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Taxonomic Significance of the Occurrence and Distribution of Secretory Canals and Tanned Cells in Tissues of some Members of the Nigerian Clusiaceae

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Abstract

The occurrence and distribution of secretory cavities and tanniniferous cells in the tissues of four species of Nigerian Clusiaceae were investigated by light microscopy (LM), with respect to their taxonomic values. These species were *Harungana madagascariensis* (Lam.) *ex* Poir., *Garcinia kola* Heckel., *Allanblackia floribunda* Oliv. and *Pentadesma butyracea* Sabine. Results showed significant variations in the location, distribution and density of these micro-anatomical features in these species. Tanned cells and secretory cavities were observed in the epidermis, collenchyma, parenchyma, phloem and pith tissues of all the species except for *H. madagascariensis*. Correlation analysis between these variables were very highly significant at ($P > 0.01$) and correlated positively ($r = 0.97$), ($r = 0.73$) and ($r = 0.75$) for *G. kola, A. floribunda* and *P. butyracea,* respectively. Similarly, simple linear regression analysis were very highly significant at ($p < 0.001$) and positive with ($r^2 = 0.96$), ($r^2 = 0.86$) and ($r^2 = 0.97$) for *G. kola*, *A. floribunda* and *P. butyracea*, respectively, while these were neither significant non-positive for *H. madagascariensis* at ($p < 0.447$) and ($r^2 = 0.17$), respectively. High level of tanned cells in epidermal and phloem tissues suggested its protective functions, potential source of dye for tanning industry and the presence of bioactive component for ethno medicinal potency. The taxonomic values of these features are discussed.

Keyword: Taxonomic values, Secretory cavities, Tanned cells, Nigerian Clusiaceae

1. Introduction

The Clusiaceae (Alt. Guttiferae) belongs to the Class Magnoliopsida, Sub- Class Dilleniidae and Order Malpighales (Angiosperm Phylogeny Group, 2003, Judd *et al*., 2003) or Guttiferales (Keay, 1954, Benson, 1957). It is a family of approximately 1,200 species distributed in 40 genera (Kokwaro, 1976; Beentje, 1994, Samuel and Luchsinger, 1979). In Nigeria, the family is represented by 16 species distributed in 5 genera, consisting of *Symphonia* L., *Allanblackia* Oliv., *Pentadesma* Sabine., *Mammea* L. and *Garcinia* L. (Keay, 1954; Gill, 1988). However, a broader scope was proposed by Keay (1989), who recorded 8 genera to include, such as *Vismia* Vand., *Harungana* Lam *ex* Poir. and *Endodesmia* Benth. represented by 18 species. The inclusion of *Harungana* as a genus in the Clusiaceae has been a source of controversy to both taxonomists and other researchers. The family is very important economically, with some of its members having very high socio-economic, industrial, ethno medicinal and pharmacological values. The Igbo and Efik cultures in Southeastern Nigeria use *H. madagascariensis* medicinally for the treatment of ringworm, conjunctivitis, rashes and fevers (Madubunyi, 1995). Fresh leaf juice is applied topically to treat abscesses, while young leaves are boiled in water and the resulting “tea” administered orally three times a day as an analgesic and to treat whooping cough. Dried leaf infusion is administered orally in Nigeria to treat stomach problems, leprosy, and it equally serves as oxytocic and abortifacient medicine (Bisby *et al.*, 2007), while leaf powder is used to treat ulcer (Inyang, 2003). Economically, a mature tree *G. kola* can yield about 500 fruits, providing 1700 nuts per year. The above could yield about N50- 60,000.00 proceed annually. It serves as a very good source of income for women and men in Igbo land (Adebisi, 2002, S.E. Okeke, (personal communication, 2010).

Seeds are widely eaten in Igbo land as masticant and as kola (Nwosu, 1990). It produces the most valuable commercial resin or gum and well-known edible seed of high commercial values (Okafor, 2001; Nzegbule and Mbakwe, 2001). The bark is used for tanning while in the last 3- 4 years, the seeds are used as industrial bittering agent in some Nigerian breweries (Aniche and Uwahwe, 2010). In Ibadan area of Southern Nigeria, *G. kola* is used in natural dental care to prevent dental caries (Eyogi *et al.*, 2007). In herbal medicine, fruit pulp is used in the treatment of jaundice while nuts are dried, ground and mixed with honey to make a traditional cough mixture (Adebisi, 2002, Esimone *et al.*, 2002). A cold-water extract of root-bark with salt is administered to ease off bronchial asthma and vomiting (Farombi *et al.*, 2005). Fruits are eaten in Nigeria as a cure for general head and back aches as well as a vermifuge while the latex from stem is applied externally to treat parasitic skin diseases (Liu *et al.*, 2005). Seed is an aphrodisiac and palm wine tappers use it when tapping to scar away snakes and as antidote to poison (ASICUMPON, 2005).
Anti-inflammatory, antimicrobial, anti-diabetic and antiviral properties of the seeds have equally been established (Aduradola, 1999). Who further stated that the water extract and the dried powder of seeds have been formulated into various dosage forms including tablets, lozenges, creams, vials and tooth paste. *P. butyracea* seed has been analyzed for its chemical and physical constancy and when compared with those of the known cocoa butter and Shea butter was discovered to contain 50.0, 52.1, and 53.4% of fat, respectively. Their fats are similar in many of their characteristics, particularly in their slip point, saponification number, solidification point and fatty acid composition. Butter tree fat has a much lower unsaponification matter content of 1.5 - 1.8% than Shea butter of 7.3- 9.0% (Tchobo *et al*., 2007).

Secretory canal in the leaves and stems of vascular plants is a relevant taxonomic character and important anatomical feature that has been utilized in distantly and closely related families (Vieira *et al.,* 2001). Metcalfe and Chalk (1956) stated that the presence of foliar secretory cavities is the paramount anatomical feature and good taxonomic character that identifies certain families. They listed 40 families with "sacs or cavities with unspecified contents," 16 families with "cavities with mucilaginous contents," and 14 families with "cavities with tanniferous contents," with the Clusiaceae listed in the last categories. In a note before these lists, they stressed that these "lists have been compiled because of the proven taxonomic values of these secretory structures. Studies on the significance of this structure in many selected genera of the angiosperm families such as Rutaceae, Myrtaceae, Myoporaceae, Fabaceae, Anacardiaceae, Asteraceae, Bombacaceae, Olacaceae, Scrophulariaceae, Menispermaceae, Ebenaceae, Myrsinaceae, and Cactaceae have showen the taxonomic values of secretory cavities (Metcalfe and Chalk, 1957). The inclusion of *H. madagascariensis* in the Clusiaceae has been a source of confusion to both botanists and other researchers, this because it shears many morphological features with members of this family. In this present study an account was made to evaluate the distribution, density of secretory canals and tanned cells in these species in order to strengthen their recognition. The major objectives of this work were to, 1) determine the distribution of secretory canal and tanned cells in the stem and leaf tissues of these species 2), evaluate the density of these features and 3) to correlate the interdependency of these variables.

2. Materials and Methods

2.1. Sample Collections

Specimens were collected from Southern and Southeastern parts of the country which served as the population rang. *G. kola* was collected from Ohum Orba in Udenu Local Government Area of Enugu State, Enugu State Forest Reserve, Enugu. *H. madagascariensis* samples were collected from Odenigbo in Nsukka Local Government Area and Staff farm in Faculty of Biological Sciences, Ebonyi State University, Abakaliki while *P. butyracea* samples were collected from Adada Forest along Adada River at Nkpologu, Uzo Uwani Local Government Area of Enugu State while *A. floribunda* plants were collected from Forestry Research Institute of Nigeria (FRIN) at Benin in Edo State and Forestry Research Institute of Nigeria (FRIN) at Ikom in Cross River State.

Specimens were identified with the aid of Keay, 1954; Benson, 1957; Keay, 1989). Voucher specimens of these plants are deposited in the Herbaria of the Department of Botany University of Nigeria Nsukka and Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria.

2.2. Determination of the Secretory Canals

The presence and or absence of these micro-anatomical features were recorded from the transverse sections of the stem. Tissues where they appear to be more in abundance were recorded and their percentage density determined for each of the species by counting. Data derived from these were further subjected to statistical analysis.

2.3. Determination of Tanned Cells

The density of tanned cells was determined by counting out 100 cells from the various tissues in four slides from each sample. The numbers of tanned cells within were recorded. The following device was employed in determining their abundance in each of the tissues according to (Ndukwe, 1999), where - = complete absence of tanned cells, + = 1- 20 tanned cells per 100 cells, + + = 21 - 40 tanned cells per 100 cells, + + + = 41 – 60 tanned cells per 100 cells while + + + + = 61 - 80 tanned cells per 100 cells and + + + + + = 81-
100 tanned cells per 100 cells.

2.4. Statistical Analysis

Data collected were subjected to average, mean percentages and their mean standard errors were determined. Means of those traits which showed significant differences between taxa were separated by Least Significant Difference (LSD) tests at P = 0.05. Furthermore, Pearson Product Movement Correlation was sought for some of the quantitative data to find out the interdependencies of some of the tanned cells on the density of secretary canals. The same statistical techniques were employed to trace the level of regressions among these variables using SPSS 15 .0 windows Evaluation Version and SAS.

3. Results

3.1. Distribution of Secretory Canals in Tissues of the Four Species

Secretory canals are distributed only within the phloem tissues *H. madagascariensis* while it is found distributed within the collenchyma, parenchyma, phloem and pith tissues of *G. kola, A florinbunda and P. butyracea,* The percentage density of the secretory canals were 18., 23.1, 17.6 and 50.42 % for *H. madagascariensis, G. kola, A florinbunda and P. butyracea,* respectively (Fig. 1).

Correlation analysis between the two variables was very highly significant at ($P > 0.01$) and correlated positively at ($r = 0.97$) for *G. kola*, ($r = 0.73$) for *A. floribunda* and ($r = 0.75$) for *P. butyracea*. Similarly, simple linear regression on these features were very highly significant at ($p < 0.0001$) and positive with ($r^2 = 0.96$) ($r^2 = 0.86$) and ($r^2 = 0.97$) for *G. kola*, *A. floribunda* and *P. butyracea*, respectively (Fig. 2B, C and D) while correlation and simple linear regression were neither significant non-positive for *H. madagascariensis* at ($p < 0.447$) and ($r^2 = 0.17$), respectively (Table.2).

3.2. Distribution of Tanned Cells in Tissues of the Four Species

High numbers of tanned cells were observed in the T/S stem of *H. madagascariensis A. floribunda and P. butyracea* at the range of 81- 94% in their epidermal tissues except for *, G. kola,* that had 55-60% tanned cells out of the 100 cells counted. However, small amount of tanned cells were equally observed in their collenchyma, parenchyma and pith tissues, while there was complete absence of these tanned cells in the sclerenchyma; medullary rays and xylem tissues (Fig. 1). The percentages of tanned cells in these species were 15.4 %, 29.01%, 14.27% and 67.92 % for *H. madagascariensis, G. kola, A. floribunda and P. butyracea,* respectively.

Discussion

Secretory Canals

Secretory canals were distributed consistently within the tissues of the collenchyma, parenchyma, phloem and pith in *G. kola, A. floribunda and P. butyracea*, but were restricted in phloem tissues only in *H. madagascariensis.* This observation was in agreement with the report by Metcalfe and Chalk (1957).

However, the localization of this micro anatomical structure within the phloem tissues of *H. madagascariensis* only is of taxonomic value. This outstanding observation was in accordance with the findings of Metcalfe and Chalk (1957) and Norman, (2006) who reported same in *Hypericium,* the type genus of the Hypericaceae where they placed *H. madagascariensis*. The interdependency between the secretory canals and abundance of tanned cells in *G. kola, A. floribunda* and *P. butyracea* was highly significant and positive. The result indicated that as the number of secretory canal increases, there will be a corresponding increase in the number of tanniniferous cells in *G. kola, A. floribunda* and *P. butyracea*. On the hand such relationship does not exist in *H. madagascariensis*. The above variation is taxonomically relevant in that it excluded the genus *H. madagascariensis* from the rest of the group.

Tanniniferous Cell

Metcalfe and Chalk (1957) considered the presence or abundance of tanned cells and secretory canals in the Clusiaceae as two principal features in this family which were characterized especially by the presence of schizogenous secretory receptacles in their various tissues. However, this present study that was based on a standard procedure with fresh specimen from their natural habitat is consistent with the above reports, but with noteworthy variations in their abundance and localizations. The intensity and distribution patterns of this feature vary significantly in the various tissues of the four species. Tanned cells were highly dense and virtually in all the tissues of *P. butyracea* while in *G. kola* the intensity and distribution were equally high but not as high as in *P. butyracea*. This result is in accordance with Metcalfe and Chalk (1957) who reported that members of the Clusiaceae are known to have laticiferous cells which secrete tannin and that the distributions of these canals or cavities where these fluids are found are of great value in the identification and classification of families in angiosperms. However, while the occurrence of tanned cells do not provide much taxonomic information because of their relative presence in all the tissues of the four species, their distribution pattern could serve as a good taxonomic tool. Thus while *P. butyracea* and *G. kola* show similarities in the patterns of distribution of tanned cell, signifying their close relatedness, as against the other two taxa. This is in conformity with the report of Ndukwe (1999) and Vega *et al.,* (2002) who pointed out that taxa which show similar pattern of distribution of secondary metabolites are usually phylogenetically related. Ndukwe stressed that, this is because these compounds follow similar biosynthetic pathways in related groups of plants and that their distribution can thus form a vital taxonomic tool with which grouping difficult genera or family could be resolved. In this same line, the increased presence of tanned cells in the epidermal cells and phloem tissues as against other tissues in these species studied is noteworthy. This revelation is in accordance with the report of Ndukwu (1999), who recognized this in his work on Combretaceae and suggested that tannins are metabolized in the leaves and are probably mobilized to the stem through the phloem for excretion and storage. He concluded that the high level of tanned cells observed from the phloem regions further strengthened this suggestion since the phloem is the route of translocation of organic compounds in vascular plants. Okoli *et al* (1989) suggested that the biological functions of this compound coupled with its consistent presence in the epidermis of most plants suggest their functions to include protection from harmful organisms, browsing animals and even environmental stress. The taxonomic significance of this secondary metabolite is of high value and noteworthy, especially since they show uneven patterns in distribution and localization in the four species studied. Thus, it can form vital taxonomic evidence with which the puzzle regarding the inclusion of *H. madagascariensis* in the Clusiaceae could be resolved.

Conclusively, the significant variations in the distribution of these micro anatomical features have been highlighted. The presence of foliar secretory cavities and tanned cells in *H. madagascariensis, G. kola, A. floribunda* and *P. butyracea* have aided their classification and have shown to be a good significant anatomical feature and good taxonomic character that identifies certain families. There appears to be a close relationship existing between the presence of secretory cavity and the frequency and density of tanned cells in this family. There is every indication that as the number of secretory canal increases, there will be a corresponding increase in the number of tanniniferous cells in those related genera such as *G. kola, A. floribunda* and *P. butyracea*. The above variation is taxonomically relevant in that it excluded the genus *H. madagascariensis* from the rest of the group. Finally, the widespread occurrence of these tanned cells and orange-yellow exudates in the various tissues of these species as recorded in this study suggests the secretory potentials of members of this family, which could be utilized by the dyeing industries as source of raw material.

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Footnotes
Conflict of Interest: None declared.

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Table1. Distribution of Tanniniferous Cells in T/S Stem of the Four Species

Plant Species	Epi	Com	Pam	Scm	M. rays	Phloem	Xylem	Pith
H .madagascariensis	+++++	+	++	-	-	++++	-	+
G. kola	+++	++	+	-	-	+++++	-	+++
A. floribunda	+++++	++	+	_	-	+	-	++
P.butyracea	+++++	++++	++++	-	-	+++	-	+++++

Epi - epidermis, Com - collenchyma; Pam - parenchyma; Scm - sclerenchyma; M. rays - medullary rays

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Table2: Linear Correlation Matrix between Secretory Canals and Tanned Cells in the Four Species

	SCH	TCH	SCG	TCG	SCA	TCA	SCP	TCP
SCH								
	-							
	22							
TCH	.171							
	.447	-						
	22	22						
SCG	.149	.934(**)						
	.509	.000	-					
	22	22	22					
TCG	.148	.962(**)	.967(**)					
	.491	.000	.000	-				
	22	22	22	22				
SCA	.232	.944(**)	.959(**)	.955(**)				
	.299	.000	.000	.000	-			
	22	22	22	22	22			
TCA	.055	.796(**)	.735(**)	.813(**)	.725(**)			
	.808	.000	.000	.000	.000	-		
	22	22	22	22	22	22		
SCP	.155	.964(**)	.980(**)	.970(**)	.979(**)	.754(**)		
	.490	.000	.000	.000	.000	.000	-	
	22	22	22	22	22	22	22	
TCP	.192	.959(**)	.955(**)	.970(**)	.967(**)	.832(**)	.967(**)	
	.393	.000	.000	.000	.000	.000	.000	-
	22	22	22	22	22	22	22	22

**= P< 0.01 Correlation significant; features not followed with asterisk are not significant. SCH- secretory canals for *H. madagascariensis*; TCH- tanned cell for *H. madagascariensis*; SC G- secretory canals for *G kola,* TCG - tanned cell for *G kola,* SCA - secretory canals for *A. floribunda*, TCA- tanned cell for *A. floribunda* and SCP-secretory canals for *P. butyracea* and TCP- tanned cell for *P. butyracea*

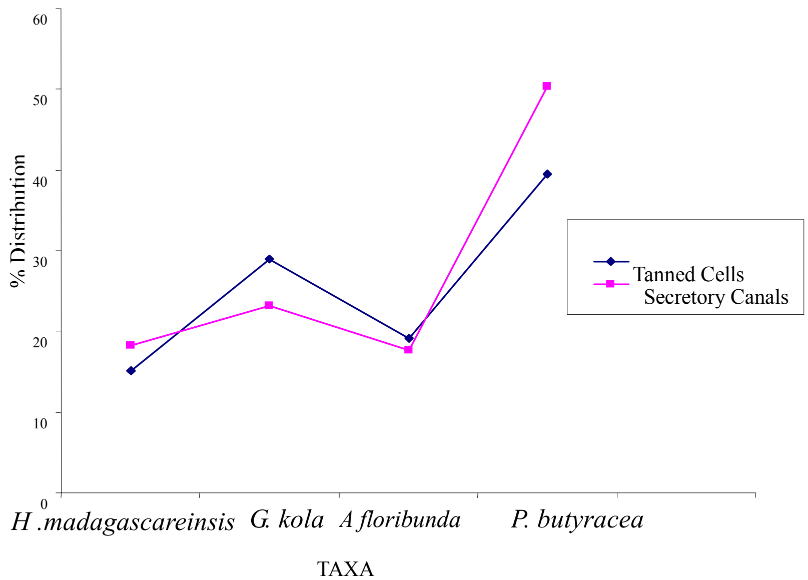
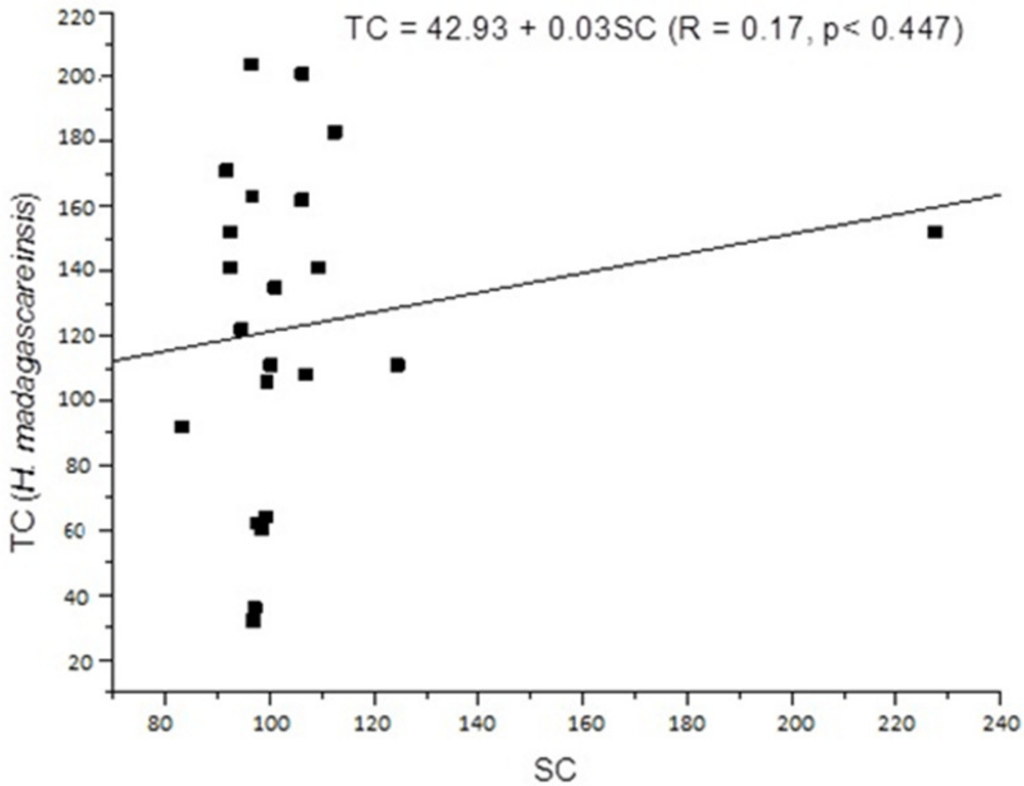
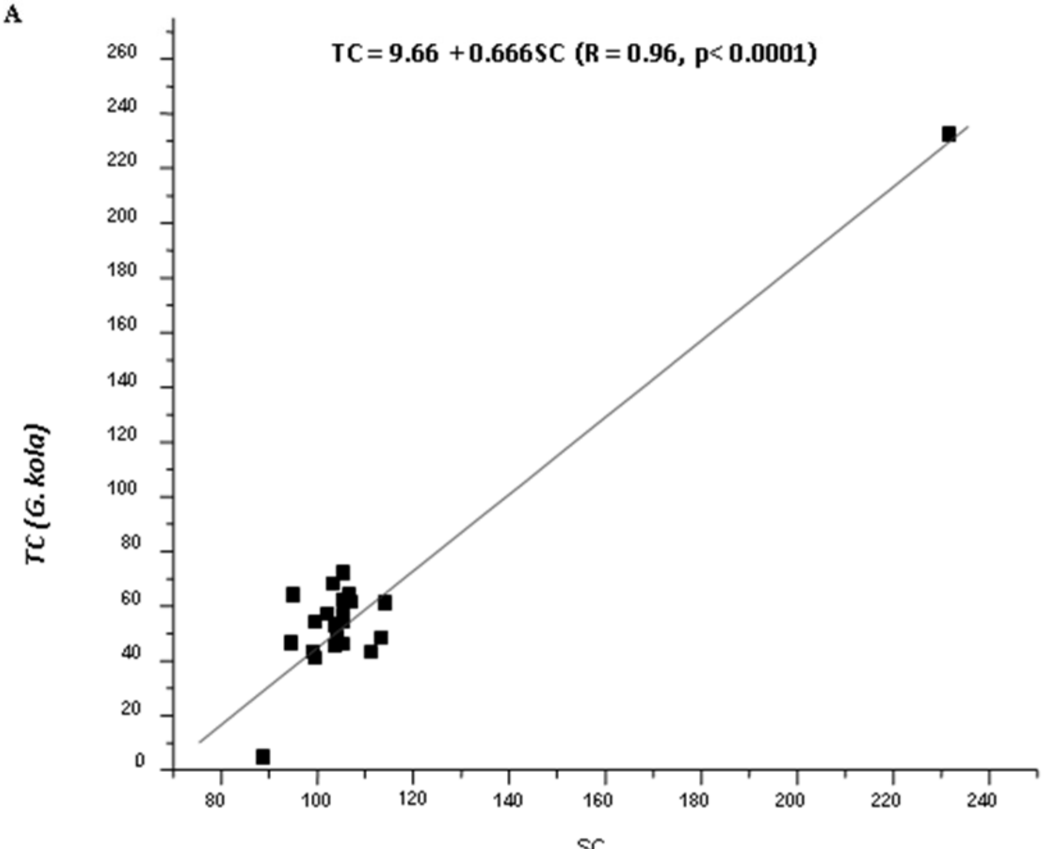


Figure1: Percentage Distribution of Tanned Cells and Secretory Canals in the T/S Stem of the Four Species


TC = 42.93 + 0.03SC (R = 0.17, p< 0.447)
TC (H. madagascareinsis)
SC

A


TC = 9.66 + 0.666SC (R = 0.96, p< 0.0001)
TC (G. kola)
SC

B

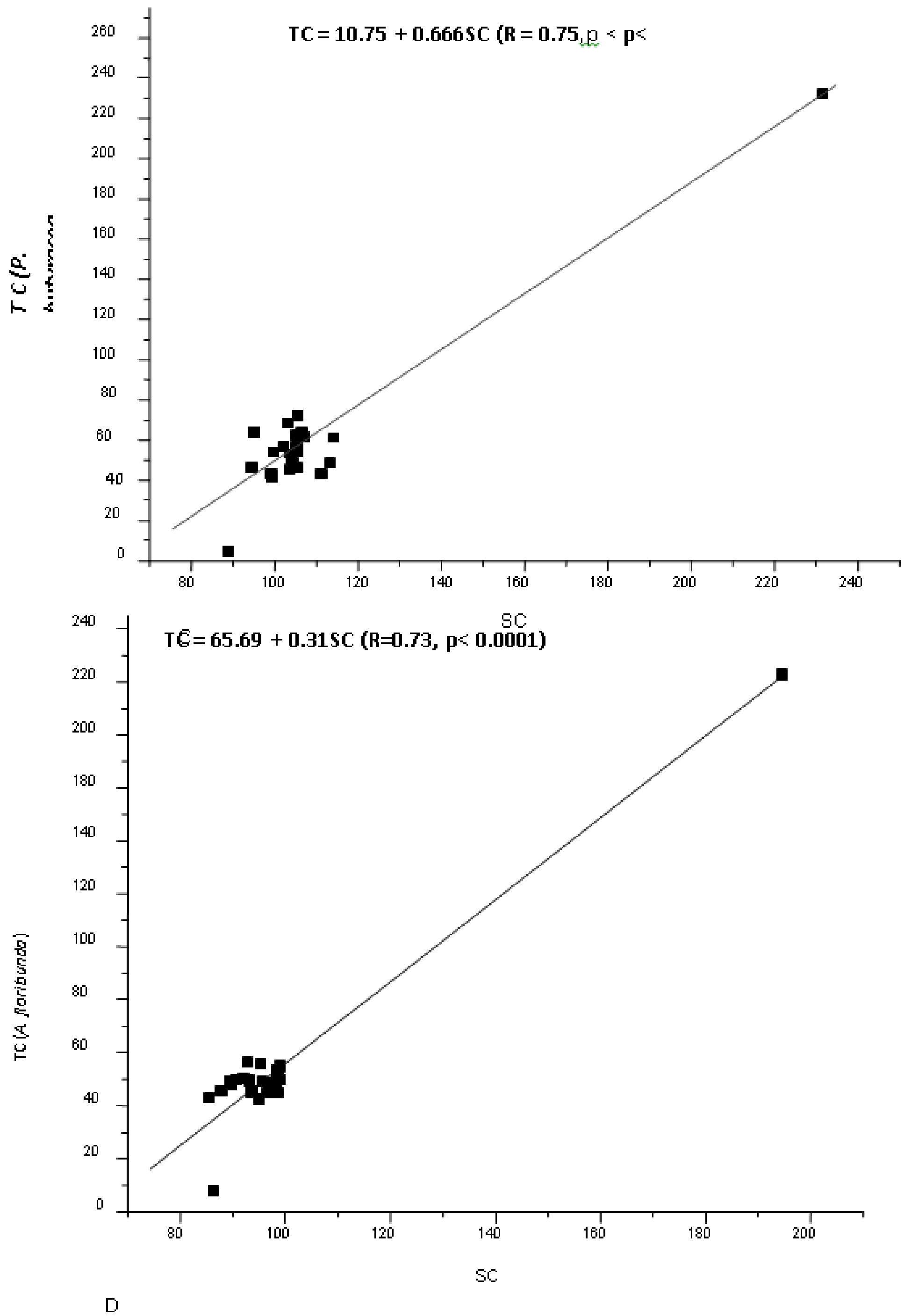


Figure 2: Scatter diagram on the relationship between secretory canals and tanned cells of A=*Harungana madagascareinsis*, B = *Garcinia kola*, C= *A. floribunda* and D= *P. butyracea.*