Leaf epidermal studies of some medicinal plant species of the family Annonaceae in the Pra - Suhien forest in the central region of Ghana

Y. AMEYAW^{1*} and H.K. AKOTOYE²

Centre for Scientific Research into Plant Medicine, P. O. Box 73, Mampong-Akuapem, Eastern Region, (Ghana). Department of Environmental Science, University of Cape Coast, Cape Coast, Central Region, (Ghana).

(Received: October 19, 2007; Accepted: November 25, 2007)

ABSTRACT

Histomorphological studies of five forest medicinal plant species of the family Annonaceae have been studied; the reported plant species are *Cleistopholis patens* (Benth.) Engl. and Diels, *Enantia polycarpa* Engl. and Diels, *Monodora myristica* Dunal, *Xylopia aethiopica* (Dunal) A. Rich and *Xylopia quintasii* Engl. and Diels. The studies revealed that leaves are hypostomatic with paracytic stomata found only in the lower epidermis. The epidermal cell walls are straight to wavy. The stomatal index of the plant species ranges from 0.086 ± 0.001 in *E. polycarpa* to 0.199 ± 0.001 in *X. quintasii*. Epidermal hairs – multicellular, non-glandular and stellate – were noticed only in *E. polycarpa*. Epidermal crystals observed include – cluster crystals, which were present in all the plant species and trihydric crystals found only in *E. polycarpa*.

Keywords: Annonaceae, hypostomatic, paracytic.

INTRODUCTION

Annonaceae, is a typical woody (dicotyledonous) family effectively used for treating diseases; such as diarrhea, fatigue, fever, menstrual pains and wounds. Reports on the medicinal significance of the family have been given by Abbiw 1990; Hufford *et. al.* 1987; Burkhill 1985; Hocquemiller *et. al.* 1982.

The family has found use in traditional societies both economically and medicinally. For example, the reddish wood of *Hexalobus monopetalus* Engl. and Diels has been used in the production of handles of picks and tools including knives in East and West Africa, and hut building in Uganda (Irvine 1961). Likewise, *H. crispiflorus* A. Rich is also useful in roofing as well as paddles, gunsticks and for house beams (Irvine 1961).

Medicinally, *H. crispiflorus* A. Rich is useful in the treatment of venereal diseases. In the Cote d'Ivoire, *H. crispiflorus* A. Rich is employed in steambaths for children with fever and baths for skin diseases (Bouquet and Debray 1974).

Remarkable pharmacological effects have been exhibited by the family either in the form of crude extracts or pure isolates. *X. aethiopica* (Dunal.) A. Rich is a common ingredient in several Ghanaian folklore medicines and has anti-microbial activity (Fiagbe 1983). Alcoholic extracts of the leaves and stem of *Annona squamosa* Linn. have been reported to show anti-cancer effects (Hufford *et al.* 1987). The alkaloidal extracts of *A. crassiflora* have shown analgesic, spasmolytic and limited anti-bacterial effects (Hocquemiller *et al.* 1982).

From the fore-going, it is evident that the family Annonaceae plays an important role in

traditional healthcare of the rural populace. *C. patens, E. polycarpa, M. myristica, X. aethiopica* and *X. quintasii* of the family Annonaceae are among some the tree species of medicinal use to the Lower Denkyeras living on the fringes of the Pra – Suhien Forest in the Central Region of Ghana. Our knowledge of these taxa based primarily on gross morphology is inadequate for better authentication so as to avoid wrongful identification that may results in fatalities.

In making accurate plant authentication, due attention is now being paid in addition to gross morphological characters and bark characteristics to foliar epidermal characters. Leaf epidermal 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.

A plethora of literature is available on phytodermology of medicinal plants. Chandra 1967

studied the leaf epidermal structure of some Solanaceous plant species. The leaf epidermal structure of some members of the Apocynaceae have been studied significantly (Chandra et al. 1972). Chaudhuri 1963 under took comparative pharmcognostic studies of the leaves of Catharanthus roseus and C. pusillus. The microscopical and macroscopical studies of the leaves of Digitalis have also been studied extensively. All the above examples or studies have helped in the identification of some species of medicinal interest. Despite the long list of plants, which have studied pharmcognostically, the only evidence that Annonaceae has received such a study is provided by Olowokudejo 1988, who reported on the epidermal morphology of the genus Annona.

Therefore, this research is aimed at the determination of some leaf epidermal features by which the plant species understudies could be recognized in the field and in the laboratory.

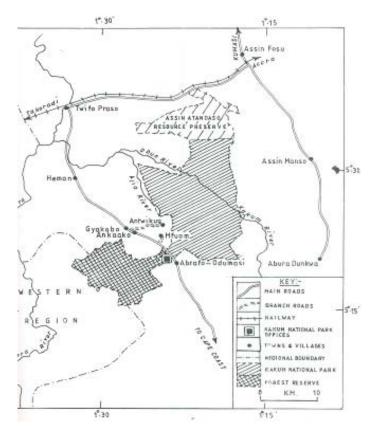


Fig. 1: Map of Pra Suhien forest reserve

MATERIAL AND METHODS

Foliar materials of the plant species used in the study were obtained fresh from the Pra- Suhien Forest Reserve portion in the Central Region of Ghana (Fig. 1). *C. patens, E. polycarpa, M. myristica, X. aethiopica* and *X. quintasii* were selected because two other members of the family; *Annona muricata* (Sour sop) and *A. squamosa* (Sweet sop) are being enjoyed by everybody.

Peels were made from both the adaxial and abaxial surfaces of each sample using a thin layer of clear nail-vanish spread and allowed to dry, and latter peeled off using a fine forceps. The epidermal strips obtained were cleared of chlorophyll in chloralhydrate. The strips were thoroughly washed using distilled water, stained with Safranin O, and mounted in drop of pure glycerol on a glass slide. Twenty slides were prepared for each of the plant species, examined under the (epidermal cells; nature of trichomes (if present); nature of distribution and dimension of stomata. The stomatal dimensions considered were the length and breadth, and also the Stomatal Index (SI), which is given as

$$SI = \frac{S}{S + E}$$
 (Metcalfe and chalf 1979)

Where: S = the number of stomata per field of view, E = the corresponding number of epidermal cells.

RESULTS

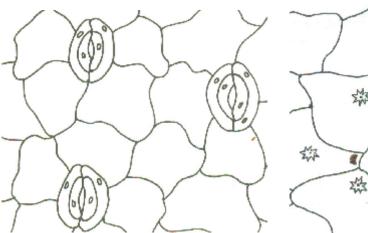
The structure of the leaf epidermis of the presently investigated taxa is enumerated below:

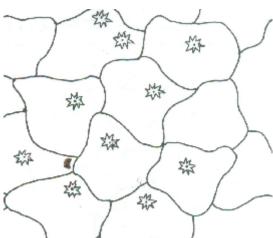
Cleistopholis patens (benth.) engl. and diels

The cell walls of both the upper and lower epidermis are slightly sinuous. Cluster crystals are present on the upper epidermis. The leaves hypostomatic with stomata restricted to the lower epidermis. Mature stomata are paracytic (Fig. 2). Mature stomata measure $22.2 \pm 0.3 \times 13.0 \pm 0.2$ µm in size. Stomatal Index is 0.178 ± 0.001 µm.

Enantia polycarpa engl. and diels

In *E. polycarpa*, the cells of both the upper and lower epidermis are straight-walled. Cluster crystals, i.e. druses, are present in the lower



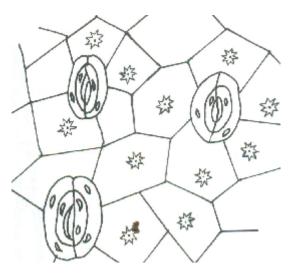




Lower epidermis with paracytic stomata (x400).

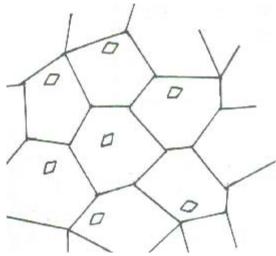
Upper epidermis with Star-shaped crystals (x400).

epidermis. The crystals on the upper surface are of the trihydric type. Two types of trichomes have been observed on the lower epidermis. These are simple unbranched hairs and stellate hairs (Fig. 3b). Leaves hypostomatic; stomata found only on the lower epidermis. Mature stomata measure $21.9 \pm 0.2 \ \mu m$ x 12.6 \pm 0.2 μm in size (Fig. 3a). Stomatal index is 0.086 \pm 0.001.



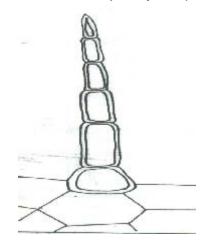
Monodora myristica dunal

Epidermal cell walls of *M. myristica* are sinuous with cluster crystals in the epidermis. The leaves are hypostomatic with stomata restricted to the lower epidermis. Mature stomata are paracytic, and measure $22.6 \pm 0.5 \ \mu m \times 10.7 \pm 0.1 \ \mu m$ in size (Fig. 4). Stomatal Index is 0.119 ± 0.001 .





Lower epidermis with paracytic stomata and star-shaped crystals (X400).



Upper epidermis with prismatic crystals (x400).

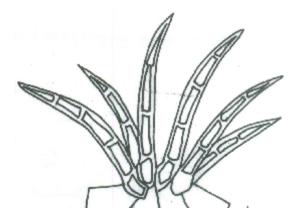


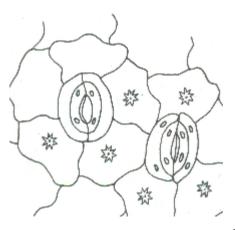
Fig. 3b:

A non-glandular trichome (x400)

A stellate trichome (x400)

444

Ameyaw & Akotoye, Biosci., Biotech. Res. Asia, Vol. 4(2), 441-447 (2007)



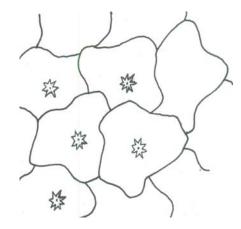
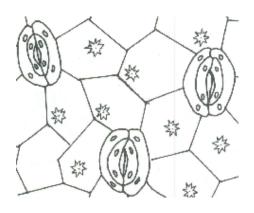
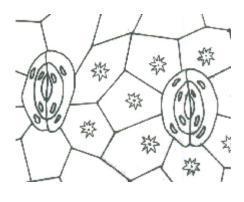


Fig. 4:

Lower epidermis with paracytic stomata and star-shaped crystals (x400).



Lower epidermis with paracytic Stomata and star-shaped crystals (x400).



Lower epidermis with paracytic stomata and star-shaped crystals (x400).

Upper epidermis with star-shaped crystals (x400).

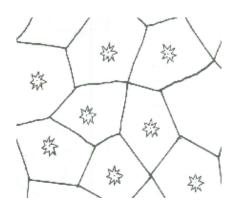
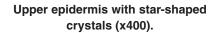


Fig. 5:



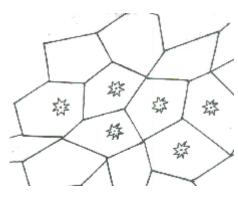


Fig. 6:

Upper epidermis with star-shaped crystals (x400).

Xylopia aethiopica (dunal) a. rich

In *X. aethiopica*, the cells of both the upper and lower epidermis are straight-walled. Cluster crystals are on both the lower and upper epidermis. The leaves are hypostomatic with stomata restricted to the lower epidermis. Mature stomata measure $23.1 \pm 0.3 \ \mu m \ x \ 11.7 \pm 0.1 \ \mu m$ (Fig. 5). Stomatal Index is 0.105 ± 0.001 .

Xylopia quintasii engl. and diels

The cell walls of both the upper and lower epidermis in *X. quintasii* are straight. Cluster crystals are found in both the lower and upper epidermis. The leaves are hypostomatic with stomata restricted to the lower epidermis. Mature stomata are paracytic, and measure $20.9 \pm 0.2 \ \mu m \ x \ 11.9 \pm 0.1 \ \mu m$ (Fig. 6). The Stomatal Index is 0.199 ± 0.001 .

DISCUSSION

Leaves are probably the most varied anatomically organs of Angiosperms (Carlquist 1961). The size and shape of epidermal cells are useful in classification at different levels (Sivarajan 1991). Metcalfe and Chalk 1979 have reported the use of epidermal characters such as leaf surface, epidermal cell wall pattern as identifiable aid of some families and genera, and sometimes for species. The epidermal cell walls of *C. patens* and *M. myristica* are sinuously-walled in both lower and upper epidermis. In *E. polycarpa, X. aethiopica* and *X. quintasii*, the epidermal cell walls are straight-walled in both epidermis.

Crystals, especially calcium oxalate crystals, are widely distributed among plants. They are of different types, e.g., styloid, prismatic and odioblasts (Sivarajan 1991). Their distribution in a given taxon is very specific and hence taxonomically useful. The epidermis of all the presently investigated taxa revealed the presence of cluster crystals on the lower and upper epidermis. There were no crystals in the lower epidermis of *C. patens*. Trihydric crystals occurred in the upper epidermis of *E. polycarpa*.

According to Stace 1984, distribution of stomata is of considerable taxonomic value though occasionally it is influenced by ecological factors under which a taxon grows. The leaf stomatal distributions are hypostomatic, i.e. restricted to the lower epidermis in *C. patens*, *E. polycarpa*, *M. myristica*, *X. aethiopica* and *X. quintasii*.

The mature stomatal types for the tree species investigated are paracytic. According to Stace 1965, stomatal size may vary on the same leaf but this may not prevent it from being used as an important taxonomic character in delimiting different species of a genus. From the present investigation, it is apparent that the stomatal size does not reveal much variation among the different genera or among different species of the same genus which could be of very taxonomic value. The stomatal measurement taken for all the tree species, show the stomatal length to be somehow twice the stomatal breadth in all cases.

Stomatal frequency is one of the most widely used characters in pharmacognostic studies(Krishnamurthy and Sundaram 1970), but its taxonomic significance has not yet been fully realized. From the present study, it is apparent that stomatal frequency does not vary much among the five species; therefore, the use of stomatal frequency as a delimiting factor is not possible for the plant species.

Trichomes have been found to be a great systematic value in the classification of certain members of Capparaceae and Caesalpiniaceae (Leelavathi and Ramayya 1983; Aleykutty and Inamder 1978). Gornall 1986 utilised anatomical characters of the trichomes of *Saxifraga* to elucidate the taxonomy of that genus. In this study, simple unbranched and stellate trichomes are observed on the lower epidermis of *E. polycarpa*. Trichomes were not observed in the other four plant species. This makes *E. polycarpa* to be unique to the other species.

CONCLUSION

The five species are important to the human communities living on the forest considering the extent to which they make use of them in the treatment of ailments. Again, it is a common observation to see parts of these species being sold as crude drugs or spices. With the ever increasing application of herbal medicines, plant collectors, ethnobotanists or taxonomists should make use of a wide range of taxonomic characters in the collection of plant raw materials in order to avoid fatalities in the preparation herbal medicines.

ACKNOWLEDGMENTS

The main author would like to thank the Department of Environmental Science, University of Cape Coast for making their anatomical laboratory available to him.

REFERENCES

- 1. Abbiw, D., Intermediate Technology . *Royal Bot. Gard.* Kew, 337 (1990).
- Aleykutty, K. M. and Inamder, J. A., Structure, Ontogeny and Taxonomic Significance of Trichomes and Stomata in some Capparidaceae. Feddes Report. 89 (1978).
- Bouquet, A. and Debray, M., Plantes Medicinelle de la Cote d'Ivoire, Trav. Doc. O. R. S. T. O. M. 32 (1974).
- Burkhill, H. M., Useful Plants of West Tropical Africa. White Friars Press Ltd. Great Britain 960 (1985).
- Carlquist, S., Comparative Plant Anatomy. Holt, Rinehart and Winston, New York, In: Plant Anatomy (J. D. Mau Seth, 1988). The Benjamin/Cummings Publishing Co. Sand Hill Road, California., 560 (1961).
- Chandra, V. R., Epidermal Studies of some Solanaceous Plants. *Ind. J. Pharm.* 29: 227-229 (1967).
- Chandra, V. R., Mitra, R., Kapoor, L. D., Epidermal and Venation Studies In Apocynaceae 14: 76-82 (1972).
- Chaudhuri, R. H. N., Comparative Pharmacognostic Studies in the Leaves of *Catharanthus roseus* G. Don and *C. pusillus* (Mur.)G. Don, *J. Pharm.* 25: 338-341 (1963).
- Fiagbe, N. I. Y., Syrup Xylopica. Proceeding of the 5th International Semminar on Medicinal Plants. Uni. of Ife, Nigeria., 121-122 (1983).
- Gornall, R. J., Trichome Anatomy and taxonomy of *Saxifraga* (Saxifragaceae). *Nod. J. Bot.* 6: 257-275 (1986).
- 11. Hawley, C., The Encyclopaedia of Chemistry

2nd Ed. Van Nostrand Reinhold Company, New York, USA 802 (1966).

- 12. Hocquemiller, R., Rasamizafy, S. and Cave, A., *Tetrahedron Letter*. New York, USA **38**: 911 (1982).
- Hufford, C. D., Liu, S. and Clark, A. N., *Natural Products*. New York, USA **50**: 1961 (1987).
- Irvine, F. R., Woody Plants of Ghana. Oxford Uni. Press, American House, London., 863 (1961).
- Krishnamurthy, K. H. and Sundaram, R., Foliar Epidermis and Pharmacognosy in some members of Asclepiadaceae. *J. Ind. Bot. Soc.* 46: 160 -168 (1970).
- Leelavathi, P. and Ramayya, N., Structure, Distribution and Classification of Plant Trichomes in Relation to Taxonomy II (Caesalpinioideae). *Ind. J. For.* 6: 43-56 (1983).
- 17. Metcalfe, C. R. and Chalk, L., Anatomy of the Dicotyldons. 1: 276 (1979).
- Olowokudejo, J. D., Taxonomy of Medicinal Plants III: Epidermal Morphology of the genus Annona (Annonaceae) in West Africa. Abstract Proceedings, 2nd Annual Conference of the Botanical Society of Nigeriia., 7-8 (1988).
- Stace, C. A., Taxonomic importance of the leaf surface. In: "Current Concepts in Plants Taxonomy", (Ed. V. H. Heywood and D. M. Moore) London., 67-94 (1984).
- Sivarajan, V. V., Introduction to the Principles of Plant Taxonomy, 2nd Ed. Cambridge Uni. Press, Great Britain pp. 292 (1991).