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MORPHO-HISTOLOGICAL CHARACTERS FOR THE IDENTIFICATION OF *CRYPTOLEPIS SANGUINOLENTA* (LINDL.) SCHTR.

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ABSTRACT
An investigation was conducted into the morphological and histological characters of *Cryptolepis sanguinolenta* (Lindl.) Schtr., a traditional medicinal plant species harvested from three sampling sites, namely; Abonse, Mamfe and Pepease all in the Eastern Region of Ghana. The plant materials collected from the locations were subjected to various morphological and histological laboratory studies quantitatively. Statistically, SPSS version 16.0 was fitted to the dataset to determine whether variations do exist in the quantified morphological and histological dimensions of the identified characters. Results gathered showed that identified morphological characters were more variable than the histological characters.

KEYWORDS: *Cryptolepis sanguinolenta*, morphological, histological, Abonse, Mamfe, Pepease

INTRODUCTION

Cryptolepis sanguinolenta (Lindl.) Schtr. belongs to the family Periploaceae, although it had earlier been classified as a member of the family Ascelpiadeceace (Hutchinson and Dalziel, 1963). According to Hutchinson and Dalziel (1963), the new family to which the plant belongs can be distinguished from the Ascelpsiadeceace by the presence of granular pollen borne on spathulate glandular carriers. *Cryptolepis* belongs to the order Apocyales. The genus, *Cryptolepis* includes *Cryptolepis brazzei*, *C. deciduas*, *C. oblongifolia, C. nigritana, C. triangularis* and *C. sanguinolenta*, which are common in West Africa. It is also known as *Pergularia sanguinolenta* Lindl. (Hutchinson and Dalziel, 1963). In Ghana, the plant is also known locally as "Ghana quinine", although different localities have different local names for it; such as: nurubima (Guans), kadze (Ewe) and nibima (Twi).

The plant is a tropical shrub indigenous to West Africa, from Senegal to Nigeria and found where rainfall is not very high. It avoids the wet rain forest where there is abundant shade. It does well in areas where there is adequate supply of sunshine and water; hence it is totally absent from the marshy and salty swamps of coastal regions. In Ghana, the plant is commonly found in some districts in particular months of the year. On the Aburi hills, in the Akuapim area, it is found in June when there is enough rainfall for optimum growth. In the Ejura District of Ashanti Region, the plant thrives well in the woody savanna vegetation and flourishes well in June. Around Lake Bosomotwe in the Kuntanase-Bosomtwe District (also in the Ashanti Region), the plant grows well in April and sometimes it is the dominant vegetation cover on deserted farmlands (Hutchinson and Dalziel, 1963).

C. sanguinolenta (Lindl.) Schtr is a twining and scrambling thin-stemmed shrub with blood-red sap. It is indigenous to tropical Africa, and is used locally as a medicinal plant. The leaves of *C. sanguinolenta* are glabrous, oblano-elliptic or ovate (Plate 1). The leaf apex is acute to shortly acuminate. The leaf base is symmetrical, petiolate, and up to 2.2 – 7 cm long. The plant has a cymose inflorescence. There may be yellow flowers on the shoot and seeds 10 – 12 mm long with a tuft of silky hairs at the end.

The roots are rather tortuous and branches with little or no rootlets. The outer surface is yellowish brown and when dry, show longitudinal ridges with occasional cracks (Plate 2). The roots break easily with fractures leaving a smooth transverse surface, which is yellow in colour. The sap is extremely bitter and is characterized by the rapidity with which it turns deep red on exposure to air (Boye, 1992; Sofowora, 1984).

A concoction of the stem and roots serve as herbal medicine in Ghana, West Africa to treat malaria, venereal disease and rheumatism (Boakye-Yiadom, 1979; Boye and Oku-Ampofo, 1983).

The root decoction of *C. sanguinolenta,* has been used clinically by Oku Ampofo at the Centre for Scientific Research into Plant Medicine, Mampong-Akuapim since 1974 for the treatment of malaria fever, urinary and upper respiratory tract infections (Dwuma-Badu, Ayim, Fiagbe, Knapp, Schiff, and Slatkin., 1978). In folklore tradition, the roots of *C. sanguinolenta* have been used as a bitter stomachic. In the Peoples' Democratic Republic of Congo and in Cesamace, Senegal, infusions of the root serve for the treatment of stomach and intestinal disorders and rheumatism (Sofowora, 1984). The root is also sold and used as a yellow dyestuff amongst the Hausa of Northern Nigeria and other parts of West Africa and South Angola (Sofowora, 1984; Dalziel, 1956). The whole root water extract has been used by Ghanaian Traditional Healers for the treatment of fevers.

PLATE 1: Photograph of the morphological features of *C. sanguinolenta* (x1).

The identification of plant species in the field is often difficult. In some cases, there may not be good key characters or these may not be apparent at the time of observation. Accurate identification is always important but particularly so, when wild relatives of crop species are being collected, conserved and eventually utilised in breeding programmes. It is clear that some of the material held in plant germplasm collections bears an incorrect specific name; for example, confusion over identification of taxa has been reported in collections of rice (*Oryza*: Vaughan, 1994; Martin, Juliano, Newbury, Jackson and Ford-Lloyd, 1997) and beet (*Beta*: Ford-Lloyd, 1986). The genus *Beta* is divided into four sections: *Beta*, *Corollinae*, *Nanae*, and *Procumbentes* and includes more than 10 species (Ford-Lloyd, 1986; Letschert, Frese and Van Der Bergrg, 1994). Several groups of species or subspecies are often misidentified using traditional methods (Ford-Lloyd, 1986; Reamon-Büttner, Wricke and Frese, 1996).

In recent years data from such diverse fields as anatomy, biochemistry, cytology, embryology, palynology and reproductive biology have strengthened the concepts of identification. For example, the qualitative and quantitative variation in birch secondary chemistry has been found to be taxonomically significant as a useful tool for species recognition (Julkunen-Tiitto, Rousi, Bryant, Sorsa, Keinänen and Sikanen, 1996). In making accurate plant identification, due attention is now also being paid in addition to gross morphological characters, to bark characteristics as well as foliar epidermal characters.

According to Metcalfe and Chalk (1979), the term bark is applied to all secondary tissues external to the xylem in stems and roots. The taxonomic importance of bark has been stressed by various authors, such as Cudjoe (1970); Parameswaran and Liese (1969) and Whitmore (1963; 1962). The dimensions of the fibres and other elements of the bark are useful in the identification of tree species (Metcalfe and Chalk, 1979).

Crystals, especially calcium oxalate crystals, are widely distributed among plants. They are of different types; for example: styloid, prismatic and idioblasts (Sivarajan, 1991). Their distribution in a given taxon is very specific and hence taxonomically useful. Nayar, Rai and Vatsala (1977) discovered that the size and shape of the crystals in the abaxial epidermis of the leaf of *Myristica fragrans* widely cultivated in India for nutmeg and mace, enabled the resaerchers to distinguish between male and female trees, even at the sapling stage.

Leaf and seed morphological characters are very useful in pharmacognosy, i.e. the science of crude drug (Hawley, 1966) for the identification of plants used in herbal medicine and diagnosing the foliar drugs. The importance of leaf morphological characters in the identification of flowering plants has been stressed by various authors: Stace (1984); Barthlott (1981); Dehgan (1980); Tomlinson (1974) and Van Cottham (1973).

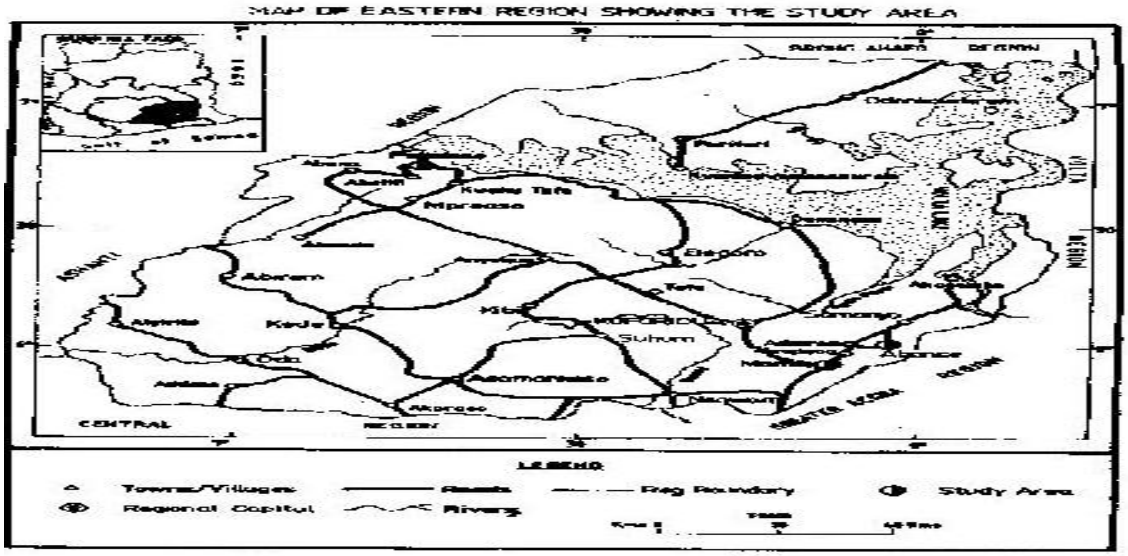
A plethora of literature is available on phytodermology of medicinal plants. Chandra, Mitra, Kapoor, and Kapoor (1972) studied the leaf morphology of some Solanaceous and Apocynaceae plants. Chaudhuri (1963) undertook comparative pharmacognostic studies in the leaves of *Catharanthus roseus* and *C. pusillus*. Dewar (1933) reported on the histology of the leaves of *Digitalis thapsi*. Dewar and Willis (1935) studied the microscopic and macroscopical characters, potencies and constituents of *Digitalis* leaf. Ghani and Pindiga (1985) undertook comparative pharmacognostic studies of two *Datura* species. Krishnamurthy and Sundaram (1967) reported on the foliar epidermis and pharmacognosy in some members of Asclepiadaceae and Owonubi (1986) also reported on the pharmacognosy of *Blighia sapida*.

Despite the long list of plants which have been studied pharmacognostically, there has not been any report on the morphology and anatomy of the genus *Cryptolepis*. From the immerse importance of the plant species in traditional medicine, and the lack report on the morphology and anatomy of the genus *Cryptolepis*., there is the need for the correct authentication in order to avoid vitalities. This research focuses on the establishment of some diagnostic

features by which *C. sanguinolenta* could be recognised both in the field and in the laboratory.

MATERIALS AND METHODS

Plant materials for morphological and anatomical studies were collected from three (3) locations; namely; Abonse, Mamfe and Pepease all in the Eastern Region of Ghana (Fig. 1). Morphological studies carried out include: leaf index, leaf petiole, pod index, seed index, seed and hairy appendage.



Leaf index

The length and width (broadest width) of 100 leaves were measured. The length was taken from the base of the leaf lamina to the leaf apex while the width was from one end of the broadest section to the other as shown in Plate 2.

Leaf petiole

The petiole length of each of the 100 leaves was measured. Measurement was taken from the leaf base to the node as presented in Plate 2.

Pod index

Measurement of the length and width of 100 pods harvested from each of the locations were recorded. The length of each pod was taken from the tip to the base while the width was taken from one end of the widest section to the other of the pod as indicated in Plate 3.

Seed index

Hundred seeds obtained from each of the locations were used for the measurement. The length of each seed was taken from the tip to the base while the width was taken from one end of the widest section to the other of the seed as indicated in Plate 4.

Seed and hair length

The seed and hair length was measured for 100 seeds obtained from each of the locations. The measurement was taken from the tip of the longest hair to the base of the seed (Plate 4).

Hair length

The hair length was measured for 100 hairs obtained from each of the locations. The measurement was taken from one end to the other of the longest hairy appendage (Plate 4).

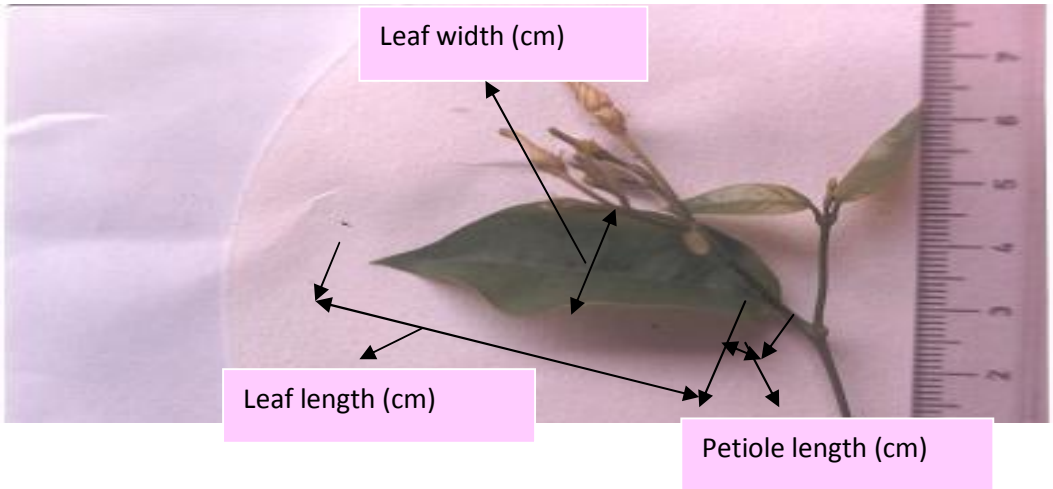


PLATE 2. Leaf morphology of *C. sanguinolenta* (X 1).

Anatomical studies of the root were carried out on plant materials harvested from the various locations. Root materials harvested from the various locations were macerated using hydrogen peroxide and acetic acid in a ratio 2:1v/v. Maceration was carried out by heating gently on a water-bath, until the tissues were softened and turned whitish. The contents of the beaker were thoroughly washed with distilled water. Twenty slides were prepared from each of the macerated plant materials obtained from the locations. Clear slides viewed at X100 were photographed. The following measurements were taken: fibre index, vessel element index and prismatic crystal index.

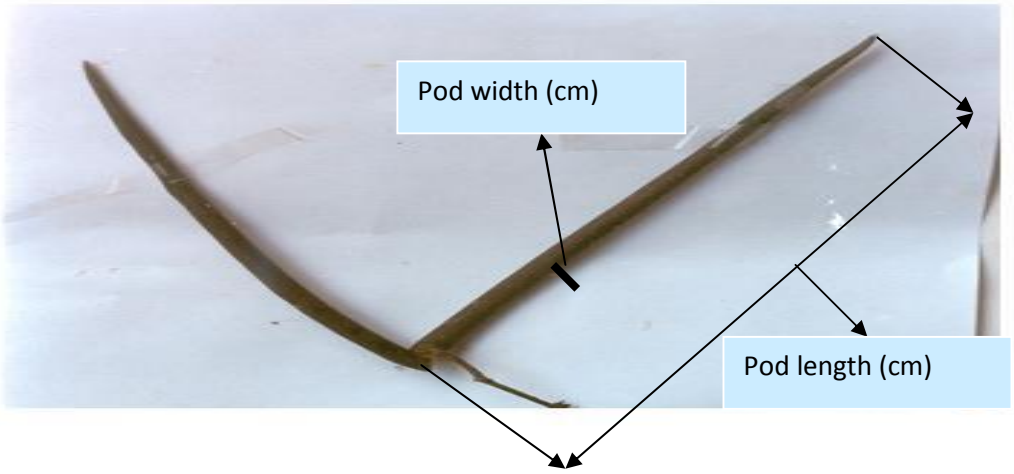


Plate 3. Pod morphology of *C. sanguinolenta* (X 1).

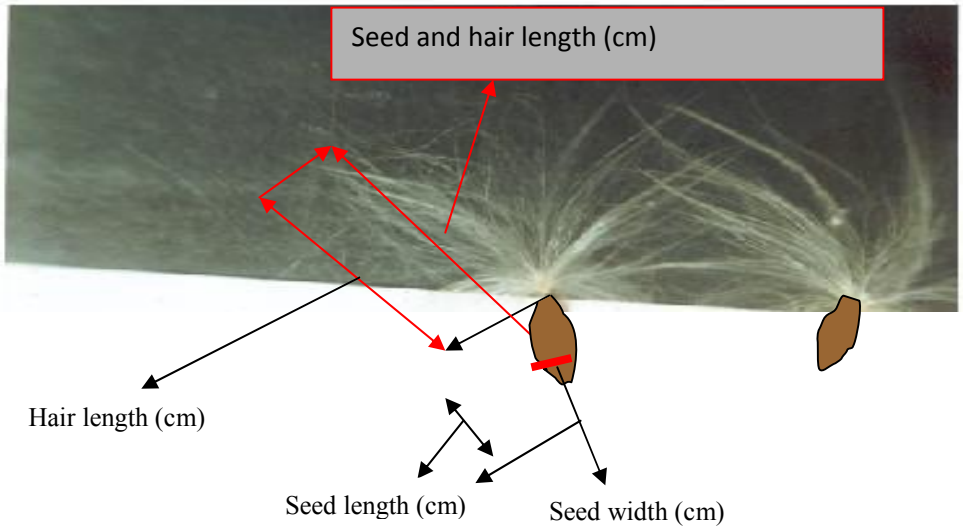


PLATE 4. Seed morphology of *C. sanguinolenta* (X 1).

Fibre index

The length and width of 10 fibres on each prepared slide were measured using a pre-calibrated ocular micrometer. Length measurement was taken from one tip of a fibre to the other whilst the width was also taken at the widest section of the fibre as shown in Plate 5.

Prismatic crystal index

A pre-calibrated ocular micrometer was used in taking the length and width of the prismatic crystals identified. Ten prismatic crystals of each of the 15 slides were recorded. The longest section was taken as the length and the broadest section also as the width as shown in Plate 6.

Vessel element index

Measurement of the length and width of 10 vessel elements of each prepared slide was recorded using a pre-calibrated ocular micrometer as shown in Plate 7. The length was taken from one pointed tip to the other whilst the width was measured at the widest section of the fibre.

Statistical analysis

The SPSS version 16.0 was fitted to the dataset to analysis the quantified parameters obtained from the leaf

morphological characters, pod characteristics, seed characteristics and the histological characters.

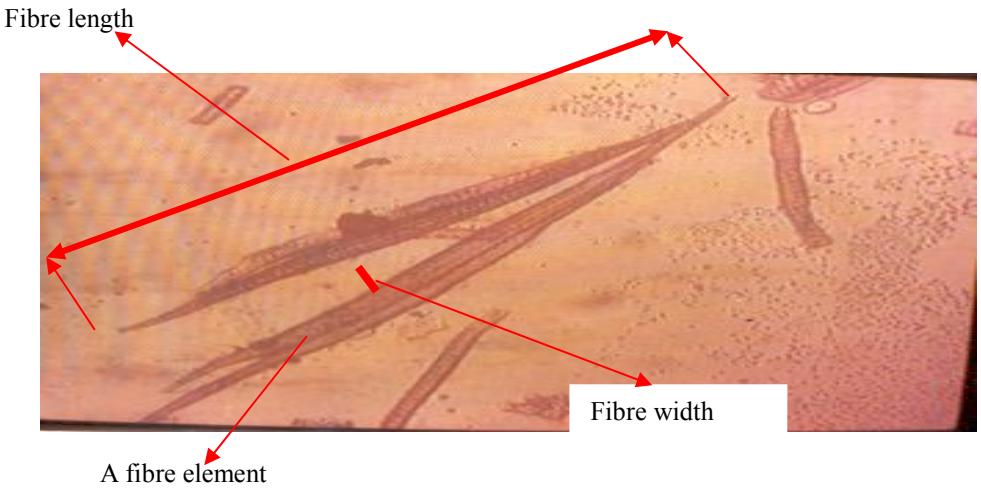


PLATE 5. Fibres isolated from the root of *C. sanguinolenta* (X100).

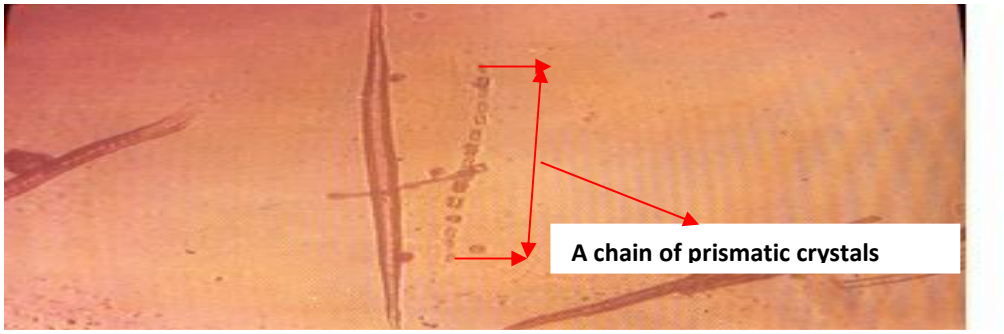


PLATE 6. A fibre and a chain of prismatic crystals isolated from the roots of *C. sanguinolenta* (X100).

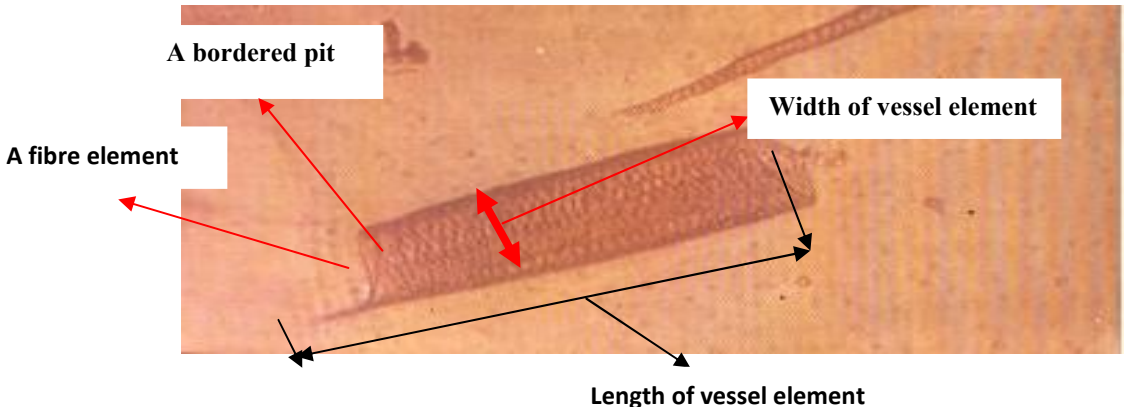


PLATE 7. Vessel element isolated from the roots *C. sanguinolenta* (X100).

A vessel element

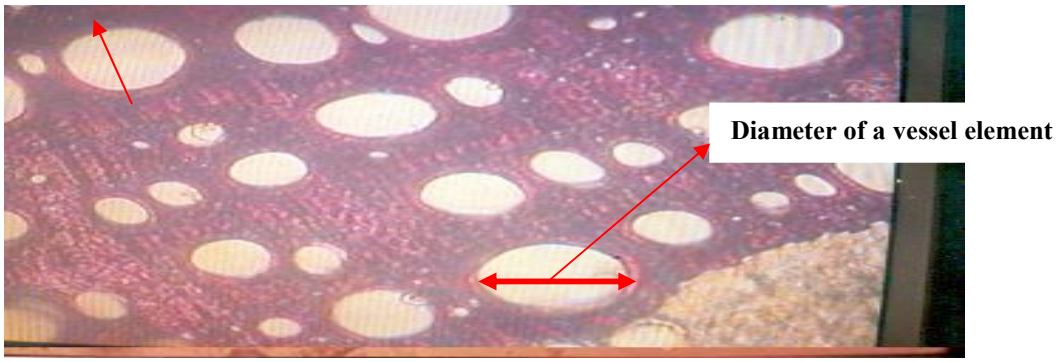


PLATE 8. Transverse section (TS) of vessel element of the root of *C. sanguinolenta* (X100).

RESULTS

The results gathered from the morpho-anatomical characters of the plant species, *C. sanguinolenta* (Lindl.) Schter have been presented below:

Variations in the morphological characters

Variations within the morphological features of the plant species, *C. sanguinolenta* obtained from the different locations based on the mean dimensions of the following: Leaf length, leaf width, leaf petiole length, pod length, pod width, seed length, seed width, seed and hair length and hair length have been presented in Tables 1 - 2: Twelve (12) of the characters were significantly different at the following probability levels; $p < 0.001$; $p < 0.01$ and at $p < 0.05$ (Appendix 4).

Table 1: Mean dimensions of the leaf index, petiole length and pod index.

Location	Leaf length (cm)	Leaf width (cm)	Leaf petiole length (cm)	Pod length (cm)	Pod width (cm)
Pepease	7.9 a ± 1.0483	4.2 a ± 0.7970	0.9 c ± 0.1545	18.6 b ± 3.5	0.8 a ± 0.17
Mamfe	6.8 b ± 1.0131	3.2 b ± 0.4849	1.4 a ± 0.2429	15.9 c ± 2.44	0.7 b ± 0.17
Abonse	6.1 c ± 0.6254	3.0 c ± 0.3621	1.1 b ± 0.3172	18.7 a ± 2.74	0.7 b ± 0.17

Generally, the results presented in Table 1, show that the leaf index, leaf petiole and pod length of the plant species obtained from the three locations were statistically different from one another. The pod width of the plant species harvested from Pepease was statistically at variance with those of Mamfe and Abonse while again; statistically there was no significant difference between the last two locations as shown in Table 1.

TABLE 2: Mean dimensions of the seed index, seed and hair length and hair length only.

Location	Seed length (cm)	Seed width (cm)	Seed/ hair length (cm)	Hair length (cm)
Pepease	0.8 c ± 0.18	0.4 a ± 0.16	5.3 c ± 0.81	4.5 b ± 0.73
Mamfe	1.0 a ± 0.23	0.3 b ± 0.17	6.0 a ± 0.81	4.8 a ± 0.86
Abonse	0.9 b ± 0.17	0.3 b ± 0.14	5.4 b ± 0.96	4.8 a ± 1.03

The seed length and seed and hair length of *C. sanguinolenta* obtained from the locations were statistically at variance (Table 2). The seed width and hair appendage of the plant species obtained from Pepease were statistically different

from those from Mamfe and Abonse while there was no statistical difference between those of *C. sanguinolenta* collected from the last two locations in both cases (Table 2). The plants collected from Pepease recorded the highest leaf index of 6.0 – 9.8cm for the length and 3.0 – 5.3cm for the width while the shortest was noted at Abonse ranging from 5.0 – 7.2cm in length and 2.4 – 3.5cm in width (Table 1). As presented in the above table, the petiole was longest at Mamfe (1.0 – 1.7cm) with the shortest occurring at Pepease ranging between 0.6 – 1.1cm (Table 1). The pod length was longest at Abonse (14.8 – 22.5cm) and shortest at Mamfe ranging 10.5 – 21.3cm, and the maximum width occurred at Pepease (0.5 – 9.5cm) with the minimum occurring at Mamfe (0.4 – 0.9cm) (Table 2). In Table 2, the longest seed length was recorded at Pepease (0.6 – 1.1cm) and the shortest was at Abonse (0.6 – 1.3cm). Again, the maximum seed and hair length was recorded at Mamfe (0.6 – 1.3cm) with the minimum being at Pepease (0.6 – 1.0cm) (Table 2). Lastly, the longest hair length was recorded at Mamfe with a range of 3.4 – 6.1cm with the shortest also occurring at Pepease (3.6 – 5.4cm) (Table 2).

Variations of the histological features

Table 3 summarizes the level of variation or significant differences within the plant species based on histological features such as: fibre index, vessel element index and prismatic crystal index of the root.

As presented in Table 3, the fibre index and vessel element length of the root of the plant species harvested from the three locations were statistically different from each other. The width of the root vessel element of the root of the plant species obtained from Pepease was statistically different from those collected from Mamfe and Abonse while no statistical difference was observed between those of the last two locations in both cases (Table 3).

TABLE 3: Mean dimensions of the fibre index, vessel element index and prismatic crystal index

Location	Fibre index		Vessel element index		Prismatic crystal index	
	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)	Width (µm)
Pepease	52.5 a ± 0.53	17.6 a ± 0.203	34.5 a ± 3.541	7.0 a ± 0.04	2.25 a ± 0.032	1.00 a ± 0.004
Mamfe	37.0 c ± 0.43	10.5 c ± 0.134	32.6 b ±3.230	6.8 b ± 0.03	2.25 a ± 0.032	1.00 a ± 0.004
Abonse	40.6 b ± 0.27	15.3 b ± 0.153	30.2 c ± 2.862	6.8 b ± 0.03	2.21 a ± 0.031	0.65 b ± 0.002

Statistically, there was no significant difference between the locations in terms of the prismatic crystal length while the prismatic crystal of the root of the plant species obtained from Abonse was also statistically different from those of Pepease and Mamfe (Table 3).

The structure of the fibres, vessel elements and prismatic crystals obtained from the macerated root materials were morphologically alike. Therefore, a sample each of the histological features obtained from Pepease had been shown in Plates. 5 - 8 to represent the other two locations. The fibres were pointed in both ends with distinct bordered pits (Plates 5 and 6) with the longest ranging 52.4 – 52.6µm and shortest 34.6 – 39.4 µm occurring at Pepease and Mamfe, respectively. The width of the fibres followed a similar order with Pepease being the widest (17.5 – 17.7µm) and the least (10.5 – 10.6µm) occurring at Mamfe. The maximum diameter of the root vessel element (Plates 7 and 8) of the plant species, *C. sanguinolenta* occurred at Pepease (6.9 – 7.1µm) with the minimum at Abonse (15.1 – 15.5µm). The longest length of the root vessel element occurred at Pepease (34.4 – 34.6µm) with the shortest occurring at Abonse (30.1 – 30.3µm. The crystals of *C. sanguinolenta* obtained from the different locations were all prismatic in nature (Plate 6) with those of Pepease and Mamfe being the longest and equal in length ranging from 2.24 to 2.26µm.

DISCUSSION

Morphological characters (leaf index, leaf petiole, pod index, seed index, seed and hairy appendage) of the plant species, *C. sanguinolenta* harvested from the locations were more variable or significantly different as shown in Tables 1 - 2. The variations or significant differences of the leaf index, leaf petiole, pod index, seed index, seed and hairy appendage within the plant species (Tables 1 – 2) obtained from the different study locations may be attributed to edaphic and environmental factors. Thus the morphological variations of the plant species, *C. sanguinolenta* in Tables 1 – 2, may be assigned to the significant differences of the soil characteristics and some of the environmental factors prevailing in the different locations. Therefore, soil differences may have reflected in the morphological outlook of the plant species, as results of the significant differences of the soil nutrients and environmental factors. Bradshaw (1959) and Gregor (1938) reported similarly on the morphological variations in *Agrostis tenuis* Sibth and *Plantago maritima* Linn. as a result of edaphic and environmental factors. Again, morphological variations in plant species have been linked to the varying nature of edaphic factors by Snaydon (1973) and Clausena *et al.*, (1940).

The resultant significant differences or variations of the leaf index, leaf petiole, pod index, seed index, seed and hairy appendage in Tables 1 – 2, may be useful in the correct identification of the plant species both on the field and in the herbarium, so as to avoid fatalities in the preparation of

herbal medicine. The application of leaf and seed morphology in the identification of plants, for example, in some Solanaceous and Apocynaceae plants, *Catharanthus roseus*, *C. pusillus* and *Digitalis thapsi* have been reported by Chandra (1972), Chaudhuri (1963) and Dewar (1933) respectively. Again, other authors like Owonubi (1986) Ghani *et al*., (1985) and Dewar and Willis (1935) have all proved the usefulness of correct identification in pharmacognostic studies, i.e. the science of crude drug through the application of any of the following; the microscopic and macroscopical characters of *Digitalis* and *Datura* species.

The resultant variations or differences of the root histological characters (Table 3) especially, the fibre index and vessel element index (Figs 9a – d), have proved as useful taxonomic tools in bringing about a clear distinction of variations within the plant species, *C. sanguinolenta*. The fibres were pointed in both ends with distinct bordered pits and the vessel elements were also pointed with oval openings, pointed ends with distinct pits. Although differences within the crystals were not significant, they were noted to be prismatic in nature. Even though the root histological features of the plant species had similar taxonomic structures, there were variations in terms of their dimensions; decreasing in the order of Abonse, Mamfe and Pepease for the vessel elements. The fibres were different lengths decreasing in the following order: Pepease, Abonse and Mamfe. Similar application of the use of components of the bark of plants as taxonomic tools in plant identification have been reported by Sivarajan (1991), who looked at the distribution of different types of crystals and classified them as styloid, prismatic and idioblasts. Metcalfe and Chalk (1979) made use of the fibre length to group some tree species into various classes as short, medium-sized and long based on some ranges. Nayar, *et al*.(1977) also discovered the use of the size and shape of the crystals in the abaxial epidermis of the leaf of *Myristica fragrans* widely cultivated in India in bringing about a distinction between male and female trees, even at the sapling stage.

CONCLUSION

Morphological characters and histological characters obtained from the different locations were generally unrelated. Some of the characters were more influential or weighted more than others, although, all the characters were statistically, treated equally (Tables 1, 2 and 3).

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