Advances in Applied Sciences
2017; 2(5): 60-63
http://www.sciencepublishinggroup.com/j/aas
doi: 10.11648/j.aas.20170205.11

Investigation of Phytochemical and Proximate Components in Different Parts of *Boerhavia diffusa* L. and *B. erecta* L.

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To cite this article:

Chinelo Anthonia Ezeabara, Ujunwa Collete Nwiyi. Investigation of Phytochemical and Proximate Components in Different Parts of *Boerhavia diffusa* L. and *B. erecta* L. *Advances in Applied Sciences*. Vol. 2, No. 5, 2017, pp. 60-63. doi: 10.11648/j.aas.20170205.11

Received: March 21, 2017; **Accepted**: June 29, 2017; **Published**: September 18, 2017

Abstract: The phytochemical and proximate constituents of leaves, stem and root of *Boerhavia diffusa* L. and *B. erecta* L. were examined in order to determine the biochemical compositions of the two species of *Boerhavia*. The significant difference was established at $p<0.05$. The greatest level of anthraquinone and hydrogen cyanide were found in the leaves of *B. diffusa* and there were no significant differences in the amounts found in the root. Leaves and stem of *B. diffusa* had the greater values of tannin, saponin, phenol and terpenoid in comparison to *B. erecta,* while higher concentrations of alkaloid were detected in the leaves and stem of *B. erecta*. Considerable great percentages of crude protein were present in the leaves of *B. diffusa* and *B. erecta*, being 16.41±0.04 and 15.89±0.03 respectively. The various parts of *B. diffusa* and *B. erecta* could be regarded as good sources of bioactive compounds for drug formulations.

Keywords: Biochemicals, Tannin, Saponin, Phenol, Terpenoid, Alkaloid, Anthraquinone, Hydrogen Cyanide

1. Introduction

The genus *Boerhavia* L. is a member of Nyctaginaceae family. It is a genus of about 40 species distributed in the tropical and subtropical regions of the world and is especially diverse in south-western North America [1]. They are annual or perennial diffuse herbs, flowers in capitula or lax cymes; fruits narrowly obovoid with 3-5 prominent ribs, smooth or minutely and densely glandular [2]. Moreover, stems of *B. diffusa* L. are erect or ascending; leaves usually more than 2.5 cm long; flowers more than 1 mm in diameter; ultimately forming lax, much-branched, leafless; flowers rich magenta or crimson; stems usually glabrous; young leaves fringed with long brown, septate hairs, while for *B. erecta* L., the fruits are eglandular, not glutinous; inflorescences erect and much branched and leafless. In addition, four species of the genus were reported in Nigeria, namely: *Boerhavia coccinea* Mill., *B. erecta* L., *B. diffusa* L. and *B. repens* L. *B. coccinea* is currently known as *B. viscosa* Lag. & Rodr. [3]. *B. erecta* has similar properties to *B. diffusa*, and can be distinguished by its ascending to erect habit, multi-branched inflorescence with pale pinkish-white flowers and obconical glabrous fruit [3, 4]. The distinction between these two species is based on the morphology.

The literature is replete with the medicinal applications of these plants around the world. The whole plant parts of *B. diffusa* and *B. erecta* are traditionally used as medicine. Various ethnomedicinal uses of the plants in Nigeria were documented. The root of *B. erecta* is used in the treatment of whitlow by Lala people of Nigeria [5]. *B. erecta* plant infusion is used as a mild laxative, while *B. diffusa* is instilled into the eye for conjunctivitis [6]. The leaves of *B. diffusa* are used for treatment of diabetes, anti inflammatory, abscess and boils [7]. The leaves are also used for treatment of dysmenorrhoea and prevent abortion. In addition, the leaves and root tubers are used as antidote for scorpion bites and for treatment of hepatitis and liver problems, while the whole plant is used to prevent miscarriage [8]. *Boerhavia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values [9]. The aim of this study therefore, was to compare *B. diffusa* and *B. erecta* biochemically; while the objectives were to investigate the phytochemical and proximate compositions of the leaves, stem and root of both species.

2. Materials and Methods

2.1. Collection of Plant Materials

The leaves, stems and roots of *Boerhavia diffusa* and *B. erecta* were collected in the month of May at flowering stage. Both plants were collected from Nnamdi Azikiwe University Awka, Anambra State. The voucher specimens were deposited in the herbarium of Department of Botany of the same institute after proper authentication.

2.2. Preparation of Samples

The plant parts (leaves, stems and roots) were air dried at room temperature for three weeks and crushed with sterilized mortar and pestle. Electric grinder was used to grind the plant parts into powder and sieved with a muslin cloth. The powdered samples were stored in an air tight container for analyses.

2.3. Qualitative Phytochemical Screening

Qualitative phytochemical tests were conducted with the methods described by Ezeabara and Okonkwo [10].

2.4. Quantitative Phytochemical Screening

The concentrations of tannin and phenol were determined using the Folin Dennis spectrophotometric and folin-cio caltean colorimetric methods described by Pearson [11]. The alkaloid, flavonoid, saponin and steroid contents of the plants were determined using the gravimetric and double extraction gravimetric methods described by Harborne [12]. Hydrogen cyanide was determined by Alkaline Pikrate Colorimeter method of Trease and Evan [13]. Anthraquinone was determined with the method outlined by Ezeabara and Egwuoba [14]. The total terpenoid content of the two plant species was determined by the method described by Ferguson [15].

2.5. Proximate Analysis of Plant Materials

Fat content of the sample was determined by the continuous solvent extraction method using a soxhlex apparatus [11]. Carbohydrate, crude fibre, moisture and protein contents were determined by the difference, Wende, gravimetric and Kjeldahl digestion methods respectively [16]. Total Ash was done using the incineration gravimetric method [17].

2.6. Statistical Analysis

One–Way–Anova (F-Test) was used to statistically analyze the data at $p<0.05$. Then Duncan's multiple range test (DMRT) was used to compare the means and the values were expressed as mean ± standard error of three replicates.

3. Results and Discussion

Results showed that alkaloids, tannin, saponin, steroid, phenol, anthraquinone, flavonoid, terpenoid and hydrogen cyanide were present in the leaves, stem and root of both *B. diffusa* and *B. erecta* (Table 1). This explained the reason various parts of these plants are used as medicine for treatment of wide array of diseases worldwide. Alkaloid content was higher in leaves and stem of *B. erecta*, being 1.86±0.00 and 1.64±0.00% respectively, in comparison with those of *B. diffusa*. There was no significant difference between the levels of alkaloid in the root of both plants. The *in vitro* anticancer [18]; antiestrogenic [19]; antiamoebic [20]; immunomodulatory [21] and anti metastatic [22] activities of Punarnavine, an alkaloid isolated from *B. diffusa* has been reported [23]. There were also significant differences ($p<0.05$) between the concentrations of flavonoid found in the leaves of both plants as well as in the stem. Meanwhile, the root of *B. diffusa* had the higher flavonoid. Alkaloids, flavonoids, quinines, terpenes, triterpenoids, polyphenols, and to a lesser extend sterols are the most common phytochemicals from extracts of West African plants, which were reported for antiplasmodial activities [24]. The leaves and stem of *B. diffusa* contained the greater values of tannin. Higher levels of tannin were also found in leaves of *Mimosa invisa* and *M. pudica*, being 3.46±1.00 and 2.62±0.02% respectively [25]. Tannins have shown varieties of medicinal activities in animals and humans. Saponin contents were greater in the leaves and stem of *B. diffusa*, being 2.71±0.01 and 2.86±0.04% respectively, when compared with *B. erecta*. No significant difference ($p>0.05$) in the saponin level of the root of both plants. There were also no significant differences among the steroid concentrations of the leaves, stem and root of both species. It was reported that the extract of *B. diffusa* leaves produced hypoglycemic effect which might be as a result of the glycosides, flavonoids, tannins and saponins present in the extract [26]. Phenol contents of the leaves and the stem were higher in *B. erecta* at 0.18±0.01 and 0.17±0.00% respectively. Meanwhile, significant difference ($p<0.05$) in the level of phenol contents was found in the root of both plants. The levels of phenol were also greatest in the leaves and the stem of *M. invisa*, being 0.18±0.02 and 0.08±0.01%, respectively and *M. pudica*, being 0.17±0.01 and 0.08±0.03% respectively [25]. The concentrations of anthraquinone and hydrogen cyanide were greater in the leaves of *B. diffusa*, being 0.51±0.02 and 2.24±0.00% respectively and there was no significant difference among the values detected in the stem as well as the root. Hydrogen cyanide is a chemical present in some plants and is deadly to animals and humans when ingested in high dosage. High levels of hydrogen cyanide were also reported in the leaves of three species of *Stachytarpheta*: *S. cayannensis* (L. C. Rich.) Schau and *S. indica* (L.) Vahl, being 5.64±0.104 and 6.93±0.017 ml/kg respectively [27]; as well as *S. angustifolia* (Mill.) Vahl, being 6.36±0.05% [28]. In addition, great values were detected in the leaves of *Croton hirtus* L'Herit and *C. lobatus* Linn., being 2.53±0.11 and 2.20±0.44 mg/kg respectively [10]; leaves of *Oldenlandia corymbosa* L. and *O. herbacea* (L.) Roxb., being 2.29±0.01 and 3.41±0.01 mg/kg respectively [14]. Moreover, anthraquinone levels were high

in the leaves of *Oldenlandia corymbosa* and *O. herbacea*, being 2.78±0.03 and 2.26±0.00% respectively; as well as leaves of *Croton hirtus* and *C. lobatus*, being 3.42±0.00 and 1.86±0.21 mg/100g respectively [10]. These indicated that the leaves of plants probably synthesize greater values of anthraquinone and hydrogen cyanide. There is scanty of information on the therapeutic application of anthraquinone but it could be used as dyes. Terpenoid contents were higher in the leaves and stem of *B. diffusa*, being 0.51±0.02 and 0.41±0.01% and there was no significant difference between the quantities in the root of both plants. High values of terpenoid were also detected in the leaves of *Croton hirtus*, being 0.59±0.01 and the stem of *C. lobatus*, being 3.54±0.08 mg/100g [10].

The moisture content, dry matter, ash, crude fibre, fat, crude protein and carbohydrate were present in the parts of *B. diffusa and B. erecta* (Table 2). The moisture content of leaves and root of *B. diffusa* were higher than those of *B. erecta*, while the moisture content of the stem was greater in *B. erecta*, being 9.66±0.04%. The value of the dry matter was higher in stem of *B. diffusa* whereas those of leaves and the root were greater in *B. erecta.* Concentrations of ash were greater in the leaves and the stem of *B. erecta,* while the root of *B. diffusa* contained the higher amount. *B. erecta* had the higher crude fibre content in the leaves, stem and root. The fat levels of *B. diffusa* were higher in the leaves and the stem, being 5.45±0.02 and 3.17±0.01% respectively whereas; there was no significant difference in the fat contents of the root of both plants. The values of crude protein and carbohydrate were greater in the leaves and the stem of *B. diffusa,* while *B. erecta* had the higher quantities in the root. As a result of the nutritive contents of parts of *B. diffusa* and *B. erecta*, they could be considered as food.

Table 1. *Quantitative phytochemical compositions (dry matter) of leaves, stems and roots of B. diffusa and B. erecta.*

	B. diffusa			***B. erecta***		
Constituents (%)	**Leaves**	**Stem**	**Root**	**Leaves**	**Stem**	**Root**
Alkaloid	1.79 ± 0.01^{d}	1.53 ± 0.01^{b}	1.31 ± 0.01^{a}	1.86 ± 0.00^{c}	1.64 ± 0.00^{c}	1.24 ± 0.01^{a}
Flavonoid	1.14 ± 0.02^{c}	1.34 ± 0.02^{d}	0.91 ± 0.01^{b}	1.07 ± 0.01^{c}	1.26 ± 0.03^{d}	0.78 ± 0.02^{a}
Tannin	0.91 ± 0.02^{d}	0.96 ± 0.00^{d}	0.45 ± 0.02^{a}	0.75 ± 0.01^{c}	0.85 ± 0.00^{c}	0.57 ± 0.04^{b}
Saponin	2.71 ± 0.01^{c}	2.86 ± 0.04^{d}	0.77 ± 0.02^{a}	2.29 ± 0.01^{b}	2.66 ± 0.04^{c}	0.72 ± 0.00^{a}
Steriod	0.09 ± 0.01^{b}	0.13 ± 0.00^{c}	0.05 ± 0.01^{a}	0.07 ± 0.01^{b}	0.10 ± 0.00^{c}	0.05 ± 0.00^{a}
Phenol	0.18 ± 0.01^{c}	0.17 ± 0.00^{c}	0.09 ± 0.00^{a}	0.13 ± 0.01^{b}	0.16 ± 0.00^{b}	0.08 ± 0.00^{a}
HCN (Mg/kg)	2.24 ± 0.00^{d}	1.67 ± 0.04^{b}	0.53 ± 0.01^{a}	2.17 ± 0.01^{c}	1.78 ± 0.03^{b}	0.49 ± 0.01^{a}
Anthra.	0.51 ± 0.02^{d}	0.33 ± 0.02^{b}	0.15 ± 0.00^{a}	0.36 ± 0.00^{b}	0.28 ± 0.01^{b}	0.14 ± 0.02^{a}
Terpenoid	0.51 ± 0.02^{c}	0.41 ± 0.01^{c}	0.15 ± 0.00^{a}	0.36 ± 0.00^{b}	0.28 ± 0.01^{b}	0.14 ± 0.02^{a}

Anthra. = Anthraquinone; HCN = Hydrogen cyanide. Values are mean ± standard error of three determinations. Rows with different superscripts are significantly different at $p<0.05$.

Table 2. *Proximate contents (dry matter) of leaves, stems and roots of B. diffusa and B. erecta.*

	B. diffusa			***B. erecta***		
Compositions (%)	**Leaves**	**Stem**	**Root**	**Leaves**	**Stem**	**Root**
Moisture content	11.53 ± 0.08^{f}	8.78 ± 0.00^{b}	9.16 ± 0.02^{c}	10.31 ± 0.03^{e}	9.66 ± 0.04^{a}	8.67 ± 0.04^{a}
Dry matter	88.48 ± 0.01^{a}	91.22 ± 0.00^{d}	90.84 ± 0.03^{d}	89.69 ± 0.04^{b}	90.34 ± 0.06^{c}	91.34 ± 0.05^{e}
Ash	20.42 ± 0.07^{e}	16.38 ± 0.07^{d}	16.63 ± 0.18^{d}	22.53 ± 0.08^{f}	16.52 ± 0.02^{c}	14.89 ± 0.03^{a}
Crude fibre	12.43 ± 0.17^{a}	14.56 ± 0.04^{c}	15.91 ± 0.01^{d}	13.51 ± 0.09^{b}	15.83 ± 0.03^{d}	16.34 ± 0.06^{e}
Fat	5.45 ± 0.02^{e}	3.17 ± 0.01^{c}	2.84 ± 0.02^{b}	4.17 ± 0.01^{d}	1.88 ± 0.02^{a}	2.78 ± 0.01^{b}
Crude protein	16.41 ± 0.04^{f}	8.47 ± 0.02^{d}	6.38 ± 0.01^{a}	15.89 ± 0.03^{e}	7.61 ± 0.01^{c}	6.92 ± 0.02^{b}
CHO	33.78 ± 0.20^{b}	48.65 ± 0.14^{d}	49.08 ± 0.23^{e}	33.62 ± 0.24^{a}	48.50 ± 0.12^{c}	50.41 ± 0.11^{f}

CHO =Carbohydrate. Values are mean ± standard error of triplicate determinations. Row with same superscript is not significantly different ($p>0.05$).

4. Conclusion

There were differences in the levels of phytochemicals and nutrients in the various parts of *B. diffusa* and *B. erecta*, implying that their degree of syntheses and accumulations in parts of these plants varied. Furthermore, the findings of this study indicated that these plants could serve as sources of raw materials for manufacturing of fotableod and drugs.

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