Asian Plant Research Journal

9(3): 8-16, 2022; Article no.APRJ.87802
ISSN: 2581-9992

Evaluation of Phytochemicals and Bioactive Properties in Leaf and Root Parts of *Cyathula prostrata* (Pasture Weed) – A Qualitative and Quantitative Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/APRJ/2022/v9i330207

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/87802

Original Research Article

Received 23 March 2022
Accepted 01 June 2022
Published 06 June 2022

ABSTRACT

Cyathula prostrata (Blume) L, a member of the Amaranthaceae family, has long been used in tropical regions of the world for a variety of diseases, and study has shown that it is beneficial in the treatment of rheumatism, dysentery, wounds, and urethral discharges. The goal of this study was to use standard conventional methods to investigate the phytochemical content of this plant's leaf and root parts. The presence or absence of several secondary metabolites was examined qualitatively and quantitatively in the crude extract. Phytochemical components were discovered to be more abundant in the leaves of *Cyathula prostrata* than in the root, according to the findings. Flavonoids, tannins, saponins, cardiac glycosides, steroids, and terpenoids were found in the leaf and root sections of the plant. These phytochemicals were found to be higher in the leaf than in the root, according to quantitative analysis of the leaf and root parts. Flavonoids were discovered to have the highest concentration of phytochemicals, followed by tannins, alkaloids, steroids, saponins, and terpenoids in that order. The findings revealed that the leaf and root of *Cyathula prostrata* have excellent nutritional and therapeutic values, suggesting that it could be used for medical purposes.

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Keywords: Phytochemicals; Cyathula prostrata; leaves and roots; bioactivities; ethnomedicine.

1. INTRODUCTION

Pasture weed, Fula Fulfulde (Hausa – Northern Nigeria), Cawere pepe (Yoruba – Western Nigeria), and Agbi ri gba (Igbo – Eastern Nigeria) are all names for *Cyathula prostrata*. *Cyathula prostrata* (L.) Blume belongs to the Amaranthaceae family and is a tropical annual slender herbaceous plant [1,2,3]. Pigweed, pasture weed, and the prickly chaff-flower plant are all names for the same plant [4]. It grows in tropical and subtropical farmed regions, wasteland, and forest boundaries, and it is used for fundamental traditional therapeutic purposes in many tropical and subtropical nations, including Nigeria, Cameron, Ghana, Ivory Coast, China, and Australia [5,6,7].

It was once only found in tropical Africa and Asia (Nigeria, Mozambique, Uganda, China, India, and Vietnam), but it has now spread to tropical America, Australia, and the Pacific Islands [8-13].

Traditionally, various preparations of the leaves, stems and roots of this plant are used to treat a range of illnesses including articular rheumatism, cough, skin diseases, scabies, craw-craw, snake bites, bruises, liver problem, dysentery, diarrhea, nausea, cholera vomiting blood, and many others in Nigeria and other African countries [5,14,15]. Among the Kurichayas tribe of Kannur District, a tea spoon of the dried powdered root is boiled in water and taken thrice daily as cure for fever [16]. When mixed with other plants (*Synedrella nodiflora* and *Aframomum melegueta*), and clay, it is used to treat heart trouble and bronchial infections while the fruit has been claimed to prevent miscarriages [5]. Scientifically, the methanolic extract of *Cyathula prostrata* has been documented to be relatively non toxic in albino mice [15]. Also, it was recently documented that the methanolic extract of this plant possesses anti-inflammatory and analgesic properties, justifying its application in the traditional management of ailments associated with pains among others [17]. Despite the arrays of traditional applications to which the leaf, stem and root of *Cyathula prostrata* are subjected to, available literature revealed that there is paucity of information on the scientific elucidation of these plants as remedy for the acclaimed related ailments. Hence, this present investigation seeks to scientifically evaluate the phytochemical properties present in the leaves and roots of the plant for which it has been able to have these acclaimed medical properties.

2. MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Samples:

The leaves and roots were collected from Rumuosi Community in Obio/Akpor Local Government Area of Rivers State, Nigeria. The plant was authenticated at the Herbarium unit of the Department of Plant Science and Biotechnology of the University of Port Harcourt, Choba, Rivers State, Nigeria by comparing it to existing specimens at the herbarium section. The leaves and roots were oven-dried at 80ºC for 2 hours and ground using mortar and pestle till a uniform powdery form was achieved.

Fig. 1. Leaves and Root Part of *Cyathula prostrata* (Pasture Weed)

Chemicals:

In the present study, all the chemicals were purchased from De-Integrated Laboratories Limited, Alakahia, Rivers State. The chemicals were of analytical grade.

Sample Extraction Procedure:

Cold water extraction:

Each powdered sample (200g) was soaked in 500ml of sterile distilled water, manually agitated, and allowed to extract for 48 hours before being filtered using Whatmann No 1 Filter paper. The filtrates were dried in a water bath at 50 degrees Celsius. The extracts were kept at 4 degrees Celsius and utilized as needed.

Hot water extraction:

200g of each weighted plant material was soaked for 48 hours in 500ml of hot water that had been heated for 30 minutes. Each extract was filtered through filter paper and dried in a water bath at 50 degrees Celsius. The extracts were kept at 4 degrees Celsius and utilized as needed.

Ethanol extraction:

200g of plant samples were soaked for 48 hours at room temperature in 500ml of 100% ethanol with intermittent stirring. The content was filtered and dried in a water bath at 50 degrees Celsius. The extracts were collected and kept at 4°C until they were needed for the experiment.

Qualitative Phytochemical Examination:

Phytochemicals such as alkaloids, flavonoids, saponins, tannins, phenols, steroids, terpenoids, cardiac glycoside, and phytosterols were identified in the different extracts of *Cyathula prostrata* using the standard procedures described by Odebiyi and Sofowora [18], Trease and Evans [19], Onwuka [20], Ogu et al. [21] and Olayinka et al. [22].

Test for alkaloids:

0.5g of the sample was accurately weighed and defatted with 5% ethyl ether for 15mins. The defatted sample was extracted for 20 minutes with 5.0ml of aqueous HCL on a steam bath. The resulting mixture was centrifuged for 10 minutes at 3000rpm to remove filtrate (Supernatant). 1.0ml of the filtrate was treated with a few drops of Mayer‘s reagent and a second 1.0ml portion was treated similarly with Dragendorff‘s reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids.

Test for flavonoids:

1.0ml of 10% lead acetate was added to 1.0ml of the extract contained in a test-tube. A formation of a yellow precipitate was taken as positive for flavonoids.

Test for tannins:

5.0 g of dried extract was stirred with 10.0ml of distilled water. This was filtered and ferric chloride reagent was added to the filtrate. A blue-black precipitate was taken as evidence for the presence of tannins.

Test for phenols:

5.0 g of dried extract was stirred with 20ml of distilled water in a test tube, boiled and then filtered. 3-4 drops of 0.1% v/v Ferric chloride was added to the filtered sample. The appearance of a brownish green or blue colour indicated presences of phenols.

Test for saponins:

The ability of saponins to produce frothing in aqueous solution was used as screening test for the sample. 0.5g of dried extract was shaken with water in a test tube, frothing which persist on warming was taken as evidence for the presence of saponins.

Test for cardiac glycosides:

0.5g of dried extract was dissolved in 2.0ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under laid with 1.0ml of concentrated H_2SO_4. A brown ring obtained at the interface indicated the presence of a cardenolides.

Test for steroids:

0.5g of the dried extract was extracted with 2.5ml of chloroform in a test tube and 1ml of concentrated sulphuric acid added to form a

lower layer. A reddish-brown interface indicated the presence of steroids.

Test for Terpenoids:

0.5ml of the chloroform extract of the dried extracts was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicated the presence of terpenoids.

Test for phytosterols:

The plant extract was mixed with chloroform and filtered. 5-6 drops of concentrated sulphuric acid was added to the filtrate and shaken gently and then allowed to stand for 1 to 5 minutes. The presence of triterpens (phytosterol) was indicated by the appearance of yellow or golden yellow colour.

Quantitative Phytochemical Screening by Gas Chromatography:

The phytochemicals in the extract were quantified using BUCK M910 Gas Chromatography (BUCK Scientific, USA). A flame ionization detector and a RESTEK 15m MKT-1 column (15m x 20m x 0.15um) were used in the gas chromatography. With a 20cL split less infusion of sample, the injection temperature was set at 280^{o}C and the injection velocity was 30cm/s. The carrier gas was helium (5.0pa) at a flow rate of 40ml/min. The oven's initial temperature will be 200°C; the oven will be heated at a rate of 3°C/min until it reaches 330°C, with the detector set to 320°C. The area and mass of the internal standard were compared to the area of the detected phytochemicals to identify them. The concentrations of each phytochemicals were reported in g/mL [23].

Method of Data Analysis:

As a result of the analytical process, the results were expressed in figures. During qualitative screening, the presence or lack of phytochemical was indicated by (+) for the presence of phytochemical and (-) for the absence of phytochemical.

3. RESULTS

Table 1. Cold and hot extract yield of *Cyathula prostrata*

Plant part	Cold water extract yield (% w/w)	Hot water extract yield (% w/w)	Ethanol extract yield (% w/w)
Leaf	13.6	14.1	14.8
Root	14.0	13.8	14.0

Table 2. Qualitative analysis of the phytochemicals in Leaf and Root extracts of *Cyathula prostrata*

Phytochemicals	Leaf	Root	Test	Observation
Alkaloids	+	-	Filtrate + Mayer's reagent	Pale yellow precipitate. Alkaloid present
Flavonoids	+	+	1ml of 10% lead acetate + 1ml of extract	Cloudy solution or yellow precipitate. Flavonoid present
Tannins	+	+	Extract + 4 drops of FeCl3	Blue-black precipitate. Tannin present
Saponins	+	+	Foam test	Foam/Frothing seen. Saponin present
Cardiac Glycosides	+	+	Extract + 2ml glacial acetic acid + drop of Ferric chloride laid under conc. H_2SO_4	Brown-ring indicates the presence of a glycoside. No reaction indicates the absence of glycosides.
Steroids	+	+	Extract + 2.5ml chloroform + 1ml conc. H_2SO_4	Appearance of a reddish-brown colour indicates the presence of steroid.
Terpenoids	+	+	Extract + 0.5ml chloroform + Heated to dryness + 3ml	Appearance of a grey colour indicates the presence of

Phytochemicals	Leaf	Root	Test	Observation
			of conc. H_2SO_4	terpenoid.
Phytosterols	-	+	Filtrate of extract + chloroform + drops of conc. H_2SO_4	Appearance of a yellow or golden-yellow colour indicates the presence of phytosterol.
Phenols	+	-	Ferric chloride (5% FeCl3)	Appearance of a brownish green or blue colour indicates the presence of phenol.

Key: + = Deteected, - = Not detected

Table 3. Quantitative phytochemical screening of *Cyathula prostrata* using GC-FID

PK No.	RT	Parameters	Concentration (µg/g)		Class of compound	Pharmacological activity
			Leaf	Root		
1	8.21	Naringin	0.19	0.12		Anti-inflammatory, anti-
2	10.13	Proanthocyanin	0.07	0.03		oxidant activity, inhibition
3	10.25	Anthocyanin	8.84	5.46		of cancer cell invasion,
4	10.48	Naringenin	13.20	10.18		potent inhibitory effect on
5	11.06	Flavonones	23.01	24.62		in vitro bone resorption.
6	11.62	Epicatechin	27.83	26.72		Anti-mutagenic and
7	12.11	Kaenpferol	30.45	30.51	Flavonoid	antiangiogenic activity.
8	12.67	Flavones	32.66	30.02		
9	13.09	Catechin	36.52	35.35		
10	13.54	Rutin	38.68	40.27		
11	13.98	Resveratrol	40.11	38.46		
12	14.27	Flavan-3-ol	40.57	39.17		
13	14.84	Lunamarin	32.92	27.66		Anti-epileptic activity.
14	14.92	Quinine	5.02	0.81		Anti-hypoxic, immune-
15	15.10	Ribalindine	10.98	2.52		modulating and anti-
16	15.35	Spartein	13.93	1.83	Alkaloid	inflammatory activity.
17	15.52	Epihedrine	40.76	12.04		Exhibit nootropic, cytotoxic and sedative activity.
18	15.78	Sapogenin	16.57	14.28	Saponin	Cardiovascular effect and anti-cancer effect
19	15.86	Oxalate	38.41	2.05	Dicarboxylic acid	Antimicrobial activity
20	16.01	Steroids	24.82	18.31	Steroid	Anti-inflammatory and anti-cancer activity
21	16.33	Tannin	42.04	36.26	Tannin	Antioxidant, antibacterial, antiviral, antiparasitic and antidiarrheal activities.
22	16.68	Phenol	20.15	0.02	Phenol	Antioxidant potency
23	16.92	Paracyclophane	12.16	12.04	Terpenes	Antioxidant, Antibacterial, anti-inflammatory
24	17.13	Eicosane	8.72	6.95		
25	17.32	Phytol	33.16	10.41	Diterpene	Anti-inflammatory Anticancer, Diuretic, Antimicrobial activity.

Note: PK No. = Peak Number, RT = Retention Time

4. DISCUSSION

The presence of phytochemicals such as flavonoids, tannins, saponins, cardiac glycosides, steroids, and terpenoids in the leaf and root parts of *Cyathula prostrata* was discovered by qualitative screening, and these phytochemicals have been determined to have great medicinal potential [24]. As demonstrated in Table 1, alkaloid and phenol were absent from the root, while phytosterol was absent from the leaf of *Cyathula prostrata*. Phytochemical elements

were found to be more present in the leaves of *Cyathula prostrata* than in the root. Antimicrobial, antihypertensive, antioxidant, anti-inflammatory, antidiarrheal, and analgesic properties are all known for these phytochemicals. The biological activity of *Cyathula prostrata* extracts can be attributed to the presence of these constituents. Ogu et al. [21] investigated the antimicrobial and phytochemical properties of *Cyathula prostrata* leaf, stem bark, and root extracts against a variety of human pathogens, and discovered the presence of terpenoid, tannin, flavonoid, cardiac glycoside, and steroid in the leaf and root parts of the plant in both aqueous and ethanolic extracts. In addition, in their study of the antihypertensive potentials of *Cyathula prostrata*, Ojekale et al. [25] looked for phytochemicals in the plant and found alkaloids, flavonoids, tannins, cardiac glycosides, cyanogenic glycosides, terpenoids, anthraquinones glycosides, saponins, anthocyanosides, phlobatannins, and reducing sugars. As a result, our investigation supports the findings of Ojekale et al. [25] and Ogu et al. [21].

Plant-derived phytochemicals, such as flavonoids, have long been utilized in traditional medicine for their anti-inflammatory, anti-hypertensive, and anti-diabetic properties, particularly in the treatment of chronic inflammatory and allergy illnesses, breast cancer, and coronary artery disease [26]. Flavonoids have also been proven to be an effective antibacterial agent against a variety of pathogens in vitro, with their ability to combine with bacterial extracellular and soluble proteins being linked to their action [27]. Terpenes and steroids in general have attracted a lot of attention because of their physiological importance [28]. Several studies have shown that terpenoids and steroids have anti-malarial properties [28,29]. Saponins have anti-inflammatory capabilities, according to research [30]. In medicine, saponins are employed as emulsifiers, whereas tannins have been discovered to have anti-oxidant properties [31]. Alkaloids have been claimed to offer anti-malaria and pain-relieving properties [23].

Table 3 shows the findings of additional quantification of several extract components using a Gas Chromatography Flame Ionization Detector (GC-FID). The results revealed a large amount of flavonoids in both the leaf and root parts of *C. prostrata*, including resveratrol (40.11 µg/g, 38.46 µg/g), flavan-3-ol (40.57 µg/g, 39.17 µg/g), rutin (38.68 µg/g, 40.27 µg/g), catechin (36.52 µg/g, 35.35 µg/g), kaempferol (30.45g/ Naringenin (13.20 µg/g, 10.18 µg/g), anthocyanin (8.84 µg/g, 5.46 µg/g), naringin (0.19 µg/g, 0.12 µg/g), and proanthocyanin (0.07 µg/g, 0.03 µg/g) are some of the other flavonoids that have been found and quantified. From the foregoing, the measured chemicals were higher in the leaf than in the root. Flavonoids are recognized to have antifungal, antibacterial, antioxidant, anti-inflammatory, and anti-cancerous properties. The action of enzymes like alpha-rhamondase and beta-glucosidase in the body quickly converts naringin to naringenin [26]. Naringin, also known as naringenin, has a wide range of pharmacological and therapeutic activities, including antibacterial, antimutagenic, anti-inflammatory, free radical scavenging, anticancer, and cholesterol-lowering qualities [26]. Visual acuity, heart disease, cancer treatment, age-related neurodegenerative illnesses, and angiogenesis are all affected by anthocyanins [26]. Resveratrol has been linked to the treatment of high cholesterol, cancer, and heart disease, as well as its antioxidant properties, making it a promising supplement for decreasing blood pressure [31]. Proanthocyanins are antioxidants that protect the heart and circulatory system. Antioxidant, anti-inflammatory, antioxidant, and anticancer properties are among them. Resveratrol has been demonstrated to have antibacterial activity against gram-positive bacteria, and time-kill studies revealed that it had a bacteriostatic effect [32]. In addition to its cardioprotective, antioxidant, anticancer, neuroprotective, anti-inflammatory, anti-dyslipidemia, and antidiabetic activities, resveratrol exhibits antiproliferative and androgen-lowering actions on the interstitial cells of the ovary [33].

Lunamarin (32.92µg/g, 27.66µg/g), quinine (5.02µg/g, 0.81µg/g), ribalindine (10.98µg/g, 2.52µg/g), spartein (13.93µg/g, 1.83µg/g), and epihedrine (40.76µg/g, 12.04µg/g) were also found and measured in the leaf and root parts of *C. prostrata* in the study. Ojekale et al. [25] used standard phytochemistry procedures to determine the existence of secondary metabolites in *C. prostrata* extracts, which were then validated using GC-MS. They found 13 plant secondary metabolites in the class of essential oils, terpenes, palmitic acid, ester, and diterpene. The existence of terpenes, diterpenes, flavonoids, alkaloids, steroids, tannin, saponin, and phenol class compounds was investigated in this study. Alkaloids have a wide range of physiological effects in the body. Quinine's

antimalarial properties have been reported multiple times as a medication candidate for malaria treatment; however it has been stopped due to its negative effects [30]. Lunamarine has been claimed to have anti-amoebic and radical scavenging properties [29]. Ribalimdine, lunamarine, and spartein have all been linked to possible health advantages [32]. It has been suggested that the plant under investigation is used to treat diarrhea. The presence of tannins and saponins may be to blame for this habit. Saponins are a family of chemicals that have a cardiotonic action [34]. Sapogenins are utilized as a precursor in the production of medications or pharmacological analogues that are used to treat heart issues [35]. Tannins have been described as antioxidants, metal chelators, and regulators of lipid peroxidation and pre-oxidative enzymes. Tannins have recently been discovered to have antibacterial, anticarcinogenic, anti-inflammatory, anticancer, and antidiabetic properties [27]. Tannins are also used to treat diarrhea, inflammation, and burns in the medical field [27]. Another secondary metabolite found in the sample was phytol (33.16µg/g, 10.41µg/g). Antilipidemic and anti-inflammatory properties have been reported for phytol [27]. Phytic acids, on the other hand, are the most effective nutrition in the body. They act as metal chelators [27]. Tannins are helpful in the treatment of gastritis, esophagitis, enteritis, and bowel imitating disorders. Tannins have the ability to heal burns, stop bleeding, and prevent infection while also healing the wound internally [36]. This could be due to the traditional use of the plant's leaf in dressing sharp cut wounds and stopping the flow of blood in a fresh wound. Oxalate was also measured in the sample (38.41µg/g, 2.05µg/g). It has been used to treat cardiovascular ailments and as an antibacterial and anticancer agent. A significant level of steroid was measured (24.87µg/g, 18.31µg/g). Stress, immune response, glucose metabolism, protein catabolism, blood electrolyte levels, inflammation, and behavior control are all steroid-related physiological processes. Plant steroids include a variety of medicinal, pharmacological, and agrochemical qualities, including anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, anthelminthic, cytotoxic, and cardiotonic effects.

Steroid substances have also been shown to have hypocholesterolemic and anti-inflammatory properties. Kaempferol was discovered in the sample, and it has been shown to reduce the risk of chronic illnesses, including cancer. It strengthens the body's antioxidant defenses against free radicals. It has an impact on apoptosis, angiogenesis, inflammation, and metastasis. Rutin was also discovered as a major flavonoid in the sample. It's widely used to treat arthritis pain and minimize oxidative stress in arthritis patients. This could be because of rutin's anti-inflammatory and antioxidant properties. Rutin has also been shown to help patients with arthritis improve their knee function. This is proof of the plant's use in the treatment of pain and inflammatory disease such as arthritis. Phenol (20.15µg/g, 0.02µg/g) has long been used to clean the skin and relieve itching. It's also used to treat pharyngitis as an analgesic or anesthetic in products like chloroacetic. It also functions as an antiseptic. Other pharmacological properties have been described, including antioxidant, antiviral, anticancer, and anti-inflammatory properties [23].

5. CONCLUSION

The current research revealed that the concentrations of both qualitative and quantitative phytochemical components in *C. prostrata* leaf and root parts vary. Furthermore, the leaf contained far more of these constituents than the root. All of these phytochemicals are active and are responsible for the plant's various effects. More research is needed to determine specific phytochemicals that are responsible for the plant's antidiarrheal, antihypertensive, antimalarial, anti-inflammatory, and other biological properties. These would lend firm credence to the ethnomedicinal uses of the leaf and root of this plant.

6. RECOMMENDATION

The present study revealed that *C. prostrata* is rich in phytochemicals. These phytochemicals are responsible for the plant's ethnomedical usage. More research is needed to better demonstrate the pharmacological potentials of this plant, as drugs made from *Cyathula prostrata* could be an active ingredient in the treatment of certain diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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