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TAXONOMIC STUDIES ON *Solanum macrocarpon* LINN. AND *Solanum incanum* LINN.

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ABSTRACT

The present study investigated the comparative taxonomic properties of S. macrocarpon Linn. and S. incanum Linn. members of Solanaceae in the Niger Delta Tropics, Nigeria. They are used as vegetable and medicine. The plants are erect annual sub-wood, attain up to 120cm in height. The leaves are ovate to lanceolate and glabrous up to 18.5 ±5.0cm in length and 8 ± 3.0cm wide for S. macrocarpon Linn. while that for S. incanum Linn. is glabrous, obovate and cuneate base, acute apex and variegated shiny surface up to 27 ± 6cm in length and 15 ± 4.5cm wide with alternate phyllotaxy. The inflorescence has 4 to 6 flowers for S. macrocarpon Linn. but a panicle of 4 to 5 flowers for S. incanum Linn.. The petals are whitish up to 0.6cm in length and 0.3cm wide with greenish sepals for the former whereas the latter has very large flowers having violet coloured petals measuring 1.3 to 2.0cm in length and 0.3cm wide with long greenish sepals. The berry fruit is greenish when unripe and yellowish when ripe up to 4.5cm in diameter for the formerand darkish green up to 5.5cm in diameter for the latter. The epidermis revealed anisocytic stomata whereas the trichomes aresimple uniseriate and the flowers are axile in placentation. The anatomy of mid-ribs and petioles showed bicollateral vascular systems. There are 3 vascular traces at primary growth phases and node is unilacunar.At secondary growth phases, their mid-ribs and petioles revealed vascular arcs and the stems have rings of open vascular systems. The cytological studies showed a diploid chromosome number of 2n = 24. Alkaloids, saponins, tannins, flavonoids, combined anthraquinones and cardiac glycosides are present in both specieswhile free anthraquinones is absent only in Solanum macrocarpon Linn. and phlobatannin is absent in both species.

Key words: Morphological, Anatomical, Cytological, Phytochemical, Studies.

INTRODUCTION

Solanaceae, the egg plants family comprises 95 genera Watson and Dallwitz (1992). It is widely distributed in temperate and tropical regions, but the center of distribution is Central and South America. In West Africa however, there are 8 genera and 53 species of Solanaceae (Gill, 1987). *Solanum incanum* Linn. and *Solanum microcarpon* Linn. are annual sub-shrubs(Akobundu and Agyakwa, 1998).According to Van Cottham (1970) had defined the differenttypes of stomatal complexes. Stace (1980) exploited the variations in stomatal complex for

taxonomic delimitations. *Solanum incanum Linn*. has simple uniseriate trichomes and *Solanum microcarpon* Linn. has rather stellate trichomes (Purseglove, 1968).Trichomes are termed 'simple' when unbranched. Simple trichomes could be unicellular or multicellular .The type of hair can be of diagnostic value at species level, sometimes also at generic level, but rarely at family level (Cutler, 1977). Metcalfe and Chalk (1979) stated that members of Solanaceae have unilacunar node.The primary vascular tissues of Solanaceae are bicollateral(Metcalfe and Chalk,1979). Most members of Solanaceae are diploids for example the genus *Solanum* Linn. where 2n = 24 (Omidiji, 1985; Okoli, and Osuji, 2008).Woody plants can accumulate in their cells a great variety of phytochemicals including alkaloids, flavonoids, tannins, saponins, cyanogenic glycosides, phenolic compounds, lignin and lignans (Okwu and Josiah, 2006).

The relevance of the study is focused on providing more information to existing literature and taxonomic characteristics of *Solanum incanum* Linn. and *Solanum microcarpon*Linn. Thus, the objectives of the study is aimed at providing additional data based on morphology, anatomy, cytology and phytochemistry that could enhance the taxonomic delimitation of the species of *Solanum*

MATERIALS AND METHODS

Collection of Plant Material

The GPS was used to read the geographic position of the plants. The materials used for this study were collected from the wild and raised from seeds. A study of the macro-morphological features of the species was made using a 30cm ruler. The plants' parts measured included: leaf length, leaf width, petiole length, stamen length, style length, fruit diameter and average plant height. The presence or absences of trichomes were observed painstakingly under a light microscope, and microphotographs were taken where relevant.

Flower Anthesis Study

The opening and closing times of the flowers were studied.The arrangement pattern of the petals and sepals (that is the aestivation type) was observed and the insect pollinators noted.

Epidermal Studies

Fresh materials (leaves and stem epidermal peels) were collected for this study; the fresh leaves were peeled and bleached using sodium hypochlorite for about 2 minutes following the method of .Cutler (1977). The clear epidermal layers obtained were then washed in several changes of distilled water and stained with 1% Alcian blue or safranin and temporarily mounted in aqueous glycerol solution. Microphotographs were taken from good preparations. Stomatal indices were done from the cleared leaves. The length and width of the guard cells were measured using a calibrated eye piece graticule following the method of Arnold (1973). The stomata were observed with the light microscope and the calculation in unit area was done using the stomatal index [S.I.] formula as shown below: S.I. = $\frac{S}{E+S} x \frac{100}{1}$ where S and E mean numbers of stomata and epidermal cells within the particular area under investigation. The same formula was applicable for the calculation of trichome indices (T.I.), in this case, trichomes (T) were used instead of stomata: T.I = $\frac{T}{E+T} x \frac{100}{1}$.

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Anatomical Study

Seeds of the plants were plated in petri dishes containing wetted 110mm Whatman filter paper and germination tests were calculated using similar formula as applied to stomatal indices but based on the percentage of the number that germinated divided by total number of seeds plated. After three days to two weeks, harvested stems and roots were fixed, alongside with mature leaves, flowers, fruits and petioles from mature plants, in FAA in the ratio of 1:1:18 of 40% formaldehyde, acetic acid and 70% alcohol for at least 48% hours following the method of Johansen (1940).Also the free hand section using a systematic arrangement of 5 razor blades as described byWahua*et al*. (2013) was also used.The slides prepared from both methods were comparatively good. These slides were viewed using the light microscope and microphotographs were taken after careful examination, from good preparations.

Cytological Study

Healthy root tips for mitotic study were obtained from seeds of *Solanum incanum* Linn. and *Solanum microcarpon* Linn. grown in petri dish containing 110mm Whatman filter paper wetted 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. 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 crystals dissolved in 100ml of a solution of equal parts of formic acid, lactic acid, propanoic acid and water) under a coverslip, flattened out and examined under a light microscope, following the method of Okoli (1983).Microphotographs of the chromosomes were taken from good temporary slides using a Sony digital camera (7.2 Mega pixels). For the meiotic chromosomes, in-mature flower buds were used.These were treated with Carnoy's fluid for 24 hours and preserved in 70% alcohol and kept in the refrigerator, or used immediately as already described.

Phytochemical Study

Qualitative analysis of each species studied was carried out. The leaves were sun dried for 72 hours (3 days) and weighed. 50g of the leaves were macerated in 96% ethanol using a pestle and a mortar. The extract was thereafter 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 test was done following the method described by Wall*et al.* (1952 and 1954) 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 (Harborne, 1973; Trease and Evans, 1989). 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 (1962) 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 vacuo 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 (Trease and Evans, 1989).

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 color in the ammonia (lower) phase indicated the presence of free hydroxyanthraquinones.

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 (Trease and Evans, 1989).

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 (Trease and Evans, 1989).

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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. 1ml tetraoxosulphate VI acid was carefully added. A color change from violet to blue to green indicated the presence of a steroidal nucleus (i.e. aglycone portion of the cardiac glycoside) (Shoppee, 1964).

RESULTS

The Geographical Distribution Study
The geographic location of the parent plants studied were $04^{0}52^{1}3445^{11}$N and $006^{0}54^{1}864^{11}$E at 17m altitude for *Solanum macrocarpon* Linn. and $04^{0}52^{1}34331^{11}$N and $006^{0}54^{1}878^{11}$E at 19m altitude for *Solanum incanum* Linn..

Flower Anthesis Study
The opening and closing times of the flowers was studied. It was revealed that the flowers commenced opening at 4:45 a.m. and opened completely at 7:45 a.m. while the closing time started at 4:45 p.m. and closed completely at 8:35 p.m. for *S. macrocarpon* Linn. while *S. incanum* Linn. flowers started opening at 4:30 a.m., opened completely at 8:00 a.m. and commenced closing at 3:05p.m. and closed completely at 7:30p.m.This feature is of taxonomic relevance as it aids supply information patterning the breeding status of the plants.

Germination Study
The germination test conducted was 35% for *S. macrocarpon* Linn. and 10% for *S. incanum*Linn.

Morphological Study
The distributional pattern of the species has been recorded by Hutchinson and Dalziel (1958). *S. incanum* Linn. Plate 1 and *S.macrocarpon*Linn. Plate 2.

Plate 1: *Solanumincanum*Linn.Plate 2: *Solanummacrocarpon*Linn.

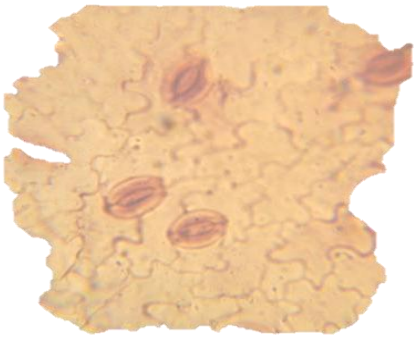
The morphological features of *S. macrocarpon* Linn. are as thus: The plant attains up to heights of 120cm or more. The petiolate glabrous leaves are simple lobate, ovate to lanceolate, acuminate at apex, cuneate to rounded at base, measuring 15 ± 6.00cm in length and 6.50 ± .3.50cm in width with alternate phyllotaxy while *S. incanum* Linn. is ovate to lanceolate with variegated shiny glabrescent appearance measuring up to 26 ± 4.00cm in length and 12± 3.00cm in width with alternate

phyllotaxy. The inflorescence of*S. macrocarpon* Linn. is a panicle of 4 to 5 flowers while those of *S. incanum* Linn. have 4 to 6 flowers per inflorescence. The pentamerous yellowish petals are up to 0.6 to 0.7cm in length and 0.3 to 0.4cm in width and sepals greenish measuring 0.3 to 0.5cm long, 0.2 to 0.4cm wide, a stamen of 0.5 to 0.6cm in length and a berry fruit of 4.5 to 4.7cm in diameter which is greenish with stripes of greener patches when unripe while yellowish to reddish color when ripe having numerous seeds ranging from 0.2 to 0.3cm in diameter for *S. macrocarpon* Linn. while for *S. incanum* Linn. the stamens are 0.5 to 0.6cm long, the inflorescence has 4 to 5 flowers. The pentamerous violet flowers are not separated, very large up to 1.30 to 2.00cm long, 0.30 to 0.40cm wide with greenish sepals up to 1.30 to 1.50cm in length, 0.20 to 0.80cm in width and the berry fruits are dark green with darker greenish patches when unripeand yellowish to dark red color when ripe, not directly eaten as those of *S. macrocarpon* Linn.because they are very hard to break rather they are first cooked before eating or used in some African dishes, and measured up to 5.50 to 6.00cm in diameter with very numerous seeds up to 0.3 to 0.4cm in diameter. 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 sometimes about 2:00 p.m., and sometimes resurfaced later in the day.

Epidermal Study

The foliar epidermal study for both species revealed the presence of anisocytic stomata. Presence of stellate trichomes at both the adaxial and abaxial foliar surfaces of *S. macrocarpon* Linn. were observed. Plates 3 and 4. It is shown that the adaxial foliar layer has 23.26% stomatal index and

3



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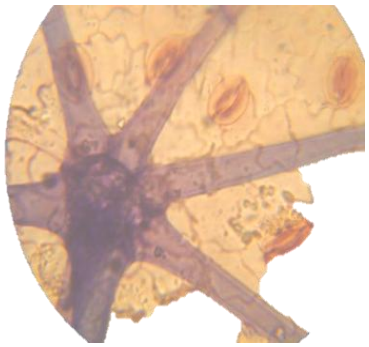

Plate 3: Adaxial Foliar Epidermis of *Solanummacrocarpon*Linn.Plate 4: Abaxial Foliar Epidermis of *Solanummacrocarpon*Linn.

19.57% for the abaxial surface. Trichome index is also studied revealing 12.04% for the adaxial and 9.63% for the abaxial surfaces, being stellate or star-shapedtrichome type; stomatal characteristics showed that adaxialstomatal length is 5.9±0.292µm with 4.95% coefficient of variation (C.V.) and width of 3.8±0.306µm with 8.06% C.V. and abaxialstomatal length as 5.7±0.274µm with 4.81% C.V. and width of 3.4±0.233µm with 6.86% C.V. while *S. incanum* Linn. revealed anisocytic stomata, and the stomatalcharacteristics showed

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adaxialstomatal length of 6.7±0.274µm with 4.09% C.V. and width of 3.9±0292µm with 7.49% C.V. and that of abaxialstomatal length of 5.9±0.246µm with 4.17% C.V. and width of 4.0±0.351µm with 8.79% C.V. respectively. The trichome types are simple uniseriate forms at both adaxial and abaxial surfaces. See plates 5 and 6.

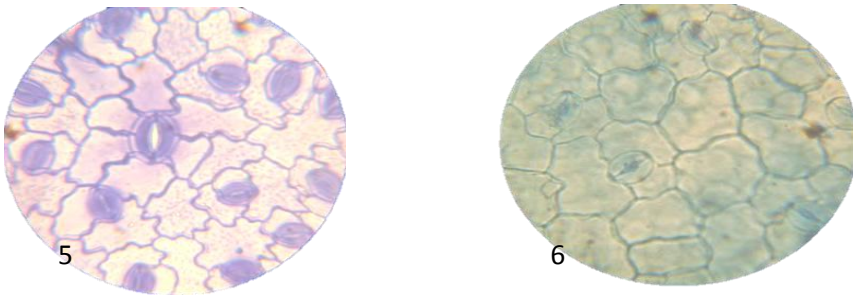
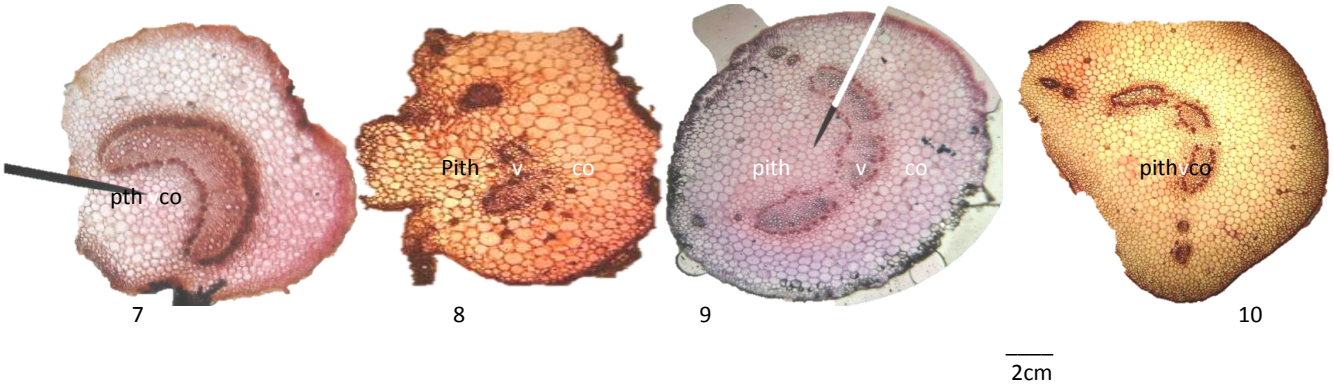


Plate 5: Adaxial Foliar Epidermis of *Solanumincanum* Linn. Plate 6: Abaxial Foliar Epidermis of *Solanumincanum* Linn.

Anatomical Study

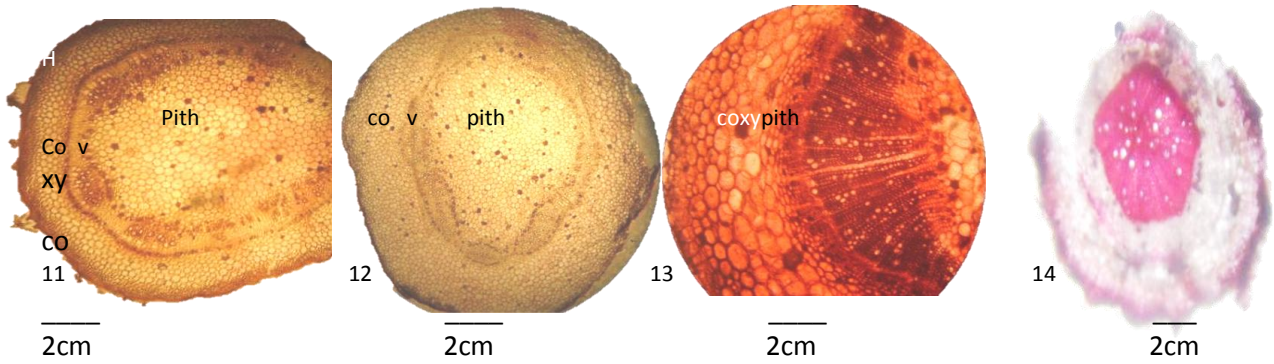
Anatomy of *S. macrocarpon* Linn. mid-rib revealed a layer of cells composition of the epidermis. The collenchymatous cells occupied the region of the hypodermis. Parenchymatous cells occupied the ground meristem. The primary growth phase revealed 3 vascular traces with no rib bundle in both growth phases. Plate 7. The mid-rib of *S. incanum* Linn. is similar to that of *S. macrocarpon* Linn. plate 8. The petiole of *S. macrocarpon* Linn. epidermis is made of a layer of cells, 2 to 4 layers of collenchyma wings in the hypodermis; the general cortex is predominated by parenchymatous cells. The primary growth phase revealed 3 vascular traces with 2 rib bundle wings. Plate 9. The petiole anatomy for *S. incanum* Linn. is as described for *S. macrocarpon* Linn. Plate 10.



KEY: co- represents cortex, pth- pith, v- vascular bundle.
Plate 7: Mid-rib Anatomy of *Solanummacrocarpon* Linn. Plate 8: Mid-rib Anatomy of *Solanumincanum*Linn. Plate 9: Petiole Anatomy of *Solanummacrocarpon* Linn. Plate 10: Petiole Anatomy of *Solanumincanum* Linn.

The intermodal anatomy of *S. macrocarpon* Linn. showed a circular structure with 7 vascular bundles. The epidermis is made of a layer of cells. The hypodermis is made of about 1 to 2 layers of collenchymatous cells, and the general cortex comprises 2 to 3

layers of parenchyma of thin walls. The endodermis is made of a layer of barrel-shaped cells clearly-marked. The pericycle just below the endodermis is composed of 1 to 2 cell-layers. The pith region is made of large parenchymatous cells when present. The intermodal or stem anatomy is shown in Plate 11. Thenodal pattern is unilacunar for both species. *S. incanum* Linn.stem anatomy revealed same structural arrangement from the epidermis to the pith as in the former see plate12. Root anatomy of*S. marcrocarpon* Linn.and *S. incanum* Linn. haveexarch xylary structure. The piliferous layer is single-cell thick. The vascular bundles are radially symmetrical. Centralized parenchymatous cells occupied the pith region of the root. (Plate 13 and 14). The ovary anatomy of both species revealed the placentation as axiletype. Their ovaries are trilocular and 3-celled.



KEY: co- represents cortex, H- hypodermis, pth- pith, v- vascular bundle, ry- xylary rays. Plate 11: Stem Anatomy of *Solanummacrocarpon* Linn. Plate 12:Stem Anatomy of *Solanumincanum* Linn. Plate 13: Root Anatomy of *Solanummacrocarpon* Linn. Plate 14: Root Anatomy of of*Solanumincanum* Linn.

Cytological Investigation

Cytological Studies of *S. macrocarpon* Linn. and *S.incanum* Linn. showed the mitotic chromosome number as 2n=24 at late prophase (Plates 15 and 16).

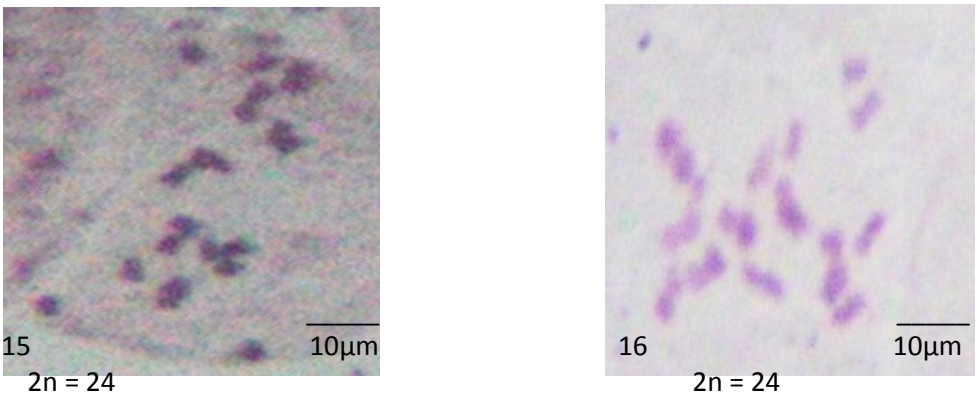


Plate 15:Diploid chromosome study of *Solanummacrocarpon* Linn. Plate 16: Diploid chromosome study of *Solanumincanum* Linn.

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Phytochemical Studies

Qualitative analysis carried out revealed the presence of the following phytochemical constituents: alkaloids, saponins, tannins, flavonoids, combined anthraquinones and cardiac glycosides are present in both species while free anthraquinones is absent only in *Solanum macrocarpon* Linn., phlobatannin is absent in both species, table 1.

Table 1: Phytochemical properties of *Solanummacrocarpon* Linn. and *Solanumincanum*Linn. within the Niger Delta, Nigeria.

Reference No.	Species	Alkaloids	Saponins	Tannins	Phlobatannins	Flavonoids	Combined Anthraquinones	Free Anthraquinones	Cardiac Glycosides
CW004	*S. macrocarpon* Linn.	+	+	+	_	+	+	_	+
CW006	*S. incanum* Linn.	+	+	+	_	+	+	+	+

Presence of phytochemical +
Absence of phytochemical _

DISCUSSION

Observation on vegetative and floral features of *S. macrocarpon* Linn. and *S. incanum* Linn. revealed the habits of the species as annual herbs as also recorded by Hutchinson and Dalziel (1958). *S. incanum* Linn. possesses simple uniseriate trichomes and anisocytic stomata for both species except that *S. macrocarpon* Linn. has stellate trichome. The structure of the stamens and carpels, especially their basi fixed nature, are of taxonomic relevance in delimitations at the generic and species level. The nodal pattern is unilacunar for both species and the roots' vascular system revealed radial symmetry. The species investigated are bisexual, hypogenous and placentation is axile which is also in accordance to the observation of Hutchinson and Dalziel (1958). Anatomically, studies on the primary growth phase revealed the mid-ribs and petioles ofthe species are observed with 3 vascular traces and also have bicollateral vascular system. The secondary growth phase revealed vascular arc structure in the mid-ribs and petioles, while the stems and roots showed a complete ring structure of an open vascular system in both the species investigation. Cytologically, the basic chromosome number for members of Solanaceae is x = 12 Omidiji (1985). Okoli and Osuji (2008) also supported the chromosome basic number as x = 12, and diploids of 2n = 24.

S. macrocarpon Linn. and*S. incanum* Linn. are used as vegetables in most African dishes. They are useful in tradomedicine. Research works in morphological, anatomical, cytological, and phytochemical properties are not altogether new; other areas of interest need are DNA barcoding, Palynology, proximate analysis and quantitative aspect of phytochemistry.

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