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Original Article

Pharmacognostic Studies of the Leaves, Stem and Root of *Capparis erythrocarpos* Isert (Capparaceae)

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ABSTRACT

Introduction: The roots of *Capparis erythrocarpos* are used traditionally across Africa for the management of pain, arthritis and other forms of inflammatory conditions. Owing to its proven efficacy, it has gained commercial value, particularly as a key ingredient in several herbal products and alcoholic beverages. The increased scarcity owing to demand outstripping supply lend the roots of *C. erythrocarpos* to adulteration. This paper presents a detailed pharmacognostic evaluation of the leaf, stem and root of *C. erythrocarpos* which will be used in its identification and consequent standardization. **Methods:** The leaf, stem and root were evaluated for their macroscopic and microscopic features as were the physicochemical parameters and phytochemical screening done. **Results:** Leaves are alternately arranged, have collateral vascular bundle, crystal sheaths and a pericyclic fibre. Actinocytic stomata and secretory cells were contained in powdered leaves. The stem showed lenticels and thorns, stellate and branched trichomes which leave off cicatrices in older stems. The powdered stem and roots contained stone cells, secretory cells and scalariform vessels. However, the roots lacked thorns, trichomes and had smaller secretory cells. Aqueous and ethanolic extracts of the leaves, stem and roots were slightly acidic to neutral. Ash values of leaves, stem and roots are (16.58 ± 0.09) % w/w, (5.01 ± 0.09) % w/w and (6.53 ± 0.19) % w/w respectively. Preliminary phytochemical screening of the leaves, stem and roots showed the presence of glycosides, flavonoids and tannins. **Conclusion:** The determined parameters for the leaf, stem and root of *C. erythrocarpos* constitute quality parameters for their unequivocal identification.

Key words: Capers, crystal sheaths, Cicatrices, physicochemical parameters, Morphological features, Herbal medicine.

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INTRODUCTION

Natural products hold potential sources of novel compounds with varied bioactivities. Bioactivity studies should be preceded with a clear cut standardization of the plant to avoid intentional or non-intentional adulteration as this can lead to treatment failure, sub therapeutic effect or even death. *Capparis erythrocarpos*, an important medicinal plant in herbal medicine used globally in the management of pain and other inflammatory conditions lack standards for quality control.

Globally, there are about 650 species present in the family and 250 species present in the genus Capparis. Though such a huge number globally, just a few of them are found locally.[1] In Ghana, five species have been identified.[1-2] Among these species, *C. erythrocarpos*, commonly referred to as capers[3] has been found to be the most commonly used for the management of several ailments. A mention of the plant in Ayurveda states the use of the stem bark and root for its analgesic and other inflammatory disorders.[4] Across Africa, the roots are used for the treatment of conjunctivitis, headache[5] and chronic diarrhoea.[6] In Tanzania, capers is used in the management of child convulsive fever and inflammation of the connective tissue of the eye.[7] In Uganda, the whole shrub is used for the management of menstrual pains, infertility and anemia.[8] In Ethiopia, it is used traditionally for the management of symptoms of cyst and skin infections. In Kenya, it is used as an antidiarrheal and anthelmintic agent.[4] In Ghana, it is used for the management of pain, arthritis and other forms of inflammatory conditions.[5]

Pharmacological investigations have been conducted on *C. erythrocarpos* and it possesses anti-arthritic effects[7-10] without any organ specific toxic effects.[11] Phytochemical analysis of the ethanol extract of *C. erythrocarpos* root revealed the presence of alkaloids and flavonoids.[9]

In Ghana, *Capparis erythrocarpos* roots and its formulations are in high demand commercially. It is used by Kasapreko Group of Companies for their Alomo bitters brand variants which have received global acclaim[12] and is marketed as an alcoholic beverage and appetite stimulant. The Centre for Plant Medicine Research (CPMR) uses the root in

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an oral formulation, Sirrapac, as an antiarthritic medication.[11] Due to their popularity and high value, *C. erythrocarpos* containing products are a target for economically driven adulteration. In the face of demand outstripping supply and the economic motives for adulteration being considerable, *Capparis erythrocarpos* is at risk of an admixture or complete substitution with closely related species. This is made worse, by the use of the roots (which requires the destruction of the whole plant). A study in 2007 highlighted the unnoticed threatened state of *C. erythrocarpos* and the need for its sustainable use and conservation.[13]

Despite its wide use and potential for adulteration, no report exists of a pharmacognostic study aimed at unequivocally identifying *C. erythrocarpos* from its closely related species. Authentication of *C. erythrocarpos* is of primary importance for both consumers and the industries that manufacture products containing *C. erythrocarpos*. For the industries, product authentication is essential to maintain trust of consumers and avoid unfair competition that can eventually create a destabilized market and disrupt regional or national economies. Consumers also will have the full benefit of quality products that promote their health and well-being. Clearly, a study that addresses this gap in knowledge holds numerous benefits for the herbal medicine industry.

Consequently, this paper aims at establishing quality standards for the unequivocal identification of *Capparis erythrocarpos*. The macro- and micro morphological features, physicochemical properties and fluorescence characteristics were determined using standard methods.

MATERIALS AND METHODS

Materials

Reagents and chemicals of analytical grade were purchased from Sigma Aldrich Co. Ltd. A Leica DM. 700 light microscope fitted with camera (Leica ICC50 HD) was used for all the microscopy. Fluorescence analysis was performed with UVGL58 UV lamp (Cambridge, UK).

Plant Collection and Authentication

Leaves, stem and roots of *Capparis erythrocarpos* were collected from Ayikuma, Ghana (5°55'2.384"N, 0°0'40.3092"W). Parts collected were authenticated by Dr. George Henry Sam of the Department of Herbal Medicine.

Leaf samples were pressed while the stem and root samples were stored in formaldehyde (37%) solution. They were deposited at the herbarium of the Herbal Medicine Department, Faculty of Pharmacy and Pharmaceutical Sciences (FPPS), Kwame Nkrumah University of Science and Technology (KNUST) with the identification codes KNUST/HM1/2017/L002, KNUST/HM1/2017/S012 and KNUST/HM1/2017/R004 for leaf, stem and root respectively.

Methods

Macromorphology and micromorphology of leaf

The color, taste, odor and texture of the leaves of *Capparis erythrocarpos* were determined and recorded as per standard procedures.[14]

The leaves of *C. erythrocarpos* were macro-morphologically characterized.[14]

For histological studies, the transverse and longitudinal sections of the leaf midrib were prepared and stained as per standard procedures.[14-15]

Powdered leaves was mounted in appropriate reagents. Cell contents and inclusions were described by standard procedures.[14-16]

Quantitative evaluations (stomatal number, stomatal index, palisade ratio, vein-islet number and veinlet termination numbers) and measurements were done. Area measurements and particle counting were achieved using Image J (National Institute of Health, USA, http://imagejnih.gov/ij) and Microsoft Excel, Microsoft Office Professional Plus 2013.

Macromorphology and micromorphology of stem

Similar standard procedures were performed as described for leaves. Quantitative evaluations were however not performed. Powdered stem was mounted in appropriate reagents and observed under the microscope.

Macromorphology and micromorphology of roots

Similar standard procedures were performed as described for the leaves. Powdered roots was mounted in appropriate reagents and observed under the microscope. Quantitative evaluations were not performed.

Physicochemical and phytochemical characters

Physicochemical parameters, extractive and ash values determinations as well as phytochemical screening of powdered samples were performed by standard procedures.[14-15] Test samples were run in triplicates.

Fluorescence analysis

The leaves, stem and root powders were observed for characteristic colors. Strong acids, alkalis and organic solvents were added and thereafter observed under the visible, short (254 nm) and long wavelengths (366 nm).

Plant extraction

The method of extraction was carried out as per the protocol employed by Centre for Plant Medicine Research (CPMR), Akuapim-Mampong, Eastern Region. Plant material (roots, stem and leaves) was warm macerated in water for three hours. It was filtered, concentrated under low temperature (40°C) for 6 h and freeze dried to obtain a powdery mass. The yield obtained for the roots, stem and leaves were 0.79 %, 0.59 % and 2.73 % respectively.

Fourier Transform Infrared (FTIR) fingerprint

The spectra of the extracts were measured as a solid film using a PerkinElmer Spectrum Two Universal Attenuated Total Reflectance (UATR) FTIR spectrometer. They were recorded with 32 scans at a resolution of 4 cm^{-1} over a wavenumber range of 4000 – 400 cm^{-1}.

RESULTS

Macromorphology and micromorphology of leaf

The fresh matured leaves were green colored, elliptical to ovate shaped, bitter to taste and musty in odour. The simple leaves were alternately arranged with pinnate venation, sinuate margin, acute apex, short stalked petiole and has two spiny stipules on the symmetrical base (Figure 1**A**). They are fleshy and have a glossy appearance. The striking difference between the young and old leaves is the light green lanceolate shaped young leaves (4.2×0.8) cm (Figure 1**A**) as opposed to dark green elliptical to ovate old leaves (7.6×3.8) cm.

The transverse section of the midrib of *C. erythrocarpos* young leaf showed a simple arrangement consisting of a layer of upper and lower epidermal cells each respectively overlaid with a thick cuticle. The abaxial surface had no palisade cells (Figure 2**A**). Dispersed in the collenchyma and parenchyma cells are crystal sheaths and starch grains. Also present was an arc- shaped unicollateral vascular bundle (Figure 2**A**). The lamina showed wavy epidermal cells with stomata scattered in them. Attached to the lamina were branched and stellate trichomes (Figure 3). Worth noting is the more advanced cellular structures: higher degree of lignification and pericyclic fibers (Figure 2B) in the older leaf. Also present in the older leaves were branched and/or unicellular clothing trichomes.

In the section of the young and old leaves, dark blue stained starch grains and pink lignified xylem vessels were observed. Safranin stained the cuticle bright red and the lignified pericyclic vessel purplish violet (Figure 2**B**).

Figure 1: A-Leaves (X 0.7) showing alternate arrangement, B-stem-showing thorns (X 0.1) and C- roots (0.3) of *Capparis erythrocarpos*; Leaves, stem and root of *Capparis erythrocarpos*

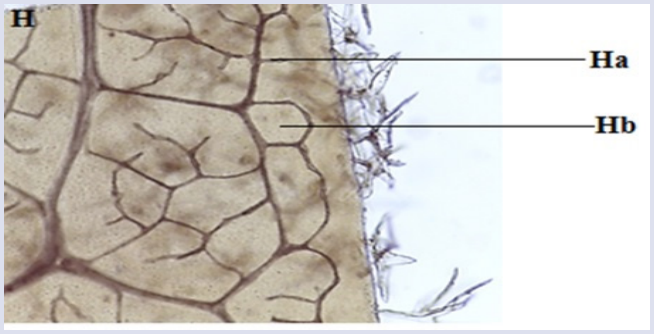

Figure 3: Whole leaf of *C. erythrocarpos* (X 100) showing Ha=vein Islet terminations Hb=Vein islets; Whole leaf of *C. erythrocarpos*

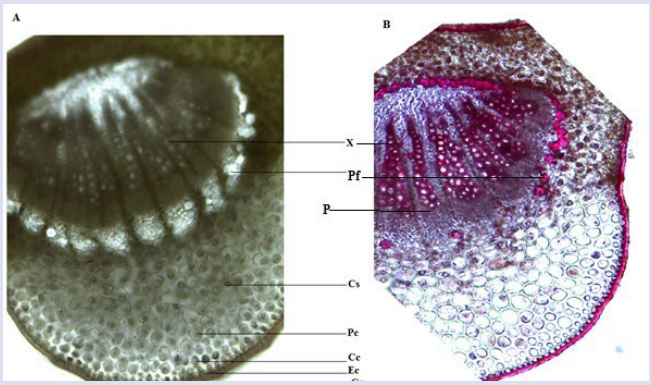

Figure 2: Transverse section of the midrib of a leaf of *C. erythrocarpos* showing A (X 400): section with Cu=cuticle, Ec=epidermal cell, Cs=Crystal sheath, Cc=collenchyma cell, Pc=Parenchyma cell, X=Xylem, P=Phloem, Pf=Pericyclic fiber, B (X 400)=Safranin stained section; Midrib of leaf of *C. erythrocarpos*

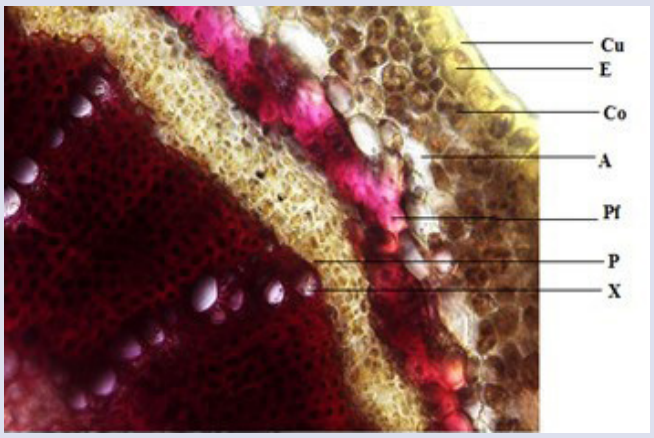

Figure 4: Transverse section of the stem (X 100) showing Cu=cuticle, E=epidermal cell, Co=collenchyma, A=aerenchyma, Pf= pericyclic fibre, P=phloem, X=xylem; Transverse section of stem.

Powdered leaves have prismatic calcium oxalate crystals, crystal sheaths, stomata with straight to wavy walled epidermal cells, unicellular and branched trichomes, secretory cells, lignified vessels and fibers.

Leaf surface data determinations

Epidermal cells on both surfaces of *C. erythrocarpos* leaf are slightly wavy walled. *C. erythrocarpos* has a palisade ratio of 6. The main type of stomata present on both surfaces is the anomocytic stomata. Other types of stomata present include anisocytic and actinocytic. The leaf is amphistomatic with the adaxial surface bearing a lower number (12.4 to **29.7** to 49.5) than the abaxial surface (24.8 to **42.1** to 49.5). The stomatal index ranges for the adaxial and the abaxial surfaces are 4.8 to **14.2** to 25.0 and 11.1 to **15.5** to 25.0 respectively. The stomata is 0.018 ± 0.004 mm long and 0.019 ± 0.003 mm wide.

The vein-islet number and the veinlet terminations were 3.96 to 4.46 and 1.86 to 3.47 respectively. The vein islets are polygonal and the vein islet terminations are fork shaped (Figure 3).

Macromorphology and micromorphology of stem

The woody stem of *C. erythrocarpos* organoleptically assessed has bitter taste and a musty odor. The young stems are green, old stems are greenish brown (Figure 1**B**) and the inner parts are creamish white. The stem showed white patches, lenticels and wrinkles which were well distributed all over the outer bark. The pattern of fracture is splintery and it assumes a channeled curvature.

A transverse section of the stem of *C. erythrocarpos* reveals a lot of branched and stellate trichomes attached to a thick layer of cuticle. Sections from older stems have cicatrices (stumps from broken trichomes). Present was a single layer of epidermal cells (Figure 4), isodiametric parenchyma cells and a pith containing prismatic calcium oxalate crystals and numerous starch grains. The vascular bundle has lignified vessels with outer phloem (Figure 4).

The powdered stem contains a lot of starch grains, numerous vessels and lignified sclerenchymatous cells.

Macromorphology and microscopy of root

The root of *C. erythrocarpos* has bitter taste. Macromorphological features of the root are similar to the stem (Figure 1**C**).

The root has one to two layer(s) of epidermal cells overlaid with a cork with no trichome and four to seven layers of cortical collenchyma cells. Highly dispersed through the isodiametric parenchyma cells are large starch grains and stone cells. Present is a collateral vascular bundle and lignified medullary rays consisting of 15-25 rows of parenchyma cells (Figure 5). In the middle lies a pith. All throughout the cells in the section (from the epidermis to the pith) are oil deposits (Figure 5).

The powdered root showed similar structures as the stem but it lacked trichomes and had smaller secretory cells.

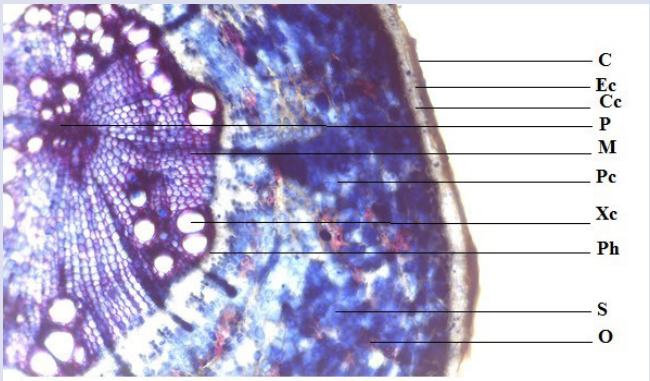

Figure 5: Transverse section of root (X100) showing C=cork, Ec=epidermal cell, Cc=collenchyma cell, P=pith, M=Medullary ray, Pc=parenchyma cell, Xc=Xylem cell, Ph=phloem, S=starch grain.

Table 1: Physicochemical analysis of the leaves, stem and root of *C. erythrocarpos* (n=3).

Physical parameters	Total ash value (% w/w)	Acid insoluble ash value (% w/w)	Water soluble ash value (% w/w)
Leaves	16.58±0.09	2.26±0.03	10.91±2.92
Stem	5.01±0.09	1.68±0.40	2.39±0.93
Roots	6.53±0.19	2.30±0.88	2.22±1.34

Table 2: pH readings and extractive values of alcohol and water extracts of *C. erythrocarpos* (n=3).

Plant sample	pH Reading		Extractive value (% w/w)	
	Alcohol	Water	Alcohol (90 %)	Water
Leaves	6.54±0.04	5.84±0.05	29.86±0.04	54.78±0.06
Stem	6.17±0.06	5.44±0.05	9.5±0.04	10.82±0.05
Root	6.3±0.05	4.55±0.05	18.3±0.04	36.80±0.05

Physicochemical principles

Results on ash values have been presented in Table 1 while that of extractive values and pH readings have been presented in Table 2.

Phytochemical Screening

Saponins, tannins, glycosides, flavonols, triterpenoids, coumarins and alkaloids were detected in the powdered leaves, stem and roots of *C. erythrocarpos*.

Characteristic fluorescence of C. erythrocarpos

This result is as presented in Table 3.

Fourier Transform Infrared fingerprint (FTIR)

The FTIR spectra of the leaf, stem and roots extracts of *C. erythrocarpos* has been presented in Figure 6.

DISCUSSION

This paper focused on filling the gap in knowledge of standards that guides the correct collection and quality control of *C. erythrocarpos* parts.

Table 3: Characteristic fluorescence of the leaves, stem and root powders in dry state and different reagents under visible light, long, short wavelengths.

Powder	Visible light	Long wavelength	Short wavelength
Leaves	Green	Light green	No fluorescence
Stem	Light green	Light Green	No fluorescence
Root	Cream	Light Green	No fluorescence
Powder+ Methanol			
Leaves	Green	Light yellow	No fluorescence
Stem	Green	Light yellow	No fluorescence
Root	Cream	White	Cream
Powder+ Petroleum ether			
Leaves	Green	Light brown	No fluorescence
Stem	Light green	Cream	No fluorescence
Root	Cream	Light blue	Light brown
Powder+ 50% HCl			
Leaves	Green	No fluorescence	No fluorescence
Stem	Light green	No fluorescence	No fluorescence
Root	Cream	Light green	No fluorescence
Powder+ 50% H_2SO_4			
Leaves	Green	No fluorescence	No fluorescence
Stem	Light green	Brown	No fluorescence
Root	Cream	Light green	No fluorescence
Powder +KOH			
Leaves	Brown	Light yellow	No fluorescence
Stem	Yellow	Light yellow	No fluorescence
Root	Light green	Light green	No fluorescence

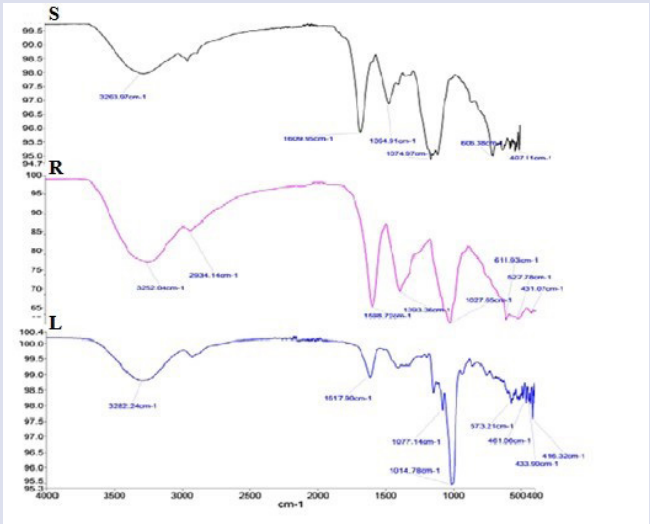

Figure 6: FT-IR fingerprints of the S (stem), R (roots) and L (leaves) of *C. erythrocarpos* showing different stretches of characteristic functional groups and unique fingerprint. Fingerprints of *C. erythrocarpos*.

Macromorphology and micromorphology of leaf

Leaf dimensions of *C. erythrocarpos* are macroscopically similar to those of another member in the genus; *Capparis zeylanica*. However, *C. zeylanica* leaves have a rounded base and a mucronate apex.[17] *C. erythrocarpos* has small amphistomatic leaves [18-19] which shorten travel distance of carbon dioxide to mesophyll cells in the leaves.[20] This enhances photosynthesis in *Capparis erythrocarpos*.[21-22] Thick outer epidermal walls, a feature of xerophytes which was confirmed in *C. erythrocarpos*[23-26] protects it against drought.[26] These observed features are essential for easy identification and authentication of the plant.

Safranin, a stain for detecting different types of cells; lignified, cutinized or suberized cell wall[25] confirmed the presence of lignin and suberin in *C. erythrocarpos* leaves.

Leaf surface data determinations

The stomata of *C. erythrocarpos* is shorter (0.018 ± 0.004 mm) but broader (0.019 ± 0.003 mm) than another member of the genus, *C. spinosa* (0.028mm long, 0.010 mm wide).[26] Another distinctive feature about *C. erythrocarpos* leaves is its hyperstomata. Stomatal index, a distinct feature which shows less variability for a given species over stomatal number was determined to aid in *C. erythrocarpos*' identification.[14] The ratio of stomatal number on the adaxial and abaxial surfaces was determined since it is of diagnostic importance.[14] The presence of different types of stomata in *C. erythrocarpos* is essential for unique physiological regulation of water as these stomatal types respond appropriately to changing climatic conditions.[27]

The vein-islet number and the veinlet termination values were determined as part of identification parameters of *C. erythrocarpos*.[14] Polygonal shaped vein islets and the fork shaped vein islet terminations seen for *C. erythrocarpos* are similar to those observed in *C. sepieria*.[24]

Macromorphology and micromorphology of stem

C. erythrocarpos stem, similar to *C. spinosa* possesses thorns.[3,23] Also, comparable to *C. sepieria*, it has thick fibre, vessels arranged within the collateral vascular bundle and small, compact parenchymatous cells.[24] Similar to *C. erythrocarpos*, *C spinosa* has cortical sclereids but not pericyclic fibres.[24]

Numerous starch grains in stem serves as energy reserves for the plant in adverse weather conditions.[28]

Macromorphology and microscopy of root

The observed features are essential for easy identification and authentication of the plant.

The powdered root has smaller secretory cells as compared to the stem.

Physicochemical principles

Ash values of *C. erythrocarpos* (Table 1) are essential in determination of quality; which focuses on grade, presence of earthy matter and purity of drug.[29] pH readings of water and alcohol extracts are weakly acidic to neutral (4.0 - 6.54) (Table 2). Extractive values are useful in crude drug evaluation. Values are useful as quality parameters and detection of adulteration in a claimed *C. erythrocarpos* sample. Physicochemical characterization revealed that water extraction gave a better yield as compared to ethanol (Table 2).

Examination of Phytochemical Constituents

Secondary metabolites present agrees with documented literature of members of this genus and species, *C. erythrocarpos*.[10,30] *Capparis erythrocarpos* have been reported to possess analgesic, anti-inflammatory, antimicrobial, anthelminthic and hepatoprotective properties.[31] This is mainly due to the secondary metabolites present.

Characteristic fluorescence of C. erythrocarpos

Characteristic color produced due to the presence of fluorochromes can be used in characterization and subsequent evaluation of crude drug samples (Table 3).

Fourier Transform Infrared Fingerprinting

Fourier transform Infrared was done to obtain fingerprint for quality control of leaves, stem and roots of *C. erythrocarpos*. Figure 6 reveals unique fingerprints which could be used as reference standard in the authentication of the leaf, stem and root of *C. erythrocarpos*.

CONCLUSION

This research work has laid down morphological and physicochemical standards that can be used in the quality control of the leaves, stems and roots of *C. erythrocarpos*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

CPMR: Centre for Plant Medicine Research; **FTIR:** Fourier transform Infrared; **FPPS:** Faculty of Pharmacy and Pharmaceutical Sciences; **KNUST:** Kwame Nkrumah University of Science and Technology; **UATR:** Universal Attenuated Total reflectance; **UV:** Ultraviolet.

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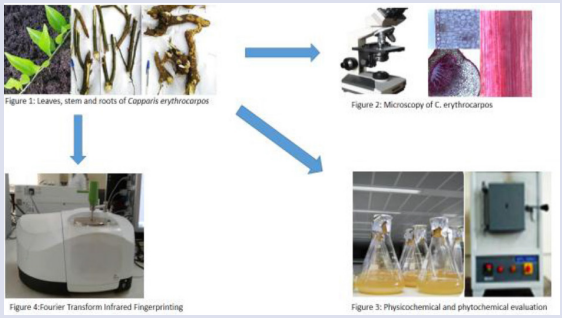
GRAPHICAL ABSTRACT



SUMMARY

- *Capparis erythrocarpos* commonly referred to as capers is used across Africa and several Ayurvedic systems in treating inflammatory conditions such as pain and arthritis. Though the caper plant is widely used, there are no standards to aid in its identification and consequent standardization. This paper established pharmacognostic characters of the leaves, stem and roots of capers focusing on macromorphological, micromorphological, physicochemical, phytochemical and fluorescence parameters. Fingerprints of powdered capers were also generated. This study has laid down pharmacognostic features for unequivocal standardization and consequent quality control of the leaves, stem and roots of capers.

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