Pharmacognostic and free radical scavenging Evaluation of *Cyathula prostata* (Blume) L.

Mubo Adeola Sonibare* and Oluwaseun Victoria Olatubosun

Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.

ABSTRACT

Background: *Cyathula prostrata* (Blume) L. from the family Amaranthaceae has been used traditionally for rheumatism, dysentery, wounds and urethral discharges in the tropical regions of the world. **Aim:** The present study was undertaken to perform quality control standardization and to evaluate antioxidant activity of the leaf, stem, root and the whole plant of *Cyathula prostrata*. **Methods:** Macroscopic and microscopic evaluations were carried out on the plant using standard procedures. Powdered sample of the leaf was evaluated with various organic solvents for fluorescence. The chloroform, ethyl-acetate and methanolic extracts of the leaf, stem, root and whole plant were subjected to various pharmacognostic analyses and evaluated for *in vitro* antioxidant activity using DPPH assay.Further, thin layer chromatoghraphy was used to evaluate the chloroform extract. **Results:** Important epidermal features in the plant include: coastal cells, unbranched, uniseriate, multicellular and non-glandular trichomes. Leaves are amphistomatic showing mostly anomocytic and actinocytic stomata. Starch grains are restricted to the adaxial surface. Vascular bundles are mainly collateral and well-developed bundle sheath. The transverse section of stem is circular, hypodermis (1-3 layers). Cross section of the root is described in detail for the plant. Cortex has angular cells. Fluorescence studies showed different colours. Physico-chemical results are comparable with standards. The TLC profile showed presence of at least seven compounds in the leaf, root and the whole plant extracts, while nine components were obtained from the stem extract. The ethyl acetate extract of the root and ethanol extract of the stem gave the highest phenolic contents (30.09 ± 3.768 mg GAE/g) and DPPH free radical scavenging activity (87.0 ± 0.208), respectively. **Conclusion:** The distinctive features established in this study are steps in identification, standardization and quality control of this medicinal plant.

Key words: *Cyathula prostrata*, standardization, microscopy, physicochemical parameters, antioxidant.

INTRODUCTION

The World Health Assembly in resolutions has emphasized the need to ensure the quality of medicinal plant products by using current control techniques and applying appropriate standards.[1] The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs.[2] With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as a medicine. Thus, in recent years, there has been an emphasis on standardization of medicinal plants of therapeutic potential.[3,4,5]According to the World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken. In order to standardize a drug, various studies such as macroscopic, microscopic, physico-chemical, phytochemical screening and fluorescence analysis are done. *Cyathula prostrata* of the family Amaranthaceae selected for this study has several medicinal applications especially in developing countries. The medicinal applications of *C. prostrata* include its use for treatment of sores, articular rheumatism, dysentery, wounds and urethral discharges.[6] The plant has also been reported to demonstrate antibacterial activity[7] and cytotoxic activity against breast and cervical cancer.[8,9] The need for proper identification, standardization and quality control

***Corresponding author:**
Dr. Mubo A. Sonibare,
Department of Pharmacognosy,
Faculty of Pharmacy,
University of Ibadan, Ibadan, Nigeria.
Phone: +2348134901273
E-mail: sonibaredeola@yahoo.com

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of its crude drugs becomes imperative with the paucity of information on the aspect of its standardization in literature. The aim of the present study was therefore to establish the pharmacognostic profile of the plant and analyze the plant for free radical scavenging activity.

MATERIAL AND METHODS

Plant material

Cyathula prostrata was collected from the premises of Federal College of Forestry, Jericho, Ibadan and Idi-Ishin quarters, Jericho area, Ibadan from May-June 2012. Plant identification and authentication were carried out at Forest Herbarium Ibadan (FHI) of the Forestry Research Institute of Nigeria, Ibadan by Mr. E. C. Chukwuma. Voucher specimen were deposited at FHI (FHI 109590) and at Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI). Macroscopic and microscopic studies were carried out on fresh plant. For phytochemical analysis, the leaves, stem, root and whole plants of *Cyathula prostrata* were rinsed in water to remove earth materials attached to them. They were air dried for two weeks, oven dried at 40^0C for 2-3 h to make them crispy and pulverized using an electric blender. The powdered samples were stored in tight sacs until use. Extraction of powdered samples (200 g each) was done successively with analytical grades of chloroform (3x), ethyl acetate (3x) and ethanol (3x) for a period of 72 h with periodic shaking and filtered. Each filtrate collected was concentrated dryness under vacuum at 40°C using a rotary evaporator (Buchi, rotavapor R – 210) and kept in a refrigerator at 4°C until use.

Macroscopic evaluation

Macroscopic analysis of *Cyathula prostrata* was done according to methods reported in literature and the one outlined by the World Health Organization.[10,11] The macro morphological characters studied and assessed include; plant height, leaf length, width, arrangement, apex, margin, base, shape, composition. Ten measurements of each character were made and the mean range was calculated.

Microscopic evaluation

For the microscopic studies of leaf epidermises, previously reported methods were followed.[12,13] Pieces of fresh leaves of *C. prostrata* were infused in 100% nitric acid and boiled for 3-5 min in a petri dish on a water bath. The formation of bubbles in the leaves surfaces indicated the separation of the upper and the lower epidermis from the mesophyll. Adaxial and abaxial surface peels carefully separated using camel hair brush were dehydrated by graded series of 70%, 85%, and 100% ethyl alcohol and mounted with safranin O and or lactophenol. Observations and measurements were made with a light microscope using micrometer eye piece. Ten measurements of each character were randomly made from each specimen and the mean and standard error calculated. Photomicrographs of specimens were taken using Zeiss Standard 25 photomicroscope.

Transverse section of the leaf, stem median portion and root of *C.* were prepared with the aid of Reichert Austria NF 369 143 sliding microtome with thickness between (10-12) µm using pawpaw tissue fastened into the microtome clamp as support and 95% ethanol to keep the tissue moist using camel brush. Sections were rinsed in distilled water three times to remove alcohol and stained with safranin O for 5-10 min. They were rinsed in distilled water thrice and few drops of Alcian blue was added to counter stain for 10-15 min. Specimens stained and mounted in Canada balsam, slides were then examined for diagnostic features using Zeiss Standard 25 Photomicroscope.

Fluorescence analysis

The fluorescence properties of plants samples were studied under ultra violet (UV) light adopting previously described methods.[14,15] A small quantity of powdered leaf, stem, root and whole plant of *Cyathula prostrata* was placed on a clean glass slide and 1 mL of freshly prepared fluorescence reagents *viz.* 1 N HCl, 1 N NaOH, 50% HNO_3, conc. HNO_3, 50% H_2SO_4, conc. H_2SO_4, acetic acid, Iodine water, $FeCl_3$, Picric acid was added, followed by gentle mixing. The behaviour of the sample with different chemical reagents was studied and fluorescence characters were observed in daylight and under Ultra Violet lamp at 254 and 365 after 1-2 min.

Physico-chemical evaluation

The determination of physico-chemical parameters was done according to standard procedures.[16] The parameters studied include: moisture content, loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble extractive and alcohol soluble extractive. The pH of aqueous solution was measured by suspending the powdered materials each in a glass of distilled water. After 2 h, the mixture was filtered and the clear solution was measured for pH value.

Thin Layer Chromatography

Thin layer chromatography (TLC) was used to screen

the extracts of the root, stem, root and whole plant of *C. prostrata* for important secondary metabolites using pre-coated TLC plates (Silica gel G 60 F_{254} sheets 20 X 20 cm, 0.5 mm thickness, Merck Darmstadt, Germany). The plates were activated for 1 h before they were used. The concentrated extracts were spotted on TLC plates, developed in suitable solvent system containing ethyl acetate, methanol, ethanol and water in different ratio. The plates were dried, visualized in daylight and under UV lamp fluorescence at 254 nm and 365 nm before they were sprayed with 1% anisaldehyde in glacial acetic acid, vannin in sulphuric acid and 5% Ferric chloride in 0.5N HCl. Spots were marked and recorded accordingly for the calculation of retardation factor.

Determination of Total Phenolic Content (TPC)

The total phenolic content of the different solvent extracts of leaf, stem, root and whole plant of *C. prostrata* were determined using the reported method with slight modifications[17]. Calibration curve was prepared by mixing Gallic acid (1 mL: 0.001 - 0.005 mg/mL) with 0.2 mL of Folin-Ciocalteu reagent (undiluted), 2 mL of distilled water and 1 mL of 15% Na_2CO_3. Mixture was made to 50 mL volume using distilled water and then allowed to stand for 2 h. Ten milligram of different solvent extracts of the leaf, stem, root and whole plant of *C. prostrata* were weighed in a conical flask and dissolved with 100 mL of distilled water to make the stock solution after which 0.2 mL of Folin-Ciocalteau (undiluted), 2 mL of distilled water and 1 mL of 15% Na_2CO_3 were added to 0.5 mL of the sample stock solution. Mixtures were allowed to stand for 2 h. Mixtures turned to blue on adding 1 mL of 15% Na_2CO_3 indicating presence of phenolics. Absorbance values were measured at 760 nm using Ultraviolet visible Spectrophotometer (Ultraviolet Grating spectrophotometer 725s) and the standard curve was drawn by plotting the values of absorbance against concentration. All determinations were carried out in triplicate. The total phenolic content was calculated in milligrams of Gallic acid equivalents (GAE) per gram of extract.

Determination of Total Flavonoid Content

The aluminum chloride colorimetric method that was followed in this study was modified from the procedure reported in literature.[18] Quercetin was used to make the standard calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 25 and 50 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 1% aluminium chloride, 0.1 mL of 1M Potassium Acetate and 2.8 mL distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction was measured at 415 nm with Ultraviolet visible Spectrophotometer. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank. Ten milligram of each different solvent extracts of the leaf, stem, root and whole plant of *Cyathula prostrata* were weighed and transferred to 10 mL volumetric flask and made up with 80% ethanol. Similarly 0.5 mL of ethanol extracts of leaf, stem, root and whole plant of *C. prostrata* were treated with aluminium chloride for determination of flavonoid content as described above. Each sample extract was repeated three times. Based on the measured absorbance, the concentration of flavonoids was read on the calibration curve.The content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of quercetin/g of extract).[19]

DPPH Radical Scavenging Assay

The radical scavenging activity of the plant part extracts of *C. prostrata* in different solvents against 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was carried out by a slight modification of reported procedure.[13] The DPPH powder (18 mg) was dissolved in 150 mL methanol (Analytical, BDH) to make a stock solution. Powdered plant (0.1 g of leaf, stem, root and whole plant of *C. prostrata* in different solvents were dissolved in 100 mL methanol (Analytical, BDH) to make a stock solution each. The solution (3 mL each) was taken from the stock solution and 1 mL of DPPH solution was added to the solution in a test tube. These mixtures were shaken and kept at room temperature in the dark for 30 min (incubation period). The blank solution was prepared using methanol in place of the extract solution. Ascorbic acid and Gallic acid were used as standard reference drugs. Absorbance was read with a UV spectrophotometer at 517 nm. The experiment was carried out in triplicate. The radical scavenging activity of extract was calculated using the % inhibition formula:

$$\% \text{ inhibition} = 100 \times (1 - AE/AD).$$

Where AE is the absorbance of the sample solution and AD is the absorbance of the blank. Lower absorbance of the reaction mixture indicated higher free radical.[13]

RESULTS

Macroscopic Evaluation

Cyathula prostrata plant grows to 26.0 (66.3 ± 42.5) 122.0

cm, leaves are small, exstipulate, pubescent, opposite in arrangement, 2.0 (2.4 ± 0.32) 2.8 cm long and 1.5 (1.5 ± 0.2) 1.8 cm wide. They are ovate in shape with acuminate apex and asymmetrical base.

Microscopic Evaluation

Leaf

Epidermal peel of *C. prostrata* consists of trichomes, stomata and epidermal cells. Trichomes of both surfaces are simple, uniseriate, multicellular and non-glandular. Adaxial surface has few trichomes compared to abaxial surface. *C. prostrata* is amphistomatic. Adaxial surface consists of anomocytic stomata while abaxial surface consists of actinocytic stomata. Adaxial surface consists of few stomata compared to abaxial surface (Figure 1 A-D). The shape of epidermal cells of both surfaces is curve to undulate. The cell of adaxial surface is thick and slightly curved to undulated type. The cell of abaxial surface is thick with pitted periclinal wall and wavy anticlinal wall pattern. The epidermal cells of abaxial surface also contain costal cell as shown in Figure 1 D. Trichome density on adaxial surface is 12 while on abaxial surface is 6. Trichome length and width on the adaxial surface are 1110 (208 ± 339) 60 µm x 100 (59.0 ± 17.9) 40 µm while abaxial shows 490 (189 ± 153) 50 µm x 70 (53.0 ± 11.6) 410 µm. Stomatal index is 40.98 on the the adaxial surface and 22.72 on the abaxial surface. Stomata numbers on the adaxial and abaxial surfaces are 25 and 20 per field of view, respectively. The length and width of stomata on the adaxial and abaxial surfaces are 22.5 (15.5 ± 3.87) 10.0 x 15.0 (12.8 ± 1.80) 10.0 and 20.0 (14.8 ± 3.48) 10.0 x 17.5 (14.1 ± 1.81) 12.5, respectively.

Transverse Section of *C. prostrata* leaf passing through the midrib has undulated outline on the lower side and convex shaped at the upper side with epidermal hairs. Vascular bundle is centrally located with a well-developed bundle sheath (Figure 2A). Epidermis is single layered followed by thin cuticle and well developed starch grains distributed mainly at the upper side of the lamina. Mesophyll tissue consists of collenchymatous cells. It has prominent intercellular spaces present underneath the bundle sheath (Figure 2A&B)

The lamina showed 1-2 layered palisade mesophyll cell that are elongated shaped and 2-5 layered spongy mesophyll cell. Epidermis is single layered while hypodermis is double layered. Starch grains are confined to the upper side of lamina (Figure 2B).

Stem

Transverse Section of stem of *C. prostrata* has circular outline. It shows epidermis covered with thin cuticle. Hypodermis is 2-4 layered collenchymatous followed by 3-5 layered parenchyma cortex. Cells of cortex are well developed and with intercellular spaces compactly arranged and well differentiated from other cells. Cells of cortex are elongated and angular in shape. Sclerenchyma cell is 1-2 layered. Middle portion shows four ring closely arranged collateral vascular bundles with intercellular spaces at the region. Pith is well developed. (Figure 2C).

Root

Transverse section of root of *C. prostrata* is circular in shape. Cork is well developed, thick and about 6-10 layers which covers the thin layer epidermis. Cortex is developed, angular in shape and about 8-10 layers. Pericyclic fibres are present in patches and well outlined. Xylem is well developed while phloem consists of sieve tubes that are prominent in this region. Vascular bundles are arranged in a regular fashion with parenchymatous pith centrally located (Figure 2D).

Fluorescence Analysis

The characteristic fluorescence properties or colours recorded in this study are presented in Table 1. This could be used as a standard in the identification and authentication of the leaf, stem, root and whole plant of *Cyathula prostrata* in crude form. The results presented could aid in checking adulteration, where the adulterated samples would show variation or difference in the emission of colours when compared with the genuine samples.

Physico-chemical Analysis

Physico- chemical parameter is a valuable analytical tool in the identification of plant samples and crude drugs. Table 2 shows the results of physico-chemical parameters. Loss on drying, Moisture content, Ash values, Extractive values and pH of studied samples were all recorded. Moisture contents according to British Herbal Pharmacopoeia should not be more than 14% in medicinal plants.The results obtained from this study indicates that the moisture contents is within the limits as *C. prostrata* leaf, stem and root have moisture content values of 12.50%, 12.50%, 7.5%, respectively. The moisture content (14.50%) of the whole plant is slightly higher than the recommended value.

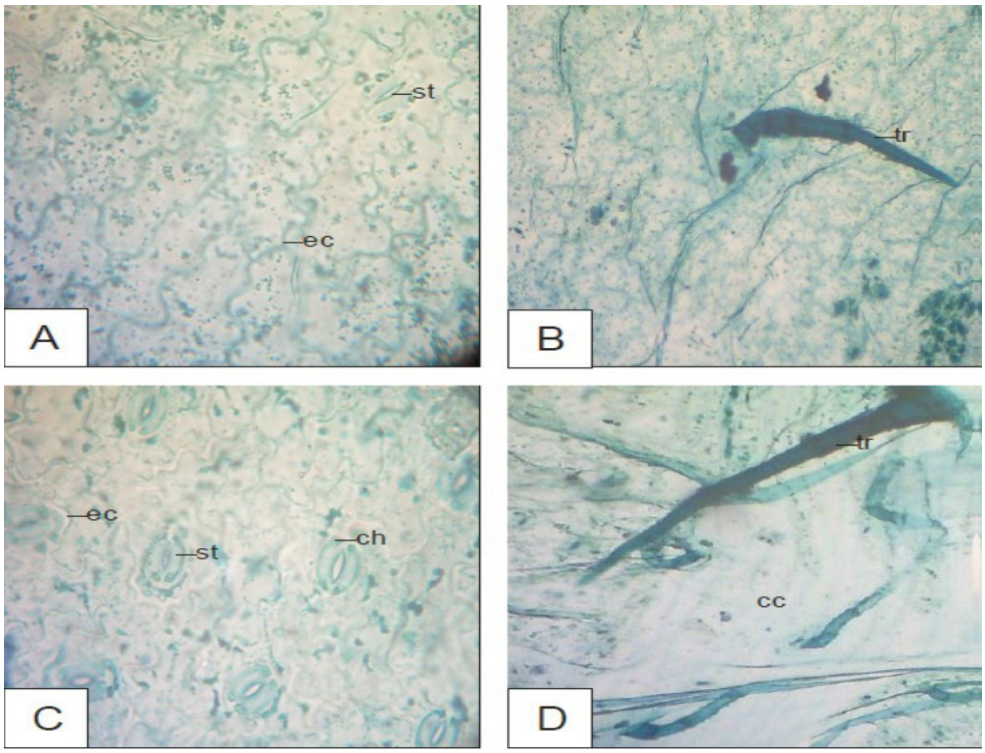

Figure 1: Photomicrograph of leaf surface epidermis of *Cyathula prostrata* x400:

(A) Adaxial surface showing stomata and epidermal cells; (B) Adaxial surface showing trichome; (C) Abaxial surface showing epidermal cells and stomata; (D) Adaxial surface showing trichome and coastal cells (cc: coastal cells, ch: chloroplast, ec: epidermal cell, st: stomata, tr: trichome)

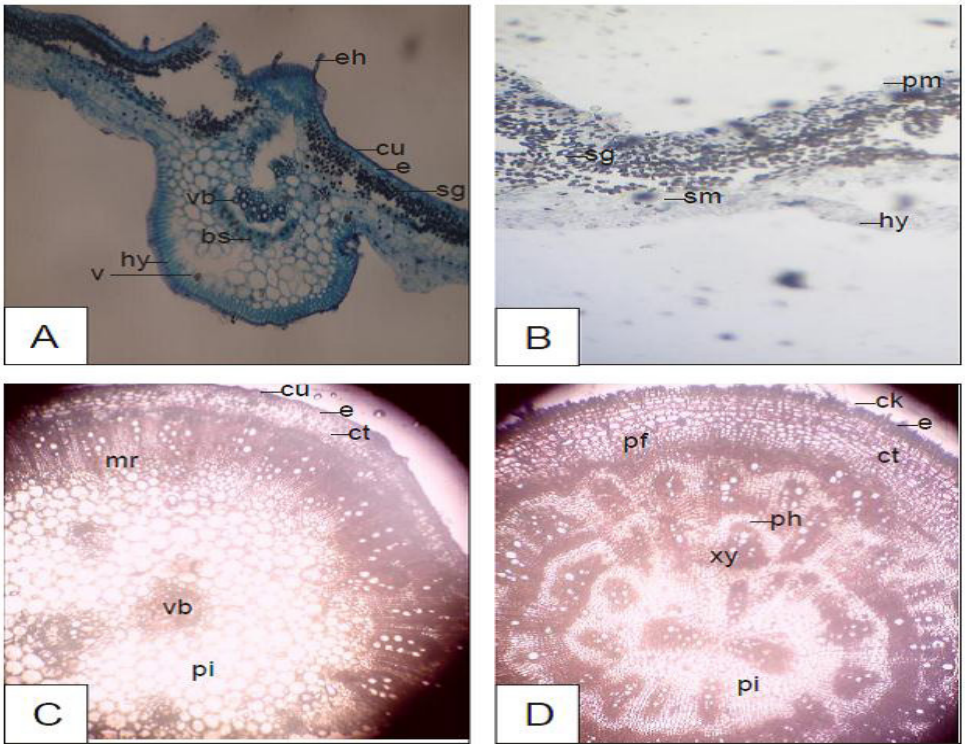

Figure 2: Photomicrograph of Transverse section (TS) of leaf, stem and root of *Cyathula prostrata* x400:

(A) TS of leaf showing midrib portion; (B) of leaf showing lamina portion; (C) TS of stem; (D) TS of root [bs: bundle sheath, ck: cork, ct: cortex, cu: cuticle, e: epidermis, eh: epidermal hair, hy: hypodermis, mr: medullary ray, pf: pericyclic fibre, pm: palisade mesophyll, ph: phloem, pi: pith, sg: starch grain, sm: spongy mesophyll, v: vein, vb: vascular bundle, xy: xylem]

Table 1: Fluorescence Characters of the powdered samples of *Cyathula prostrata*

***C. prostrata* leaves**			
Particulars of Treatment	**Under Visible Light**	**Under UV Light**	
		254	365
Powder as such	Deep Green	Deep Green	Dark Green
1 N HCL	Deep Green	Deep Green	Dark Brown
1 N NaOH in H_2O	Dark Green	Dark Green	Brownish Red
1 N NaOH in 1m MeoH	Dark Green	Deep Green	Warm Red
50% KoH	Dark Green	Dark Green	Dark Green
50% HNO_3	Dark Green	Deep Green	Brown
Conc. HNO3	Deep Brown	Dark Green	Warm Red
50% H_2SO_4	Dark Green	Green	Brownish Red
Conc. H_2SO_4	Deep Green	Dark Green	Brownish Red
Acetic Acid	Dark Green	Brownish Green	Dazzling Red
Iodine in Water	Deep Green	Dark Green	Deep Green
$FeCl_3$	Light Brown	Lemon Yellow	Cherry Red
***C. prostrata* stem**			
Particulars of Treatment	**Under Visible Light**	**Under UV Light**	
		254	365
Powder as such	Light Brown	Light Brown	Deep Brown
1 N HCL	Light Brown	Deep Green	Light Brown
1 N NaOH in H_2O	Brownish Green	Deep Green	Deep Green
1 N NaOH in 1m MeoH	Brown	Dark Green	Golden Yellow
50% KoH	Brownish Green	Dark Green	Deep Brown
50% HNO_3	Creamy Brown	Forest Green	Brown
Conc. HNO_3	Brown	Green	Dark Brown
50% H_2SO_4	Brown	Dark Green	Dark Brown
Conc. H_2SO_4	Black	Dark Green	Dazzling Brown
Acetic Acid	Light Green	Light Green	Dark Green
Iodine in Water	Brown	Dark Green	Dark Green
$FeCl_3$	Light Brown	Dark Green	Deep Red
***C. prostrata* root**			
Particulars of Treatment	**Under Visible Light**	**Under UV Light**	
		254	365
Powder as such	Brown	Creamy Brown	Brown
1 N HCL	Light Brown	Brown	Fire Red
1 N NaOH in H_2O	Brownish Green	Deep Green	Deep Green
1 N NaOH in 1m MeoH	Brown	Deep Green	Creamy Brown
50% KoH	Brown	Dark Green	Dark Green
50% HNO_3	Creamy Brown	Forest Green	Brown
Conc. HNO_3	Brown	Dark Green	Warm Red
50% H_2SO_4	Brown	Forest Green	Deep Red
Conc. H_2SO_4	Black	Dark Brown	Warm Red
Acetic Acid	Dark Green	Light Green	Green
Iodine in Water	Brown	Deep Green	Green
$FeCl_3$	Light Brown	Lemon Yellow	Cherry Red

***C. prostrata* whole plant**			
Particulars of Treatment	**Under Visible Light**	**Under UV Light**	
		254	365
Powder as such	Deep Green	Deep Green	Dark Green
1 N HCL	Dark Green	Dark Green	Dark Brown
1 N NaOH in H_2O	Light Green	Lemon Yellow	Dark Green
1 N NaOH in 1m MeoH	Dark Green	Dark Green	Dark Brown
50% KoH	Dark Green	Dark Green	Dark Green
50% HNO_3	Dark Green	Deep Green	Dark Brown
Conc. HNO_3	Deep Brown	Dark Green	Dark Red
50% H_2SO_4	Dark Green	Dark Green	Deep Red
Conc. H_2SO_4	Deep Green	Dark Green	Dazzling Brown
Acetic Acid	Dark Brown	Dark Green	Fire Red
Iodine in Water	Dark Green	Dark Green	Dark Green
$FeCl_3$	Green	Dark Green	Deep Red

Table 2: Physicochemical parameters of leaf, stem, root and whole plant of *Cyathula prostrata*

Parameters	**Values**			
	Leaf	**Stem**	**Root**	**Whole plant**
Loss on Drying (%)	5.0	6.0	7.0	6.5
Moisture Content (%)	12.5	12.5	7.5	14.5
Alcohol-soluble Extractive (%)	1.1	0.5	0.8	0.6
Water-soluble Extractive (%)	0.2	0.5	0.3	0.4
Total Ash (%)	14.8	9.5	13.5	15.5
pH	6.6	4.8	7.0	7.1

Table 3: Preliminary phytochemical screening of *Cyathula prostrata* powdered parts

Phytoconstituents	**C. prostrata Leaf**	**C. prostrata Stem**	**C. prostrata Root**	**C. prostrata Whole plant**
Anthraquinones	-	-	-	-
Saponins	+++	++	+	+++
Alkaloids	-	+	++	+
Tannins	+	++	+++	++
Phlobatannins	-	-	-	-
Flavonoids	++	+	++ +	++
Cardiac glycosides	++	+	+	++
Glycosides	+	-	+	+
Steroids	++	+	+	+++

+++ = abundant, ++ = present, + = present in trace amount, - = absent

Phytochemical Analysis, Thin Layer Chromatography and antioxidant activity

Preliminary analysis revealed the presence of saponins, alkaloids, tannins, flavonoids, cardiac glycosides and steroids in varying amount (Table 3). The TLC analysis of the chloroform extracts of the leaf, stem, root and whole plant of *C. prostrata* was used to evaluate the presence of secondary metabolites in the plant extracts for uniformity and reproducibility of the detected classes of secondary metabolites. The results showed the presence of at least seven components in the leaf, root and whole plant of *C. prostrata* while up to nine components were observed in the stem (Figure 3; Table 4).Total phenols results show that ethyl acetate extract of the roots of *C. prostrata* is the most responsive plant parts (Table 5). Total phenol highest mean value is 30.09 mg GAE/g. The result of the free radical scavenging activity of the plant against DPPH

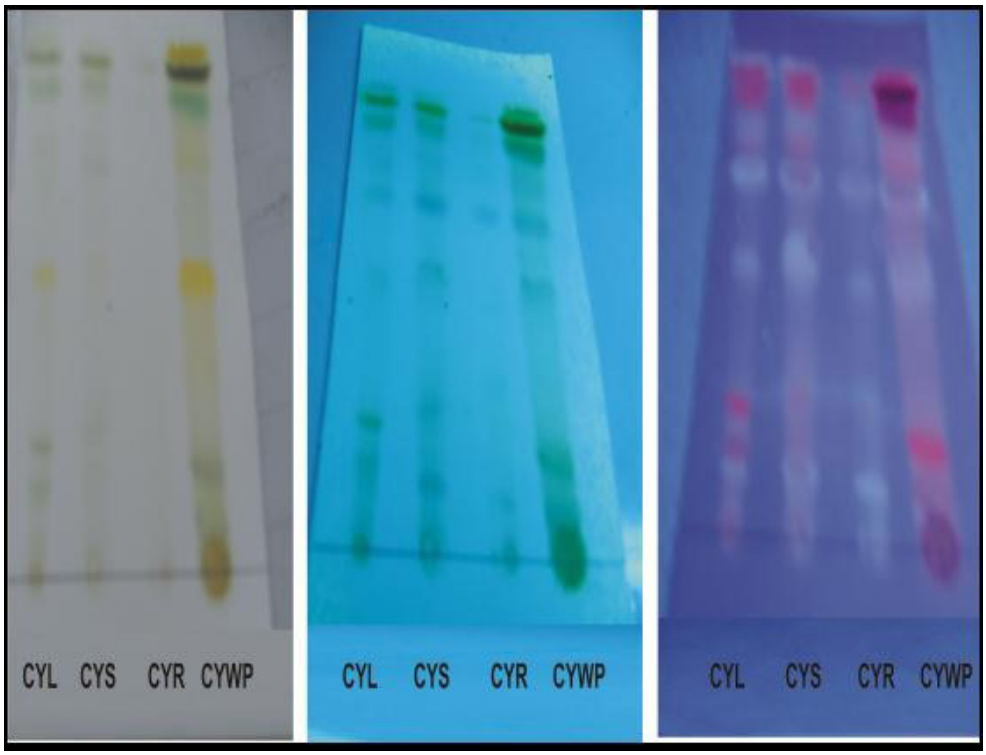

Figure 3: Thin Layer chromatographic profile of extracts of *C. prostrata* (Solvent system: Chloroform: methanol 8:2).

A. Visualization under UV 254, B. Visualization under UV 365, C. Visualization after spraying with Anisaldehyde-sulphuric acid reagent. CYL: *Cyathula prostrata* leaves, CYS: C*yathula prostrata* stem, CYR: *Cyathula prostrata* root, CYWP: *Cyathula prostrata* whole plant

Table 4: Retardation Factors (Rf) values of compunds obtained by thin layer chromatography of chloroform extract of *Cyathula prostrata*

Spot	Leaf	Stem	Root	Whole plant
1	0.40	0.22	0.43	0.46
2	0.60	0.24	0.47	0.61
3	0.68	0.43	0.63	0.70
4	0.73	0.60	0.73	0.83
5	0.80	0.70	0.84	0.89
6	0.87	0.80	0.89	0.92
7	0.91	0.84	0.95	0.95
8	-	0.87	-	-
9	-	0.92	-	-

Mobile phase solvent system for thin layer chromatography: chloroform-methanol (8:2), Spray reagent: Anisaldehyde in Sulphuric acid

is presented in Table 5 for chloroform, ethyl acetate and ethanolic extracts. Percentage inhibition of free radicals was significant at $P<0.05$ in ethanol extract of the root of the studied plant with a mean value of 87.0 ± 0.208.

DISCUSSION

The relevance of macroscopic and microscopic techniques in the standardization of medicinal plants is well documented in literature.[20,21,22,23,24] Macroscopy involves the use of vegetative and or floral characters to discriminate the desired plant species and plant part or morphologically similar species that might be included in recipes as adulterants while microscopy relates to assessment of micromorphological characters. The macroscopic and microscopic description of *C. prostrata* given in this study is the first step towards proper identification and authentication of the plant for purity.[25]

Fluorescence analysis is one of the pharmacognostic procedures useful in the identification of authentic samples and recognizing adulterants.[26] In the fluorescence

Table 5: Total phenolics content, total flavonoids content and DPPH free radical scavenging activityof *Cyathula prostrata*

Plant Samples	Total phenol mg GAE/g	Total flavonoids mg Quercetin/g	% DPPH Inhibition
Chloroform			
Leaf	9.389±0.362	1.407±0.143	7.380±1.955
Stem	7.183±0.698	3.225±0.703	3.187±2.303
Root	20.49±0.770	3.028±0.167	27.46±2.898
Whole plant	13.91±2.165	2.611±0.539	3.053±1.871
Ethyl acetate			
Leaf	8.434±1.738	2.334±0.053	11.77±0.991
Stem	8.618±0.645	2.216±0.147	11.9±4.82
Root	30.09±3.768	2.492±0.008	7.343±1.814
Whole plant	18.07±0.607	2.463±0.019	18.9±4.81
Ethanol			
Leaf	4.904±2.097	2.625±0.027	67.34±4.515
Stem	12.85±2.263	2.381±0.023	87.0±0.21
Root	22.62±0.414	2.268±0.028	32.56±5.326
Whole plant	15.16±2.131	2.427±0.142	42.0±2.88

Results are expressed as means ± SD (n = 3).

analysis, the plant parts or crude drugs may be examined as such, or in their powdered form or in solution or as extracts. Although, in most of the cases the actual substances responsible for the fluorescence properties has not been identified, the merits of simplicity and rapidity of the process makes it a valuable analytical tool in the identification of plant samples and crude drugs.[27] The fluorescence analysis of *C. prostrata* displayed an array of colours that could be employed for identification of probable classes of compounds in the plant.

The range of the moisture content of the plant parts is within the acceptable limit except in the whole plant part. For the extract of the leaf, stem and root of *C. prostrata*, there is less probability of degradation due to microbial growth as excess moisture in crude drug may lead to the breakdown of important active constituents.[28]

The preliminary phytochemical and TLC analyses of solvent extracts of different parts of *C. prostrate* are in support of the presence of several metabolites of interest in the plant. The probable classes of compounds detected are mainly terpenes and steroids. This claim could be substantiated as a result of the observation of an array of colours, which include violet, blue, green, pink, bluish green, brown or grey, indicating presence of different classes of compounds through spraying with anisaldehyde in sulphuric acid. Some of these classes of metabolites have been reported in literature to be responsible for various biological activities in many plants. Many plant terpenoids have been reported to have cytotoxic activities to tumor cells, making them useful chemotherapeutic or chemopreventive compounds. Paclitaxel (Taxol®) and related taxanes are the most well-known anticancer agents. These compounds bind to tubulin and stabilize microtubles, thus inhibiting cell division.[29] Mezerein and the phorbol esters activate protein kinase C isoforms through the diacylglycerol regulatory site and are potent second stage tumor promoters. Phytosterols, which are among plant steroids of note in the plant kingdom have been reported to possess hypocholesterolemic activity.[30]A large number of phenolic compounds present in vegetable foods, such as fruits and nuts, have been reported to possess good antioxidant properties.[31] Flavonoids, natural polyhydroxylated aromatic compounds, are widely distributed in the plant kingdom, including fruits and vegetables. About two-third of the polyphenols obtained in diet are flavonoids. Flavonoids display pronounced biological effects. Various investigations have established a relationship between the structure of different flavonoids and their relative efficiencies as antioxidants.[32] The antioxidant activity observed in *C. prostrata* could therefore be related to the presence of phenolic compounds such as flavonoids and tannins in the different parts of the plant. In some of the studies on antioxidants, the potential beneficial effects on health have been related to the polyphenol content of plants.[33] The reduction of oxidative stress elicited by the antioxidants aid in the prevention of cancer, cardiovascular diseases and complications of diabetes, among others.[34]

CONCLUSION

The study presents important diagnostic characters of *C. prostrata* that may be employed in correct identification of the plant. The study also reports the free radical scavenging activity of the plant that may be due to the presence of phenolic compounds in the different parts of the plant.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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REFERENCES

1. Radha C, Kumar PR, Prakash V. Enzymatic modification as a tool to injure the functional properties of heat processed soy flour. J Sci Food Agric. 2008; 88(2): 336-43.
2. Calixto JB, Barz J. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Med Biol Res. 2000; 33: 179-89.
3. Bhutani KK. Herbal medicines enigma and a challenge for science and guidelines for new initiatives. J Nat Product. 2003; 19(1): 3-8.
4. Patel PM, Patel NM, Goyal RK. Quality control of herbal products. The Indian Pharmacist 2006; 5(45): 26-30.
5. Sharma SK, Singh H. Pharmacognostical standardization of *Jatropha integerrima* Jacq. (Euphorbiaceae) roots. Der Pharmacia Lettre 2013; 5(1): 155-9.
6. Burkill HM. The Useful Plants of West Tropical Africa, vol. 1. Royal Botanic Gardens, Kew; 1995 pp. 58–9.
7. Oladimeji OH, Nia R, Ajibesin K, Igboasoiyi AC, Dalandi B. Phytochemical and antibacterial studies on the aqueous and non-aqueous extracts of *Cyathula prostrata* L. Blume. Global J Med Sci. 2005; 4: 9-12.
8. Sowemimo A, van de Venter M, Baatjies L, Koekemoer T. Cytotoxic activity of selected Nigerian plants. Afr J Tradit Compl Alt Med. 2009; 6(4): 526-8.
9. Sowemimo A, van de Venter M, Baatjies L, Koekemoer T. Cytotoxicity evaluation of selected Nigerian plants used in traditional cancer treatment. J Med Plant Res. 2011; 5(11): 2442-4.
10. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright-scienctechnica, Bristol; 1975. p 4-9.
11. WHO. Quality control methods for medicinal plant materials. Geneva, Switzerland; 1998.
12. Ayodele AE, Olowokudejo JD. The family Polygonaceae in West Africa: Taxonomic significance of leaf epidermal characters. S African J Bot. 2006; 72(3): 442-59.
13. Ahmad M, Khan MA, Zafar M, Arshad M, Sultana S, Abbasi BH, Din SU. Use of chemotaxonomic markers for misidentified medicinal plants used in traditional medicines. J Med Plant Res. 2010; 4(13): 1244-52.
14. Kokoski CJ, Kokoski RJ, Slama FJ. Fluoresecence of powdered vegetable drugs under ultraviolet Radiation. J Am Pharm Assoc. 1958; 47(10): 715-8.
15. Chase CR., Pratt RF. Fluorescence of powdered vegetable drugs with particular reference to the development of systems of identification. J Am Pharm Assoc. 1949; 38(6): 324-33.
16. Audu AA, Lawal AO. Variation in Metal Contents of Plants in Vegetable garden sites in KanoMetropolis. J Appl Sci Environ Management 2005; 10(2): 105-9.
17. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999; 299: 152-78.
18. Woisky R, Salatino A. Analysis of propolis; Some parameters and procedures for chemicals quality control. J Agric Res.1998; 37(2): 99-105.
19. Quiettieret AL, Betranda C, Habricot Y, Miginiac E, Agnes C. Flavonoid, hesperidine, total phenolic content and antioxidant. Academic J. 2000; 18: 1.
20. Kadiri AB, Ayodele AE. Comparative leaf micromorphological characters of the Nigerian species of *Rauvolfia* L. (*Apocynaceae*). Biosci Res Commn. 2003; 15(6): 35-41.
21. Sonibare MA, Jayeola AA, Egunyomi A. A survey of epidermal morphology of the genus *Ficus* Linn. (Moraceae). Bot Bull Acad Sinica, 2005; 46: 231-8.
22. Smillie TJ, Khan IA. A comprehensive approach to identifying and authenticating botanical products. Clin Pharmacol Therapeu. 2009; 87(2): 175-86.
23. Kahraman A, Celep F. Anatomical properties of *Colchicum kurdicum* (Bornm.) Stef. Colchicaceae). Aust J Crop Sci. 2010; 4(5): 369-71.
24. Nurit-Silva K, Costa-Silva R, Coelho VPM, Agra MF. A Pharmacobotanical study of vegetative organs of *Solanum torvum*. Brazilian J Pharmacogno. 2011; 21(4): 568-74.
25. Singh S, Manchawal L, Chauhan MG. Pharmacognostic study of male leaves of Trichosanthes dioica Roxb. With special emphasis on microscopic technique. J Pharmacogno Pharmacother 2010; 2(5): 71-5.
26. Tyler VE, Brady LR, Rubber JF. "Pharmacognosy". Lea and Febiger, Philadephia; 1976. p 24.
27. Denston TC. A textbook of Pharmacognosy, Sir Isaac Pitman and sons, Ltd., London; 1946. p 46-51.
28. Adeshina GO, Jegede IA, Kunle OF, Odama LE, Ehinmidu JO, Onaolapo JA. Pharmacognostic studies of the leaf of *Alchornea cordifolia* (Euphorbiaceae) found in Abuja. Nig J Pharm Sci. 2008; 7(1): 29-35.
29. Bhalla KN. Microtubule-targeted anticancer agents and apoptosis. *Oncogene*. 2003; 22(56): 9075-86.
30. Gunaherath BG, MK, Gunatilaka, AAL. Plant Steroids: Occurrence, Biological Significance and their Analysis. Encyclopedia of Analytical Chemistry; 2014. p 1–26.
31. Maestri DM, Nepote V, Lamarque AL, Zygadlo JA. *Natural products as antioxidants*. Phytochemistry: Advances in Research 2006. p 105-35.
32. Bors W, Heller W, Muchel C, Saran M. Structural Principles of Flavonoid Antioxidants, in Free Radical and the Liver, ed. by G. Csomós and J. Fehér, Springer; 1992. Berlin, 77.
33. Shieh HL, Hansen H, Zhu J, Riedel H. Differential protein kinase C ligand regulation detected *in vivo* by phenotypic yeast assay. Mol Carcinog, 1995; 12(3): 166-76.
34. Walter M, Marchesan E. Phenolic compounds and antioxidant activity of rice. Brazilian Arch Biol Technol. 2011; 54(2): Curitiba Mar./Apr. 2011.