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Research Article
Comparative Phytochemical, Morphological and Anatomical Studies of *Amaranthus hybridus* L. and *Amaranthus spinosus* L. (Amaranthaceae)

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Abstract
Background and Objective: *Amaranthus hybridus* and *Amaranthus spinosus* are widely distributed in Nigeria and are being used as medicinal plants. The comparative morphological, anatomical and phytochemical studies were carried out on 2 species of the genus *Amaranthus* L. (*A. hybridus* and *A. spinosus*) to determine their differences to easy their identification and potential sources of raw materials for pharmaceuticals. **Materials and Methods:** Standard HPLC method was used for phytochemical screening. For anatomical study, fresh specimens were dehydrated, wax embedded, sectioned, mounted and micro-photographed using Optika B-1000 FL LED fitted with digital camera and morphological study was done by visual observation. **Results:** The presence of spines on *A. spinosus* distinguishes it from *A. hybridus.* Also, the stem of *A. spinosus* is reddish-brown while that of *A. hybridus* is light green. The number of vascular bundles in the midrib, petiole, stem and root were different and could be used to differentiate them. Data obtained from the quantitative phytochemical analysis showed that their concentrations varied among the 2 species. *Amaranthus hybridus* had 72.56 and 61.79% vitamin A in the root and leaves, respectively while compared to *A. spinosus.* Leaves of *A. hybridus* had higher total flavonoids concentration of 56.46% while *A. spinosus* had the highest phenolics of 19.27 g/100 g and 18.63 g/100 g in the leaves and roots, respectively. Also, the roots of *A. spinosus* had the highest concentration of alkaloids, glycosides and phenolics. These explain why they are used in ethno-botany in Nigeria. **Conclusion:** Based on the phytochemical constituents, both plants could be valuable sources of dietary vitamins and potential sources of phytochemicals if properly exploited.

Key words: *Amaranthus,* alkaloids, secondary metabolites, phytochemicals, dietary vitamin

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

From the origin, plants have provided a variety of resources that contribute to the fundamental needs of food, clothing and shelter to man. Among plants of economic importance, medicinal and aromatic plants have played vital roles in alleviating human suffering. Plants are utilized as therapeutic agents since time immemorial in both organized and unorganized (folk, tribal, native)[1,2]. The genus *Amaranthus* L. belongs to the family Amaranthaceae in the order Caryophyllales and is native to tropical America and Africa. Members of this family are mainly herbs, but also include vines, shrubs and trees and comprised approximately 800 species represented by more than 60 genera and is broadly divided into 2 sub-families Amaranthoideae and Gomphrenoideae[3]. The cultivated grain Amaranths include *A. caudatus*, *A. cruentus* and *A. hypochondriacus* and their parental wild species are thought to be *A. hybridus* L.*,* *A. quitensis* and *A. powellii* S. Other *Amaranthus* species like *A. dubious* L., *A. hybridus* and *A. ticolor* L. are consumed as leafy vegetables. Meanwhile*, A. retro exus* L. (redroot pigweed), *A. albus* (tumbleweed), *A. palmeri* S. Wats. (Palmer amaranth) and *A. spinosus* (spiny amaranthus) represent weed species[4].

According to Akubugwo *et al.*[5] leaves extract of *A. spinosus* produces about 73% inhibition of prostaglandin biosynthesis *in vitro*. It has been discovered that the leaves and roots boiled together are also used traditionally as a diuretic, anti-diabetic, antipyretic, anti-snake venom, antileprotic and antigonorrheal[6]. The plant has a long history of usage in traditional medicine against various ailments around the World[7]. Traditionally boiled leaves and roots of *A. spinosus* are given to children as a laxative. Leaves and stems are collected, cooked, steamed or fried and consumed. It is used as forage for livestock. The ash is a tenderizer in cooking vegetables and pigeon peas. The root is effective to treat gonorrhea, applied externally to treat eczema, burns wounds, boils, earache, sores, ophthalmia and convulsions[6]. *Amaranthus spinosus* is a potential agent for accumulation and translocation of heavy metals[8]. In Nigeria*, A. hybridus* leaves combined with condiments are used to prepare soup[9]. Leaves are also eaten as green vegetables. *Amaranthus hybridus* grown on dumpsites possessed a higher concentration of heavy metals[10].

Amaranthus hyridus also known as 'green' in Nigeria, is mostly cultivated and used as leafy vegetables to prepare various meals such as; yam and soup in southern Nigeria[9]. Other species of *Amaranthus* such as; *A. spinosus* is also eaten in Nigeria and it is also used for other ethnobotanical purposes by the local communities. Despite the economic importance of these species, only a few or little information is available about the potentials of these species and the taxonomy of some *Amaranthus* still problematic, although some methods have been employed to classify them[11-16]. Also, proper identification of these species is important. This work aimed at conducting morpho-anatomical studies of two *Amaranthus* species (*A. hybridus* and *A. spinosus*) widely distributed in Nigeria for easy identification and to analyze and compare the phytochemical and vitamin contents of both species as potential raw materials for pharmaceutical industries.

MATERIALS AND METHODS

Study area: The study was carried out at Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria from April, 2018-August, 2019.

Sample collection: Materials used for this research work were sourced from in and around the University of Port Harcourt. Fresh plant parts from *A. spinosus* were collected from the botanical garden of the Faculty of Agriculture, University of Port Harcourt while *A. hybridus* was collected from Igbogo road in Choba community, Obio-Akpor Local Government, Rivers State. The plant specimens were properly identified, confirmed and deposited in the Department of Plant Science and Biotechnology Herbarium, University of Port Harcourt with herbarium number *A. spinosus* (UPH/V/1354) and *A. hybridus* (UPH/V/1403). The mature vegetative parts including the stem root and leaves of both species were examined for morphological characteristics, anatomical studies and phytochemicals (flavonoids, alkaloids, glycoside and phenolic) properties.

Morphology: Morphological studies were carried out by visual observation of the vegetative parts, stem and roots of *A. spinosus* and *A. hybridus* growing in the wild. The morphological observations were described.

Epidermal investigation: Fresh samples of the mature leaves were obtained. Adaxial and abaxial epidermal peels were peeled and were fixed by passing through alcohol series (30, 50, 70 and 100%) solution for 2 h each after which they were washed with distilled water. The strips were first stained with safranin O and counter stained with Alcian blue, it was mounted on a slide and a drop of glycerine was put on it then it was covered with a coverslip and viewed under the microscope using $\times$40. Photomicrographs were taken with the aid of a camera.

Anatomical investigation: The fresh parts (stem, petiole, midrib and root) were fixed in Formalin Acetic Acid (FAA) for 24 h after which they passed through alcohol series (30,50, 70 and 100%) solution and stored in 100% ethanol until use. These specimens were embedded in paraffin wax and sectioned. Thin sections of the stem, petiole, midrib and root were obtained by free-hand sectioning using a new razor blade. These selected thin sections were de-waxed, stained with safranin O and counter stained with Alcian blue, rinsed with distilled water and mounted on a clean slide with a drop of glycerine then covered with a coverslip and viewed under microscope. Photographs of selected good sections were taken using camera.

Phytochemical investigation

Alkaloids determination: Five grams of the sample was weighed into a flask. Hundred milliliters of 12% alcohol was added, shake and filtered. Thereafter, washed with 20 mL of industrial alcohol. The extracted residue was washed into flasks with 50 mL of ammonia-water (i.e., use ultrapure water), heated in boiling water for 20 min and cooled. Diastase (0.1 g+water) was added and the temperature maintained at 50-55°C for 2 h. At the expiration of 2 h, the sample was allowed to cool and made up to 250 mL with ultrapure water, swirled and filtered. Filtrate (200 mL) was mixed with 20 mL hydrochloric acid (sp.g. 1.125), heated in boiling water for 3 h, cooled, neutralize with sodium hydroxide solution, made up to 250 mL, shook, centrifuged and decanted. The supernatant was used for alkaloid determination using water 616/626 HPLC with the nitrogen gas flow rate of 40 mL min^{-1}, detector temperature of 170°C, injection port temperature of 190°C and column temperature[17] of 125°C.

Phenolics determination: Sample (2 g) was weighed into a set of test tubes. Three milliliters of 70% acetone and water were added to the test tube, placed in an ultrasonic water bath at 10°C for 5 min. The sample was stirred occasionally with a glass rod and filtered through a 50-60 μ Gooch crucible into a 50 mL Erlenmeyer flask. Steps 2 and 3 were repeated 3 times and the test tubes with the final rinsed with a 3 mL portion of 70% acetone in water and emptied into the test tubes. About 2 mL of 0.1 M acetate and 15 mL of 0.1 M triethylamine (TEA) reagent were added into the filtrate. Thereafter, the contents of the test tube were transferred into a volumetric flask, closed with a rubber stopper, swirled, shook for 20 min and allowed to settle for 4 h. The supernatants were collected for analysis using HPLC (Water 616/626) with the argon gas flow rate of 60 mL min^{-1}, detector temperature of 120°C, injection port temperature of 155°C and column temperature[18] of 117°C.

Glycosides determination: Half (0.5 g) of the sample was weighed into a set of digestive tubes. About 5 mL of 0.1 M HCl was added and warm gently for 15 min at 105°C and transferred into a 50 mL volumetric flask. Steps 1 and 2 above were repeated twice, rinsed with 2-3 additional aliquots, allowed for complete filtration and the filtrate made up to 100 mL mark with the extractant solution and mixed thoroughly. Extract (5 mL) solution from the 100 mL flask was purified by running it through a 2 cm layer (the resin is packed on a macro pipette tip) cation exchange resin (CEC). The glycoside compounds were eluded with 10 mL of absolute ethanol, the ethanol washed from the column with ultrapure water (10 mL), supernatant transferred to a sample vial and ran on HPLC (Water 616/626) with the nitrogen gas flow rate of 38 mL min^{-1}, detector temperature of 167°C, injection port temperature of 183°C and column temperature[18] of 130°C.

Flavonoids determination: Sample (1.5 g) was weighed into a set of extraction tube. Boiled (20 mL) ultra-pure water dispensed into each extraction tubes, allowed to stand for 1½ h, a vertex for 5 min and transferred to a set of centrifuge tubes, shook for 15 min and centrifuged for 5 min at 3000 rpm. Thereafter, the supernatant was transferred to a set of vials and determined on water 616/626 HPLC with the nitrogen gas flow rate of 60 mL min^{-1}, detector temperature of 147°C, injection port temperature of 166°C and column temperature[19] of 115°C.

Determination of vitamins: The extraction and determination of vitamin A, B_2, B_6, B_{12} and E were performed according to the method described by Ezeonu and Ejikeme[20] and Gaafar *et al.*[21] while vitamin C was determined using the titrimetric method[22].

Vitamin A (extraction and determination): Plant sample (0.5 g) was weighed into a conical flask, 20 mL of 0.2 N HCl dispensed and allowed to stand for 1.5 h. The solution was cooled and the pH adjusted to pH 6, using NaOH. Also, 1 N HCl added to lower the pH to 4.5. The solution was made up to 50 mL, shook and centrifuged for 10 min at 3000 rpm. The supernatant was separated, 1 mL of acetic acid (CH_3COOH) added and mixed properly. Also, 0.5 mL of 3% H_2O_2 added and mixed well. Finally, 20 mg of sodium hydrogen sulphate was added and then shook properly. The extract was run on

HPLC (Waters 616/626). Water 616/626 accessories used had Merck Lichrospher WOCH-18/2 (5 μm) at 40°C column (stationary phase) and mobile phase (Solvent 'A' was 30 mM sodium acetate, pH 6.5 containing 5% dimethylformamide and solvent 'B' was acetonitrile) with fluorescence detector, range of working standard (0, 2, 4, 6 and 8 ppm) and determination was carried out at a wavelength of 328 nm.

Vitamin B_1, B_2, B_3 , B_6, B_9, B_{12} (extraction and determination): Plant sample (2.5 g) was weighed into a set of digestion tubes and an extraction solution (Ultra-pure water: HCl: 0.1N H_2SO_4 in the ratio 5:2:3) dispensed. The tube was warmed at the temperature of 40°C for 2 h, allowed to cool to room temperature and transferred to a set of plastic centrifuged tubes. The latter was shaken for 10 min and centrifuged at 3000 rpm. The supernatant was set in auto-analyser tubes and ran on HPLC. Water 616/626 accessories used had Merck Lichrospher WOCH-18/2 (5 μm) at 40°C column (Stationary phase) and mobile phase (Solvent 'A' was 30 mM sodium acetate, pH 6.5 containing 5% dimethylformamide and solvent 'B' was acetonitrile) with fluorescence detector, range of working standard (0, 0.2, 0.4, 0.6 and 0.8 ppm) and determination was carried out at wavelength range of 240- 465 nm.

Vitamin E (extraction and determination): Plant sample (0.5 g) each was weighed into a set of digestion tubes, 20 mL of diluted Hydrochloric Acid (HCl) added and shook vigorously for 2 h. The extract was further treated with phosphatase to liberate free vitamin E into the solution. The extract was purified by passing through base exchange silicate alkaline column to remove interfering compounds. Thereafter, the extract was stored in a set of vials for analysis using HPLC. Water 616/626 accessories used had Merck Lichrospher WOCH-18/2 (5 μm) at 40°C column (stationary phase) and mobile phase (Solvent 'A' was 30 mM sodium acetate, pH 6.5 containing 5% dimethylformamide and solvent 'B' was acetonitrile) with fluorescence detector, range of working standard (0, 0.2, 0.4, 0.6 and 0.8 ppm) and determination was carried out at a wavelength of 356 nm.

RESULTS

Macro-morphology: The results showed that both species have similar leaf shape, leaf arrangement, leaf type, leaf venation, root colour and hairy leaves. However, they differ in the presence of spines in *A. spinosus* and absent in *A. hybridus* and in stem colour which is reddish-brown in *A. spinosus* and light green in *A. hybridus* (Table 1).

Micro-morphological (anatomy) characteristics

Epidermal studies: The results of the epidermal investigation in Table 2 showed that both species have similar epidermal wall and cell shape in both adaxial and abaxial surfaces and amphistomatic, however, they differ in their stomata type. The adaxial surface of *A. hybridus* has tetracyclic, anisocytic and contiguous stomata while *A. spinosus* have stomata of anisocytic and isotricytic type. The abaxial surface is the same in both *A. hybridus* and *A. spinosus* with regards to stomata types as shown in Fig 1.

Midrib and petiole anatomy: The midrib cuticle in *A. hybridus* is V-shaped while the cuticle outline of *A. spinosus* is relatively straight or flat (Table 3, Fig. 2(a-d). The result of the petiole as seen in Table 3 and Fig. 2 showed that both species have similar vascular bundle types and shape of parenchyma. The adaxial cuticle region of *A. hybridus* has pronounced collenchymatous cells (Fig. 2d) while that of *A. spinosus* is not visible (Fig. 2c). The petiolar protuberances in *A. spinosus* have thick layers of collenchymatous cells (Fig. 2c) while this feature is minimized or absent in *A. hybridus.* The leaf trace in

Table 1: Results for macro-morphological characteristics of *A. spinosus* and *A. hybridus*

Characters	*A. hybridus*	*A. spinosus*
Leaf shape	Ovate	Ovate
Leaf arrangement	Alternate	Alternate
Leaf type	Simple	Simple
Leaf venation	Pinnately veined	Pinnately veined
Stem colour	Light green	Reddish-brown
Root colour	Brown	Brown
Presence of spines	Absent	Present
Leave surface	Hairy	Hairy

Table 2: Summary of the epidermal attributes of *A. hybridus* and *A. spinosus*

Species	Surface	Epidermal wall pattern	Epidermal cell shape	Stomata types
A. hybridus	Abaxial	Straight, curved	Polygonal	Tetracytic, anisocytic and contiguous stomata
	Adaxial	Undulating	Irregular	Anisocytic, isotricytic and tetracytic
A. spinosus	Abaxial	Straight, curved	Polygonal	Anisocytic and isocitric
	Adaxial	Undulating	Irregular	Anisocytic and tetracytic

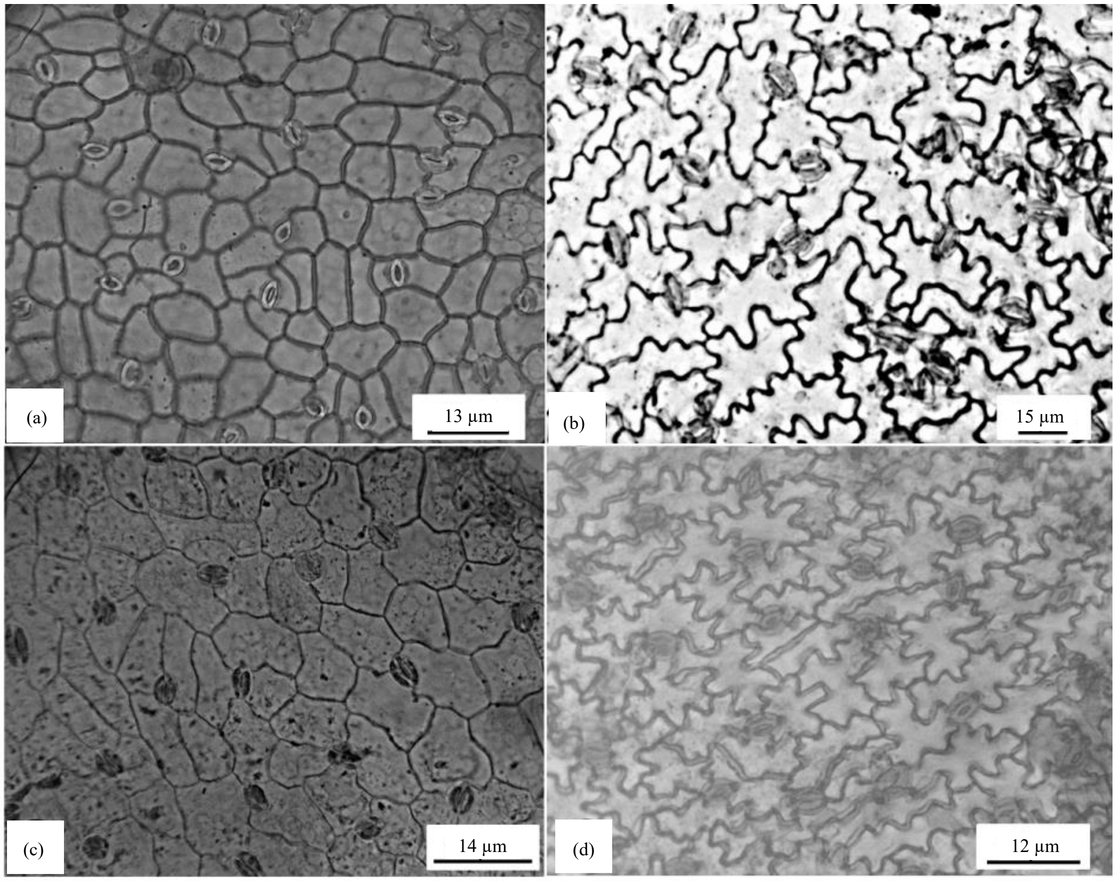

Fig. 1(a-d): Leaf epidermal characteristics of *Amarathus,* (a) Abaxial surface of *A. hybridus*, (b) Adaxial surface of *A. hybridus,* (c) Abaxial surface of *A. spinosus* and (d) Leaf adaxial surface of *A. spinosus*

Table 3: Summary of the anatomy of petiole and midrib of *A. hybridus* and *A. spinosus*

Characters	Plant parts	*A. hybridus*	*A. spinosus*
Vascular bundle types	Petiole	Collateral with accessory vascular bundles	Collateral with accessory vascular bundles
	Midrib	Collateral	Collateral
Number of vascular bundles	Petiole	7 with accessory ones	5 with accessory ones
	Midrib	4 (one on the abaxial region)	5 (2 on the abaxial region)
Shape of parenchyma	Petiole	Pentagonal-hexagonal	Pentagonal-hexagonal
	Midrib	Pentagonal-hexagonal	Pentagonal-hexagonal
Cuticle outline	Petiole	U-shaped	U-shaped
	Midrib	V-shaped	Flat
Kranz anatomy	Midrib	Present	Present

both species comprised of bundle sheaths. Also, the leaf lamina of both species has bundle sheath (kranz) anatomy. Druse (calcium oxalate) crystals were conspicuous in *A. spinosus* than *A. hybridus.*

Stem and root anatomy: The stem and root of both species studied have similar anatomical features (Table 4) but the stem of *A. spinosus* has narrow cambial ring while the cambial ring is pronounced in *A. hybridus* (Fig. 3). Also, the number of vascular bundle in the stem of *A. spinosus* was 52 while that of *A. hybridus* is less than 52. Furthermore, the root of *A. spinosus* has 16 vascular bundles while that of *A. hybridus* has 18 vascular bundles.

Vitamins: The results of vitamins in different parts of the plants studied are shown in Table 5. The vitamins (A, B1, B2, B3, B6, B12, C and E) occurred in the leaves and roots of both *A. hybridus* and *A. spinosus*; however, the concentration varied. The concentration of vitamin A varied from 27.46% in *A. spinosus* root to 72.56% in *A. hybridus* root and from

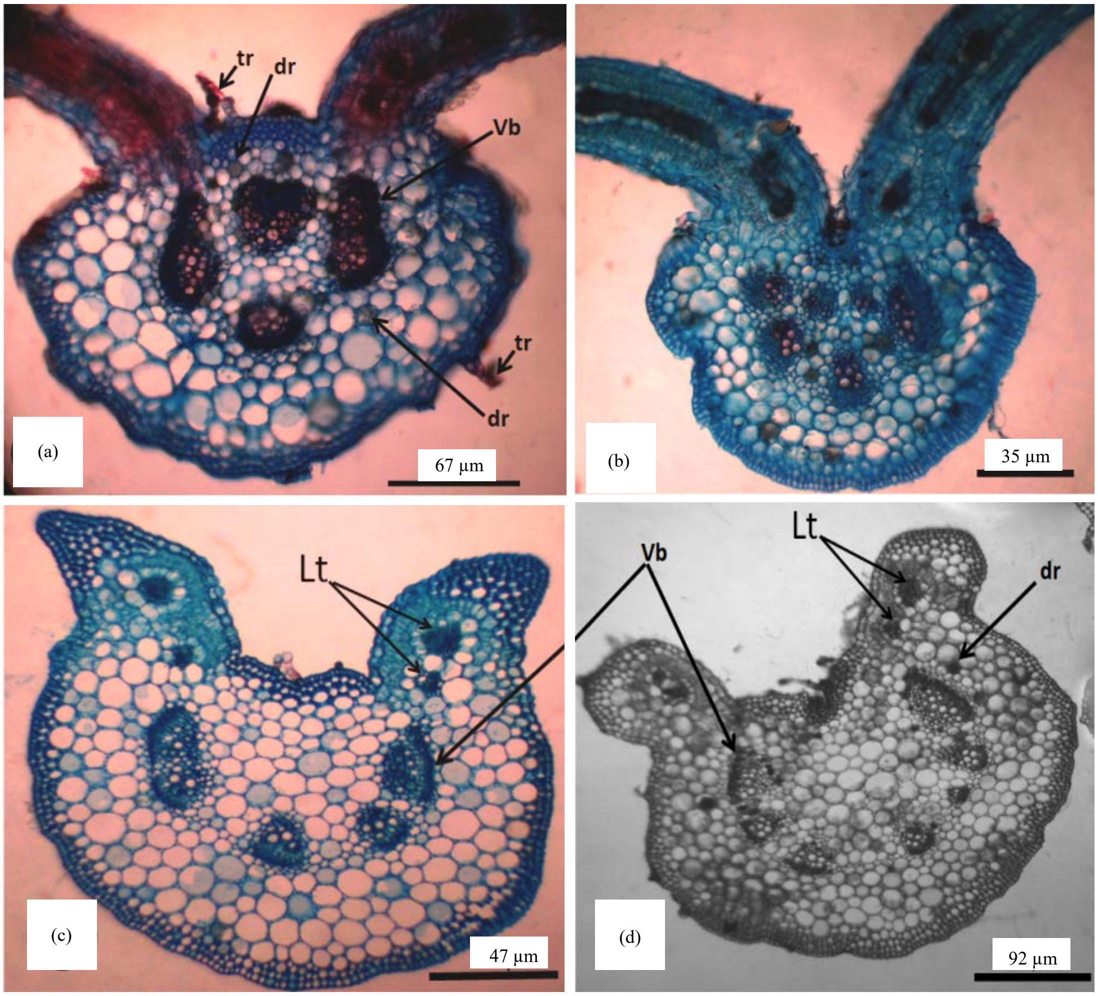

Fig. 2(a-d): Midrib and petiole anatomy, (a) Midrib of *A. hybridus*, (b) Midrib *A. spinosus,* (c) Petiole of *A. spinosus* and (d) Petiole of *A. hybridus*
Vb: Vascular bundle, dr: Druses, tr: Trichome, Lt: Leaf traces

Table 4: Summary of root and stem anatomy

Plant parts	*A. hybridus*	*A. spinosus*
Stem	<52, scattered, open and collateral vascular bundles	52, scattered, open and collateral vascular bundles
	Possess an anomalous cambium ring	Possess an anomalous cambium ring
Root	18, endarch xylem and no cambium ring	16, endarch xylem and no cambium ring
Stem	Parenchymatous cells oval and widespread throughout the cortex	Parenchymatous cells oval and widespread throughout the cortex
Root	With 2 separate parenchymatous rays, parenchymatous cells oval in shape	With 2 separate parenchymatous rays, parenchymatous cells oval in shape

Table 5: Quantitative vitamin composition in *A. hybridus* and *A. spinosus*

	A. hybridus		*A. spinosus*	
Vitamins (%)	Leaves	Root	Leaves	Root
Vitamin A	61.8	72.56	32.7	27.46
Vitamin B1	2.16	2.45	2.35	1.95
Vitamin B2	0.04	0.04	0.03	0.03
Vitamin B3	1.47	1.63	1.57	1.35
Vitamin B6	0.62	0.67	0.65	0.58
Vitamin B12	0.10	0.11	0.11	0.09
Vitamin C	0.07	0.07	0.07	0.06
Vitamin E	0.04	0.05	0.04	0.04

32.70% in *A. spinosus* leaves to 61.80% in *A. hybridus* leaves. These concentrations of vitamin A are relatively high. The percentage vitamins B1, B2, B3, B6, B12, C and E in the species are fairly similar.

Phytochemical results: The data obtained for the phytochemical investigation showed that they are similar in the composition, however, they differ in their concentrations. The total alkaloids, flavonoids, phenolics and glycosides are presented in Table 6. The total alkaloids contents varied form

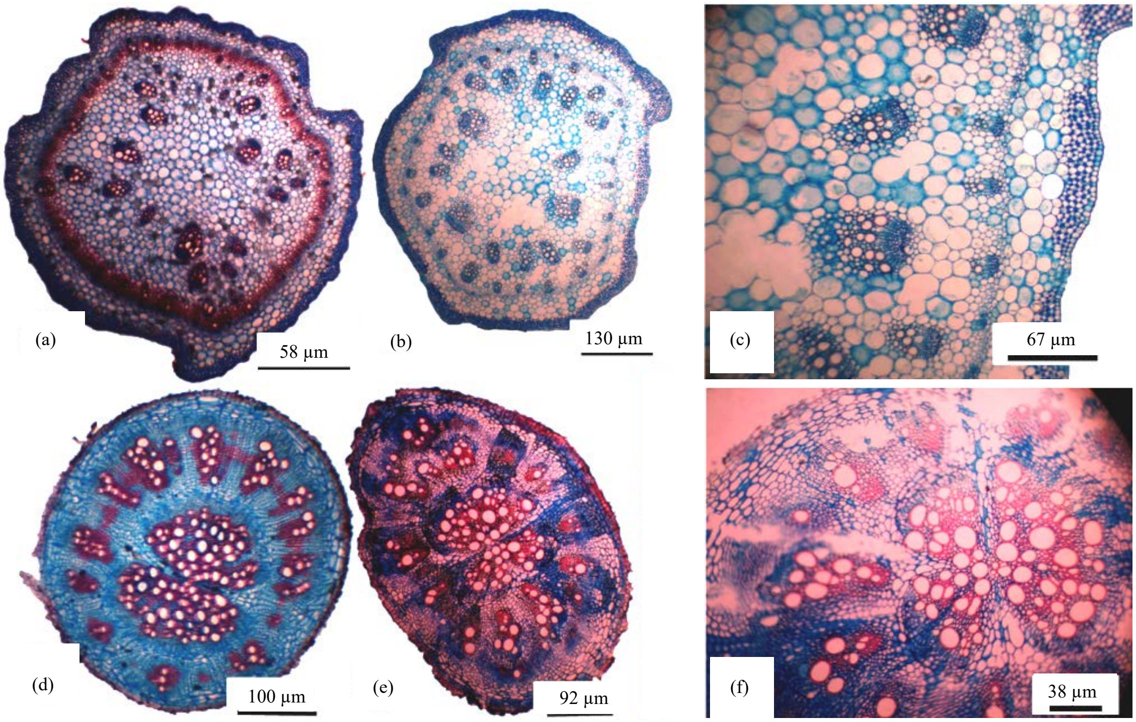

Fig. 3(a-f): Stem and root anatomy, (a) Stem of *A. hybridus*, (b-c) Stem of *A. spinosus,* (d) Root of *A. hybridus* and (e-f) Root of *A. spinosus*

Table 6: Summary of phytochemicals in *A. hybridus* and *A. spinosus*

	A. hybridus		*A. spinosus*	
Phytochemicals	Leaves	Root	Leaves	Root
Alkaloids (g/100 g)	50.42	29.69	50.82	50.89
Flavonoids (g/100 g)	56.46	23.08	50.88	16.70
Phenolics (g/100 g)	12.29	13.27	19.27	18.63
Glucosides (g/100 g)	8.590	11.67	10.07	18.95
Oxalate (ppm)	2.935	2.009	2.843	2.086
Tannin (ppm)	8.219	5.626	7.961	5.842
Saponin(ppm)	5.284	3.617	5.118	3.756
Trypsin-inhibitor (ppm)	2.046	1.400	1.982	1.454

29.69 g/100 g in the root of *A. hybridus* to 50.89 g/100 g in the root of *A. spinosus.* It is worthy to note that there is relatively no variation in the concentration of the alkaloids in the leaves of the species. Flavonoids varied from 16.70 g/100 g in *A. spinosus* root to 23.08 g/100 g in *A. hybridus*. Also, the concentration of phenolics ranged from 12.29 g/100 g in *A. hybridus* to 19.27 g/100g in *A. spinosus* (Table 6).

Anti-nutrients: The anti-nutrient contents of the plant species studied is presented in Table 6. The oxalate maximum (2.935 ppm) and minimum (2.009 ppm) contents were recorded in the root and leaves of *A. hybridus*, respectively. The concentrations of the tannin, saponin and Trypsin-inhibitor followed the same sequence. Generally, the concentrations of the anti-nutrients in the leaves are lower than the concentrations in the roots.

DISCUSSION

Comparative studies carried out on 2 species of the genus *Amaranthus* (*A. hybridus* and *A. spinosus*) for their morphological, phytochemical and anatomical characteristics. The macro-morphology evaluation of these species showed that they are monoecious erect herbs which grow up to 1-2 m. These species showed major similarities in the leaf arrangement, root colour, leaf type and leaf shape. They both have glabrous on leaves. One major distinctive difference in the macro-morphology of these species is the presence of spines. *Amaranthus spinosus* possess spines on the stem at the base of the petiole while *A. hybridus* does not. Thus the results obtained correspond with the morphological studies on different *Amaranthus* species[23,24].

Most of the members of Amaranthaceae are amphistomatic[25-31] and many stomata types have been reported among the members of this family such as; Anomocytic and diacytic in *A. braslilina*[32], anisocytic, paracytic and anomocytic in *A. spinosus* and *A. hybridus*[26]. The epidermal characteristic of *A. hybridus* and *A. spinosus* studied showed that both species are amphistomatic with similar epidermal cell shape and wall pattern. However, adaxial surface of *A. hybridus* had anisocytic, tetracytic and contiguous stomata while *A. spinosus* had anisocytic and isotricytic. The abaxial surfaces of both species are similar having anisocytic and tetracytic stomata with irregular shape and undulating walls. This study agreed with the stomata types[26-31], observed on the leaves of the members of this family but differed in his report on the anticlinal cell wall pattern[26].

Amaranthus species are used in traditional medicine due to their phytochemical constituent properties[33]. Pharmacological analysis has shown that most *Amaranthus* species have anti-cancer, anti-bacterial, anti-fungal, antioxidant and, anti-inflammatory properties[33], analgesic and antipyretic activity[34], antioxidant activity[35], immunological effects[36], antidepressant activity[37], antifertility activity[38], hepatoprotective activity[39], antiulcer activity[40-43], haematological activity[44] and diuretic activity[45,46].

*Amaranthus spinosus*is an extremely interesting crop because its vegetable and seeds are known to be highly nutritious and, therefore, consumed by human as well as animals, as nearly all essential nutrients for humans are available in plants[47-49] and high in vitamins[50]. Other species in this genus are edible and some are cultivated for their leaves[51]. In Nepal, *Amaranth* seeds are eaten as gruel called "sattoo" or milled into a flour to make chappatis[52] and *Amaranthus* leaves contain 3 times more vitamin C, calcium and niacin than spinach. In Ethiopia, the root of *A. caudatus* and *A. sylvestris* are used as a laxative and the seed for expelling tapeworms and for treating eye diseases, amoebic dysentery and breast complaints[53]. Research showed that *A. caudatus* plants grown on dump sites contain a higher concentration of heavy metals[54]. *Amaranthus tricolor* has a high cadmium-accumulating ability[55]. *Amaranthus viridis* can concentrate heavy metals especially Pb and Cd in its tissues[56]. This suggested that *Amaranthus* species can serve as phyto-accumulators of heavy metals and can be used for the purpose of phytoremediation[56].

Phenolic compounds have anti-cancer, anti-bacterial and anti-fungal properties. Also, it has antioxidant, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory actions[57,58]. Flavonoids (phenolic compounds) have been reported to have antioxidant, protect cell from degradation, stress, act as signaling molecules, phytoalexins, detoxifying agents, reduce toxic effects and stimulants, triggers the production of natural enzymes that fight disease, hence reduce the risk of certain cancers, heart disease and age-related degenerative diseases and play chemopreventive role in cancer[59,60]. Catechin a naturally occurring phenolic compound has anti-oxidant activity and has the potential to reduce cardiovascular disease, stroke, obesity and cancer.

Although high intake of flavonols is associated with reduced risk of cancer and stroke, promote bone health, prevent osteoporosis and have anti-inflammatory properties[60,61]. Also, flavonols promote a healthy brain as they possess neuroprotective properties. Hence, the consumption of food rich in flavonols is associated with long-term health benefits[58].

Alkaloids are used in drug production and has antifungal and bactericidal properties[62] can inactivate enzymes, block ion channels, interfere with neurotransmission and cause loss of electrical coordination (ataxia) in affected organisms[63] and have anticancer, antibacterial, antiviral and antifungal properties[64].

Cardiac glycosides induce strong specific effects on the myocardium and enhance the strength of cardiac contractions[65,66]. Tannins have anti-malaria, antimicrobial, antifungal, allelopathic activities, dyes and spices[67] and astringents[68]. Saponins are immunostimulant and possess phytoanticipins or phytoprotectants properties[69].

Phytochemical studies revealed that the plant *A. spinosus* has several active constituents like alkaloids, flavonoids, glycosides, phenolic acids, terpenoids, tannins and saponins. It also shows that kaempferol glycosides, amaranthoside, a lignan glycoside, etc. are also contained in the stem bark of *A. spinosus*[70]. Barku *et al.*[11] also stated that the roots contain α-spinasterol octacosanoate and saponin and have antibacterial activity[71].

From present study, *A. spinosus* and *A. hybridus* have similar phytochemical constituents though the concentrations varied. According to Walton *et al.*[72] and Wang[73], *A. hybridus* and *A. spinosus* contain similar phytochemicals as reported in this current work i.e., vitamins (vitamin A, B1, B2, B3, B6, B12, C and E) and secondary metabolites (alkaloids, flavonoids, glycosides and phenolics). These species showed major difference amongst them. *Amaranthus hybridus* had a higher concentration of vitamin A compared to *A. Spinosus* in both leaves and root. Also, the leaves *A. hybridus* had a higher concentration of flavonoids than *A. spinosus* while phenolics are higher in *A. spinosus* than *A. hybridus*. Also in the roots, *A. spinosus* had a higher concentration of alkaloids, glycosides and phenolics than *A. hybridus* while flavonoids are higher in *A. hybridus* than *A. spinosus*. Presence of these phytochemicals demonstrates while they are used as food[47-49] and different pharmacological purposes[52,73]. Therefore, these species can be used for antimicrobial, antioxidant, antidiuretic, etc. activities due to the presence of these phytochemical properties. *Amaranthus hybridus* leaves contain appreciable values of β-carotene, thiamine, riboflavin, niacin, pyridoxine and ascorbic acid but low value of β-tocopherol and as such are good sources of these vitamins[74]. This could be the reason why *A. hybridus* is used more domestically compared to *A. spinosus*. Therefore, both plants could be a valuable source of dietary vitamins in human nutrition.

CONCLUSION

Based on this study, it is evident that *A. hybridus* and *A. spinosus* have similar morphological, anatomical and phytochemical characters. The number of vascular bundles in the midrib, petiole, stem and root are different and could be used to differentiate them. The data obtained from the quantitative phytochemical analysis showed that their concentrations varied among the 2 species. They are potentials sources of phytochemicals if properly exploited.

SIGNIFICANCE STATEMENT

This study discovered the anatomical difference among the two species for easy identification and high vitamin contents and other phytochemicals in the plant species that can be beneficial. These made *A. hybridus* and *A. spinosus* potential sources of raw materials for pharmaceutical industries.

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