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European Journal of Experimental Biology, 2014, 4(1):464-471

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Morphological, anatomical, cytological and phytochemical studies on *Capsicum annuum* Linn. (Solanaceae)

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ABSTRACT

The present study is set to investigate the micro- and macro-morphological, anatomical, cytological and phytochemical properties of Capsicum annuum Linn. a member of the family Solanaceae predominantly found in the Niger Delta Tropics, Nigeria. It is used as spice, vegetable and medicine. It is one of the extremely pungent pepper. The habit is an annual sub woody plant which attains up to 65cm or more in height. The leaves are simple, glabrous, lanceolate to ovate with apex being acutely acuminate and the base being cuneate or abruptly acute and petiolate measuring 8.2 ± 1.67cm in length and 4.1 ± 0.322cm in width. The glabrous stem is angular and the inflorescence terminal, flowers are axile in placentation borne at nodes. The corolla is whitish and calyx greenish. The berry fruits are many seeded, globose shaped and borne at nodes. The epidermal studies reveal anomocytic stomata and 6.02% for adaxial and 25.95% abaxial stomatal indices whereas the trichomes are simple uniseriate forms having 1.61% for adaxial and 7.69% for abaxial trichome indices. The stomatal characteristics showed that the adaxialstomatal length of 12.7±3.20µm with 25.18% coefficient of variation (C.V.) and width of 8.70±1.98µm with 21.71% C.V., while the abaxial stomatal length of 12.0±1.83µm with 28.56% C.V. and width of 7.6±1.27µm with 16.64% C.V. The anatomy of mid-rib and petiole revealed open vascular system and node is unilacunar having 2 vascular traces from one gap. The stem has 5 to 6 vascular bundles. The petiole is associated with 2 rib traces at primary growth phase. At secondary growth phase, the mid-rib and petiole revealed vascular arcs. The cytological study showed a diploid chromosome number of 2n = 24 and the karyotype revealed a set of telocentric chromosomes. The phytochemical studies revealed the presence of the following secondary metabolites: alkaloids, saponins, tannins, phlobatannins, flavonoids, combined anthraquinones, free anthraquinones and cardiac glycosides.

Keywords: Morphological, Anatomical, Cytological, Phytochemical.

INTRODUCTION

The family Solanaceae is composed of 95 genera [1]. It is widely distributed in temperate and tropical regions, but the centre of distribution is Central and South America. In West Africa however, there are 8 genera and 53 species of Solanaceae [2]. *Capsicum annuum* Linn. is mostly annual sub-shrubs [3]. *Capsicum annuum* Linn. has simple uniseriate trichomes [4]. Trichomes are termed 'simple' when unbranched. Simple trichomes could be unicellular or multicellular [5]. The type of hair can be of diagnostic value at species level, sometimes also at generic level, but rarely at family level [6].The word 'uniseriate' is really an anatomical term and applies to morphological properties and does not describe the shape. 'Multiseriate' is not unique to trichomes, it could also mean multi layers as in epidermal and hypodermal axial parenchyma. Metcalfe and Chalk [5]; Watson and Dallwitz [1] stated that members of Solanaceae have unilacunar node. The primary vascular tissues of Solanaceae are bicollateral [1]. Most members of Solanaceae are diploids for example the genus *Solanum* Linn. where 2n = 24 [7, 8].The use of *Capsicum annuum* Linn. in trado-medicine is due to the presence of relevant phytochemical properties. The relevance of the study is to

enhance information on the existing literature and taxonomic characteristics of *Capsicum annuum* Linn, this is due to the fact that it is an economic plant of high repute. Thus, the objectives of the study are therefore aimed at considering: the morphological investigation with a view of looking at both the macro- and micro-morphological, anatomical, cytological (including the karyotype) and phytochemical properties.

MATERIALS AND METHODS

The materials used for this study were collected from both cultivated and domesticated species and raised from seeds purchased from the fruit and vegetable markets in Rivers State.

Macro-morphological features of the species were made using a 30cm rule. The plant parts measured included: leaf length, leaf width, petiole length, sepal length, petal length, stamen length, style length, fruit diameter, flower stalk length, and average plant height. The presence or absence of trichomes was observed under a light microscope, and photomicrographs were taken.

Floral biology: The opening and closing time of the flowers of species in question was studied. The arrangement pattern of the petals and sepals was also observed and the insect pollinators noted.

Epidermal Studies: Fresh materials (leaves and stem peels) collected for this study were peeled and bleached using sodium hypochlorite for about 2 minutes following the method of Cutler [9] with some modifications. The clear epidermal layers obtained were then washed in several changes of distilled water and stained with Alcian blue or safranin and temporarily mounted in aqueous glycerol solution [9]. Photomicrographs were taken from good preparations. Stomatal study (Stomatal indices) was done from the cleared leaves. The length and width of the stomatal complexes were measured using a calibrated eye piece graticule following the method of Arnold [10]. The stomata observed were viewed with the light microscope and were measured or calculated in unit area using the Stomatal Index [S.I.] formula as shown below:

$$\text{S.I.} = \frac{S}{E+S} x \frac{100}{1}$$

where S = numbers of stomata and E = epidermal cells within the particular area under investigation. The same formula was applicable for the calculation of Trichome Index (T.I.), in this case, trichome (T) was used instead of stomata:

$$\text{T.I} = \frac{T}{E+T} x \frac{100}{1} \quad .$$

Anatomical Studies: Seeds of the plant materials were plated out in petri dishes containing moist Whatman (110mm) filter paper and the germination test was calculated using similar formula as applied to stomatal index but based on the percentage of the number that germinated divided by total number of seeds plated. Some seeds were planted in labelled containers. Three days to two weeks after growth had occurred, stem and root systems were fixed, alongside with mature leaves, flowers, fruits and petioles harvested from mature plant, in FAA in the ratio of 1:1:18 of 40% formaldehyde, glacial acetic acid and 70% alcohol for at least 48% hours following the method of Johansen [11].

Free hand sectioning using a systematic arrangement of 5 razor blades, with 2 sets (nacet and tiger blades) crossed and a central vertical one (nacet) lying in between the 2 sets crossed. The blades were adjusted until the holes in them synchronized. The plant part to be sectioned was placed in the hole and using the first two fingers of the left hand to hold the vertical blade sets, while pressing down the 2 crossed sets with the first two fingers of the right hand to make a transverse section of about 20 to 25µm thick. The sections made were passed through alcohol solutions in the order: 30%, 50% 70%, 95% and absolute alcohol, allowing them for 5 minutes in each solution. The dehydrated materials were cleared of their natural wax by passing them through different proportions of alcohol and chloroform in the following ratios (3:1; 1:1; 1:3) v/v for 10 minutes in each, and as the chloroform gradually replaced the alcohol, the process was repeated from the pure chloroform and down the series again within same time interval. These were rehydrated in alcohol series starting with absolute then 95%, 70%, 50%, 30% and stained with 1% Alcian blue for 2 minutes, washed off with water before counter-staining with 1% safranin for 2 minutes. The stain was washed off and placed on clean glass slide with a drop of glycerol and a clean cover slip placed on it. The slides so prepared are as good as those of microtomy and are near permanent ones. These slides were viewed with the light microscope and photomicrographs taken from good preparations after proper examination.

Cytological Study: Healthy root tips for mitotic study were obtained from seeds of *Capsicum annuum* Linn. grown in a petri dish containing 110mm Whatman filter paper moist with water for a period of three days to one week. The early germinated roots were transferred to solution of 0.002M of 8- hydroxyquinoline for 3 hours specifically to suspend the spindle fibres or to accumulate chromosomes at metaphase between 9 and 10 a.m. to be precise. The roots were treated with Carnoy's fluid (3:1 ethanol/acetic acid v/v) for 12 to 24 hours aimed at killing the cells. The roots were then preserved in 70% alcohol and kept in the refrigerator until when needed or used immediately by hydrolyzing in 9% HCl for 8 minutes and passing them through 70% ethanol for 10 minutes. 1mm of the root tip studied was excised from the apex and squashed in a drop of FLP-orcein stain(2g of orcein dissolved in 100ml of a solution of equal parts of formic acid, lactic acid, propanoic acid and water) under a cover slip, flattened out and examined under a light microscope, following the method of Okoli [12]. Photomicrographs of the chromosomes were taken from good temporary slides, using a Sony digital camera (7.2 Mega pixels).

Phytochemical Studies (Qualitative analyses): The leaves of the species were sun dried for 72 hours (3 days) and weighed. Fifty grams (50g) of the leaves were macerated in 96% ethanol using a pestle and a mortar. The extract was there after filtered and evaporated to dryness using a rotary evaporator set at 45^0C to constant weight and later, an exhort extraction machine. Residue yields were noted and a portion was used for the phytochemical screening. Phytochemical screening for saponin, frothing tests, was done following the method described by Wall *et al.* [13, 14] as shown below:

The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening test for these compounds. 0.5g of each plant extract was shaken with water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for the presence of saponins. In order to remove 'false-positive' results, the blood haemolysis test was performed on those extracts that frothed in water. 0.5g of each extract was boiled briefly with 50ml phosphate buffer, pH 7.4, and then allowed to cool and filtered; 5ml of the filtrate was passed for 3 hours through an asbestos disc (1.5mm thick and about 7mm in diameter), which had been previously soaked with two or three drops of 1 percent cholesterol in ether and dried. After filtration the disc was washed with 0.5ml of distilled water, dried and boiled in 20ml of oxylol for 2 hours to decompose the complex formed between cholesterol and any saponins in the extract. The disc was then washed in ether, dried and placed on a 7 percent blood nutrient agar. Complete haemolysis of red blood cells around the disc after 6 hours was taken as further evidence of presence of saponins.

Test for alkaloids: 0.5g of each extract was stirred with 5ml of 1 percent aqueous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated [15, 16]. A confirmatory test designed to remove non-alkaloidal compounds capable of eliciting 'false-positive' results was carried out as follows with all extracts which gave preliminary positive tests for alkaloids. A modified form of the tin-layer chromatography (TLC) method as described by Farnsworth and Euer [17] was used. 1g of the extract was treated with 40 percent calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10ml portions of chloroform. The extracts were combined and concentrated in vacuole to 5ml. The chloroform extract was then spotted on thin-layer plates. Four different solvent systems (of widely varying polarity) were used to develop each plant extract. The presence of alkaloids in the developed chromatograms was detected by spraying the chromatograms with freshly prepared Dragendorff's spray reagent. A positive reaction on the chromatograms (indicated by an orange or darker coloured spot against a pale yellow background) was confirmatory evidence that the plant extract contained an alkaloid.

Test for tannins: 5g of each portion of plant extract was stirred with 10ml of distilled water, filtered, and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins [16].

Test for anthraquinones: Borntrager's test was used for the detection of anthraquinones. 5g of each plant extract was shaken with 10ml benzene, filtered and 5ml of 10 per cent ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet colour in the ammonia (lower) phase indicated the presence of free hydroxyanthraquinones.

For combined anthraquinones, 5g of each plant extract was boiled with 10ml aqueous tetraoxosulphate (vi) acid and filtered while hot. The filtrate was shaken with 5ml of benzene, the benzene layer separated and half its own volume of 10 per cent ammonia solution added. A pink, red, or violet coloration in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extract [16].

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1 per cent aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins [16].

Test for cardiac glycosides: Lieberman's test was used. 0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled well in ice. Tetraoxosulphate (vi) acid was carefully added. A colour change from violet to blue to green indicated the presence of a steroidal nucleus (i.e. aglycone portion of the cardiac glycoside) [18].

RESULTS

Macro-morphology

The geographic location of the parent plant studied was $04^{0}52^{1}3377^{11}$N and $006^{0}54^{1}860^{11}$E at 18m altitude. The opening and closing times of the flowers were studied. It was revealed that the flowers commence opening at 6:50 a.m. and open completely at 8:30 a.m. while the closing time start at 5:00 p.m. and close completely at 8:50 p.m. .These features are of taxonomic relevance. The germination test conducted was 60%. The distributional pattern of the species has been recorded by Hutchinson and Dalziel [3]. Pepper as commonly known is an annual stout branched sub woody plant attaining up to a height of 65cm. The sub-sessile leaves are simply ovate, apex acutely acuminate and cuneate or abruptly acute at base measuring up to 19cm in length and 4cm wide. The petioles are 0.2cm in length. The glabrous stem is also angular in shape. Inflorescence is terminal and flowers are borne singly at nodes and measuring up to 0.4cm in diameter. The petals are 5 and 6 in number, pale whitish and averagely 0.5cm long and 0.3cm wide. The greenish sepals are 5 and 6 in number but not separated, averagely 0.2cm long and 0.1cm wide. The stamens are also 5 and 6 in number up to 0.3cm long. It is discovered that the plant is both pentamerous and hexamerous. The fruits comprised many seeded berry, borne singly at nodes, globose in shape and when unripe green, and when ripe are red, orange, yellow, brown, or purplish up to 1 to 2.5cm in diameter. The seeds are 0.2 to 0.3cm in diameter (FIG. 1). The morphology and quantitative characteristic of the plant is revealed Tables 1 and 2. Aestivation type for the species studied is valvate. Insect pollinators are ants, spiders, house flies, bees and caterpillars. Pollinators started appearing at 7:00 a.m. and were not seen at 2:20 p.m., and sometimes resurfaced later in the day.

TABLE-1: Morphological Characteristics of *Capsicum annuum* Linn.

Collector's No	Name of Taxa	Stem	Leaf	Inflorescence	Plant Height (m)
CW015	*Capsicum annuum* Linn.	Glabrous less woody with nodal swellings	Simple lanceolate to ovate leaf, acutely acuminate at apex and cuneate or abruptly acute at base 10cm long and 4cm wide	Single flower at nodal positions	Usually annual less woody under shrub up to 65cm in height

TABLE-2: Quantitative Characteristics of *Capsicum annuum* Linn.

Plant Height (cm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Flowers Diameters (cm)	Petal (cm)	Sepals (cm)	Stamens length (cm)	Carpel length (cm)	Inflorescence composition	Fruit size (cm) in diameter	Seed size (cm) in diameter
65	10	4	2.0	0.4	0.5	0.2	0.3	0.5	Solitary axillary	1 to 2.5	0.3

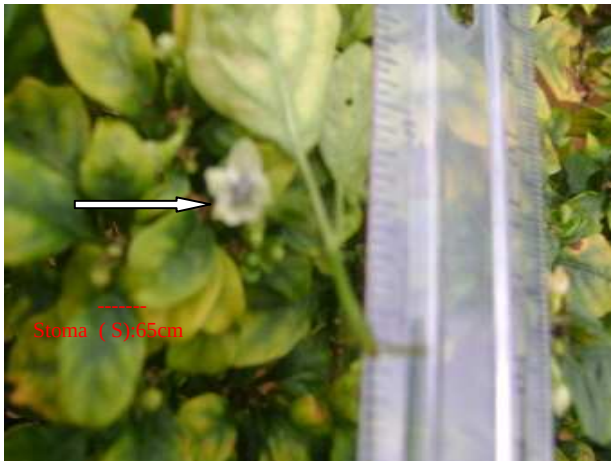

FIG. 1: *Capsicm. Annuum* Linn. Arrow shows hexamerous flower

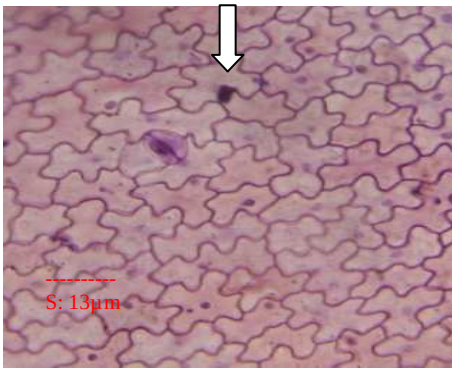

FIG. 2: Adaxial surface
Arrow reveals glandular trichome

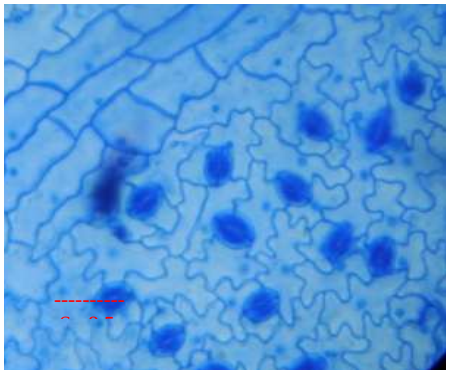

FIG. 3: Abaxial surface- Anomocytic stomata

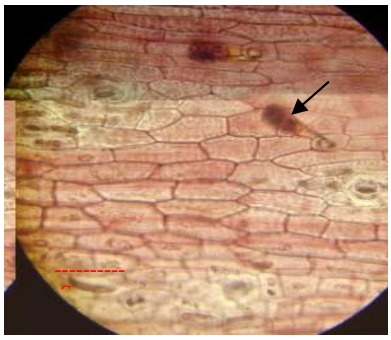

FIG. 4: Stem epidermis- Arrow shows biseriate trichome

Micro- morphology

Capsicum annuum Linn. foliar epidermal study revealed the presence of anomocytic stomata and uniseriate trichomes at both the adaxial and abaxial foliar surfaces Tables 3 and 4. It is shown that the adaxial foliar layer has 6.02% stomatal index and 25.95% for the abaxial surface, in other words, there are more stomata in the abaxial foliar surface than as observed in the adaxial foliar surface, see FIGs 2 and 3. Trichome index is also studied revealing 1.61% for the adaxial and 7.69% for the abaxial surfaces. Stem epidermal study showed paracytic stomata, uniseriate trichomes and irregularly-shaped cells (FIG. 4). The stomatal characteristics showed that the adaxial stomatal length of 12.7±3.20µm with 25.18% coefficient of variation (C.V.) and width of 8.70±1.98µm with 21.71% C.V., while the abaxial stomatal length of 12.0±1.83µm with 28.56% C.V. and width of 7.6±1.27µm with 16.64% C.V. (Table 5).

TABLE - 3: Stomatal Indices of *Capsicum annuum* Linn.

Collector's No	Adaxial Foliar Surface				Abaxial Foliar Surface			
CWO15	No of Stomatal Cells	No of Epidermal Cells	Stomatal Index (SI)	Stomatal Type	No of Stomatal Cells	No of Epidermal Cells	Stomatal Index (SI)	Stomatal Type
	41	117	25.95%	Anomocytic	5	78	6.02%	Anomocytic

TABLE - 4: Trichome Indices of *Capsicum annuum* Linn.

Collector's No	Adaxial Foliar Surface				Abaxial Foliar Surface			
CWO15	No of Trichomes Cells	No of Epidermal Cells	Trichome Index (SI)	Trichome Type	No of Trichomes Cells	No of Epidermal Cells	Tirchome Index (SI)	Trichome Type
	5	315	01.61%	Uniseriate	25	300	07.69%	Uniseriate

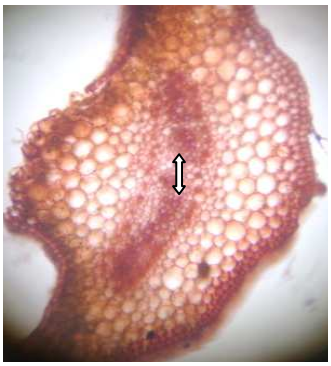

Fig-5: Mid – rib anatomy pattern unilacunar. Double – way arrow between 2 vascular traces

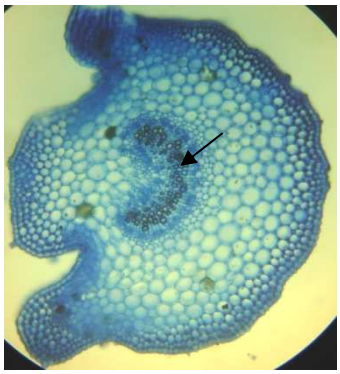

Fig. 6: Petiole anatomy. Arrow point at vascular arc

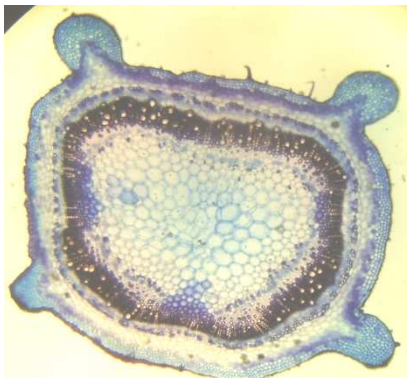

Fig-7: Stem inter modal anatomy

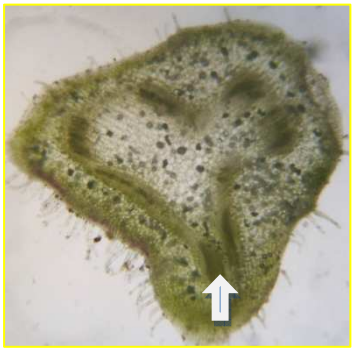

Fig-8: Nodal; arrow reveals 2 vascular traces from 1 gap

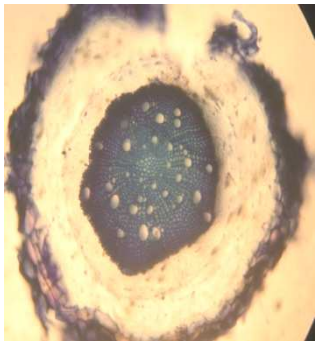

Fig-9: Root anatomy with vascular system at radial symmetry

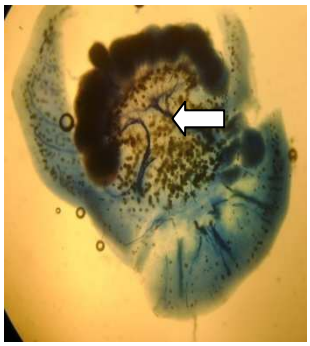

Fig-10: Ovary anatomy; arrow at the position of placenta

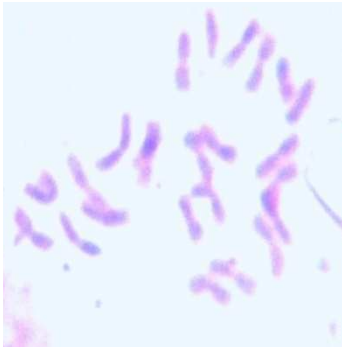

Fig-11: Mitotic chromosomes. Arrow reveals a set of Telecentric chromosome

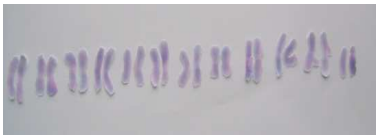

Fig. 12 : Karyotype

TABLE-5: Stomatal Characteristics of *Capsicum annuum* Linn.

Collector's No.	Leaf surface	Stomatal length (x±SD)	Stomatal width (x±SD)	Co-efficient of variance - length	Co-efficient of variance – width
CW 015	Adaxial	12.7±3.20	8.7±1.98	25.18%	21.71%
	Abaxial	12.0±1.83	7.6±1.27	28.56%	16.64%

Anatomy

Table 6 gives the summary anatomy of *Capsicum annuum* Linn. The mid-rib shows uniseriate trichomes in epidermis made of a layer of cells. The collenchymatous cells occupy the region of the hypodermis. Parenchymatous

cells occupy the ground meristem. The primary growth phase reveals 2 vascular traces. The vascular bundles have bicollateral arrangement, no rib bundle wings observed in the mid-ribs of both primary and secondary growth phases (FIG. 5). The petiole of *Capsicum annuum* Linn. is made of a layer of cells in the epidermis, 2 to 4 layers of collenchyma in the hypodermis, the general cortex is predominated by parenchymatous cells. The primary growth phase revealed 2 vascular traces and 2 rib bundle wings observed at each wing position (FIG. 6). Internodal anatomy of *Capsicum annuum* Linn. showed a four-sided or rectangular structure with swollen protuberances at each end. The hypodermis is made of 5 layers of collenchymatous cells, and the general cortex has 3 layers of parenchyma of thin walls. The endodermis is made of a layer of barrel-shaped cells. The pericycle just below the endodermis is composed of 3 to 4 cell-layers. The pith region is made of large parenchymatous cells (FIG. 7). The nodal pattern is unilacunar having 2 vascular traces from 1 gap (FIG. 8). Root anatomy of *Capsicum annuum* Linn. revealed the piliferous layer as single-cell thick. The vascular bundles are radially symmetrical with exarch xylary cells. Centralized parenchymatous cells occupy the pith region of the root (FIG. 9). Ovary anatomy of *Capsicum annuum* Linn. revealed the placentation as axile type . It is bilocular and 2-celled (FIG. 10).

Cytology

Cytological Studies of *Capsicum annuum* Linn. showed the mitotic chromosome number as 2n=24 at side metaphase (FIG. 11). The karyotype revealed 2 telocentric chromosomes (FIG. 12).

The phytochemical studies revealed the presence of the following secondary metabolites: alkaloids, saponins, tannins, phlobatannins, flavonoids, combined anthraquinones, free anthraquinones and cardiac glycosides respectively (Table 7).

TABLE- 6: Anatomy of *Capsicum annuum* Linn.

Epidermis	Hypodermis Made of collenchyma	Ground-meristem Comprises parenchyma	Endodermis, barrel-shaped cells	Pericycle	Primary Mid-rib, Petiole, Stem number of Vascular traces(V.T.); vascular gaps (VG.) and number of Rib bundle wings (RBW)	Secondary Mid-rib, Petiole, Stem one Vascular arc (V. Ar.) and number of rib bundle wings (RBW)	Open Vascular System Type For Primary Tissues	Root Vascular System	Pattern of Radial Primary Xylem Maturation
Composed of a layer of sclerenchyma	Mid-rib:3 layers Petiole: 3 ,, Node: 4 ,, Root: 3 ,,	6 layers 7 ,, 3 ,, 5 ,,	1-cell layer	Multi-layered	2 V.T.from 1 V.G, no RBW 2 V.T.from 2 V.G with 2 RBW. Bilacunar,, Actinostele with xylem star-shaped	1 V. Ar.,no RBW. 1 V. Ar.2 RBW. Bilacunardial symmetry,	Collateral ,, ,, ,,	Radially symmetrical	Maturation progresses centripetally, exarch

TABLE-7: Phytochemical Properties of *Capsicum annuum* Linn.

Collector's No	Alkaloids	Saponins	Tannins	Phlobatannins	Flavonoids	Combined anthraquinones	Free anthraquinones	Cardiac glycosides (Lieberman test)
CWO15	+	+	+	+	+	+	+	+

Note: Presence = +; Absence = -

DISCUSSION

Observations on vegetative and floral features of *Capsicum annuum* Linn. reveal the habit of the species as either annual herbs or short-lived perennial sub-woody plant which is of taxonomic relevance. *Capsicum annuum* Linn. is mostly annual sub-shrub as also recorded by Hutchinson and Dalziel [3]. Fruits of *Capsicum annuum* Linn. are very pungent and as such are used as spices and preservatives, as supported by Gill [2]. *Capsicum annuum* Linn. possesses simple uniseriate trichomes. The stem epidermal study revealed paracytic stomata. *Capsicum annuum* Linn. was observed having pentamerous and hexamerous flowers. The structure of the stamens and carpels, and

mostly their pilose nature are of taxonomic importance in the delimitations at generic and species level. The stem investigation revealed *Capsicumannuum* Linn. as angular or tetra-angular structure, also possessing swollen protuberances at the tetra angular ends. The primary node of *Capsicum annuum* Linn. Is unilacunar and the roots have radially symmetrical vascular system. The species investigated is bisexual, hypogynous and placentation is axile which is also in accordance to the observation of Hutchinson and Dalziel [3]. The fruit is globose as supported by the findings of Hutchinson and Dalziel [3].

Anatomically, studies on the primary growth phase revealed the mid-rib and petiole of *Capsicum annuum* Linn. are observed with 2 vascular traces and also have bicollateral vascular system. It was observed that the departure of the rib-bundle wings is towards the position of the open vascular system. The secondary growth phase revealed vascular arc structure in the mid-rib and petiole, while the stem and root showed a complete ring structure of an open vascular system.

Cytologically, the basic chromosome number for members of Solanaceae is x = 12 Omidiji [7] Okoli and Osuji [8] also supported the chromosome basic number as x = 12, and diploids of 2n = 24.The presence of phytochemical constituents account for the therapeutic effect of the plant in trado-medicine.

Acknowledgement
I appreciate my project supervisor Prof. B. E. Okoli for his guidance and advice. My honest gratitude to Prof. (Mrs) L. A. Akonye for her indelible role in my career and DR. N.L. Edwin-Wosu for the authentication of the plant species in the Herbarium. My unreserved thanks to my wife for her encouragement and support. Above all things, I thank my Lord and Saviour Jesus Christ, whose wonderful protection, love and grace have strengthened me at all times.

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