Research Article

Phytochemical screening, quantification and correlation matrix of Nigerian medicinal plant: *Waltheria americana*.

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Abstract

Background: Plant's kingdom provides new and important leads against various pharmacological targets due to the current wide spread of belief that green medicine is safe and more dependable than the costly synthetic drugs. The medicinal property of plants step from their ability to synthesize aromatic substances and secondary metabolites that are potent bioactive compounds found in medicinal plant parts that are precursors for the synthesis of useful drugs. In the present study, the leaf, stem, and root extracts of *Waltheria americana* were evaluated for phytochemical compositions and their correlation matrix. **Methods**: Quantitative and quantitative standard methods of analysis were used to evaluate the presence, amount, and the correlationships of the different phytochemicals in the leaf, root and stem of *W. americana* plant. **Results**: The quantitative phytochemicals percentage composition of *W. americana* varied with large ranges for alkaloids, tannins, flavonoids, but short ranges occurred of terpenes and cardiac glycosides. Alkaloids had the highest percentage composition and cardiac glycosides showed the lowest for all the plant parts. The stem seems to be the major area of phytochemical production than other parts of the plant, indicating that the stem of *W. americana* could serve as a major source of phytochemicals in any herbal concoction. "The correlation" of phytochemical constituents, alkaloids and tannins in the leaf were positively and significantly correlated with cardiac glycosides in the stem at 95% confidence respectiely. However, no correlation was observed of any phytochemicals in the other plant. **Conclusion**: These findings indicated that the production, quantification, and distribution of these phytochemicals were complimentary in nature in *Waltheria americana* plant, and the shoot may have played a major role in this regard

Keyword: *Phytochemical; Waltheria americana; Herbal plant; Correlation Matrix; Quantification.*

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1.0 Introduction

In a constant attempt to improve the quality of life, men have used plants as source of food, shelter, clothing, medicine, cosmetics and for seeking relief from hardship of life [1]. According to world health organization (WHO) traditional medicine is becoming more popular in the developed countries, and more than 80% of people in the developing countries use traditional medicine as part of their primary healthcare [1]. Plant's kingdom provides new and important leads against various pharmacological targets [2] and many pharmaceutical companies have shown interest in plant derived drugs mainly due to the current wide spread of belief that green medicine is safe and more dependable than the costly synthetic drugs which have adverse side effects [3, 4].

The medicinal property of plants step from their ability to synthesize aromatic substances and secondary metabolites which leads to drug development [5]. Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "secondary metabolites" which are of several classes, including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids [6, 7]. Most of these phytochemical constituents are potent bioactive compounds found in medicinal plant parts which are precursors for the synthesis of useful drugs [8].

Waltheria americana belongs to Sterculiaceae or Cacao family. It is commonly called barulad, sleepy morning, velvet leaf, monkey bush, uhaloa, marshmallow, buff coat, leather coat and many other names [9]. It is a tropical and

subtropical genus with 60 species, 53 of them American and one species distributed worldwide, six species are extra american from either North West Australia or Asia or Madagascar or West Africa [10]. It is widely applied in traditional medicine by various cultures worldwide although applications vary by region. It is used in Northern Nigeria by the Hausas for the treatment of skin diseases, impotence, and infertility and as children medicine at birth and during teething [11].

Much research has been conducted to assess the presence of phytochemicals in medicinal plants, but little have been reported on the quantity and correlation matrix of the phytochemicals in plants. Leaf, stem, and root parts of plants are commonly used by traditional medicinal practitioners [1] as concoction for herbal treatment of various ailments. This work is aimed at quantifying the levels of different phytochemicals and to correlate them with respect to the root, stem and leaves of *Waltheria americana* plant.

2.0 Materials and Methods

2.1 Sample Location and Collection

Matured fresh leaf, root and stem of *Waltheria americana* was identified and collected from rural area of Gerei, in Gerei Local Government Area of Adamawa State, Nigeria. The sampling method of Ponaruselvam *et al.* [12], was adopted. The plant was identified and confirmed by a taxonomist from Plant Science Department of Moddibo Adama University, Yola, Nigeria. The fresh plant samples collected were numbered with a voucher specimen identification number and kept in the Chemistry Research Laboratory of the University for Further Reference.

2.2 Sample pre-treatment

The plant sample was freed from soil, grit, sand and dirt by washing shaking off and thoroughly washed with water under a running tap and rinsed with distilled water. It was shade dried at 26 ^{o}C temperature for 15 days. The dried sample was ground into fine powder using ceramic pestle and mortar.

2.3 Phytochemical Screening

Qualitative and quantitative phytochemical analysis of *Waltheria americana* root, stem and leaf powdered samples were determined as follows:

2.4 Qualitative analyses of phytochemicals

The phytochemical screening of the phytochemical constituents of the plant's three different parts were determined as follows: Crude plant filtrates were prepared by boiling 20 g of the plant powdered sample in distilled water. The solution was filtered through whatman filter paper No. 42 using vacuum pump and was kept for further analysis. The phytochemical screening for flavonoids, steroids, cardiac glycosides, tanins, alkaloids, saponins, anthroquinones and carbohydrates was qualitatively carried using various methods reported in literature with little modifications [8, 12 - 17].

2.5 Quantitative analyses of phytochemicals

The evaluation of the quantity and the number of phytochemical constituents present in the plant parts were determined as follows:

2.5.1 Flavonoid quantification

The method for the determination of flavonoid reported by Ejikeme *etal.* [18]; Boham and Kocipai [19] was adopted. Exactly 50 mL of 80% aqueous methanol was added to 2.50 g of the sample in a 250 mL beaker, covered, and allowed to stand for 24 hrs. at room temperature. After discarding the supernatant, the residue was extracted with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of each plant sample. Each plant sample filtrate was later transferred into a crucible and evaporated to dryness. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoid was calculated as

$$\% \text{ Flavonoid} = \frac{\text{Weight of flavinoid}}{\text{Weight of sample}} \times 100$$

2.5.2 Alkaloids quantification

Alkaloid was determined according to the method used by Harborne, [6]. About 200 mL of 10 % acetic acid in ethanol was added to each wood powder sample (2.50 g) in a 250 mL beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by

addition of 15 drops of concentrated ammonium hydroxide dropwise to the extract until the precipitation was complete immediately after filtration. After 3 hrs of mixture sedimentation, the supernatant was discarded, and the precipitates were washed with 20 mL of 0.1M of ammonium hydroxide and then filtered using Gem filter paper (12.5 mL). Using electronic weighing balance Model B 218, the residue was dried in an oven and the percentage of alkaloid was determined as

$$\%\ \text{Alkaloid} = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \times 100$$

2.5.3 Cardiac Glucosides quantification

The method of legal test and the Killer-kiliani was adopted, where 0.5g of the extract was added to 2 mL of acetic anhydrate plus H_2SO_4 [15].

2.5.4 Tannins quantification

The method of analysis that was reported by Temitope et al., [20] was adopted for the determination. About 500 mg of the plant sample was shaken inside a 50 mL plastic bottle with 50 mL distilled water for 1 hr. on a mechanical shaker. It was filtered into a 50 mL volumetric flask and made up to the marked. To 5 mL of the filtrate in a test tube 2 mL of 0.1 M FeCl in 0.1 M HCl and 0.008 M potassium ferrocyanide was added and mixed thoroughly. The absorbance of the resultant solution was measured at 120 nm within 10 min of mixing. The tannins content was calculated using a standard curve.

2.5.5 Terpenoids quantification

Powdered plant sample (10g) was soaked in methanol for 24 hrs. It was filtered and the filtrate extracted with petroleum ether. The ether phase was evaporated and weighed as total terpenoids [21].

$$\%\ \text{Terpenoid} = \frac{\text{Weight of terpenes}}{\text{Weight of sample}} \times 100$$

2.6 Statistical analysis

The correlation matrix analysis (descriptive and partial correlation analysis) was carried out using IBM SPSS statistics 22 package.

3.0 Results and Discussion

3.1 Qualitative phytochemical compositions

The result of phytochemical screening of *W. americana* leaf, stem and root presented in **Table 1** revealed the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, terpenes, anthraquinones and cardiac glycosides in different parts of the plant at various concentrations. Saponins, flavonoids and carbohydrates were predominantly present in the root sample, and cardiac glycoside in the stem sample than other phytochemicals. However, tannins and alkaloids were moderately present in all the plant parts, and flavonoids were moderately present in the stem and leaf, whereas anthroquinones were moderately present only in the root. Nevertheless, anthroquinones and carbohydrates were present in trace amount in the leaf and stem respectively. Terpenes were absent in all the plant parts, cardiac glycosides were absent in the leaf and root, anthraquinones was absent only in the stem whereas saponins was absent in both the stem and leaf.

Table 1: Phytochemical screening of *Waltheria americana* leaf (WAL), stem (WAS) and root (WAR)

Phytochemicals	WAS	WAL	WAR
Alkaloids	++	++	++
Saponins	-	-	+++
Tannins	++	++	++
Flavonoids	++	++	+++
Carbohydrates	+	++	+++
Steroids	++	++	-
Terpenes	-	-	-
Anthraquinones	-	+	++
Cardiac glycosides	+++	-	-

Key: Absent (-), Trace (+), Moderately present (++), Abundantly Present (+++)

3.2 Compositions and descriptive statistics of the phytochemical

The results of phytochemicals descriptive statistics of root, stem and leaf of *W. americana* plant samples in tables 2-4 showed that alkaloids had a maximum value of 7.573, 6.460 and 5.840 g in the root, leaf and stem samples respectively than other phytochemicals. Tannins moderate values of 5.963, 5.353 and 4.647 g were observed in the root, leaf and stem respectively. The result of the quantitative phytochemical percentage composition of *W. americana* stem, leaf and root in **Table 5** showed that the percentage concentrations of alkaloids and tannins follow the order stem > leaf > root and on the contrary flavonoids followed the order root > stem > leaf. The percentage composition of steroid in root was not detected and that of cardiac glycoside in leaf and root was also not detected **Figure 1**.

The steroids /terpenes showed the least values in the stem and leaf whereas cardiac glycoside showed the lowest value in the stem. These phytochemical compounds are known to have antibacterial activity against pathogens and could be used traditionally for therapeutic purposes [22]. Plants are rich source of wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids which have been found *in vitro* to have antimicrobial properties [5, 23]. Generally, from the results of *W. americana* root, stem and leaf obtained, alkaloids and tannins showed the highest phytochemical concentrations in the stem, indicating that the stem is the major area of production of these phytochemicals than other parts of the plant. This may have informed the high antimicrobial activity of the stem than the leaves and the roots reported in literature [24 - 25]. Our finding also agrees with the work of Kolapo *et al*. [26] who reported the stem barks of medicinal plants to generally showed high antimicrobial activity than other parts. In a related research on *Waltheria* family it was reported that W. indica contain steroid derivatives and alkaloids that makes it physiologically active. Its extracts were used as standard febrifugal, purgative, emollient (softening skin), tonic analgesic and astringent herbal medicine in Africa [9].

Waltheria contains a wide variety of secondary metabolites such as tannins, terpenoids, alkaloid and flavonoids which have been found in vitro to have antimicrobial properties [5, 23]. These antibacterial activities against both gram-negative and the gram-positive is due to the presence of broad-spectrum antibiotic compound or general metabolic toxins in addition to the pharmacologically active metabolites in *Waltheria* [27, 28].

Table 2: Phytochemicals descriptive statistics for *Waltheria americana* stem extract

Phytochemicals	N	Mean (g)	Range	Std. Deviation
Flavonoids	3	1.733	1.440-2.160	0.378
Alkaloids	3	5.840	4.900-6.750	0.925
Steroids	3	0.000	0.000-0.000	0.000
Tannins	3	4.647	3.850-5.130	0.695
Cardiac glycosides	3	0.000	0.000-0.000	0.000

Table 3: Phytochemicals descriptive statistics for Waltheria americana leaf extract

Phytochemicals	N	Mean (g)	Range	Std. Deviation
Flavonoid	3	1.680	1.240 - 2.000	0.394
Alkaloid	3	6.460	5.500 - 7.250	0.887
Steroid	3	0.004	0.004 - 0.006	0.001
Tannins	3	5.353	3.470 - 6.730	1.688
Cardiac glycosides	3	0.000	0.000 - 0.000	0.000

Table 4: Phytochemicals descriptive statistics for *Waltheria americana* root extract

Phytochemicals	N	Mean (g)	Range	Std. Deviation
Flavonoids	3	1.811	1.372 - 2.240	0.434
Alkaloids	3	7.573	6.080 - 9.350	1.653
Steroids	3	0.480	0.390 - 0.630	0.131
Tannins	3	5.963	5.430 - 6.580	0.580
Cardiac glycosides	3	0.250	0.160 - 0.320	0.082

Table 5: Percentage composition of phytochemicals in stem, leaf and root

Phytochemicals	Stem (%)	Leaf (%)	Root (%)
Flavonoids	3.94	3.84	4.24
Alkaloids	16.49	14.55	13.02
Steroids	1.02	1.05	0.00
Tannins	13.97	11.57	10.52
Cardiac glycosides	0.54	0.00	0.00

Despite these common findings, the work of Olajuyigbe *et al.* [1] reported that *Waltheria americana* contain high amount of saponins and anthraquinones in the three different parts of the plant than other phytochemicals which differ from our finding where saponins, flavonoids and carbohydrates were the phytochemicals that were predominantly present in the root, and similarly cardiac glycoside in the stem were higher than other phytochemicals in the plant parts. Cardiac glycosides were only present in the stem which was reported to be predominant in the root and leaf extracts than in the stem extracts [1]. These variations in the phytochemical constituents of plant parts in this study and those reported by Olajuyigbe et al. [1] may have arisen from different geographical location and soil type of the sourced *W. americana* samples. Tannins, terpenoids, alkaloids and flavonoids have antimicrobial properties [5, 23]. Also, tannin have been shown to selectively inhibit HIV replication and are widely known to make trees and shrubs a difficult meal for caterpillars, due to its astringent taste [11]. In addition, tannins prevent the development of micro-organisms by precipitating microbial protein making nutritional protein unavailable [10]. Moreover, it may hasten the healing of wounds and inflamed mucous membrane [29]. The presence of saponin was found in the root of *W. americana* and its detergent properties enable it to serve as lytic (destruction of cell) agents and exhibit anti-inflammatory properties [30]. Cardiac glycosides work by inhibiting the (Na^+/K^+) pump, thereby increasing the amount of Ca^{2+} ions available for the contraction of heart muscles which improves cardiac output and reduces the distension of heart, thus, used in the treatment of congestive heart failure and cardiac arrhythmia [31]. Alkaloids are useful as analgesic, antispasmodiac, bactericidal effect [6, 7, 32]. Kandaswami *et al.* [33] and Manikandan *et al.* [34] reported that flavonoids are good antioxidants, anticarcinogens, antimicrobial and antitumor. The result of our finding on alkaloids and tannins showed they were highest phytochemicals in the stem of *W. americana* as such could serve as a major source of phytochemicals in any herbal concoction.

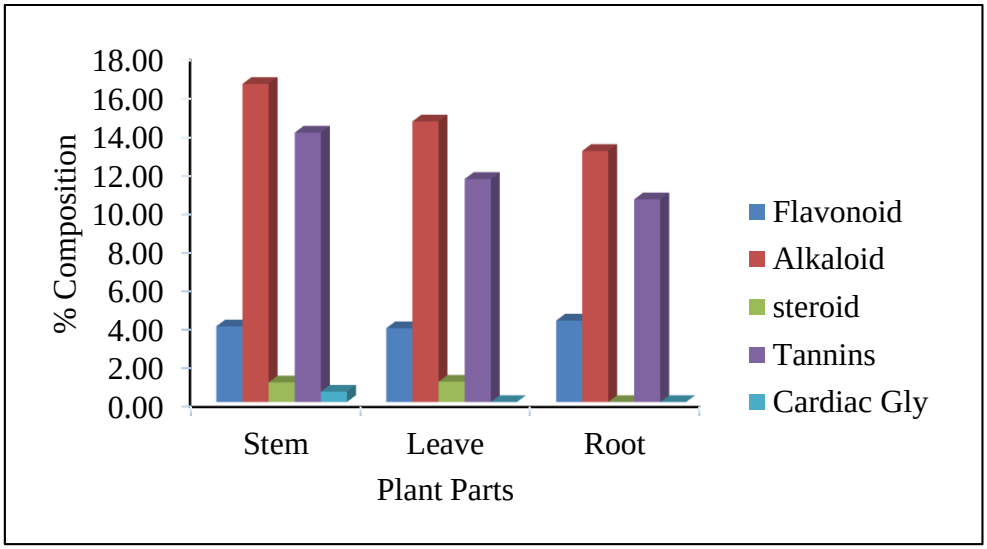

Figure 1: Percentage composition of phytochemicals in stem, leaf and root of *W. Americana.*

3.3 Correlation matrix of the mixture of phytochemical constituents

Correlation matrix of the mixture of phytochemical constituents in *W. americana* plants showed that there was positive correlation among some phytochemicals in the leaf and stem at 95% confidence (Table 6). However, no correlation was observed among phytochemicals in the root constituents. But leaf alkaloids (WALA) positively correlated with stem cardiac glycoside (WASC) while the leaf tannins (WALT) significantly correlated with the stem cardiac glycoside (WASC) at 0.05 level (95% confidence) as shown in table 6. These observations indicated that the production and distribution of these phytochemicals are complimentary in nature in *W. americana* leaf and stem plant parts.

Table 6: Correlation matrix of phytochemicals of stem, leaf and root extract of *Waltheria americana*.

	WASF	WASA	WASS	WAST	WASC	WALF	WALA	WALS	WALT
WASA	0.986	1							
WASS	0.792	0.883	1						
WAST	0.405	0.247	-0.238	1					
WASC	0.981	0.935	0.659	0.574	1				
WALF	0.724	0.598	0.151	0.924	0.844	1			
WALA	0.989	0.951	0.693	0.536	0.999*	0.818	1		
WALS	0.898	0.959	0.98	-0.038	0.796	0.346	0.823	1	
WALT	0.97	0.916	0.62	0.614	0.999*	0.869	0.995	0.765	1

Key: * Correlation is significant at 0.05 level (2-tailed), WASA= *Waltheria americana* stem alkaloids, WASS= *Waltheria americana* stem steroid, WAST= *Waltheria americana* stem tannins, WASC= *Waltheria americana* stem cardiac glycoside, WALF= *Waltheria americana* leaf flavonoid, WALA= *Waltheria americana* leaf alkaloids, WALS= *Waltheria americana* leaf steroids and WALT= *Waltheria americana* leaf tannin

4.0 Conclusion

The qualitative and quantitative phytochemical studies of root, stem and leaves of *Waltheria americana* plant is reported here. The Percentage composition of the plant alkaloids, tannins, flavonoids, terpenes, cardiac glycosides were also reported. The correlation matrix of the phytochemical constituents, alkaloids and tannins in the leaf were positively and significantly correlated with cardiac glycosides in the stem at 95% confidence respectively. The production, quantification and distribution of these phytochemicals might be complimentary and the shoot may have played a major role in this regards.

Conflict of interest: The authors declare that they have no conflict of interest

Authors Contributions: Authors PMD and FPA were responsible for conceptualization, project administration, supervision, review and final editing. JJD was responsible for conceptualization, data collection and analysis, literature scouting, writing of original draft for the paper. BMW carried out some of the analysis and final editing.

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