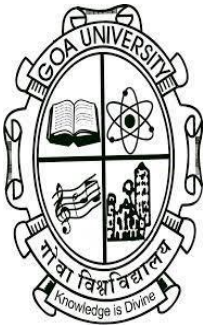
ANATOMICAL, HISTOCHEMICAL AND PHYTOCHEMICAL STUDIES OF FOUR MEDICINAL PLANTS

Dissertation submitted to the Goa University
in partial fulfillment for requirement of

THE DEGREE OF MASTER IN SCIENCE IN BOTANY

By
MISS GUNJAN GURUDAS KERKAR

Under the guidance of
PROF. S. KRISHNAN, M.Phil., Ph.D.



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CERTIFICATE

This is to certify that this dissertation is a bonafide and an authentic record of this research entitled **"Anatomical, Histochemical and Phytochemical Studies of Four Medicinal Plants"** carried out by **Gunjan Gurudas Kerkar**, student of Department of Botany, Goa University. This work is carried out under my supervision and guidance at the Department of Botany, Goa University, Taleigao Plateau, Goa, in partial fulfillment for the requirement for the award of 'MASTER OF SCIENCE IN BOTANY' degree of the University and that no part, therefore has been presented before in any other degree or diploma of any University.

Date: Signature of Guide

(Prof. S. Krishnan)

DECLARATION

I hereby declare that this dissertation entitled **"Anatomical, Histochemical and Phytochemical Studies of Four Medicinal Plants"** submitted for the degree of **Master of Science in Botany** to Goa University is carried out by me under the supervision of **Prof. S. Krishnan**, Department of Botany, Goa University. The work is authentic and had not been submitted in any part or whole by me for any other degree or diploma in any other University.

Date: Signature of the student

(Ms. Gunjan Gurudas Kerkar)

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CONTENTS

INTRODUCTION

A medicinal plant is a plant that contains different substances in its organs that can be used in the treatment of various diseases or which act as precursors for the production of valuable drugs. Since ancient times, these have been used in traditional medicine to cure various health problems (Hao 2019; Kumar *et al*., 2009). These medicinal plants integrate and accumulate secondary metabolites like alkaloids, flavonoids, glycosides, saponins, sterols, terpenes, quinines, tannins, etc. In many developed countries, traditional medicine is still the main component of healthcare, and most of the drugs are the product of plants. Since many people are switching to herbal remedies, the use of medicinal plants for extraction has increased, which is why these plants are on the verge of extinction. As estimated by World Health Organization (WHO), 80% of the world's population is partially dependent on herbal medicines for treating minor ailments (Motaleb 2011).

Generally, medicinal plants are classified based on active principles in their storage organs like roots, leaves, flowers, seeds, etc. These principles are precious to humankind in treating various ailments (Mani 2016). Around the world, there are many medicinal plants whose medicinal potential has not been studied so far, and their medical activities could be pivotal in the treatment of innumerable diseases.

Importance of Medicinal plants

In the olden times, our ancestors used plants to flavor food, lessen the pain, treat headaches, and even prevent diseases, including epidemics. The knowledge of their curative properties has been delivered over the centuries among the human communities. The active

substances produced in plants during the secondary metabolism are primarily responsible for the induction of medicinal properties in the plants used throughout the world for different purposes, including contagious diseases (Singh 2015). The term "Medicinal Plant" comprises the plants employed in herbalism. Herbalism utilizes plants for medicinal purposes (Shah 2021). Medicinal plants are used as a vital source for extracting important bioactive compounds due to their role in curative and preventive medical therapy preparations for human beings (Lambert *et al*., 1997; Thirumalai *et al*., 2009; Rasool 2012). Almost 80% of the world's total population is dependent on traditional medicine for its healthcare needs, and many people from developing regions take a combination of conventional medicine and traditional medicine (Musila 2000; Mahwasane 2000; Kinyanjui 2014).

Medicinal plants worldwide

Medicinal plants and medicines obtained from plants are extensively used worldwide and are progressively becoming popular in modern society as alternatives to man-made chemical drugs (Wyk and Wink 2017). Medicinal plants have immense importance around the world. They have been consumed for nutrition and treating illnesses, such as anticancer new drugs (Dehkordy *et al.*, 2017).

Medicinal plants in India

Several undeveloped countries and developed countries use plant-based medicines to maintain human welfare and personal health conditions and treat specific ailments such as cough. Some of these plants may include Ginger, Garlic, Ginkgo, Gingseng, and others (Rakotoarivelo *et al*., 2015). Amidst the ancient civilizations, India is notably a rich repository of medicinal

plants. The forest in India is the capital repository of many medicinal and aromatic plants gathered mainly as raw materials for the production of therapeutic drugs and perfumes. Nearly 8000 herbal remedies have been categorized in AYUSH systems in India. Ayurveda, Unani, Siddha, and Folk medicines are the crucial systems of indigenous medicines. Ayurveda and Unani are most evolved and extensively practiced in India (Zahid 2016).

Weeds as Medicinal Plants

Weeds can also be utilized as medicinal plants and have a lot of scopes; many plants can be brought under cultivation (Soi 2022). Weeds enormously reduce agricultural productivity by competing with the crop plants for resources like water, mineral nutrients, space, and light and thus are hazardous for the cultivated crops like vegetables. It is necessary to eradicate these weeds to get maximum yield from the crop plants. As per the earlier studies on weeds, it is shown that there is no substitute for a chemical treatment to control the growth of these weeds, but the rising use of chemicals in weed control affects the nutritive values of crops. Hence, it is proposed to utilize these weeds instead of destroying them (Sahu 1984).

Some of the prominent weeds are used widely in traditional medicines because certain phytochemicals like alkaloids, flavonoids, phenols, terpenes, saponins, etc., are used to treat various health disorders. These weeds are based on the treatment in olden times and experiences from past generations (Rizki *et al*., 2019).

Some Medicinal Plants from Goa

***Lantana camara* L.**

Lantana camara, commonly known as Lantana, wild sage, or shrub verbena, is an erect, frost-tender shrub native to Central and South America. It belongs to the family Verbenaceae. The stem is quadrilateral in outline and covered with bristly hairs when green. Having a strong root system, the roots give a new flush of shoots even after repeated cuttings (Prianka and Joshi 2013). The leaves are bright green and rough with fine hairs and serrate margins and emit a pungent odor when crushed. Nevertheless, *Lantana camara* escaped the custody of human cultivation and took the form of weed by spreading rapidly and occupying almost every type of land, viz., forests, grasslands, agricultural lands, and even the wastelands (Sabu and Kuttan 2000).

Lantana camara is a notable medicinal plant in the traditional therapeutic system, and recent scientific research has highlighted the possible use of *Lantana camara* in modern medicine (Rajkumar 2009). The leaves are used to relieve itching. Sometimes, the Lantana oil is used to treat skin itches as an antiseptic for wounds and is externally applied to treat leprosy and scabies (Adama *et al*., 2009). The roots are known to treat gonorrhea. The plant extracts are used to treat cancers, chickenpox, measles, ulcers, eczema, tumors, and high blood pressure (Dwivedi and Kumar 2018).

***Solanum anguivi* Lam.**

Solanum anguivi is a rare medicinal herb belonging to the family Solanaceae. The plant is erect, highly-branched, prickly undershrubs with stout prickles having a broad base. The leaves are sinuately lobed, having prickles on the mid-nerve. Simple hairs are present on the

leaves mixed with stellate ones. The flowers exist in extra-axillary, racemose cymes with purple corolla clothed outside with purple stellate hairs. The berries are globose, glabrous, and yellow, having flat and minutely pitted seeds (Yadav and Sardesai 2002). The plant is used as a curative for various diseases. The roots are carminative and expectorant, valuable for coughs, dysuria, colic, nasal ulcers, asthma, toothache, cardiac disorder, worm complaints, spinal guard disorder, nervous disorder, and fever. In the case of skin itches, the fruits and leaves are rubbed with sugar and are applied externally (Johnson *et al.,* 2010). Being rich in essential minerals and vitamins, the plant is recommended as a dietary staple or supplement for nursing mothers, young ones, the aged, and even the anemic patients (Elekofehinti *et al*., 2020; Denton and Nwangaburuka, 2011).

***Tabarnaemontana alternifolia* L.**

Tabarnaemontana alternifolia L. is a small shrub belonging to Apocynaceae, known as *Kundalam Paalai* in Tamil. It possesses antimicrobial and antihelminthic properties against skin, venereal, respiratory, and nervous disorders (Ignacimuthu *et al*., 2006). The decoction of the stem bark has been used for cleaning cuts and wounds. A mixture of leaf and stem powder and the stem bark of *Ficus racemosa* Linn., *Ficus benghalensis* Linn. and *Madhuca longifolia* (Koenig.) heated with coconut oil can be applied externally as a remedy for skin diseases (Ignacimuthu *et al*., 2005). Earlier reports on this plant have shown the presence of alkaloids like 15-β-stemmadenine, tabernoxidine, coronaridine, voacangine, and ibogaine (Roy *et al*., 2002). The presently available scientific documentation reveals the presence of tannins in the bark of *T. heyneana,* which act as defensive compounds and thereby providing the plant with a broad habitat tolerance. Recently, a study has reported the presence of prominent natural polyphenolics

like rutin and quercetin-related compounds and a few unknown phenolic acids from the ethanolic leaf extracts of *T. alternifolia* (Sathishkumar *et al*., 2008).

***Rauwolfia verticillata* (Lour.) Baill.**

Rauwolfia verticillata, formerly known as *Rauwolfia densiflora*, is a large evergreen shrub up to 9m high with milky juice, obovate leaves, and red or white flowers obliquely ellipsoid, brownish or purple drupes belonging to the family Apocynaceae. Commonly, it is known as Jeyasembagam in Tamil, Kattamalpori in Malayalam, dieng larkei, dieng latyrking, dieng latyrkai, dieng sohbu blang in Assam, and Dense-flowered snakeroot in English. It is primarily found in Dajipur, Hassane, Patgaon, Tambyachiwadi, Khandala, Mahabaleshwar, Matheran, Lonavala, Amboli (Maharashtra), the Himalayas, Khasi and Aka hills, and the Western and the Eastern Ghats. *Rauwolfia verticillata* is an economically significant medicinal plant because of various indole alkaloids (Yadav and Sardesai 2002; Almeida 1996; Alfred 1992; Manjunatha and Krishna 2004; Vadakkemuriyil *et al*., 2012).

Ethnobotanical Uses of *Rauwolfia verticillata*

The Ethnobotanical study on *Rauwolfia verticillata* manifested that the plant exhibits various significant activities and is primarily used by South Indian Tribes. The fresh leaves of the plant and the leaf galls are eaten by the *Malapandaram* tribe of the Idukki district of Kerala to expulse the placenta and facilitate childbirth. In other instances, the crushed bark of the plant is mixed with coconut and is ingested to induce sterility and as a contraceptive. The ground green fruit mixed with coconut oil is applied to overboils, carbuncles, cuts, and sores. Ulladan tribe of Waynadu district of Kerala has been using the unripe fruit to remedy ringworm and psoriasis. In

addition, they have been taking the sap from a green fruit as a medicine for asthma and shortness of breath. The grounded leaves are taken with salt for coughs, and the juice extracted from the rhizome is used for diabetes, coughs, colds, peptic ulcers, stomachache, and mouth infections. The local inhabitants from Wayanadu have used the leaf juice in water for treating fever, and root bark is squeezed in saltwater and is ingested as a remedy for hypertension.

Kaani tribe of Wayanadu district of Kerala has used the sap squeezed from the bark to treat ringworm. The leaf sap mixed with water is drunk to treat hypotension or anemia, various plant parts are utilized to treat cough, tuberculosis, and stings of the stingray, the stalk of the plant can either be chewed, or the healer himself chews the young leaves and spits them into the patients face to treat headache.

The Kurumba tribe of the Wayanadu district of Kerala has been treating earache by applying leaf juice or latex to the ear. Alternatively, the healer spits into the infected ear using the warmed stalk like a straw. In the cases of Snakebites, beetle bites, and scorpion bites, the leaf paste is applied topically. Also, the powdered leaf is mixed with cow or goat's milk and has been orally taken to treat diabetes and remedy snake bites and hypertension (Iqbal *et al*., 2013).

Anatomical Studies

Plant anatomy is the study related to the cellular structure and tissue of the plant organs. A plant is a complex structure comprising several parts that constitute the whole plant. The plant

anatomy study helps in understanding how the plant works as a whole, which can be of great importance to aromatherapists who need to be aware of the part of the plant from which essential oil is derived as there is often a connection between the location of oils in the plant and its therapeutic action. Sanghvi *et al*. (2011) anatomically characterized *Solanum pseudocapsicum* L. by uniseriate trichomes, anomocytic stomata, calcium oxalate needles in leaves, and the presence of starch grains, angular vessels, uni-biseriate rays and intercalary phloem with differentiation of internal cambium and protoxylem in the stem. Inter-xylary secondary phloem was composed of sieve tube elements, companion cells, and axial parenchyma cells.

Histochemical Studies

Histochemistry is the technique used for the visualization of biological structures. It involves identifying and distributing various chemical compounds of tissues by using stains and indicators. Histochemical methods are used to identify a specific compound, density of its accumulation, and its distribution in biological cells and tissues in different organs under the microscope. These involve the preparation of fixed, stained specimens and their examination under the microscope. Active cell constituents such as starch, proteins, lipids, nucleic acids, and a range of ionic elements are successfully detected and localized in the cells (Badria and Aboelmaaty 2019). Naidoo *et al.* (2019) conducted a histochemical analysis and fluorescence microscopy on fresh leaf material of *Tabarnaemontana ventricosa* using Sudan IV for lipids, Ferric trichloride for Phenolics, Ruthenium Red for mucilage, Nile Blue for neutral and acidic lipids, Mercuric Bromophenol Blue for proteins, NADI reagent for essential oils and Wagners's reagent for alkaloids. They detected intense staining of significant compounds such as alkaloids, phenolics, acidic lipids, and essential oils in the laticifers and latex.

Antioxidant Assay

Antioxidants have a protective role in food and pharmaceutical products against oxidative deterioration and oxidative stress-mediated pathological processes. The screening of plants' antioxidant properties requires specific methods employed in evaluating the antioxidant activity of samples at various research levels (Gulcin 2020). Living organisms are complex systems in which plenty of enzymatic reactions require oxygen. Hence, molecular oxygen (O_2) is a central component of metabolism. However, it may also be present as ROS (Reactive Oxygen Species) such as superoxide ($0_2^{\bullet-}$), Hydrogen peroxide (H_2O_2), and the hydroxyl radical (•OH). These molecules can cause cell damage (Wickens 2001). To counterpoise such damages, organisms have developed various antioxidant defense systems. However, the efficiency of these protective systems decreases with age, and the accumulation of these Reactive Oxygen Species can cause the development of various diseases (Harman 1992; Huang 2016; Ullah *et al*., 2016).

The antioxidant activity can be screened by different methods, viz. 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical ($ABTS^+$) scavenging, 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging, Fe^{3+}-Fe^{2+} transformation assay, ferric reducing antioxidant power (FRAP) assay, cupric ions (Cu^{2+}) reducing power assay (Cuprac), Folin Ciocalteu reducing capacity (FCR assay), peroxyl radical (ROO•), superoxide radical anion (O_2-). Hydrogen peroxide (H_2O_2) scavenging assay, hydroxyl radical (OH•) scavenging assay, singlet oxygen (O_2) quenching assay, nitric oxide radical (NO•) scavenging assay, and chemiluminescence assay. Among these, the DPPH• based is probably the most popular due to its simplicity, speed, and low cost (Alam *et al*., 2013). DPPH• (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical

which can be reduced by transferring hydrogen from another compound. Antioxidants can react with DPPH• by proving an electron or hydrogen atom, thus reducing it to 2,2-diphenyl-1-hydrazine (DPPH-H), characterized by the pale-yellow color that could be easily monitored with a spectrophotometer (Njoya 2021).

OBJECTIVES OF THE PRESENT INVESTIGATION

1. Collection of plant samples from different regions of Goa and adjoining places.
2. To study the anatomical characteristics of Stem, Leaf, and Petiole of selected medicinal plants, *viz., Rauwolfia verticillata*, *Solanum anguivi*, *Lantana camara*, *Taebarnamontana heyneana*, using general staining techniques.
3. To study the histochemical localization of the selected plant species using different stains.
4. To analyze the phytochemical profile of the chosen plant species by preliminary phytochemical analysis and antioxidant assay.

REVIEW OF LITERATURE

The relevant literature on the present study has been briefly reviewed to understand the different parameters of the study done on the mentioned objectives. Hernandez *et al*. (2015) determined the *in-situ* localization of Acetogenins (ACGs) in the endosperm of *Annona macroprophyllata* seeds using Kedde's reagent. In addition, they also analyzed the co-localization of ACGs with other storage molecules. They observed positive reactions with Kedde's Reagent in fresh, unfixed sections that were preserved in water, and staining was found only in the giant cells (the idioblasts) at the periphery of the endosperm. Lugol's iodine, which is similar in chemical composition to Wagner's reagent, caused a golden-brown reaction product in the cytoplasm of the idioblasts, which may indicate the presence of alkaloids. The results concluded that Kedde's reagent is an appropriate histochemical stain for detecting ACGs *in situ* in idioblasts and that idioblasts store ACGs and probably alkaloids.

Bunkar (2017) screened the ethanolic extract of powder root of *Rauwolfia serpentina* to observe the effects of the extracted phytochemicals on combating the oxidation stresses and the free radicals. They carried out the antibacterial activity by analyzing the action of the ethanolic extract against the bacterial infections caused by two-gram (+ve) and three-gram (-ve) bacteria. Out of 5 bacteria tested, only three bacteria were found susceptible, including *Klebsiella pneumonia>Staphylococcus (local)>Bacillus subtilis*. Hence this study shows that the bacterial infections appear to be treatable using ethanolic extract of *Rauwolfia serpentina.*

Patil *et al*. (2019) carried out the morphological and phytochemical investigation of *Solanum xanthocarpum*, commonly known as Kantakari, a valuable medicinal plant in

Ayurvedic medicines. The fresh plant was collected from Aurangabad, Maharashtra (India), in November 2018. The investigation was done using UV Spectroscopy. The Phytochemical analysis through UV Spectroscopy showed the presence of alkaloids and flavonoids. Fruits of these plants showed the presence of phenols or tannic acid, terpenes, saponins, proteins, and alkaloids.

Matias *et al.* (2015) performed the structural analysis and histochemical tests to locate and identify the classes of chemical compounds in the *Solanum* species in the Cerrado (Brazilian savanna). The root, stem barks, leaves, and pericarp samples of *Solanum agrarium* Sendtn., *S. lycocarpum* A., *S. palinacanthum* Dunal, *S. paniculatum* L., and *S. stipulaceum* Roem. &Schult was processed according to standard light microscopy techniques. They identified a low concentration of crystals in the root and stem, terpene resin in the root, and an absence of hypodermis in the leaf. In the case of *S. lycocarpum*, *it showed bright spots,* the group of sclereids in the root. The exocarp had a high concentration of crystal sand in the root and stem, oval-shaped limb, and isolated crystals. In *S. palinacanthum,* strong sclerification and rays were seen with great height in root and stem. *S. paniculatum* showed the accumulation of soluble protein in the root and stem and the presence of conspicuous membranous stipules. The distinctive features identified were low concentration of crystal sand in the root and stem, presence of terpene resin in the root, and absence of hypodermis in the leaf, in *S. agrarium*; bright spots (group of sclereids) in the root, isobilateral mesophyll, thickened cell walls with hemicelluloses and strong aroma in the fruit, in *S. lycocarpum*; high concentration of crystal sand in the root and stem, oval-shaped limb, presence of isolated crystals in the exocarp, in *S. palinacanthum*; strong sclerification and rays with great height in the root and stem, in S.

paniculatum; and accumulation of soluble protein in the root and stem, presence of conspicuous membraneous stipules, absence of spiniform trichomes, in *S. stipulaceum*. This study identified distinctive structural features and their ecological importance and determined the distribution of secondary compounds associated with the medicinal properties.

Udayakumar *et al.* (2016) examined the antibacterial variety of various parts (stem, leaf, and fruits) of different solvent extracts (Petroleum Ether, Alcohol, and Acetone) of *Solanum xanthocarpum* against *Escherichia coli, Klebsiella,* and *Bacillus cereus.* In this study, *Solanum xanthocarpum* showed high sensitivity to *Escherichia coli* and less sensitivity and resistance to *Bacillus cereus*.

Hussain *et al*. (2015) carried out the phytochemical analysis of methanolic root extracts of *R. serpentina* to test the presence of different phytoconstituents like alkaloids, flavonoids, sterols, phenols, and so on. The extraction was performed with n-hexane and methanol. The quantitative determination of phytochemical constituents of *Rauwolfia serpentina* determined the presence of 2% fat, 12.4% alkaloids, and 7.35% saponins in the plant sample. The qualitative analysis of root extracts from *R. serpentina* revealed the presence of alkaloids, saponins, tannins, steroids, flavonoids, and phenols.

Hong *et al*. (2013) described a high-performance liquid chromatography assay using ultraviolet detection to measure five bioactive indole alkaloids: Sarpagine, Yohimbine, Ajmaline, Ajmalicine, and Reserpine in Rauwolfia. Their detection was conducted at 280 nm. They identified and assayed more than 39 compounds by qualitative analysis. The results showed that

the combination of qualitative and quantitative analysis provided an efficient way to evaluate the quality of *Rauwolfia verticillata*.

Preet and Gupta (2018) detected triterpenoids and phytosterols in different plant parts, viz., fruit, stem, leaf, and root of *Solanum xathocarpum* Schrad. and Wendl. Such a type of analysis was done for the first time. The results showed that the fruits contained the highest number of phytochemicals. They concluded that the method they followed could be an essential tool to ensure the therapeutic dose in medicinal formulations and standardization.

Pathak and Mia (1965) reported the results of histochemical tests for cytochrome oxidase activity in four species of *Rauwolfia.* The histochemical tests showed high enzymatic activity in the cells that would differentiate as sclereid initials. It was also reported that the parenchymatous ground tissue of the pith and leaf base exhibited very little enzymatic activity of cytochrome oxidase. In the control sections, there was no appearance of characteristic indophenol blue. They also recorded intensified cytochrome oxidase activity in the meristematic tissues of the shoot apex, procambium, axillary buds, and the laticiferous cells of *Rauwolfia.*

Sousa *et al.* (2015) carried out the phytochemical analysis of phenolic compounds and antioxidant activities of ethanolic extracts of *Lantana camara* and *Lantana montevidensis*. They used the Folin-Ciocalteu and aluminum chloride methods to analyze the phenolic compounds. They identified and quantified phenolic compounds such as Quercetin, Rutin, Gallic acid, chlorogenic acid, and caffeic acid by HPLC-DAD. Also, the antioxidant activity of these species was determined using different assay systems. The ethanolic extracts exhibited more antioxidant

activity. These results suggested the potential use of *L. camara* and *L. montevidensis* to treat several diseases due to their ability to act as antioxidants.

Mbuni *et al*. (2020) aimed to find and make a record of plants that were used for medicinal therapy by three communities living in Cherangini Hills. They obtained the ethnobotanical data by interviewing the informants using semi-structured questionnaires and extracting information from journals and books. Overall, they identified 296 plant species from 80 families and 191 genera. The Asteraceae family was the most dominant, representing about 10.7% of the total recorded plant species. Roots meant the most commonly used parts of the plant being 35.9%. The decoction was the most frequently used preparation method (54.9%). The therapeutic use of the compiled plants provided essential information that can aid scientists in conducting additional research dedicated to species conservation.

Abubakar and Haque (2020) carried out a study to evaluate various methods used in the preparation and screening of medicinal plants in research studies. They reported that the techniques involved in producing multiple bioactive fractions and compounds are generally the same, though used for different purposes. The major stages in acquiring quality bioactive molecules are the selection of an appropriate solvent, extraction methods, phytochemical screening procedures, fractionation methods, and identification techniques. Solvents commonly used in the extraction of medicinal plants are polar solvents such as water and alcohols, intermediate polar such as acetone and dichloromethane, and non-polar such as n-hexane, ether, and chloroform. The extraction procedures included maceration, digestion, decoction, infusion, percolation, Soxhlet extraction, superficial extraction, and ultrasound-assisted and microwave-

assisted extractions. Fractionation and purification of phytochemicals were obtained through paper chromatography, thin-layer chromatography, gas chromatography, and high-performance liquid chromatography. The compounds obtained were characterized using mass spectroscopy, infrared spectroscopy, ultraviolet spectroscopy, and nuclear magnetic resonance spectroscopy.

Sathishkumar *et al*. (2012) investigated the in vitro antibacterial and antifungal activities of different leaf extracts of *Tabarnaemontana heyneana* Wall. *Klebsiella pneumonia* and *Salmonella typhi* were inhibited maximally and minimally by the *T. heyneana* leaves, respectively. The maximum and minimum antifungal effect was observed against *Rhizopusmucor* and *Trichodermaviridins* respectively. Methanol was proved to be the best solvent for extracting antimicrobial compounds. Ethanol and aqueous systems were reported to be moderate, whereas chloroform was proved to be a poor solvent in extracting the antimicrobial compounds. They concluded that *T. heyneana* leaves possess significant antibacterial and poor antifungal activities.

Srivastava *et al*. (2001) isolated a new alkaloid designated as ervatine along with seven known alkaloids, viz. tabersonine, coronaridine, heyneanine, voacristine, voacristine hydroxy indolenine, hydroxy ibogamine and coronaridinehydroxyindolenine from the fruit of *Tabarnaemontana heyneana* Wall. Based on the spectral analyses, they did the Characterization and structure elucidation of the compounds. It was found that the ethanolic extract and isolated alkaloids heyneanine and voacristineactedas a contraceptive, thus preventing pregnancy when administered during the preimplantation period in Sprague-Dawley rats.

Sathishkumar *et al.* (2008) carried out a study to investigate the effects of temperature, extraction time, the concentration of ethanol, material ratio, and a number of extractions on the contents of flavonoids present in the leaves of *Tabarnaemontana heyneana* Wall. On this basis, they adopted an L_{16} orthogonal design of the experiment to determine the optimal conditions for the extraction of flavonoids. It was observed that the number of flavonoids extracted reached its maxima when removed at 85°C for 2 hours by using 75% ethanol with a material ratio of 1:5 and four times of extraction. The TLC results showed the presence of rutin, quercetin-related compounds.

Seenu *et al*. (2019) investigated the anatomical description of vegetative parts of *Tabarnaemontana alternifolia* L. belonging to the family Apocynaceae. The samples were collected from mature plants of *T. alternifolia* during February from Nadugani, Gudalur taluk of Nilgiri district, Tamil Nadu, India. The leaves of *T. alternifolia* were found to be hypostomatic with paracytic stomata. The transverse section through the leaves had shown the presence of uniseriate epidermis of thin-walled parenchymatous cells covered with thin cuticles on both the upper and lower surfaces. The hypodermis was comprised of angular collenchyma cells. Mesophyll had shown the presence of silica bodies, and vascular bundles were collateral. The petiole was found to be flattened adaxially and arch-shaped abaxially with a uniseriate epidermis that was covered by a thin cuticle. The hypodermis was reported to be 7-8 layered with parenchymatous angular collenchyma cells consisting of laticifers. They also observed the presence of silica bodies and thick-walled fibers in the cortex. The vascular bundles were collateral and U-shaped. The periderm formation and thick-walled fibers in the vascular tissues revealed secondary growth in stems. Some sclerenchymatous patches had covered the collateral

vascular bundles. In the case of root anatomy, they observed unicellular root hairs on the uniseriate epidermis, 16-18 layered cortex containing silica bodies and indistinct endodermis. The vascular bundles in roots were radially arranged with 14-16 arched xylem. They observed lignin deposition in the root stellar region. The pith was found to be absent.

Karthika and Vijaykumar (2017) aimed to study the medicinal and nutritional characteristics of *Solanum anguivi* L. fruit. They investigated the phytochemical constituents qualitatively, and the results revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, steroids, and glycosides. The fruit was composed of 83% of moisture, and the proximate analysis on a dry basis indicated that the fruit was rich in protein, crude fiber, and ash. The fruit had also shown a certain amount of minerals like calcium, sodium, potassium, phosphorus, zinc, iron, and copper. Hence, they concluded that *Solanum anguivi* L. could be utilized as an herbal medicine for treating anemia and other oxidative stress.

Kirimuhuzya *et al*. (2009) screened the chloroform and methanolic extracts of *Lantana camara* against three strains of *Mycobacterium tuberculosis* viz. H37Rv, TMC-331(rifampicin-resistant), and 28-25271(a non-resistant wild strain) using the agar well diffusion method. The plant samples were collected from Southwestern Uganda. Using the Agar dilution method, they determined the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration). It was reported that the methanolic extract exhibited the highest activity against all the three strains mentioned, with zones of inhibition of 18.0-22.5mm and MIC values of 20 µg/mL for H37Rv and 15 µg/mL for both TMC-331 and wild stain. The MBC value of the methanolic extract of *Lantana camara* was found to be 30 µg/mL for the H37Rv and 20 µg/mL

for both the TMC-331 and wild strain. Hence, based on these results, they concluded that Lantana camara contains active compounds against *Mycobacterium tuberculosis*.

Deshwal and Vig (2012) studied the antibacterial effect of *Rauwolfia serpentina* on *Staphylococcus aureus.* They prepared two extracts with *R. serpentina* viz. aqueous extract and ethanolic extract, and Norfloxacin was taken as the control drug for the study. Agar well diffusion method was employed to detect the antibacterial activity of *Rauwolfia serpentina.* They observed that the ethanolic extract showed more antibacterial activity than the aqueous extract and the control drug against *Staphylococcus aureus.* Maximum inhibition was reported with ethanolic extract (30 mg/mL), which was 32.3% more than Norflaxin (30 mg/mL). Hence, based on these results, they concluded that *Rauwolfia serpentina* is highly effective against *Staphylococcus aureus.*

Gandhiappan and Rengasamy (2012) extracted the metabolites from the leaves of *Solanum anguivi* using Ethyl acetate. They collected the cytotoxic HEpG-2 and MCF-7 cell lines from the National Centre for Cell Sciences (NCCS). The antiproliferative activity of the ethyl acetate extract of the *Solanum anguivi* was screened against the cell lines using MTT (methyl thiazolyl diphenyl-tetrazolium bromide) assay at various concentrations. The cell viability was observed to be reduced up to 50% at the concentration of 0.625 mg/mL for the HEpG2 cell line and 1.25 mg/mL MCF-7 cell line. They have also performed the DNA fragmentation assay to identify the DNA fragmenting pattern prior to and after treatment with the plant extract. The present study revealed that the ethyl acetate extract of *Solanum anguivi* exhibits anticancer activity.

Arage *et al*. (2022) investigated the antimicrobial activity and acute toxicity of 80% methanolic extracts of leaves of *Artemisia absinthium*, seeds of *Datura stramonium*, and fruit of *Solanum anguivi*. The extracts were prepared by cold maceration and used at 125, 250 and 500 mg/mL concentrations to evaluate the antimicrobial activity against five bacterial species using the agar well diffusion method. In this study, they reported that the 80% methanolic extract of the fruit of *Solanum anguivi* showed better activity against most of the bacterial strains, while the seed extract of *Datura stramonium* exhibited the most minor activity against most of the organisms. This study indicated that the methanolic extracts of the mentioned three plants had different degrees of antibacterial activity against the selected bacterial pathogens, thus being of great potential to be used as antibacterial agents.

Karanje *et al*. (2022) evaluated the extraction methods like maceration, ultrasonication, vortex mixer, Soxhlet extraction, and microwave-assisted extraction (MAE) for the extraction of Camptothecin (CPT) from the leaves and stem of *Tabarnaemontana alternifolia, Tabarnaemontana divaricata,* and *Tabarnaemontana citrifolia.* High-Performance Thin Layer Chromatography (HPTLC) was used for analyzing the extracts. The results revealed that the leaves of *Tabarnaemontana alternifolia* exhibited the highest yield of Camptothecin as compared to *Tabarnaemontana divaricata* and *Tabarnaemontana citrifolia*. Also, microwave-assisted extraction (MAE) was found to be the most efficient extraction method, followed by Soxhlet extraction, sonication, maceration, and vortex extraction.

Gandhiappan and Rengasamy (2012) compared the antioxidant activity of the ethyl acetate extract of *Solanum anguivi* Lam. with that of *Solanum pubescens* Willd, *Solanum torvum* Swartz, *Solanum trilobatum* Linn., *Solanum nigrum* Linn. and *Solanum surratense* Burm. F. The antioxidant assay was done using the DPPH radical scavenging method. It was found that the antioxidant activity of the plant extracts decreased in the order: of *S. anguivi* > *S. pubescens* > S. *torvum* > *S. trilobatum* > *S. nigrum* > *S. surratense*. Thus, this study concluded that *Solanum anguivi* could be considered a potential source of natural antioxidants as strong inhibitions of free radicals were caused by the ethyl acetate extract of *Solanum anguivi*.

Al-Hakeim *et al*. (2021) isolated positively charged alkaloids from the methanolic extract of *Lantana camara* leaves using magnetic nanoparticles (MNPs). The fractionization of the crude alkaloid was done using HPLC to separate the highest peak of the alkaloid fraction (HPAF). The crude alkaloids (CA) and HPAF were analyzed for their antiproliferative effect against cell lines (MCF-7, HCT-116, and HeLa) and an endothelial cell line (EA. hy926) as a standard cell line. Rat aortic ring assay was used to examine the antiangiogenic properties. HPAF was exhibiting a profound anticancer effect against MCF-7 and HeLa cell lines, while it showed reasonably mild cytotoxicity against the HCT-116 cell line. The CA also demonstrated a remarkable anticancer effect against MSF-7 and HeLa. It was also reported that the cationic alkaloids showed selective antiproliferative activity against HeLa, while it was safe on the normal test cell line. Hence, they concluded that the use of magnetic nanoparticles (MNPs) to separate precious compounds is convenient and cost-effective.

MATERIALS AND METHODS

The present study describes the dissertation work performed in the Department of Botany, Goa University, during the period from August 2021 to April 2022. The details of the methodology followed in conducting the study are presented in this chapter.

Study Area and Plant Collection

The collection field trips were carried out in the various regions of Goa and Western Ghats of Maharashtra, India. The plant species collected were *Rauwolfia verticillata* **(Plate 1),** *Solanum* anguivi **(Plate 2),** *Lantana camara* **(Plate 3)** and *Tabarnaemontana alternifolia* **(Plate 4)**. The Plant specimens were collected and pressed into herbarium using standard methods described by Maiden (2004) and were identified for correct taxonomic identification of species using the Flora of The Presidency of Bombay by Theodore Cooke, C.I.E. (1901,1902,1903). The taxonomic authentication of the collected samples was performed by Prof. S. Krishnan and Prof. M. K. Janarthanam.

Study of Anatomical characterization

Systematic anatomical characterization of collected plant species was carried out for stem, leaf, and petiole. Thin free-hand sections were taken from the matured stem of the collected plant species with a sharp razor blade for anatomical characterization. All the anatomical characters were studied from the sections of the stem. These freehand sections were stained using 0.1% Safranin or 0.1% Toluidine Blue O and were mounted on a Bright-field Nikon Eclipse E200 microscope. The mounted sections were photographed using Nikon digital compact camera and were analyzed.

Leaf anatomy

Thin free-hand sections were taken from the matured leaf, specifically from the middle of the lamina with the central vein, for anatomical characterization. All the anatomical characters were studied from the sections of the leaf. These freehand sections were stained using 0.1% Safranin and were mounted on a Bright-field Nikon Eclipse E200 microscope. The mounted sections were photographed using Nikon digital compact camera and were analyzed.

Petiole anatomy

Thin free-hand sections were taken from the petiole of the leaf for anatomical characterization. All the anatomical characters were studied from the sections of the petiole. These freehand sections were stained using 0.1% Safranin and were mounted on a Bright-field Nikon Eclipse E200 microscope. The mounted sections were photographed using Nikon digital compact camera and were analyzed.

Histochemical Localization in Plant sections

Histochemical tests were performed on sections obtained from fresh material as described by Krishnamurthy (1998). Fresh stem and leaf tissue were used to take thin free-hand sections and were stained using specific stains for the histochemical localization of primary and secondary metabolites.

1) **Localization of Starch**

Iodine potassium iodide (I_2KI) staining reaction

Preparation of Stain: I_2KI was prepared by dissolving 2g of KI and 0.2 g of Iodine in 100 mL of distilled water.

Protocol: Sections were stained for 1-2 minutes and excess stain was removed with water. The sections were mounted with dilute glycerine and observed under bright-field microscope. Starch granules appears blue to black.

2) **Localization of Lipids**

Sudan IV Method

Preparation of Stain: Sudan IV stain was prepared by dissolving 0.7 g of Sudan IV in 100 mL of 95% Ethanol.

Protocol: Sections were placed in 50% Ethanol. These sections were then stained with filtered solution of Sudan IV for 5-20 minutes and washed with 50% Ethanol for few seconds. Sections were then mounted with dilute glycerine and observed under bright-field microscope. Lipids stains red.

3) **Localization of Alkaloids**

a. **Dragendorff's Test**

Preparation of reagent: The stock solution was prepared by boiling 5.2 g of Bismuth carbonate and 4 g of Sodium iodide with 50 mL of glacial acetic acid for few minutes. After 12 hours, the precipitated sodium acetate crystals were filtered off using sintered glass funnel. The clear, red-brown filtrate was mixed with 160 mL of Ethyl acetate and 1 mL of water and stored in an amber-coloured bottle. The working solution was prepared

by mixing 10 mL of Stock solution with 20 mL of acetic acid and made up to 100 mL with distilled water.

Protocol: Sections were placed in Dragendorff's Reagent for 10-20 minutes. These were then cleared in 5% Sodium nitrite solution for 1 minute and then mounted with dilute glycerine. The yellow color in the section indicates the presence of Alkaloids.

b. Mayer's Test

Preparation of Stain: Mayer's Reagent was prepared by dissolving 1.358 g of Mercuric chloride in 60 mL of distilled water 5.0 g of Potassium iodide in 10 mL of distilled water. The two solutions were then mixed and made up to 100 mL with distilled water.

Protocol: Sections were placed in Mayer's Reagent for 10-20 minutes and washed in distilled water. These sections were then mounted with glycerine and observed under the Bright-field microscope. The Grey colour in the section reveals the presence of alkaloids.

c. Wagner's Test

Preparation of Stain: Wagner's Reagent was prepared by dissolving 1.27 g of Iodine and 2g of Potassium iodine in 5 mL of distilled water and made up to 100 mL with distilled water.

Protocol: Sections were placed in Wagner's Reagent for 10 minutes and washed in distilled water for 30 seconds. These sections were then mounted with dilute glycerine and observed under a Bright-field microscope. The brown color in the section indicates the presence of Alkaloids.

4) Localization of Phenolic compounds

a. Ferric sulphate or Ferric chloride Method

Protocol: Sections were placed in 10% Formalin solution containing 2% Ferric sulphate or Ferric chloride and washed with water. The sections were then mounted with dilute glycerin and observed under a Bright-field microscope. The Blue or Blue-green color indicates the presence of Phenolic compounds.

Biochemical analysis in selected species

The plants were washed under the tap water multiple times until the soil particles were washed off. Fresh and healthy leaf and stem material were put for drying under the shade. After 14 days, when the samples were thoroughly dried, they were powdered using a grinder and stored in an airtight container. The leaf extracts of each plant were prepared using Soxhlet extraction and Maceration techniques. 5g of each dry powdered material was subjected to extraction with three solvents of different polarities: Methanol, Ethyl acetate, and n-hexane. Soxhlet extraction was carried out for 8 hours at 45^0C, completing eight cycles, and maceration was carried out for 72 hours at 45^0C on an orbital shaker. The extracts were filtered using Whatman No.1 filter paper and concentrated using Rotary Evaporator under a vacuum at 45^0C. The dried solvent-free extracts were kept at 4^0C for further analysis.

Preliminary qualitative phytochemical analysis

The extracts obtained with different solvents were used for the preliminary qualitative estimation, which was carried out according to the methods described by Raaman (2006).

Test for Alkaloids

50 mg of extract was stirred with a few mL of dilute hydrochloric acid and filtered. The filtrate is tested with alkaloidal reagents.

1. **Wagner's Test**

   The side of the test tube added a few drops of Wagner's Reagent to a few mL of filtrate. A reddish-brown precipitate reveals the presence of Alkaloids.

2. **Hager's Test**

   1 or 2 mL of Hager's Reagent were added to a few mL of filtrate. A prominent yellow precipitate reveals the presence of Alkaloids.

Test for Carbohydrates

100 mg of extract was dissolved in 5 mL of water, filtered, and used in the following tests.

1. **Fehling's Test**

   1 mL of filtrate was boiled with 1 ml of Fehling's solutions A and B in a a water bath. A red precipitate reveals the presence of Carbohydrates.

2. **Benedict's Test**

   0.5 mL of Benedict's Reagent was added to 0.5 mL of filtrate and heated in a boiling water bath for 2 minutes. A brown color precipitate reveals the presence of sugars.

Test for Glycosides

50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and hydrolysate was used for the following test.

1. **Borntrager's Test**

3 mL of Chloroform was added to 2 mL of hydrolysate and shaken. The chloroform layer was separated and 10% ammonia solution was added to it. A pink color reveals the presence of Glycosides.

Test for Saponins

50 mg of extract was diluted with 20 mL of distilled water, and this suspension was shaken in a graduated cylinder for 15 minutes. A two cm layer of foam reveals the presence of Saponins.

Test for Proteins

100 mg of extract was dissolved in 10 mL of distilled water and filtered. The filtrate was used for the following test.

Biuret Test

2 mL of filtrate was treated with a 2% Copper sulphate solution. 1 mL of 95% Ethanol was added, followed by an excess Potassium hydroxide pellet. The pink color in the ethanolic layer reveals the presence of Proteins.

Test for Amino Acids

100 mg of extract was dissolved in 10 mL of distilled water and filtered. The filtrate was used for the following test.

Ninhydrin Test

Two drops of Ninhydrin solution were added to 2 mL of aqueous filtrate. The purple color reveals the presence of Amino Acids.

Test for Phytosterols

1. **Libermann-Burchard's Test**

50 mg of extract was dissolved in 2 mL Acetic anhydride. One or two drops of concentrated sulphuric acid were added slowly along the sides of the test tube. Change in color reveals the presence of Phytosterols.

Test for Fixed Oils and Fats

1. **Saponification Test**

A few drops of 0.5N Alcoholic potassium hydroxide solution were added to a small quantity of extract and a drop of phenolphthalein. This mixture was heated in a water bath for 2 hours. Soap formation reveals the presence of Saponins.

Test for Phenolic compounds and Tannins

1. **Ferric chloride Test**

50 mg of extract was dissolved in 5 mL of distilled water. Few drops of 5% Ferric chloride solution were added to this. White precipitate reveals the presence of Phenolic compounds.

Test for Gums and Mucilages

100 mg of extract was dissolved in 10 mL of distilled water and 25 mL of absolute alcohol was added to this with constant stirring. White precipitate reveals the presence of Gums and Mucilages.

Extractive values with different solvents

The leaf extracts of the two plant species, *Rauwolfia verticillata,* and *Solanum anguivi* were prepared using Soxhlet Extraction and Maceration. 5 g of each powdered plant material was subjected to extraction with three different solvents, namely, Methanol, Ethyl acetate, and n-hexane. Soxhlet extraction was carried out for 8 hours at 45^0C and maceration for 72 hours at 45^0C. The extracts were filtered and concentrated using Rotary Evaporator under a vacuum at 45^0C. The extracts were kept at 4^0C for further analysis. The percent extractive yield of the leaf methanolic, ethyl acetate, and n-hexane extracts was calculated by the following formula,

Extractive value (%) = weight of dried extract/ weight of plant material X 100

Antioxidant studies

The antioxidant studies in selected plant species for leaves were carried out using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.

A. **Preparation of DPPH:** Stock solution was prepared by dissolving 24 mg of DPPH 100 mL of methanol in the dark and stored in an Amber-colored bottle. The working solution was prepared by adding 10 ml of the Stock solution to 45mL of methanol.
B. **Preparation of L-ascorbic acid solution:** 10 mg of ascorbic acid was dissolved in 10 mL of distilled water. Serial dilution was performed to prepare solutions with different concentrations (12.5 µg/mL – 200 µg/mL).
C. **Preparation of Test solution:** 10 mg of methanolic extract of leaf was dissolved in 10 mL of methanol, and then serial dilution was performed to prepare the required concentrations (12.5 µg/mL – 200 µg/mL).

D. Preparation of control: 3 mL DPPH was used as a negative control.

In the reaction mixture, 3 mL of DPPH working solution was added to 250µl of leaf extract of different concentrations and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517nm. The following equation calculated the percent inhibition:

Inhibition % = $(A_0-A_1)/A_0$ x 100

Here, $\mathbf{A_o}$ is the absorbance of the control and

$\mathbf{A_1}$ is the absorbance of the extract.

The Antioxidant assay was performed in triplicate, and the mean value was expressed. IC_{50} values were calculated by plotting percent (%) inhibition against the concentration (µg/mL) of the extract.

RESULTS AND DISCUSSION

The results of the present study carried out on the Anatomical, Histochemical and Phytochemical studies of selected medicinal plant species from Goa and its adjoining regions are reported in this chapter.

Morphological Characteristics

Rauwolfia verticillata is a large evergreen shrub up with milky juice, obovate leaves in whorls, and red or white flowers obliquely ellipsoid, brownish or purple drupes **(Plate 1)**. *Solanum anguivi*, commonly known as forest bitterberry, is a erect, highly-branched, prickly undershrubs with stout prickles, purplish flowers and yellow to orange fruits with minute pitted seeds **(Plate 2).** *Lantana camara*, commonly known as Lantana, wild sage, or shrub verbena, is an erect, frost-tender shrub with quadrilateral stem covered with bristly hairs, bright green leaves with rough and fine hairs and flowers in heads of many small tubular flowers **(Plate 3)**. *Tabrenaemontana alternifolia,* commonly called Nag Kuda is a small tree with elliptic and long leaves, white flowers in corymb cymes and fruits consisting of two boat-shaped orange pods **(Plate 4).**

Study of anatomical characterization

Systematic anatomical characterization of four selected medicinal plants viz. *Rauwolfia verticillata*, *Solanum anguivi*, *Lantana camara*,and *Tabarnaemontana alternifolia* was carried out for three vegetative parts stem, leaf, and petiole.

Stem anatomy of *Rauwolfia verticillata*

A study on the transverse section of stem of *Rauwolfia verticillata* revealed the following features as shown in **Plate 5**.

1. **Epidermis**

The epidermis was single-layered, made up of barrel-shaped, parenchymatous cells,and covered with a thin cuticle. Few lenticels were found on the epidermis.

2. **Periderm**

Periderm was present inner to the epidermis, which was made up of three parts: Phellem, Phellogen, and Phelloderm. The phellem (cork) was 4-5 layered, composed of tightly packed uniform rectangular cells with no intercellular spaces. Inner to the phellum was present the phellogen (cork cambium) consisting of 1-3 layers of radially elongated rectangular cells. The phellogen was followed by 4-5 layers of loosely arranged oval-shaped cells comprising the phelloderm. It forms the secondary cortex of the stem. Cells of the phellogen were placed in a radial manner as that of the phellogen. These cells showed the presence of chloroplasts **(Plate 5-C).**

3. **Cortex**

Cortex comprises 7-8 layers of loosely arranged oval-shaped, thin-walled parenchymatous cells with many intercellular spaces. Abundant chloroplasts were present in the cortical cells; hence the cells were chlorenchymatous in nature. (Plate 1- E,F). At the end of the cortex, it showed the presence of patches of non-lignified fibers. (Plate 2-A,B). Few druce crystals were also present **(Plate 6- C)**.

4. **Vascular tissues**

Vascular bundles were bicollateral, i.e., a single strand of xylem was covered on both the inner and outer side by the inner phloem strand and outer phloem strand, respectively. Xylem

was diffuse-porous. Vessels were arranged in vertical rows. Xylem fibers were well-developed and arranged in vertical rows. Both the phloem strands were composed of 4-5 layers of cells **(Plate 6-D)**.

5. **Pith**

The innermost and central tissue of the stem was composed of parenchymatous cells. Within the pith, it showed prominent sclerenchymatous cells called the stone cells (Brachysclereids). Pith also showed the presence of abundant starch granules **(Plate 6-E, F)**.

Leaf anatomy of *Rauwolfia verticillata*

A study on the transverse section of the leaf of *Rauwolfia verticillata* revealed the following features as shown in **Plate 7**.

1. **Epidermis**

Single layered epidermis was present on both the surfaces of the leaf. Cells were more or less rectangular. Upper Epidermis was covered with a thick cuticle. The upper epidermis was continuous. The lower epidermis was punctured at certain places with stomata.

2. **Hypodermis**

In the leaf midrib region, below the upper epidermis, there was the presence of a well-defined hypodermis. It comprised 7-8 layers of annular collenchymatous cells that were darkly stained. These cells were smaller than the parenchymatous cells. Angular collenchymatous cells were present above the lower epidermis. Raphides were found scattered in these collenchymatous cells on upper and lower surfaces **(Plate 7-E)**.

3. **Ground tissue**

The ground tissue in the midrib region was composed of tightly packed parenchymatous cells, which were oval in shape and irregular in size. Laticifers were found arranged in the ground tissue of the leaf **(Plate 7-C)**.

4. **Mesophyll tissue**

The mesophyll tissue is comprised of the upper palisade layer and lower spongy tissue. The palisade tissue was single-layered, and its columnar cells contained abundant chloroplasts. The lower part of the mesophyll tissue was composed of loosely arranged parenchymatous cells with intercellular spaces. These cells also contained chloroplasts in abundance **(Plate 7-D)**.

5. **Vascular tissues**

Vascular bundles were arranged in a V-shaped form. It consisted of a xylem in the center, and phloem was present on both the sides of the xylem; hence the vascular bundles were found to be bicollateral **(Plate 7-C)**.

Petiole anatomy of *Rauwolfia verticillata*

A study on the transverse section of the leaf of *Rauwolfia verticillata* revealed the following features as shown in **Plate 7-F**.

1. **Epidermis**

Single layered epidermis was present on both the surfaces of the petiole. Cells were more or less rectangular in shape. Upper Epidermis was covered with a thick cuticle.

2. **Hypodermis**

A well-defined hypodermis comprised the annular collenchyma on the upper surface and angular collenchymas on the lower surface. The hypodermal cells were cubical to round in shape. Raphides were found scattered in these collenchymatous cells in more abundance than that present in the hypodermis of the leaf.

3. **Ground tissue**

Ground tissue was composed of tightly packed parenchymatous cells, oval in shape and irregular in size. Laticifers were found more in the cells surrounding the vascular bundles thanin the rest of the cortical cells.

4. **Vascular tissue**

Vascular bundles were arranged in a V-shaped form. It consisted of xylem in the center, and phloem was present on both the sides of the xylem; hence the vascular bundles were found to be bicollateral.

Stem anatomy of *Solanum anguivi*

A study on the transverse section of the stem of *Solanum anguivi* revealed the following features as shown in **Plate 8**.

1. **Epidermis**

The epidermis was single-layered made up of barrel-shaped, parenchymatous cells and covered with a thick cuticle. The epidermis showed the presence of prominent stellate trichomes **(Plate 8-C, D)**.

2. **Hypodermis**

Inner to the epidermis, hypodermis was present, comprised of 4-5 layers of annular collenchymatous cells. These cells showed the presence of chloroplasts.

3. **Cortex**

The hypodermis followed 4-5 layers of oval-shaped parenchymatous cells, which comprised the cortical tissue. These cells were relatively larger as compared to the hypodermal cells. **(Plate 8-E)**. Few laticifers were seen scattered in these cells. On its inner side, it showed the presence of patches of lignified sclereids lining the outer phloem strand. **(Plate 9-C)**.

4. **Vascular tissues**

Vascular bundles are bicollateral, i.e., a single strand of xylem was covered on both the inner and outer side by phloem. The external phloem was present in the form of a continuous ring, while the inner phloem was discontinuous in the form of patches. Xylem was diffuse-porous. Vessels were arranged in vertical rows. Xylem fibers were well-developed and arranged in vertical rows. Both the phloem strands were composed of 4-5 layers of cells. Both the inner and outer phloem were lined by patches of lignified sclereids on the inner and outer side of the phloem **(Plate 9-A, B)**.

5. **Pith**

Pith was well developed, comprising oval-shaped parenchymatous cells that were irregular in size. Few laticifers were also present scattered throughout the pith **(Plate 9-D)**.

Leaf anatomy of *Solanum anguivi*

A study on the transverse section of the leaf of *Solanum anguivi* revealed the following features as shown in Plate 9 and Plate 10.

1. **Epidermis**

A single-layered epidermis was present on both the surfaces of the leaf. Cells were oval. Both the upper and lower epidermis was covered with a thick cuticle. The upper epidermis was continuous. The lower epidermis was punctured at certain places with stomata. The stellate trichomes were present on both the upper and lower epidermis.

2. **Hypodermis**

In the leaf midrib region, below the upper epidermis, there was the presence of a well-defined hypodermis. It comprised 4-5 layers of angular collenchymatous cells that were darkly

stained. These cells were smaller than the parenchymatous cells. Similar collenchymatous cells were also present above the lower epidermis **(Plate 10-B)**.

3. Ground tissue

The ground tissue in the midrib region was composed of tightly packed parenchymatous cells, which were hexagonal in shape and irregular in size. Laticifers were found arranged in the ground tissue of the leaf.

4. Mesophyll tissue

The mesophyll tissue comprises the upper palisade layer and lower spongy tissue. The palisade tissue was single-layered, and its columnar cells contained abundant chloroplasts. The lower part of the mesophyll tissue was composed of loosely arranged parenchymatous cells with intercellular spaces. These cells also contained chloroplasts **(Plate 9-F)**.

5. Vascular tissues

Vascular bundles were arranged in a crescent-shaped form. It consisted of a xylem in the center, and phloem was present on both the sides of the xylem; hence the vascular bundles were bicollateral **(Plate 10-A)**.

Petiole anatomy of *Solanum anguivi*

Study on transverse section of petiole of *Solanum anguivi* revealed the following features as shown in **Plate 10.**

1. Epidermis

A single-layered epidermis was present on both the surfaces of the petiole. Cells were cuboidal to oval in shape. Both the upper and lower epidermis was covered with a thick cuticle. The stellate trichomes were present on both the upper and lower epidermis.

2. **Hypodermis**

A well-defined hypodermis was present, comprising 4-5 layers of angular collenchymatous cells that were darkly stained. These cells were smaller than the parenchymatous cells. Similar collenchymatous cells were also present above the lower epidermis.

3. **Ground tissue**

Ground tissue was composed of tightly packed parenchymatous cells, which were hexagonal in shape and irregular in size. Laticifers were found arranged in the ground tissue of the petiole.

4. **Vascular tissues**

Vascular bundles were arranged in a crescent-shaped form. It consisted of a xylem in the center, and phloem was present on both the sides of the xylem; hence the vascular bundles were bicollateral.

Stem anatomy of *Lantana camara*

Study on transverse section of stem of *Lantana camara* revealed the following features as shown in Plate 10 and Plate 11.

1. **Epidermis**

The epidermis was single-layered and made up of barrel-shaped, parenchymatous cells of varying size covered with a thick cuticle. The epidermis showed the presence of prominent glandular and non-glandular trichomes **(Plate 11-A, B)**.

2. **Hypodermis**

Below the epidermis, a few layers of annular collenchymatous cells were present, becoming angular as they moved towards the inner side. The cells were compactly arranged These collenchymatous cells were 5-6 layered at the four edges of the quadrilateral stem, forming a cap over the resin canals, while on the rest of the stem portion, these cells were in 2-3 layers **(Plate 10-F)**.

3. Cortex

Cortex is represented by 5-6 layers of loosely arranged parenchymatous cells with many intercellular spaces. In the cortical region, at the four edges of the stem, four prominent resin canals were present with inclusions **(Plate 11-C)**.

4. Vascular tissues

Vascular bundles were collateral, i.e., a single xylem strand was covered on the outside by a single phloem strand. Xylem was diffuse-porous. Xylem vessels and fibers were well-developed **(Plate 11-D, E)**.

5. Pith

The innermost part of the stem comprised the large star-shaped pith and was well developed with loosely arranged hexagonal parenchymatous cells of irregular sizes **(Plate 11-F)**.

Leaf anatomy of *Lantana camara*

Study on transverse section of leaf of *Lantana camara* revealed the following features as shown in **Plate 12**.

1. Epidermis

A single-layered epidermis was present on both the surfaces of the leaf. Cells were oval. Both the upper and lower epidermis was covered with a thick cuticle. The upper epidermis was

continuous. The lower epidermis was punctured at certain places with stomata. Both the upper and lower epidermis possessed several glandular and non-glandular trichomes. Trichomes were more abundant on the adaxial surface than that of the abaxial surface **(Plate 12-A)**.

2. Hypodermis

In the leaf midrib region, below the upper epidermis, 2-3 layered angular collenchymatous cells formed the hypodermis. These cells were smaller than the parenchymatous cells. Similar collenchymatous cells were also present above the lower epidermis **(Plate 12-A)**.

3. Ground tissue

The ground tissue in the midrib region was composed of loosely arranged parenchymatous cells, hexagonal in shape and irregular in size.

4. Mesophyll tissue

The mesophyll tissue comprises the upper palisade layer and lower spongy tissue. The palisade tissue was single-layered, and its columnar cells contained abundant chloroplasts. The lower part of the mesophyll tissue was composed of loosely arranged parenchymatous cells with intercellular spaces comprising the spongy tissue. These cells also contained chloroplasts. Secretory idioblasts were also present within the spongy tissue **(Plate 12-C)**.

5. Vascular tissues

The vascular bundles were collateral, forming an arch in the U-shaped form with xylem towards the upper and phloem towards the lower surface. One or two accessory bundles were present dorsally **(Plate 12-B)**.

Petiole anatomy of *Lantana camara*

Study on transverse section of petiole of *Lantana camara* revealed the following features as shown in **Plate 12**.

1. **Epidermis**

A single-layered epidermis was present on both the surfaces of the leaf. Cells were oval. Both the upper and lower epidermis was covered with a thick cuticle. The adaxial surface was abundantly covered with glandular and non-glandular trichomes. Few trichomes were present on the adaxial surface.

2. **Hypodermis**

Below the upper epidermis, there was the presence of 2-3 layered, angular collenchymatous cells forming the hypodermis. These cells were smaller than the parenchymatous cells. Similar collenchymatous cells were also present above the lower epidermis.

3. **Ground tissue**

The ground tissue was composed of loosely arranged parenchymatous cells, which were hexagonal in shape and irregular in size.

4. **Vascular tissues**

The vascular bundles were collateral, forming an arch in the U-shaped form with xylem towards the upper and phloem towards the lower surface. One or two accessory bundles were present dorsally.

Stem anatomy of *Tabarnaemontana alternifolia*

Study on transverse section of stem of *Tabarnaemontana alternifolia* revealed the following features as shown in **Plate 13.**

1. **Epidermis**

The epidermis was single-layered made up of barrel-shaped, parenchymatous cells and covered with a thin cuticle. Few lenticels were found on the epidermis.

2. **Periderm**

Periderm was present inner to the epidermis, which was made up of three parts: Phellem, Phellogen, and Phelloderm. The phellem (cork) was 4-5 layered, composed of tightly packed uniform rectangular cells with no intercellular spaces. Inner to the phellum was present the phellogen (cork cambium) composed of 1-3 layers of radially elongated rectangular cells. The phellogen was followed by 4-5 layers of loosely arranged oval-shaped cells comprising the phelloderm. It forms the secondary cortex of the stem. Cells of the phellogen were arranged radially as that of the phellogen. A ring of lignified sclerenchymatous cells lined the outer side of the phelloderm **(Plate 13-C)**.

3. **Cortex**

Cortex comprises 7-8 layers of loosely arranged oval-shaped, thin-walled parenchymatous cells with many intercellular spaces. At the end of the cortex, it showed large patches of non-lignified fibers. The lower side of the cortex was thoroughly embedded with scattered laticifers **(Plate 13-D)**.

4. **Vascular tissues**

Vascular bundles were bicollateral i.e., single strand of xylem was covered on both the inner and outer side by inner phloem strand and outer phloem strand, respectively. Xylem was diffuse-porous. Vessels were arranged in vertical rows. Xylem fibers were well-developed and arranged in vertical rows. Both the phloem strands were composed of 4-5 layers of cells **(Plate 13-E).**

5. **Pith**

The innermost and central tissue of the stem was composed of parenchymatous cells. Within the pith, it showed the presence of prominent sclerenchymatous cells called the stone cells (Brachysclereids). Pith also showed the presence of abundant starch granules **(Plate 13-F).**

Leaf anatomy of *Tabarnaemontana alternifolia*

Study on transverse section of leaf of *Tabarnaemontana alternifolia* revealed the following features as shown in **Plate 14.**

1. **Epidermis**

A single layered epidermis was present on both the surfaces of the leaf. Cells were more or less rectangular. Upper Epidermis was covered with a thin cuticle. The upper epidermis was continuous, while the lower epidermis was punctured at certain places with stomata **(Plate 14-B)**.

2. **Hypodermis**

In the leaf midrib region, below the upper epidermis, there was the presence of a well-defined hypodermis. It comprised 7-8 layers of annular collenchymatous cells that were darkly stained. Raphides were observed in these cells. **(Plate 14-C)**. These cells were smaller than the

parenchymatous cells. Angular collenchymatous cells were present above the lower epidermis **(Plate 14-B)**.

3. **Ground tissue**

The ground tissue in the midrib region was composed of tightly packed parenchymatous cells, which were oval in shape and irregular in size. Laticifers were found arranged in the ground tissue of the leaf. The cortical region of the dorsal surface was filled with abundant chloroplasts **(Plate 14-B)**.

4. **Mesophyll tissue**

The mesophyll tissue comprises of upper palisade layer and lowers spongy tissue. The palisade tissue was single-layered, and its columnar cells contained abundant chloroplasts. The lower part of the mesophyll tissue was composed of loosely arranged parenchymatous cells with intercellular spaces. These cells also contained chloroplasts in abundance **(Plate 14-D)**.

5. **Vascular tissues**

Vascular bundles were bicollateral arranged in a V-shaped form consisting of a xylem in the center and a phloem on both sides of the xylem **(Plate 14-E)**.

Petiole anatomy of *Tabarnaemontana alternifolia*

A study on transverse section of petiole of *Tabarnaemontana alternifolia* revealed the following features as shown in **Plate 14**.

1. **Epidermis**

A single-layered epidermis was present on both the surfaces of the petiole. Cells were more or less rectangular. Upper Epidermis was covered with a thick cuticle.

2. Hypodermis

A well-defined hypodermis comprised the annular collenchyma on the upper surface and angular collenchymas on the lower surface. The hypodermal cells were cubical to round in shape.

3. Ground tissue

Ground tissue was composed of tightly packed parenchymatous cells, which were oval in shape and irregular in size. Laticifers were found arranged in the cortical cells of the petiole. The cortical region of the dorsal surface was filled with abundant chloroplasts.

4. Vascular tissues

Vascular bundles were bicollateral arranged in a V-shaped form consisting of a xylem in the center and a phloem on both sides of the xylem.

In the present study, two plants from the family Apocynaceae and one plant each from the family Solanaceae and Verbenaceae were taken for the anatomical studies.

Seenu *et al*. (2019) investigated the anatomical description of vegetative parts of *Tabarnaemontana alternifolia* L. in which they found similar results as obtained in this study. Their results showed that the leaves of *T. alternifolia* possessed a uniseriate epidermis made up of thin-walled parenchymatous cells and was covered by a thin cuticle on both the adaxial and abaxial surfaces. The secondary growth in the stem was characterized by the formation of periderm and thick-walled fibers in the vascular tissues.

Both the plant species belonging to the family Apocynaceae viz. *R.verticillata* and *T. alternifolia* showed the presence of lenticels in the periderm of their stems. Lenticels are circular structures that protrude from the surface of stems and roots. They possess small openings

through which the gas exchange takes place. El-Fiki*et al*. (2019) also observed lenticels in *Pachypodium lamerei* and *Alstonia scholaris,* both of which belong to the Apocynaceae family.

In the case of *Solanum anguivi,* stellate trichomes were seen on the surface of both the stem and the leaf. Wahua and Edwin-Wosu (2016) observed the stellate trichomes in *Solanum* species viz. *S. aethiopicum* Linn., *S. torvum* Swartz., *S. anomalum* Thonn., *S. erianthum* D. Don.

Passos*et al.* (2009) reported that the petioles of the two species *Lantanacamara* and *Lantana radula* were covered with several types of glandular and non-glandular trichomes throughout their extension. Similar trichomes were also observed in this study on the stem, leaves and petioles of *Lantana camara.*

Study of histochemical localization

Histochemical localization for primary and secondary metabolites of four selected medicinal plants viz. *Rauwolfia verticillata*, *Solanum anguivi*, *Lantana camara* and *Tabarnaemontana alternifolia* was carried out.

Localization of Starch

Freehand thin sections of the vegetative parts of the plant were stained with I_2KI solution and observed under the bright field microscope.

In the the stem of *Rauwolfia verticillata,* the starch granules were scattered throughout the pith, cortex, and phloem region, stained black. Leaves also showed the presence of starch granules around the vascular bundles as well as in spongy tissue of mesophyll **(Plate 15).** In the case of *Solanum anguivi,* the starch granules were present in the cortex of the stem and stained

blue to black in color **(Plate 16).** In *Lantana camara*, the starch granules were concentrated in the mesophyll tissue of the leaf and phloem part of the stem **(Plate 16,17)**. In the case of *Tabarnaemontana alternifolia* the starch granules were scattered throughout the stem portion, more abundantly in the pith region. Few granules were found scattered in the cortical cells below the vascular tissue in the leaf **(Plate 17)**.

Localization of Lipids

Free-hand thin sections of the vegetative parts of the plant were stained with Sudan IV stain and observed under the bright field microscope. In the stem of *Rauwolfia verticillata* the lipids were present in the pith and scattered throughout the cortex Few lipid globules were also seen in the secondary cortex. Lipids-stained bright red **(Plate 18,19).** In the leaf of *Solanum anguivi*, the lipids were embedded throughout the mesophyll tissue **(Plate 19).** The mesophyll tissue in the leaf of *Lantana camara* showed the presence of lipid globules. Its stem also showed the presence of a few lipid globules in the cortex as shown in **Plate 19,20**. In *Tabarnaemontana alternifolia*, lipids were present in the pith, phloem, and cortex region of the stem and mesophyll tissue of the leaves **(Plate 20).**

Localization of Alkaloids

Free-hand thin sections of the vegetative parts of the plant were stained with Dragendorff's or Wagners's reagent and observed under the bright field microscope. **Plate 21** shows presence of abundant alkaloids present in the stem of *Rauwolfia verticillata*, mainly concentrated in the cortex and phloem region. In the case of leaf and petiole, the alkaloids were present in the hypodermis and ground tissue. *Solanum anguivi* showed the presence of alkaloids

in hypodermis and the cortex of the stem and the mesophyll tissue of the leaf **(Plate 21-E)**. In *Lantana camara,* the alkaloids were present in the phloem, hypodermis, cortex region of the stem **(Plate 22).** In the case of *Tabarnaemontana alternifolia,* alkaloids were present in thecortex of the stem and the ground tissue of the leaf, as shown in **Plate 22.**

Localization of Phenolic compounds

Free-hand thin sections of the vegetative parts of the plant were stained with 10% formalin solution containing 2% ferric chloride and observed under the bright field microscope.

Phenolic compounds were found in the hypodermis of the leaf of *Rauwolfia verticillata,* as depicted in **Plate 23-A**. *In the* case of *Solanum anguivi,* these phenolic compounds were present in the pith, cortex, and phloem region of the stem and hypodermis of the leaf **(Plate 23-B,C)**. In *Lantana camara,* the hypodermis and the cortex in the stem showed the presence of phenolic compounds, as shown in **Plate 23 - D,E.** *Tabarnaemontana alternifolia* showed a few phenolic compounds in the cortex portion of the stem **(Plate 23-F).**

Extractive values with different solvents

The leaf extracts of the two plant species, *Rauwolfia verticillata,* and *Solanum anguivi* were prepared using Soxhlet Extraction and Maceration and are shown in **Table 1**. 5 g of each powdered plant material was subjected to extraction with three different solvents: Methanol, Ethyl acetate, and n-hexane. The extracts were filtered and concentrated using Rotary Evaporator under a vacuum at 45^0C. The concentrated extracts were weighed, and the percent extractive yield was calculated. It was found that the percent extractive yield was higher in Soxhlet extraction than in the Maceration. Also, the percent extractive yield decreased with a decrease in

the solvent's polarity in the order Water > Methanol > Ethyl acetate > n-hexane. These results showed that solvents took an essential role in the extraction yield.

Preliminary qualitative phytochemical analysis in selected plant species

The phytochemical analysis of leaf extracts of *R. verticillata* and *S. anguivi* revealed the presence of medicinal bioactive constituents. The extracts extracted with both Soxhlet and Maceration showed similar results. However, the use of different solvents showed variations in the results.

The phytochemical tests indicated the presence of alkaloids, carbohydrates, glycosides, proteins, phytosterols, saponins, fixed oils and fats, phenolic compounds, and tannins in methanolic and ethyl acetate extracts of *R. verticillata* shown in **Table 2,3**. It showed negative results for amino acids and gums, and mucilages. However, the aqueous extract of *R. verticillata* showed positive results for Gums and mucilages.

The phytochemical tests for methanolic and aqueous extracts of *S. anguivi* showed the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, phytosterols, fixed oils, and fats, phenolic compounds, tannins and gums and mucilages and absence of amino acids as depicted in **Table 4,5.** The ethyl acetate extract showed the presence of carbohydrates, proteins, phytosterols, phenolics, gums, and mucilage but showed the lack of alkaloids, amino acids, fixed fats, and oils, and phenolics.

The n-hexane extracts of both plants showed the absence of Alkaloids, proteins, amino acids, saponins, phytosterols, phenolic compounds, gums, and mucilage. However, n-hexane extract of *S. anguivi* showed a slight presence of carbohydrates.

These results showed that methanol is the best solvent for the extraction of phytochemicals compared to ethyl acetate and n-hexane. N-hexane being the solvent of lowest polarity,was found to be not ideal for the extraction of phytochemicals. Hexane is generally for extracting edible oils from seeds and vegetables as a special-use solvent and as a cleaning agent.

Dhawan and Gupta (2017) also observed similar results when comparing different solvents for phytochemical extraction potential from *Datura metel* plant leaves. They used distilled water, methanol, acetone, chloroform, ethyl acetate, and hexane. Their results showed that methanol worked best to extract various active phytochemicals with high flavonoid and phenol concentration compared to the other solvents used.

Antioxidant studies in selected medicinal plants

The antioxidant studies in two selected medicinal plants, *Rauwolfia verticillata* and *Solanum anguivi*were carried out using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. This method measures the decrease in the absorption of the DPPH solution after adding an antioxidant at 517 nm. Ascorbic acid (10mg/mL distilled water) was used as a positive control.

The antioxidant activity of methanolic extract of *Rauwolfia verticillata* and *Solanum anguivi*was estimated by DPPH scavenging activity. 1, 1 Diphenyl 2- Picryl Hydrazyl is a stable free radical in powder form that was red in color and turned yellow when scavenged.

The scavenging reaction between DPPH and an antioxidant can be written as,

(DPPH) + (H-A) → DPPH-H + (A)

Here, the antioxidants reacted with the DPPH and reduced it to DPPH-H, and thus the absorbance decreased with an increase in the concentration of the extract. This indicated the scavenging potential of the antioxidant compounds in the extracts in terms of hydrogen donating ability.

The results showed that both the plants extracted with two different extraction methods exhibited antioxidant activity. The highest free radical scavenging activity was shown by *R. verticillata* leaf extract extracted by the Soxhlet extraction method with an IC_{50} value of 79.46 µg/mL, while, the *S. anguivi* leaf extract exhibited the lowest antioxidant activity with an IC_{50} value of 182.82 µg/mL. The maximum percentage increased with the concentration,i.e., 250 µg/mL. L-ascorbic acid was used asa standardto compare the radical scavenging activity of the extracts, and its IC_{50} value was 68.07 µg/mL **(Table 6, 7)**.

IC_{50} value represents the concentration at which a substance exerts half of its maximal inhibitory effect. Thus, IC_{50} value is used to represent the effectiveness of an antagonist in inhibiting a specific biochemical process.

CONCLUSION

The present study on the anatomical characterization of the four medicinal plants viz. *Rauwolfia verticillata*, *Solanum anguivi*, *Lantana camara* and *Tabarnaemontana alternifolia*. revealed the unique anatomical features. The most important secondary metabolites, such as alkaloids and phenolic compounds, and primary metabolites like starch and lipids were localized in various vegetative parts using histochemistry. *R. verticillata* and *S. anguivi* showed the highest alkaloid levels localized in their cells. Phytochemical parameters were also studied. The effect of two different extraction methods on the yield of extraction was studied, wherein soxhlet extraction was found to be the better method than a maceration. Also, among the solvent used for extraction, methanol gave better results for the phytochemical analysis. Phytochemicals tests revealed the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, phytosterols, fats and oils, phenolic compounds and tannins; and gums and mucilages. The antioxidant assay showed that *R. verticillata* exhibited the highest antioxidant activity. Thus, it can be concluded that among the four plants studied, *R. verticillata* possessed more medicinal properties than *S. anguivi*, *L. camara,* and *T. alternifolia.* The scope for future studies lies in the field of micropropagation as the chosen plant *R. verticillata* is an endemic plant to the Western Ghats. Since it is proven that it exhibits enhanced medicinal properties, detailed study into its bioactive compound such as isolation and characterization of the lead molecule and enhancing the production via micro-propagation studies would yield promising results.

SUMMARY

A total of four medicinal plants were collected from various regions of Goa and its adjacents, namely, *Rauwolfia verticillata*, *Solanum anguivi*, *Lantana camara* and *Tabarnaemontana alternifolia*. *Rauwolfia verticillata* was endemic to the Western Ghats of Maharashtra. The other species were collected from various regions of Goa.

R. verticillata is a medicinal plant belonging to the family Apocynaceae and is not much studied on its anatomical characterization. It is distinct with its whorled leaves (species names *"verticillata"*) and persistent lenticels all over the stem. Therefore, identification of *R. verticillata* can be made in its vegetative state. *Solanum anguivi* is a medicinal shrub of the family Solanaceae with prickly stems and leaves. *L. camara* is a flowering ornamental plant belonging to the family Verbenaceae. *Tabarnaemontana alternifolia* is a species in the family Apocynaceae with various medicinal properties and is endemic to India.

The anatomical study of the stem, leaf, and petiole of the four medicinal plant species were carried out. This study was done to find distinct anatomical characters among the four mentioned plants. It revealed the unique anatomical characters in the cellular structures of these plants. The stem of *R. verticillata* and *T. alternifolia* showed the presence of periderm with lenticels. Also, both the plants showed the presence of druce crystals and non-lignified fibers. There is no much difference in the anatomical features of the stem in *R. verticillata* and *T. alternifolia*. The stem, leaf, and petiole of *S. anguivi* showed the presence of stellate trichomes on the surface. The stem in *L. camara* showed the presence of glandular and non-glandular trichomes on the surface of the stem.

The histochemical localization of primary and secondary metabolites was carried out in the four plants' stem, leaf, and petiole. The primary metabolites like starch and lipids were successfully localized in all the parts studied. The starch grains were mainly found deposited in the pith and cortex region of the stem and in the ground tissue of the leaves. Lipids were abundant in the mesophyll tissue of the leaves. The alkaloids were concentrated in the cortex region of the stem and the ground tissue of the leaf and petiole. Out of the four plants examined, *R. verticillata* and *S. anguivi* showed the highest quantity of the alkaloids in their cells whereas *L. camara* showed its lowest concentrations. Phenolic compounds were localized in small amounts in the cortical cells of *S. anguivi* and *L. camara.*

Based on the abundance of the secondary metabolites, the leaf extracts were prepared from the two plant species, viz. *R. verticillata* and *S. anguivi,* for the biochemical analysis. Two methods were employed for the extraction, viz. Soxhlet and Maceration. To analyze the effects of different solvents on the yield of extraction, solvents with different polarities were used in the extraction, namely, water, methanol, ethyl acetate, and n-hexane. The soxhlet extraction method showed better extraction, resulting in more yield than the maceration method. Among the solvents used, methanol was found to be the best solvent for extraction delivering higher yields followed by water, ethyl acetate, and n-hexane.

Preliminary phytochemical analysis of the methanolic extracts of *R. verticillata* and *S. anguivi* revealed the presence of alkaloids, carbohydrates, glycosides, proteins, phytosterols, fixed oils, and fats, phenolic compounds, tannins and gums, and mucilages. However, the ethyl acetate and n-hexane extracts of both the plants showed the absence of most of the

phytochemicals. Both the extraction methods i.e., soxhlet and maceration, showed similar results concerning the phytochemical analysis.

The antioxidant studies were carried out for the methanolic extracts of *R. verticillata* and *S. anguivi* using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Ascorbic acid was used as a positive control. Both the plants extracted with two different extraction methods exhibited antioxidant activity. The highest free radical scavenging activity was shown by *R. verticillata* leaf extract extracted by the soxhlet extraction method with an IC_{50} value of 79.46 µg/mL, while, the *S. anguivi* leaf extract exhibited the lowest antioxidant activity with an IC_{50} value of 182.82 µg/mL.

The histochemical and phytochemical analysis revealed that the plants exhibit medicinal properties due to phytochemicals in their various vegetative parts. Hence this study can be helpful in drug discovery, and utilizing these plants as potential drug sources can aid in treating various ailments.

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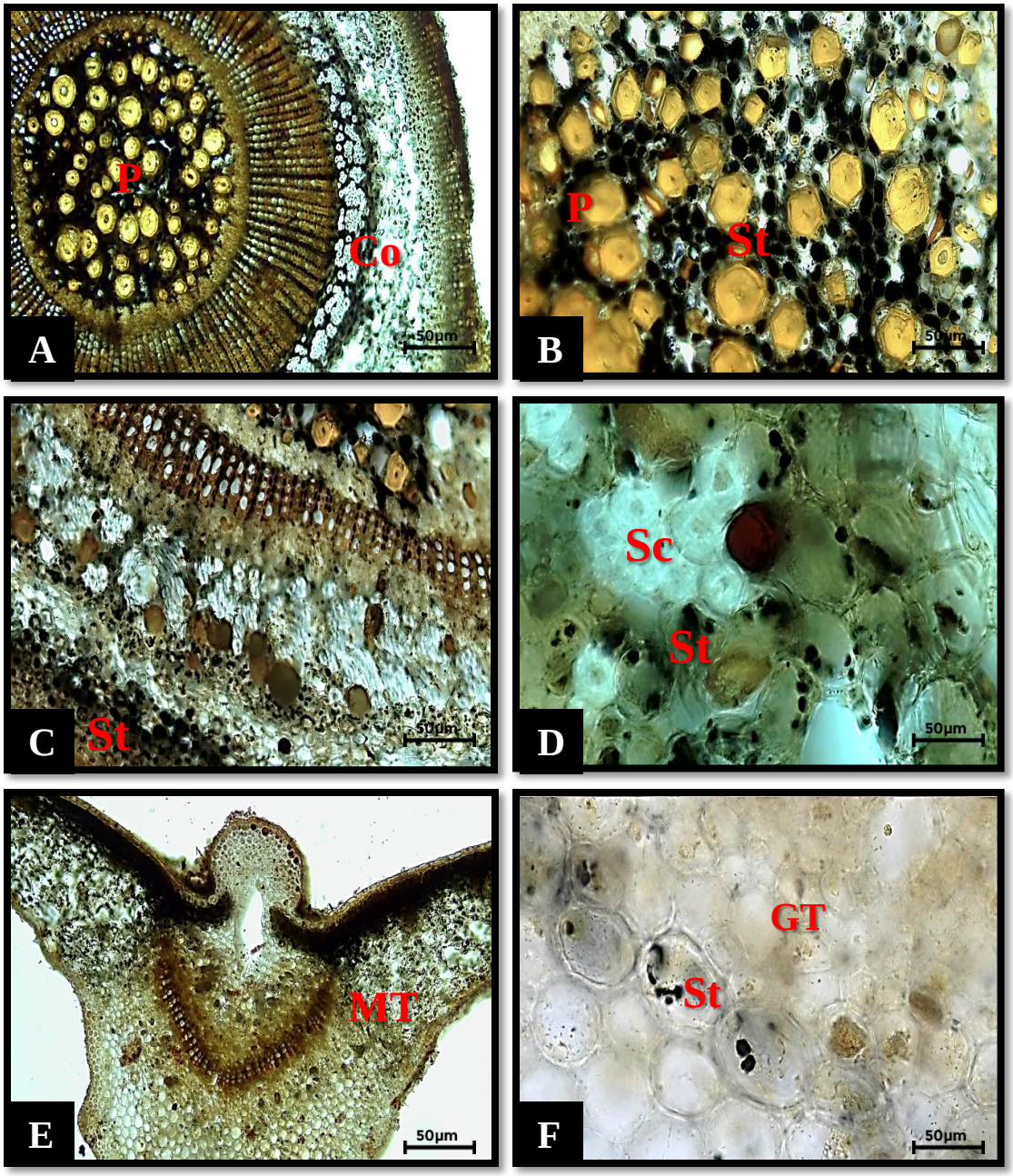


Plate 15. Localization of Starch

A. Portion of the stem of *R. verticillata* stained with I_2KI solution; **B.** Starch granules in pith of the stem of *R. verticillata ;* **C.** Starch granules in phloem and cortex of the stem of *R. verticillata;* **D**. Starch granules around the sclereids in the stem of *R. verticillata;*

E. Starch granules in the mesophyll tissue of the leaf of *R. verticillata ;* **F.** Starch granules in the ground tissue of the leaf of *R. verticillata*.

St=Starch,Sc=Sclereids, P=Pith, Co=Cortex, MT=Mesophyll tissue, GT=Ground tissue

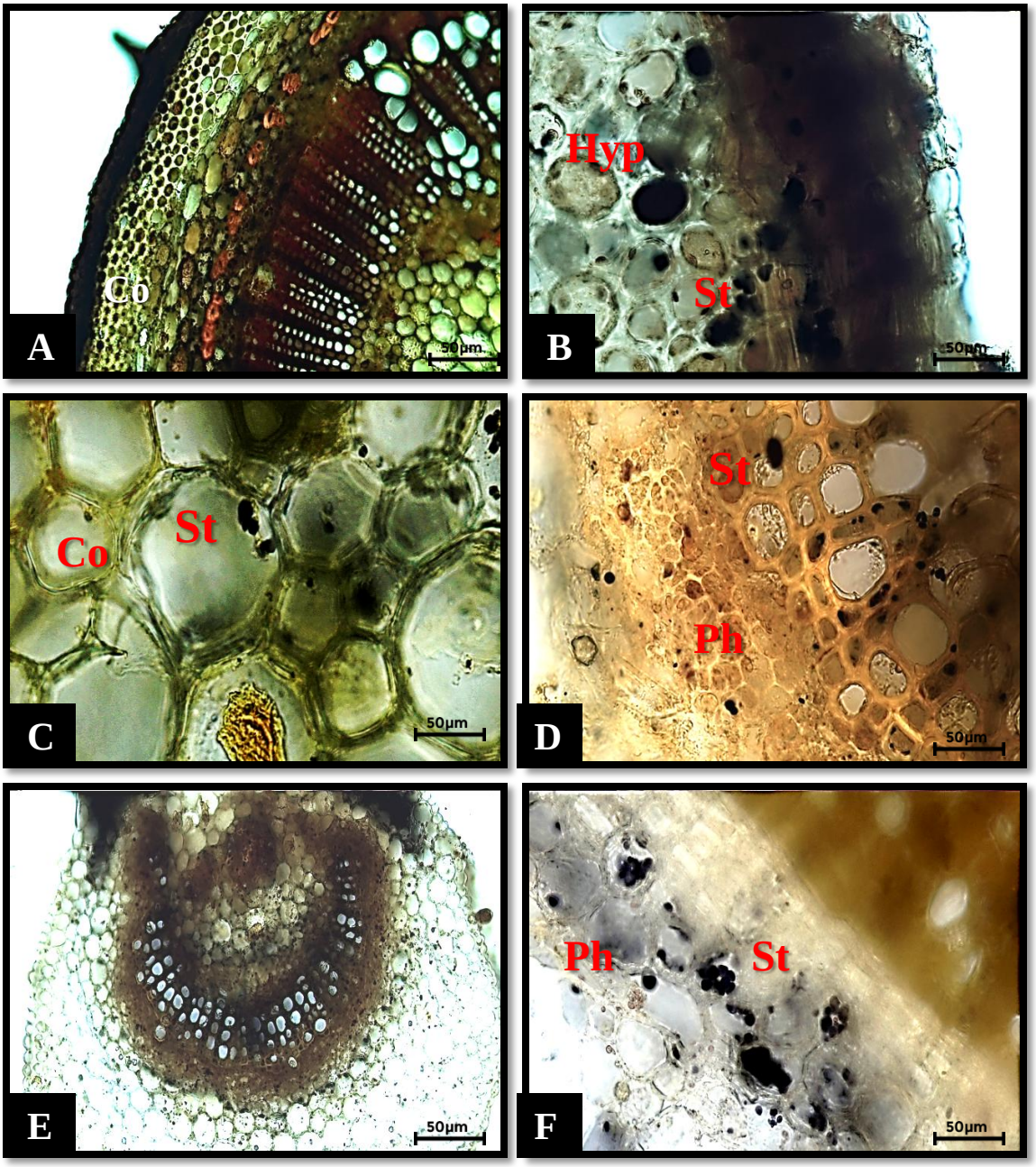


Plate 16. Localization of Starch

A. Portion of the stem of *S.anguivi* stained with I_2KI solution; **B.** Starch granules in hypodermis of the stem in *S. anguivi ;* **C.** Starch granules in cortex of the stem of *S. anguivi;* **D.** Starch granules in the phloem of the stem in *S. anguivi;* **E**. Portion of the leaf of *S. anguivi* stained with I_2KI solution; **F**. Starch granules in phloem of the leaf of *S. anguivi*
St=Starch, Co=Cortex, Hyp=Hypodermis, Ph=Phloem

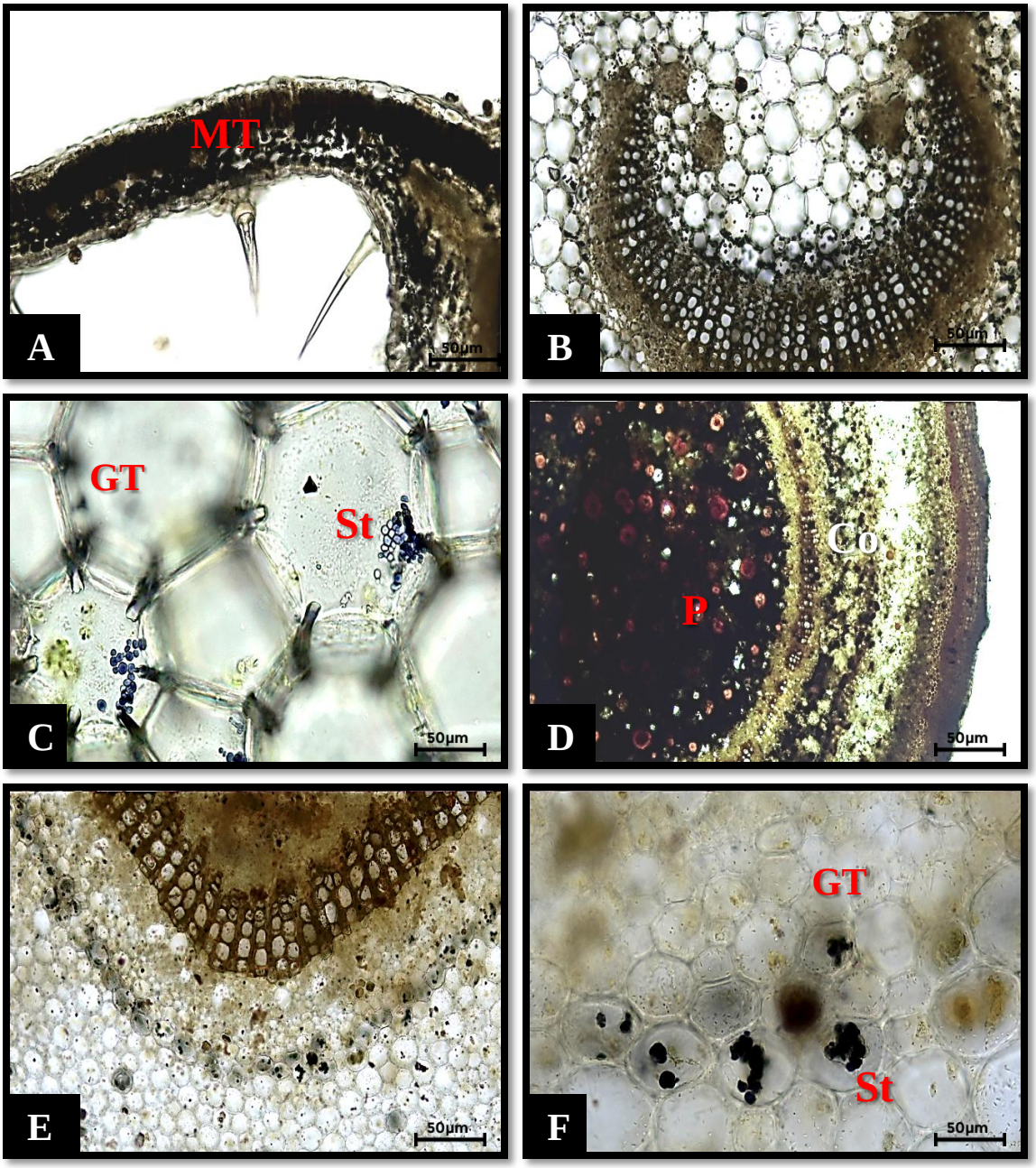


Plate 17 . Localization of Starch

A. Starch granules in the mesophyll tissue of the leaf of *L. camara;* **B**. Portion of the petiole of *L. camara* stained with I_2KI solution; **C**. Starch granules in the ground tissue of the petiole of *L. camara;* **D.** Starch granules in the pith and cortex of the stem of *L. camara;* **E**. Portion of the leaf of *T. alternifolia* stained with I_2KI solution; **F.** Starch granules in the ground tissue of the leaf of *T. alternifolia*

St=Starch, GT=Ground tissue, MT=Mesophyll tissue, Co=Cortex, P=Pith

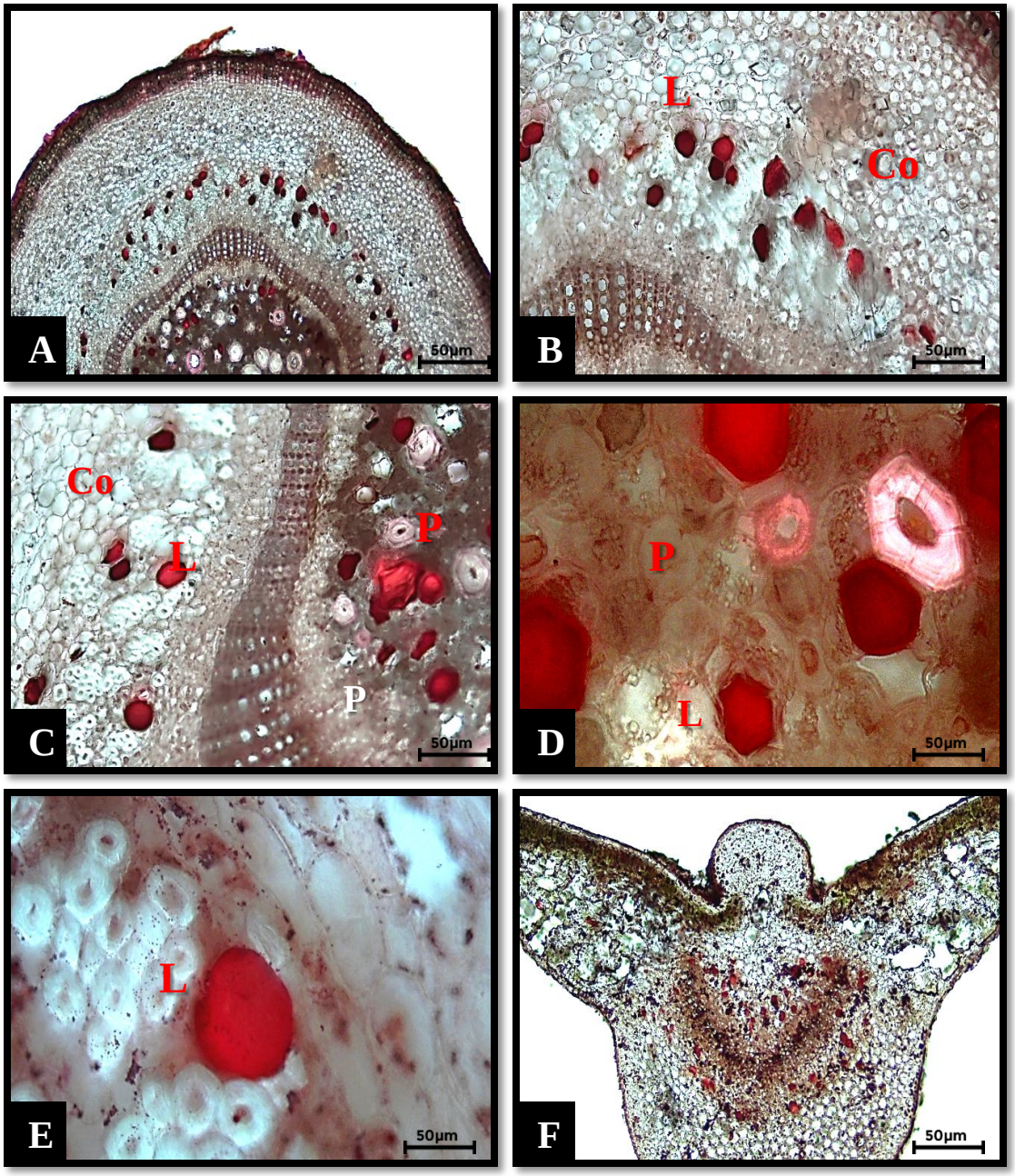


Plate 18. Localization of Lipids

A. Portion of the stem of *R. verticillata* stained with Sudan IV; **B**. Lipid globules in the cortex of the stem of *R. verticillata;* **C.** Lipid globules in the pith and cortex of the stem of *R. verticillata;* **D.** Lipid globules in the pith of the stem of *R. verticillata;* **E.** Lipid globule in cortex of the stem of *R. verticillata;* **F.** Transverse section of the leaf of *R. verticillata* stained with Sudan IV

L=Lipid, Co=Cortex, P=Pith

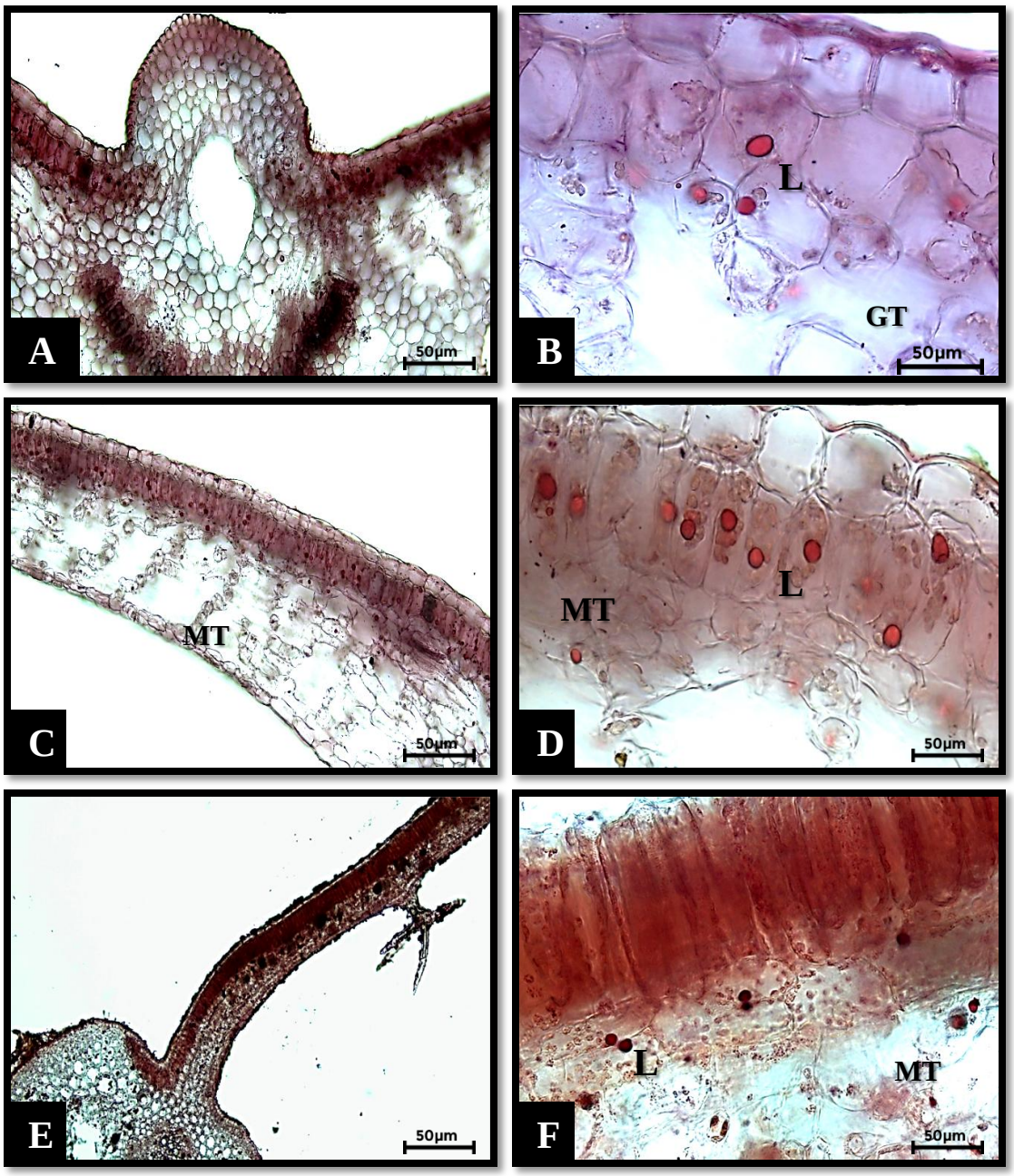


Plate 19 . Localization of Lipids

A. Midrib portion of the leaf of *R. verticillata* stained with Sudan IV; **B**. Lipid globules in ground tissue of the leaf of *R. verticillata;* **C**. Portion of the leaf lamina of *R. verticillata* stained with Sudan IV*;* **D**.Lipid globules in the mesophyll tissue of the leaf of *R. verticillata;* **E**. Portion of the leaf lamina of *S. anguivi* stained with Sudan IV*;* **F**. Lipid globules in mesophyll tissue of the leaf of *S.anguivi.*

L=Lipid, GT=Ground tissue, MT=Mesophyll tissue

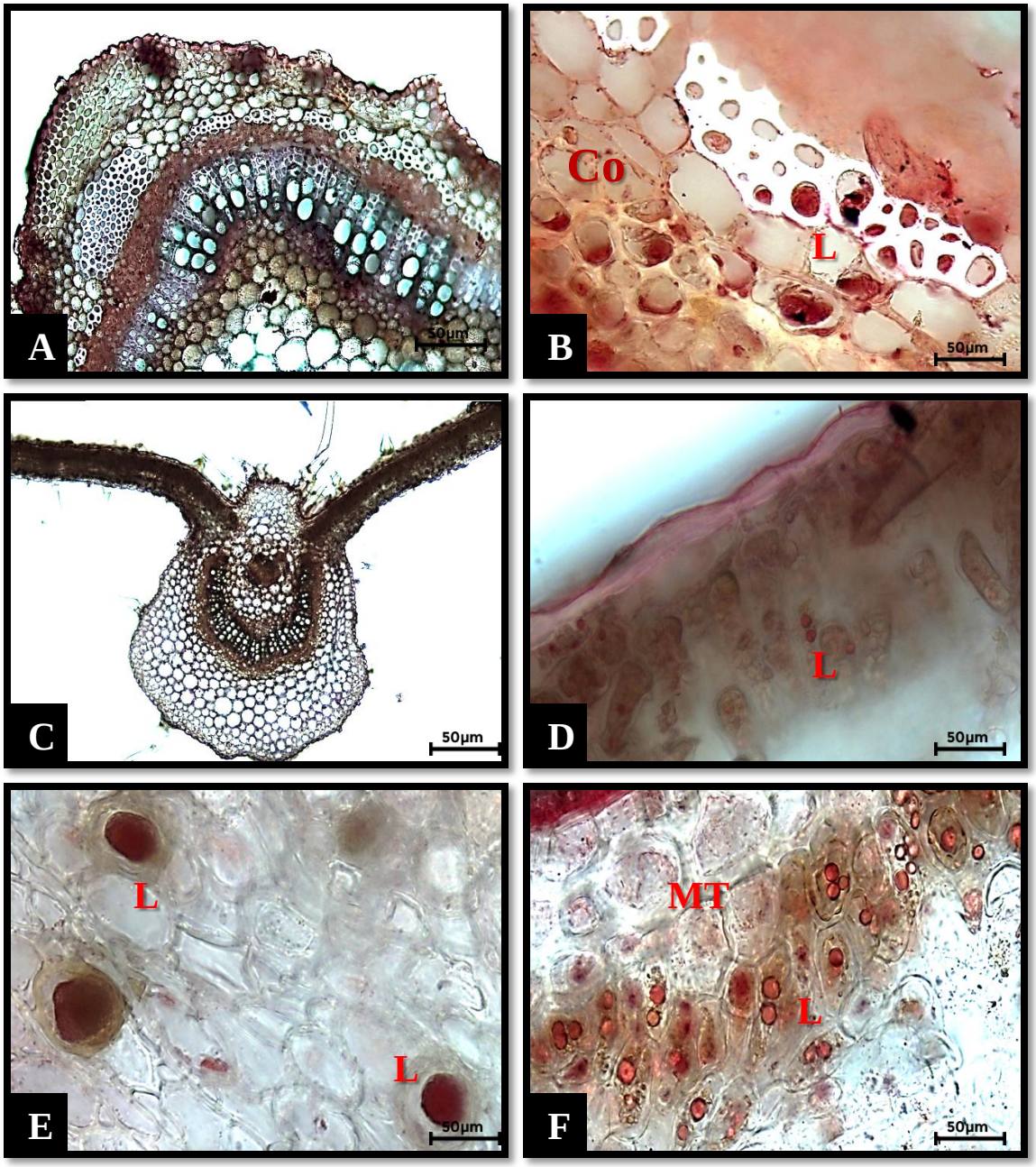


Plate 20 . Localization of Lipids

A. Portion of the stem of *L. camara* stained with Sudan IV; **B.** Lipid globules in cortex region of the stem of *L. camara;* **C.** Transverse section of the leaf of *L. camara* stained with Sudan IV*;* **D.** Lipid globules in the mesophyll tissue of the leaf of *L. camara;* **E.** Lipid globules in the cortex of the stem of *T. alternifolia;* **F.** Lipid globules in mesophyll tissue of the leaf of *T. alternifolia*

L=Lipid, GT=Ground tissue, MT=Mesophyll tissue

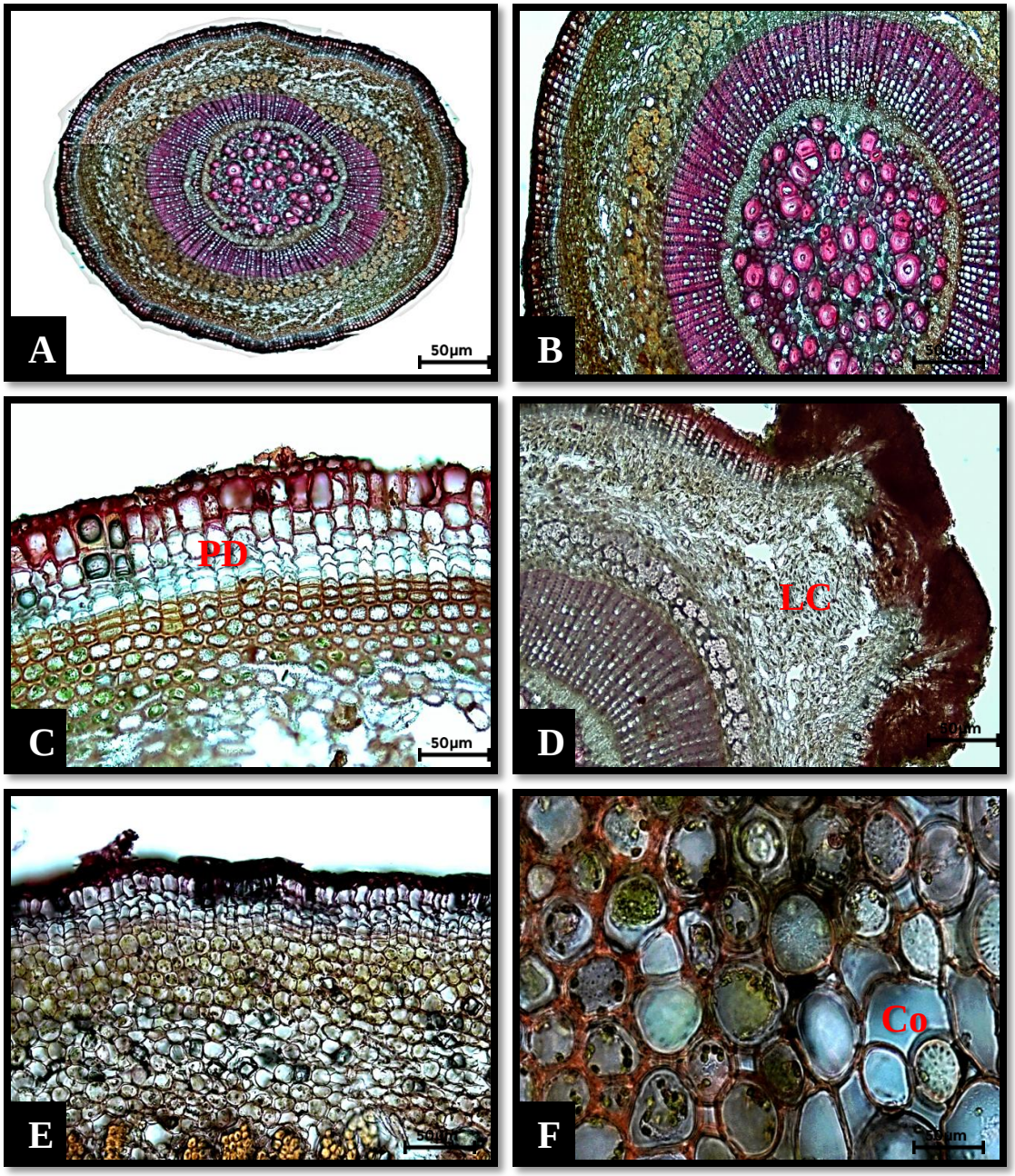


Plate 5: A. Overview of the Stem of *R.verticillata*; **B.** Portion of the stem of *R. verticillata;* **C.** Enlarged view of periderm in the stem of *R.verticillata;* **D.** Enlarged view of lenticel in the periderm in the stem of *R. verticillata*; **E.** Portion of the stem of *R. verticillata* showing chlorenchymatous cortex; **F.** Enlarged view of the cortical cells in the stem of *R. verticillata.*
PD=Periderm, LC= Lenticel, Co= Cortex.

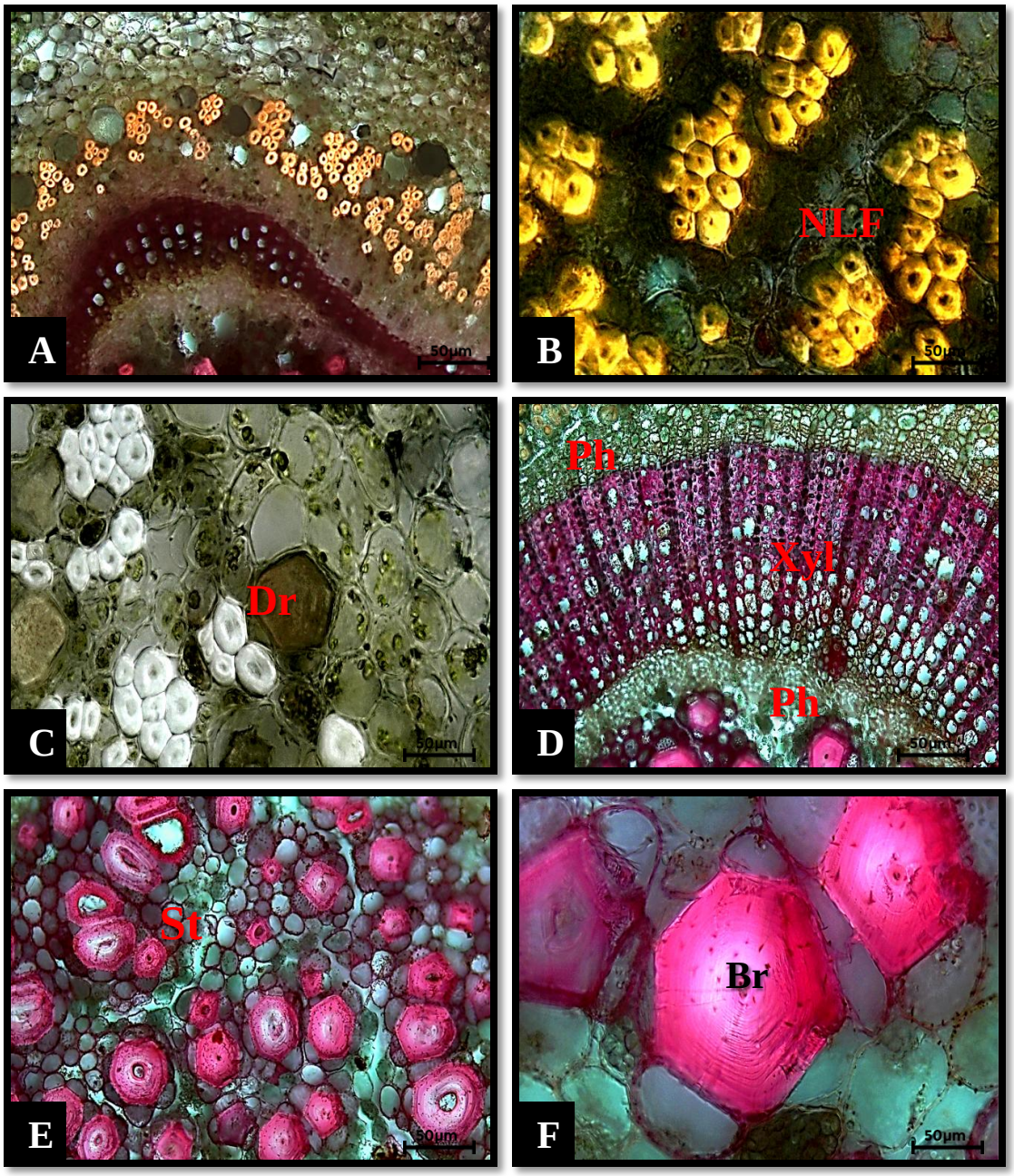


Plate 6: A. Portion of the stem of *R. verticillata* showing non-lignified fibers and druce crystals; **B.** Enlarged view of non-lignified fibers**; C.** Enlarged view of a druce crystal; **D.** Bicollateral vascular bundle in the stem of *R. verticillata*; **E.** Pith showing presence of Brachysclereids and starch granules; **F.** Enlarged view of Brachysclereids.
NLF= Non-lignified fibers, Dr= Druce crystals, Xyl= Xylem, Ph= Phloem, St= Starch, Br=Brachysclereids

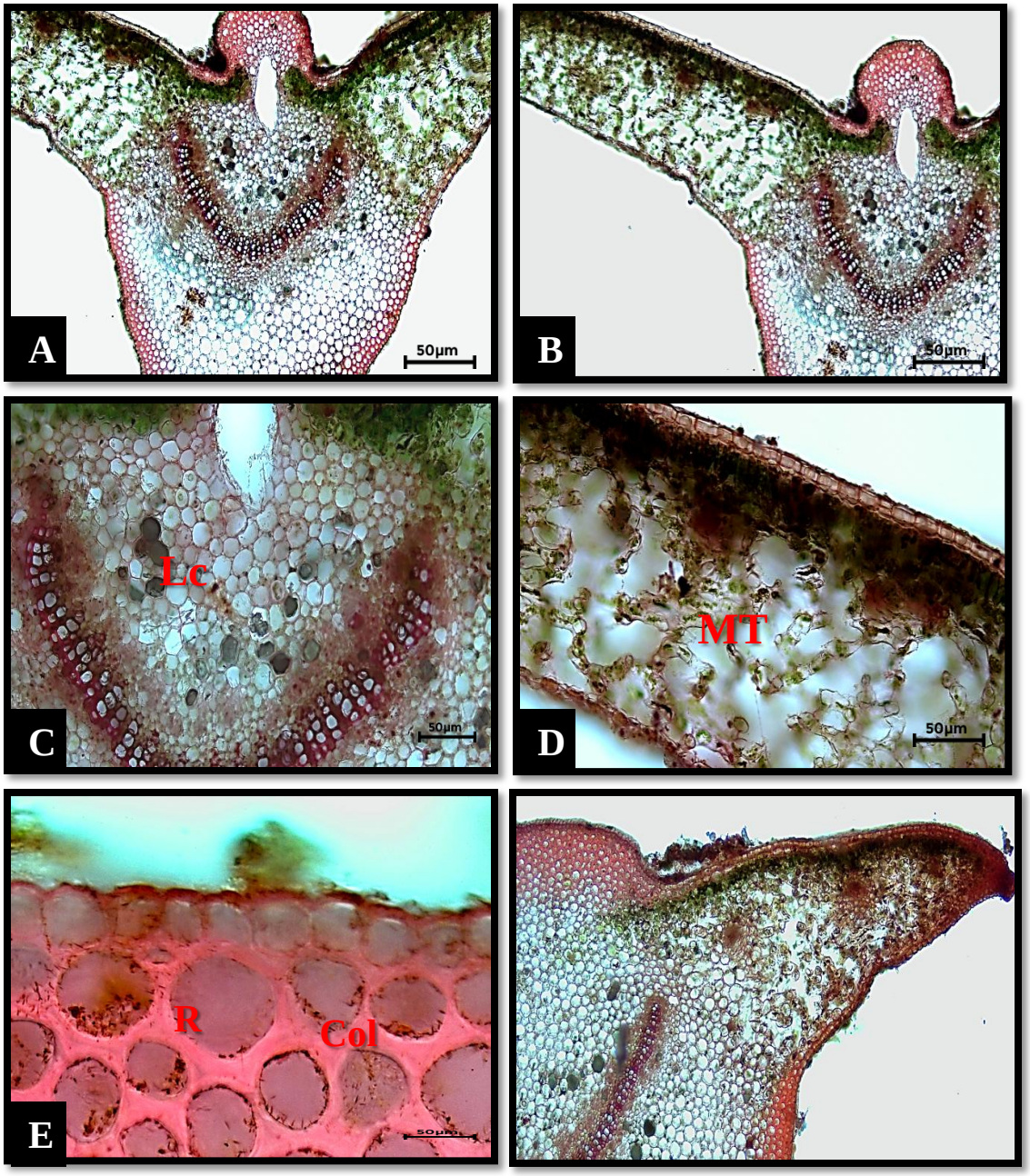


Plate 7: A. Overview of the leaf of *R. verticillata*; **B**. Leaf lamina of *R. verticillata*; **C.** Laticifers in ground tissue of the leaf; **D.** Mesophyll tissue in the leaf lamina of *R. verticillata*; **E.** Raphides in the collenchymatous tissue of the leaf; **F.** Portion of the petiole of the leaf of *R. verticillata*.
Lc=Laticifers, MT=Mesophyll tissue, R=Raphides, Col=Collenchymatous cells.

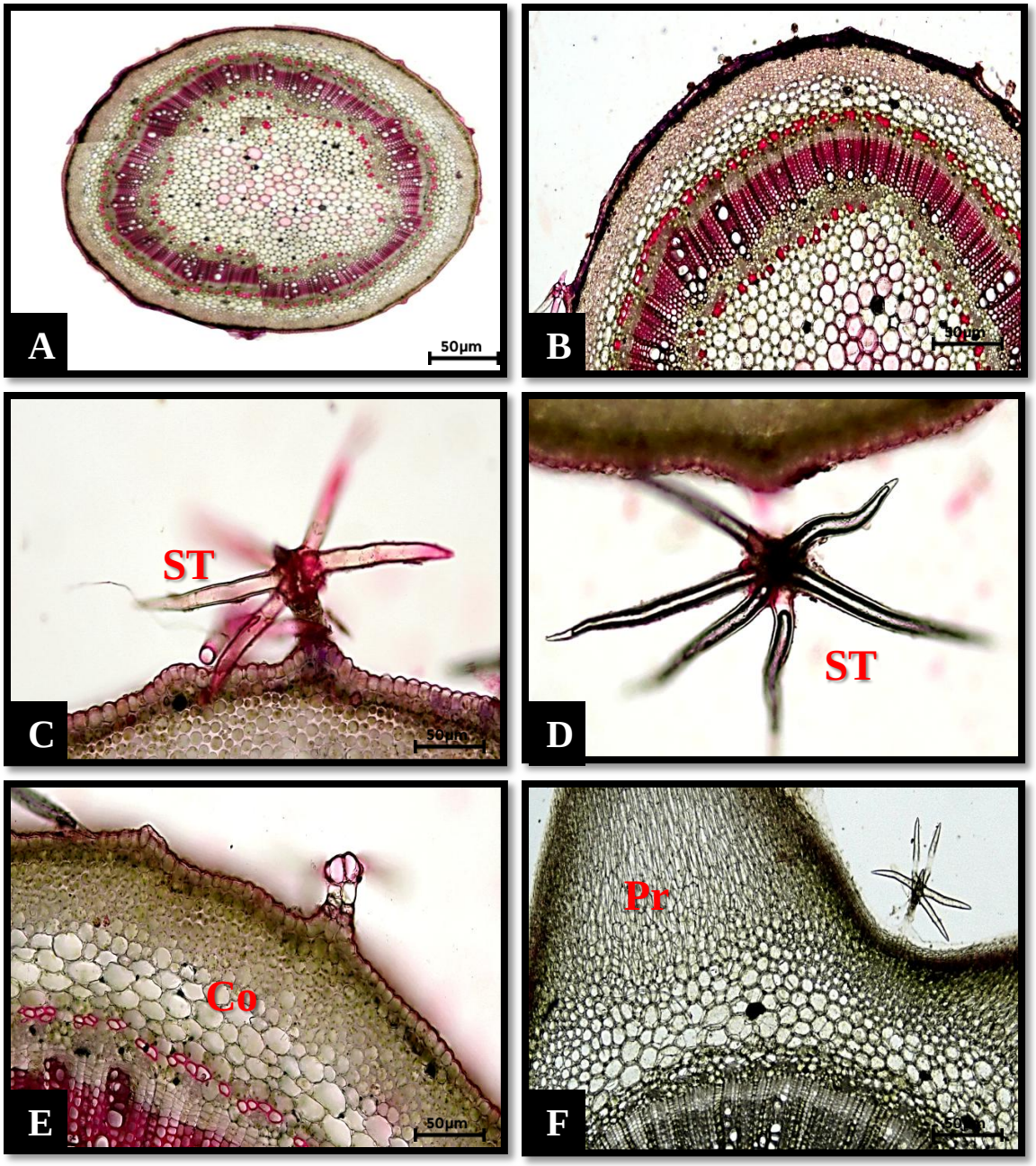


Plate 8: A. Overview of the stem of S. anguivi; **B.** Portion of the stem of S. anguivi; **C,D.** Stellate trichome on the epidermis of the stem; **E.** Portion of the stem showing hypodermis and cortex of the stem; **F.** Transverse section of the stem passing through the prickle.
ST=Stellate Trichome, Co=Cortex, Pr= Prickle

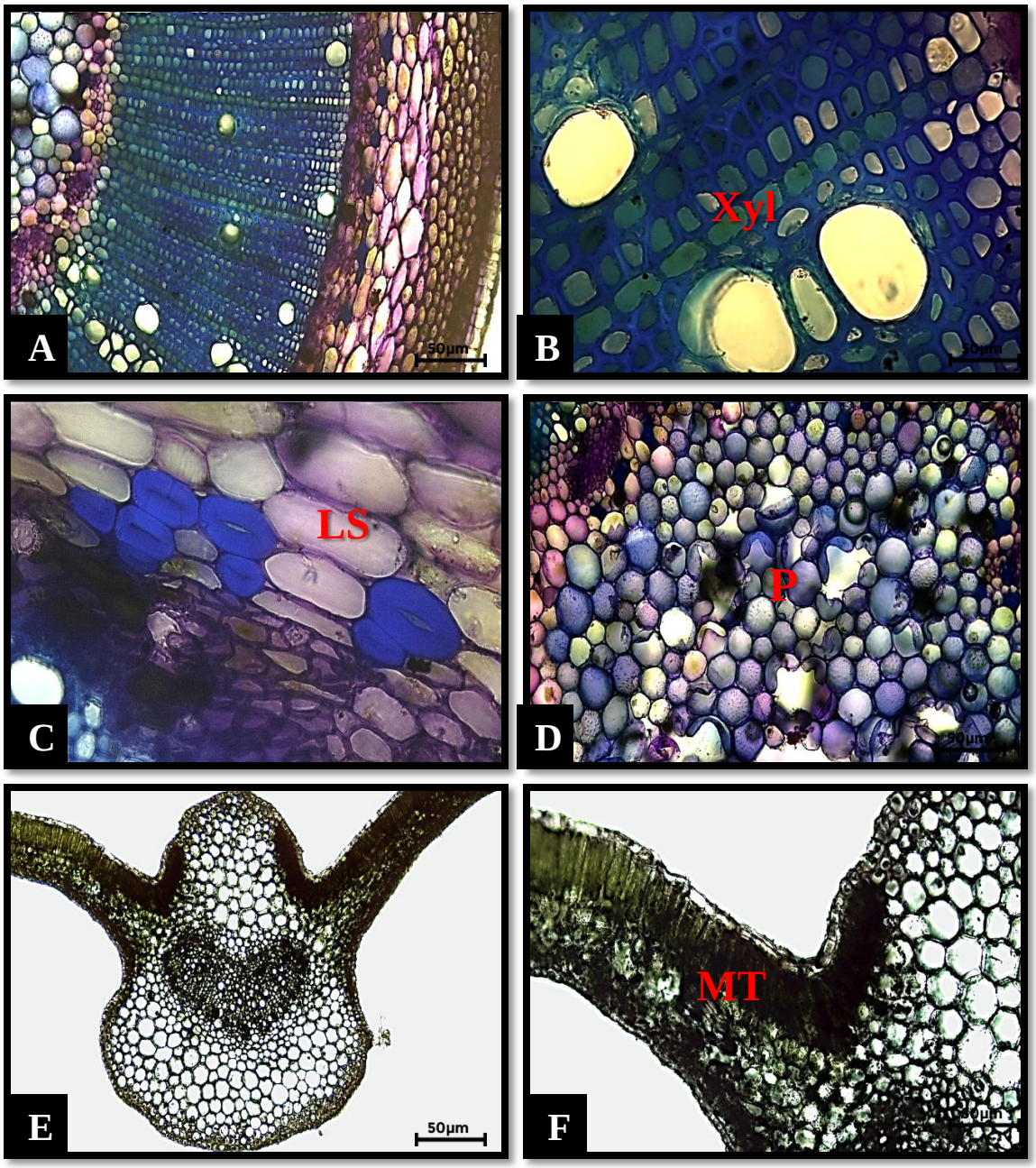


Plate 9: A. Bicollateral vascular bundles in the stem of *S. anguivi*; **B.** Enlarged view of the vascular bundles; **C.** Lignified sclereids; **D.** Pith in the stem of S. anguivi. **E.** Overview of the leaf of *S. anguivi*; **F.** Leaf lamina of *S. anguivi* showing mesophyll tissue.
Xyl=Xylem, LS= Lignified sclereids, P=Pith, MT=Mesophyll tissue

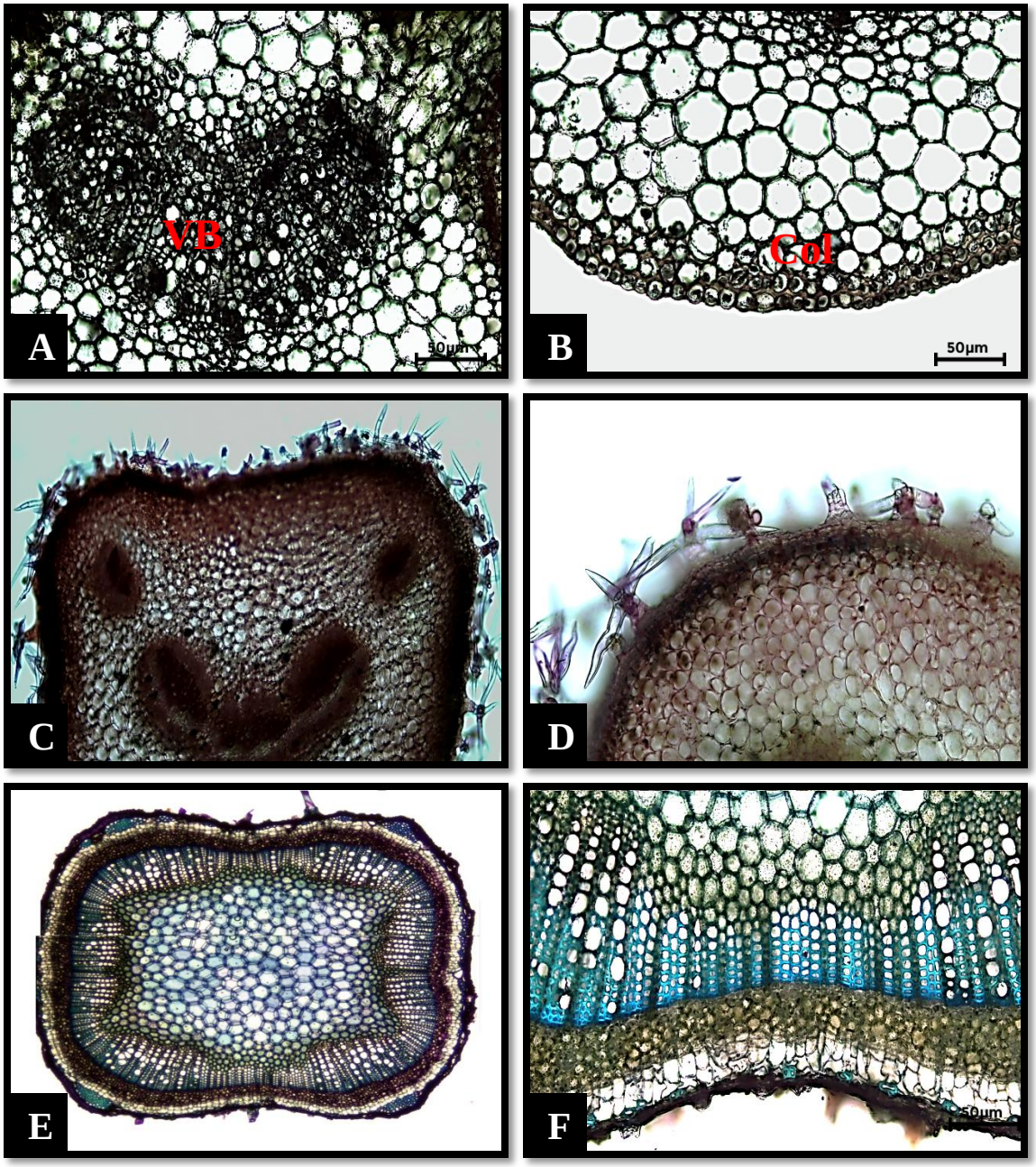


Plate 10: A. Bicollateral vascular bundles in the leaf of *S. anguivi*; **B.** Collenchymatous hypodermis in the leaf of *S. anguivi*; **C.** Portion of the petiole of leaf in *S.anguivi;* **D.** Portion of the petiole showing stellate trichomes; **E.** Overview of the stem of *L. camara;* **F.** Portion of the stem of *L. camara*.
VB=Vascular Bundle, Col=Collenchymatous cells.

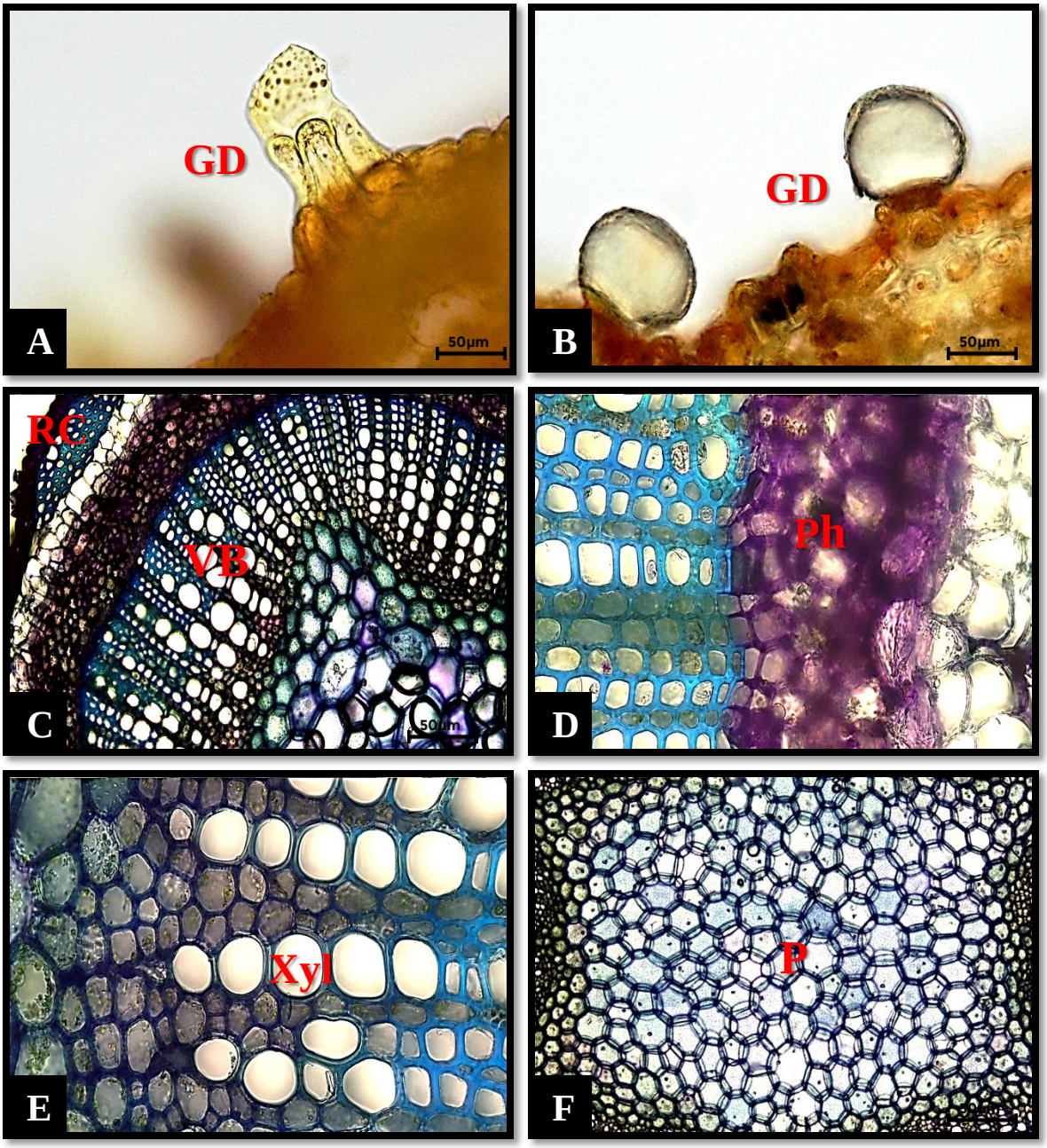


Plate 11: A,B. Glandular trichomes on the surface of the stem in *L. camara;* **C.** Portion of the stem in *L. camara* showing a resin canal and vascular bundles; **D,E.** Collateral vascular bundles in the stem of *L. camara*; **F.** Star-shaped pith in the stem of *L. camara.*

GD=Glandular trichomes, RC=Resin canal, VB=Vascular bundle, Xyl=Xylem, Ph=Phloem, P=Pith.

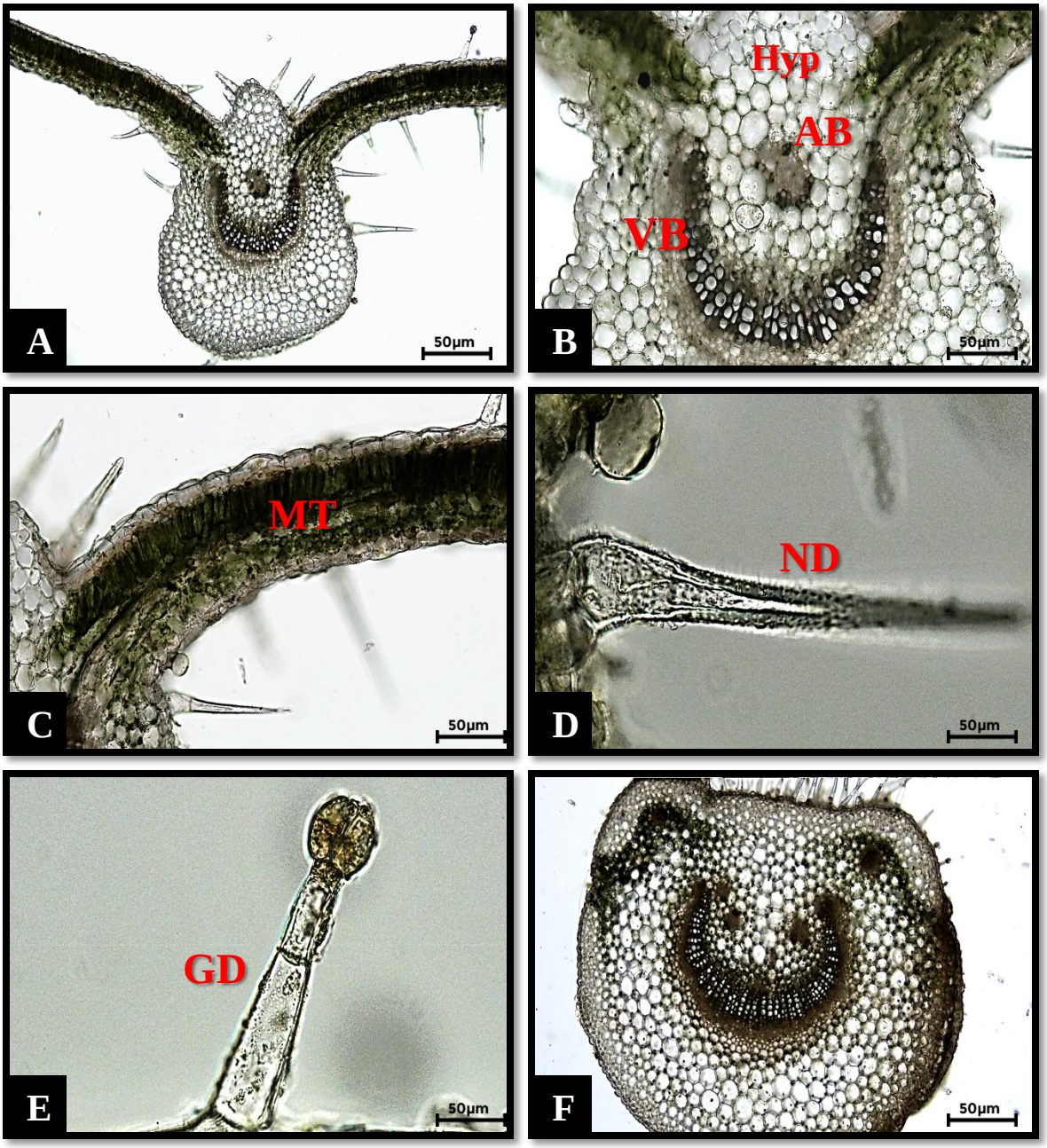


Plate 12: A. Overview of the leaf of *L. camara*; **B.** U-shaped vascular bundles in the midrib of the leaf in *L. camara;* **C.** Leaf lamina showing mesophyll tissue; **D.** Glandular trichome on the surface of the leaf; **E.** Glandular trichome on the surface of the leaf; **F.** Overview of the petiole of the leaf of *L. camara*.
Hyp=Hypodermis, VB=Vascular bundles, AB=Accessory bundle, MT=Mesophyll tissue, ND=Non-glandular trichome, GD=Glandular trichome.

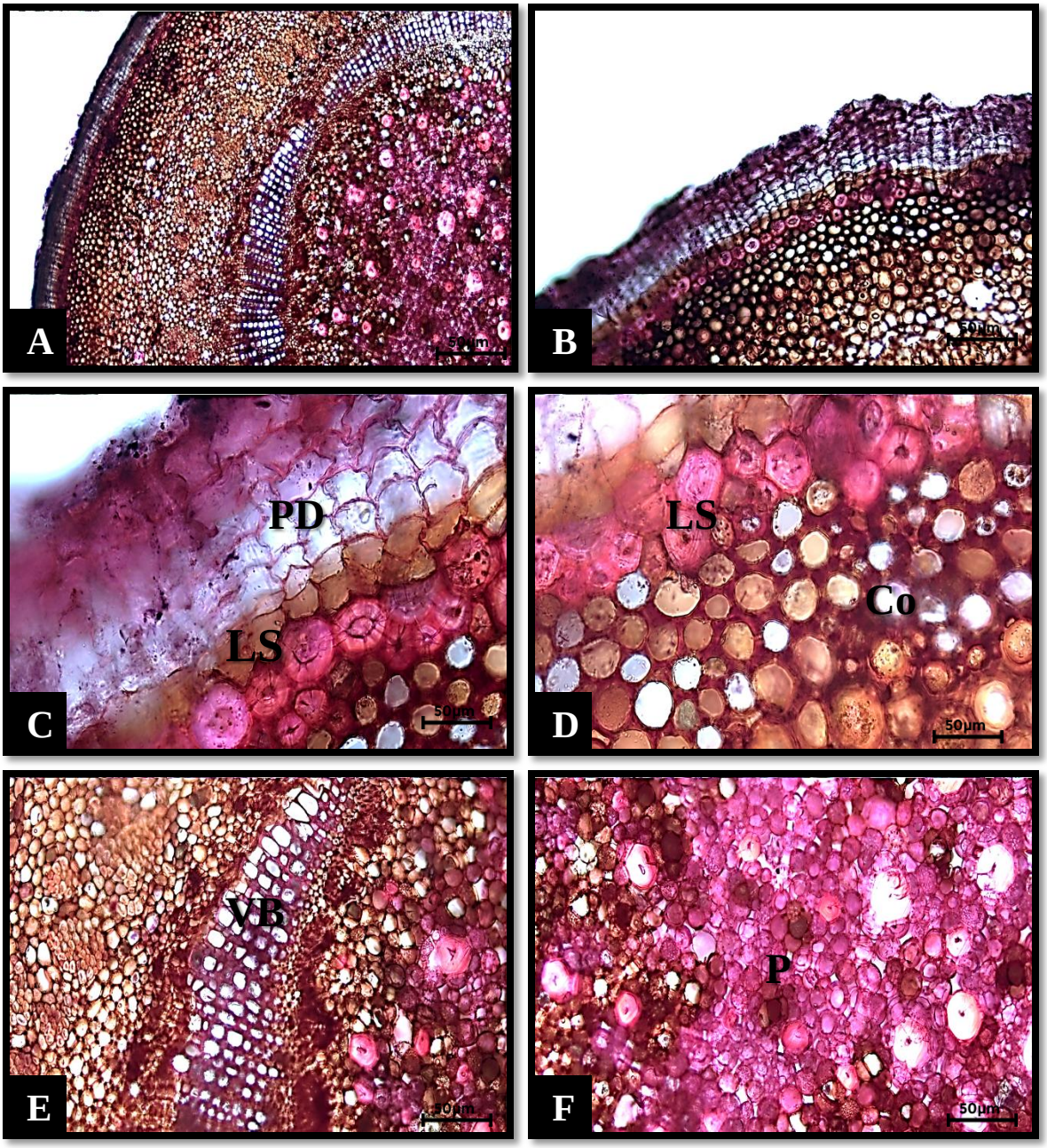


Plate 13: A. Portion of the stem of *T. alternifolia;* **B.** Portion of the stem showing outer cellular layers; **C.** Enlarged portion of the periderm; **D.** Portion of the stem showing cortical cells and lignified sclereids; **E.** Bicollateral vascular bundles in the stem of T. *alternifolia*; **F.** Pith with brachysclereids and starch granules.
PD=Periderm, LS= Lignified sclereids, VB=Vascular bundles, P=Pith.

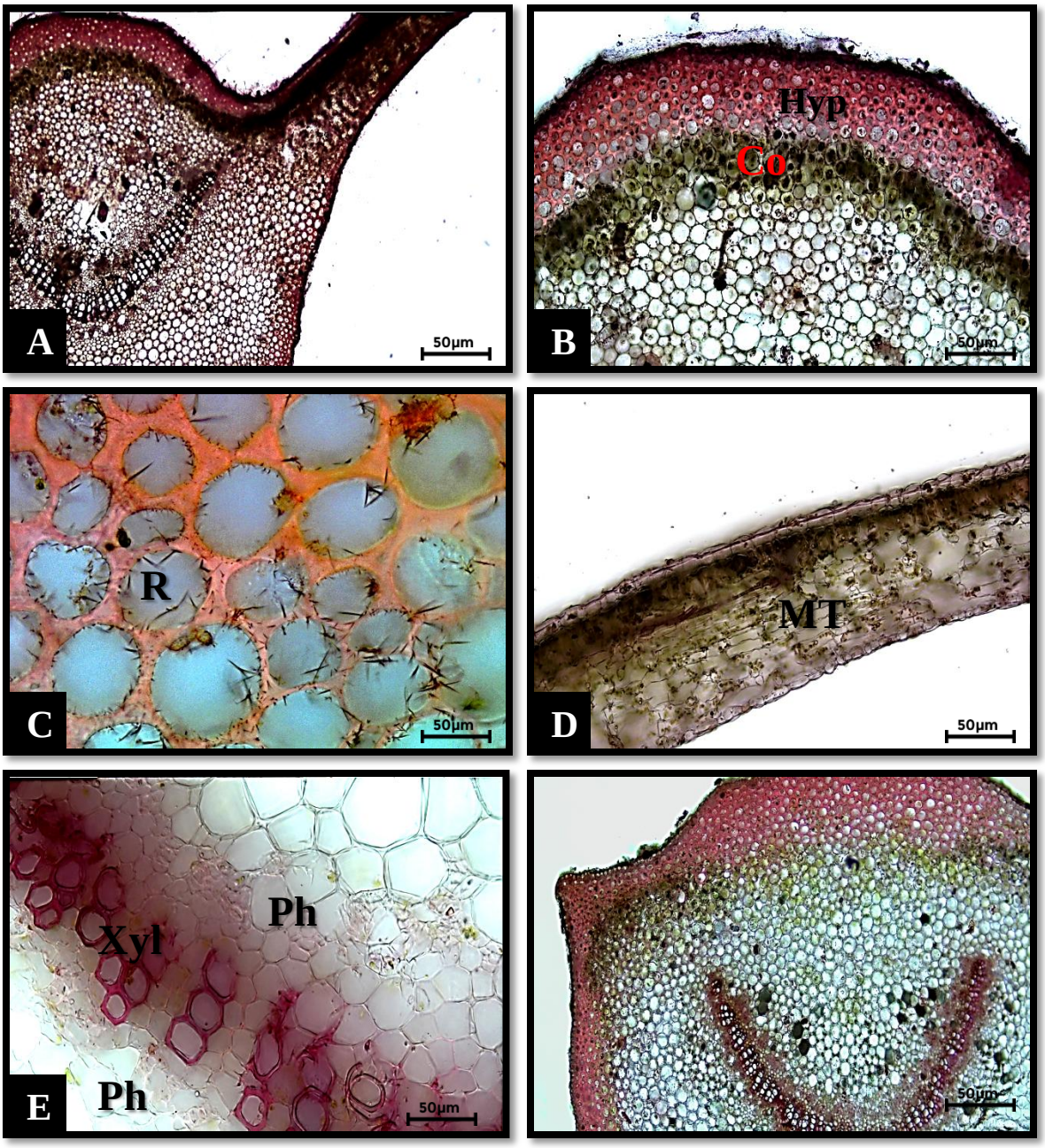


Plate 14: A. Portion of the leaf of *T. alternifolia*; **B.** Annular collenchymatous hypodermis in the leaf; **C.** Raphides in the hypodermis of the leaf; **D.** Mesophyll tissue in the leaf lamina of *T. alternifolia*; **E.** Bicollateral vascular bundles in the leaf; **F.** Portion of the petiole of T. *alternifolia.*

Hyp=Hypodermis, Co=Cortex, R=Raphides, MT=Mesophyll tissue, Xyl=Xylem, Ph=Phloem.

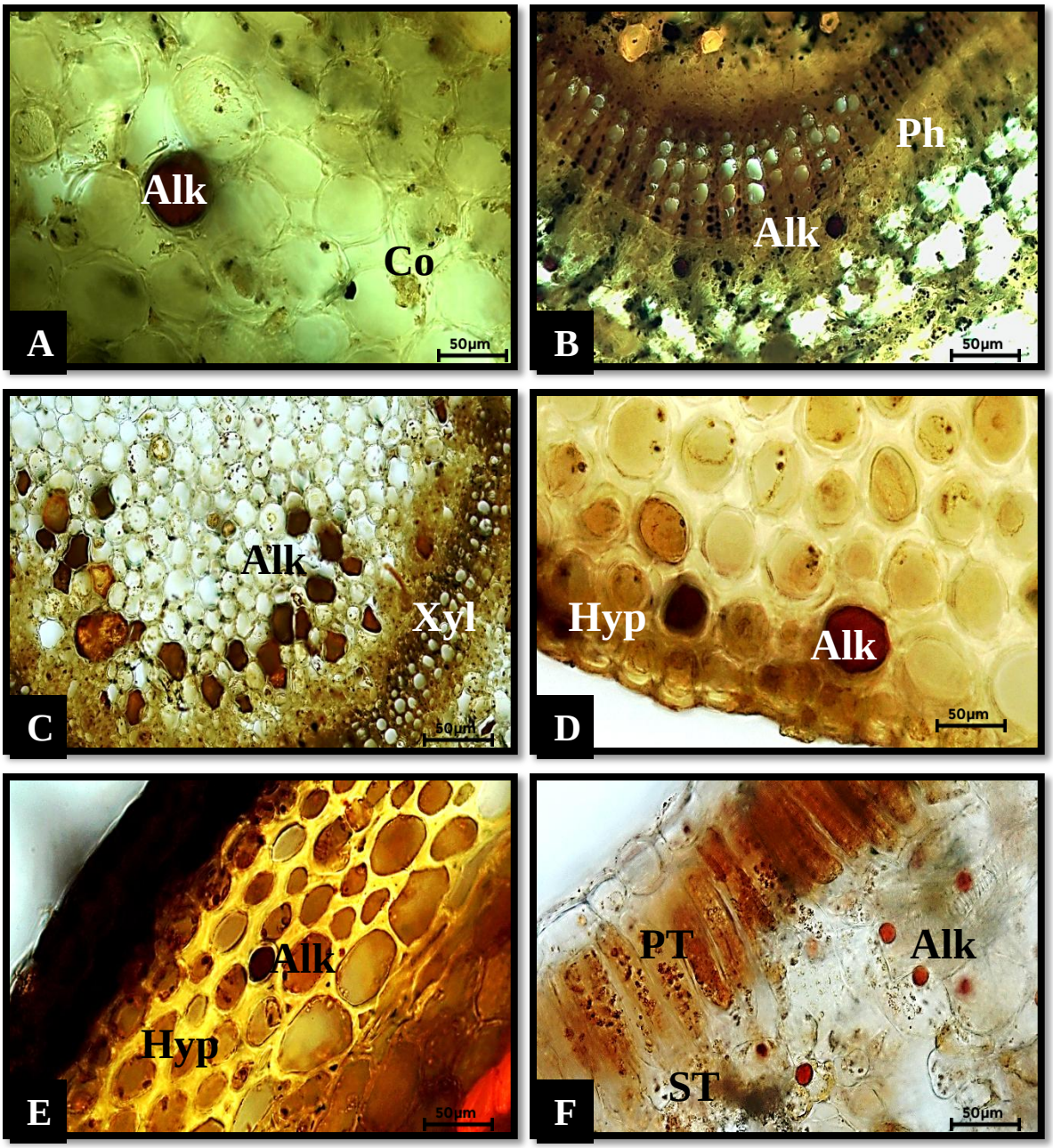


Plate 21. Localization of Alkaloids.
A-B. Stem of *R. verticillata*; **C.** Leaf of *R. verticillata*; **D.** Petiole of *R. verticillata*; **E**. Stem of *S. anguivi;* **F.** Leaf of *S. anguivi*.
Alk=Alkaloids, Co=Cortex, Xyl= Xylem, Ph=Phloem, Hyp=Hpodermis, PT=Palisade tissue, ST=Spongy tissue.

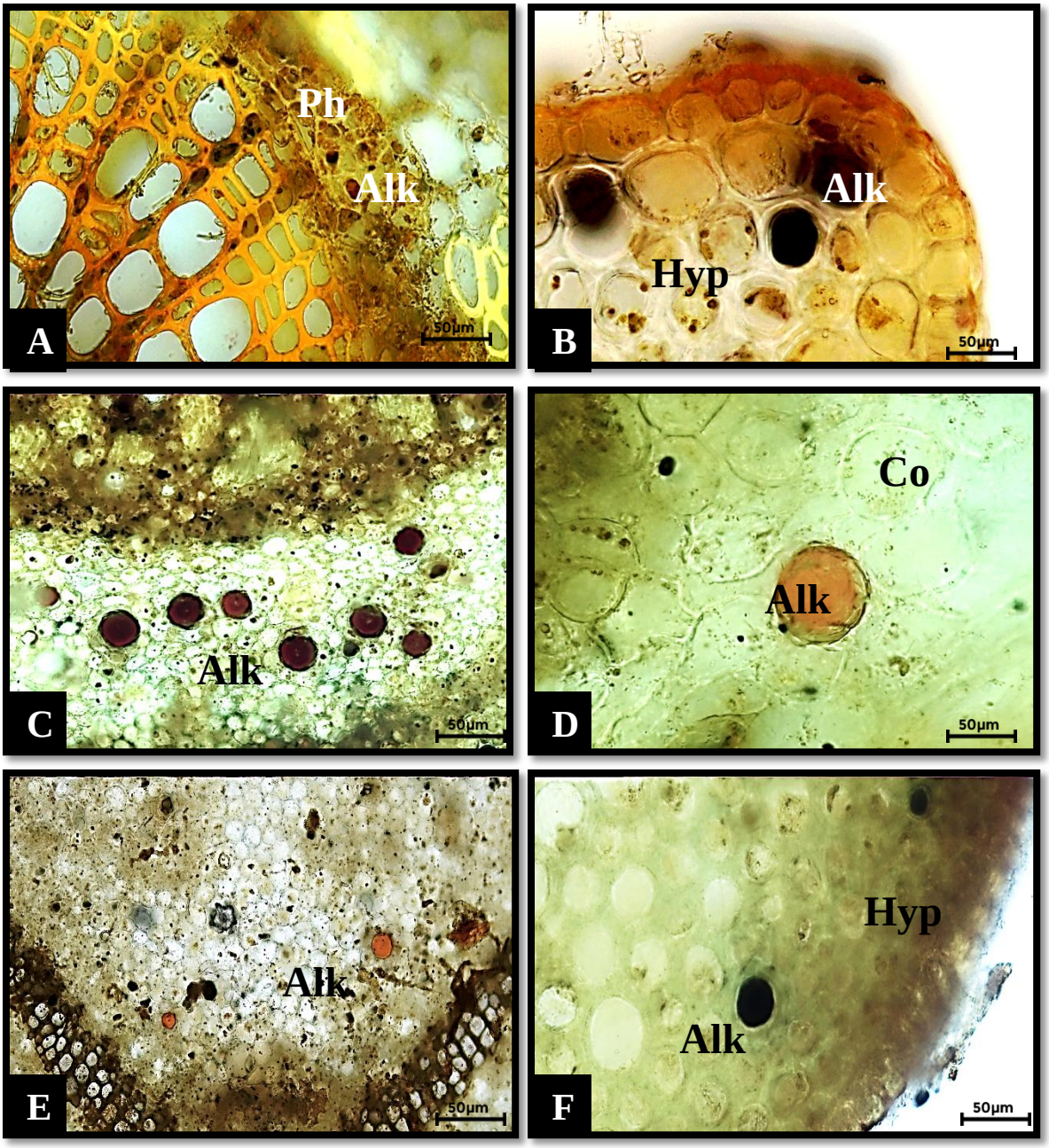


Plate 22. Localization of Alkaloids

A,B. Stem of *L. camara;* **C,D**. Stem of *T. alternifolia;* **E.** Leaf of *T. alternifolia;* **F**. Petiole of *T. alternifolia*

Alk=Alkaloids, Ph=Phloem, Hyp=Hypodermis, Co=Cortex, GT=Ground tissue

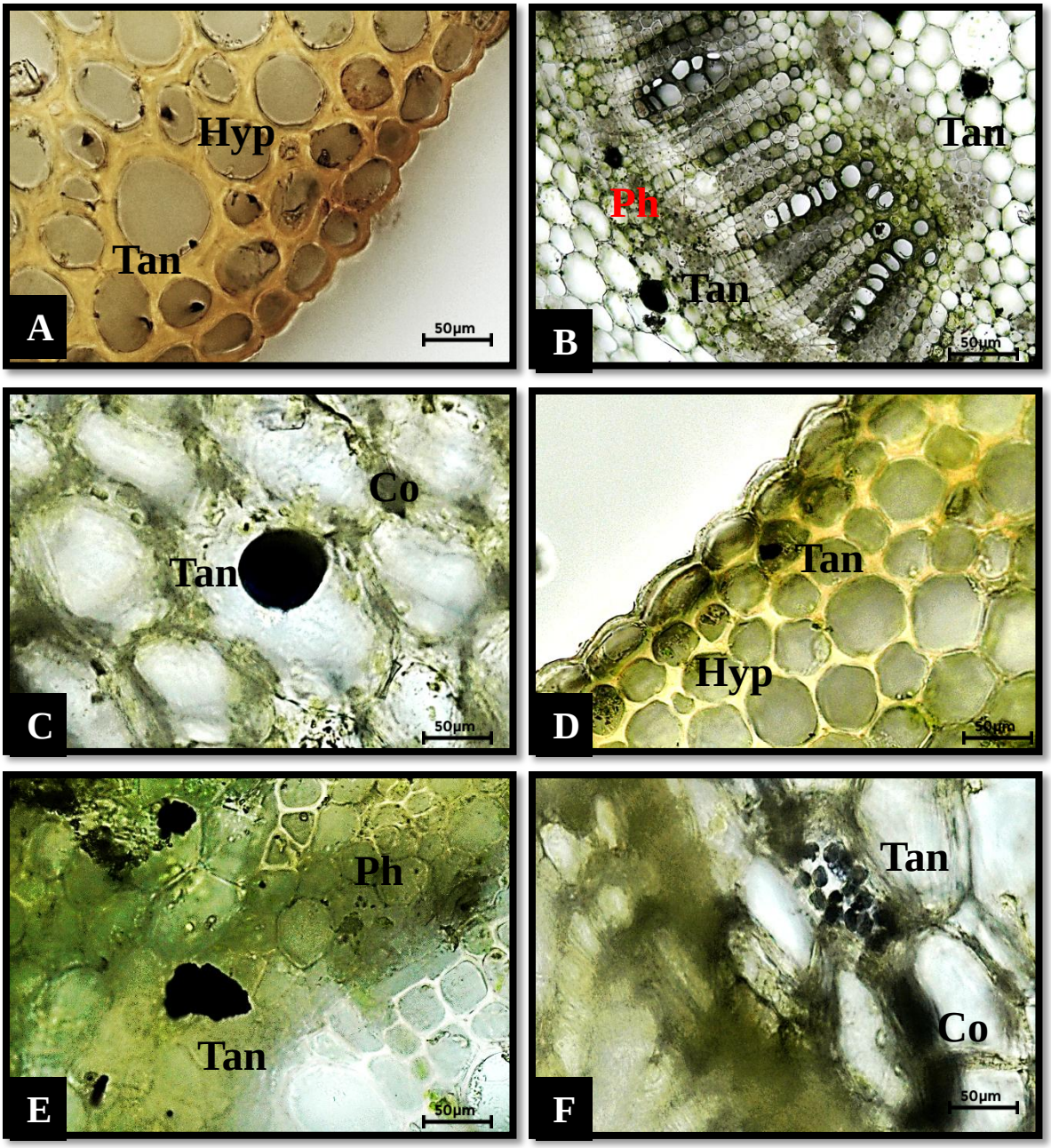


Plate 23. Localization of Phenolic compounds

A. Leaf of *R. verticillata;* **B-C**. Stem of *S. anguivi;* **D-E**. Leaf of *L. camara;* **F.** Stem of *T. alternifolia.*

Tan=Tannins, Hyp=Hypodermis, P=Pith, Ph=Phloem, Co=Cortex

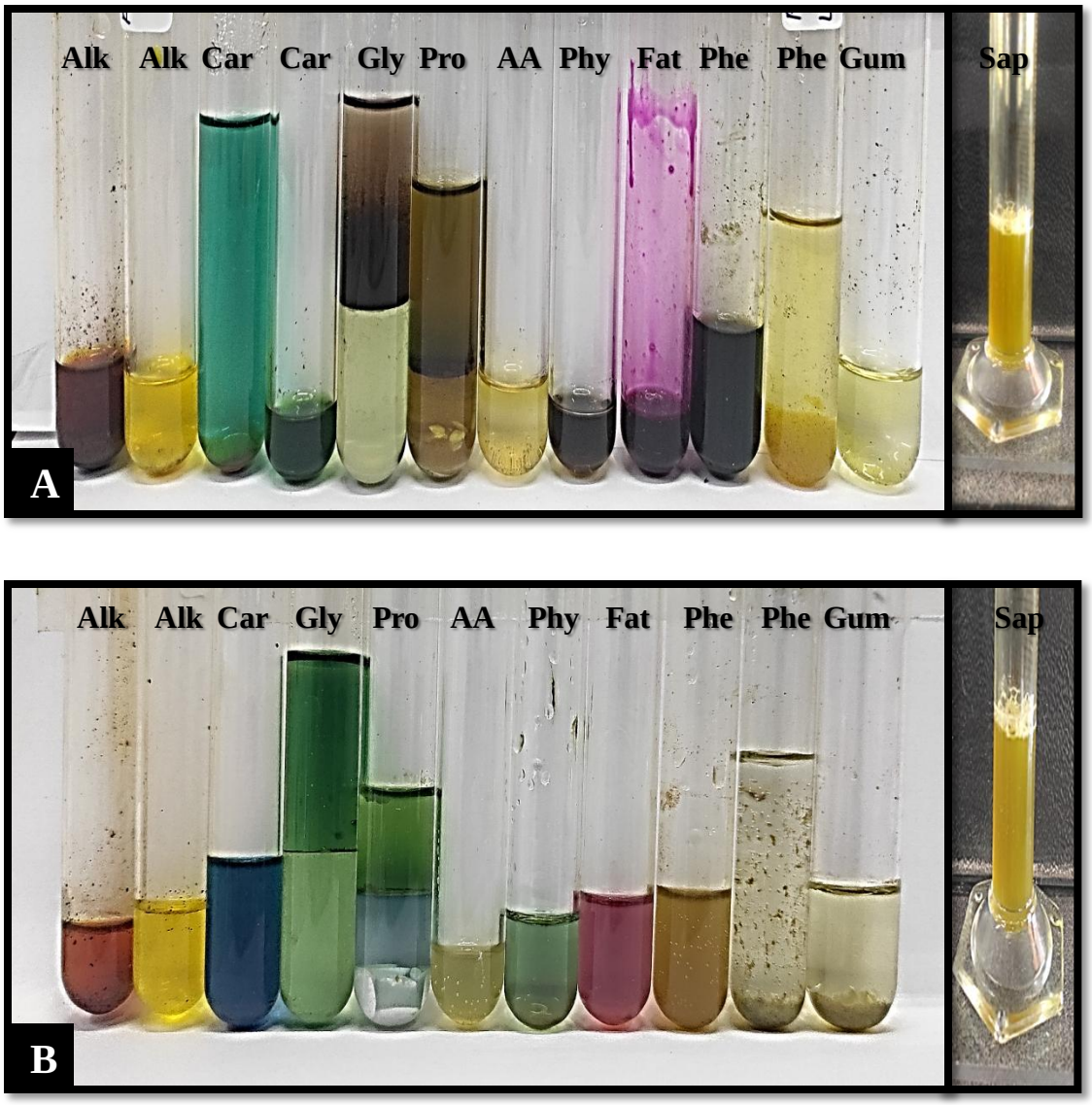
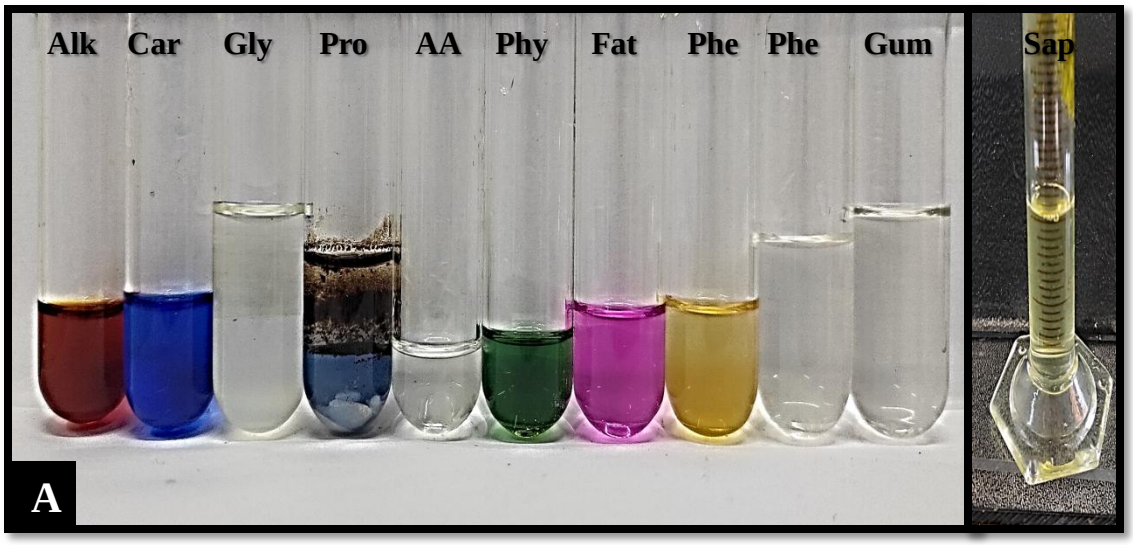


Plate 24: A. Preliminary qualitative phytochemical analysis of *R. verticillata* (soxhlet) with methanol. **B.** Preliminary qualitative phytochemical analysis of R. *verticillata* (soxhlet) with Ethyl acetate. Alk=Alkaloids, Car=Carbohydrates, Gly=Glycosides, Pro=Proteins, AA=Amino acids, Phy=Phytosterols, Fat=Fats and Oils, Phe=Phenolics and tannins, Gum=Gums and Mucilages.



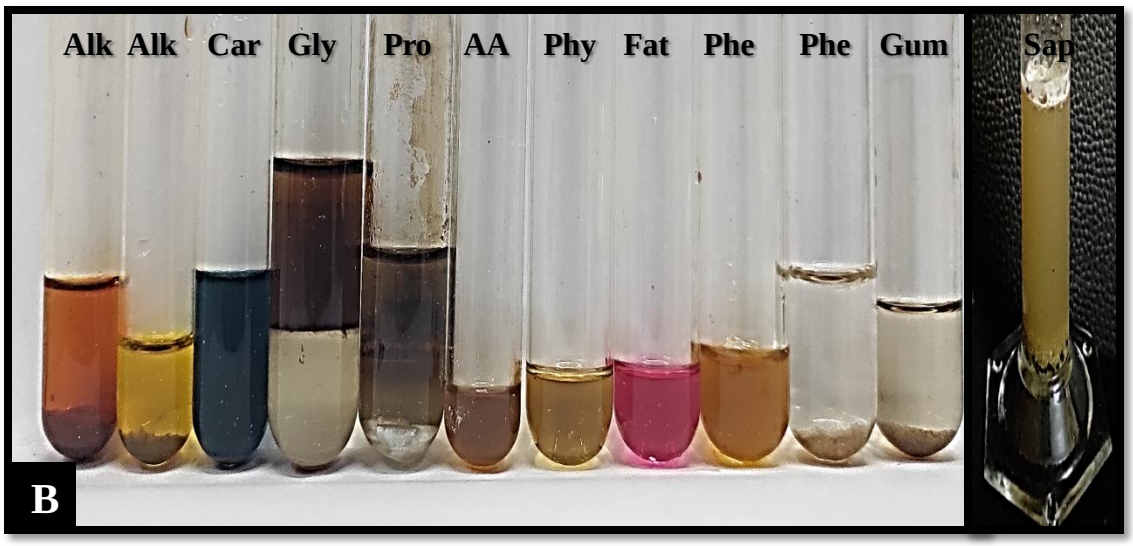


Plate 25: A. Preliminary qualitative phytochemical analysis of *R. verticillata* (soxhlet) with n-hexane. **B.** Preliminary qualitative phytochemical analysis of *R. verticillata* (soxhlet) with water.
Alk=Alkaloids, Car=Carbohydrates, Gly=Glycosides, Pro=Proteins, AA=Amino acids, Phy=Phytosterols, Fat=Fats and Oils, Phe=Phenolics and tannins, Gum=Gums and Mucilages.

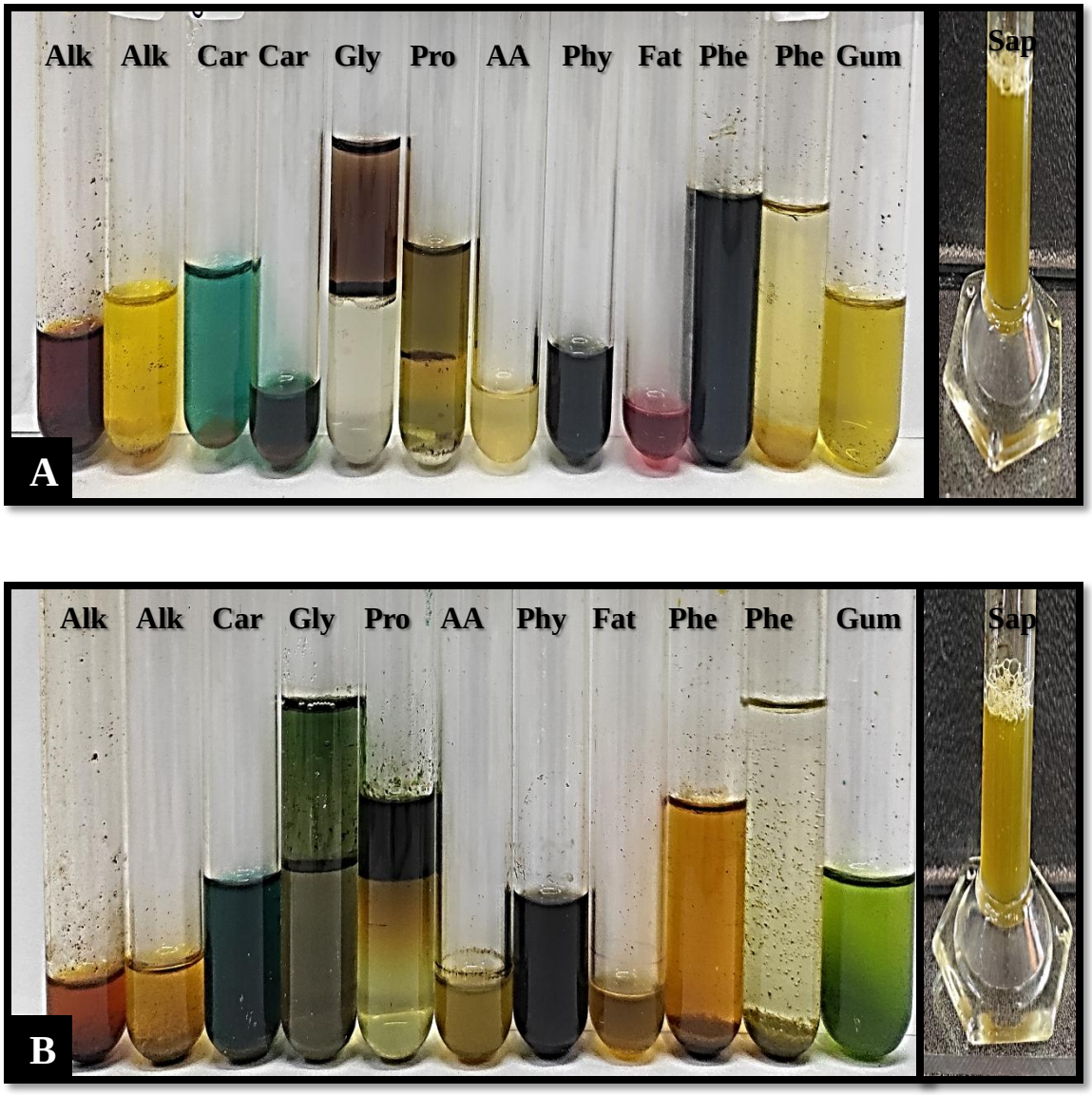


Plate 26: A. Preliminary qualitative phytochemical analysis of *R. verticillata* (maceration) with methanol**.** **B**. Preliminary qualitative phytochemical analysis of *R. verticillata* (maceration) with ethyl acetate.

Alk=Alkaloids, Car=Carbohydrates, Gly=Glycosides, Pro=Proteins, AA=Amino acids, Phy=Phytosterols, Fat=Fats and Oils, Phe=Phenolics and tannins, Gum=Gums and Mucilages.

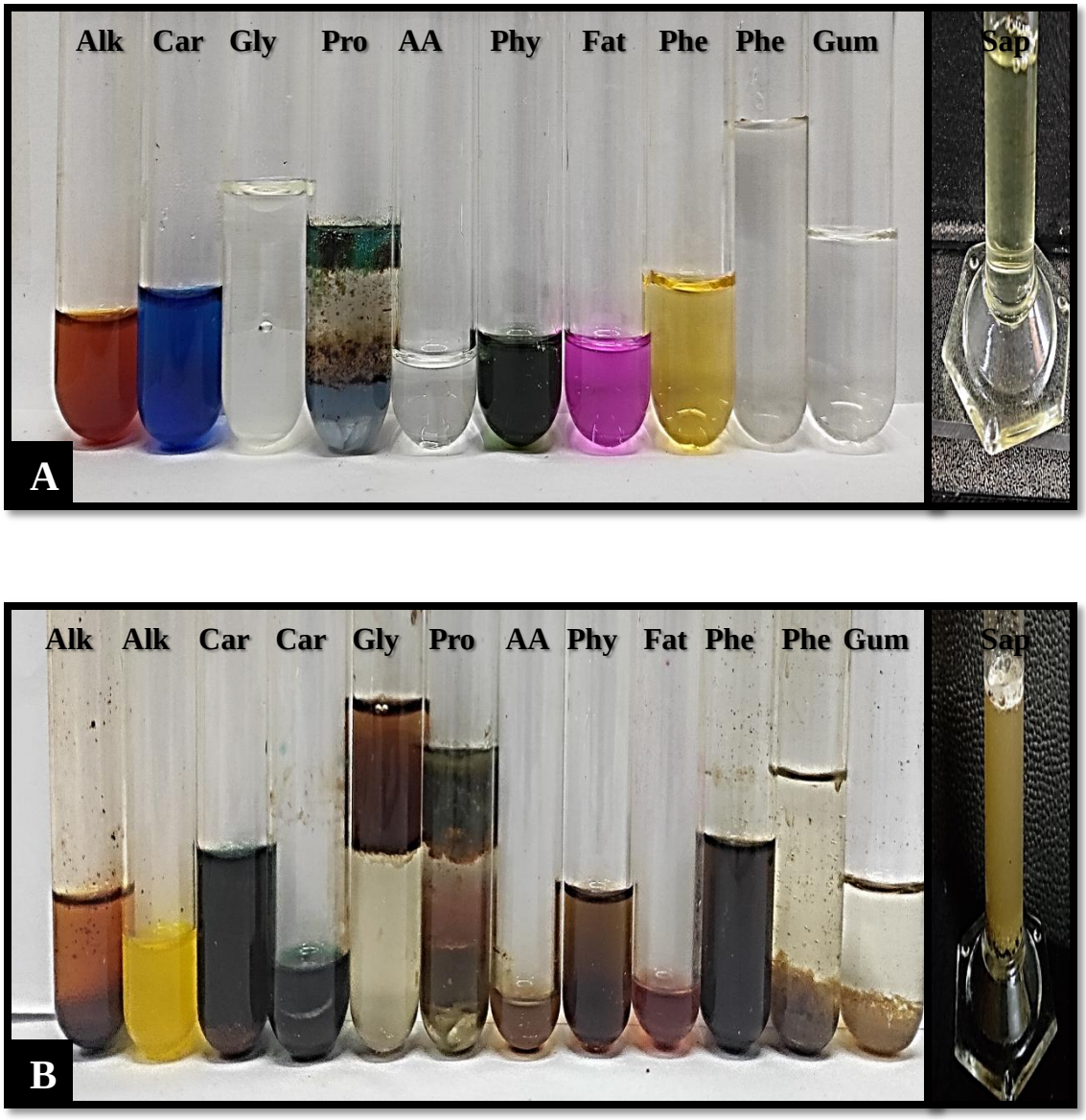
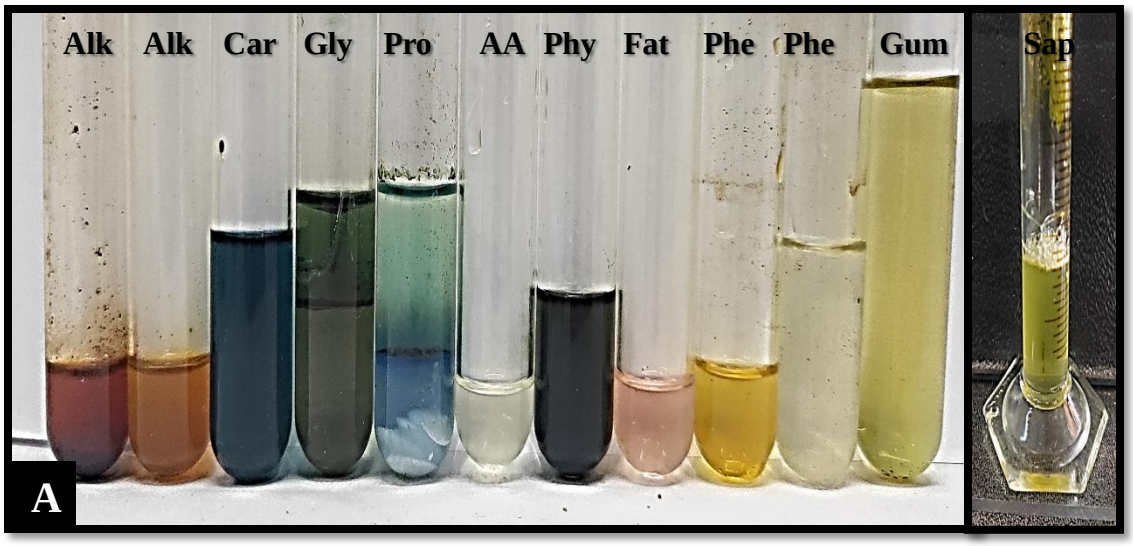


Plate 27: A. Preliminary qualitative phytochemical analysis of *R. verticillata* (maceration) with n-hexane. **B**. Preliminary qualitative phytochemical analysis of *R. verticillata* (maceration) with water
Alk=Alkaloids, Car=Carbohydrates, Gly=Glycosides, Pro=Proteins, AA=Amino acids, Phy=Phytosterols, Fat=Fats and Oils, Phe=Phenolics and tannins, Gum=Gums and Mucilages.



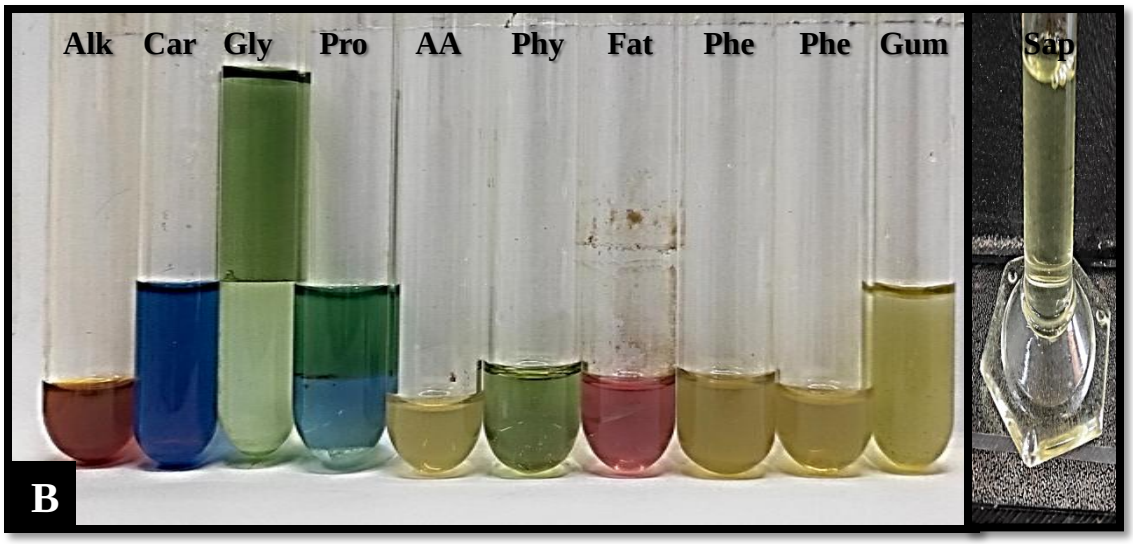


Plate 28: A. Preliminary qualitative phytochemical analysis of *S. anguivi* (soxhlet) with methanol. **B**. Preliminary qualitative phytochemical analysis of *S. anguivi* (soxhlet) with ethyl acetate.
Alk=Alkaloids, Car=Carbohydrates, Gly=Glycosides, Pro=Proteins, AA=Amino acids, Phy=Phytosterols, Fat=Fats and Oils, Phe=Phenolics and tannins, Gum=Gums and Mucilages.

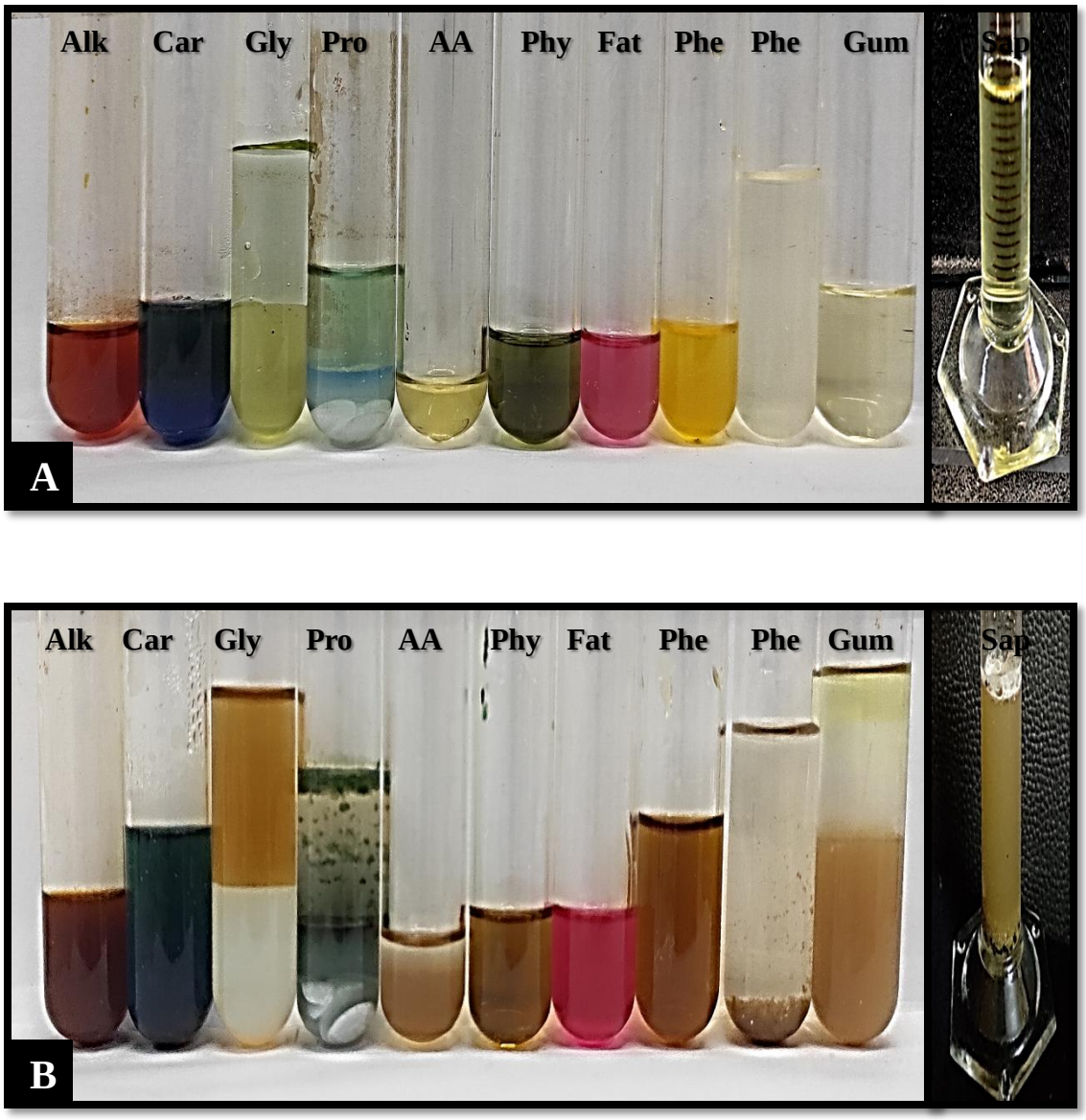


Plate 29: A. Preliminary qualitative phytochemical analysis of *S. anguivi* (soxhlet) with n-hexane. **B**. Preliminary qualitative phytochemical analysis of *S. anguivi* (soxhlet) with water.
Alk=Alkaloids, Car=Carbohydrates, Gly=Glycosides, Pro=Proteins, AA=Amino acids, Phy=Phytosterols, Fat=Fats and Oils, Phe=Phenolics and tannins, Gum=Gums and Mucilages.

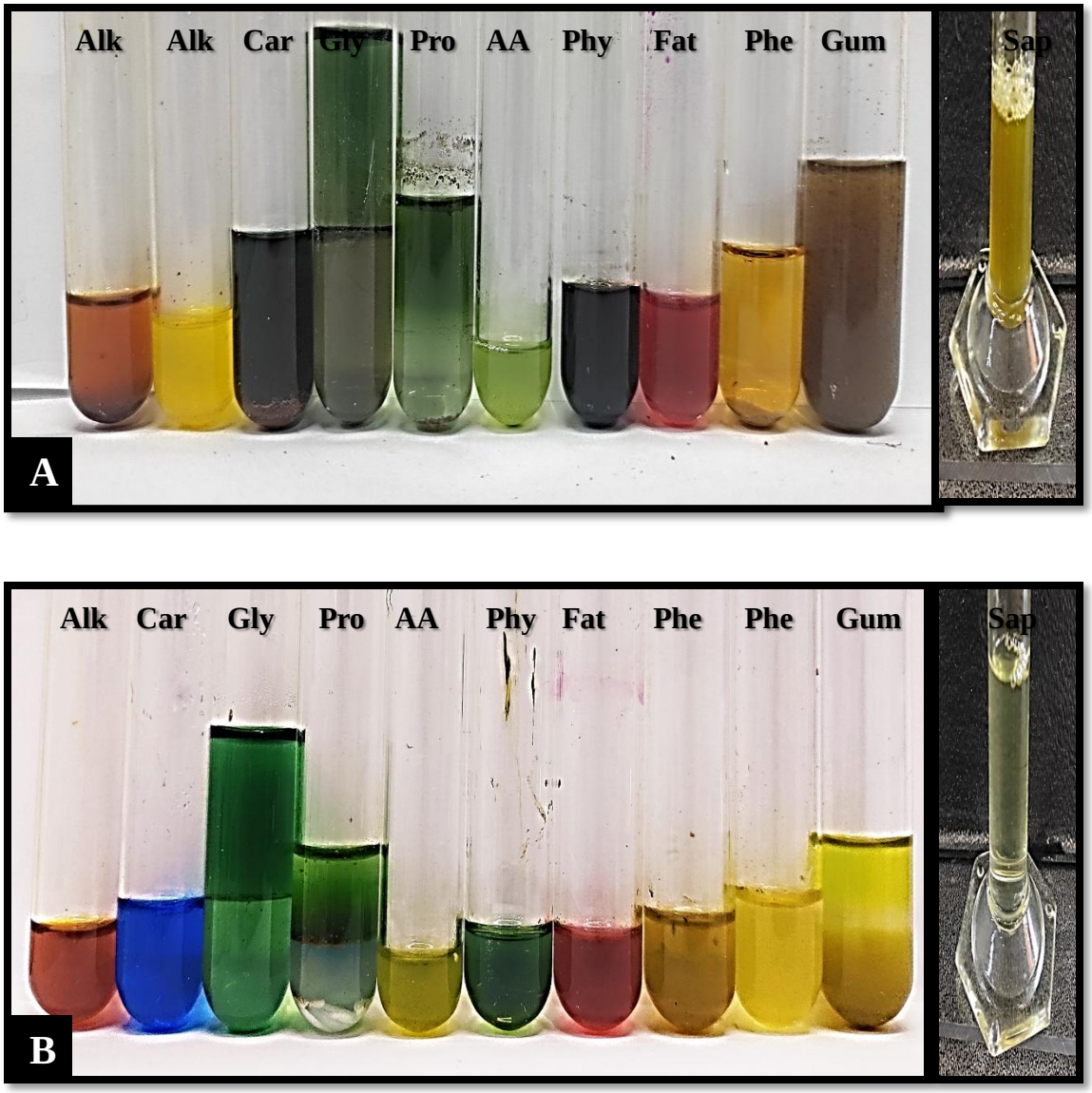
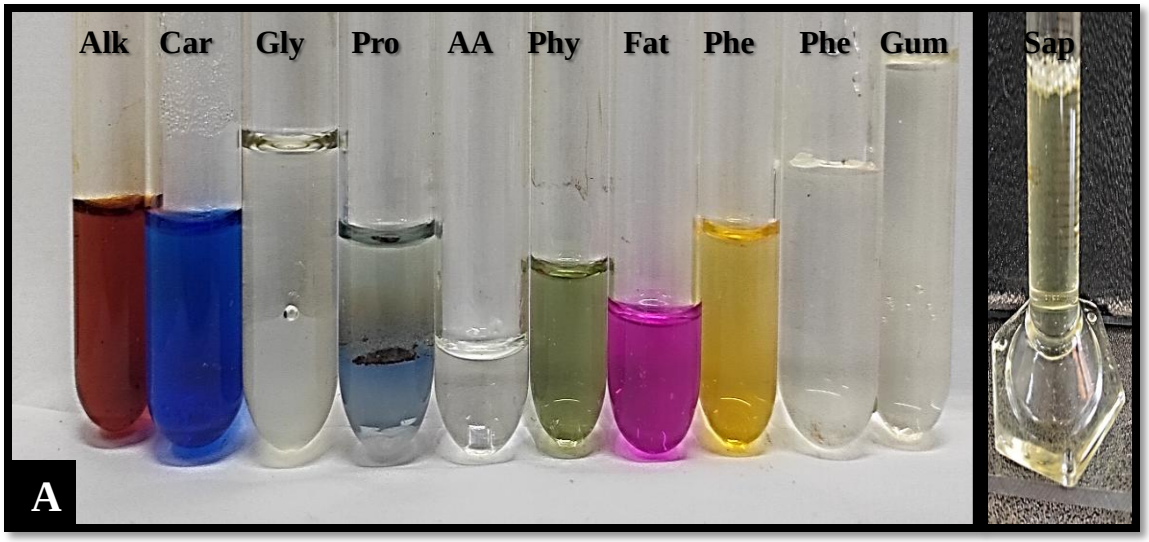


Plate 30: A. Preliminary qualitative phytochemical analysis of *S. anguivi* (maceration) with methanol. **B**. Preliminary qualitative phytochemical analysis of *S. anguivi* (maceration) with ethyl acetate. Alk=Alkaloids, Car=Carbohydrates, Gly=Glycosides, Pro=Proteins, AA=Amino acids, Phy=Phytosterols, Fat=Fats and Oils, Phe=Phenolics and tannins, Gum=Gums and Mucilages.



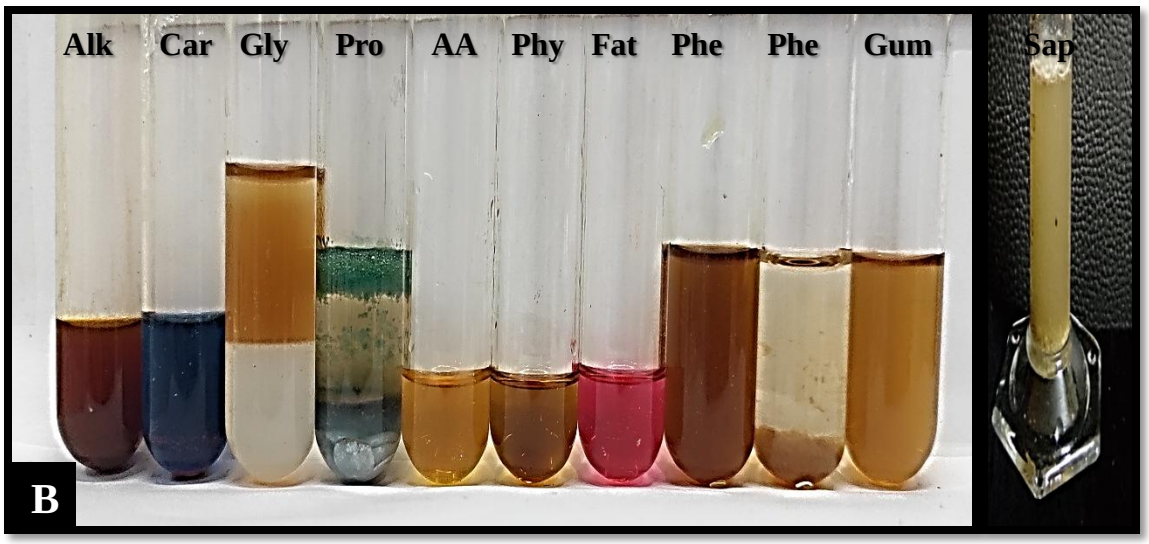
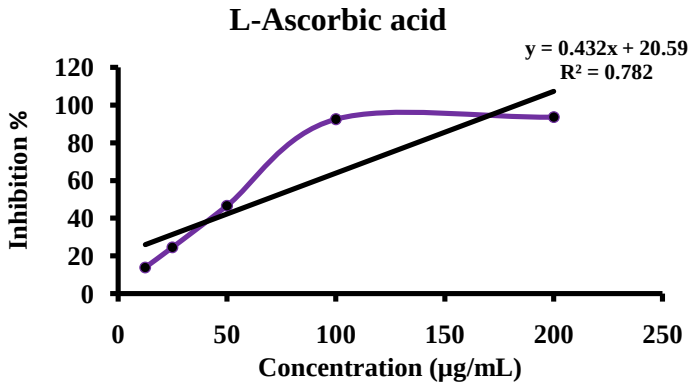
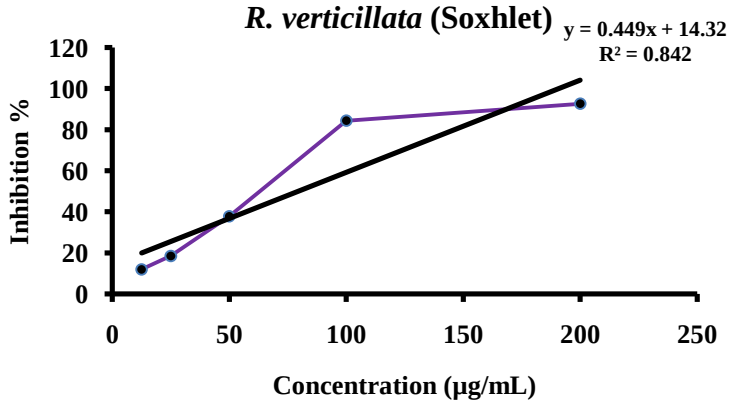


Plate 31: A. Preliminary qualitative phytochemical analysis of *S. anguivi* (maceration) with n-hexane. **B**. Preliminary qualitative phytochemical analysis of *S. anguivi* (maceration) with water.
Alk=Alkaloids, Car=Carbohydrates, Gly=Glycosides, Pro=Proteins, AA=Amino acids, Phy=Phytosterols, Fat=Fats and Oils, Phe=Phenolics and tannins, Gum=Gums and Mucilages.





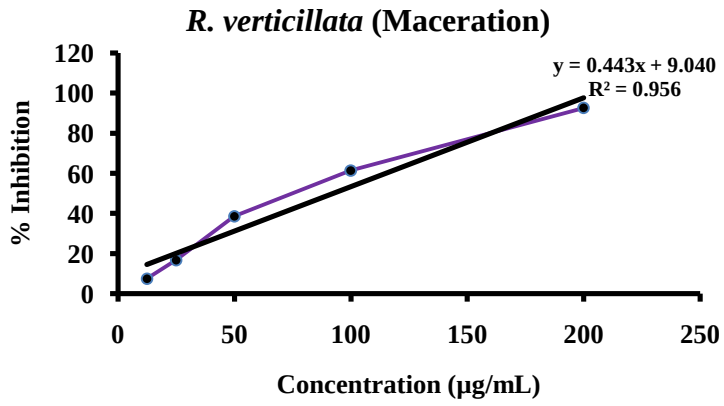


Figure 1. DPPH radical scavenging activity of *R. verticillata* methanol extract (Soxhlet and maceration technique)

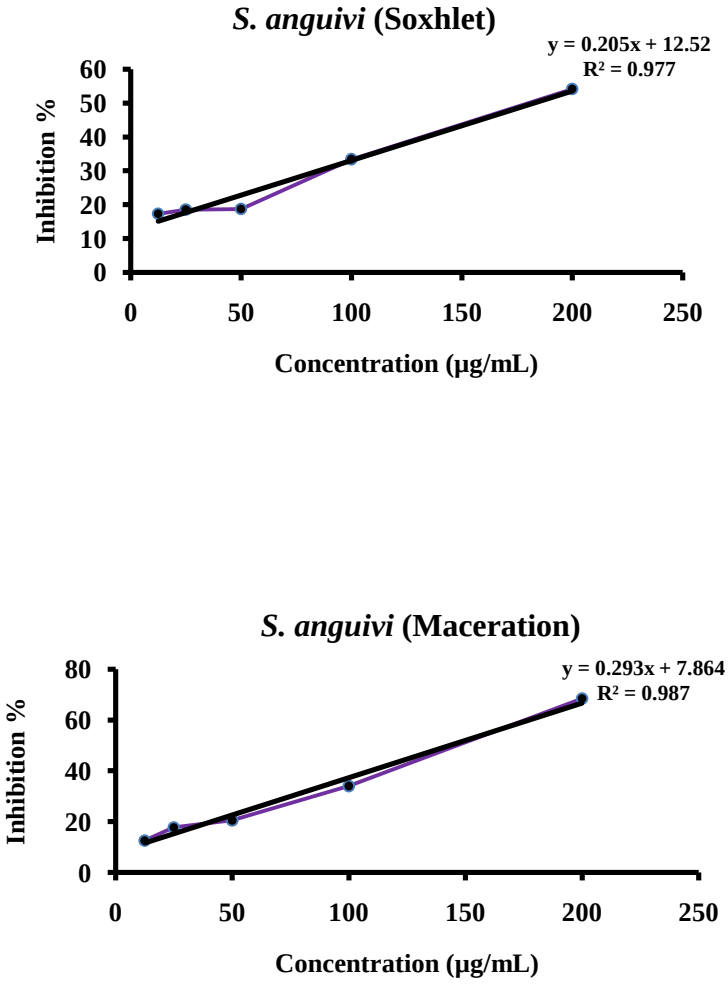


Figure 2. DPPH radical scavenging activity of *S.anguivi*

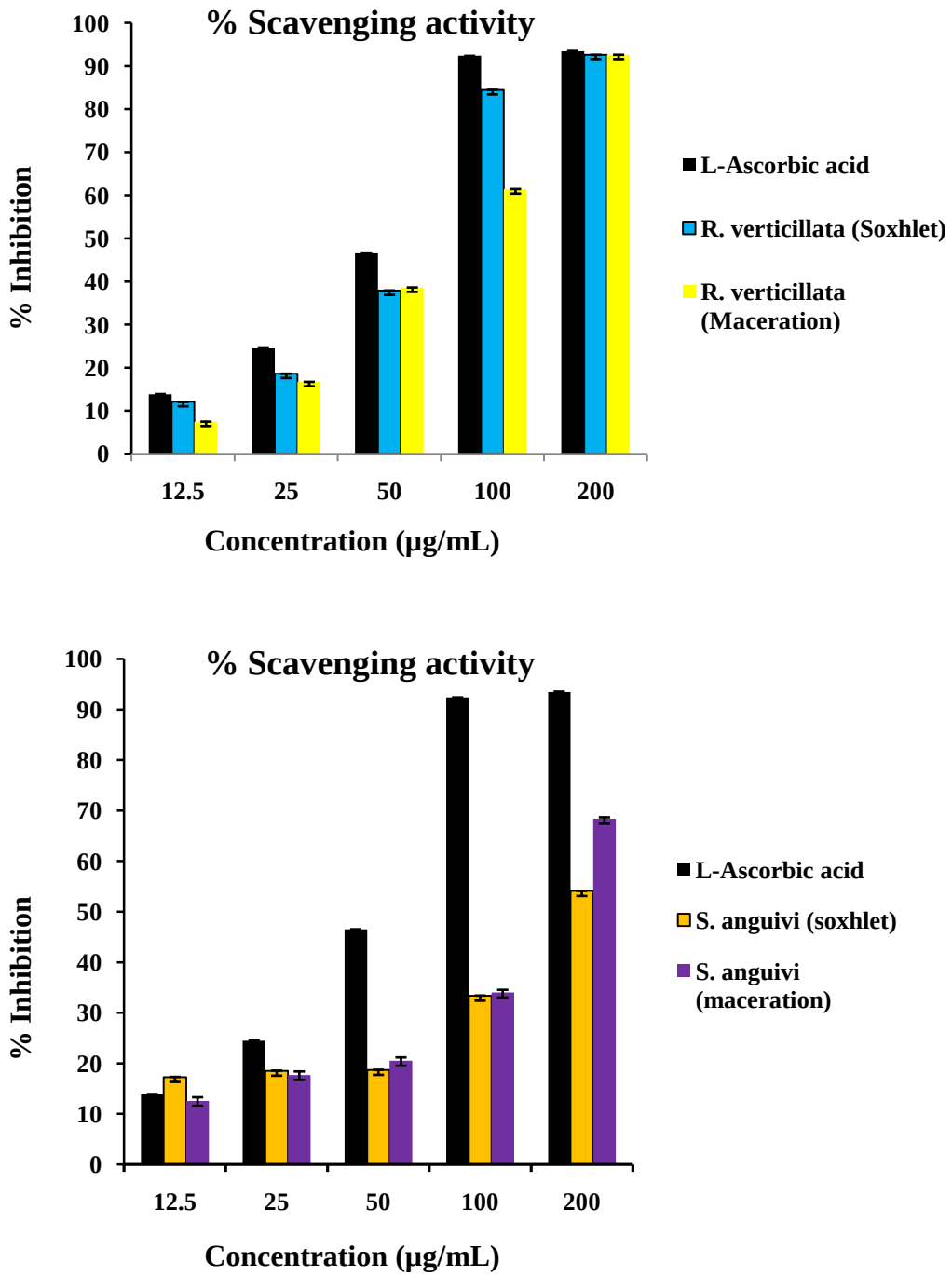


Figure 3: percent scavenging activity of L-Ascorbic acid (stadard) with *R. verticillata* and *S. anguivi*

Table 1. Percent extractive values of R. *verticillata* and *S. anguivi* using different solvents

Solvents used for Extraction	**Plants used in extraction with the extraction method employed**			
	R. verticillata **(Soxhlet)**	***R.verticillata*** **(Maceration)**	***S. anguivi*** **(Soxhlet)**	***S.anguivi*** **(Maceration)**
Water	17.18%	12.4%	13.64%	12.24%
Methanol	13.64%	10.5%	10.25%	11.8%
Ethyl acetate	6.52%	5.60%	7.3%	4.84%
n-hexane	3.5%	2.52%	4.02%	1.48%

Table 7: IC_{50} values of *R. verticillata* and *S. anguivi*

Sample	**IC_{50} value (µg/mL)**
L-ascorbic acid	68.07
R. verticillata (soxhlet)	79.46
R. verticillata (maceration)	92.46
S. anguivi (soxhlet)	182.82
S. anguivi (maceration)	143.8

Table 2: Preliminary phytochemical analysis of *R. verticillata* extracted using Soxhlet extraction

Phytochemicals	**Solvents used in extraction**			
	Water	Methanol	Ethyl acetate	n-hexane
Alkaloids	++	++	+	-
Carbohydrates	++	++	+	-
Glycosides	+	+	+	-
Saponins	+	+	+	-
Proteins	+	+	+	-
Amino acids	-	-	-	-
Phytosterols	+	+	-	-
Fats and oils	+	+	-	+
Phenolics	+	+	+	-
Gums & Mucilages	-	+	-	-

++: very intense, + : intense, - : absent

Table 3: Preliminary phytochemical analysis of *R. verticillata* extracted using Maceration extraction.

Phytochemicals	**Solvents used in extraction**			
	Water	Methanol	Ethyl acetate	n-hexane
Alkaloids	++	++	+	-
Carbohydrates	++	+	-	-
Glycosides	+	+	-	-
Saponins	++	+	-	-
Proteins	+	+	-	-
Amino acids	-	-	-	-
Phytosterols	+	+	-	-
Fats and oils	+	+	-	+
Phenolics	+	+	-	-
Gums & Mucilages	+	+	-	-

++: very intense, + : intense, - : absent

Table 4: Preliminary phytochemical analysis of *S. anguivi e*xtracted using Soxhlet extraction.

Phytochemicals	**Solvents used in extraction**			
	Water	Methanol	Ethyl acetate	n-hexane
Alkaloids	++	++	+	-
Carbohydrates	++	++	+	-
Glycosides	+	+	-	-
Saponins	++	+	-	-
Proteins	+	+	-	-
Amino acids	-	-	-	-
Phytosterols	+	++	-	-
Fats and oils	+	+	-	-
Phenolics	++	+	-	-
Gums & Mucilages	+	+	-	-

++: very intense, + : intense, - : absent

Table 5: Preliminary phytochemical analysis of *S. anguivi e*xtracted using Maceration extraction.

Phytochemicals	**Solvents used in extraction**			
	Water	Methanol	Ethyl acetate	n-hexane
Alkaloids	++	++	+	-
Carbohydrates	++	++	+	-
Glycosides	+	+	-	-
Saponins	++	+	-	-
Proteins	+	+	-	-
Amino acids	-	-	-	-
Phytosterols	+	++	-	-
Fats and oils	+	+	-	-
Phenolics	++	+	-	-
Gums & Mucilages	+	+	-	-

++: very intense, + : intense, - : absent

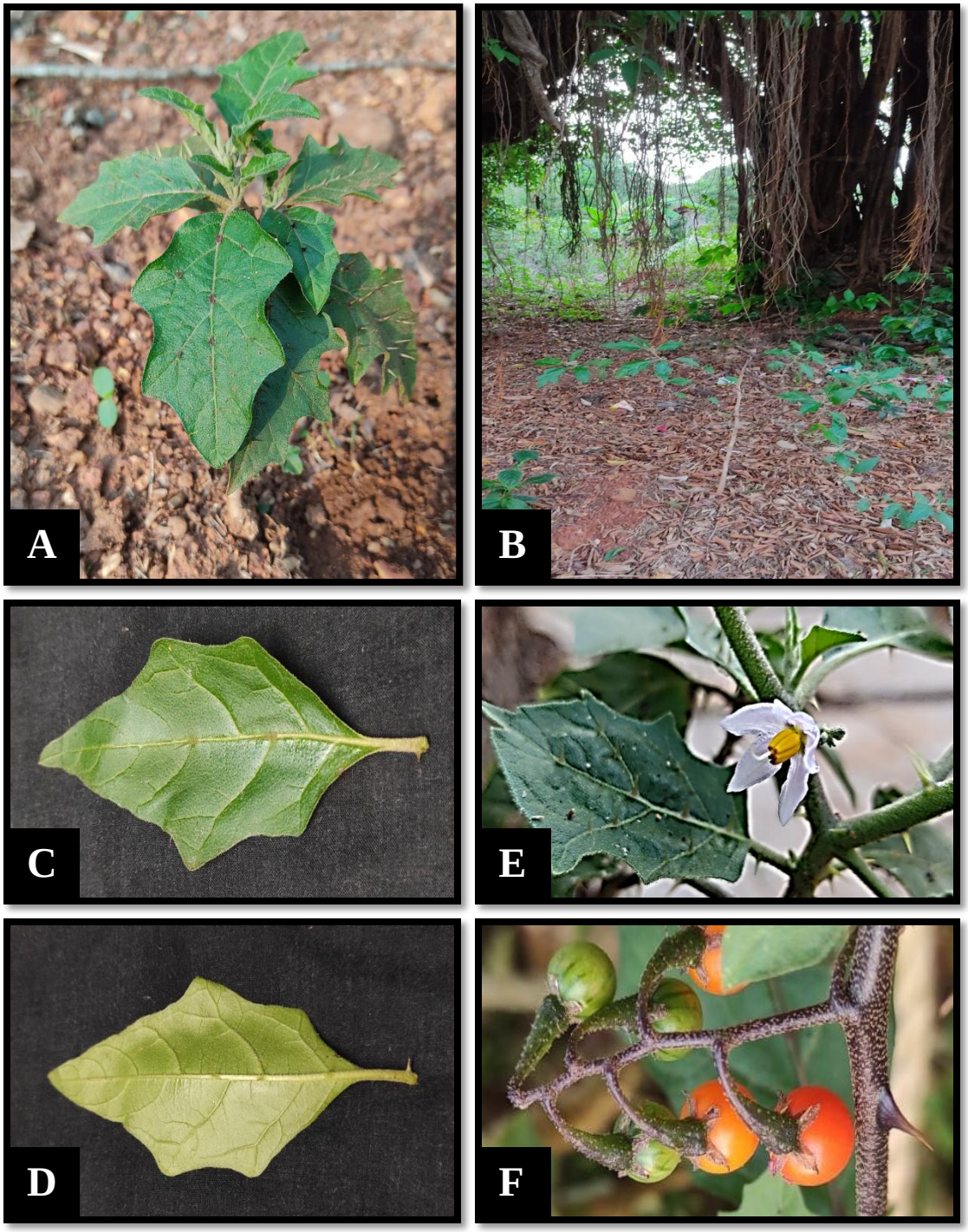


Plate 2: ***Solanum anguivi*** . **A.** Habit. **B.** Habitat. **C.** Leaf (Adaxial). **D.** Leaf (Abaxial). **E.** Flower. **F.** Fruit

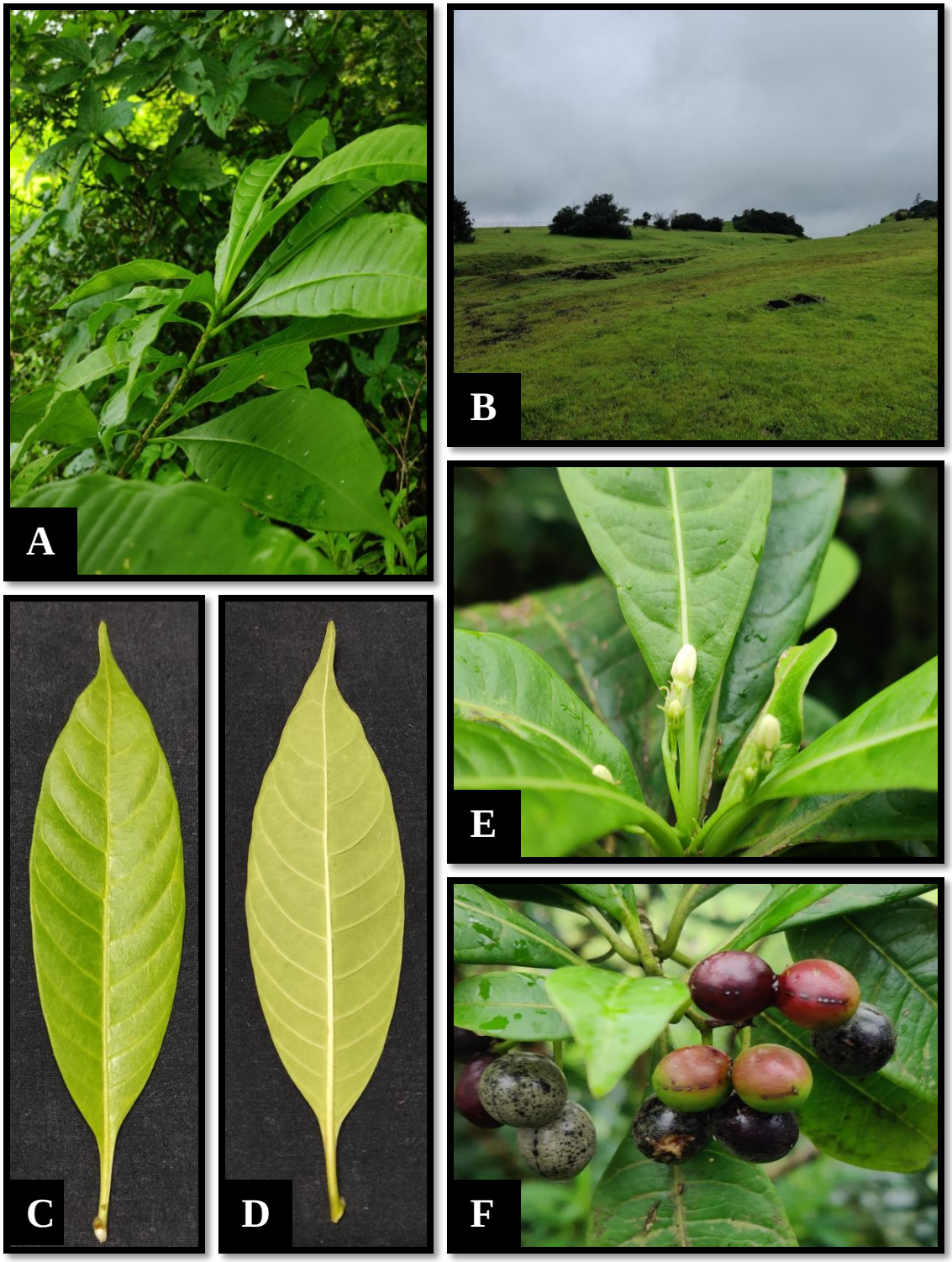


Plate 1: ***Rauwolfia verticillata.*** **A.** Habit. **B.** Habitat. C**.** Leaf (adaxial). **D.** Leaf (abaxial). **E.** Flower. **F.** Fruit.

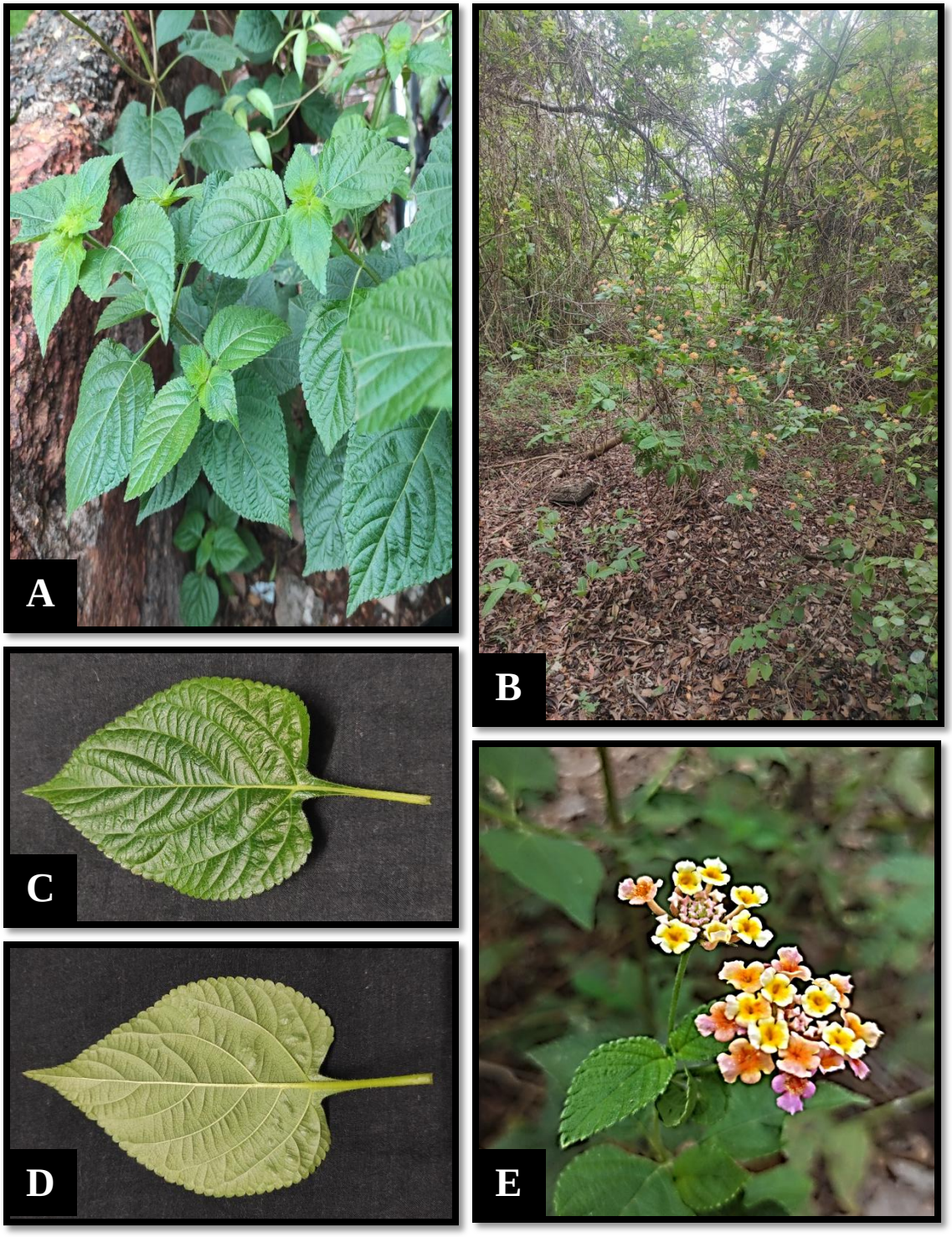


Plate 3: ***Lantana camara*****. A.** Habit. **B.** Habitat. **C.** Leaf (adaxial). **D.** Leaf (abaxial). **E.** Flower.

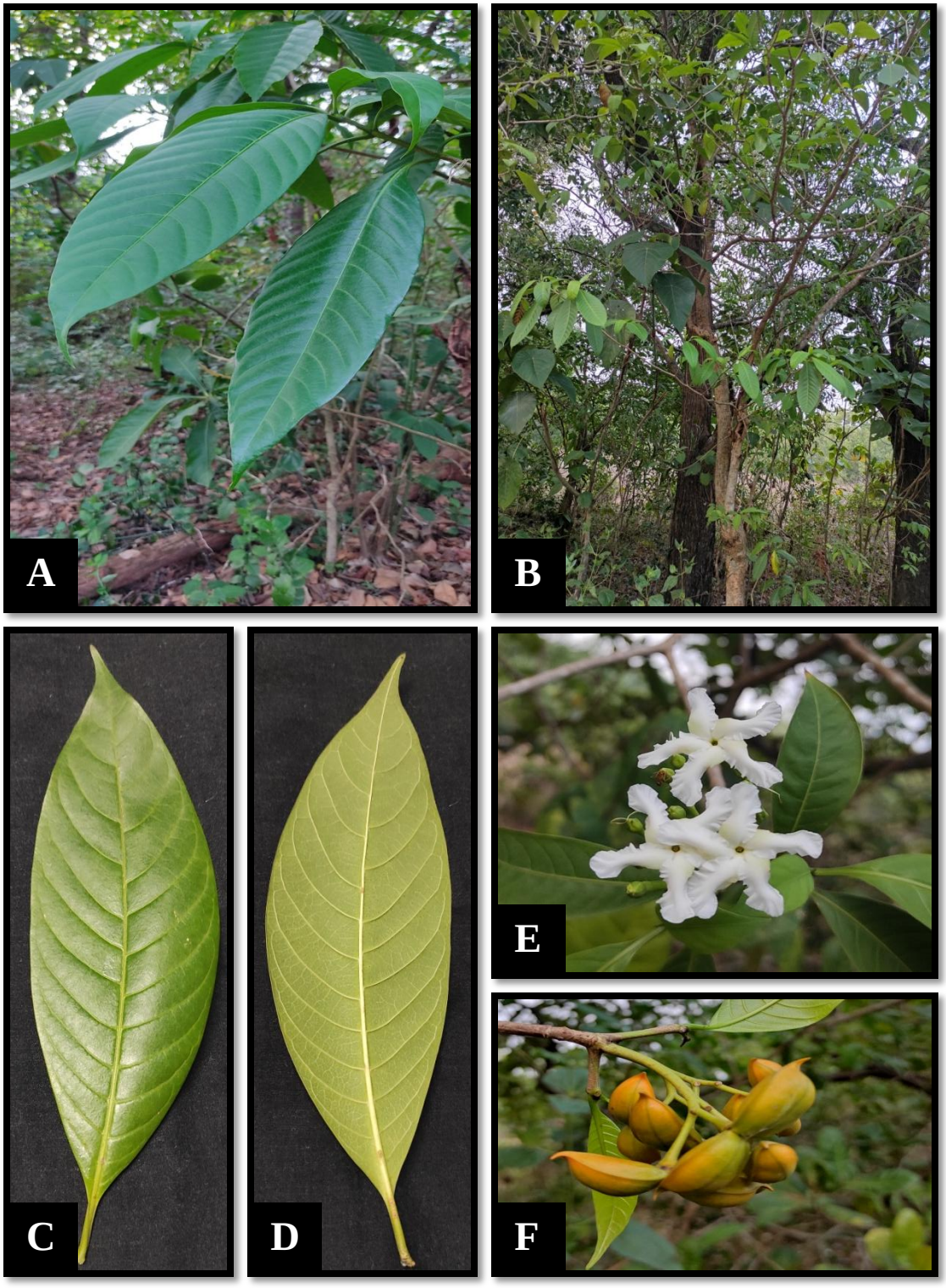


Plate 4: ***Tabarnaemontana alternifolia.*** **A.** Habit. **B.** Habitat. **C.** Leaf (adaxial). **D.** Leaf (abaxial). **E.** Flower. **F.** Fruit.