.5ml of 20mM $FeCl_3.6H_2O$ was mixed with 1ml of

extract and the absorbance read at 593mM. The calibration curve was prepared with $FeSO_4.7H_2O$ and was linear between concentration 25 and 500µM. Results obtained were expressed in µM Fe^{2+}/ g of dry plant material and compared with that of ascorbic acid.

RESULTS

The phytochemical screening of the methanol extract of *Seneco biafrae* stem revealed the presence of alkaloids, reducing sugars, cardiac glycoside, steroids, flavonoids and saponins while tannins and anthraquinones were absent as shown in (Table 1).

The weight of the extract obtained was 6.92 g which corresponds to 8.65%w/w percentage yield.

The DPPH assay showed percentage inhibition range from 40.08±0.26– 47.27±0.02% for *Seneco biafrae* stem extract, 96.64±0.07 – 98.76±0.01% for ascorbic acid and 54.62±0.06 – 58.99±0.06% for alpha tocopherol (Figure 1), with IC_{50} 112.58 µg/ml, 5.66 µg/ml and 18.51 µg/ml respectively. The Ferric Reducing Antioxidant Power assay (FRAP) of the extract was 107 µM Fe^{2+}/g dry extract while the positive control (ascorbic acid) had a value of 272.5 µM$Fe^{2+/}$g dry material (Table 2).

Table 1: Qualitative phytochemical constituents of *Senecio biafrae* stem extracts

Phytochemicals	Extract
Alkaloids	+ ve
Reducing sugars	+ ve
Anthraquinone	- ve
Cardiac glycoside	+ ve
Steroids	+ ve
Tannins	- ve
Flavonoids	+ ve
Saponins	+ ve

+ve = present -ve = absent

Table 2: Total antioxidant activity (FRAP assay) of *Senecio biafrae* stem extracts

Sample/standard	FRAP (µM Fe^{2+}/g extract)
Senecio biafrae leaves extracts	147.4
Ascorbic acid	265.4

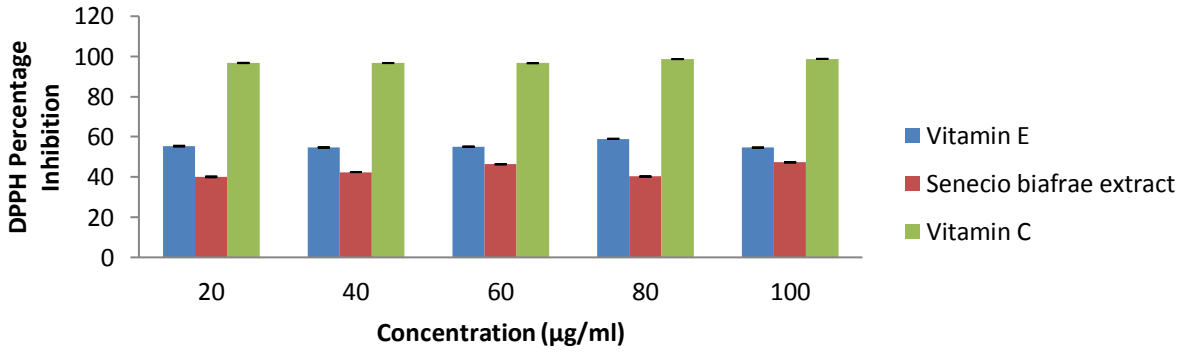

Figure 1: Percentage inhibition of DPPH against concentration of *Senecio biafae* stem extract

Standard error of mean (*n* = 3/group).

DISCUSSION

Most medicinal plants contain secondary metabolites responsible for observed biological activities. Phytochemical screening of the methanol extract of *Senecio biafrae* stem shows the presence of saponins, alkaloids, reducing sugars, cardiac glycoside, steroids, flavonoids while tannins and anthraquinones were absent as shown in (Table 1). Previous studies had shown the presence of alkaloids, tannins, phlobatannins, phenols, saponins, terpenes, steroids, flavonoids, glycosides and chalcones in the leaves of *S. biafrae* consumed in Ekiti State, Nigeria (Ajiboye *et al*., 2013b). While the roots of *Senecio biafrae were* rich in alkaloids, saponins, tannins, glycosides, phenols, flavonoids and steroids (Okoro *et al*., 2014). The presence of saponins in the stem extract supports the potentials of the plant to fight infections caused by parasites and in humans, saponins serves as immune system booster. The non-sugar part of saponins has a direct antioxidant activity which may result in reduced risk of cancer and heart diseases (Prohp and Onoagbe, 2012). Flavonoids have antioxidant activities as well as health promoting effects such as antiallergic, anticancer, antioxidant, antiinflammatory, antithrombotic, vasoprotective and antiviral effects. These effects have been associated with the influence of flavonoids on arachidonic acid metabolism (Trease and Evans, 2002). Alkaloids are the most effective phytochemical compounds in therapeutic uses (Okwu, 2005; Ayoola and Adeyeye, 2010). Several authors have reported the analgesic (Okwu and Okwu, 2004), antispasmodic and antibacterial activities of alkaloids (Stray, 1998). Actions of alkaloids are also felt in respiratory system, gastrointestinal tract, malignant diseases and malaria (Trease and Evans, 2002). Cardiac glycosides have a strong and direct action on the heart, helps in supporting its strength and rate of contraction when it is failing (Persinos and Quinmbly, 1967).

DPPH test shows that the ability of the plant extracts to act as a free radical scavenger, however it was less potent when compared to ascorbic acid and alpha-tocopherol (see figure 1). The higher the percentage inhibition of DPPH absorbance the higher the free radical scavenging activity. Previous studies on the ethanol extract of *S.biafrae* root reveals percentage inhibition of 17.72±1.29% to 42.15±1.30% (Okoro *et al*., 2014) while *S. biafrae* leaves extract showed percentage inhibition of 64.15% based on DPPH assay at 1 mg/ml (Azeez *et al.*, 2015). It is well known that vegetables are rich in various antioxidants, including ascorbic acid, carotenoids, and phenolic and can be considered as source of natural antioxidant. Many plants including vegetables had been categorized as sources of natural antioxidants that can protect against oxidative stress and thus play an important role in the chemoprevention of diseases that have their aetiology and pathophysiology in reactive oxygen species (Odukoya *et al.*, 2001; Dragland *et al.*, 2003). FRAP (Ferric reducing antioxidant power) is one of the most rapid test and very useful for routine analysis. The antioxidant activity was estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent. The reducing ability of the extract (107 μM Fe^{2+}/g) shows that it is less active when compared to ascorbic acid (272.5 μMFe^{2+}/g). This is expected from a crude extract. Isolation of active compounds from the extract may yield compounds with better antioxidant activity.

CONCLUSION

This study shows that the methanol extract of *Senecio biafrae* stem possesses various phytochemical constituents, has antioxidant potential and could be very useful in the prevention and treatment of various diseases and ailments.

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