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Phytochemical and Elemental Analysis of *Sida corymbosa* (Broom Weed or Wire Weed) Leaf and Root Extracts

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Authors' contributions

Author CCD did a literature search, designed the work, carried out the bench analysis, statistical analysis and wrote the manuscript. Author FCE redesigned, supervised and edited the work. Author HCCM edited the work while author ENE did sample collection. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate if *Sida corymbosa* leaf and root ethanolic extracts have some medicinal values and whether they can be used as a supplement to some mineral elements.
Study Design: This work was designed to identify the presence of some phytochemicals and mineral elements in leaf and root samples of the plant and to determine which samples contain more of the above mentioned parameters.
Place and Duration of Study: This work took place at the Department of Human Biochemistry Research Laboratory, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus and Spring Board. Research Laboratory, Awka, Anambra State. The duration of study is eight weeks.

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Methodology: Qualitative phytochemical screening was done by colour identification method while the quantitative phytochemical analysis was done using the gas chromatographic method. Elemental analysis was done using Atomic absorption spectrophotometric method
Results: It was observed that the extracts contained more of flavonoids which include; epicatechin, kaempferol, catechin, naringin and rutin (Table 1). Tannin level was observed to be significantly higher (42.546 ±3.59, P<0.05) in aqueous root than ethanolic root, aqueous and ethanolic leaf extracts. Phenol and epicateclum were higher in aqueous and ethanolic root and leaf extracts (7.053 and 1.329 mg/ml). The results of mineral elements analyzed showed that the amount of Na+, Fe2+, CU^{2+}, SO^{2-} and Cl^{-} were significantly higher in the leaves than the roots. (P<0.05). Highest extraction yield was also witnessed in ethanolic leaf extract (19.57±0.32) than other extracts.
Conclusion: *Sida corymbosa* leaves and roots have many medicinal values as is claimed claimed by some traditional medicine practitioners. The plant leaf and root extracts may have some antioxidants and anti malaria properties since it contained many flavonoids and alkaloids The plant leaves may serve as a better supplement to Na^{+}, $Fe^{2+,}$ $CU^{2+,}$ Mn^{-}, CO^{2+}, SO_4^{2-} and Cl^{-} than the root.

Keywords: Sida corymbosa; phytochemical studies and mineral element.

1. INTRODUCTION

The phytochemical investigation is among the ways used for ascertaining the medicinal values of plants [1,2]. The use of herbal drugs in treating diseases has been the tradition of Africans over a long period of time. Traditional medical practices have been on before the inception of orthodox medicine [3]. It is older than civilization itself. From the beginning, a human being has been looking for traditional ways to be used by him to take care of the diseases that have been ravaging mankind in society [4,5]. According to Basey and Effiong [6], man has been using plant-based products to survive from disease or hunger. This is because plants are easily accessible and cheaper. The knowledge of the importance of plants is acquired by most traditional medical practitioners by trial and error. This is passed on from generation to generation without documentation. Nature has been a source of agents of medicine many years ago. Many orthodox drugs have been isolated from natural sources [6]. Medicinal plants play an essential role in human health c are delivery [2,7,8,9,10].

Sida species are among the ethnomedicinally important genus of plants which belong to the family called Malvaceae [2,11,12].There are over 200 known species of sida among which is *sida corymbosa*. The species are used in the treatment of diseases such as diarrhoea,ulcer,gonorrhea,and heptic diseases in most parts of Asia and Africa. *Sida corymbosa* or *Sida garckeana* plant is an erect, basal, perennial shrubs with hairy system of up to two meters high [12]. It is found in most Northern, South Western and South Eastern parts of Nigeria as common weeds growing in the streets and roadsides [11].

There is a lack of information on the phytochemical properties of *Sida corymbosa* plants. This work has addressed itself to investigate the phytochemical content and elemental composition of crude extracts of the leaf and roots *Sida corymbosa*. This is to ascertain if the leaves and roots of the plants have some medicinal values as being claimed by some traditional medicine practitioners in the South Eastern parts of Nigeria.

Fig. 1. Photograph of *Sida corymbosa*

2. MATERIALS AND METHODS

All chemicals that were used in this work were of analytical grade and were obtained from the

British Drug House (BDH) Ltd, Poole, England through their sales representative in Lagos State, Nigeria. These include methanol (50%), magnesium metal, potassium hydroxide (10%), tetrasulphate six acid (H_2SO_4, 10%), ferric chloride ($FeCL_3.6H_2O$, 20 mM, acetic anhydride, chloroform, hexane, Wagner's reagent (2g iodine + 6g potassium iodide in 100 ml of distilled water) and benzene. Others are ammonia (10%), absolute ethanol, potassium dichromate (5%), pyridine, anhydrous sodium sulphate, concentrated nitric acid, silver nitrate and phosphate standard, Phenolpthaline indicator (1%) and barium chloride (10%).

2.1 Collection of Plant Samples

Sida corymbosa plant was obtained from Otolo, Nnewi, in Nnewi North Local Government Area of Anambra State, behind College of Health Sciences,Nnamdi Azikiwe University, Nnewi Campus. It was identified and authenticated by Prof. J.C. Okafor of Botany Department, Enugu State University of Science and Technology (ESUT), Enugu, Nigeria. It was reauthenticated at the Department of Botany, Nnamdi Azikiwe University, Awka by Dr. Ogbuozobe Gabriel Okwudili and issued with the voucher number; NAU Herbarium No 75G.

2.2 Preparation of Plant Materials

The plant leaves and root were prepared according to the method described by Sundarganapath [9]. The leaves and roots of the plant were washed with distilled water, air-dried at root temperature and powdered using a blender. The ethanol extracts were obtained by soaking 25g of each of leaves and roots respectively in round bottom flasks containing 200 ml of absolute ethanol (98%) for forty eight hours with shaking using an orbital mixer. The ethanolic extracts were filtered through 40 mm Whatman filter paper and evaporated using a rotary evaporator (Model:TT22, USA) at 65^{o}C. The crude extract was dried using the oven at 45^{o}C. The aqueous extracts were concentrated using lyophilizer at 10^{-1}Torr.

2.3 Phytochemical Analysis

Quantitative and qualitative analysis of phytochemical were conducted using routine standard methods as described.

2.3.1 Test for presence of flavonoids

This was done according to the method described by Vinata and Mammidala [13]. Four milligrams of the extract's solution were treated with 1.5 ml of 50% methanol solution. The solution was heated and metal magnesium added to the solution. This was followed by the addition of 5 drops of concentrated hydrochloric acid (HCl). The appearance of the red colour indicates the presence of flavonoids.

2.3.2 Test for presence of tannins

This was carried out according to the method of Jack and Okorosaye-Orubite [14] by adding 10% potassium hydroxide (KOH) to 1 ml of the extract. The appearance of dirty white precipitate indicates the presence of tannin.

2.3.3 Presence of cardiac glycosides

This was also determined according to the method described by Jack and Okorosaye-Orubite [14]. This was done by adding 10 ml of 50% tetraoxosulphate six acid (H_2SO_4) to 1ml of the extracts in a test tube. The mixtures were heated in a boiling water bath for 15 min. This was followed by the addition of 10 ml of Fehling's solution after which the mixtures were boiled for 5 min. A brick red precipitate indicates the presence of glycosides.

Presence of alkaloids was determined according to the method of Richa and Sharma [1]. This was done by adding few drops of Wagner's reagents to 3ml of the extracts. Formation of yellowe precipitate indicates the presence of alkaloids.

2.3.4 Test for presence of steroids

Presence of steroids was determined using the method of Jack and Okorosaye-Orubite [14]. Five drops of concentrated H_2SO_4 were added to 1ml of extract in a test tube. Non-appearance of red colour indicates the absence of steroids.

2.3.5 Test for presence of saponin

Presence of Saponin was done according to the method of Vinata and Mamidala [13] by adding 2ml of extract in a test tube. Vigorous shaking of the 2 ml of the extract in a test tube for 2 min with persistent foaming confirms the presence of saponin.

2.3.6 Test for presence of phlobatannin

Test for Phlobatannin was performed according to the method of Vinata and Mamidala [13] by adding 2ml of extract to one drop of ferric chloride reagent. The appearance of a blue colour indicates the presence of phlobatannins.

2.3.7 Test for presence of saponin

Presence of anthraquinone was determined according to the method of Vinata and Mamidala [13] by adding 3ml of extracts to 3ml of benzene. The mixture was filtered and 5ml of ammonia solution was added to the filtrate. The mixture was shaken. The appearance of pink, red or violet colour in ammonical (lower) phase indicates the presence of anthraquinone.

2.3.8 Test for presence of terpenoid

Test for presence of terpenoids was determined according to the method of Richa and Samuel [1] by treating every 4 mg of the extract with 0.5 ml of acetic anhydride and 0.5 ml of cholorform. This was followed by the addition of concentrated solution of H_2SO_4 slowly at the side of the test tubes. The appearance of red violet colour indicates the presence of terpenoid.

2.4 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was done using a standard method as described by Richa and Sharma [1] using gas chromatography technique.

Procedure:

One gram of each extract was weighed and transferred into a test tube. This was followed by the addition of 15 ml ethanol and 10 ml of 50 %w/v potassium hydroxide. The test tube was warmed in a water bath for 60 min at 100 ^{o}C. After which the solution was transferred into a separating funnel. The test tube was washed successively with 20 ml of ethanol, 10 ml of cool water, 10 ml of hot water and 3 ml of hexane respectively. The extracts were combined and washed three times with 10 ml of 10 % w/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulphate and the solvent was evaporated. The sample was solubilized in 1000ul of pyridine of which 200ul was transferred to a vial for analysis. The analysis of the phytochemicals in the extracts was performed using a BUCK M910 gas chromatograph equipped with a flame ionization detector. A RESTEK 15 m MXT-1 colunm (15 m x 250 m x 0.15 um) was used. The injector temperature was 280^{0}C with splitless injection of 2 µl of sample and a linear velocity of 30cms-1. Helium 5.0 pas was the carrier gas with a flow rate of 40 ml min^{-1}. The oven operated initially at 200^{0}C was heated to 330^{0}C at a rate of 3^{0}C min^{-1} and was kept at this temperature for 5 min. The detector operated at a temperature of 320^{0}C.Amount of Phytochemicals was determined by the ratio between the area and the mass of internal standard and the area of the identified phytochemicals. The concentration of various phytochemicals was expressed in µg/g.

The analysis of the phytochemicals in the extracts was performed using a BUCK M 910 gas chromatograph equipped with flame ionisation detector.

The extraction yield of the palnt extracts was calculated using:

$$\text{Extraction yield} = \frac{\text{Weight of extract}}{\text{Weight of raw sample}} \text{X } 100\%$$

2.5 Elemental Analysis of *Sida corymbosa* Leaf and Root

This was done using routine standard methods as described Phosphate was measured using the standard method of APHA [15]. Exactly 20 ml of the samples was pipetted into test tubes, 10 ml of the combined phosphate reagent was added, shaken and left to stand for 10 min before reading the absorbance at 690 nm spectrophotometrically using UV-VIS spectrophotometer (Model 752, China).

$$\text{Conc. Of phosphate} = \frac{\text{Abs of samples}}{\text{Abs of STD}} \text{ X Conc STD (mg/l)}$$

STD – Standard, Abs = Absorbance.

2.5.1 Chloride determination

Chloride level was determined according to the standard method of APHA, [15]. A 100 ml of each clear sample was pipetted into an Erlenmeyer flask and the pH adjusted with 2M H_2SO_4 solution. This was followed by the addition of 1ml of K_2CrO_4 indicator solution and titrated against with a standard solution of AgNO3 in a permanent reddish-brown colouration. The $AgNO_3$ titrant was standardised and reagent blank established.

Chloride conc. = Titre value (X) X 10 (Mg/l)

2.5.2 Sulphate determination

Sulphate level in the extract was determined using the standard method of APHA [15]. A 250

ml of extracts was evaporated to dryness on a dish. The residue moistened with a few drops of concentrated HCl and 30 ml distilled water. This was boiled and filtered. The dish was rinsed and the filter paper washed several times with distilled water. The filtrate and the washings were added together. This was heated to boiling and then 10 ml of 10 % $BaCl_2$ solution was added dropwise with constant stirring. The mixture was digested for about 30 mm, filtered and the filter paper washed with warm distilled water. It was then ignited, cooled and weighed in an already weighed crucible.

Conc of sulphate = Mg $BaS0_4$ X 411.5ml of sample (ppm).

2.5.3 Determination of potassium(K^+), zinc(Zn^+), sodium (Na^+), iron (Fe^{2+}), magnesium (Mg^{2+}), copper (CU^{2+}), manganese (Mn^-), cobalt (CO^{2+}) and calcium (Ca^{2+})

These were done according to the method described by Yildiz et al. [16] using flame atomic absorption spectrophotometer (Model 400, India). Atomic absorption spectrophotometer's principle is based on the sample being aspirated into the monochromator and onto the detector that measures the amount of light absorbed by the atomized element in the flame. The amount of energy of characteristic wavelength absorbed in furnance for 2h at 550^{o}C. the flame is proportional to the concentration of the element in the sample. The samples were first digested by heating 2g of both samples in the This was followed by diluting the samples with 20 ml 20 % H_2SO4 and filtered with filter paper (Whatman 110 mm). The digested samples were fed into an air-Acetylene flame and metal concentrations were determined.

2.6 Statistical Analysis of Results

The results were expressed as mean $\pm$ standard deviation of triplicate determinations, students' T-test and one-way analysis of ANOVA were used to analyze the results using SPSS statistical software (Version 21).

3. RESULTS AND DISCUSSION

3.1 Results

Tables 1 shows the results of qualitative phytochemical screening done on ethanolic leaf and root and aqueous leaf and root extracts of *Sida corymbosa* plant. Flavonoid, alkaloid, tannin, phenol, saponnin, terpenoid and anthocyanin were present in all the extract. Steroids, glycosides and phlobatanin were absent in all the extracts.

Table 2 shows the results of the quantitative phytochemical analysis done on the extracts. In all instances of significant differences at $p< 0.05$ level of significance, epicatachin was higher in ethanolic leaf than ethanolic root extracts while anthocyanin was higher in ethanolic leaf than aqueous and ethanolic root extracts. Tannin and naringin (Flavonoid) were higher in aqueous root extract than other extracts while phenol was higher in aqueous leaf than and lunamarine (Alkaloids) were higher in aqueous leaf extract than the aqueous root extract. Lunamarine was higher in aqueous leaf extract than aqueous root extract. Also lunamarine was higher in ethanolic root than aqueous root extracts.

Table 1. Qualitative phytochemical screening of *Sida corymbosa* extracts

Phytochemical	Root extract		Leaf extract	
	Aqueous Ext	Ethanolic Ext	Aqueous Ext	Ethanolic Ext
Alkaloid	+	+	+	+
Tannins	+++	+++	++	++
Saponins	+++	+++	+++	+++
Flavonoids	+++	+++	+++	+++
Steroids	-	-	-	-
Glycosides	-	-	-	-
Phenol	+	+	+	+
Terpenoids	+	+	+	+
Anthocyanin	+	+	+	+
Phlobatanins	-	-	-	-

+=Positive, ++= Moderate, +++= Very high, -=Negative; Ext= Extract

Table 2. Quantitative phytochemical analysis of *Sida corymbosa* extracts

Phytochemical (µg/ml)	Root extract		Leaf extract	
	Aqueous Ext	**Ethanolic Ext**	**Aqueous Ext**	**Ethanolic Ext**
Spartein(Alkaloid)	0.013±0.00	0.002±0.00	0.001±0.00	0.002±0.00
Epicatechin(Flavonoid)	1.160±0.034	0.573±0.01^{b}	0.961±0.34	1.329±0.01^{b}
Anthocyanin	3.277±0.66^{a}	1.342±0.38^{ab}	3.559±0.00^{b}	3.530±0.04^{a}
Tannin	42.546±3.59^{ab}	36.222±0.06^{a}	13.428±0.01^{b}	13.136±0.21^{ac}
Phytate	0.794±0.15	-	-	0.641±0.01
Phenol	3.211±1.4^{ab}	6.989±0.11^{a}	7.053±0.00^{b}	5.749±1.49
Lunamarine(Alkaloid)	6.058±1.38^{ab}	8.865±0.09^{a}	8.923±0.00^{b}	8.283±0.59^{b}
Sapogenin(Saponin)	24.516±1.38^{abc}	27.135±0.04^{a}	27.156±0.12^{b}	27.051±0.06^{c}
Naringin(Flavonoid)	25.566±0.29^{ab}	-	20.007±0.021^{b}	19.639±0.752^{a}
Catechin(Flavonoid)	31.626±5.26	30.856±9.17	25.148±0.01	25.146±0.01
Rutin(Flavonoid)	2.955±0.31	3.126±0.00	3.128±0.00	3.122±0.00
Ribalinidine	-	3.210±0.108	3.302±0.06	-
Kaempferol(Flavonoid)	10.096± 4.02	5.447±2.083	6.933±1.42	3.689±0.50
Oxalate	2.188	-	-	-

Values above are the means of triplicate results ± STD. Values in the same rows having the same letters are statistically different ($p < .05$). Results were analysed using independent "T" test analysis.

Comparism was done between aqueous and ethanolic root extracts, aqueous and ethanolic leaf extracts, between the leaf and root extracts. a= comparison between aqueous and ethanolic extracts of leaf and root.

b= comparism between aqueous root and leaf extracts. c= comparism between ethanolic root and leaf extracts

Ext= Extract

Table 3. Elemental analysis of *Sida corymbosa* leaf and root extracts

Elements	Root	Leaf
Potassium (ppm)	10.127±0.220	10.290±0.254
Zinc (ppm)	16.343±1.163	14.533±0.462
Sodium (ppm)	2.367±0.294[a]	10.100±0.173[b]
Iron (ppm)	11.143±0.644[a]	14.113±1.961[b]
Magnesium(ppm)	20.253±0.564	20.750±0.332
Copper (ppm)	0.873±0.038[a]	1.647±0.197[b]
Manganese(ppm)	0.950±0.122[a]	1.760±0.173[b]
Cobalt (ppm)	0.330±0.026[a]	0.627±0.025[b]
Calcium(ppm)	30.103±0.117	30.607±0.080
Sulphate (mg/l)	25.20±0.200[a]	64.63±0.200[b]
Chloride (mg/l)	59.00±0.000[a]	67.30±1.000[b]
Phosphate (mg/l)	4.94±0.30	4.47±0.50

Values are means of triplicate results ± STD. Values in the same raw bearing different letters are statistically different (p<.05).Students' 'T' test was used to analyze the results.

Table 4. Extraction yield of *Sida corymbosa* leaf and root extracts

Root	Aqueous Extract	17.62±0.35abcd
	Ethanol Extract	14.71±0.71abcd
Leaf	Aqueous Extract	9.170.32abcd
	Ethanol Extract	19.57±0.32abcd

Values above are the means of triplicate result ± STD. Values in the same column having the same letters are statistically different (P < 0.05). Anova analysis was used to analyze the results. The values were compared between aqueous and ethanolic extracts of leaf and root, and among the extracts.
a= comparism within the extracts
b= comparism between aqueous extract of root,aqueous extract of leaf and ethanolic extract of leaf
c= comparism between ethanolic extract of root, aqueous extract of leaf and ethanolic extract of leaf

Table 3 shows the results of elemental analysis of leaf and root of *Sida corymbosa*. These include; potassium, zinc, sodium, iron, manganese, cobalt, calcium, chloride and phosphate ions. In all cases of significant differences in values, concentrations of sodium, iron, copper, cobalt and sulphate ions were higher in the leaf than the root samples at p< .05 level of significance.

Table 4 shows the results of % extraction yield of the plant extracts. Ethanolic extract of leaf showed the highest % yield significantly (p<.05) while aqueous leaf extract showed the lowest % yield.

3.2 Discussion

This work involved investigations of the phytochemical constituents and elemental components of *Sida corymbosa* ethanolic and aqueous leaf and root extracts. The qualitative phytochemical screening carried out so far showed the presence of alkaloids, flavonoids, phenol, anthcyanin, tannins and saponins in all the extracts (Table 1). Most Sida plants have been reported by Lucy and Mercy [12] and Ayodele et al. [17] to have high pharmacological values [1,2,5]. The type of phytochemical contained in a plant will determine the type of diseases the plant will be used to cure. The above results suggest that both ethanolic and aqueous extracts of leaf and root of *Sida corymbosa* plant may be good sources of flavonoids, phenol, anthcyanin, tannin and saponin. The quantitative phytochemical analysis done indicated the type of phytochemical contained in each extract (Table 2). Phytochemical such as tannins, epicatechin (Flavonoids), naringin (Flavonoid), catachin (Flavonoid), rutin (Flavonoid), phenol and kempferol (Flavonoid) which were indicated in this work have been reported to be antioxidants and have antibacterial potentials [10,17,18]. The results therefore suggest that the extracts may have some antioxidants properties.

It was observed that the amount of naringin and tannin were significantly highest in aqueous root extract(p< 0.05) while the amount of epicatachin (Flavonoids) and phenol were highest in ethanolic leaf extract. This suggests that ethanolic leaf extracts of this plant may be the best sources of epicatechin and phenol among the extracts while aqueous root extract may be the best sources of phenol and tannins. Phenol and tannin have been reported by Kanti [19] to be strong antioxidants. Tannins have also been

reported to have potentials of inducing enzymes such as gluthathione peroxidase (GPx), catalase and superoxide dismutase [20]. Tannins and anthocyannin were observed to be higher in aqueous than ethanolic roots extracts while phenol and lunamarine were higher in aqueous leaf and ethanolic root extracts, suggesting that tannin and anthcyanin may be gotten more in the root when using aqueous solvent than ethanol while phenol and lunamarine may be gotten more in the root and leaf using aqueous and ethanolic solvents. Most antioxidants such as tannin, phenol and anthocyanin are capable of regenerating liver cells [20,21]. *Sida corymbosa* ethanolic leaf extracts have been reported to have haemostatic activities in rats [12].

The extraction yield calculated showed that the ethanolic leaf extract had the highest extraction yield (19.57% $\pm$0.32) (Table 3). This also suggests that more phytochemical may be gotten in the leaf using ethanol as an extractor. This is supported by the findings of [16].

The results of elemental analysis carried out showed the presence of potassium (K^+), Zinc (Zn^+), sodium (Na^+), Iron (Fe^{2+}), magnesium (Mg^{2+}), copper (CU^{2+}), sulphate (SO_4^{2-}), chloride (Cl^-) and phosphate ($PO4^{2-}$) in both root and leaf samples of the plant (table 4). This suggests that both root and leaf of *Sida corymbosa* plant may be used as supplements to the above mentioned elements. Concentrations of mineral element, such as Na^+, Fe^{2+}, Cu^{2+}, Co^{2+}, SO_4^{2-}, and Cl^- ions were observed to be higher in the leaf than the root of the plant. This may be as a result of translocation.This suggests that the leaves of this plant may be better sources of Na^+, Fe^{2+}, Cu^{2+}, Co^{2+}, SO_4^{2-}, and Cl^- ions than the roots. Mineral elements are required by animals for healthy growth [5,16].

4. CONCLUSION

The result so far analyzed is suggesting that all the extracts of this plant contain many phytochemicals. Most of them are known strong antioxidants and are known to have antibacterial properties. The plant may, therefore, have many medicinal values as claimed by many traditional medicine practitioners in some parts of South Eastern Nigeria and many other parts of Nigeria.

Aqueous and ethanolic solvents may be good extracting solvents for obtaining phenol and lunamarine in the roots of the plant. Aqueous solvent may be better extracting solvents than ethanol for obtaining tannin in leaf and root samples. The leaves of the plant may be used as better supplements than the roots for Na^+, Fe^{2+}, Cu^{2+}, SO_4^{2-} and Cl^-.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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