Comparative Anatomical, Histochemical and *in vitro* Antidiabetic Assay of Selected *Syzygium* species from the Western Ghats of India

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THE DEGREE OF MASTER OF SCIENCE IN BOTANY

By

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Under the guidance of

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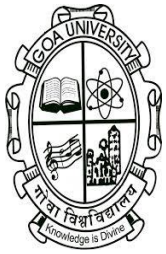

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DECLARATION

I hereby declare that this dissertation entitled **"Comparative Anatomical, Histochemical and *in vitro* Antidiabetic Assay of Selected *Syzygium* species from the Western Ghats of India"** is an authentic work done by **Ms. Nikita Vinod Gupta,** student of M.Sc., Botany, Goa University, in partial fulfilment of the requirement of **Degree of Master of Science in Botany** for the University and no part has been presented before any other degree or diploma in any University.

Date:

Signature of the student
(Ms. Nikita Vinod Gupta)

CERTIFICATE

This is to certify that the dissertation entitled **"Comparative Anatomical, Histochemical and *in vitro* Antidiabetic Assay of Selected *Syzygium* species from the Western Ghats of India"** submitted by **Ms. Nikita Vinod Gupta** in partial fulfilment for the degree of **Master of Science in Botany of Goa University** is an authentic record of the dissertation carried out by her under my supervision and guidance.

Date:

Signature of Guide
(Prof. S. Krishnan)

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(Ms. Nikita Vinod Gupta)

CONTENTS

INTRODUCTION

The Western Ghats is one of the global biodiversity hotspots, supporting an enormous vegetal wealth. It harbors considerable diversity in terms of angiosperms, of which Myrtaceae holds a paramount disposition. The family Myrtaceae is located in the order Myrtales, in the group Rosidae within the eudicotyledons. Myrtaceae includes around 5,950 species in about 132 genera (Govaerts *et al*., 2008). Its distribution is over tropical and warm-temperate regions of the world and is common in many of its biodiversity hotspots (Christenhusz *et al*., 2016). Plants belonging to Myrtaceae are trees or shrubs, frequently with conspicuous oil glands (Wilson 2010). The genera found with capsular fruits include *Eucalyptus, Corymbia, Angophora,* etc., or fleshy fruits such as *Syzygium, Melaleuca,* etc. (Govaerts *et al*., 2008).

Some of the genera which fall under Myrtaceae are *Abbevillea, Actinodium, Agnos, Astus, Barogonia, Callistemon, Corymbia, Darwinia, Eucalyptus, Eugenia, Homaranthus, Myrtus, Syzygium, Xanthostemon,* etc. (Govaerts *et al.*, 2008). *Syzygium* is the largest genus in Myrtaceae distributed in tropics and subtropics (Soh and Parnell 2011). It is a genus of flowering plants, with most species being evergreen trees and shrubs (John 2003). It comprises about 1200 species. The native range of this species extends from Africa and Madagascar through southern Asia East through the pacific (Tuiwawa *et al*., 2013). From Malaysia to northeastern Australia is where it shows the highest level of diversity. Most of the species belonging to this genus are rich in volatile oils reported for their uses in medicine and ethnobotanical practices (Reynertson *et al*., 2005). Some of the edible species of *Syzygium* are planted throughout the tropics around the world, and several have become invasive species in some island ecosystems (John 2003). The considerable economic importance of the genus is for its timber, fruit, cloves, and as a source of

medicine. Being one of the largest flowering plants, it is an essential element in the rainforest ecosystem (Soh and Parnell 2011).

***Syzygium cumini* (L.) Skeels.**

Syzygium cumini is an evergreen tropical tree native to the Indian subcontinent favored for its fruits, timber, and ornamental value. It is rapidly growing, can reach heights up to 30 meters, and has lived for more than 100 years (Robert and Paull 2017). The leaves are aromatic, are pinkish when young, then change to leathery, glossy dark green with a yellow midrib as they mature. The leaves can be used as food for livestock as they have an outstanding nutritional value (Janick *et al*., 2011). The tree starts flowering from March to April. The flowers are fragrant and small, about 5mm in diameter. By May or June, the fruits are developed, resembling large berries which are Drupaceous (Chen *et al*., 2015).

Syzygium cumini is a widely used medicinal plant to treat various diseases, particularly diabetes. The plant contains large amounts of anthocyanins, glucoside, iso-quercetin, kaempferol, ellagic acid, and myricetin (Lakshmi 2021). The bark is Acrid, sweet, digestive astringent to the bowels, is also an anthelmintic, and is used to treat sore throat, bronchitis, asthma, thirst, a good blood purifier, etc. The fruit is an astringent for bowels and removes a bad smell from the mouth, and the stomach is a diuretic and anti-diabetic. The seed has also been used as an astringent for bowel and is suitable for diabetes (Nadkarni 1976). The juice of tender leaves is used as carminatives, given to goats to treat bloody discharge (Chen *et al*., 2015). The leaves contain polyphenolic compounds connected to the target machine and organs for activating pathways and interrupted lines for inhibiting pathways. The methanolic extract of leaves was found to increase mRNA

expression of glucose transporter (GLUT)-4 and phosphatidylinositol-3-kinase (PI3 kinase), essential mediators of insulin action (Chagas *et al*., 2015).

***Syzygium caryophyllatum* (L.) Alston**

Syzygium caryophyllatum is one species that has been categorized as endangered tree species under the International Union for Conservation of Nature (IUCN) red list of threatened species (Annadurai *et al*., 2012). *Syzygium caryophyllatum* is commonly known as wild black plum. It is native to Sri Lanka and India. In India, its distribution is restricted to the forest of the Western Ghats regions (Ediriweera and Ratnasooriya 2009). It is a small evergreen tree 3-6m tall. The bark is grey or blazes brown, having white flowers and black globose berry-like fruit (Ramesh *et al*., 2014).

The fruits of *Syzygium caryophyllatum* are edible, sweet, and astringent in taste. The seeds and bark were dried, and the decoction was used for the ailment of diabetes (Herath 2020). The leaf and bark of the plant are known for their antibacterial and antioxidant efficacy (Shilpa *et al*., 2014). Roots have many phenols, flavonoids, tannins, and vitamins (Rabeque and Padmavathy 2014). The bark is used as veterinary medicine to treat tympanitis in cattle (Harsha *et al*., 2005).

***Syzygium laetum* (Buch. -Ham) Blume**

Syzygium laetum is an endangered endemic tree. Its distribution is in the western ghats and evergreen forest. It is endemic to the southern Western Ghats. It is a medium-sized tree, up to 10m tall; slender branchlets are terete. Leaves elliptical ovate or elliptic-lanceolate, gland-dotted; intra-marginal nerve near the margin. Flowers crimson or lemon yellow, 4-5 cm across, solitary or terminal cymes, petals 4, stamen numerous, yellow or

pink in color; two loculed ovary, style stronger than the stamens. Berry oblong, crowned by calyx lobes (Gandhi *et al*., 1976). The bark of the tress is white, smooth, or blaze brown color (Irulandi *et al*., 2016).

***Syzygium salicifolium* (Wight) J. Graham**

Syzygium salicifolium is a tree that grows up to 6m in height. It is found in evergreen forests and is endemic to Western Ghats (Nair *et al*., 2017). It flowers and fruits from April to May (Graham 2019). These are small trees up to 6m tall; branchlets obscurely 4 angled. Leaves are oblong or elliptic, base acute, apex obtuse or retuse, margin entire, gland dotted; lateral nerves are very slender with intra-marginal nerves. Flowers small sessile, white, in lateral or terminal cymes; calyx tube, turbinate; petals calyptrate, 3mm across; stamens many, free, bent inwards when in bud, ovary 2 loculed, style 1 stigma simple. Berry obovoid, crowed with cup-like calyx limb (Gamble *et al*., 1919).

Anatomical Studies

Plant anatomy is the study of plant organs, tissue, and cell structure. Plant anatomy is frequently investigated at the cellular level and involves sectioning and microscopy. It provides valuable characteristics that help analyze phylogenesis (Michael 2019). Anatomical structure can be used as an initial data source that can be used in plant taxonomy because anatomical characters are conserved and stable. The anatomy of vegetative organs (leaves, stems, and roots) is used more than the anatomy of reproductive organs as taxonomic features. Leaves consist of anatomical structures such as epidermis, stomata, mesophyll, and vascular bundles. Similarly, epidermis, cortex, vascular bundles, and pith in stems and roots. While studying plant taxonomy, some unique features like secretory tissues are also present (Moralita *et al*., 2020).

Most of the plants belonging to the genus *Syzygium* have secretory cavities that produce oil glands (Moralitha *et al*., 2019). Many of the plants from the Myrtaceae family were seen to have sclereids, calcium oxalate crystals, and druises in the internal structure of leaves, stems, and fruits of different types (Malaviya 1947).

Histochemical Studies

The plant is a source of many active metabolites used in medicine and a primary source for isolating natural products. The secondary metabolites present in plants give medicinal properties, including flavonoids, tannins, alkaloids, saponins, etc., possess many biological activities and are active constituents in many drugs (Oyejide 2017). Histochemistry is a branch that combines biochemistry and histology techniques in the study of the chemical components present in cells and tissues, which includes confirming the identification, the density of accumulation, and distribution of chemical compounds in the cells and tissues under a microscope using color-stain reaction techniques and photographic recordings. The procedure includes the preparation of fixed variably stained specimens and examining the sample under a microscope (Badria and Aboelmaaty 2019).

Studies on histochemical analysis of *Syzygium cumini* have shown the presence of phenolic compounds, tannins, triterpenes and steroids, alkaloids, essential oils, lipophilic compounds, starch, lignin, and calcium oxalate crystals (Moralitha *et al*., 2019). Phytochemical screening of *Syzygium caryophyllatum* showed the presence of flavonoids, phenolic compounds, alkaloids, and saponins (Raj *et al.*, 2016).

Anti-diabetic Studies

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia or an increase in blood glucose levels that disturbs carbohydrate, fat, and protein

metabolism resulting from absolute or relative lack of insulin secretion. This disorder's frequency is rising to a tremendous level globally and will likely hit 300 million by 2025, with India projected to have the most significant number of diabetic cases. The primary threat to human health is type 2 diabetes due to its increasing prevalence, chronic course, and disabling complications. Many diverse therapeutic strategies for treating Type 2 diabetes are in use. The available conventional therapies for diabetes include endogenous insulin secretion stimulation, oral hypoglycemic agents, such as biguanides and sulfonylureas, and the inhibition of the degradation of dietary starch by glycosidases such as α-amylase and α-glucosidase (Sudha *et al.,* 2011).

Alpha-glucosidase and α-amylase are the two most important enzymes involved in the metabolism of carbohydrates. α-amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides, converted into monosaccharides by α-glucosidases. Glucose, which is liberated, is absorbed by the gut, resulting in postprandial hyperglycemia. Inhibiting intestinal α-glucosidases limits postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption, making such inhibitors helpful in managing type 2 diabetes. Plant sources and microorganism shows an excellent ability to inhibition of these enzymes. For example, acarbose, 4,1-deoxynojirimycin, and genistein have all been isolated from natural sources (Shinde *et al*., 2008).

Despite the availability of anti-diabetic drugs, diabetes, and related complications continue to be significant medical problems. Many oral anti-diabetic agents have several serious adverse effects. Therefore, the search for more effective and safer drugs continues as an important area of investigation. Thus, managing diabetes without side effects is still a challenge (Modak *et al.,* 2007; Grover *et al.,* 2002). The natural medicines obtained from

plants are believed to be safe, effective, and economical. Plant-derived products play an essential role in developing new therapeutic agents and serve as sources of many bioactive substances. The antioxidant activity of plant extracts also has a beneficial effect on the conservation of *β*-cell function in diabetes mellitus (Smith *et al*., 2019).

It has also been shown that the leaf, bark, stem, and pulp of *Syzygium cumini* plants possess potent anti-diabetic activity (Chaudhary *et al*., 2012). The leaves are rich in acylated flavanol glycosides, quercetin, myricetin, myricitin, myricetin 3-*O*-4-acetyl-*L*-rhamnopyranoside, triterpenoids, esterase, galloyl carboxylase, and tannin. The stem bark is rich in betulinic acid, friedelin, epi-friedelanol, β-sitosterol, eugenin and fatty acid ester of epi-friedelanol, β-sitosterol, quercetin kaempferol, myricetin, gallic acid and ellagic acid, bergenins, flavonoids and tannins. The presence of gallo- and ellagi-tannins may be responsible for the astringent property of stem bark (Ayyanar and Babu 2012).

A Sri-Lankan study report says that the decoction of *Syzygium caryophyllatum* is used for the ailments of diabetes mellitus (Herath *et al*., 2020). The chemical anti-diabetic drugs cause a release of starch in its undigested form into the lower gastrointestinal tract, whereby abnormal fermentation of the undigested starch by the intestinal micro flora takes place, causing abdominal discomforts and diarrhea and many such side effects. Thus, moderate inhibition of α–amylase is desired, which might be effectively done with herbal medicines (Kwon *et al*., 2006).

OBJECTIVES OF THE PRESENT INVESTIGATION

1. To study comparative vegetative anatomical characters of leaf, petiole, and stem of four selected *Syzygium* species (*S. cumini*, *S. caryophyllatum*, *S. laetum*, and *S. salicifolium*) from the Western Ghats region of Goa.

2. To localize tissue-specific primary and secondary metabolites in different tissues by histochemical staining procedures.

3. To study the anti-diabetic activity of the leaf and bark methanolic extract using *in-vitro* assays like DNS (Dinitro-salicylic acid) and iodine-starch tests.

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. Soh and Parnell (2011) examined the leaf anatomy of 81 species of *Syzygium*. General generic and sub-generic descriptions of *Syzygium* leaf anatomy were given in their studies. Two major vascular systems could be differentiated by the presence or absence of adaxial phloem partition. All the leaf anatomical characters examined were homoplastic; they did not find any unique leaf anatomical characters allowing delimiting the four subgenera in *Syzygium*. The combinations of standard leaf anatomical characters included stomatal types, crystal types, frequency and the midrib vascular system (adaxial phloem partition) was diagnostic for sub-generic groups.

The study was carried out to evaluate the anatomical characteristics of *Syzygium myrtifolium* (Abdulrahman *et al.*, 2021). Macroscopic and microscopic analyses were utilized. The anatomical features revealed an open system of the vascular bundle at the midrib and a closed vascular bundle system at the petiole. Diagnostic characters from morpho-anatomy would serve as markers for the identification of the species.

Anatomical and histochemical studies on leaves of *Syzygium aromaticum* and *Clausena excavata* have been carried out (Zaman *et al*., 2018). This study was conducted to investigate the relationship between aroma production and a plant's secretory structures. They observed oil cells and secretory cavities were distributed near the adaxial and abaxial epidermal layers large in size, up to 60µm in length. Other leaf anatomical characteristics such as the shape of petiole and midrib, pattern of vascular bundle, palisade and spongy mesophyll, and the presence and absence of brachy-sclereids and crystals were also

observed. The study was also carried out to investigate the leaf's secretory structures responsible for plant's aroma production and also to detect the presence of terpenes and essential oil in secretory structures histochemically.

Rosely and Schossler (2018) conducted a study to verify the effect of *Syzygium cumini* bark extract on the pancreas of normal and diabetic rats *in-vivo*. They allocated the animals into four groups, control (C), treated control (TC), diabetic control (DC), and treated diabetic (TD). An aqueous extract from *Syzygium cumini* bark was given by gavage in a daily dose of 1g kg^{-1} of body weight. After thirty days, the animals were examined and the pancreas taken for immuno-histochemical analysis. A significant decrease in the number and size of islets, cyto-architectural alterations, and a severe reduction in the number of positive cells in diabetic animals was observed. However, there was no significant difference neither between DC and DT nor C and CT groups. These results indicated that the *Syzygium cumini* bark extract does not significantly affect the number, size, and cyto-architecture of islets, and it also does not lead to an increase in beta cell number in animals treated with the plant extract.

The *Eugenia* and *Syzygium* species belonging to the family Myrtaceae have medicinal importance and pharmacological properties; due to their chemical diversity and biological activity, the essential oils of 48 species of these two genera grow in South America and found mainly in Brazil was reported. The main volatile compounds were sesquiterpene hydrocarbons and oxygenated sesquiterpenes, mainly with caryophyllene, germacrene skeletons, and monoterpenes of mostly the pinane type were reported in the study. The oils present lead to many biological activities, especially antimicrobial (antifungal and antibacterial), anticholinesterase, anticancer (breast, gastric, melanoma,

prostate), antiprotozoal (*Leishmania* spp.), antioxidant, acaricidal, antinociceptive and anti-inflammatory making them a potent natural and alternative source to the production of new herbal medicines (Jamile *et al*., 2020).

Syzygium cumini is an essential medicinal species of the Myrtaceae family used for diabetes. This work aimed to conduct an anatomical and histochemical study of the leaf blade of *S. cumini*. They prepared semi-permanent histological slides to analyze the leaf blade in optical microscopy, polarization and scanning electron microscopy. Histochemical tests were performed in cross-sections of fresh leaf, using specific reagents for each group of metabolites. The microscopic analysis allowed the identification of essential elements in the diagnosis of the species. The histochemical tests showed the presence of phenolic compounds, tannins, triterpenes and steroids, alkaloids, essential oils, lipophilic compounds, starch, lignin, and calcium oxalate crystals. The results presented contribute to the pharmaco-botanical standardization of the species (Damascen and Randau, 2021).

The study was carried out by Stalin and Swamy (2018) to analyze the phytochemical content and evaluate *in vitro* antioxidant, antibacterial, larvicidal, and anti-diabetic activities of *S. caryophyllatum* bark, leaves, fruit pulp and seeds. The studies suggested that *S. caryophyllatum* plant parts can be used as a natural antioxidant source to prevent diseases associated with free radicals. Also, this plant can be a good source for further purification studies for isolation and characterization of compounds related to these antioxidants, anti-diabetic and antibacterial activities.

Syzygium caryophyllatum is an endangered tree species belonging to the Myrtaceae family. The antimicrobial, antioxidant, and anticancer activities of the leaf

extract were evaluated. Disc diffusion method was used for antimicrobial screening of four bacterial and three fungal strains. The 2,2-diphenyl-1-picrylhydrazyl assay was used to assess the scavenging ability of the extract. Hep2 cell line was used to evaluate the cytotoxicity by 3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide assay. They found a high zone of inhibition for the extract against all the selected strains used. Antioxidant potential was more at higher concentrations (400µg/ml). The extract showed maximum Hep2cell inhibition at higher concentration (9.80% cell viability in 1000µg/ml). This concluded that the antioxidant, antimicrobial and anticancer studies using leaves of *Syzygium caryophyllatum* showed its potential utilization in the pharmaceutical industry (Annadurai *et al*., 2012).

The chemical constituents of essential oils of six *Syzygium* species Walp., *S. caryophyllatum* (L.) Alston, *S. hemisphericum* (Wight) Alston, S. *laetum* (Bu, *S. arnottianum* ch. Ham.) Gandhi, S. *lanceolatum* (Lam.) Wight & Arn. and *S. zeylanicum* (L.) DC. var. *zeylanicum*, collected from the Western Ghats of Kerala, was reported and studied. Sesquiterpenoids were found in all the *Syzygium* species studied; caryophyllene and caryophyllene oxide were present in all the oils except *S. laetum*. The open-chain sesquiterpenoids (Z, E)-α-farnesene and (E)-nerolidol were characteristic of *S. laetum*, while phenylpropanoids were exclusively present in *S. lanceolatum* (Koranappalli *et al*., 2015).

Chatri and Mella (2019) determined the cross-sectional characteristics of leaves and stomata of three plants of S*yzygium*. They evaluated the anti-diabetic properties of *S. caryophyllatum* fruits and leaves to assess its antioxidant, antiglycation, antiamylase activities, and functional mineral element composition. The crude extracts of leaves and

fruits were fractionated into hexane, ethyl acetate, and aqueous solvents and evaluated for bioactivities. The extract amylase fraction of leaves showed the highest values for DPPH radical scavenging activity, ferric reducing antioxidant power, and oxygen radical absorbance capacity. Significantly high ABTS radical scavenging activity and iron chelating activity were observed in the hexane fraction of fruit. The composition of volatiles in leaf oil was studied with GC-MS, and 58 compounds were identified. Inductively coupled plasma-mass spectrometry data revealed the presence of biologically significant trace elements such as Fe, Zn, Mg, Cu, Se, and Sr in leaves and fruits. It is concluded that the hexane fraction of *S. caryophyllatum* fruits will be a good source for the formulation of supplements for diabetic management with further evaluation of potency and efficacy (Wathsara *et al*., 2020).

The fruits of *Syzygium caryophyllatum* and *Syzygium zeylanicum* were analyzed for their nutritional potential and pharmacological property. The disc diffusion method was used to carry out the antibacterial activity, followed by the micro-dilution method for minimum inhibitory concentration. The antioxidant activity was assessed by the 2, 2-diphenyl-1-picrylhydrazyl, free radical scavenging method. The fruits were also fermented to produce wine and were then analyzed for their physical and chemical properties. The fruit methanol extract of the *S. caryophyllatum* was independent of gram reaction inhibiting the growth of both gram-positive and gram-negative bacteria. The results highlight the importance of wild fruit species as an affordable nutrient source and as an antibacterial agent (Shilpa *et al.,* 2015).

Raj (2016) carried out the macroscopic, anatomical, powder microscopic, and phytochemical study of the leaves of *Syzygium caryophyllatum*. A microscopic

examination revealed the presence of palisade cells, parenchyma cells with tumor, vascular strands, and paracytic stomata. Preliminary phytochemical screening showed flavonoids, phenolic compounds, alkaloids, and saponins. Pentacyclic triterpenoid saponins were absent.

The study was carried out to evaluate the phytochemical and antibacterial activity of methanol, ethyl acetate, and acetone extracts of *Syzygium laetum* bark. Antibacterial activity was analyzed using the agar well diffusion method against gram-positive and gram-negative bacteria. The phytochemical analysis revealed the presence of alkaloids, coumarins, flavonoids, phenols, saponins, terpenoids, tannins, and steroids in various solvent extracts. Methanolic extract of *S. laetum* showed good antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa,* and *Klebsiella pneumoniaee*. Acetone extract revealed inhibition activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Klebsiella pneumoniae,* and *Bacillus subtilis* whereas ethyl acetate extract showed the least zone of inhibition against tested all pathogens compared with other solvent extracts. These results showed the presence of crucial Phyto-constituents and good antibacterial activity and suggested that *S. laetum* can be used in the microbial tests (Kokkaiah *et al*., 2016).

The studies reported that the effect of different doses of aqueous stem bark extracts of *Syzygium caryophyllatum* possesses potent acute antihyperglycemic activity in alloxan-induced diabetic rats, and the adequate optimum amount on glucose tolerance was found to be 1.00 g/kgb.w in diabetic rats (Attanayake *et al*., 2013).

The study of various extracts of ten medicinal plants (*Cinnamomum zeylanicum, Crataegus oxyacantha, Hibiscus sabdariffa, Morus alba, Portulaca oleracea, Rubus fruticosus, Syzygium aromaticum, Teucrium polium, Trigonella foenum-graecum,* and *Vaccinium arctostaphylos*), collected in Iran, were examined for α-glucosidase and α-amylase inhibition using DNS and PNPG reagents for an *in vitro* model (Peyman *et al*., 2013).

The aqueous extract of *S. cumini* or *Eugenia jambolana* seeds and *Psidium guajava* leaves showed higher inhibition against the porcine pancreatic-amylase among the medicinal plants studied. The amylase inhibitors from *S. cumini* seeds were separated from the extract by preparative thin-layer chromatography into fractions with different Rf values. The compounds identified from the seed extract of *S. cumini* were betulinic acid and 3,5,7,4`-tetrahydroxy flavanone, which were reported earlier from *S. formosanum* and other plants (Karthic *et al*., 2008).

Syzygium aqueum is a medicinal plant that is grown in tropical regions. This study investigated the ethanolic extracts of *S. aqueum* leaf for their antihyperglycaemic activity. The investigation revealed its effectiveness in inhibiting the carbohydrate hydrolyzing enzymes, α-glucosidase, and α-amylase at a significant level than the commercial drug acarbose. These findings provide a strong rationale for establishing *S. aqueum's* capability as an antihyperglycaemic agent (Manaharan *et al*., 2012).

A literature review revealed that scanty or no work had been conducted previously for the selected *Syzygium* species about the anatomy, histochemistry, and antidiabetic properties. Therefore, the current work was undertaken to evaluate the parameters mentioned in the below objectives for four *Syzygium* spp., viz., *Syzygium cumini*, *Syzygium caryophyllatum*, *Syzygium salicifolium*, and *Syzygium laetum*.

MATERIALS AND METHODS

Collection of Plant Materials

The selected *Syzygium spp*. (*Syzygium cumini*, *Syzygium caryophyllatum*, *Syzygium Laetum*, and *Syzygium salicifolium)* were collected from the Western Ghats regions of Goa, and northern plains, namely from Netravali, Bicholim, Taleigao, Paroda, and Satrem, with due permission from the forest department of Goa. Mature and healthy leaves and bark were collected and appropriately cleaned with distilled water and dried for 14 days under shade at room temperature.

Table 1. Names of the collected species and their location

Sr. No.	Species name	Location
1	*Syzygium cumini* (L.) skeels	Bicholim, Goa
2	*Syzygium caryophyllatum* (L.) Alston	Paroda Quepem, Goa
3	*Syzygium laetum* (Buch. -Ham) Blume	Netravali Sanguem, Goa
4	*Syzygium salicifolium* (Wight) J. Graham	Satrem Valpoi, Goa

Comparative Anatomical Studies

Mature leaves, petiole, and young stem samples were collected for anatomical studies of all four species. The free-hand sections were taken from a fresh mature sample of leaves (middle of the lamina with the main vein), stem (5-10mm), and petiole (center of the petiole). Sections were stained with 0.1% safranin for 2-3min, rinsed the sections with distilled water, mounted on a slide with 10% glycerin, 0.05% of Toluidine blue O was also used as general stain. and examined under a bright-field microscopy. The desirable

portions were photographed by using a digital compact camera attached to the microscope and images were captured on computer using TC-capture software (Fernandes and Sellappan 2019).

Histochemical Studies

1. **Test for starch** (Iodine-potassium Iodide method)

Section the material and mount the section in Iodine-Potassium Iodide solution (2g of potassium iodide in 100ml of water and dissolve 0.2g of iodide). Wash the section with distilled water and observe under bright-field microscopy. Starch appears blue to black in a few minutes, and newly formed starch may appear red to purple.

2. **Test for total lipids** (Sudan dye method)

Take the section, and place the section in pure ethylene glycol for 3-5min with occasional shaking. Transfer the section to Sudan IV and stain for 5-7 min (0.7g of dye in 100 ml of ethylene glycol.) Heat the solution to 100-110ºC and stir. Transfer to 85% ethylene glycol and water and shake gently for 2-3min. Thoroughly wash with distilled water and mount in glycerin and observe under a bright-field microscopy. Sudan-IV will stain fats, oils, and waxes orange red.

3. **Test for Alkaloids** (Dragendroff reagent)

Section the material and stain the section in Dragendroff reagent for 20min. Wash the section with 5% sodium nitrite. Mount in glycerin and observe under a bright-field microscopy. Alkaloids will appear yellow to reddish-orange in color.

4. **Test for Tannins**

Section the material and place the section in 10% formalin solution (0.1% ferrous sulfate in 1% formalin). Wash the section with distilled water for 15min. Mount in

glycerin and observe under a bright-field microscopy. Tannins will appear blue to black in color.

5. **Test for Lignin**

Stain the sections in aqueous toluidine blue O solution (0.05% toluidine in benzoic acid 0.25g and 0.29g sodium benzoate in 200ml of water pH 4.4). Wash the section with distilled water., mount in glycerin and observe under a bright-field microscope. Lignin is stained green to bluish (Krishnamurthy 1988).

***In-vitro* Anti-diabetic Activity**

Preparation of Plant Extract

Grind the leaves and bark of all four species individually into the fine powder, store in screw cap bottles, and label separately. Take 25g of leaf powder soaked in 300ml of solvent methanol using the Soxhlet extraction method for 24hrs. Concentrate the extract using a rotary vacuum evaporator under reduced pressure at 40°C until solid residues are obtained. Store the extract at -20ºC for further studies (Shettar *et al*., 2017).

The α-amylase and α-glucosidase Inhibitory Assay

1) **α-amylase inhibitory assay**

Dinitro-salicylic acid reagent method

100µl of plan extract at different concentrations with 1% starch solution, add 2mM phosphate buffer (adjust pH to 6.9 with 6mM NaCl) Incubate at 25ºC for 10min. Add 100 µl of the α-amylase enzyme (0.6mg/ml) and incubate at 25ºC for 10min. To terminate the hydrolytic reaction, 200µL of dinitro-salicylic acid reagent was added. Incubate at 100ºC for 5min. Cool down the sample at room temperature. 50µl of the

reaction mixture was transferred to test tubes and diluted by adding 200µl of distilled water. Measure the absorbance at 540nm (Singamoorthy *et al*., 2021).

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions. The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values. The IC_{50} values were defined as the concentration of the extract containing the α-amylase inhibitor that inhibited 50% of the PPA activity (Sudha *et al.*,2011).

Percentage of α-amylase inhibition (% activity)

$$Ac - \frac{As}{Ac} * 100$$

where $Abs_{(control)}$ corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α -amylase solution, and $Abs_{(extract)}$ corresponds to the solution with extract and α -amylase solution (Singamoorthy *et al*., 2021).

2) Iodine Starch

α-amylase inhibition assays Starch-Iodine color assay

Screening of plant extract for α-amylase inhibitors was carried out based on the starch-iodine test. The total assay mixture composed of 40µL 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 0.02 units of α-amylase solution and plant extracts at different concentrations were incubated at 37°C for 10 min. Then soluble starch (1%, w/v) was added to each reaction mixture and incubated at 37°C for 15 min. 1 M HCl (20µL) was added to stop the enzymatic reaction, followed by the addition of 100µL of iodine reagent (5mM I_2 and 5mM KI). The color change was noted and the absorbance was read at 620nm. The control reaction representing 100% enzyme activity did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls without the enzyme were also included. The known α-amylase

inhibitor, acarbose, was used as a positive control at a concentration. A dark-blue color indicates the presence of starch; yellow color indicates the absence of starch, while a brownish color indicates partially degraded starch in the reaction mixture. In the presence of inhibitors from the extracts, the starch added to the enzyme assay mixture is not degraded and gives a dark blue color complex, whereas no color complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolyzed by a-amylase (Sudha *et al*., 2021).

RESULTS AND DISCUSSION

Comparative Leaf Anatomy of Selected *Syzygium* species

The finding of anatomical characterization of leaf, petiole and stem of *Syzygium spp*. (*Syzygium cumini, Syzygium caryophyllatum, Syzygium Laetum,* and *Syzygium salicifolium*) have been presented and discussed in this Chapter. Anatomical characteristics of above species have been summarized in Table 2-5 and Plate. 5-7.

The transverse section of leaves (Plate.5) of all *Syzygium* species revealed dorsiventral nature containing palisade parenchyma on the adaxial surface and spongy parenchyma on the abaxial surface. The leaves are seen with cuticular layer in all four species.

Transverse section of leaves showed the presence of uniseriate layer of epidermis on both adaxial and abaxial side having rectangular cells. The cell wall of epidermal layer is well cuticularized in all the species studied, however the thickness of the cuticle varied among the species. The thinnest cuticular layer was observed in *S. caryophyllatum*, whereas the thickest was observed in *S. laetum*. Based on the sinuosity of epidermal cell walls of leaf lamina both the adaxial and abaxial epidermal cells were found to be undulated in all four species.

The mesophyll tissue in all *Syzygium* species is well developed with compactly arranged palisade parenchyma and loosely arranged spongy parenchyma cells. Both palisade and spongy parenchyma cells were filled with chloroplast. Palisade cells were observed to be elongated in all four species and appear to be arranged in rows. It was

observed that the high content of chlorophyll is present in palisade cells as compared to spongy parenchyma cells. On the basis of number of palisade layers, the following three groups could be recognized: (Plate.6)

1. Palisade parenchyma single layered: *S. laetum.*
2. Palisade parenchyma double layered: *S. cumini, S. caryophyllatum.*
3. Palisade parenchyma three layered: *S. salicifolium.*

The midrib (Plate.7) is arc-shaped having circular lower margin was observed in all the species having a concave surface. The vascular system is conjoint and collateral, where xylem is surrounded by phloem. In *S. cumini* was seen arc shaped vascular bundle with incurved distal ends, where two distal ends do not meet. On other hand in other three species two adaxial curls of vascular bundles almost fused a nd both bundle sheath and phloem both curved inwards and fused adaxially.

Surrounding the phloem is seen the sclerenchyma sheath in all the species examined. The presence of sclereids is one of the characteristic features found in most of genera of Myrtaceae family (Manju 1967). The sclerenchyma sheath was observed to be continuous in *S. caryophyllatum*, whereas in other three species it was discontinuous, where adaxial region showed one layer of sclereid cells and abaxial region varied from 1-3 layers of sclerenchyma sheath.

The number of layers of sclerenchyma tissue in the abaxial region varied in all four species. There were three layered sclereid cells seen in *S. laetum*, two layered sheaths in *S. caryophyllatum* and *S. salicifolium* and single layered sheath in *S. cumini* (Plate 6).

Oil glands are abundant and present in most of the species belonging to the genus *Syzygium*, usually located in abaxial epidermis in the palisade and spongy mesophylls layers (Soh and Parnell 2011). Similarly, there were globular oil glands seen in all four species which embedded in palisade and spongy parenchyma tissue on the outer sides.(Plate.8).

In all four species crystals were seen to be present which are usually in the form of druses and prismatic crystals. These druses were found to be embedded in palisade tissue spongy tissue, ground tissue of midrib and are usually concentrated near vascular bundles. The druses vary in frequency from few in numbers to many, there was also large number of druses in the transverse section of *S. laetum* mostly concentrated near vascular bundle, whereas the least was seen in *S. caryophyllatum* (Plate 8).

Table 2. Anatomical characterization of Leaf of Syzygium species.

Sr. no	Species	Epidermis	Cuticle	Palisade layer	Sclerenchyma sheath	Secretory canals	Druses	Vasculature
1	*S. cumini*	1 layer	Thick	2 layers	Single layered, Discontinuous	Mesophyll tissue and palisade tissue	present	Two distal ends do not meet
2	*S. caryophyllatum*	1 layer	Thin	2 layers	2- layered, Continuous	Mesophyll tissue and palisade tissue	present	Both distal ends fused
3	*S. laetum*	1 layer	Thick	1 layer	3- layered discontinuous	Mesophyll tissue and palisade tissue	Present and large in number	Both distal ends fused
4	*S. salicifolium*	1 layer	Thick	3 layers	2- layered Discontinuous	Mesophyll tissue and palisade tissue	Present	Both distal ends fused

Petiole Anatomy

The petiole from mature fresh leaves were collected and free-hand thin transverse sections were taken and observed under bright-field microscopy and the findings are summarized in Table 3 and Plate 8-10

Among the different *Syzygium* species studied, the shape, size in terms of length and width of petiole varied. There was winged outline in *S. cumini* and *S. caryophyllatum* whereas wavy outline in *S. laetum* and *S. caryophyllatum*. Epidermis is single layered in all four species, however shape of epidermal cells varied. In *S. cumini* and *S. laetum* round shaped epidermal cells were observed, in contrast to *S. caryophyllatum* and *S. salicifolium* in which rectangular epidermal cells were seen. Covered with a layer of cuticle of variable thickness, followed by parenchymatous cortex.

The cortical region showed large number of secretory canals (oil glands). These canals are arranged in ring on the outer side of cortical region close to the epidermal layer. The number of secretary cavities present varied in different species. Large number of continuous arrangements of these cavities were present in *S. laetum.* Druses were also seen in cortex, around the vascular region and in the phloem region, where again a large number of druses were seen *S. laetum* compared to other species.

The vascular bundles are conjoint and collateral. The vascular structure is incurved and have open vascular strand in *S. cumini, S. caryophyllatum, S. salicifolium*, whereas in *S. laetum* the vascular bundles are arranged in pouches in the centre which vary in number. Each bundle is surrounded by darkly pigmented cells which couldn’t be identified.

Table 3. Anatomical characteristics of petiole of *Syzygium* species.

S.R. No	*Syzygium* Species	Outline in transverse section	Vasculature	Secretory Canals	Druses
1	*S. cumini*	Winged	Incurved and open vascular strands	Present	Present
2	*S. caryophyllatum*	Winged	Incurved and open vascular strands	Present	Present
3	*S. laetum*	Wavy	Arranged in pouches	Present and large in number	Present and large in number
4	*S. salicifolium*	Wavy	Incurved and open vascular strands	Present	Present

Anatomy of Stem

Anatomical characteristics of stem of *Syzygium* species are summarized in Table 4 and Plate 10-12. The outline of transverse section varied from oval, elliptical to quadrangular in shape, *S. salicifolium* shows quadrangular shape with wings at the corner. Epidermis of the all the species is Uniseriate covered with a thin or thick layer of cuticle followed by multilayered cortex with secretory cavities. The cortex consisted of chlorenchyma cells below epidermis, remaining cortex consisted of parenchyma cells and a continuous arrangement of sclerenchyma cells in ring close to the secondary phloem in all four species. Pith is present in the centre of stem showing open type of vascular bundle. The vascular bundles are surrounded with sclerenchyma cells, where thick-walled sclerenchyma cells were seen in *S. laetum* and *S. salicifolium.*

The secretory cavities were found in the cortex region below the epidermis in all four species but were less in number as compared to leaves and petiole. The druses were seen present in the cortex region, pith and in the phloem region. Spherical secretory cavities were usually present in all species but large and oval secretory cavities were observed in *S. laetum*.

The presence of secretory cavities and druses were found in all the parts examined and in all four species. There was seen no epidermal appendages in any of the species.

Table 4. Anatomical characteristics of stem of *Syzygium* species.

S.R. No	*Syzygium* species	Outline in Transverse Section	Wings	Secretory canals	Druses	
					In cortex and phloem	In pith
1	*S. cumini*	Elliptical	Absent	Present	present	Absent
2	*S. caryophyllatum*	Oval	Absent	Present	present	Present
3	*S. laetum*	Oval	Absent	Present	present	Present
4	*S. salicifolium*	Quadrangular	Present	Present	present	Absent

Histochemical Analysis

Primary metabolites

Starch

Starch is the principle substance of the protoplast. Starch is composed of the long elastic chains of molecules, whose basic units are anhydrous glucose residue. The morphometric variation of grain is so extensive that they may be used taxonomically and pharmacologically up to a limited extend (Kadam 2013).

Localization of starch in transverse section of leaf

Starch deposition occurred widely in the transverse leaf sections. The common places of its accumulation are mesophyll tissue, parenchymatous tissue of the midrib, and in the phloem tissue if vascular bundles. In *S. cumini* there was found a great amount of starch present in the parenchymatous tissue of midrib and phloem region whereas comparatively less accumulation in other tissues. The similar condition was seen in *S. caryophyllatum* and *S. salicifolium*. The amount of starch observed in *S. salicifolium* was least among all four species examined. The highest amount of starch accumulation was observed in the leaf section of *S. laetum*, occupying almost all the tissues of the leaf except for epidermis, sclerenchyma sheath and xylem tissue. (Plate 13. Table 5)

Localization of starch in transverse section of petiole

As was observed in leaf sections, the highest accumulation of starch was seen in *S. laetum* in petiole section as well, accommodating the parenchymatous and phloem tissue. Followed by *S. cumini* and *S. caryophyllatum* where the accumulation was seen mostly around phloem tissue, and some scattered granules in parenchymatous region. The least

was observed in *S. salicifolium*, where the starch granules were seen scattered in parenchyma tissue. (Plate 14. Table 6)

Localization of starch in the transverse section of stem

In the transverse section of stem there was seen considerably similar amount of starch present in all the three species of *S. cumini*, *S. caryophyllatum* and *S. salicifolium*, with a slightly higher amount in *S. laetum*. Occupying mostly the spaces of pith, phloem and cortex region. A very unique arrangement was seen in *S. caryophyllatum* and *S. salicifolium*, where the starch granules were seen in small pouches arranged in the form of a ring around the vascular bundle just above the sclereid cells. (Plate 15. Table 7)

Lipids

The majority of plant lipids act as energy stores (e.g., triacylglycerols in seeds) or as plant membrane components (e.g., galactosyl glycerides in chloroplast membranes and phosphoglycerides in non-chloroplast membranes). Furthermore, surface lipids such as waxes and cutin provide an impervious barrier on the plant surface to reduce water loss and to provide protection against plant pathogens and toxins (McEniy and Kiely 2014)
The lipids are stained orange to red in color

Localization of lipids in transverse section of Leaf

There was seen a well distinguished layer of lipid in the leaf sections of *S. laetum* and *S. salicifolium*. In *S. caryophyllatum* and *S. laetum* lipids appeared to be present in the mesophyll and palisade tissue. (Plate 16. Table 5)

Localization of lipids in transverse section of petiole

In the petiole section of *S. cumini* and *S. caryophyllatum* and *S. laetum* globules of lipid were seen in parenchyma region towards the epidermis. *S. laetum* showed higher accumulation of lipids. In *S. salicifolium* the cell wall was fully stained for lipids. (Plate 17. Table 6)

Localization of lipids in transverse section of stem

In transverse section of stem, cell wall and phloem region appeared to be stained for lipids in *S. caryophyllatum* and *S. laetum*. However, there was not observed any lipids in stem section of *S. cumini* and *S. laetum*. (Plate 18. Table 7)

Secondary metabolites

Tannins

Tannins is a heterogenous group of phenol derivatives, usually related to glucosides. Tannins are particularly abundant in most of the species of *Syzygium*. No tissue appears to lack tannins entirely, however their concentration may vary (Kadam 2013). Tannins have a great medicinal value, because of its styptic and astringent properties, tannin has been used to treat tonsillitis, pharyngitis, hemorrhoids, and skin eruptions; it has been administered internally to check diarrhea and intestinal bleeding and as an antidote for metallic, alkaloidal, and glycosidic poisons, with which it forms insoluble precipitates (Raymond *et al*.,2012).

The tannins are stained blue to black in the test performed.

Localization of tannins in transverse section leaf

Tannins show distribution occurring in Epidermal layer,mesophyll and in the phloem region of leaf section. A large number of tannins was seen in *S. cumini*, followed by *S. caryophyllatum* and *S. laetum*. The least was seen in *S. salicifolium*. (Plate19. Table 5)

Localization of tannins in transverse section of Petiole

The epidermal layer, parenchyma tissue (mostly concentrated near epidermal layer) and some part of phloem showed the presence of accumulation of tannins. A large concentration of tannins was seen in petiole section of *S. caryophyllatum* and *S. laetum* followed by *S. salicifolium*, the least was observed in *S. cumini*. (Plate 20. Table 6)

Localization of tannins in transverse section of Stem

In stem section the cortex region of both primary and secondary growth showed the presence of tannins. There was also seen some number of tannins in the pith region of *S. caryophyllatum*. *S. cumini* showed a high amount of tannins present, whereas the least was seen in *S. caryophyllatum*. (Plate 21. Table 7)

Alkaloids

In the plant, alkaloid occurs as a soluble salt (citrate, Malate, tartarate, malonates, benzoates, iso-butyrates) or in combination with tannins. They are often localized in the peripheral tissue external layer of the bark and root or seed tegument. Alkaloids synthesis takes place at specific sites (growing root, chloroplast, laticiferous cell) (Mistry 2021).

The proposed roles of alkaloids in plant metabolism, plant catabolism, or plant physiology are end products of metabolism or waste products, storage reservoirs of nitrogen, protective agents for the plant against attack by predators, growth regulators (since structures of some of them resemble structures of known growth regulators), or substitutes for minerals in plants, such as potassium and calcium (Patel *et al*.., 2012). They exhibit antimicrobial, antifungal, antitumor, cytotoxic, antiplasmodial, antioxidant, antimutagenic, antigenotoxic and hallucinogenic properties. It acts on gamma-aminobutyric acid type A and monoamine oxidase A or B receptor, enhances insulin sensitivity and also produces vasorelaxant effect. Harmine prevents bone loss by suppressing osteoclastogenesis (Patel *et al*.,2012).

Alkaloids are stained yellow to reddish-orange with Dragendroff reagent

Localization of alkaloids in transverse section of Leaf

The epidermal layer, palisade tissue, mesophyll and phloem region show the presence of alkaloids in the leaf sections of all four species. Having more or less equal concentration of alkaloids in all the species. Though a fairy more concentration was seen in *S. cumini*. (Plate 22. Table 5)

Localization of alkaloids in transverse section of Petiole

In petiole section the epidermal layer is seen to be highly rich in alkaloids. The parenchyma and phloem region also showed the presence of alkaloids. The petiole section of *S. caryophyllatum* was observed to be highly concentrated with alkaloids. The least was seen in *S. laetum*. (Plate 23. Table 6)

Localization of alkaloids in transverse section of stem

The epidermal layer of *S. laetum* and *S. salicifolium* showed the presence of alkaloids, whereas other two species did not show alkaloids in their epidermal layer. The phloem tissue, primary and secondary cortex (mostly concentrated near epidermis) and also the pith region of all four sections taken showed the presence of alkaloids. The least accumulation was observed in *S. laetum*.(Plate 24. Table 7)

Lignin

Lignin is a substance found in vascular plants, usually within the cell walls and also between cells themselves. It is largely a supportive structure and is part of the secondary thickening of tall plants. It plays a part, along with the xylem vessels, along whose walls it is usually deposited, in controlling the transportation of liquid in plants. It prevents the walls of the xylem vessels collapsing under pressure and adds strength to the woody material of an older plant. (Liu *et al*., 2018).

Lignin's are stained blue green in toluidine blue o stain

Localization of lignin's in transverse section of Leaf

The scleral tissue of all four species was highly stained with lignin showing blue green coloration, a large amount of accumulation is seen in *S. laetum* and *S. caryophyllatum*. The phloem region of *S. cumini* and *S. caryophyllatum* also appeared to store lignin. The xylem cell wall of all four species showed the presence of lignin. (Plate 25. Table 5)

Localization of lignin's in transverse section of petiole

The epidermal and scleriedal layers showed presence of lignin in all four species. A high concentration of lignin was observed in *S. salicifolium* and *S. laetum.* (Plate 26. Table 6)

Localization of lignin in transverse section of stem

In the stem section of *S. cumini* and *S, salicifolium* the sclerenchyma cells and phloem region showed the presence of lignin. In *S. caryophyllatum* along with

sclerenchyma, epidermal was also seen lignified. In *Syzygium laetum* only the sclerenchyma layer showed the presence of lignin. (Plate 27. Table 7)

The presence of this secondary metabolites with other primary metabolites may contribute do the medicinal properties of the plants along with another ecological roles.

Table 5. Localisation of primary and secondary metabolites in transverse section of leaf in *Syzygium* species.

S.R. No	*Syzygium* species	Starch	Lipids	Tannins	Alkaloids	Lignin
1	***S. cumini***	Mesophyll, parenchyma region(midrib), phloem	Palisade tissue Mesophyll tissue	Epidermis, mesophyll, phloem	Epidermis. Palisade tissue, mesophyll and phloem	Sclerenchyma sheath, phloem and xylem cell walls
2	***S. caryophyllatum***	Mesophyll, parenchyma region(midrib), phloem	Palisade tissue Mesophyll tissue	Epidermis, mesophyll, phloem	Epidermis. Palisade tissue, mesophyll and phloem	Sclerenchyma sheath, phloem and xylem cell walls
3	***S. laetum***	Mesophyll, parenchyma region(midrib), phloem	Cell wall	Epidermis, mesophyll, phloem	Epidermis. Palisade tissue, mesophyll and phloem	Sclerenchyma sheath, xylem cell walls
4	***S. salicifolium***	Mesophyll, parenchyma region(midrib), phloem	Cell wall	Epidermis, mesophyll, phloem	Epidermis. Palisade tissue, mesophyll and phloem	Sclerenchyma sheath, xylem cell walls

Table 6. Localisation of primary and secondary metabolites in transverse section of petiole in *Syzygium* species.

S.R. No	*Syzygium* species	Starch	Lipids	Tannins	Alkaloids	Lignin
1	***S. cumini***	Parenchyma tissue, phloem	Epidermis, Parenchyma tissue	Epidermis, parenchyma tissue, phloem	Epidermis, parenchyma region and phloem	Epidermis and sclerenchyma sheath
2	***S. caryophyllatum***	Parenchyma tissue, phloem	Epidermis, Parenchyma tissue	Epidermis, parenchyma tissue, phloem	Epidermis, parenchyma region and phloem	Epidermis and sclerenchyma sheath
3	***S. laetum***	Parenchyma tissue, phloem	Epidermis, Parenchyma tissue	Epidermis, parenchyma tissue, phloem	Epidermis, parenchyma region and phloem	Epidermis and sclerenchyma sheath
4	***S. salicifolium***	Parenchyma tissue, phloem	Cell wall	Epidermis, parenchyma tissue, phloem	Epidermis, parenchyma region and phloem	Epidermis and sclerenchyma sheath

Table 7. Localisation of primary and secondary metabolites in transverse section of stem in *Syzygium* species

S.R. No	*Syzygium* species	Starch	Lipids	Tannins	Alkaloids	Lignin
1	***S. cumini***	Pith, phloem, cortex	Absent	Cortex	Phloem, cortex, pith	Sclerenchyma and phloem
2	***S. caryophyllatum***	Pith, phloem, cortex	Cell wall and phloem tissue	Cortex, pith	Phloem, cortex, pith	Epidermis sclerenchyma
3	***S. laetum***	Pith, phloem, cortex	Cell wall and phloem tissue	Cortex	Phloem, cortex, pith	Sclerenchyma sheath
4	***S. salicifolium***	Pith, phloem, cortex	Absent	Cortex	Epidermis, Phloem, cortex, pith	Sclerenchyma and phloem

Anti-diabetic Activity

Drugs that reduce hyperglycaemia by suppressing hydrolysis of starch as alpha-amylase inhibitors found useful in the control of diabetes mellitus. Many herbal extracts show the presence of anti-diabetic activity; however, they have not gained much importance due to lack of sustained scientific evidence (Sudha *et al*., 2011).

In-vitro anti-diabetic assay was carried out for all 4 mentioned species. Two analytical tests were performed *viz*., Dinitro salicylic acid reagent method and Iodine-Starch test methods, to detect the inhibitory activity of the plant extract using the enzyme α-amylase. The results of the same is recorded in the Tables 8 – 25 and the standard graphs are obtained (Fig 1-20) (Plates 28-31)

Reviewing different literatures, methanol was found to be the best polar solvent for extraction of plant extract for anti-diabetic studies. The methanol extract of leaves and bark was obtained using Soxhlet extraction method and the extract was concentrated using vacuum evaporator. The extract obtained was used for anti-diabetic studies. Different concentration of extract was used from 12.25 µg/mL to 200 µg/mL. The readings were obtained spectroscopically and percent inhibition activity and IC50 values were calculated graphically. IC50 value represents the concentration at which a substance exerts half of its maximal inhibitory effect, used to characterise effectiveness of an antagonist in inhibiting a biological process. The crude extracts of all four species of *S. cumini*, *S. caryophyllatum*, *S. laetum* and *S. salicifolium*, were observed to have α-amylase inhibitory activity that is concentration dependent.

The IC_{50} values and inhibitory effect (%) of α- amylase using DNS method is given in the Table 8-12 and 17-20 , fig 1-5 and 11-14. It was seen dose dependent effect was observed on increasing the concentrations of extract solutions, suggesting a competitive type of inhibition. Acarbose was used as reference standard. Acarbose showed highest inhibitory activity with IC50 value of 114.56µg/mL.

Anti-diabetic Activity of Leaf Extract

Methanolic leaf extracts of *S. cumini*, S. *caryophyllatum*, *S. laetum* and *S. salicifolium*, showed significant activities against α-amylase with IC50 values of 125.12, 142.09, 354.1, 127.04 µg/mL respectively (Table 8-12, fig 1-5). Indicating *S. cumini* to have the highest inhibition activity among the four with lowest IC50 value (125.12 µg/mL) and 62.97% inhibition at highest concentration of 200 µg/mL. Followed by *S. salicifolium* (127.04 µg/mL), *S. caryophyllatum* (142.09 µg/mL) and least activity was seen in *S. laetum* with highest IC_{50} value of 354.1 µg/mL, with only 28.27% inhibition at highest concentration.

The inhibitory action of methanolic plant extracts of leaves was checked against iodine test, and the findings were recorded and presented in Table 13-17. The similar trend was seen in this assay, which shows with increase in concentration resulted in greater inhibitory activity of the plant extract, with the highest in *S. cumini* showing absorbance 0.430 nm at highest concentration of 200 µg/mL and least in *S. laetum* with absorbance of 0.285nm at 200µg/mL.

Antidiabetic activity of bark extract

Similarly, methanolic extract of bark was also checked for inhibition against α-amylase using the DNS and Iodine-starch method. Which also showed dose dependent activity, showing greater inhibition activity with increase in concentration.

The IC_{50} values were obtained graphically, for bark extract using DNS method. Results are shown in the Table 18-21 and fig 11-14. The bark extract showed greater inhibition percentage for *S. cumini* with IC_{50} value of 89.64 µg/mL followed by *S. caryophyllatum* (IC50=154.86 µg/mL), *S. salicifolium* (IC_{50}=189 µg/mL) and *S. laetum* showing the least activity with IC_{50} value of 392.92 µg/mL.

For Iodine starch test of bark extract the absorbance was recorded at different concentrations and the readings are provided in Table 22-25 and fig 15-18. The graph obtained showed increase in concentration results into increase in inhibitory activity. At highest concentration i.e., at 200 µg/mL the bark extract of *S. cumini* showed the absorbance of 0.422nm indicating highest inhibition activity, whereas *S. laetum* showed the least absorbance of 0.270nm.

The comparative results given in the Fig19 shows that the leaf extracts of *S. cumini* and *S. salicifolium* showed better results than other two species, whereas for bark extract (Fig 20) *S. cumini* and *S. caryophyllatum* showed an excellent anti-diabetic activity.

Several other researchers have documented phenolic compounds exercising anti-diabetic activity by inhibiting α-amylase and α-glucosidase (Aklima *et al.*, 2014). Finding inhibitors of α-amylase from natural products may be safer than the conventional therapy

(Kim *et al*., 2005). Histochemical studies suggested that these plants are rich in tannins, and as study given by Camila Gabriel Kato *et al*. (2017) suggest that the condensed and hydrolysed tannins present are responsible for inhibitory activity of these extracts against α-amylase. Also, Gawade and Farooqui in 2017 reported the presence of alkaloids, tannin, glycosides exhibit anti-diabetic characteristics.

The α-amylase inhibition helps to increase the rate in which complex starch is broken down to glucose however, the more the inhibition for instance by Acarbose, the more the side effects like intestinal disorder and also kidney dis-functioning (Kwon *et al*., 2006). Inhibition of α-amylase in excess by Acarbose causes a release of starch in its undigested form into the lower gastrointestinal tract whereby abnormal fermentation of the undigested starch by the intestinal micro flora takes place, causing abdominal discomforts and diarrhoea. Thus, moderate inhibition of α–amylase is desired. This study therefore considers the moderate inhibition of α-amylase by methanolic extract of *S. cumini*, *S caryophyllatum*, *S. laetum* and *S. salicifolium* might not induce side effects caused by presently used prescribed medication for type 2 diabetes.

Table 8. Alpha-amylase inhibition by Acarbose using DNS method

	Conc µg/mL	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation	Inhibition %	IC_{50} value
Acarbose	**Control**	0.218	0.222	0.214	0.218	0.003		114.56
	12.5	0.191	0.201	0.211	0.201	0.008	4.95	
	25	0.12	0.125	0.109	0.118	0.007	29.15	
	50	0.104	0.1	0.108	0.104	0.003	42.6	
	100	0.01	0.099	0.011	0.04	0.042	62.39	
	200	0.0022	0.0015	0.003	0.002	0.001	62.97	

Table 9. α-amylase inhibition by *S. cumini* using DNS method (leaf extract)

	Conc µg/mL	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation	Inhibition %	IC_{50} value
S. cumini	Control	0.314	0.322	0.399	0.345	0.038		125.12
	12.5	0.3	0.298	0.311	0.303	0.006	12.17	
	25	0.245	0.29	0.31	0.282	0.027	18.55	
	50	0.114	0.281	0.245	0.213	0.072	38.26	
	100	0.111	0.268	0.211	0.197	0.065	43.18	
	200	0.085	0.123	0.109	0.106	0.016	69.56	

Table 10. α-amylase inhibition by *S. caryophyllatum* using DNS method (leaf extract)

	Conc µg/mL	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation	Inhibition %	IC_{50} value
S.caryophyllatm	Control	0.299	0.271	0.322	0.297	0.021		142.09
	12.5	0.282	0.27	0.301	0.284	0.013	4.3	
	25	0.262	0.255	0.241	0.253	0.009	15.15	
	50	0.179	0.231	0.21	0.207	0.021	30.63	
	100	0.159	0.229	0.115	0.168	0.047	43.77	
	200	0.122	0.117	0.106	0.115	0.007	62.62	

Table 11. α-amylase inhibition by *S. laetum* DNS method (leaf extract)

	Conc µg/mL	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation	inhibition %	IC_{50} value
S. laetum	control	0.342	0.356	0.333	0.344	0.009		354.1
	12.5	0.333	0.352	0.328	0.338	0.010	1.74	
	25	0.309	0.347	0.319	0.325	0.016	5.24	
	50	0.308	0.345	0.297	0.317	0.021	7.87	
	100	0.292	0.311	0.265	0.289	0.019	15.73	
	200	0.214	0.297	0.228	0.246	0.036	28.27	

Table 12. α-amylase inhibition by *S. salicifolium* DNS method (leaf extract)

	Conc µg/mL	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation	inhibition %	IC_{50} value
S. salicifolium	control	0.331	0.356	0.377	0.355	0.019		127.04
	12.5	0.312	0.265	0.356	0.311	0.037	12.53	
	25	0.299	0.261	0.325	0.295	0.026	17.2	
	50	0.209	0.211	0.221	0.214	0.005	41.1	
	100	0.169	0.208	0.119	0.165	0.036	55.1	
	200	0.152	0.152	0.125	0.143	0.013	61.51	

Table 13. α-amylase inhibition by Acarbose using Iodine starch method

	Conc ug/ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation
Acarbose	Control	0.119	0.121	0.11	0.117	0.005
	12.5	0.123	0.13	0.12	0.124	0.004
	25	0.221	0.231	0.225	0.226	0.004
	50	0.387	0.372	0.385	0.381	0.007
	100	0.401	0.442	0.433	0.425	0.018
	200	0.499	0.479	0.487	0.488	0.008

Table 14. α-amylase inhibition by *S. cumini* using Iodine starch method (leaf extract).

	Conc ug/ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation
S. cumini	control	0.115	0.12	0.156	0.130	0.018
	12.5	0.176	0.223	0.222	0.207	0.022
	25	0.268	0.275	0.297	0.280	0.012
	50	0.359	0.361	0.365	0.362	0.002
	100	0.398	0.415	0.395	0.403	0.009
	200	0.461	0.431	0.398	0.430	0.026

Table 15. α-amylase inhibition by *S. caryophyllatum* using iodine starch method (leaf extract).

	Conc µg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation
S.caryophyllatum	Control	0.133	0.121	0.102	0.119	0.013
	12.5	0.142	0.129	0.119	0.130	0.009
	25	0.169	0.145	0.12	0.145	0.020
	50	0.255	0.2	0.241	0.232	0.023
	100	0.351	0.372	0.396	0.373	0.018
	200	0.391	0.397	0.425	0.404	0.015

Table 16: α-amylase inhibition by *S. laetum* using iodine starch method (leaf extract).

	Conc ug/ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation
S. laetum	control	0.105	0.087	0.101	0.098	0.008
	12.5	0.107	0.123	0.123	0.118	0.008
	25	0.117	0.121	0.159	0.132	0.019
	50	0.117	0.189	0.205	0.170	0.038
	100	0.153	0.2	0.301	0.218	0.062
	200	0.231	0.246	0.378	0.285	0.066

Table 17. α-amylase inhibition by *S. salicifolium* using iodine starch method (leaf extract).

	Conc µg/ml	**Absorbance 1**	**Absorbance 2**	**Absorbance 3**	**Mean OD**	**Deviation**
S. salicifolium	Control	0.19	0.1	0.181	0.157	0.040
	12.5	0.203	0.201	0.296	0.233	0.044
	25	0.267	0.276	0.248	0.264	0.012
	50	0.284	0.321	0.337	0.314	0.022
	100	0.29	0.359	0.352	0.334	0.031
	200	0.418	0.38	0.4	0.399	0.016

Table 18. α-amylase inhibition by *S. cumini* using DNS method (bark extract)

	Conc µg/mL	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation	Inhibition %	IC_{50} Value
S. cumini	Control	0.394	0.382	0.449	0.408	0.03		89.64
	12.5	0.3	0.298	0.311	0.303	0.01	25	
	25	0.225	0.29	0.306	0.274	0.04	32.84	
	50	0.114	0.281	0.245	0.213	0.07	47.79	
	100	0.111	0.268	0.211	0.197	0.06	51.71	
	200	0.085	0.123	0.071	0.093	0.02	77.2	

Table 19. α-amylase inhibition by *S. caryophyllatum* using DNS method (bark)

	Conc µg/mL	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation	Inhibition %	IC_{50} Value
S. caryophyllatum	Control	0.399	0.371	0.392	0.387	0.012		154.86
	12.5	0.382	0.371	0.361	0.371	0.009	4.13	
	25	0.362	0.351	0.341	0.351	0.009	9.3	
	50	0.275	0.291	0.221	0.262	0.030	32.29	
	100	0.251	0.269	0.115	0.212	0.069	45.21	
	200	0.22	0.187	0.106	0.171	0.048	55.81	

Table 20: α-amylase inhibition by *S. laetum* DNS method (bark extract)

	Conc ug/ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation	Inhibition	IC_{50} Value
S. laetum	Control	0.329	0.351	0.33	0.337	0.010		392.92
	12.5	0.318	0.349	0.328	0.332	0.013	1.48	
	25	0.302	0.347	0.319	0.323	0.019	4.15	
	50	0.32	0.335	0.291	0.315	0.018	6.52	
	100	0.292	0.311	0.245	0.283	0.028	16.02	
	200	0.264	0.257	0.238	0.253	0.011	24.92	

Table 21: α-amylase inhibition by *S. salicifolium* DNS method (bark extract)

	Conc µg/mL	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation	Inhibition %	IC_{50}
S. salicifolium	Control	0.331	0.356	0.377	0.355	0.019		189
	12.5	0.312	0.265	0.356	0.311	0.037	12.39	
	25	0.299	0.261	0.325	0.295	0.026	16.9	
	50	0.29	0.221	0.241	0.251	0.029	29.29	
	100	0.169	0.208	0.219	0.199	0.021	43.94	
	200	0.182	0.192	0.195	0.189	0.006	46.76	

Table 22: α-amylase inhibition by *S. cumini* using Iodine starch method (bark)

	Conc µg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation
S. cumini	Control	0.115	0.106	0.12	0.114	0.006
	12.5	0.176	0.116	0.133	0.142	0.025
	25	0.268	0.198	0.202	0.223	0.032
	50	0.359	0.267	0.27	0.299	0.043
	100	0.398	0.368	0.38	0.382	0.012
	200	0.461	0.439	0.427	0.442	0.014

Table 23: α-amylase inhibition by *S. caryophyllatum* using iodine starch method (bark extract)

	Conc µg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean OD	Deviation
S. caryophyllatum	Control	0.133	0.125	0.167	0.142	0.018
	12.5	0.142	0.168	0.176	0.162	0.015
	25	0.169	0.223	0.212	0.201	0.023
	50	0.255	0.278	0.256	0.263	0.011
	100	0.301	0.359	0.325	0.328	0.024
	200	0.331	0.364	0.435	0.377	0.043

Table 24: α-amylase inhibition by *S. laetum* using iodine starch method (bark extract)

	Conc µg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean	Deviation
S. laetum	Control	0.105	0.111	0.101	0.106	0.004
	12.5	0.107	0.12	0.109	0.112	0.006
	25	0.117	0.145	0.113	0.125	0.014
	50	0.117	0.165	0.156	0.146	0.021
	100	0.153	0.178	0.234	0.188	0.034
	200	0.231	0.267	0.311	0.270	0.033

Table 25: α-amylase inhibition by *S. salicifolium* using iodine starch method (bark extract)

	Conc µg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean (OD)	Deviation
S. salicifolium	Control	0.19	0.121	0.107	0.139	0.036
	12.5	0.203	0.129	0.116	0.149	0.038
	25	0.267	0.178	0.182	0.209	0.041
	50	0.284	0.215	0.219	0.239	0.032
	100	0.411	0.332	0.355	0.366	0.033
	200	0.428	0.301	0.376	0.368	0.052

CONCLUSION

During this study comparative anatomical characterization of leaf, petiole and stem, histochemical analysis and antidiabetic *in-vitro* analysis of 4 species of *Syzygium* from the Western Ghats of India were carried out. For anatomy, the anatomical characters such as vasculature, thickness of cuticle, sinuosity in epidermal cell walls, number of palisade layers, presence of sclereids, cortical layer and secondary growth in stem, size and distribution of secretory canals and druses in different parts are of taxonomic importance.

Localizing different primary and secondary metabolites in histochemical analysis of leaf, stem and petiole, the tissues were observed to be highly occupied by tannins and alkaloids along with other primary metabolites, although their concentration varies in different parts in different species. The presence of these secondary metabolites is associated with the medicinal properties of the plants.

Alpha-amylase inhibition activity for the methanolic plant extract (extract extracted by using Soxhlet method) using DNS and Iodine starch method showed significant results for all four species examined, where for leaf extract *S. cumini* and *S. salicifolium* showed maximum anti-diabetic activity followed by *S. caryophyllatum* and *S. laetum*. Wherein for bark extract *S.cumini* and *S. caryophyllatum* showed maximum inhibition whereas *S.salicifolium* and *S.laetum* showed moderate inhibition activity. The antidiabetic activity of these plants may be associated with the presence of tannins and alkaloids and presence of other essential oils and secondary metabolites in these plants.

SUMMARY

Genus *Syzygium* belonging to the family Myrtaceae comprises around 1200 species. Wherein, fruits of some of the species are edible and also have medicinal properties. Barks and leaves are also reported to posses the medicinal properties and are used in preparations of many drugs.

The present study aimed at comparative characterization of anatomy, histochemistry and *in-vitro* antidiabetic activity of 4 *Syzygium* species from the Western Ghats of India, namely *Syzygium cumini*, *Syzygium caryophyllatum*, *Syzigium laetum* and *Syzygium salicifolium*. The specimens were collected from different parts of Western Ghats in Goa. For anatomical and histochemical analysis different parts of the plants were put into study like leaves, petiole and stem. For antidiabetic studies leaves and bark of the trees were used.

Anatomical characters for leaf, petiole and stem were observed by taking free hand sections, stained with 1% safranin and toluidine blue O stain, mounting and observing it under bright field microscope. The leaf anatomy of all four species showed a dorsiventral structure.The cuticle thickness, palisade layers, layers of sclerenchyma sheath varied among different species. In *S. cumini* was seen arc shaped vascular bundle with incurved distal ends, where two distal ends do not meet. On other hand in other three species two adaxial curls of vascular bundles almost fused and both bundle sheath and phloem both curved inwards and fused adaxially. Among the different *Syzigium* species studied, the shape, size in terms of length and width of petiole varied. *S.laetum* showed a unique anatomical character in the transverse section, where the vascular bundles were arranged in pouches at the centre of the petiole. The outline of transverse section of stem varied from oval, elliptical to quadrangular in shape where *S.salicifolium* shows quadrangular shape

with wings at the corner. Stem section showed the presence of chlorenchyma cells and a thick scelerenchyma sheath was observed in all for species. In all different parts examined of all four species there were present a large number of druses and secretory cavities embedded in different tissues of the plants. *S.laetum* showed the highest number of druses and secretory cavities.

For histochemical studies different stains were used to localize different primary and secondary metabolites present in the plant tissue. Tests for starch, lipids, tannins, alkaloids and lignins were conducted. A large number of densely packed granules of starch was seen in *S.laetum* whereas in other species moderate amount of starch was present. *S.salicifolium* and *S. leatum* showed a very well distinguished lipid stained cell wall in leaf section, whereas in other species presence of lipids was not clear. In petiole section the cell wall of *S. salicifolium* stained for lipids and in stem section the cell wall of *S.caryophyllatum* and *S. laetum* showed presence of lipids. In leaf section a large amount of tannins was seen in *S. cumini*, in petiole section *S.caryophyllatum* and *S,laetum* showed highest concentration and *S. cumini* for stem section. All species showed more or less equal amount of alkaloids present although a fairly less concentration was seen in *S.laetum*. In the test performed for lignin the cell wall, sclerenchyma layer and xylem cell wall was observed to be stained for lignins, with *S. laetum* showed highest amount of lignin. The presence of these secondary metabolites along with primary metabolites responsible for medicinal properties of the plants.

For antidiabetic studies methanolic extract of leaves and bark was extracted using Soxhlet extraction method. The extracts were checked for α-amylase inhibition activity.The two tests used were DNS method for α-amylase inhibition and Iodine-starch method. The readings were obtained spectrometrically and % inhibition activity and IC50 value was calculated graphically. For methonolic leaf and bark extract all four species

showed significant antidiabetic acticvity with cinsiderable variation in their activity percentange. For leaf extract *S.cumini* and *S.salicifolium* showed highest inhibition activity, and for bark extract *S.cumini* and *S. caryophyllatum* showed maximum inhibition..*S,laetum* showed moderately lesser inhibition activity for α-amylase. Presence of large amount of tannins and alkaloids are reported to give the plants its antidiabetic property.

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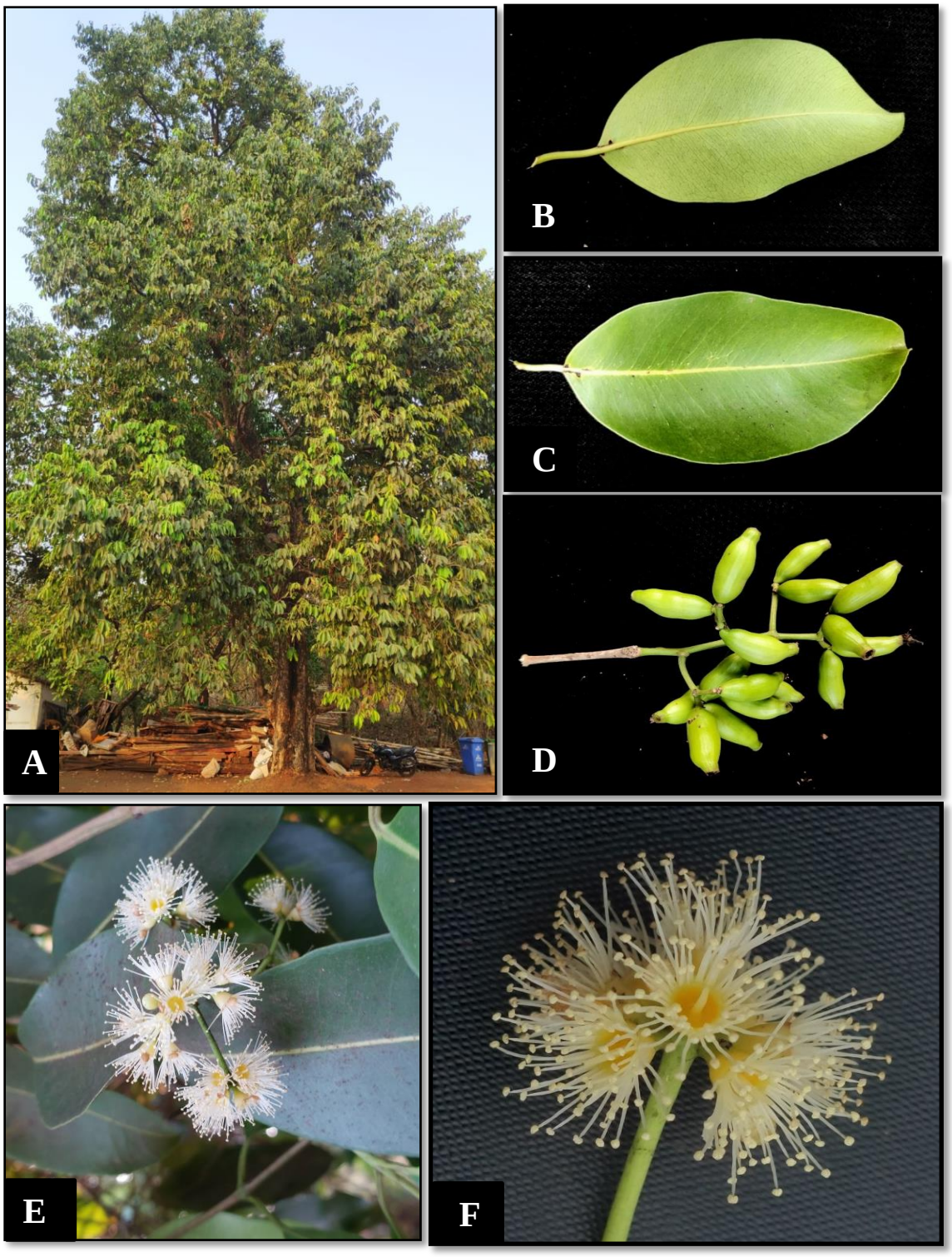

Plate1. ***Syzygium cumini*** **A) Habit B) Adaxial leaf surface C) Abaxial leaf surface D) Fruits E) Twig with flowers F) Flower**

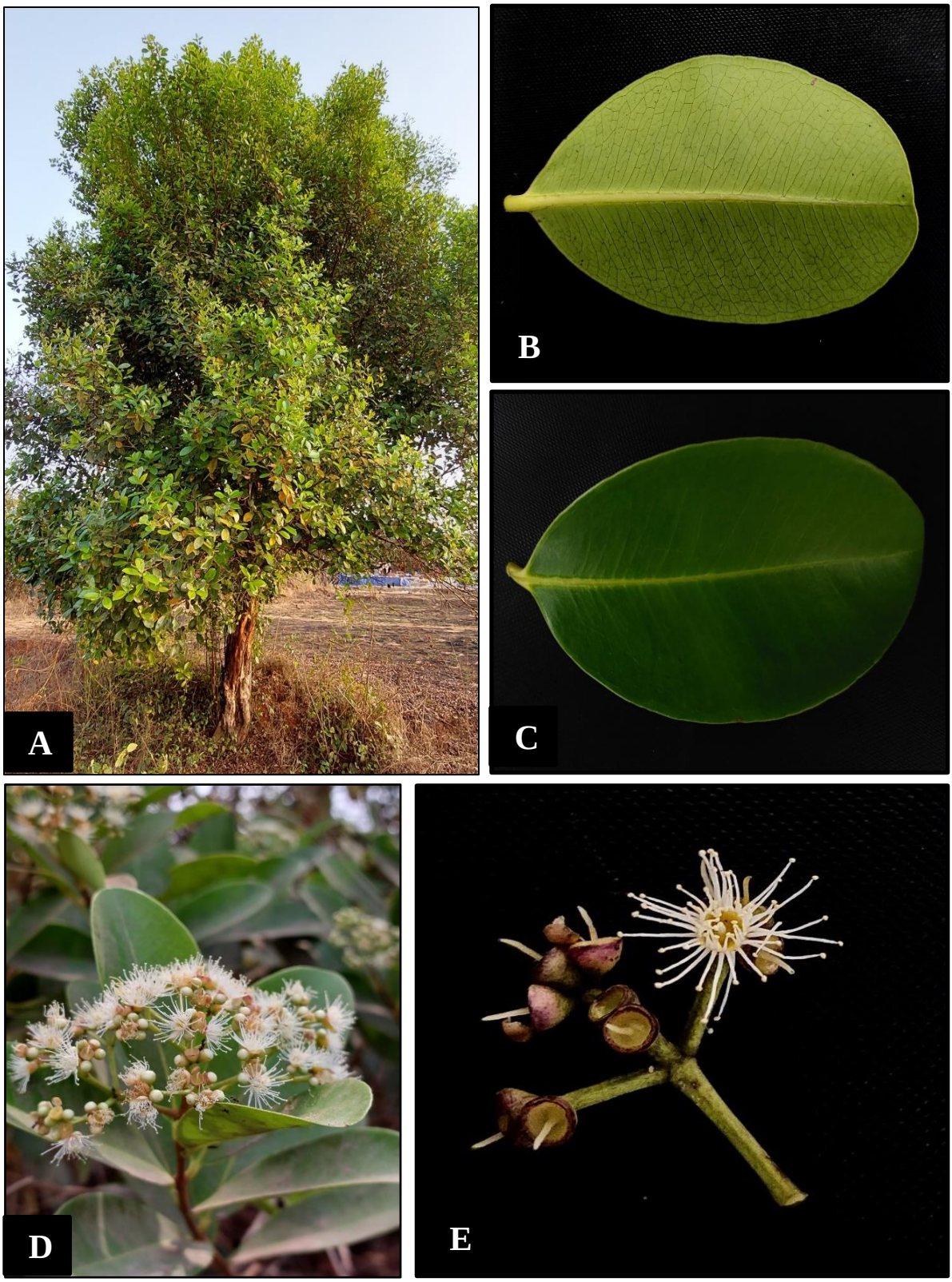

Plate 2. *Syzygium caryophyllatum* A) Habit B) Adaxial leaf surface C) Abaxial leaf surface D) Twig with flowers E) Flower

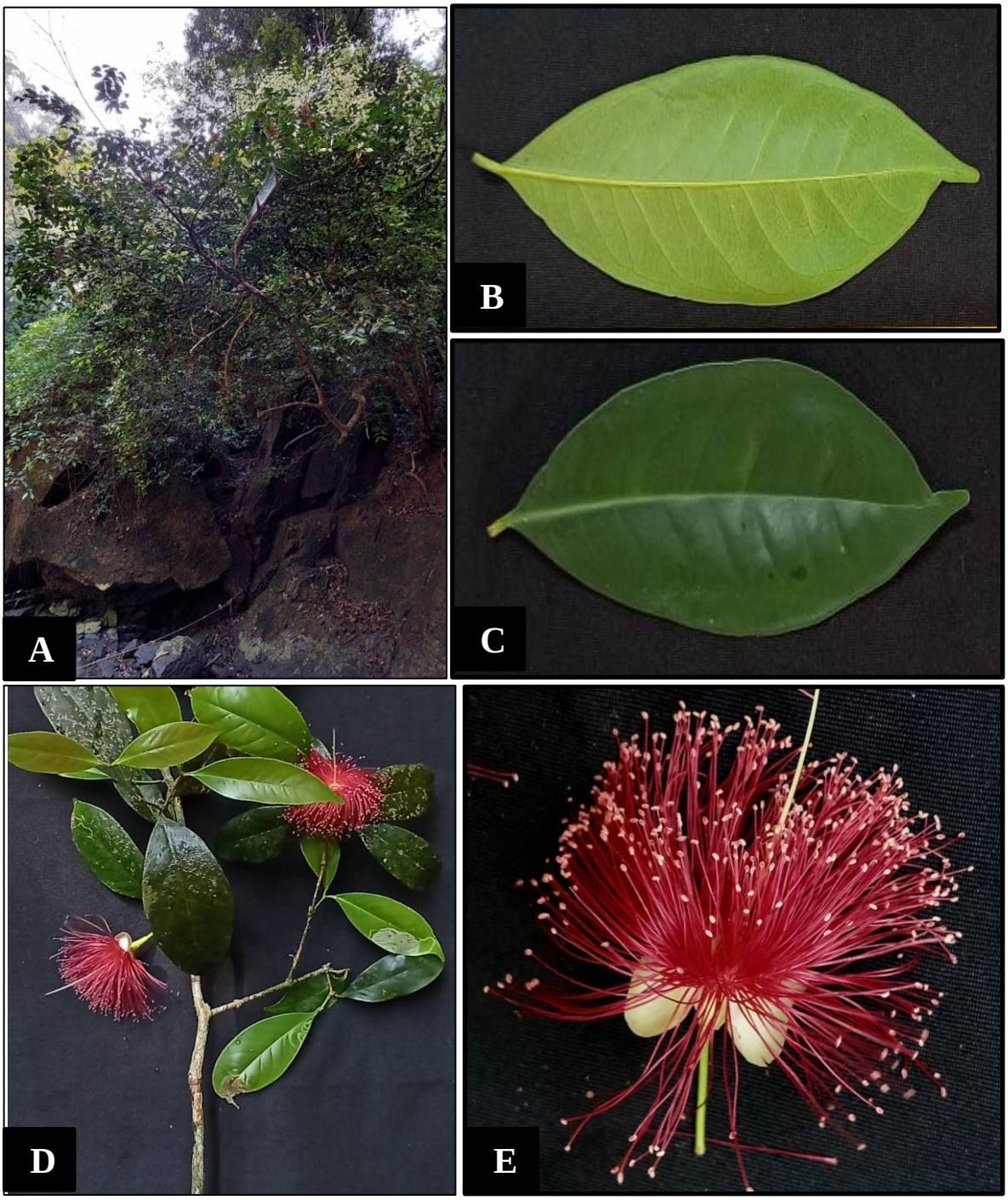

Plate 3. ***Syzygium laetum*** **A) Habit B) Adaxial leaf surface C) Abaxial leaf surface D) Twig with flowers E) Flower**

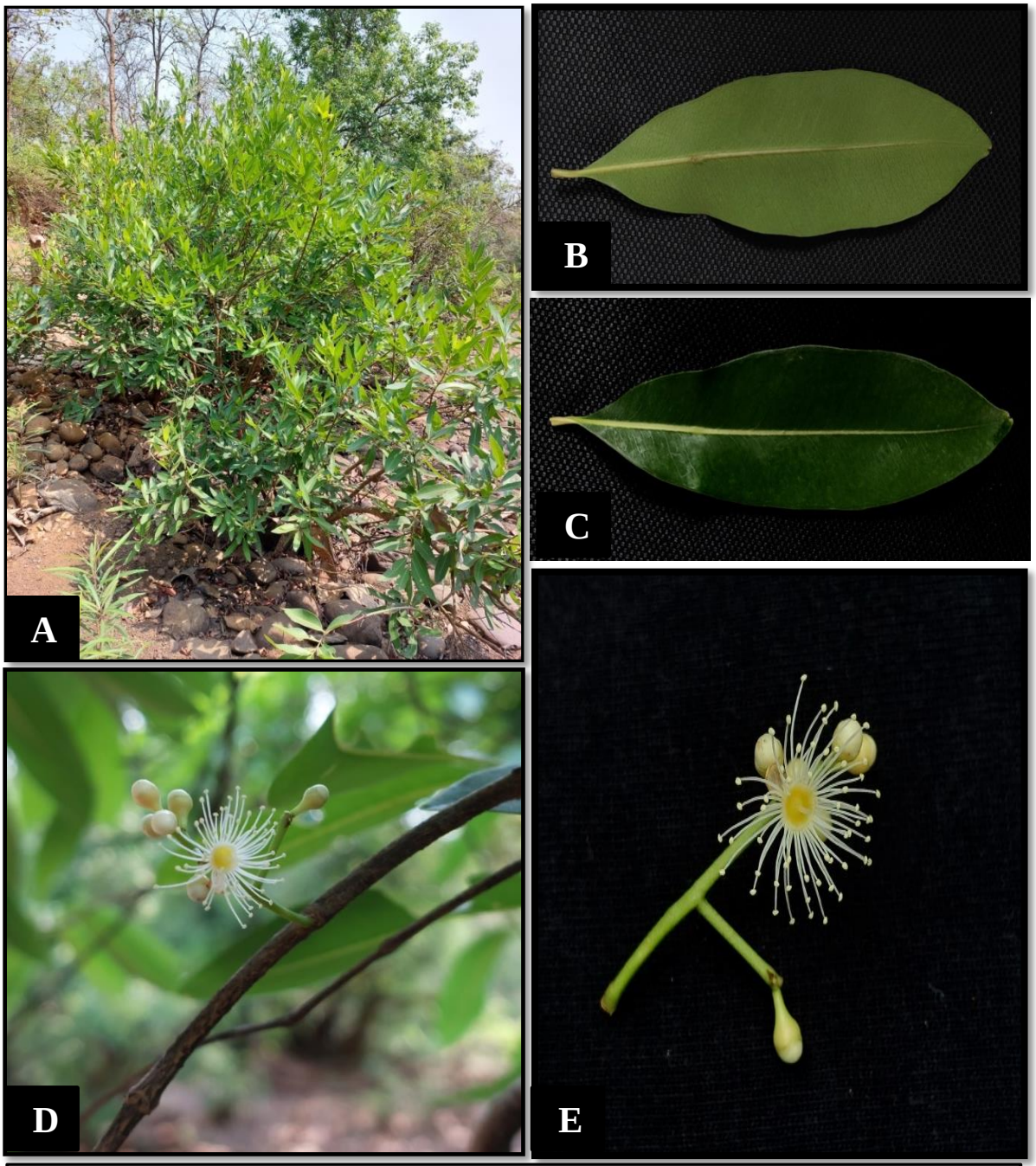

Plate 4. ***Syzygium salicifolium*** **A) Habit B) Adaxial leaf surface C) Abaxial leaf surface D) Twig with flowers E) Flower**

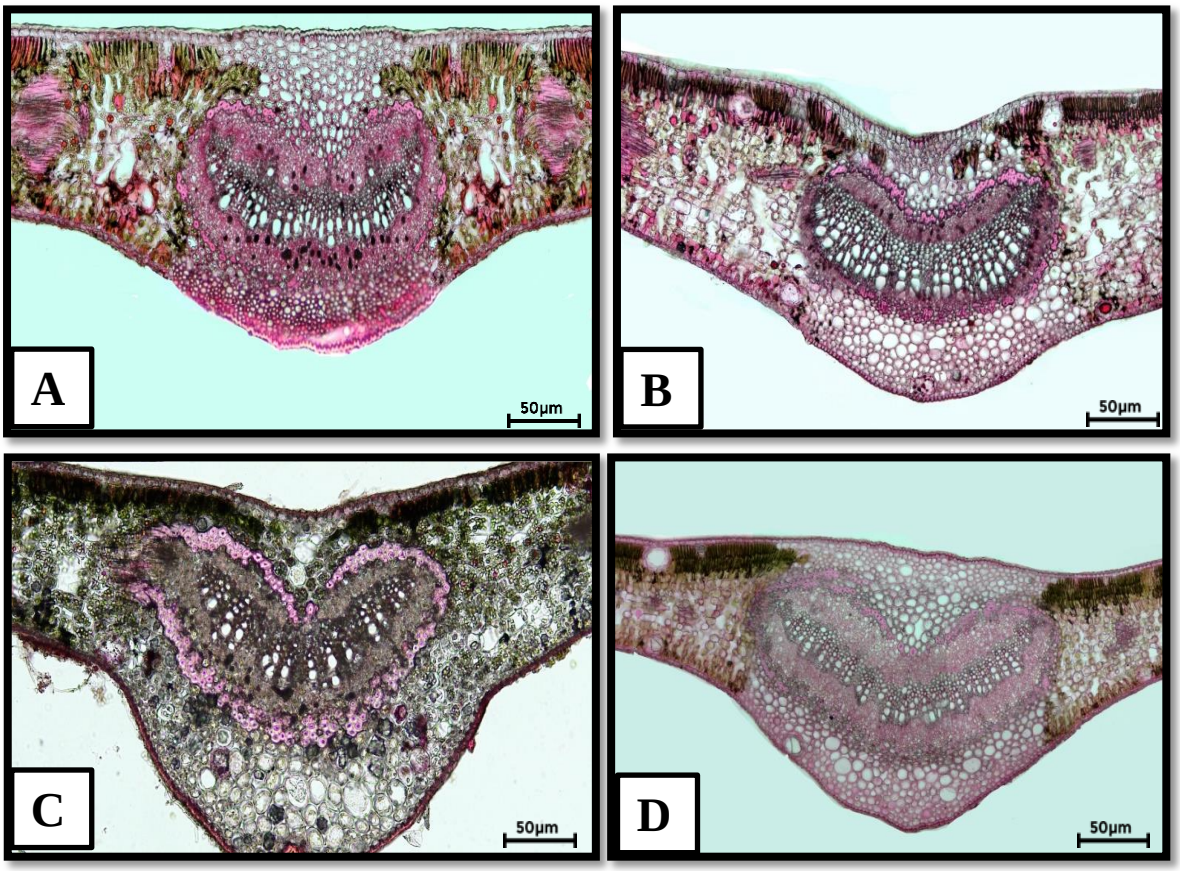

Plate 5. Overview of transverse sections of leaf of; A) *S. cumini,* B) *S. caryophyllatum* C) *S. laetum,* D) *S. salicifolium*

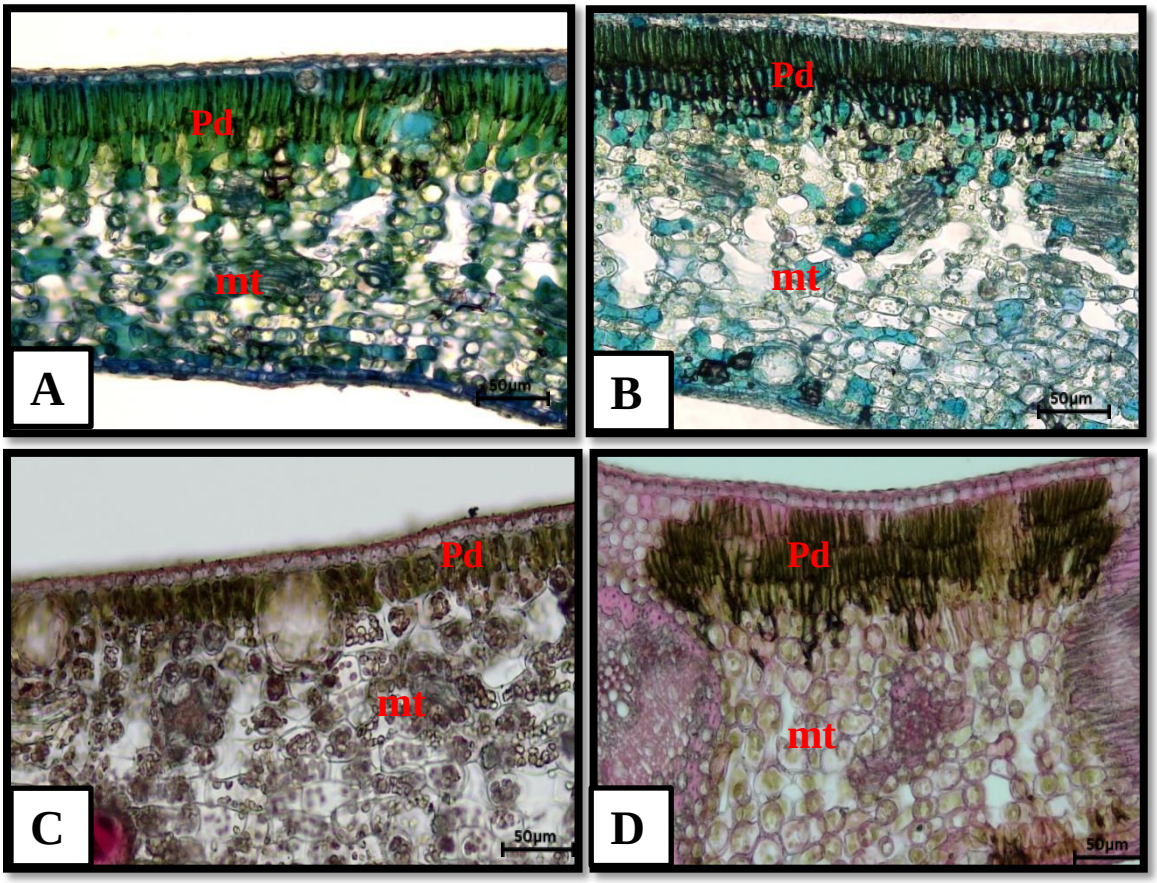

Plate 6. Transverse section showing layers of palisade cells and spongy parenchyma/Mesophyll tissue cells: A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium* *Pd- palisade tissue , Mt-mesophyll tissue.

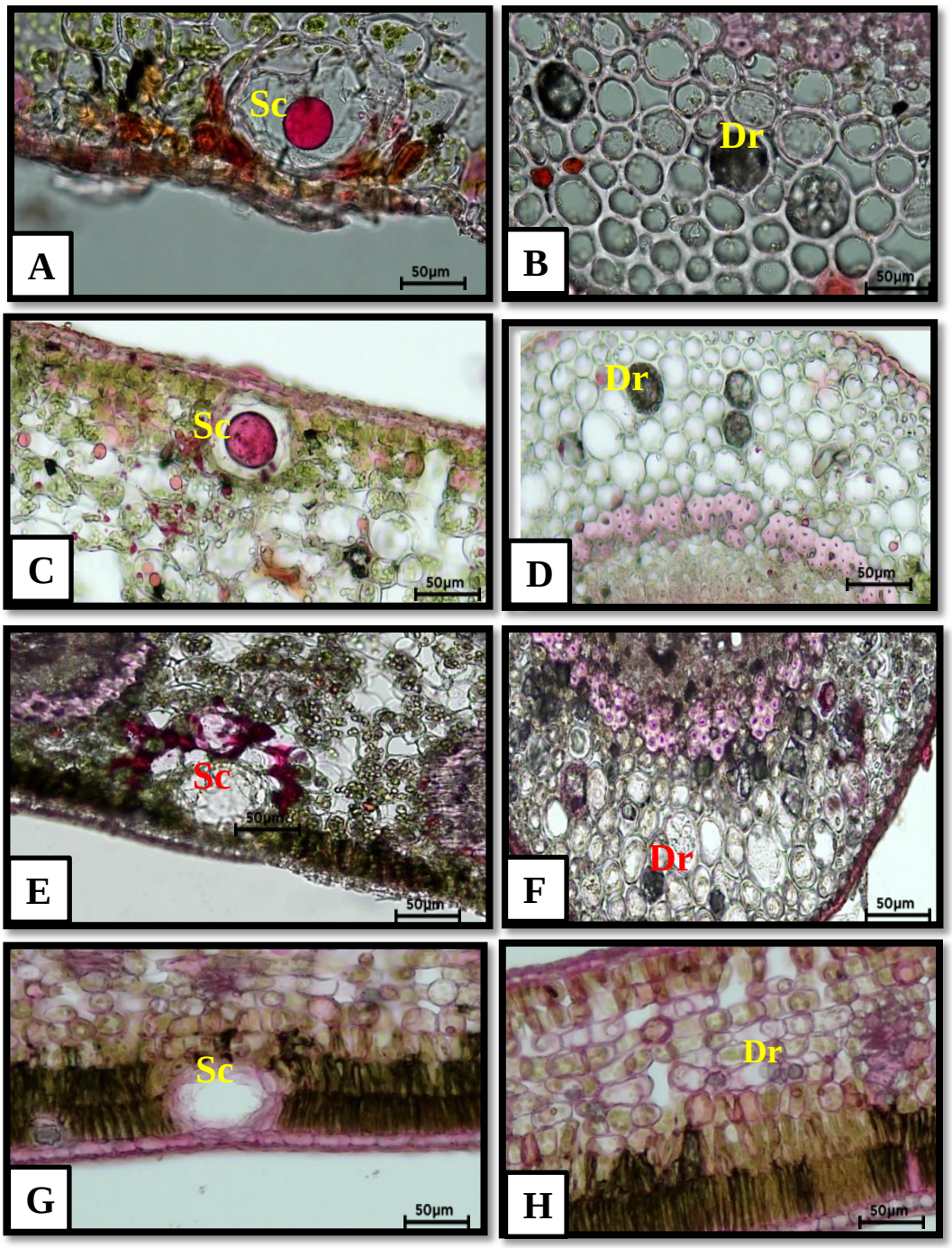

Plate 7. Presence of secretory cavities and druses in leaf section : A) *S. cumini* (S.C) B*) S. cumini* (dr*)* C) *S. caryophyllatum*(Sc*)* D) *S. caryophyllatum* (dr) E) *S. laetum* (Sc) F) *S. laetum*(dr*)* G) *S. salicifolium (Sc) .H) S. salicifolium (dr)* * dr-druses, Sc- secretory cavities.

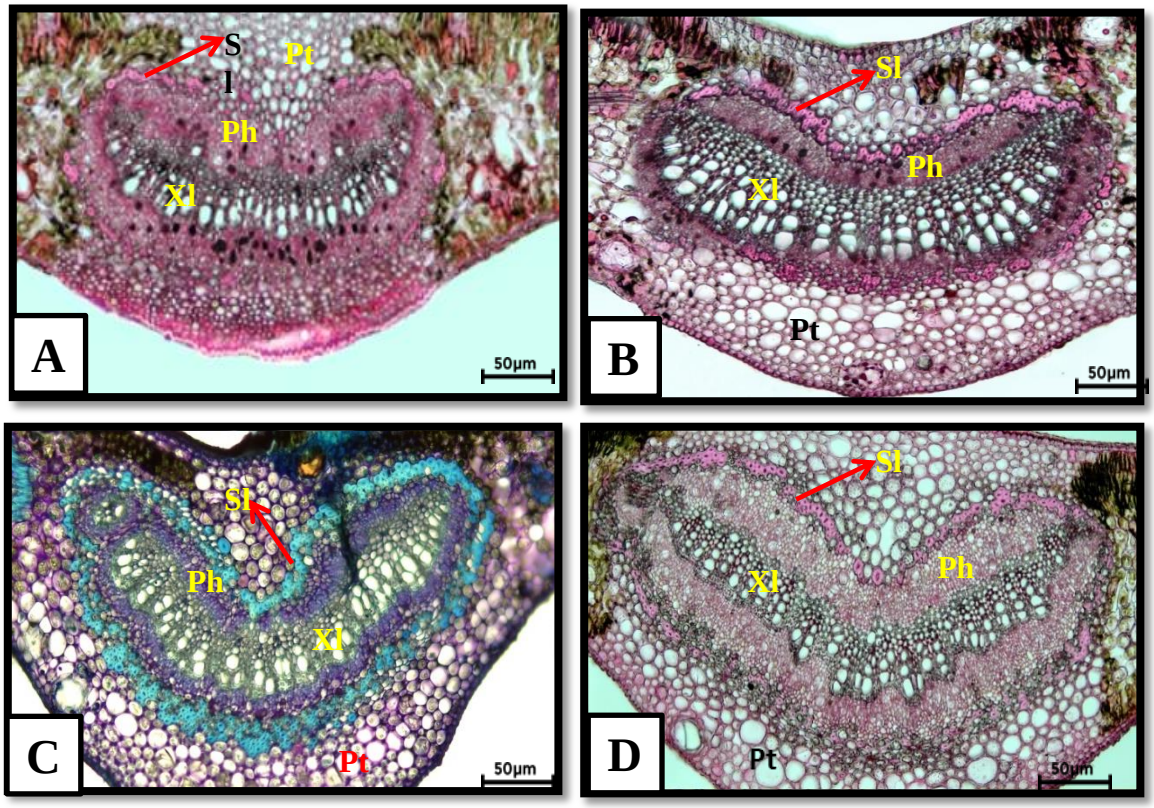

Plate 8. Transverse section of leaf showing midrib: *A) S. cumini* B) *S. caryophyllatum* C) *S.laetum* D) *S. salicifolium* *Sl- sclerieds, Pt-parenchymatous tissue ,Ph-phloem, xl-xylem

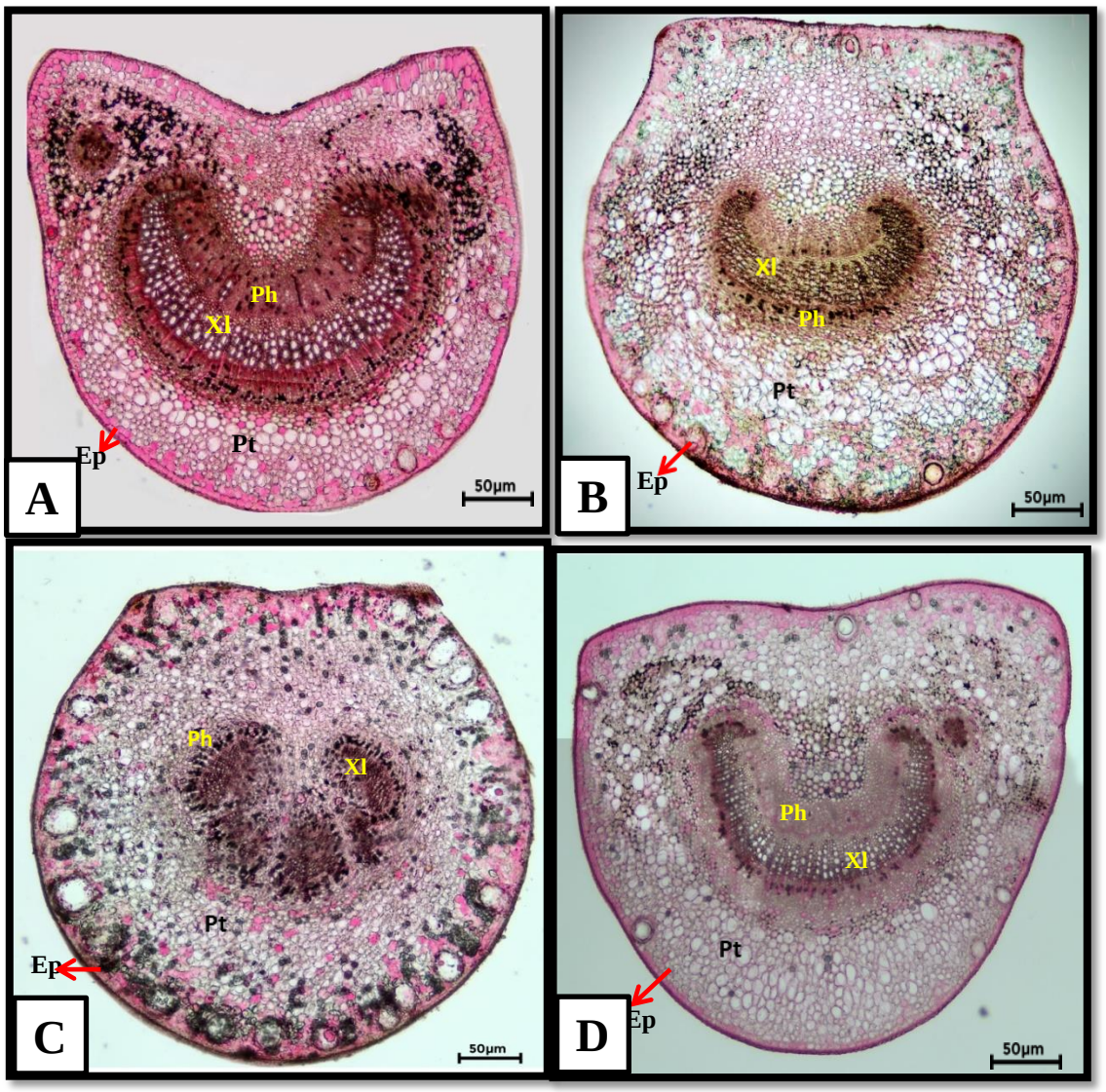

Plate 9. Transverse section of mature petiole: A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium* * Ep- epidermis, Pt-parenchyma tissue, Xl-xylem, Ph-Phloem

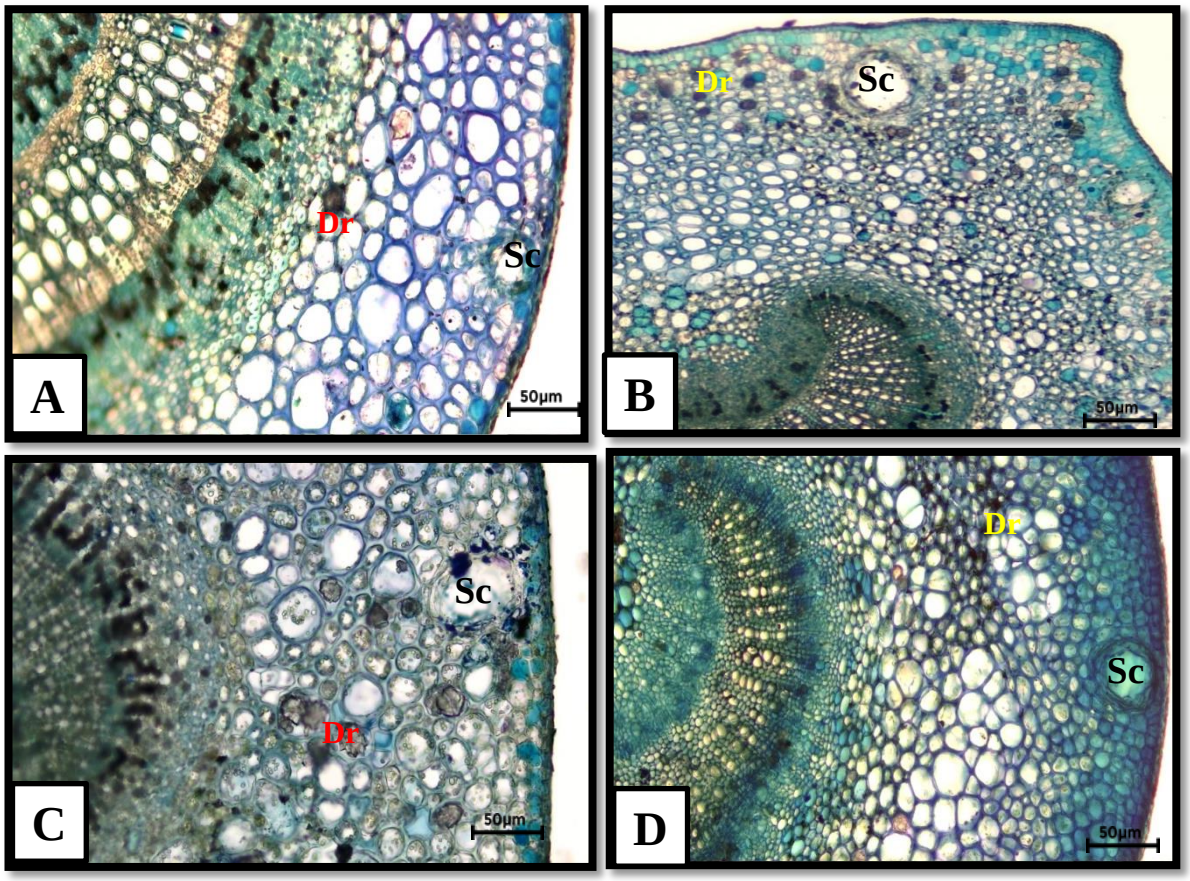

Plate 10. Transverse section of petiole showing secretory cavities and druses in: A) *S. cumini* B) *S. caryophyllatum* C) *S.laetum* D) *S. salicifolium* *Sc-secretory cavities, Dr- Druses

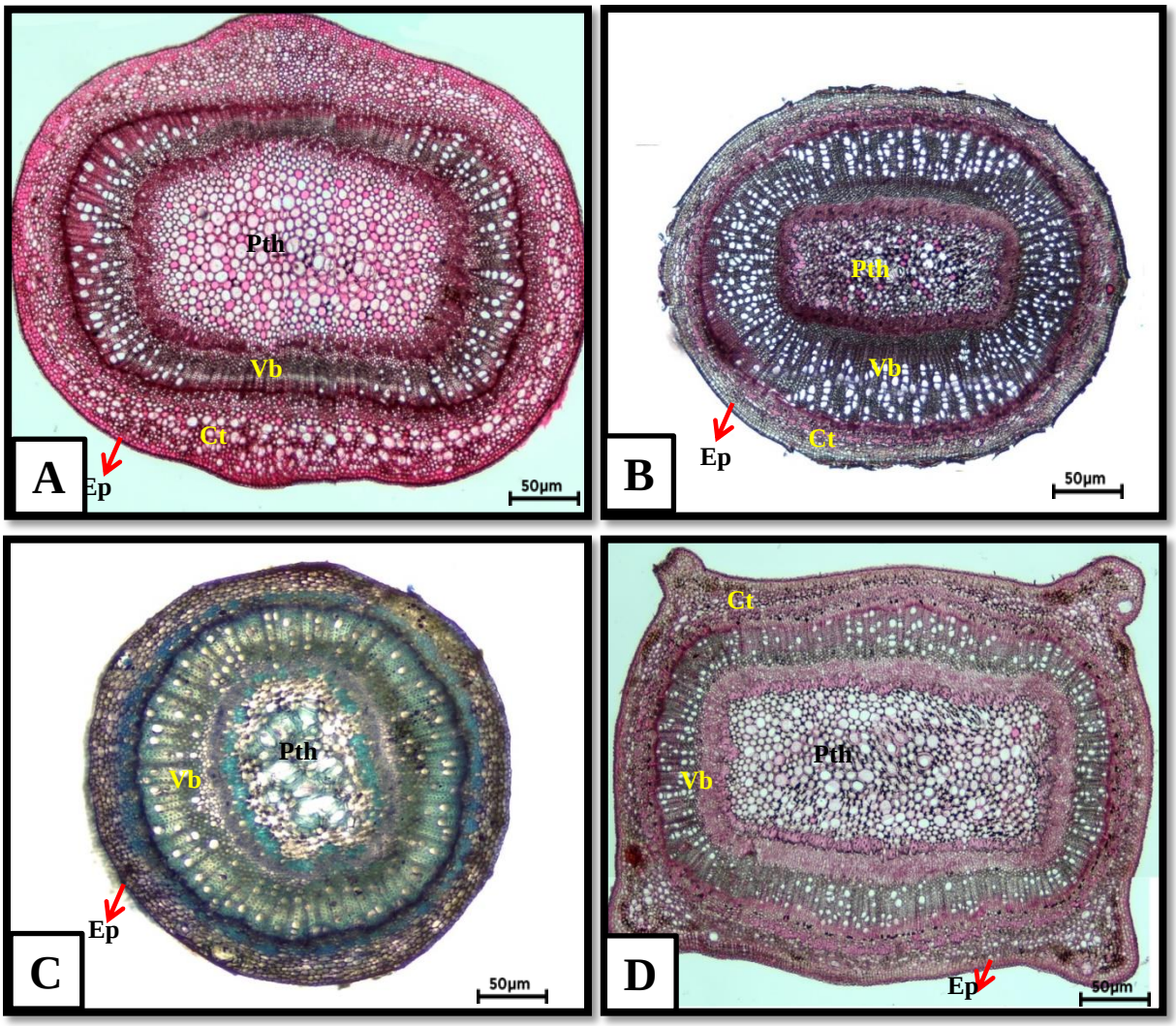

Plate 11. Overview of transverse section of stem in: A) *S. cumini* B) *S. caryophyllatum* C) *S.laetum* D) *S. salicifolium* * Ep- epidermis, pth-pith, Vb-vascular bundle

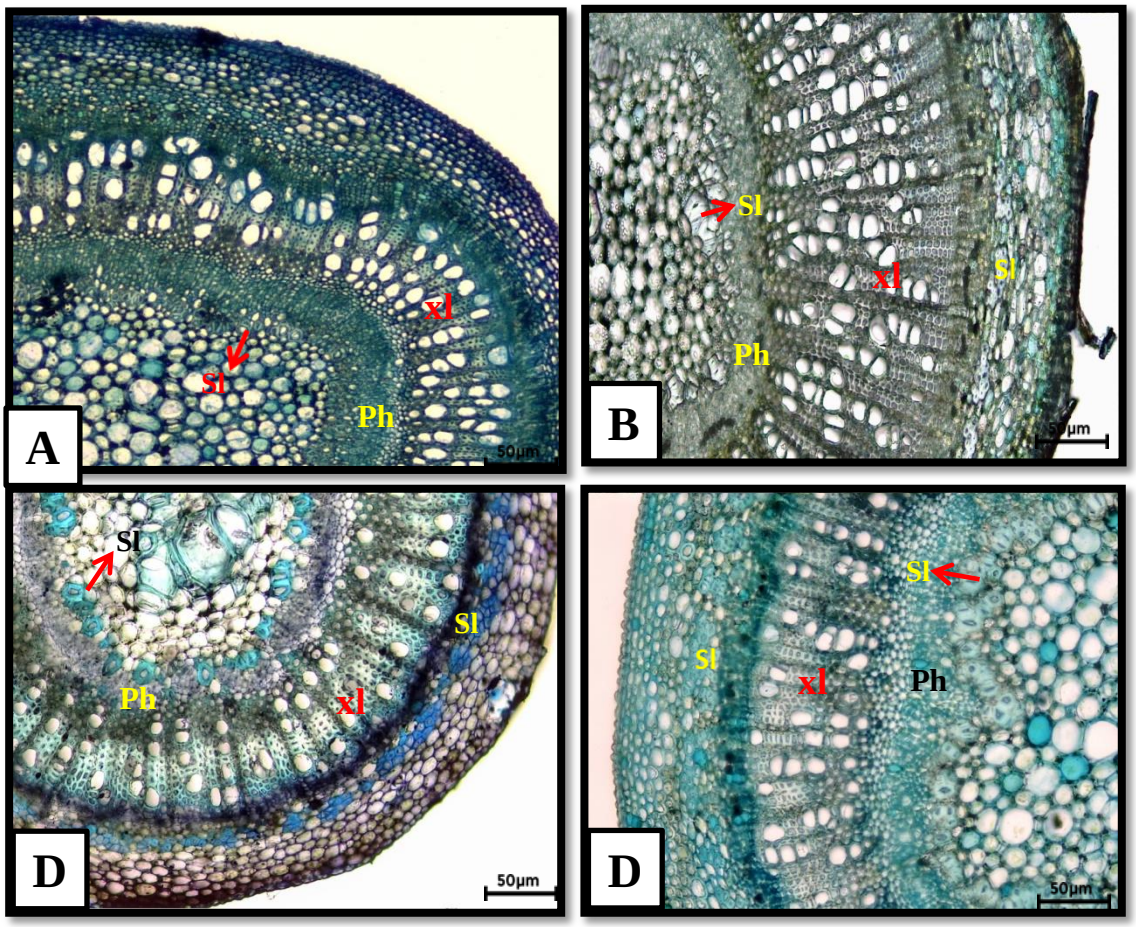

Plate 12. Transverse section of stem (detailed view): A) *S. cumini* B) *S. caryophyllatum* C) *S.laetum* D) *S. salicifolium* *Sl- sclerieds, Ph-Phloem, Xl- xylem

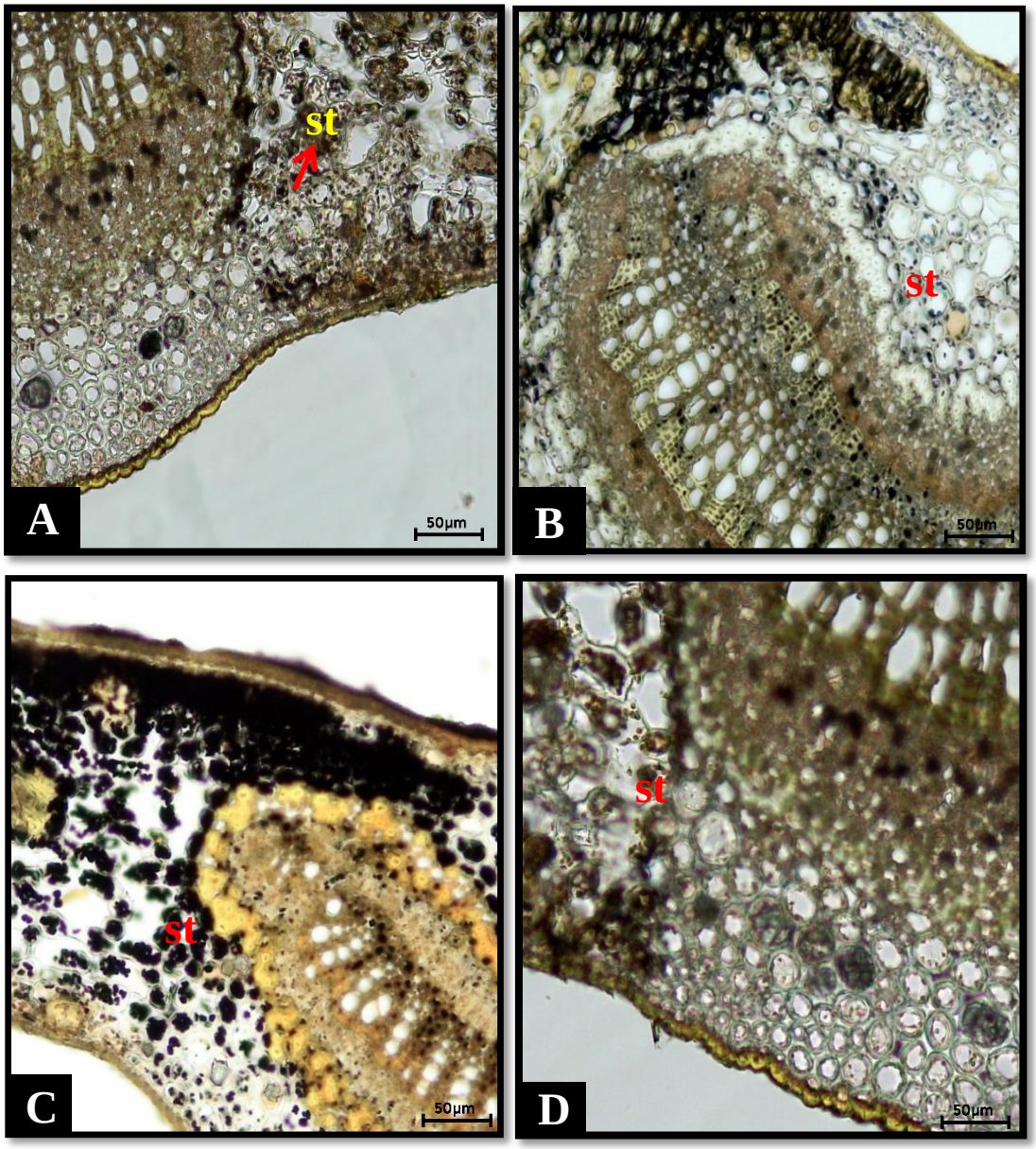

Plate 13. Localization of starch in the transverse section of leaf: A) *S. cumini* B) *S. caryophyllatum* C) *S.laetum* D) *S. salicifolium*
***st-starch**

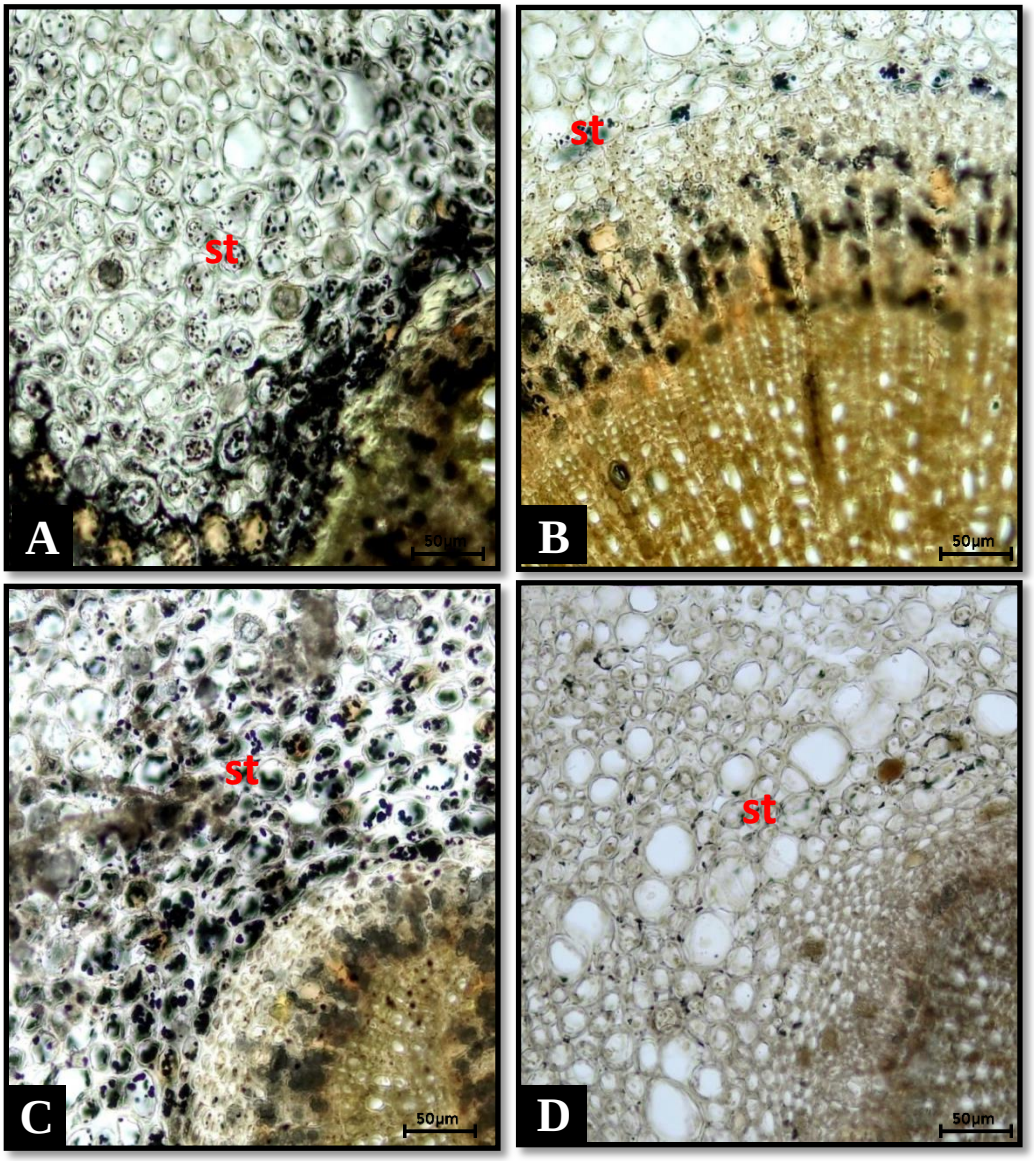

Plate 14. Localization of starch in the transverse section of petiole: A) *S. cumini* B)*S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***st-starch**

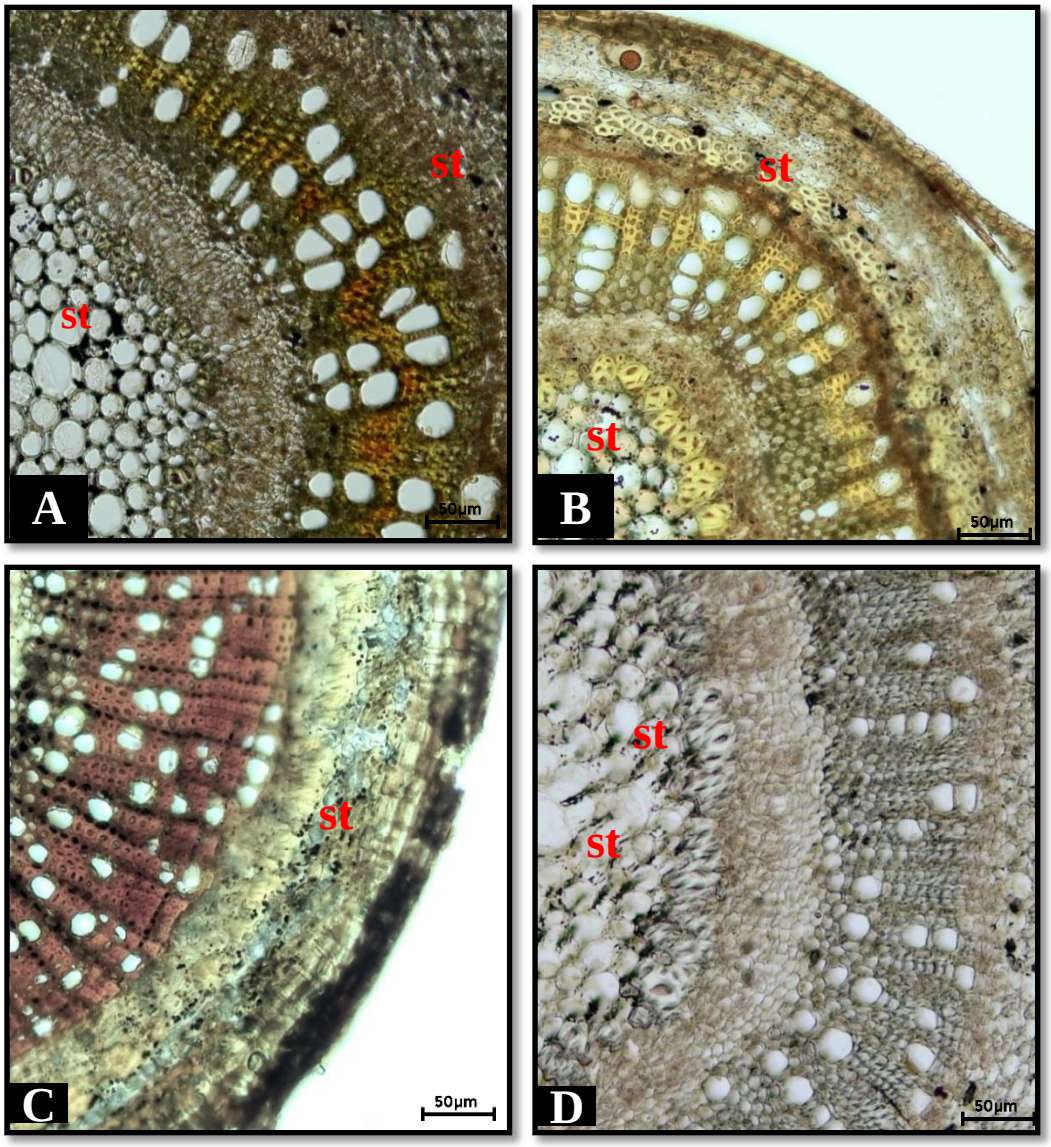

Plate 15. Localization of starch in the transverse section of stem: *A) S. cumini* B) *S. caryophyllatum* C) *S.laetum* D) *S. salicifolium*
***st-starch**

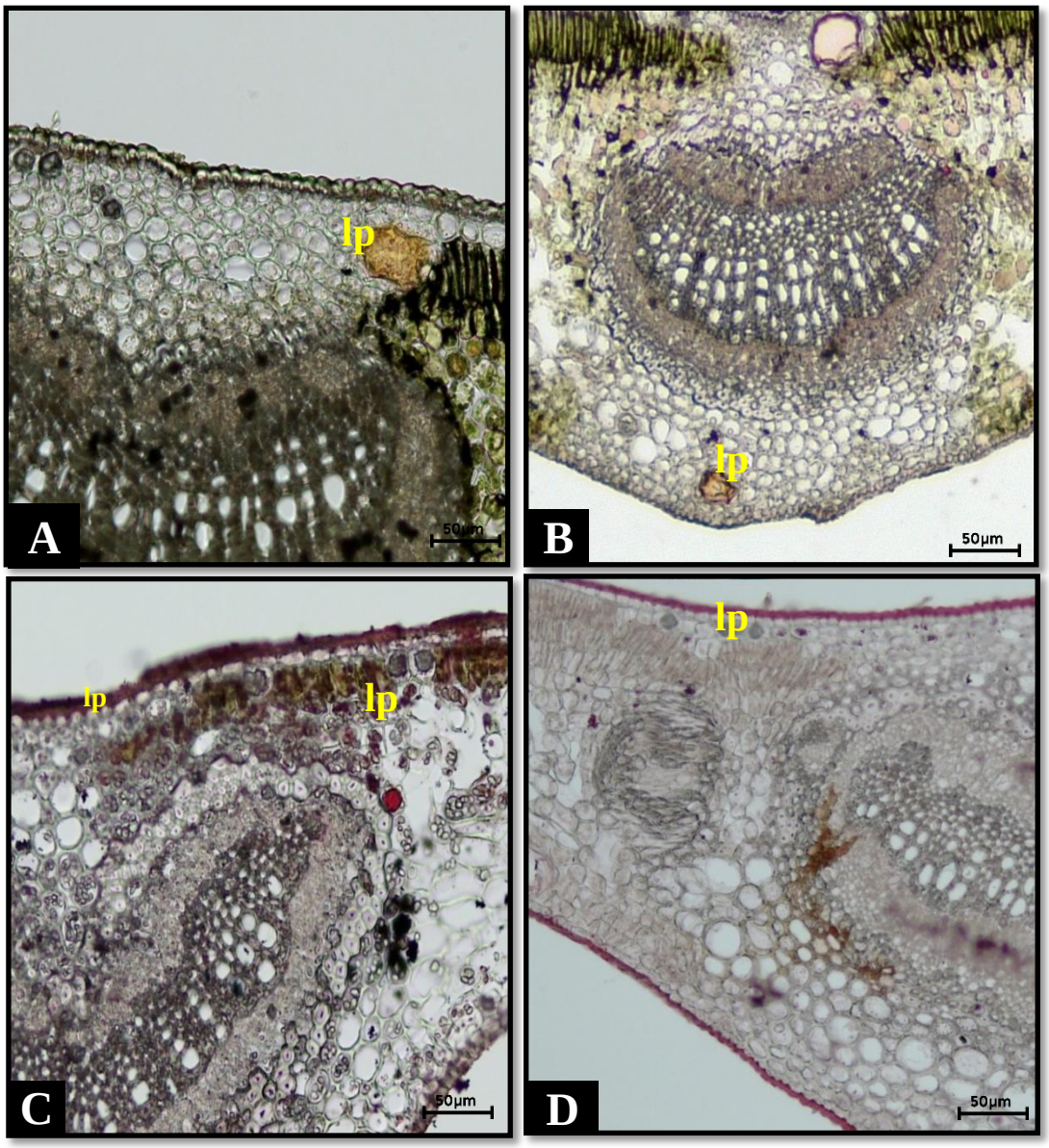

Plate 16. Localization of lipids in the transverse section of leaf: A*) S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium* *lp-lipids

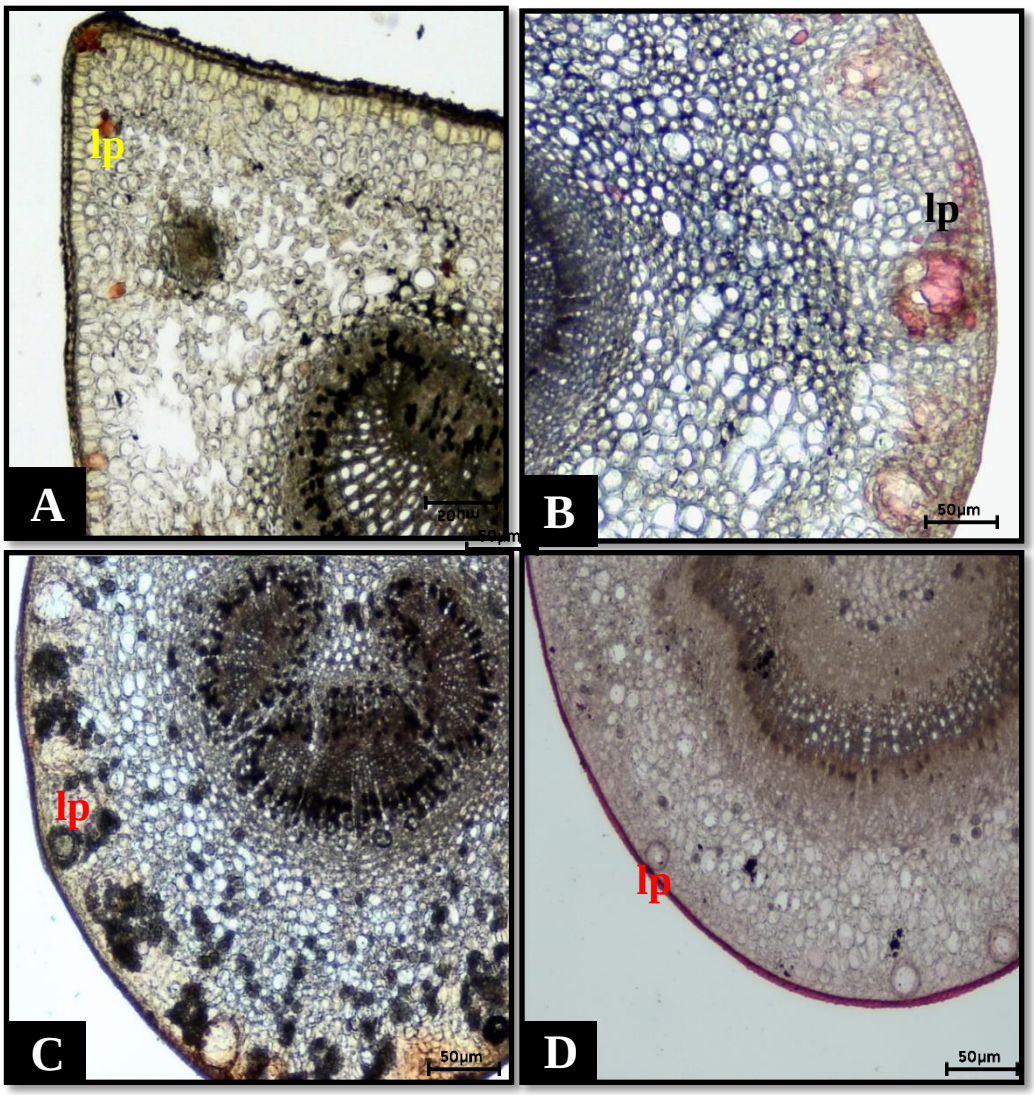

Plate 17. Localization of lipids in the transverse of section petiole: A*) S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***lp-lipids**

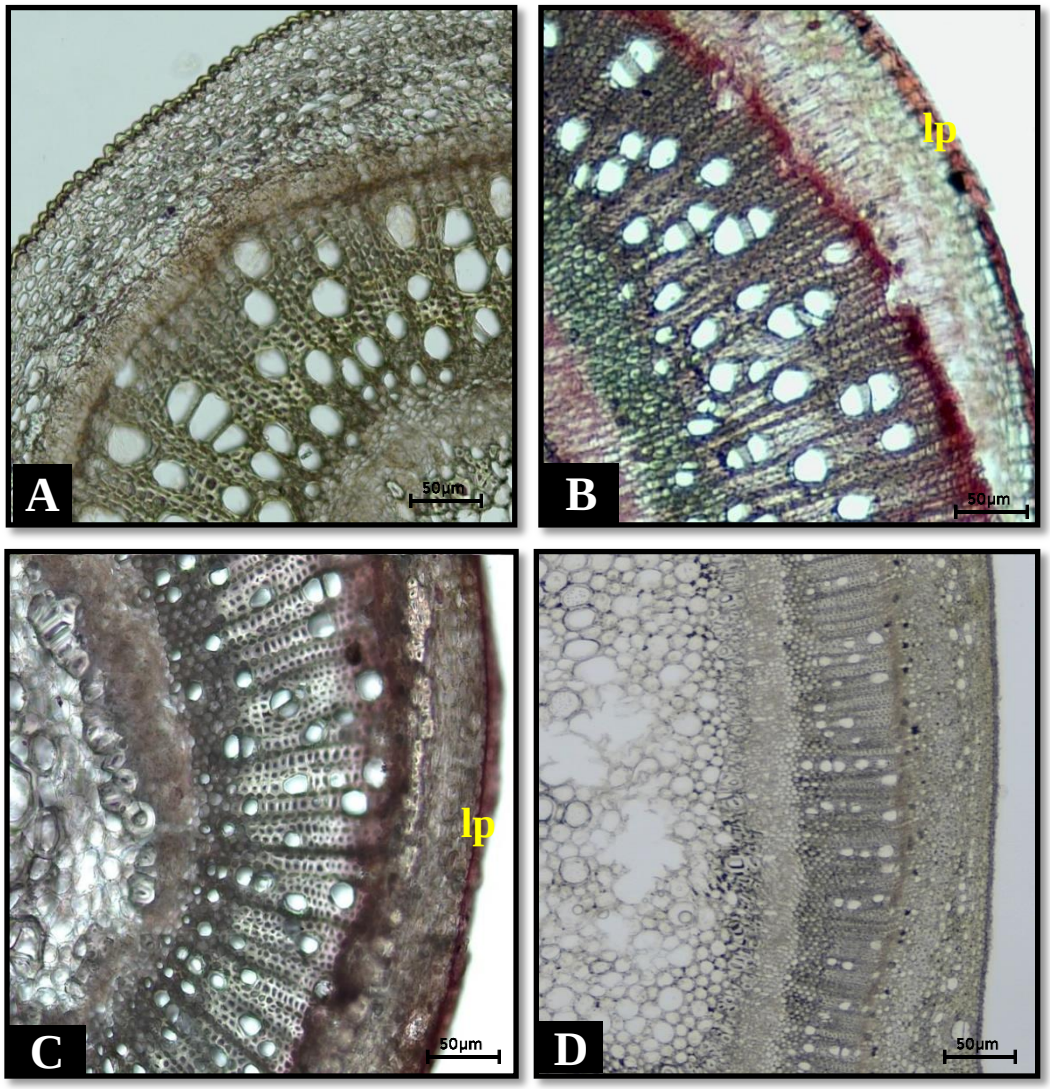

Plate 18. Localization of lipids in the transverse of section stem: A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***lp-lipids**

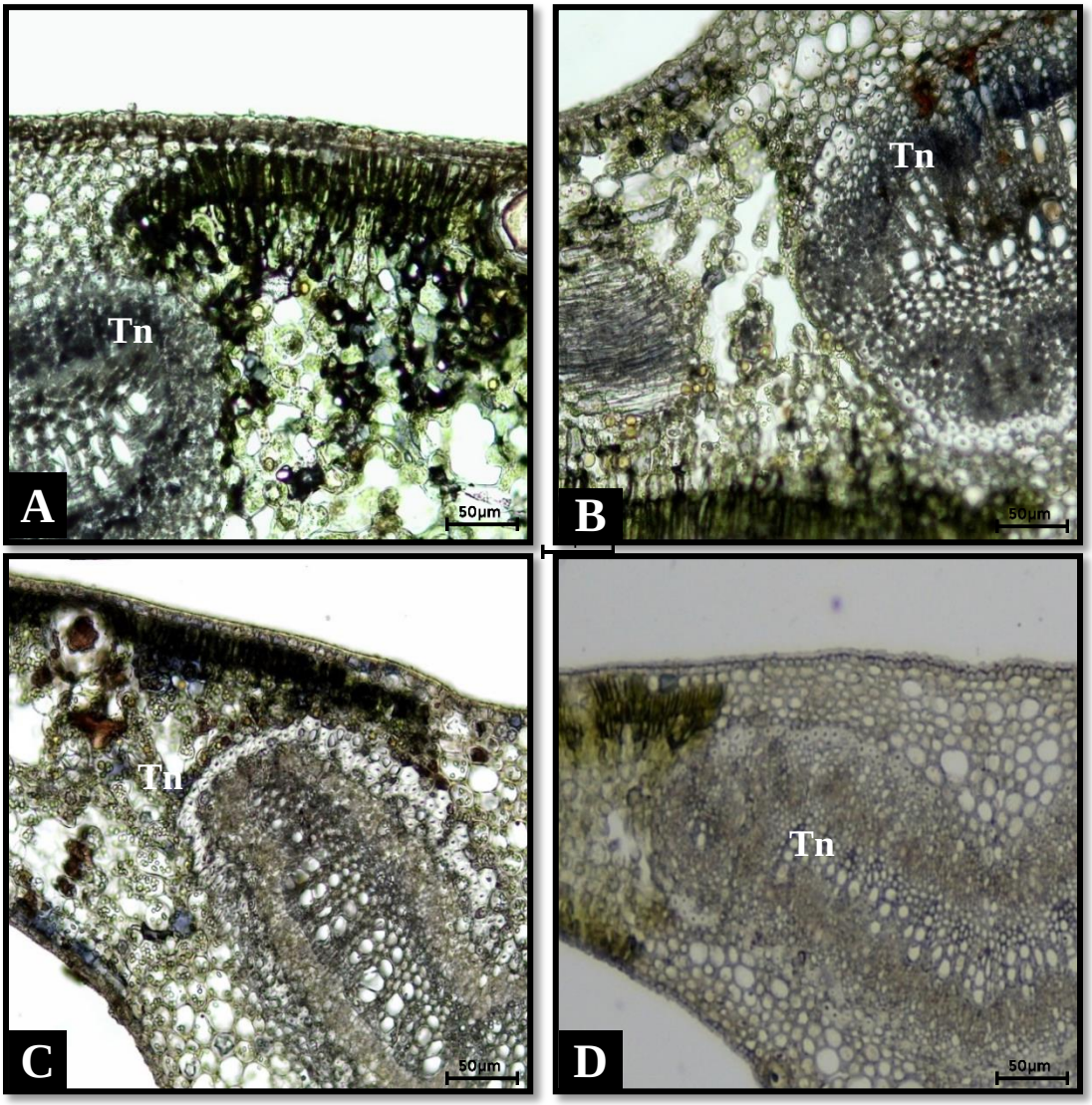

Plate 19. Localization of tannins in the transverse of section leaf: A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***Tn-tannins**

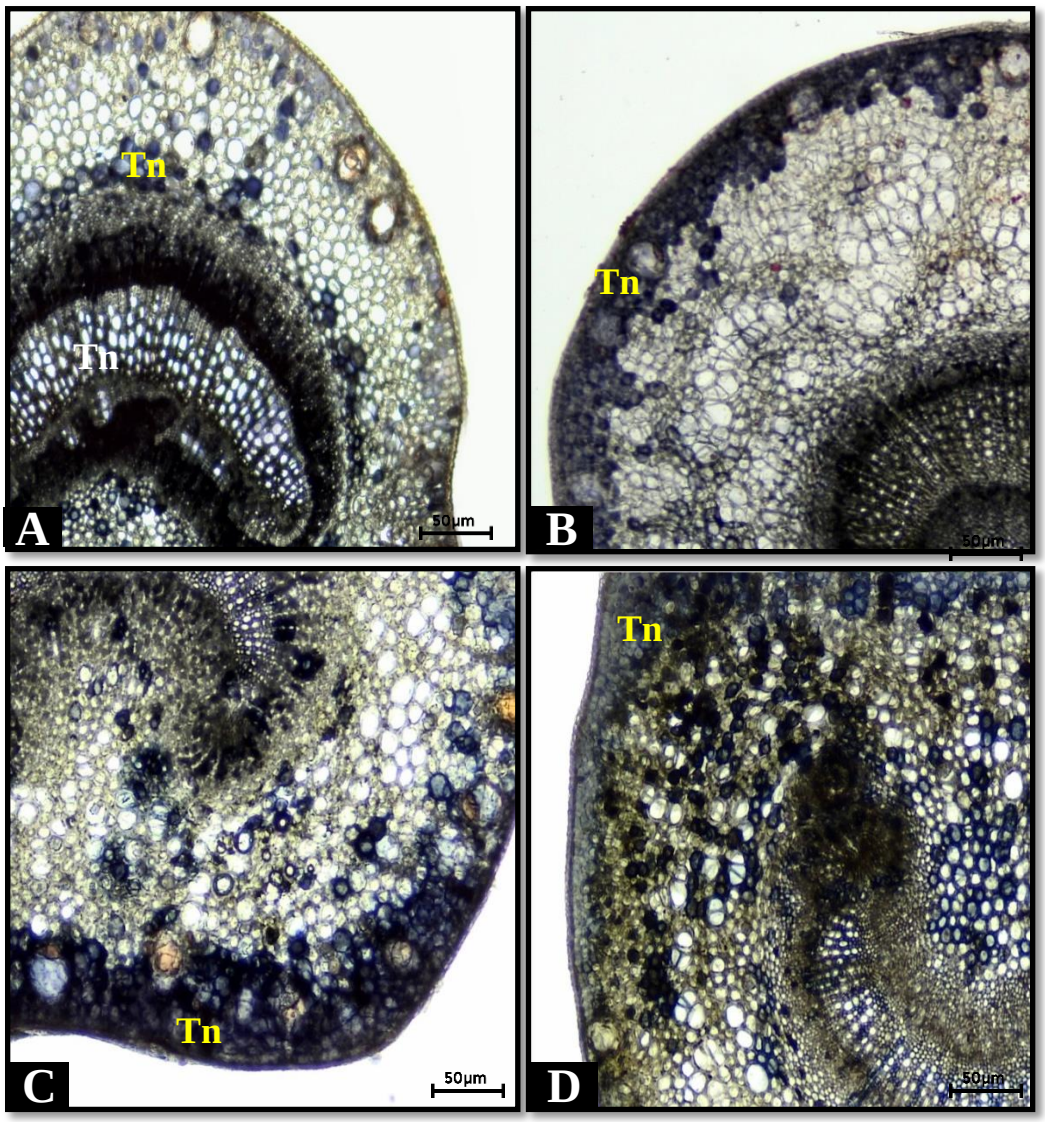

Plate 20. Localization of tannins in the transverse of section petiole: A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***Tn- tannins**

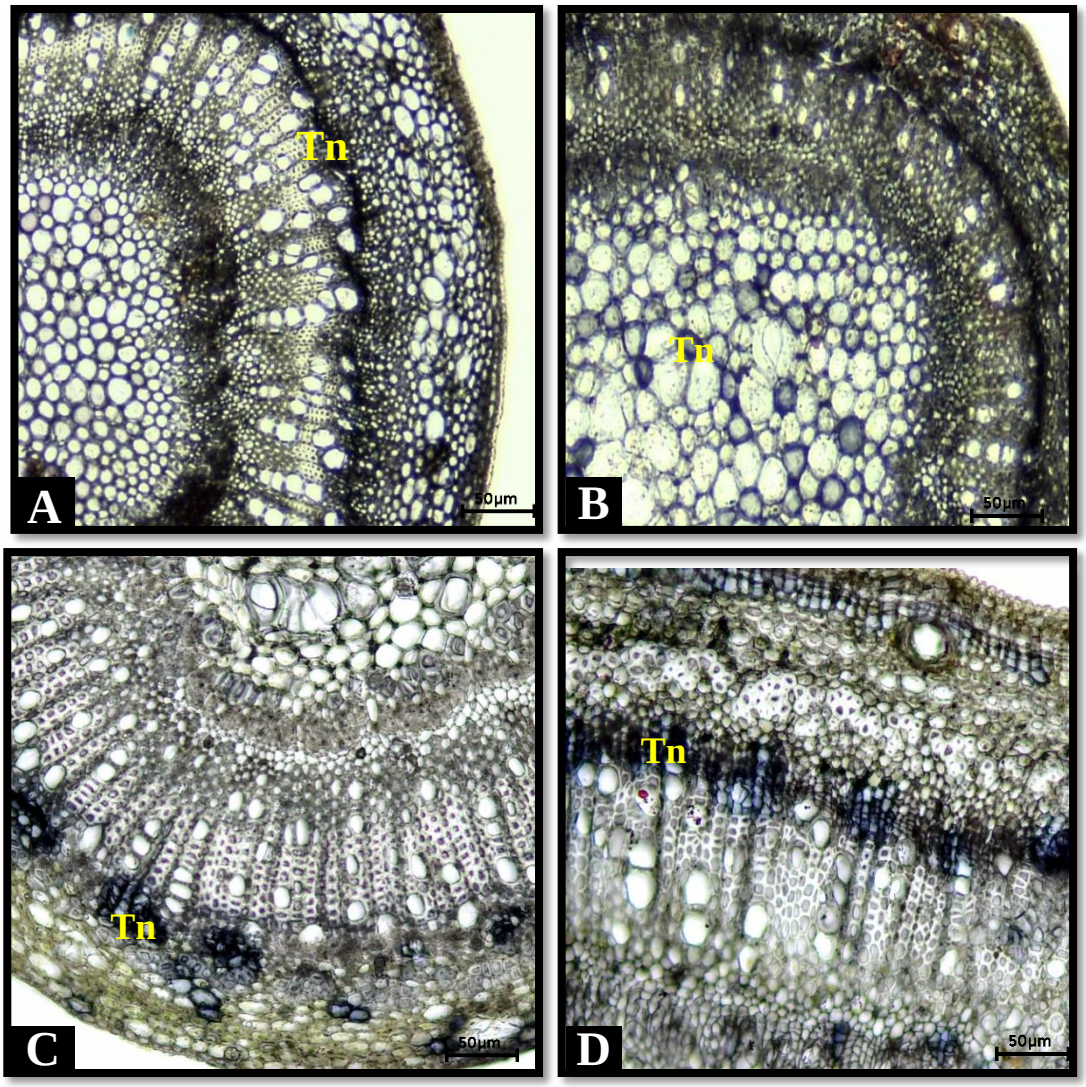

Plate 21. Localization of tannins in the transverse of section stem: A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***Tn- tannins**

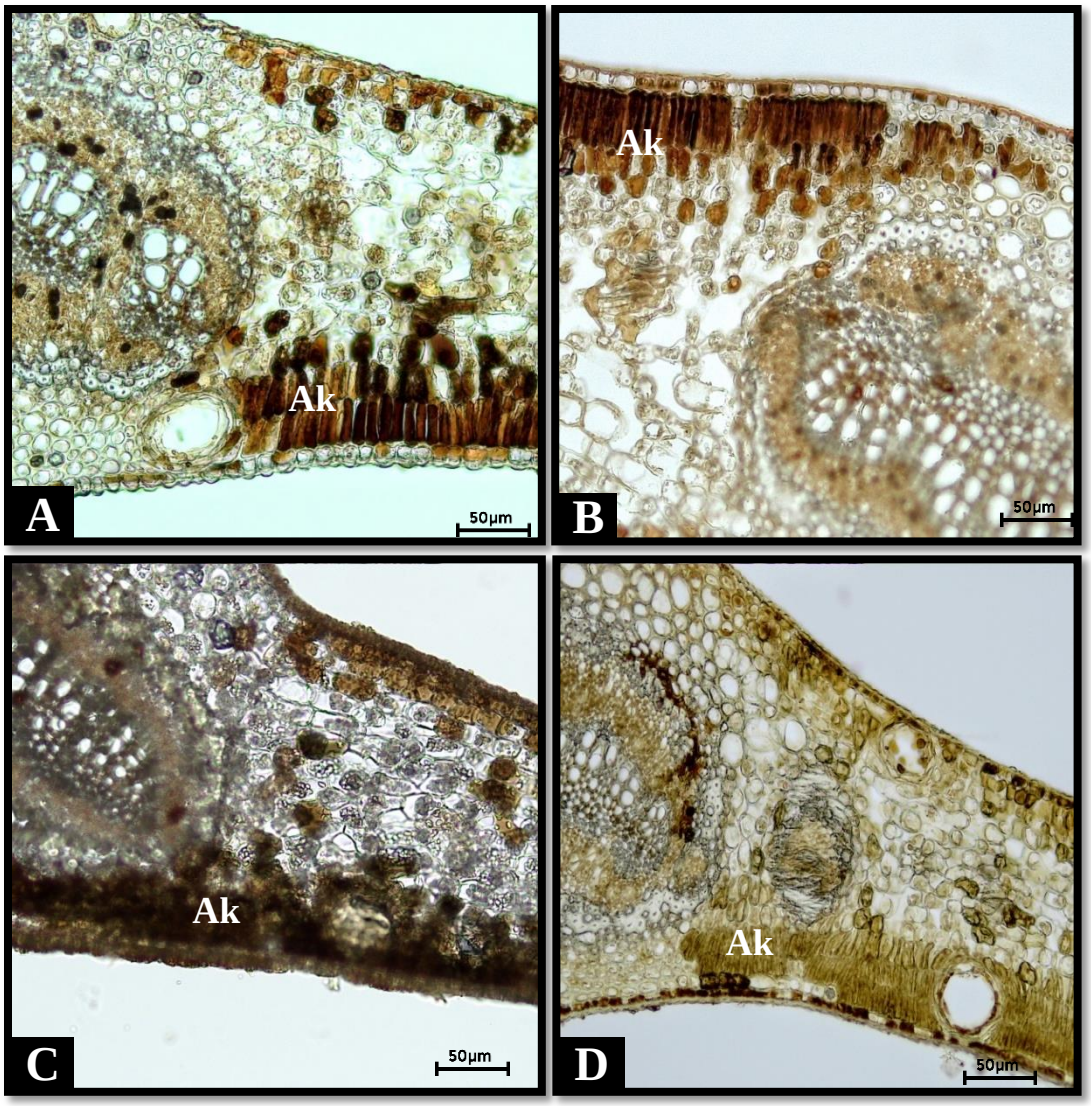

Plate 22. Localization of alkaloids in the transverse of section leaf: A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***Ak-alkaloids**

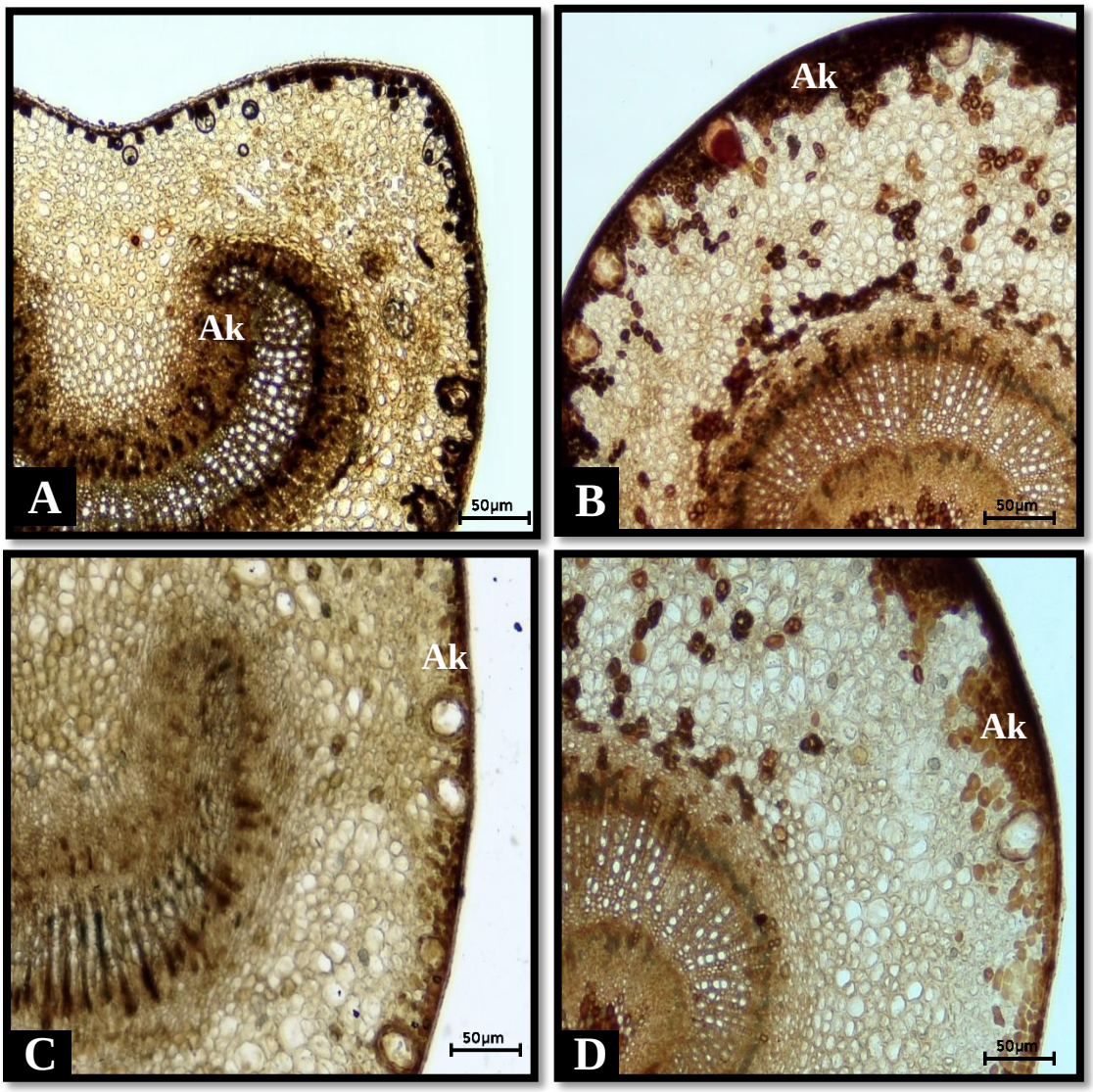

Plate 23. Localization of alkaloids in the transverse of section petiole.:A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***Ak-alkaloids**

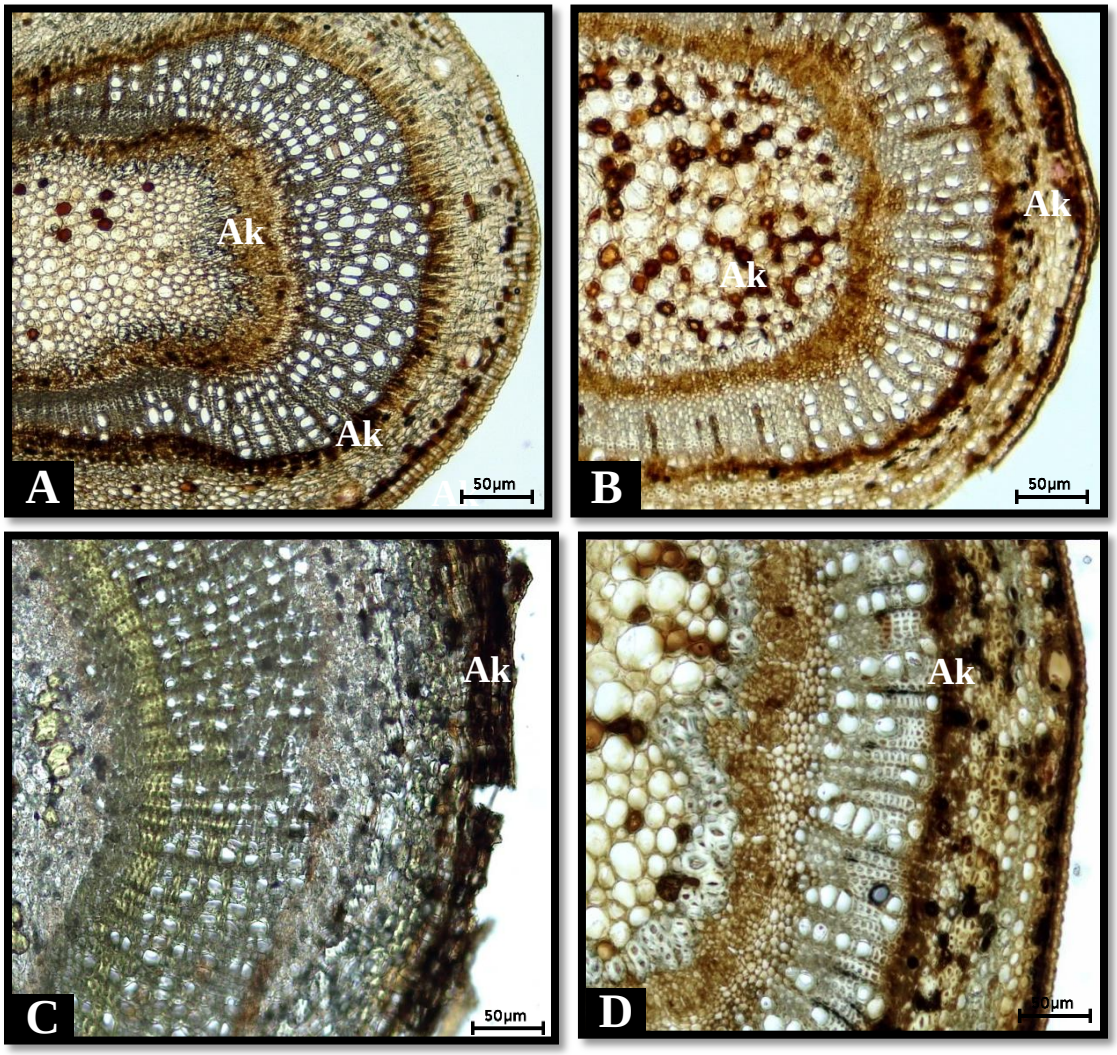

Plate 24. Localization of alkaloids in the transverse of section stem: A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***Ak-alkaloids**

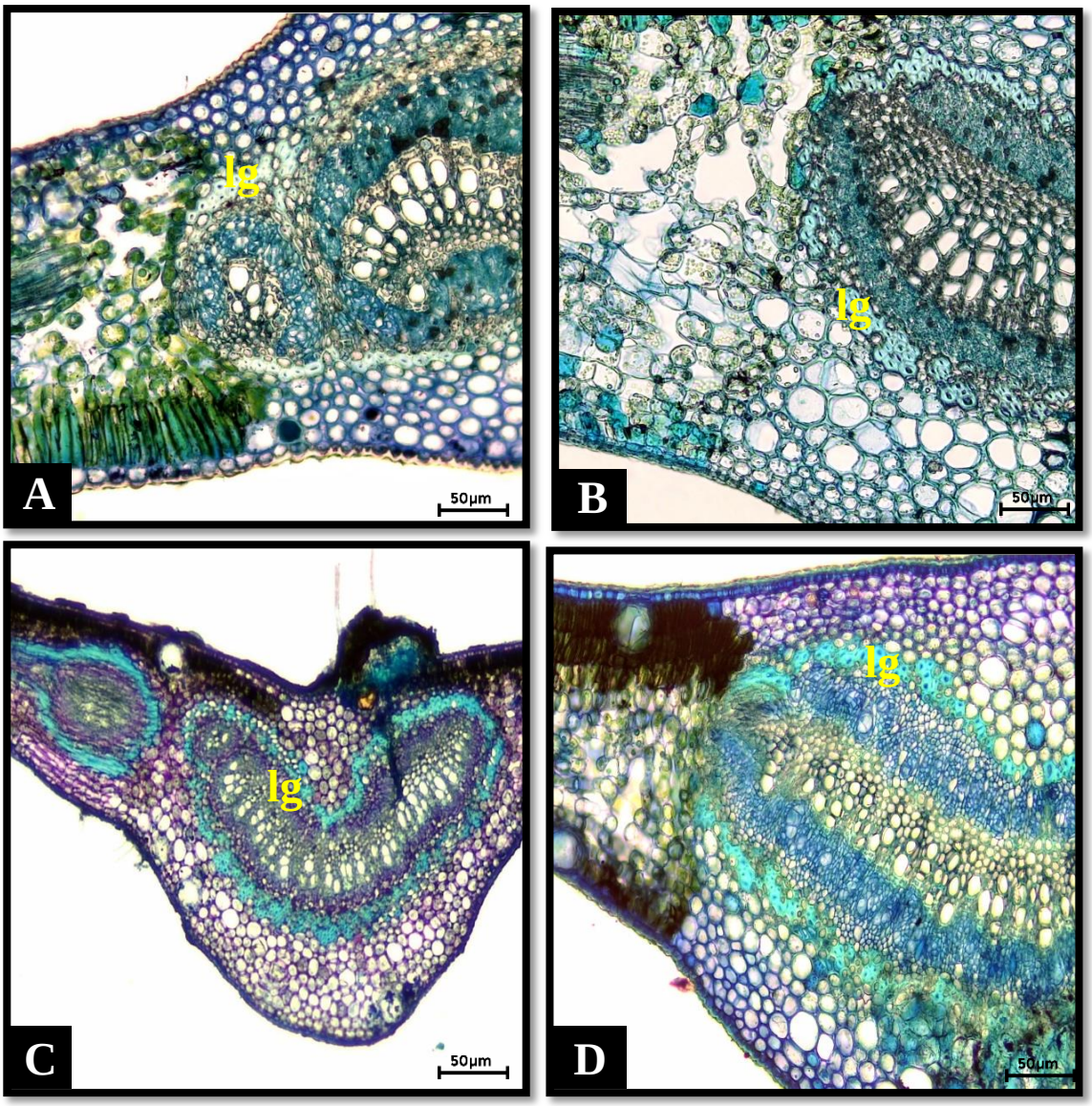

Plate 25. Localization of lignin in the transverse of section leaf : A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***lg-Lignin's**

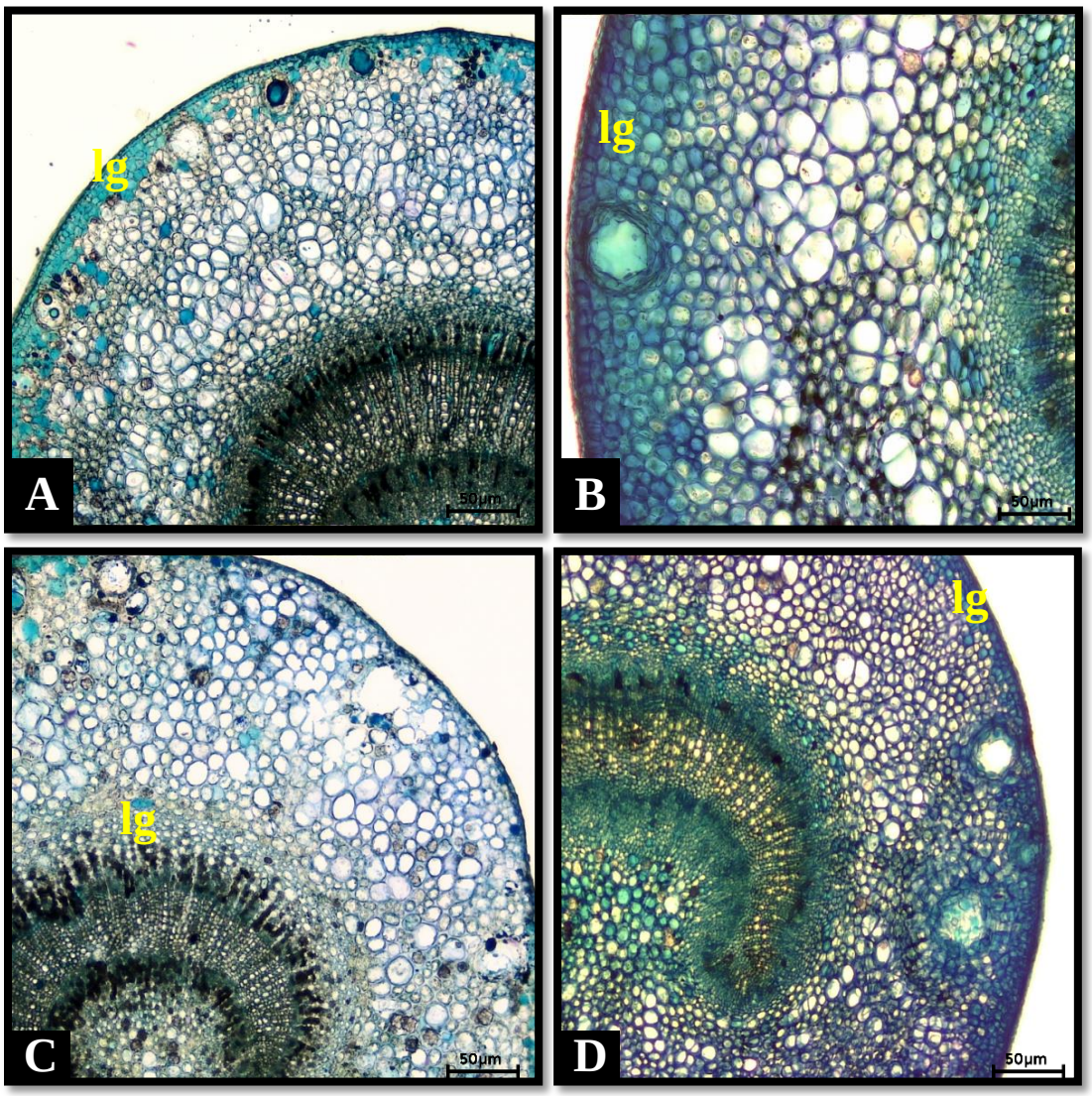

Plate 26. Localization of lignin in the transverse of section petiole: A) *S. cumini* B) *S. caryophyllatum* C) *S.laetum*
D) *S. salicifolium*
***lg-Lignins**

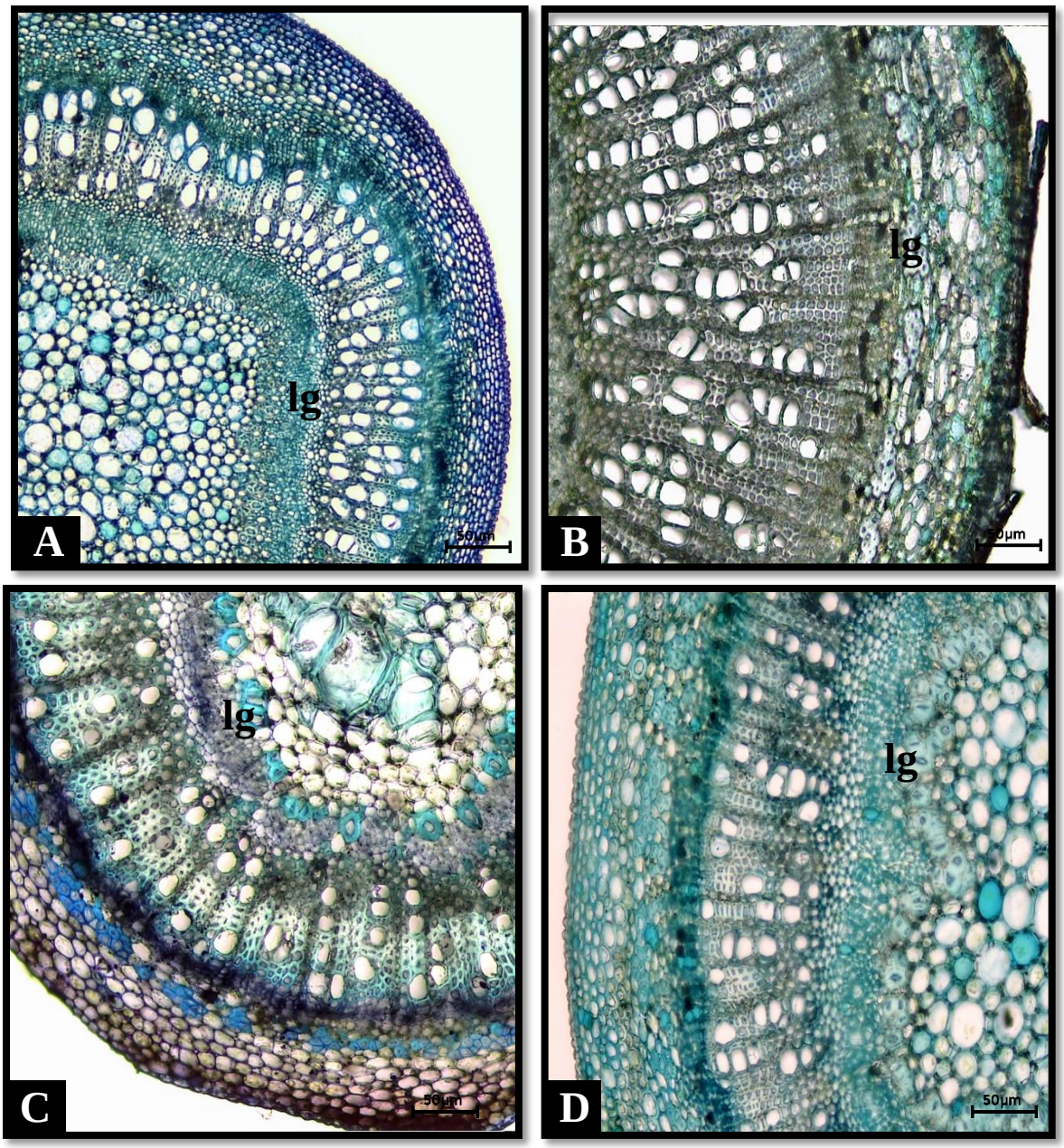

Plate 27. Localization of lignin's in the transverse of section stem: A) *S. cumini* B) *S. caryophyllatum* C) *S. laetum* D) *S. salicifolium*
***lg-Lignin's**

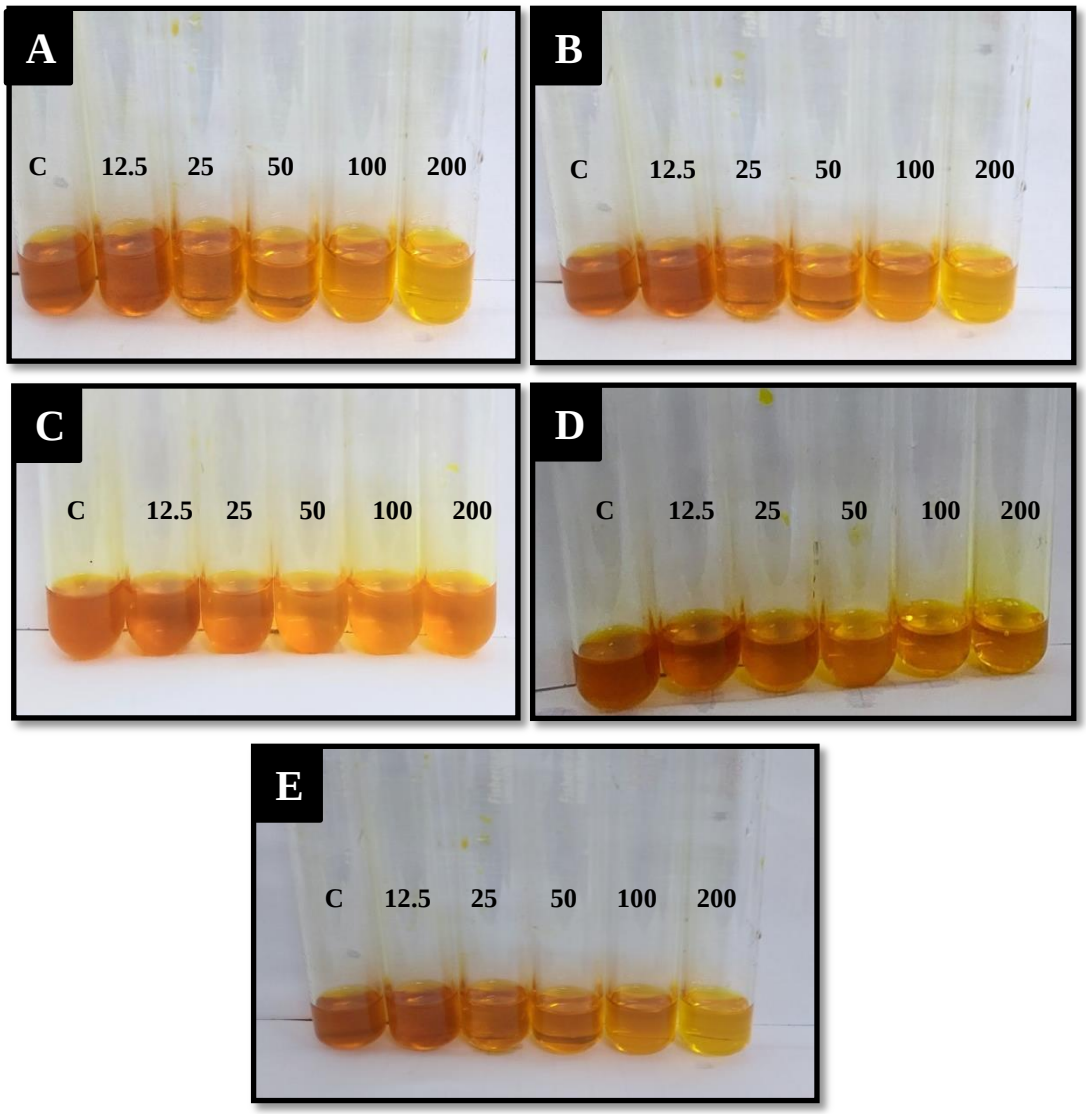

Plate 28. α -amylase inhibition activity of leaf extract of *Syzygium* species using DNS method: A)Acarbose B) *S. cumini* C) *S. caryophyllatum* D) *S. laetum* E) *S. salicifolium*

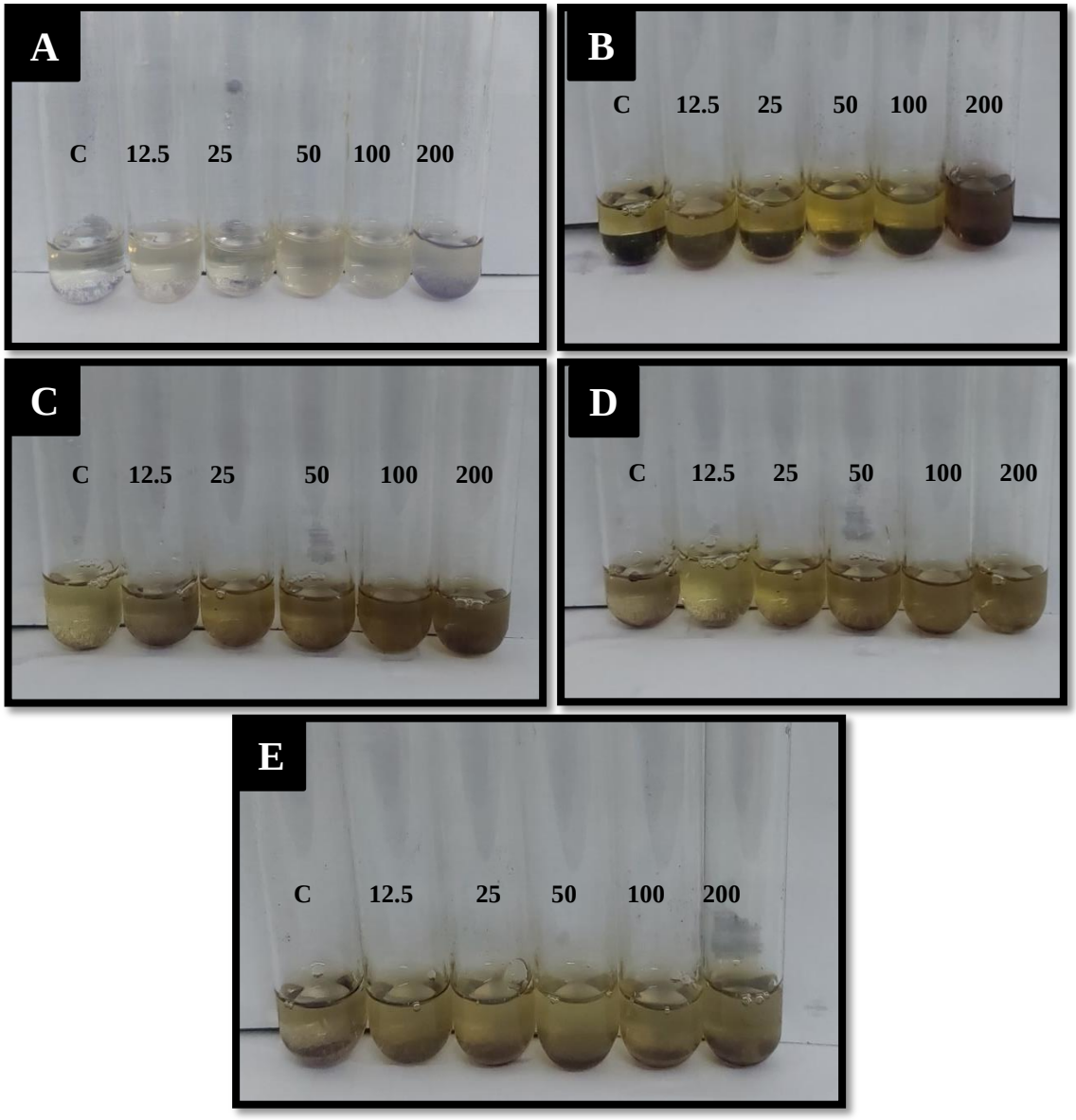

Plate 29. α -amylase inhibition activity of leaf extract of *Syzygium* species using Iodine-starch method: A)Acarbose B) *S. cumini* C*) S. caryophyllatum* D) *S. laetum* E) *S. salicifolium*

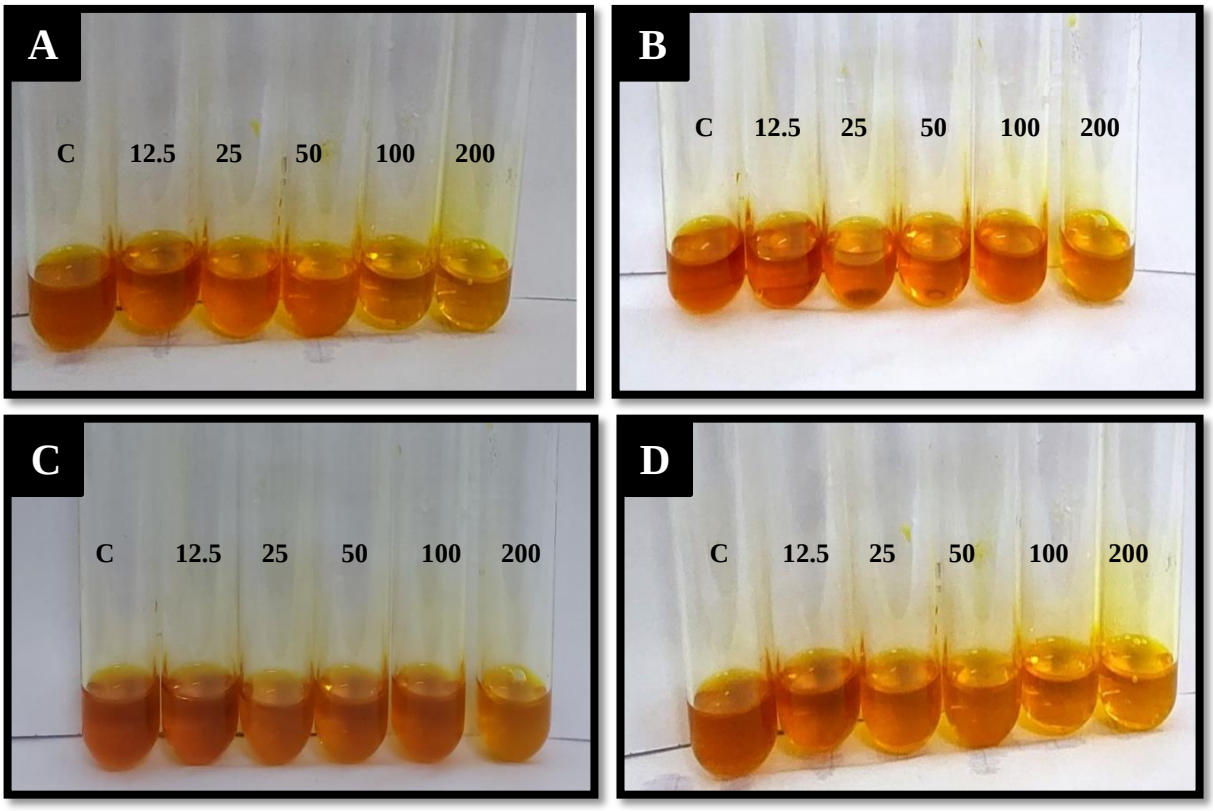

Plate 30. α -amylase inhibition activity of bark extract of *Syzygium* species using DNS method: A)Acarbose B) *S. cumini* C*)* *S. caryophyllatum* D) *S. laetum* E) *S. salicifolium*

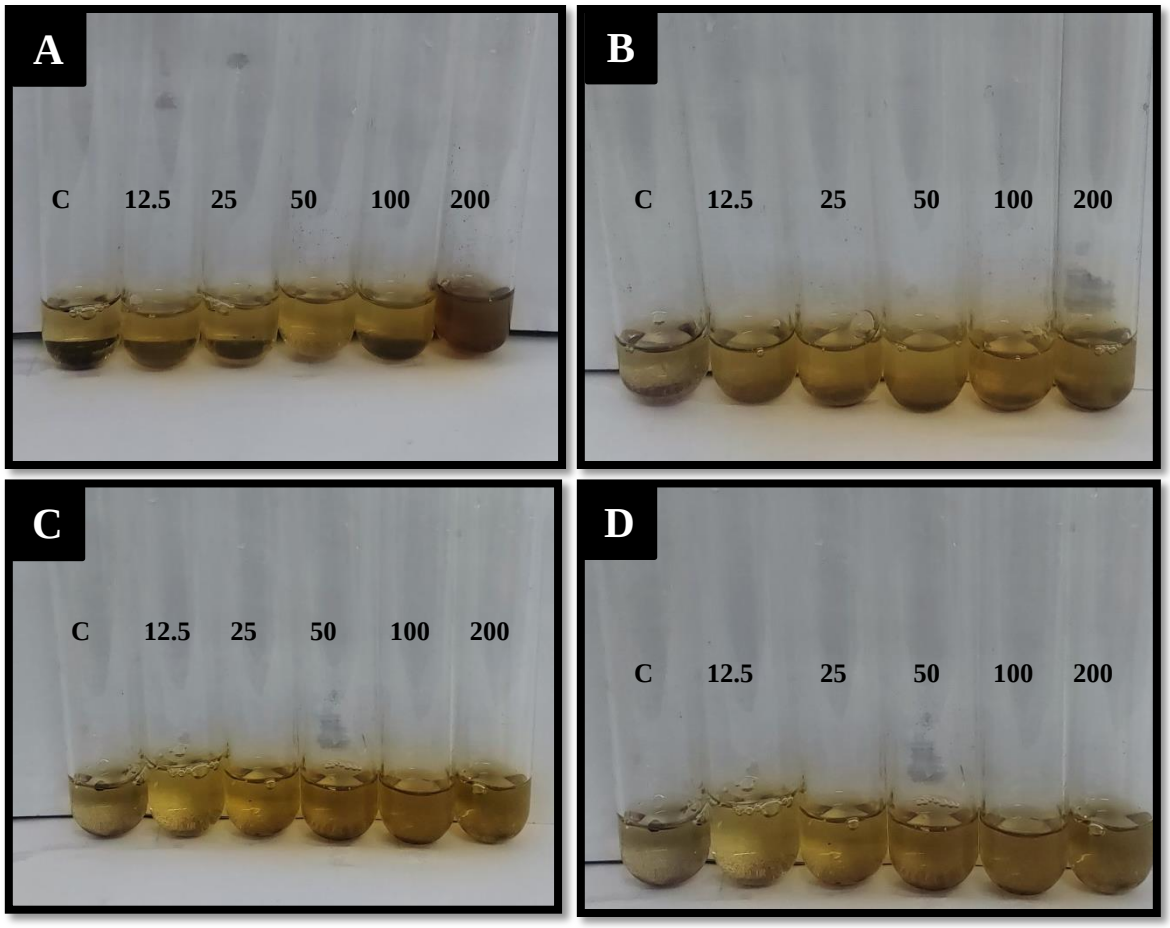

Plate 31: α -amylase inhibition activity of bark extract of *Syzygium* species using DNS method: A) Acarbose B) *S. cumini* C) *S. caryophyllatum* D) *S. laetum* E) *S. salicifolium*

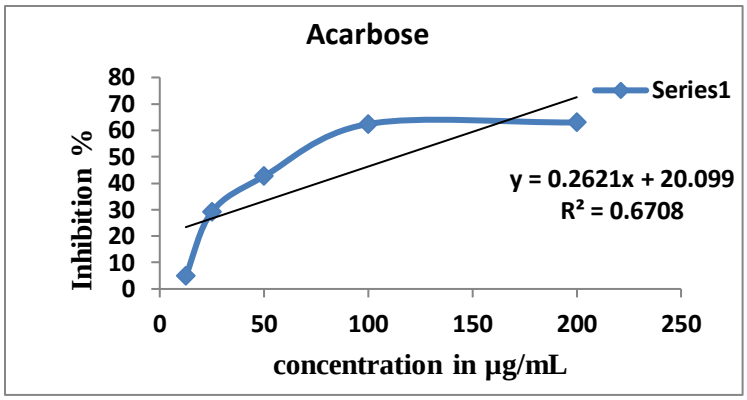

Fig1. α-amylase inhibition by Acarbose using DNS method

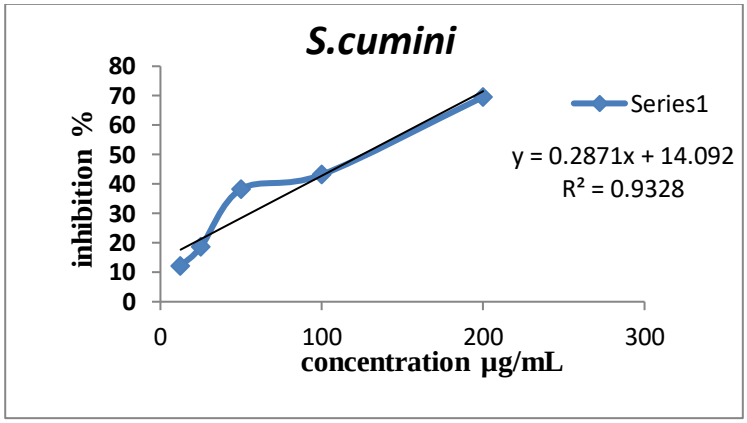

Fig 2. α-amylase inhibition by *S.cumini* using DNS method (leaf extract)

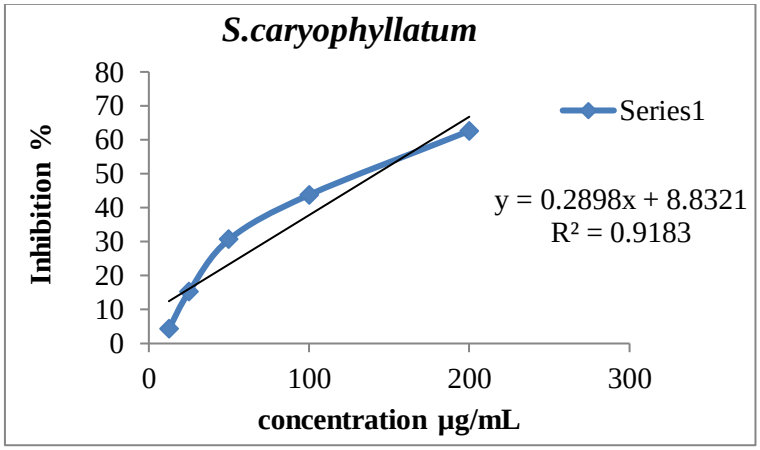

Fig 3. α-amylase inhibition by *S.caryophyllatum* using DNS method (leaf extract)

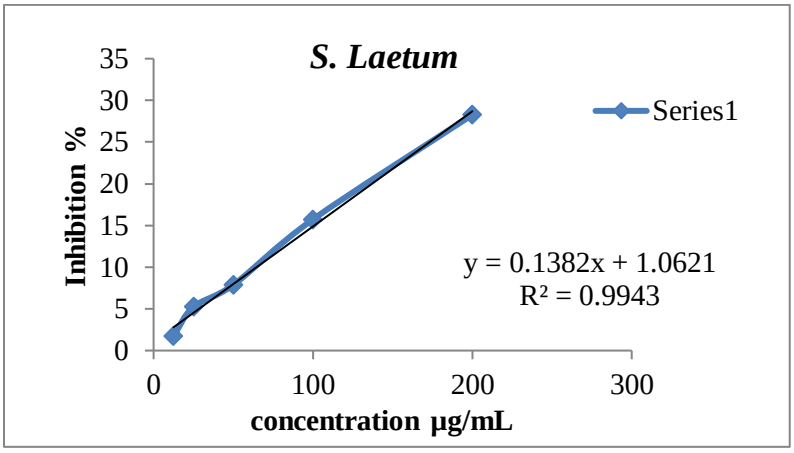

Fig 4. α-amylase inhibition by *S.laetum* DNS method (leaf extract)

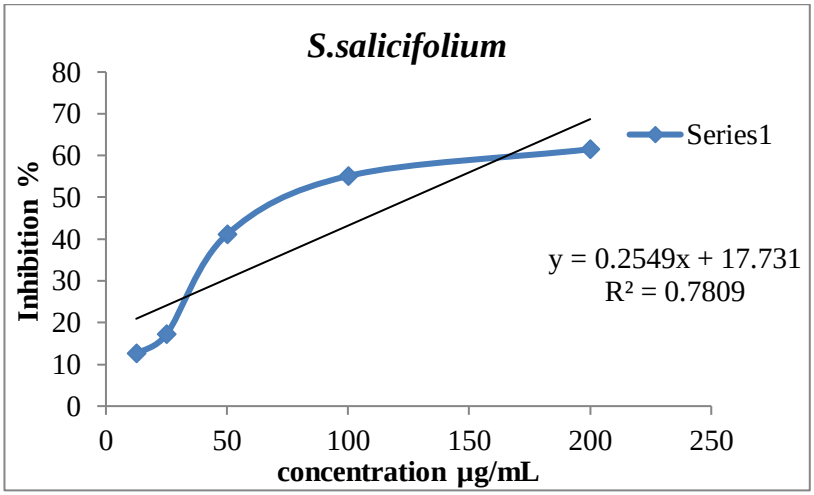

Fig 5. α-amylase inhibition by *S.salicifolium* DNS method (leaf extract)

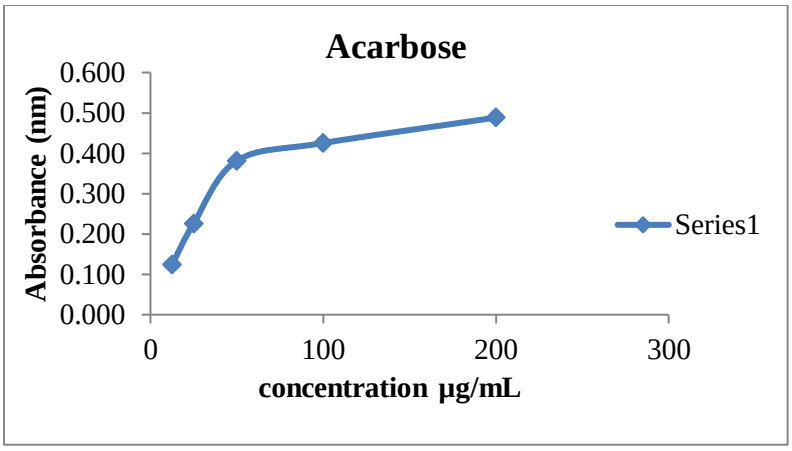

Fig 6. α-amylase inhibition by Acarbose using Iodine starch method (leaf extract)

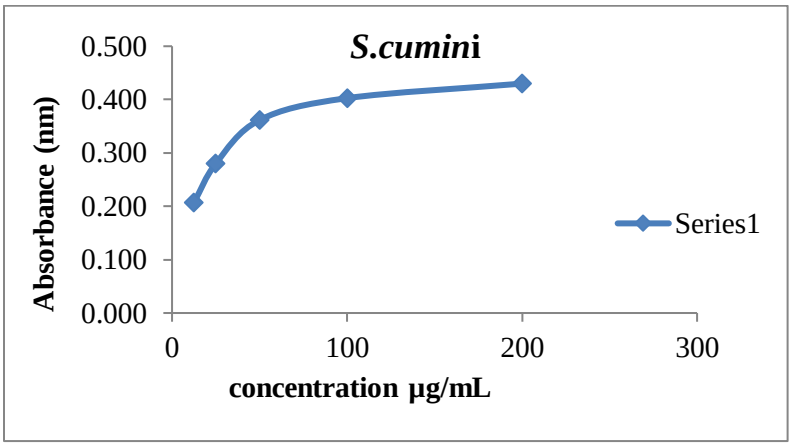

Fig 7. α-amylase inhibition by *S.cumini* using Iodine starch method (leaf extract)

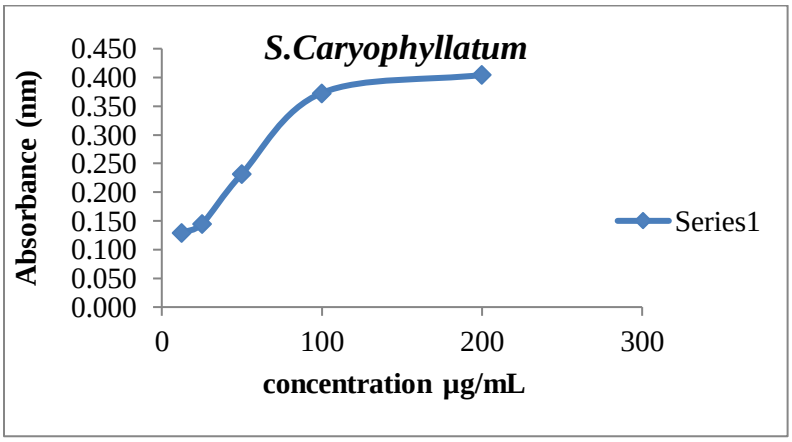

Fig 8. α-amylase inhibition by *S.caryophyllatum* using iodine starch method (leaf extract)

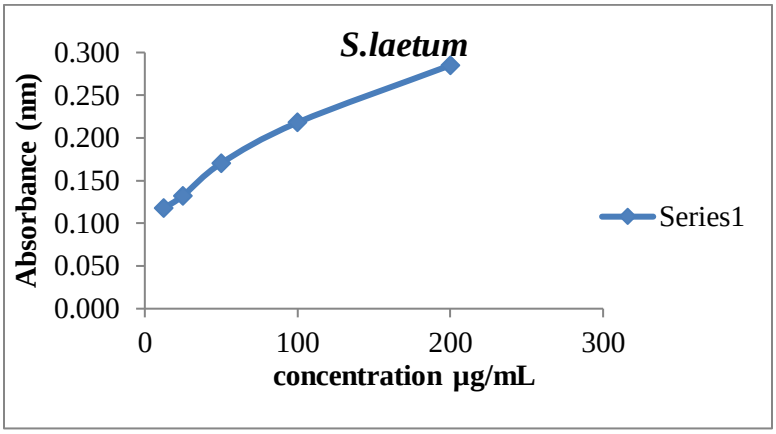

Fig 9. α-amylase inhibition by *S.laetum* using iodine starch method (leaf extract)

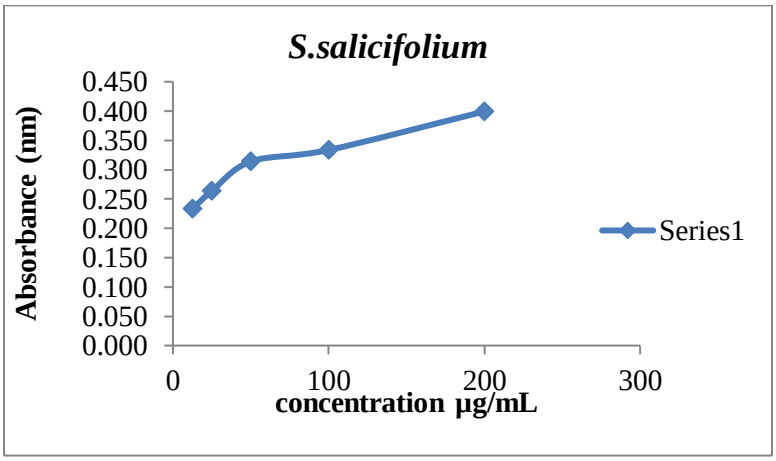

Fig 10. α-amylase inhibition by *S.salicifolium* using iodine starch method (leaf extract)

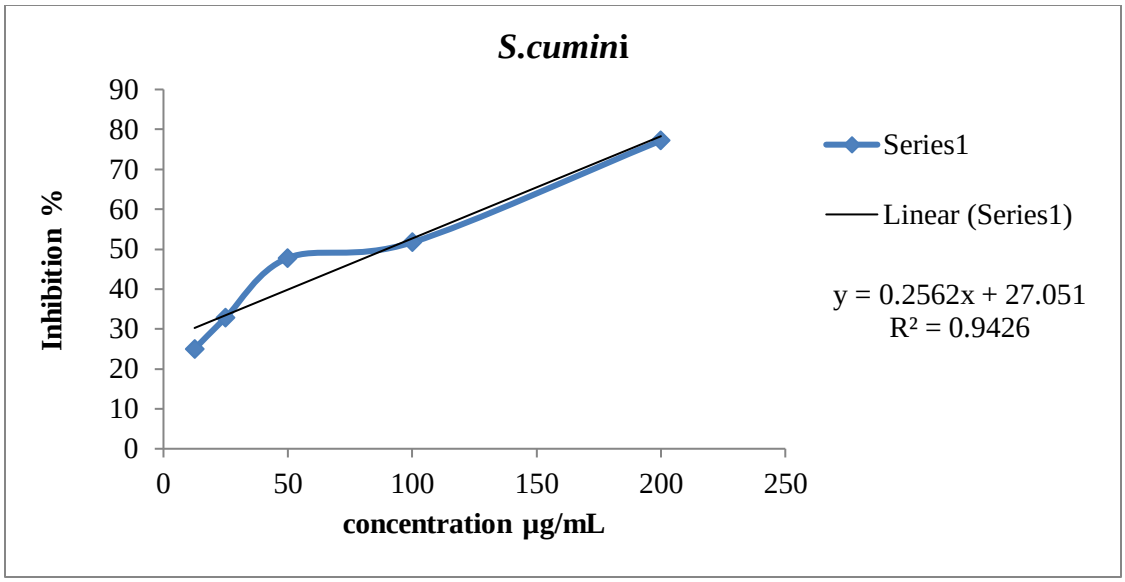

Fig 11. α-amylase inhibition by *S.cumini* using DNS method (bark extract)

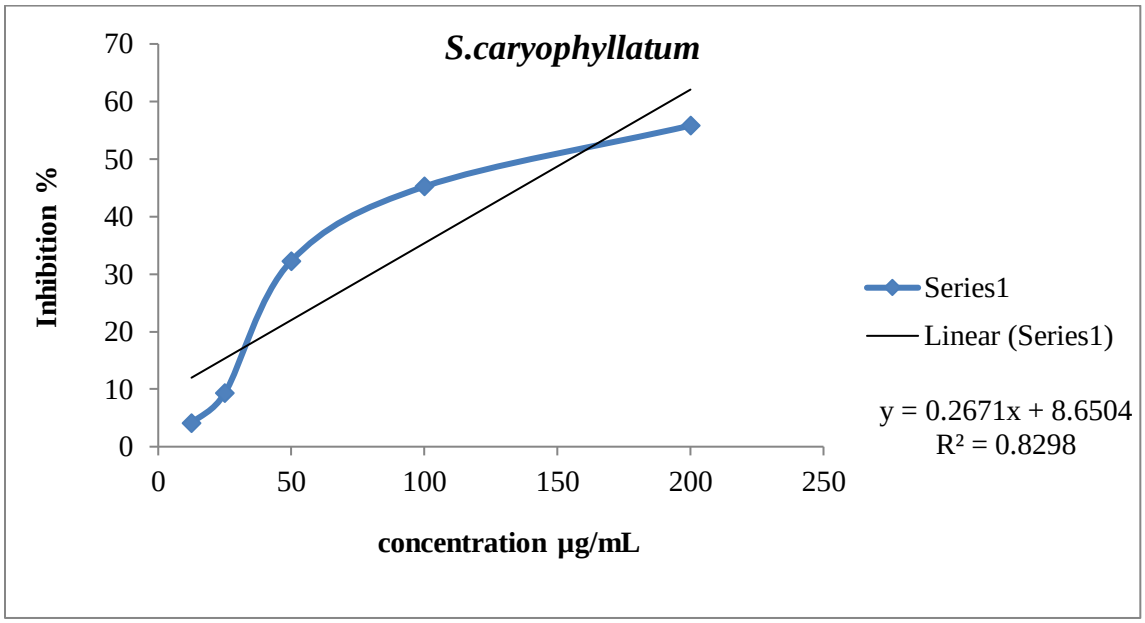

Fig 12. α-amylase inhibition by *S.caryophyllatum* using DNS method (bark extract)

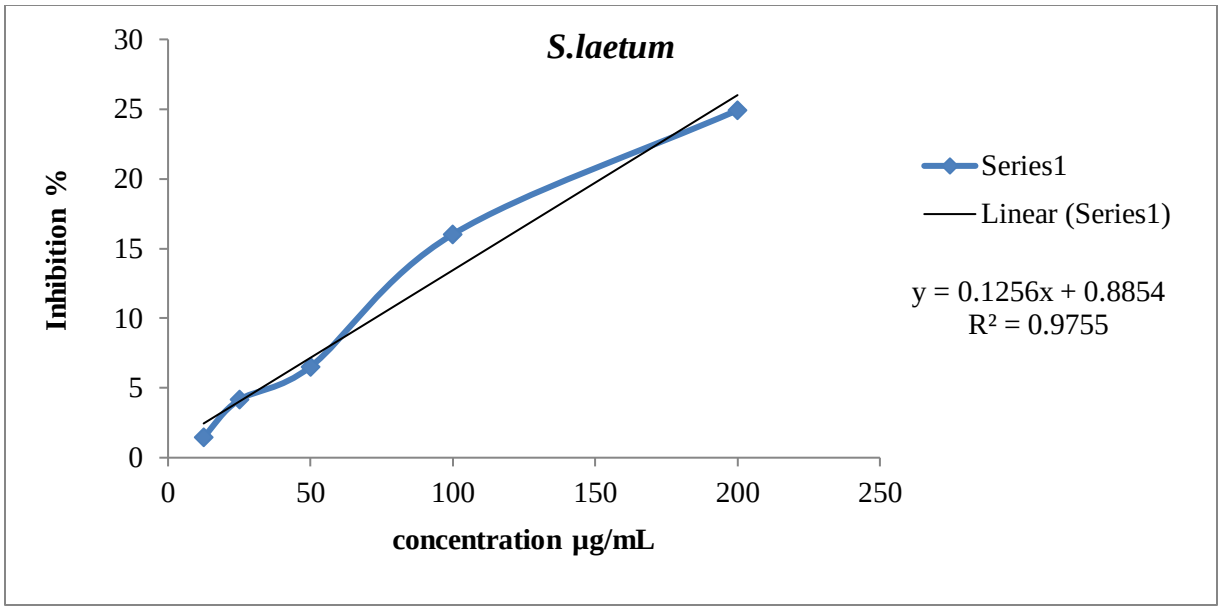

Fig 13. α-amylase inhibition by *S.laetum* DNS method (bark extract)

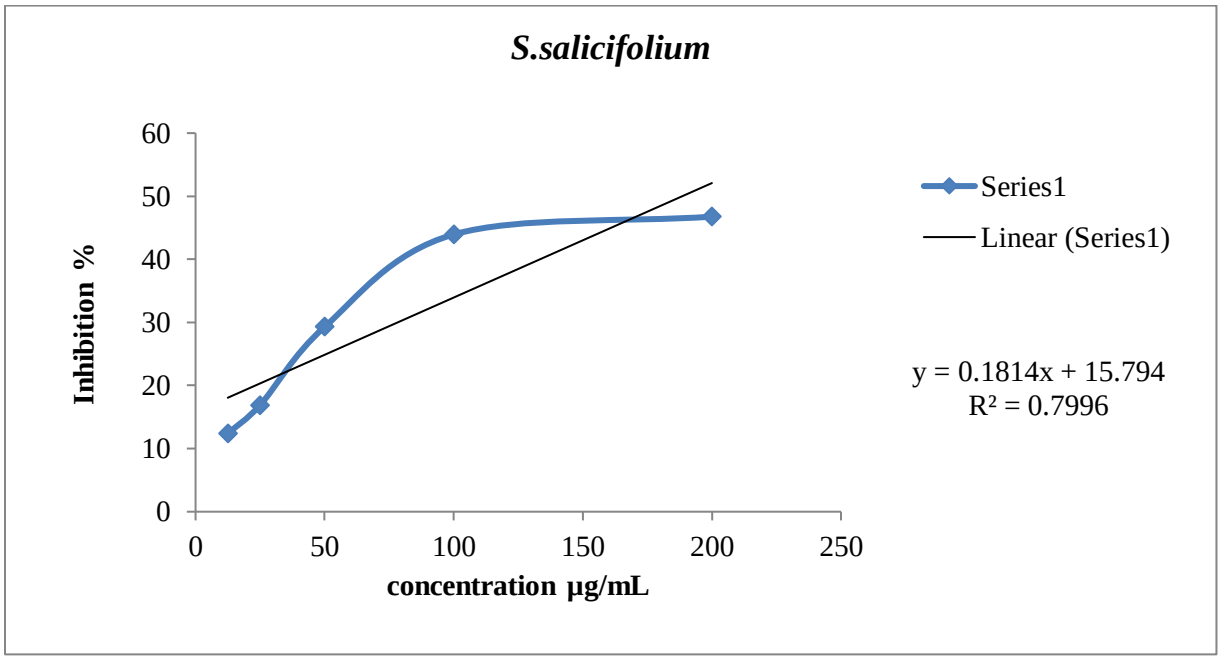

Fig 14. α-amylase inhibition by *S.salicifolium* DNS method (bark extract

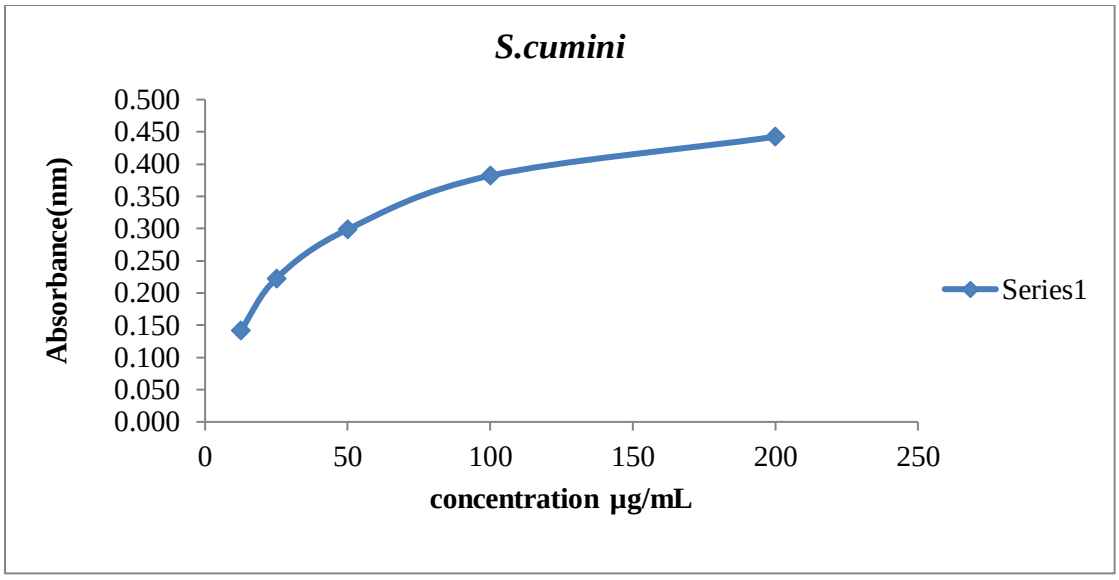

Fig 15. α-amylase inhibition by *S.cumini* using Iodine starch method (bark extract)

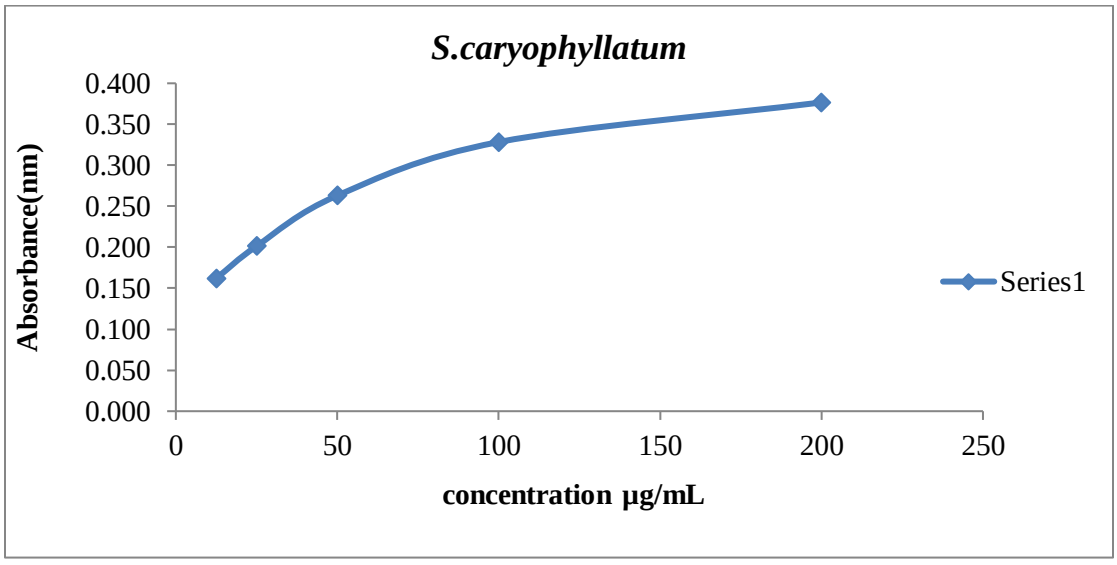

Fig 16. α-amylase inhibition by *Scryophylltumi* using Iodine starch method (bark extract)

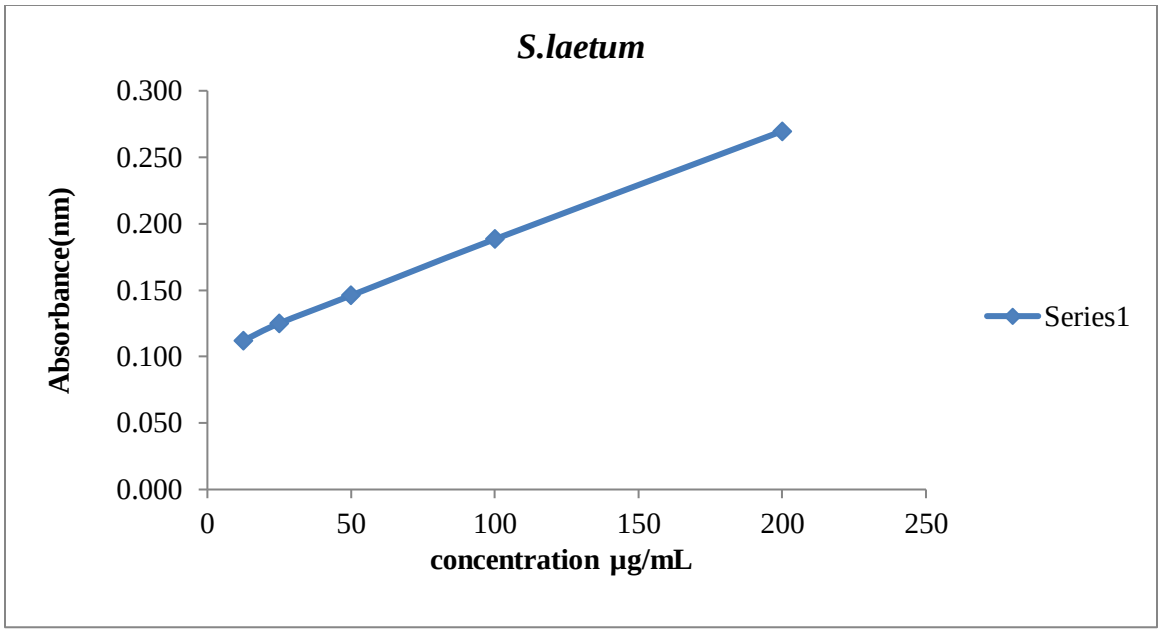

Fig 17. α-amylase inhibition by *S.laetum* using iodine starch method (bark extract)

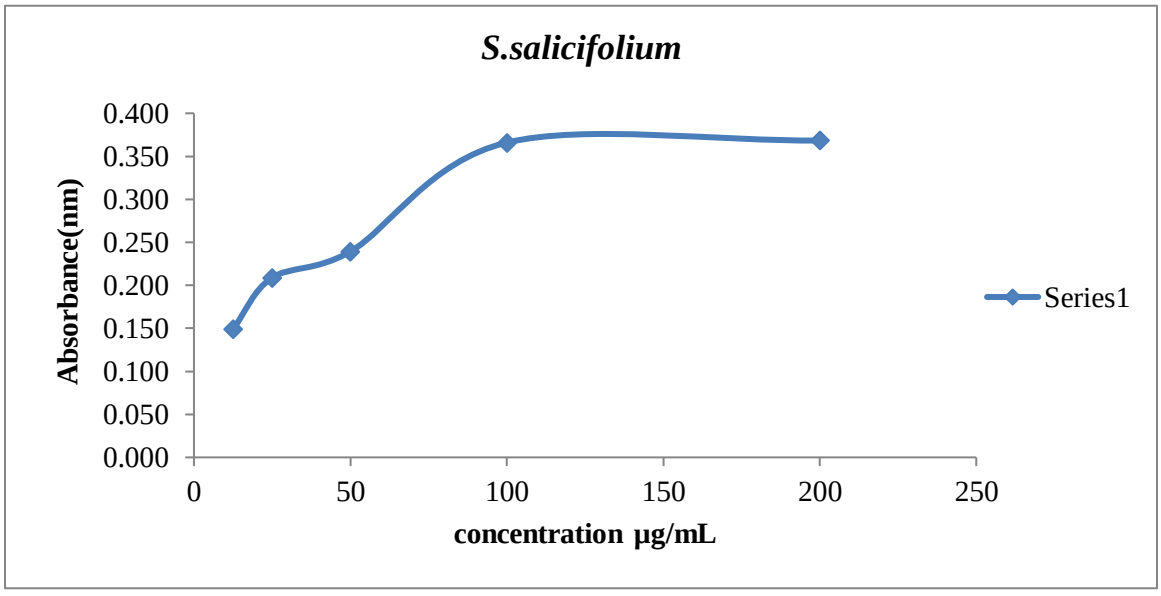

Fig 18. α-amylase inhibition by *S.salicifolium* using iodine starch method (bark extract)

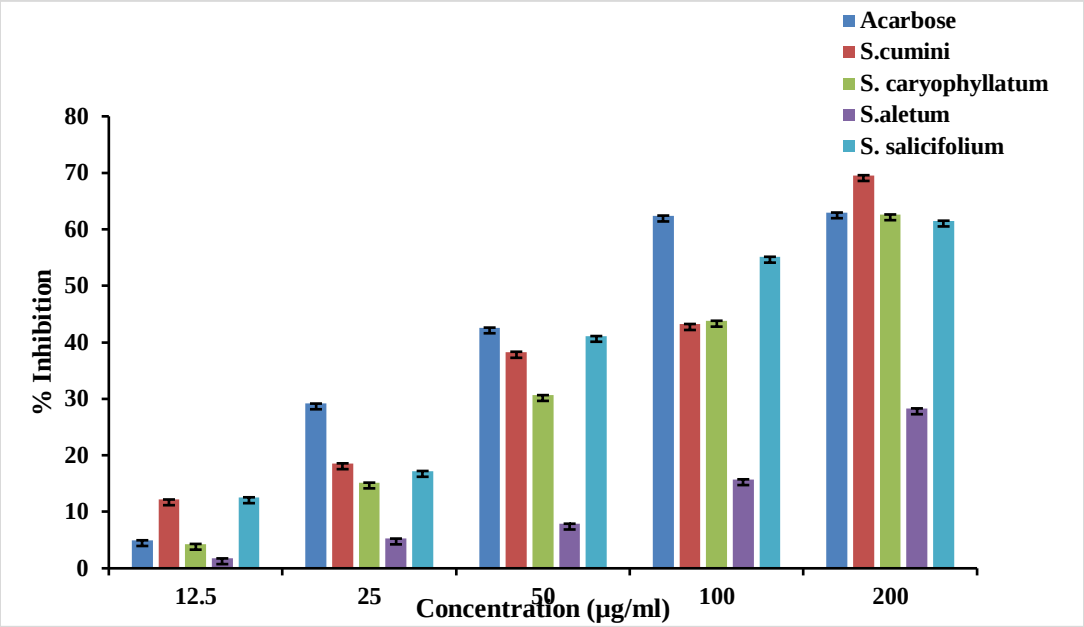

Fig19. Comparative antidiabetic activity of *S.cumini, S.caryophyllatum, S.laetum, S.salicifolium* (leaf extract)

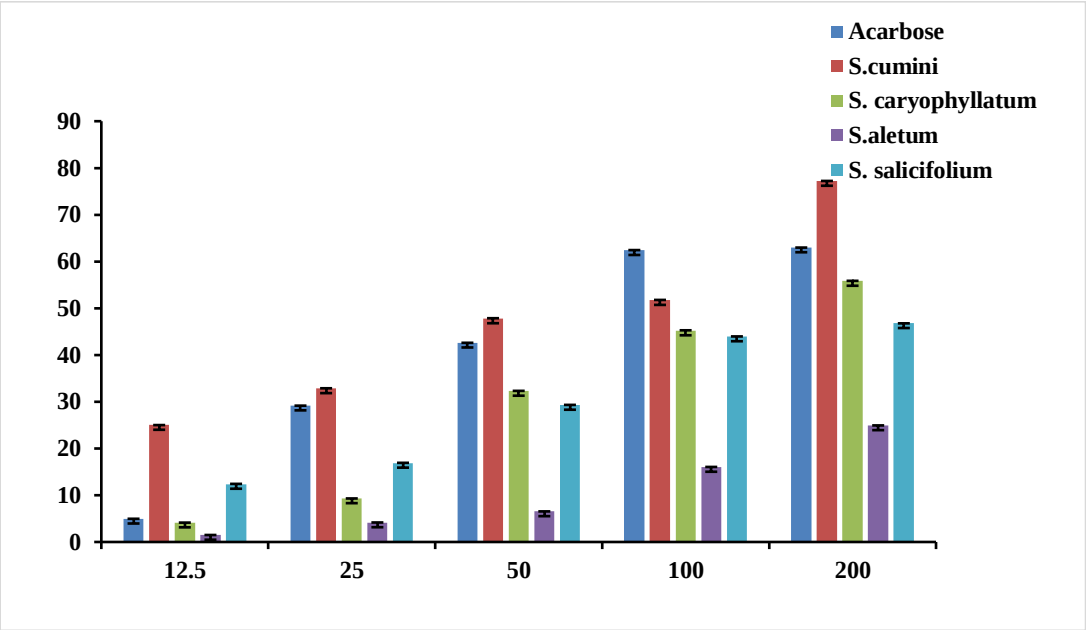

fig20. Comparative antidiabetic activity of *S.cumini, S.caryophyllatum, S.laetum, S.salicifolium* (Bark extract)