

**CYTO-MORPHOLOGICAL STUDIES OF SOME NIGERIAN SPECIES OF
ASTERACEAE (*Aspilia africana* and *Spilanthes costata* B.)**

BY

Patricia AWEH

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CERTIFICATION

We certify that this work was carried out by Patricia AWEH, of the Department Of Plant Biology And Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

Signed:

DR. I.D. Omoigui

Date

Project Supervisor

Signed:

Prof. E.O. Akpaja

Date

Head of Department

Signed:

Project Examiner

Date

DEDICATION

I dedicate these work to Almighty God whose love and mercy kept me although the completion of this research. I also want to dedicate it to the lovely memory of my Daddy Elder G.I Aweh,my Mummy and my supportive siblings. I am forever indebted.

ACKNOWLEDGEMENT

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TABLE OF CONTENTS

Title Page	-	-	-	-	-	-	-	-	-	-	i
Certification	-	-	-	-	-	-	-	-	-	-	ii
Dedication	-	-	-	-	-	-	-	-	-	-	iii
Acknowledgement	-	-	-	-	-	-	-	-	-	-	iv
Table of Content	-	-	-	-	-	-	-	-	-	-	v
List of Figures	-	-	-	-	-	-	-	-	-	-	vi
List of Plates	-	-	-	-	-	-	-	-	-	-	vii
Abstract	-	-	-	-	-	-	-	-	-	-	viii
Chapter One	-	-	-	-	-	-	-	-	-	-	1
1.0 Introduction	-	-	-	-	-	-	-	-	-	-	1
Chapter Two	-	-	-	-	-	-	-	-	-	-	7
2.0 Materials And Methods	-	-	-	-	-	-	-	-	-	-	7
Chapter Three	-	-	-	-	-	-	-	-	-	-	13
3.0 Results	-	-	-	-	-	-	-	-	-	-	13
Chapter Four	-	-	-	-	-	-	-	-	-	-	23
4.0 Discussion	-	-	-	-	-	-	-	-	-	-	23
Conclusion	-	-	-	-	-	-	-	-	-	-	25
References	-	-	-	-	-	-	-	-	-	-	26

LIST OF FIGURES

Figures								Page
1: Stomata terminology	-	-	-	-	-	-	-	9

LIST OF PLATES

Plate	Page
1: Photograph showing the whole plant of <i>A. Africana</i>	14
2: Photograph showing the leaves of <i>A. africana</i>	15
3: Photograph showing the epidermal layer of <i>A. africana</i>	16
4: Photograph showing the trichrome of <i>A. africana</i>	17
5: Photograph showing the stomata of <i>A. africana</i>	18
6: Photograph showing the whole plant of <i>Spilanthes costata Benth.</i>	20
7: Photograph showing the leaves of <i>Spilanthes costata Benth.</i>	21

ABSTRACT

Studies on the morphology and cytology of two species, *Aspilia africana* and *Spilanthes costata* B. family Asteraceae, were conducted. The departmental botanical garden served as the source for the many specimens used. The following was done: cytological study, which includes mitosis and meiosis; tests showed that it was not possible to successfully confirm the chromosomal numbers of the two specimens. Epidermal morphology results showed that both specimens had anomocytic stomata and were amphistomatic. The average specimen height, as well as the average length and breadth of the leads, were determined and reported. Fertility in pollen was noticed.

CHAPTER ONE

INTRODUCTION

1.1 *Astereceae*

The largest, most numerous and most diversified family in the plant kingdom is the Asteraceae, formerly known as Compositae and frequently dubbed the Sunflower. The plant family has the most geographic diversity among all the families, according to Harborne *et al.*(1984) and Aboul *et al.*(1991), it comprises about 1400 genera and over 25000 species, making up about 10% of all flowering plants, It possesses distinctive taxonomic traits (Muschler, 1912). Members of the family are typically perennial or annular herbs with two (2) subfamilies viz.Asteroideae and Cichorioideae, make up the family *Astereceae*. Flowers are arranged in heads called capitula that are encircled by involucre and has two types of florets, including ligulate or ray florets with star-shaped corollas and a majority of females, as well as tubular or disc florets with tubular corollas. The family is distinct in that it includes herbs, shrubs, vines and trees. The inflorescence consists of a head (capitulum) that also is surrounded by an involucre of phyllaries and the flowers can be bilabiate, disk-shaped, or ligulate. The calyx is referred to as a pappus, the androecium is syngeneous, the inferior ovary contains a single, basal ovule, and there are several achene's in the fruit.

1.2 *Ethnobotanical works of Asteraceae*

Many rituals, rites, folktales, taboos, medicines, customs, and traditions involve plants in some way. The Himalayan state of Uttarakhand in northern India is endowed with exceptional natural beauty, a plethora of wildlife and wide range of ethnic groups. The Tharu tribes(Pusalkar &

Shrivastava 2018) depend on the several species of the largest dicot family in the state—Asteraceae—for their subsistence. The objective of the current study was to investigate and record the ethnobotanical significance of Asteraceae species used by the Tharu culture. The study area was often visited, and a semi-structured questionnaire was used to conduct in-depth interviews with 104 Tharu informants from 11 villages. In the laboratory, identified plant specimens were prepared for the herbarium, utilizing use value (UV) and Factor Informant Consensus (FIC), ethnobotanical data were examined. The Tharu people have been shown to benefit from a total of 51 plants from 38 genera and 12 tribes of the Asteraceae family. The majority of the species (50.98%) were recognized to be employed in the treatment of different ailments. Numerous plants are utilized by them for a variety of functions, including feed (33.33%), adornment (27.45%), veterinary medication (9.80%), social ceremonies (5.88%), and other (13.72%). A quantitative study of the data reveals that *Tagetes erecta*, *Ageratum houstonianum*, and *Eclipta prostrata* are the species with the greatest use values, each with a score of 0.9423%.

According to the results of this study, various Asteraceae plants have a lot of practical significance and are crucial to Tharu's daily life. The state has a wide variety of valuable plant species, many of which have significant medicinal and other ethnobotanical uses. Many Asteraceae plants are utilized for food, medicine, and other things all around the world. It displays a variety of living forms, including herbs, shrubs, climbers, and occasionally trees. Around the world a great number of Asteraceae plants are used for food, medicinal, and other purposes. A review of the literature reveals that there is limited data on the ethnobotanical study of Asteraceae species, which are widely distributed in the study area.

Tribal communities living in rural areas lack knowledge about modern healthcare and medicinal facilities. They use their senses to identify the common diseases and cure their disorders with the help of various plant species. But this valuable indigenous knowledge is disappearing from generation to generation. Therefore, an attempt was made to investigate and document the ethnobotanical importance of Asteraceae plants among tharu tribe. *Spilanthes spp* is among the most common amazonian medicinal plants used by the lay population of the amazon basin for treating tuberculosis. In India, the juice inflorescence is used to treat mouth ulcers. In Western India, the flower head of *Spilanthes* is a popular remedy for stammering in children. It is also used to treat dysentery and rheumatism. Both leaves and flowers of *Spilanthes* are traditionally used to treat leucorrhoea by tribes in Bangladesh. In Cameroon, *Spilanthes* is used as a snakebite remedy and articular rheumatism treatment. Apart from its medicinal uses the Japanese use the flowers of this species as a spice for appetizers, while its extract is used as a flavoring material for dentifrices and gum. Meanwhile, the plant leaves are used as a spice in Brazilian cuisine to produce the characteristic tingling paraesthesia of the traditional regional dishes.

Historically, *A. africana*, which is widespread in Africa, is used in traditional medicine to stop bleeding from wounds, clean the surfaces of sores, in the treatment of rheumatic pains, bee and scorpion stings and for removal of opacities, conception, suggesting potential contraceptive and anti-fertility properties.

1.3 *Aspilia africana*

The genus consists of 50 species from tropical Africa, Madagascar and Latin America. The plant is commonly referred to as the 'haemorrhage plant' in some of the communities where the

plant is distributed due to its unique potential to stop bleeding. *Aspilia* species range in habit from delicate herbaceous erect and a semi-woody herb from an erect perennial woody root-stock to 25-130cm high occurring on grasslands, woodlands, forest margins and abandoned cultivated zones. Leaves are 4-12cm long and lanceolate. It is very polymorphic with at least four varieties recognized. They produce adventitious roots at the lower nodes or along branches that touch the ground enabling them to spread vegetatively. Also possess numerous bioactive phytochemicals present in it including saponins, tannins, alkaloids, flavonoids, terpenoids and phenol.

1.4 Morphology of *A. africana*

Morphologically, *A. africana* (pers.) C.D Adams is a herb measuring about 1-2 m in height covered with bristles; stem is stiff at the base with many branches and rough to touch; leaves are rough, opposite, ovate-lanceolate, creased accordion-style covered with trichomes, average 10 cm long and 5 cm wide and rounded vascular bundles with parenchymatic sheath composed by large cells (Metcalf and Chalk, 1979) at the base with petioles about 1 cm long with 3 prominent veins ; inflorescence consists of capitula which is terminal, solitary, or in lax racemes with hairy stalk of about 7 cm long on average; flowers have numerous showy-yellow forets; fruits are 4-angled achenes; Pollens are tricolporate, oblate- spheroidal and medium (media); spines with pointed ends while pores are densely situated;mature seeds usually have little endosperm. Its rapid growth characteristics make it a difficult weed in cultivated land and fallows.

1.5 *Spilanthes Costata Benth*

Spilanthes spp. has sub families which is derived from the type of genus *Aster* and examination of a wide range of recently collected material has shown that four distinct species

can be recognized in West Africa, a new combination based on *Eclipta filicaulis* Schumach. & Thonn, originally. It can also be found in a damp area, at swamp margin, on rocks near the sea, or as a road-side weed. It is an annual or short-lived prostrate plant with ascending cylindrical hairy stems and has no flower petals. All parts of the plant are unpleasantly bitter with the flower heads being the most pungent part, causing a tingling sensation and numbness.

1.6 Morphology of *S. costata* Benth

Perennial herb, creeping, decumbent or sometimes straggling and then to 1.2 m high; stems semi-succulent, glabrous to pilose. Leaves ovate, 1–9 cm long, 1–5 cm wide, base rounded to truncate, margins denticulate, apex acute, 3-veined from base, usually scabridulous. Capitula axillary, solitary, on stalks to 6 cm long, ovoid, discoid; involucre 6–12 mm long; phyllaries in 2 unequal series, the outer 6–12 mm long, the inner 4–10 mm long, glabrous or ciliate occasionally pilose. Florets many, white, 2–2.7 mm long. Achenes black, obovoid, 2.7–3.5 mm long, at maturity with cork-like margins, ciliate; pappus of 2(–4) subequal awns 0.9–2.1 mm long. The flower heads are either solitary or occur in compact or spreading inflorescences. Phytochemically, *S. costata* Benth has been reported to contain isobutyl amide derivatives amino acids, and β -amyryn esters, myricyl alcohol including sitosterol glucosides and triterpenoid.

AIM AND OBJECTIVES

- To create full taxonomic criteria, nomenclature revisions, contributions of new data to the knowledge already in existence and most significantly, to update the family *Asteraceae* in Nigeria

- To document and have better taxonomic, field status, epidermal morphology and cytology of the species.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Source of Plant materials

Living plants used were collected from several parts of University Of Benin. Different species of *A.africana* and *Spilanthes costata Benth* was examined in their cultivated habitats. Plant were collected and then planted in the departmental garden and allowed to grow.

2.2 Morphological Characteristics

Morphological Characteristics studied include the leaves, its length and width with the length of its petiole and the height of each species.

2.3 Measurement of the length and width of the leaves and petiole

The length and width of fifty leaves each of the *A.africana* and *Spilanthes costata Benth*. were measured using metre rule and recorded and the mean calculated.

2.4 Height of plants species

The heights of the different species of *A.africana* and *Spilanthes costata Benth* were measured using measuring tape and recorded.

2.5 Technique for obtaining epidermal peels

Epidermal studies were carried out on fresh materials. Sample of fresh materials taken from similar regions of the abaxial and adaxial surface of each leaf species, usually at the middle area of the leave. Epidermal peels were obtained following the method of Gill and Nyawuame (1990).

2.6 Schedule for foliate specimen

Epidermal peel was obtained by peeling off using a pair of forceps, later it was bleached and washed up with tap water. The bleached material was mounted on the slide after washing up with distilled water. A Carmel hairbrush was used to clean the bleached materials until the stomata were visible. Clear epidermal structure was then seen under the microscope using magnification x400.

2.7 Phyto—Dermological studies

Stomata distribution, epidermal cells, nature of stomata abnormalities and general morphology of the mature stomata were observed. The terminologies of matured stomata used are those of Rasmussen (1981) and (1970).

2.8 Stomatal terminology

Type A: Ranunculaceous (Anomocytic) type: Stomata surrounded by a few number of cells that are identical in form and size from and size from the rest epidermal cells.

Type B: Anisocytic (unequalled, Cruciferous) type: Stomata surrounded by three unequalled subsidiary cells of which one is distinctly smaller than the other two.

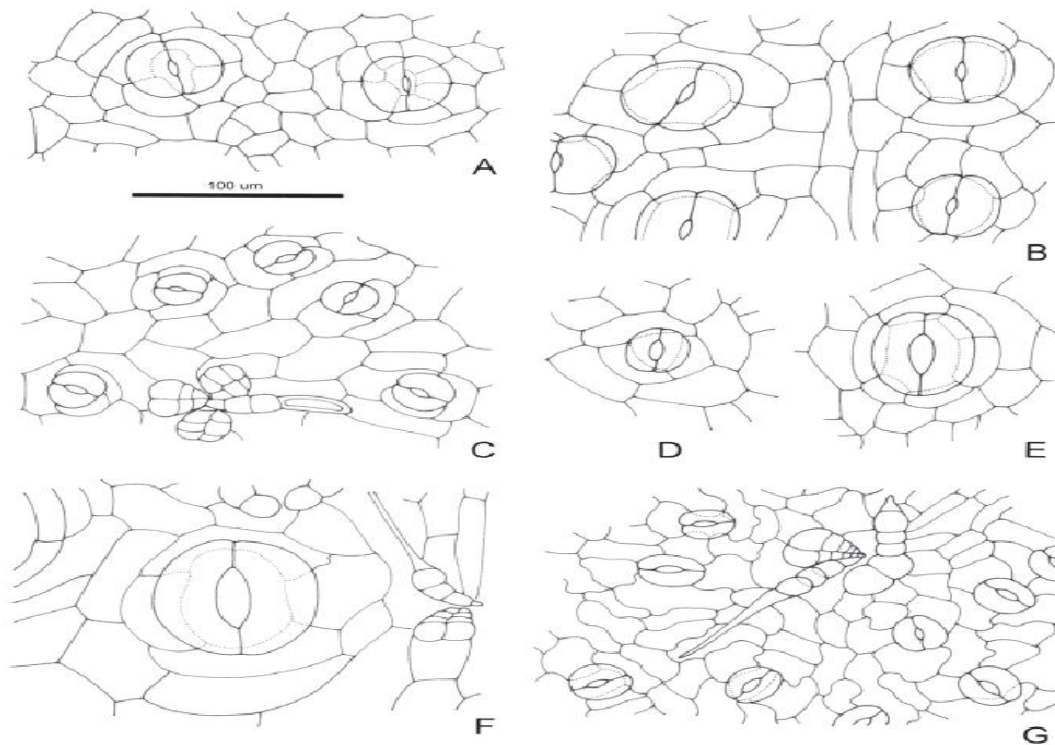
Type C: Dictytic— Paralytic (Parallel cells, Rubaceous) type: Stomata surrounded on either side by one or more subsidiary cells parallel to the long axis of the pore and guard cells.

Type D: Actinocytic (Pericytic) type: Stomata surrounded by a cuticle of radiating subsidiary cells.

Type E: Bicytic—Diacytic (Cross celled, Liabiatus - Caryophyllaceous) type :The stomata are surrounded by a plain of subsidiary cell whose common wall is at right angles to the guard cells.

Type F: Tetracytic (Monocotyledons) type: Here, Stomata are surrounded by four subsidiary cells (2 lateral and polar).It is commonly found among monocotyledon.

Type G: Hexacytic (Monocotyledons) type: Here, stomata are surrounded by six subsidiary cells (four laterals and two polar).



A: Actinocytic

B: Tetracytic

C:Anisocytic

D:Anomocytic

E:Bicytic—paracytic

F:Paracytic

G: Hexacytic

Figure 1: stomata terminology (Rasmussen,1981 and Van Cotthem,1970)

2.9 Cytological techniques:

a. Schedule of meiosis

Young flower buds were fixed in 1:3 acetic acid — alcohol for 12hours and then transferred to 70% alcohol.Staining of the chromosomes was improved by saturating the acetic acid in the fixative with ferric acetate.The anthers were subsequently squashed in 2% aceto—carmine,then debris was removed and a cover slide was placed in between filter papers and little pressure was exerted with the palm,in order to spread the content.Finally the prepared slide was observed through a microscope and the chromosomes number was confirmed by examining at least 10—20 well spread cells.The slide were made permanent by passing through acetic/absolute alcohol series and mounted in "euparal".

b. Schedule for mitosis:

Young root—tips were prefixed in saturated aqueous solution of para—dichlorobenzene for 2 hours at 16°C,then they were washed in distilled water and fixed in 1:3 acetic alcohol for

12hours.The root tips were then hydrolyzed in 1:9 concentrated hydrochloric acid in water for 10 minutes at 60°C. Root tips were then washed with distilled water and dipped in aceto-carmin solution with 1-2 drops of ferric chloride in it.The root tips were again washed and squashed in 2% acetocarmine. The chromosomes number was confirmed by examining at least 20 well-spread cells.

2.10 Schedule for Determining the Percentage of Pollen Fertility and Pollen size

Pollen Fertility was determined from the ability of pollen to stain with 50% glycerol-acetocarmine.The anthers from the open flower buds were squashed in a drop of glycerol-acetocarmine and the slides were left at 60°C for 24 hours. Grains which were filled with strained protoplasm were considered to be fertile while the small shriveled and unstained or weakly stained were considered as sterile.The grain were examined under the microscope counted,measured and finally pollen fertility was calculated.

2.11 Voucher specimens

Voucher specimens and seed specimens described during the present study and tested for ergastic substance are deposited in the herbarium of the Department of Plant Biology and Biotechnology,University of Benin,Benin—city,Nigeria.

2.12 Photographs

Different photographs were taken of the species gotten.

2.12.1 Light Microscopy

Photomicrograph of various samples of fresh materials were taken under the swift collegiate light microscope using $\times 10$ eye piece gratitude and $\times 40$ objective lens ($\times 400$) for uniformity were taken for each taxa. Camera Lucida drawings were made for each of the species studied.

CHAPTER THREE

RESULTS

3.1 Morphological results

Twelve plant taxa belonging to two different species, *Aspilia Africana* and *Spilanthes costata* *Benth* which are morphological alike were collected and identified.

Aspilia africana

- Habit: They are mainly semi -woody perennial herbs.
- Height: It has height of 3-5m
- Leaf length : It has length of 2.5 -18cm
- Leaf breadth: It has a breadth of 1.5-12cm.
- It is a toothed simple leaf
- It has an alternate phyllotaxy
- It has an acuminate leaf apex
- It has a chordate to acute base
- It has a crenate leaf margin
- A glabrous leaf surface



Plate 1: Photograph showing the whole plant of *Aspilia africana*



Plate 2: Photograph showing the leaves of *A. africana*

Epidermal morphology

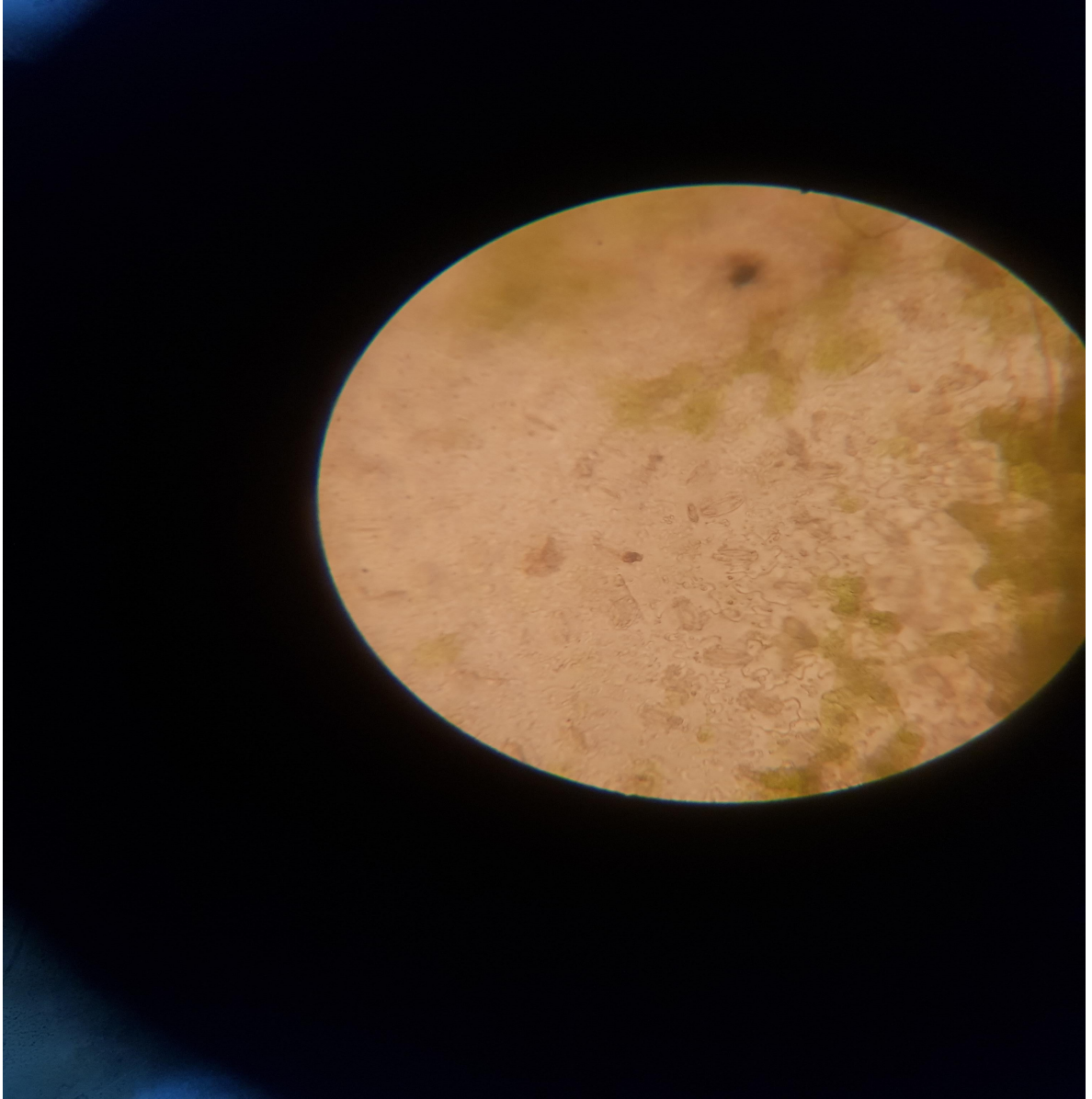


Plate 3: Photomicrograph of the epidermal layer of *A. africana* x 400

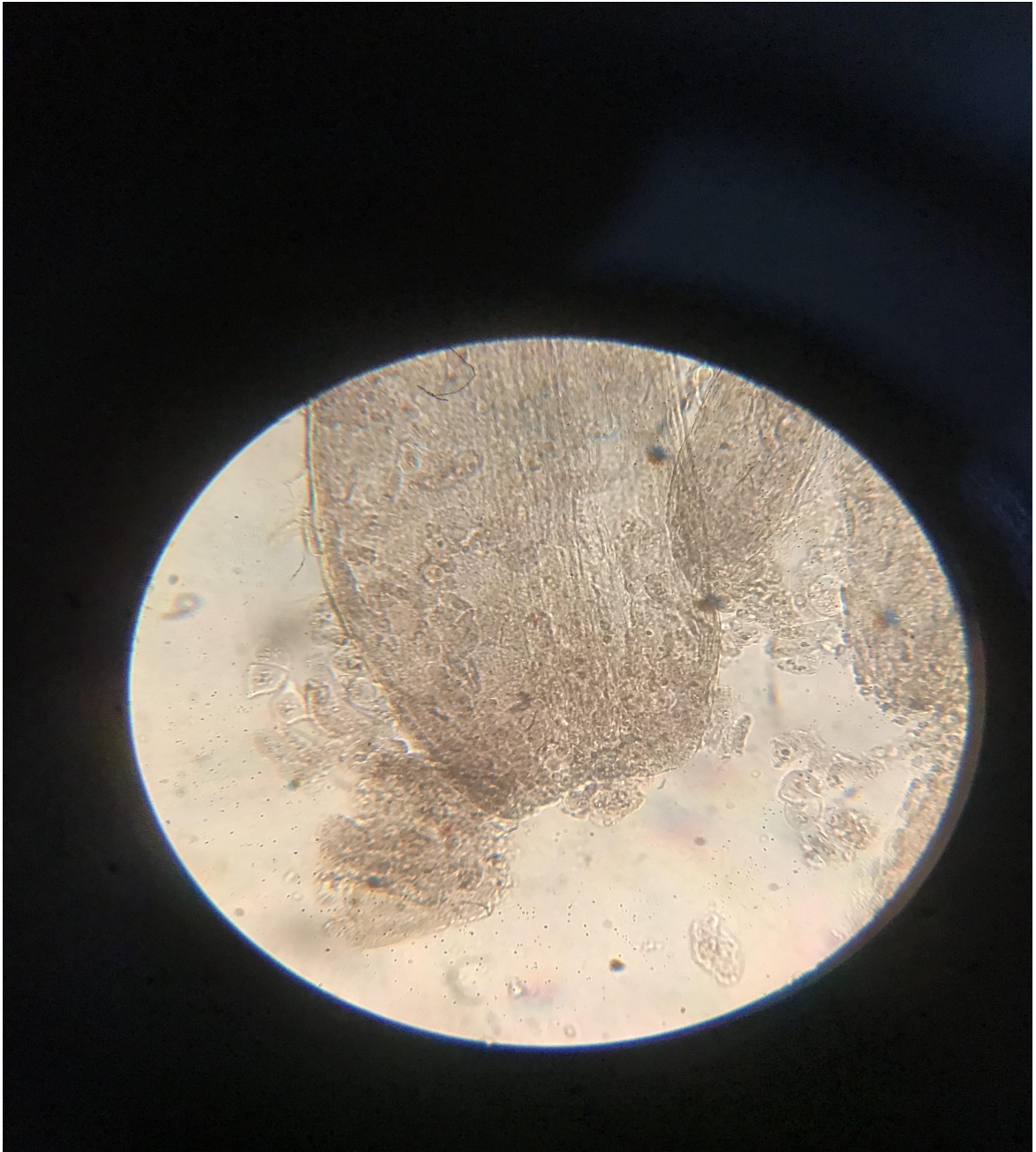


Plate 4: Photomicrograph of the Trichome of *A. africana* x 400

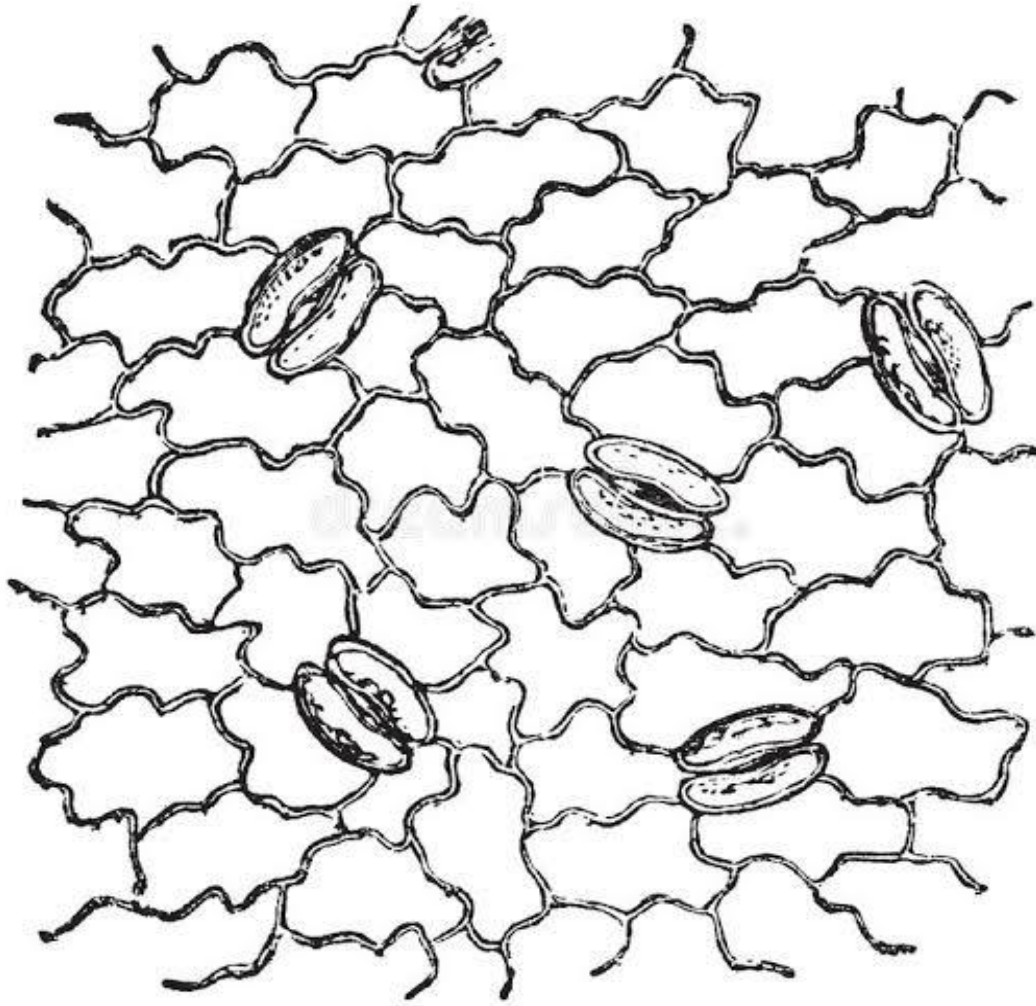


Plate 5: Stomata photomicrograph of *A. africana*

Spilanthes costata Benth

- Habit: Erect perennial herb
- Height: It has a height of 1-5m
- Leaf length: It has a leaf length of 1-9cm
- Leaf breadth: it has a leaf breadth of 1-5cm
- It has a simple leaf
- It has an alternate phyllotaxy
- It has a ovate leaf shape
- It has an acute leaf apex
- A glabrous leaf Surface



Plate 6: Photograph showing the whole plant of *Spilanthes costata* Benth



Plate 7: Photograph showing the leaves of *Spilanthes costata* Benth

3.2 Cytological result

Chromosome number

After repeated strenuous effort I was unable to determine the chromosome number of any of the species and epidermal layer of the leaves, trichome of the *S. costata* was very difficult to be seen when viewed under microscope.

From both mitotic and meiotic studies, the chromosome number of both varieties proved very difficult with no flowering or pollen of the plant.

Cytology of this species is reported for the first time. Chromosome report so far available indicate that all the known species have their chromosome number in multiples of 8 and all of them are polyploids.

CHAPTER FOUR

DISCUSSION

Due to their diagnostic usefulness in the assessment of taxonomic relationships, morphological traits are the traditional tool of the taxonomist. According to Baldauf (1965), some physical traits are the consequence of mutations, while others come about as a result of a living thing's long evolution and series of environmental adaptations.

4.1 Pollen Grains Size and Pollen Fertility

The use of pollen characters as a reliable indicator of ploidy level in plants species has been stressed by many workers (Stabbins,1950 and Baquar *et al.*,1970). The result from the pollen grain size and pollen Fertility revealed that they were stable exhibiting regular ambivalent pairing.

4.2 Epidermal Morphology

Matcalfe & Chalk noted the significance of stomatal kinds as a taxonomic characteristic (1950). Since then, the taxonomic significance of stomatal types and their phylogeny in two(2) angiospermic plants have received attention. The family Asteraceae has a lot of anomocytic stomata. Stomata size may vary on the same leaf, according to Stace (1965), but this does not exclude its use as a taxonomic trait to distinguish between distinct species within a genus. A review of the literature reveals that studies on the phytodermology of Nigerian Astereceae leaves have been conducted. The works of (Isawumi *et al.*1989) are among these masterpieces.

4.3 EPIDERMAL WALL PATTERN

The species studied had a epidermal cells on both surface and they were seriously walled.

4.4 LIMITATIONS OF THE STUDY

Chromosome Number

Chromosome number has been widely used in systematic investigations of the plant Kingdom. The information on the chromosome number when combined with other taxonomic data tends to provide a better understanding of the tribal and generic relationship with plant Kingdom (Jensen et al.,1980).

The chromosome number of both species could not be confirmed because of the low production of pollen grains from their buds which were too old or matured led to spores been seen or too young which resulted into clumping. *Spilanthes costata* plants was not flowering, therefore the Epidermal layers was unable to be determined.

CONCLUSION

The results of the investigation demonstrate that both species are distinct and have leaves with amphistomatic stomata. To fully understand the cytological and morphological distinctions between the investigated species, more research is advised.

REFERENCE

- Aboul Ela, M. A; (1984). “A Thesis of Doctor of Philosophy degree in Pharmaceutical sciences”; Faculty of Pharmacy, Alexandria University, Alexandria, Egypt. [Online] Available at:https://cdn.intechopen.com/pdfs/32953/InTech-General_introduction_on_family_asteracea.pdf [Accessed : 18/12/2022]
- Agbor, B. Ekpo, A, and B. E. and Ekanem E.B. (2021). “Antimicrobial Properties and Nutritional Composition of *Aspilia africana* C.D.Adams,” *International Journal of Applied Science and Technology*. **2:(7)**, 20 - 12.
- Barkley, T. M., Brouillet, L., Strother, J. L. (2006). Asteraceae . [Online][Available at:<https://wikideck.com/Asteraceae>] [Accessed: 12/8/2022]

- Gupta, G. C., Vij, S. P. and Grag, A.K., (1976). In IOPB chromosome number report LIV.
Taxon **25**: 631-649.
- Gupta, R. C. and Gill, B. S. (1983). Cytology of family Compositae of the Punjab plains India.
Proceedings of the Indian National Science Academy, Part B Biological Sciences **49(3)**:
356-366.
- Harborne, J. B.; Turner, B.L. (1984) "Plant Chemosystematics", Academic Press, London,
volume **21(2)**: 255-265
- Gill L.S., Nyawuame H. G. K.(1990) Phylogenetic and systematic value of stomata in
Bicarpellatae (BENTHAM et HOOKER sensu stricto)*Feddes Repertorium* volume **10(9)**:
289-295
- Jansen, R. K. (1981). Systematics of *Spilanthes* (Compositae: Heliantheae). *Systematic Botany*,
231-257.
- Lam, C.H., Lim J.K. and Jantan, B. (1993). Comparative studies of a parquat mixture and
glyphosate and/or its mixtures on the weed succession on plantation crops. *Planter* **69**:
525 - 535.
- Olorode, O. (1974). Chromosome numbers in Nigerian Compositae. *Botanical Journal of the
Linnean Society*, **68(4)**, 329 - 335.
- Omoigui, I. D. (1985). *Cytomorphological studies of some Asteraceae from Southern Nigeria*
(Doctoral dissertation, M. Sc. Thesis, University of Benin. 163p.

- Pataky, k. (1979). Leaf epidermis of *Salix* In: C.R. Metcalfe and I. Chalk (eds). *Anatomy of the dicotyledons*, ed. 2 Oxford Clarendon press, 10 -120pp.
- Paulraj, J., Govindarajan, R., & Palpu, P. (2013). The genus *Spilanthes* ethnopharmacology, phytochemistry, and pharmacological properties: a review. *Advances in pharmacological sciences*, **2**:367 – 400.
- Porter, C. L. (1969) “Taxonomy of flowering plants”, Eurasia Publishing House (Pvt.) Ltd., Ram Nagar, New Delhi, India, 410p.
- Ramsewak, R. S., Erickson, A. J., & Nair, M. G. (1999). Bioactive N-isobutylamides from the flower buds of *Spilanthes acmella*. *Phytochemistry*, **51(6)**, 729-732.
- Rasmussen, H. (1981). Terminology and classification of stomata and stomatal development—a critical survey. *Botanical Journal of the Linnean Society*, **83(3)**: 199 - 212.
- Robinson, H, Raven, R. H., King, R. M. D., Khyos, D. W., Powell, A. M. (1976). Chromosomes numbers in compositae XIII eupatorieae. *Annual Missouri Botanical Garden* **63**: 962 - 988.
- Stace, C.A. (1965) Cuticular Studies as an Aid to Plant Taxonomy. *The Bulletin of the British Museum (National History)*, **4**: 37-40.
- Stebbins, G.L and Khush, G.S. (1961). Variation in the organization of the stomatal complex in the leaf epidermis of the Monocotyledons and its bearing on the phylogeny. *American Journal of Botany* **48**: 51-59.

Vancotthem, W. R. J. (1970). A classification of stomata types. *Botanical Journal Linnaea society*. **63**: 235-246.

Wana, D., & Beierkuhnlein, C. (2011). Responses of plant functional types to environmental gradients in the south-west Ethiopian highlands. *Journal of Tropical Ecology*, **27(3)**: 289-304.

Wilkinson, H.P. (1979). Plant Surface. **In**: Metcalf and Chalk (Eds.). *Anatomy of the Dicotyledon*,. Clarendon Press, Oxford, p: 97-162.