Comparative anatomical, phytochemical, antioxidant and antimicrobial studies of three selected *Terminalia* species

A Dissertation for
Course code and Course Title: BOO-Diss & Dissertation
Credits: 8
Submitted in partial fulfilment of Master's Degree

M.Sc. in Botany

by

MS. PRACHI PRABHAKAR GAUNKAR

Roll No. 21P048011

Under the guidance of

PROF. S. KRISHNAN, M.Phil, Ph.D

School of Biological Sciences and Biotechnology
Botany Discipline

GOA UNIVERSITY
April 2023

Examined by:

Seal of the school

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation entitled, "**Comparative anatomical, phytochemical, antioxidant and antimicrobial studies of three selected *Terminalia* species**" is based on the result of investigations carried out by me in the Botany Discipline at the School of Biological Sciences and Biotechnology, Goa University under the Supervision/Mentorship of **Prof. S. Krishnan** and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations/ experimental or other findings given the dissertation.

I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

Prachi Prabhakar Gaunkar
Roll No. 21P048011
Botany Discipline
School of Biological Sciences and Biotechnology

Date: 10.04.2023
Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation "**Comparative anatomical, phytochemical, antioxidant and antimicrobial studies of three selected *Terminalia* species**" is a bonafide work carried out by **Ms. Prachi Prabhakar Gaunkar** under my supervision/mentorship in partial fulfilment of the requirement for the award of the degree of M.Sc. in the Discipline of Botany at the School of Biological Sciences and Biotechnology, Goa University.

Prof. S. Krishnan
Botany Discipline
School of Biological Sciences and Biotechnology

Date: 10.04.2023

GOA UNIVERSITY
SBSB
TALEIGAO
GOA
BOTANY DISCIPLINE

School Stamp

Prof. Savita Kerkar
Dean of the School
Botany Discipline
School of Biological Sciences and Biotechnology

Dean of School of Biological Sciences & Biotechnology
Goa University, Goa-403206
Office Ph. 8669609246

Date: 20/4/23
Place: Goa University

ACKNOWLEDGEMENTS

I am extremely thankful to the almighty Lord who has blessed and guided me throughout. I thank all the people who have helped me in the completion of my dissertation.

I express my sincere gratitude to my guide Prof. S. Krishnan, Senior Professor, Botany discipline, School of Biological Sciences and Biotechnology, Goa University, for his constant guidance, help, support and encouragement throughout my dissertation work and for providing facilities to carry out the research work.

I would also like to thank my professors, Prof. Bernard F. Rodrigues, Prof. Prabhat Kumar Sharma, Prof. Vijaya U. Kerkar, Dr. Rupali R. Bhandari, Dr. Siddhi K. Jalmi and Dr. Aditi Naik for their valuable advice and suggestions.

I would like to express my deepest gratitude to Miss Annie Princy Nadar, Miss Arti Dabholkar, Miss Akshatra Fernandes Research scholar, Goa University for their immense support, encouragement, suggestions and guidance throughout my dissertation work. I would also like to thank the research scholars Miss Vaishali Gaonkar and Miss Divya Rani for their help and advice.

I also thank Mr. Samrat Gaonkar, Mrs. Sahara Baby, Miss Shanta Baganawar from the Botany Discipline for providing me all the apparatus and chemicals required during my dissertation work.

I thank my friends for their co-operation and suggestions.

I am deeply blessed and grateful to have the support of my mother, father and brother who have been there in throughout my work.

Ms. PRACHI PRABHAKAR GAUNKAR

CONTENTS

LIST OF PLATES

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Medicinal plants have been used in traditional practices since prehistoric times. Plants are known to synthesize hundreds of chemical compounds for various functions like in defence to protect against insects, fungi, diseases, and herbivorous mammals; communication, e.g. attraction of beneficial organisms such as pollinators; antagonistic interactions, e.g. deterrent against herbivores and pathogens (Gershenzon and Ullah, 2022). As per World Health Organization (WHO) it is documented that 80% of the world population has faith in traditional medicines, mostly plant drugs, particularly for their primary healthcare (Kala *et al*., 2006). The use of plants for medicinal purposes and human sustenance has been in practice in India since the Vedic age. The earliest mention of the medicinal use of the plants is found in the Rigveda, 1500-400 BC, Atharvaveda 1500 BC, Upnishada 1000-600 BC (Chauhan, 1999). Medicinal plants contain different substances that can be used in the treatment of various diseases or which act as precursors to produce valuable drugs. Since ancient times, these have been used in traditional medicine to cure various health problems (Hao, 2019; Kumar *et al*., 2009).

Generally, medicinal plants are classified based on active principles in their storage organs like roots, leaves, flowers, seeds etc. These integrate and accumulate secondary metabolites like alkaloids, flavonoids, glycosides, saponins, sterols, terpenes, quinines, tannins, etc. These phytochemicals possess antioxidant activities, which prevent or can be used in the treatment of many diseases. The effectiveness of herbal medicine over modern medicine available in market is debatable. Herbal medicinal remedies are safer, natural, cost effective and easily available in the locality as compared to modern medicines. Majority of modern medicines are manufactured

from industrial chemical, that have been designed based on scientific research. Manufacturing of such products is high-tech, and involves lot of capital investment and subsequently prices of these products are so high that common man cannot afford it. These chemical products not only require renewable sources for their production, but also pose lot of side effects on human health as well as on environment (Sharma *et al*., 2017).

1.1. History

Preparation and application of medicines from plants has been realized through various trial and errors, allowing human societies to gradually meet their needs from environment. This information has been passed on for generations. Ayurveda is an ancient health care system which evolved in India about 5000 years ago. The literal meaning of Ayurveda is the "Science of life". It is estimated that about 7,500 plants are used in local health traditions in most rural and tribal villages in India. The plant-based traditional medicine systems continue to play a crucial role in the health care system (Shakya *et al*., 2016).

1.1.1. Wealth of medicinal plants in India

The varied topographic and climatic conditions in India offer a rich and diversified flora. Nature has bestowed with an enormous wealth of medicinal plants to India. In India, medicinal plant sector has traditionally occupied an important position in the socio-economic, cultural, and spiritual arena of rural and urban lives. It has been seen that about 8,000 flowering plants, 650 lichens, 650 algae, 200 pteridophytes and 150 bryophytes are referred with medicinal properties (Pattanaik *et al*., 2008).

1.2. Ethnobotany and medicine

Plants have traditionally served as man's most important weapon against pathogens. It is only recently due to development, modern technology and synthetic chemistry that has led to reduction of our dependence on plants as a source of medicine. Ethnobotany is broadly defined as the use of plants by humans as medicines. Ethnobotany basically deals with the study and evolution of plant-human relations in all phases and the effect of plant environment on human society (Sharma *et al*., 2011). Perhaps, ethno-pharmacology is a highly diversified approach to drug discovery involving the observation, description and experimental investigation of indigenous drugs and their biological activities. The use of plants in traditional medicine systems of many cultures has been extensively documented. These plant-based systems continue to play an essential role in health care and the WHO estimates that 80% or the world's inhabitants continue to rely mainly on traditional medicines systems for their health care (Gurib-Fakim *et al*., 2006).

1.2.1. Importance of medicinal plants

Medicinal plants are not only potential resources used in development of various drugs; they also play a critical role in the development of human cultures around the whole world. Some plants are considered as important source of nutrition and hence are recommended for their therapeutic values, examples include ginger, green tea, walnuts, aloe, pepper, turmeric, etc. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin, toothpaste, etc. A wide variety of herbs are used as tonics; they are nutritive and rejuvenate a healthy as well as diseased individual. Certain herbs are used as stimulants to increase the activity of a system or an organ, to heal wounds, sores, and boils (CSIR *et al*., 1948).

Apart from the medicinal uses, herbs are also used as natural dye source, pest control, food, perfume, tea and so on. In many countries different kinds of medicinal plants/ herbs are used to keep ants, flies, mice and flee away from homes and offices. Nowadays medicinal herbs are important sources for pharmaceutical industry wherein they are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxins. Herbs are known to have properties like antibiotic, disinfectant, which not only destroys disease causing germs but also inhibit the growth of pathogenic microbes leading to communicable diseases (Parekh *et al*., 2007).

1.3. *Terminalia*

Terminalia L. is a genus of large flowering trees of the family Combretaceae, comprising of nearly 300 species distributed in tropical regions of the world. The genus name derives from the Latin word *terminus*, referring to the fact that the leaves appear at the very tips of the shoots. *Terminalia* mainly is a deciduous or evergreen tree, found in low elevation forests with conspicuously layered branches, growing 10 to 20m tall, with an erect trunk. The tree is harvested from the wild for local use as a medicine and source of dyes and tannins. It is a good shade tree and is used in reforestation projects, often being grown as an ornamental and to provide shade along streets. The bark and wood being astringent are used in the treatment of dysentery, also they contain tannins and are used for dyeing (Fahmy *et al*., 2015).

A) *Terminalia elliptica* Willd.

T. elliptica is a large deciduous tree, 20-35m high and 1m in diameter. The bark is rough, dark grey to black in colour with deep vertical fissures & transverse cracks. Leaves are simple, sub-opposite or the uppermost alternate, thick, ovate-oblong, or elliptic-oblong, rarely obovate, with 1-2 glands (which are often turbinate or long stalked) usually on the midrib. Flowers are hermaphrodite, in axillary spikes or

terminal panicles. Fruits are 1-2 inches long and ¾ inch wide with 5 broad, coriaceous, brown, globous wings striated with numerous straight lines running vertically from the axis to the edges. The bark is bitter, useful for pitta, ulcers, vata, fractures, haemorrhages, bronchitis cardiopathy, strangury, wounds, haemoptysis, dysentery, cough, leucorrhoea, gonorrhoea & burning sensation. The plant is known to possess many pharmacological properties like antifungal, antioxidant, anti-hyperglycaemic, antidiarrheal, and anti-leukorrhoeal (Joshi *et al*., 2013).

B) *Terminalia paniculata* Roth

Terminalia paniculata is a large deciduous tree, 20-30m in height with a clear bole of about 10m, brown to dark brown, rough, bark peeling off in thin flakes; leaves simple, upper alternate, lower opposite, oblong or elliptic, acute or acuminate, pale brown with two glands near the base of the midrib; flowers reddish brown, sessile, in rusty pubescent spikes; fruits reddish brown-winged, one wing broad and the other two narrow, widely distributed in India. Traditionally, flower juice and bark of *Terminalia paniculata* have been used as a remedy for cholera, diabetes, inflamed parotid glands, menstrual disorders, cough, microbes, wounds, ulcers, worm, skin disease, leprosy and anaemia. Also, plant is used to treat cough, bronchitis, hepatitis, and diabetes and has spermicidal activity. The stem bark, leaf, and fruit possess antibacterial, antifungal, and antioxidant activity (Acharyya *et al*., 2019).

C) *Terminalia bellirica* (Gaertn.) Roxb.

Terminalia bellirica is a large, 10–20m high, deciduous tree with characteristic thick brownish grey bark and is found throughout the Indian forests and plains. The stems are straight, frequently buttressed when large. Leaves are alternate, broadly elliptic, clustered towards at the ends of branches when young but globous on maturity, with nerves being prominent on both surfaces. Flowers are greenish yellow,

borne in axillary, spender spikes longer than the petioles, having offensive odour. Fruit is a drupe about 2.5cm long, globose or narrowed at the base, silky-brownish-velvety.

Plant is used as laxative, astringent, anthelmintic and antipyretic. Fruits are useful in treatment of hepatitis, bronchitis, asthma, dyspepsia, piles, diarrhoea, coughs, hoarseness of voice, eye diseases, scorpion-sting, menstrual disorder and used as a hair tonic. Decoction of the green fruit is used for cough; pulp of the fruit is useful in dysenteric-diarrhoea, dropsy, piles, and leprosy; half ripe fruit is used as purgative; Kernel of the fruit is narcotic. Seed oil is used in rheumatism whereas, gum of the bark is demulcent and purgative. The triterpenoid presents in the fruits possess significant antimicrobial activity (Gupta *et al*., 2017).

1.4. Anatomy

"The study of gross internal structure of plant organs by the technique of section cutting is called plant anatomy (*ana* = asunder; *temnein* = to cut)." Plant anatomy deals with the study of internal structure of the various organs of the plant including the structure of cell. (Pandey, B. P. 2001). Anatomical structure can be used as initial data source that can be used in plant taxonomy because anatomical characters are conserved and stable. The anatomy of vegetative organs (leaves, stem, and root) is used more than the anatomy of reproductive organs as taxonomic features. Leaves consist of anatomical structure such as epidermis, stomata, mesophyll, and vascular bundles. Similarly, epidermis, cortex, vascular bundles, and pith in stem and roots. (Moralita *et al*., 2019). While studying plant taxonomy, some unique features like secretory tissue, trichome, stomata are also present.

1.5. Phytochemistry

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are found in plants, synthesized by primary or secondary metabolism. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research, and countless other areas. Plant products have been part of phytomedicines since time immemorial which are derived from barks, leaves, flowers, roots, fruits, seeds (Yadav *et al*., 2011). Fahmy *et al*., in 2015, conducted a survey which revealed that genus *Terminalia* is a rich source of tannins and pseudo tannins, including gallic acid and its simple gallate esters, chebulic and non-chebulic ellagitannins, ellagic acid derivatives and ellagic acid glycosides. Also, Machumi *et al*., in 2013 reported tannins, flavonoids, pentacyclic triterpenoids and glycosidic derivatives from *Terminalia*. These compounds have biological properties such as antimalarial, antifungal, antibacterial, antidiabetic, antioxidant, immunoregulatory and cytotoxic activities.

1.6. Antioxidant

Medicinal plants are an important source of antioxidants (Rice-Evans, 2004). Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior and Cao, 2000). The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots, and bark (Mathew and Abraham, 2006). The traditional

medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles (Scartezzini *et al*., 2000).

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (Yamagshi and Matsui, 2011). The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that can oxidize reduced molecules. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ($\cdot O^{2-}$) and hydroxyl radicals ($OH^{\cdot}$), as well as non-free radicals (H_2O_2) and singlet oxygen (Halliwell, 1995).

1,1 -Diphenyl- 2-picryl-hydrazyl radical scavenging (DPPH) assay, DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the nonradical form DPPH-H (Blois, 1958). This transformation results in a colour change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple colour is monitored at 517 nm.

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

(DPPH) + (H-A) ⟶ DPPH-H + (A)

Here, the antioxidant reacted with the DPPH and reduced it to DPPH-H, and thus the absorbance decreased with an increase in the concentration of the extract. This indicated the scavenging potential of the antioxidant compound in the extracts in terms of hydrogen donating ability (Kedare *et al*., 2011).

1.7. Antimicrobial

In recent years, multiple drugs resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. In addition, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune-suppression, and allergic reactions. This situation forced scientists to search and develop alternative antimicrobial drugs for the treatment of infectious diseases from the natural plant sources (Jigna *et al*., 2005). For this several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world. It revealed that medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs (Vashist *et al*., 2012). Plants are rich in secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found to have antimicrobial properties (Duraipandiyan *et al*., 2006).

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.

Jeyaprakash *et al*. (2011) identified traditional uses of medicinal plants among the tribal people in Theni District (Western Ghats), Southern India. With the help of standardized questionnaires, informants were interviewed on the medicinal use of the local flora in various tribal villages during August 2008 to July 2009 wherein a total of 86 plant species belonging to 75 genera and 45 families were reported with ethnomedicinal uses. In terms of the number of medicinal plant pecies, *Acanthaceae* (6 genera and 7 species, 8% of total collected plants) and *Cucurbitaceae* (5 species) are dominant families. Among the different plant parts used for the preparation of medicine, the leaves were most frequently used for the treatment of diseases.

Cock *et al*. (2015) investigated the medicinal properties and phytochemistry of plants of the genus *Terminalia* (Combretaceae) wherein he reported that genus *Terminalia* has multiple therapeutic bioactivities. *T. arjuna*, *T. bellirica*, *T. chebula* and *T. catappa* have been reported to have good antioxidant, anticancer, antidiabetic, antiseptic, cardiotonic and anti-inflammatory effects as well as assist in wound healing.

Akinsulire *et al*. (2018) carried out study of the structure, distribution and taxonomic significance of leaf and petiole anatomical characters in five species of *Terminalia* found in southwest Nigeria. The transverse sections of leaves and petioles of all the species were taken using Reichert Sliding Microtome at a thickness of 8-10

microns. The obtained sections were stained in Safranin O stain for 3-5 min followed by removal of excess stain and counterstaining in Alcian Blue solution for 3-5min. The sections were further washed with water and treated in series of ethanol dilution- 50%, 70%, 80%, 90% and 100% to enhance dehydration process. The dehydrated sections were transferred into absolute xylene to remove any remaining trace of water and ethanol. Finally, section was mounted in 25% glycerol containing thymol crystals. The leaf anatomy and petiole anatomy shown some variation. Anatomical characters which separate the taxa include petiole outline, epidermal features, vascular pattern, occurrence of cortical and pericyclic fibres, presence/absence of, and types of trichomes etc. It is useful in identification of different species.

Joshi *et al*. (2013) investigated the physicochemical and phytochemical parameters of stem bark of *Terminalia tomentosa*. Bark was used for physicochemical evaluation, fluorescence analysis and preparation of ethanolic extract. The ethanolic extract was prepared by maceration method. The physicochemical study revealed presence of 12.5% w/w moisture content, total ash as 19.95% w/w, acid insoluble ash as 16.35% w/w, water soluble ash as 0.9% w/w, alcohol soluble extractive as 1% w/w, water soluble extractive as 0.8% w/w, ether soluble extractive as 0.2% w/w, foaming index as less than 100 & swelling index as 1.14 cm. Also, Preliminary phytochemical screening revealed the presence of carbohydrates, flavonoids, triterpenoids, steroids, tannins & saponins in the ethanolic extract of stem bark.

Joshi *et al*. (2013) carried out phytochemical investigation of *Terminalia tomentosa* using stem bark. The plant is known to possess various pharmacological

activities like antifungal, antioxidant, antihyperglycemic, antidiarrheal, etc. The ethanolic extract was prepared by maceration method. Preliminary phytochemical screening of stem bark contains carbohydrates, flavonoids, triterpenoids, steroids, tannins and saponins. Further chemical entities isolated and characterised includes 4 – methyl - 4 - hydroxy methylene - 6β - (10 - methyl octanol) cyclohexane (Arjuna homoses quiterpenol), di-n-octyl phthalate, di isobutyl phthalate and dibutyl phthalate.

Maridass *et al*. (2010) reported the phytochemical analysis of 63 medicinal plants representing 26 genera and 25 families was collected from the Tirunelveli hills, South India. Various parts of plant such as leaf, bark, fruits, root, rhizome and tuber were used for analysis. The result of phytochemical analysis obtained showed the presence of alkaloids (58.73%), terpenoids (92.06%), flavonoids (90.48%), saponins (50.79) and tannins (31.74%) present in total of 63 plant species.

Wright *et al*. (2016) carried out qualitative phytochemical analysis and antibacterial activity evaluation of Indian *Terminalia spp*. against the Pharyngitis causing pathogen *Streptococcus pyogenes*. *Streptococcus pyogenes* is a gram-positive, pathogenic bacterium which causes a variety of diseases including streptococcal pharyngitis, impetigo and rheumatic heart disease, depending on which tissue it infects.

Gangwar *et al*. (2016) aimed to show the phytochemical screening and antimicrobial activity of *Terminalia paniculata*. Crude extract was prepared using Soxhlet apparatus, the extract was filtered, concentrated under reduced pressure to

dryness and subjected for phytochemical screening. The study shows the presence of different phytoconstituents such as alkaloids, glycosides, saponin, flavonoids, coumarins, tannins and carotenoids in the ethanol extract of *terminalia paniculata* bark. Further, the antimicrobial activity was performed by employing the pour plate as well as disc diffusion methods. The study revealed that the extract had significant antimicrobial activity against the test pathogens. The maximum and minimum inhibition of zone was observed against *Pseudomonas aeruginosa* (16mm) and *Staphylococcus aureus* (6mm) respectively.

Kannan *et al*. (2009) examined the antibacterial activity of *Terminalia chebula* fruit using ethanol extract against standard bacterial strains namely *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis*, *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Result showed maximum activity against *S. typhi*, *S. epidermidis* and *B. subtilis*. These results support the beneficial effects of *T. chebula* fruit for its antibacterial properties.

Pfundstein *et al*. (2010) carried out a study to investigate 34 polyphenolic substances in methanol extract of the fruits of three *Terminalia* species. These were identified by HPLC-ESI- MS and quantitated by analytical HPLC after column chromatography. Antioxidant capacities of raw fruit extract were determined as major isolated substance using the 1,1-diphenyl-2-pic-rylhydrazyl radical, oxygen radical absorbance capacity and ferric reducing ability of plasma in vitro assay.

Kadian *et al*. (2014) evaluated therapeutic potential of plants for diseases and phytopharmacology of *Terminalia bellirica*. It has been reported that the crude extract of various parts of plant possess multifarious medicinal properties such as analgesic activity, antibiofilm activity, anticancer activity, antidepressant activity, antidiabetic activity, antidiarrheal activity, anti-ulcer activity, immunomodulatory activity, antifertility activity, antihypertensive activity, antifungal, antimicrobial activity, anti-inflammatory activity, antioxidant activity.

Sabnis *et al*. (2014) performed the antimicrobial efficacy of *Terminalia bellirica* against virulence factors of respiratory pathogens using methanolic extract. Two respiratory pathogen strains were used namely *Staphylococcus aureus* and *Klebsiella pneumoniae*. The preliminary antimicrobial analysis was carried out using the Agar ditch method. This study indicates that *Terminalia bellirica* extract possesses potential antimicrobial activity against respiratory pathogens and can be used in treating diseases caused by these pathogens.

Suman *et al*. (2019) carried out antimicrobial and anthelmintic activity of *Terminalia paniculate* using root sample. Sample was collected from the forest of the Purba-Medinipur. Using ethanol as solvent, crude extract was prepared via Soxhlet extractor. The preliminary phytochemical studies are carried out using ethanolic extract which indicates the presence of flavonoids, terpenoids, saponins, tannins, phenol and quinones. Antibacterial activites were carried out by two ways i.e., broth dilution method and disc diffusion method. The results obtained says that ethanolic extract of *Terminalia paniculata* root have promising antibacterial activity against

gram-negative bacteria (*E. coli* and *P. aerugenosa*) as compare to gram-positive bacteria (*S. aureus* and *B. subtilis*). Also, ethanolic extract showed the anthelmintic activity in a dose-dependent manner at 10 to 25 mg/ml.

Sanjay Kumar *et al*. (2009) reported the result of histochemistry localization of metabolites and enzymes in stem galls of *Terminalia arjuna*. The metabolites such as cellulose, carbohydrates, proteins, lignin, tannins, enzymes, peroxidase and acid phosphatase were localized and documented very well. These studies revealed higher activity of various metabolites in gall tissue, especially near the nutritive zone.

Deb *et al*. (2016) reported the pharmacological activities of Baheda. The crude extracts of various parts of *Terminalia bellirica* plant have been found to contain constituents such as Glucoside, Gallo-tannic acid, Ellagic acid, gallic acid, lignans, 7-hydroxy 3'4' flavone, anolignan B, Tannins, ethyl gallate, galloyl glucose, chebulic acid, phyllemblin, β- sitosterol mannitol, glucose, fructose, rhamnose, colouring matter, resins and a greenish yellow oil. These compounds are believed to be responsible for the pharmacological activities such as antimicrobial, antioxidant, hepatoprotective, antispasmodic and bronchodilator activities. Therefore, this plant is significantly used for the treatment and prevention of diseases.

Srinivasan *et al*. (2016) evaluated antidiabetic activity of *Terminalia paniculata* bark extract in various in-vitro models and its safety evaluation using Wistar albino rats. Preliminary phytochemical studies showed that ethanolic extract contains alkaloids, glycosides, flavonoids, tannins and terpenoids. The in-vitro

antidiabetic activity was studied using Alpha amylase, Alpha glucosidase inhibition and Glucose diffusion assay. The nitric oxide radicals scavenging activity was determined using antioxidant assay. Acute and sub-acute toxicity studies were designed as per the OECD guidelines- 423. TP extract shows maximum inhibition of 94.5 % on α-Amylase at 700 µg/ml and 94 % on α-Glucosidase at 1000 µg/ml. The TP extract at 1500 µg/ml concentration showed only 34.4 mg/dl of glucose in glucose diffusion method. Nitric oxide scavenging activity and maximum percentage inhibition of 86.12±1.01 was observed at 1500 µg/ml. Acute toxicity study supported the tolerance level even at the dose of 5000 mg/kg body weight and no toxic signs and mortality were observed. The sub-acute toxicity studies did not show any significant changes.

Sumathi *et al*. (2010) examined the antimicrobial activity of some traditional medicinal plants using leaves and fruits. They used pure isolates of *E. coli* (3 strains), *Klebsiella pneumoniae* (2 strains), *Salmonella paratyphi A* (3 strains), *S. typhi* (4 strains) and *S. aureus* (3 strains). The results of the present investigation showed minimum inhibitory concentration of *T. bellirica* fruit extract against *Escherichia coli* and *S. aureus* with values being 50 and 200 µg/ml respectively.

Saxena *et al*. (2013) compared the quantitative estimation of tannins in *Terminalia chebula*, *Terminalia bellirica*, *Terminalia arjuna* and *Saraca indica* using spectrophotometer using leaves, flowers, fruits, and bark using folin-denis method. The tannin concentration was determined by the standard graph of tannic acid solution and was found to be 99.55456mg/gm, 995568mg/gm, 54.96288mg/gm and

57.4869mg/gm for harde, arjuna, baheda and Ashoka respectively. They determined the concentration curve for tannic acid and correlated coefficient was calculated and was found to be 0.998 which indicates the good linearity between the concentration and absorbance.

Wright *et al*. (2016) performed Growth Inhibitory Activity of Indian *Terminalia sp.* against the Zoonotic Bacterium Bacillus anthracis. They used three species of *terminalia* namely *Terminalia chebula*, *Terminalia catappa* and *Terminalia arjuna* for their study. Extract was prepared using 4 solvents namely methanol, deionised water, chloroform, hexane or ethyl acetate. The prepared extract was then used to display potent antibacterial activity in the disc diffusion assay against *B. anthracis*. They observed that fruit extract of methanolic *T. chebula* showed effective inhibiting microbial growth whereas aqueous extract of *T. chebula*, as well as methanolic extract of *T. catappa* and *T. arjuna* also showed good growth inhibitors.

Sumathi *et al*. (2010) performed antimicrobial activity of some traditional medicinal plants against four gram negative and one-gram positive bacteria. The results showed that the minimum inhibitory concentration (MIC) of P. niruri leaf extract was 50 µg/ml against *Salmonella typhi* and *Staphylococcus aureus*; whereas, the MICs of T. bellirica fruit extract against *Escherichia coli* and *S. aureus* were 50 and 200 µg/ml respectively. However, the leaf extracts of the *Andrographis paniculata*, *T. chebula* and *V. negundo* have not shown any antimicrobial activity in the tested concentrations.

OBJECTIVES

Genus *Terminalia* has been intensively used in traditional medicinal system to teat fever, pneumonia, diarrhea, scorpion stings, abdominal and back pain, urinary tract disorder, etc. Many species are used for their antibacterial, antifungal, antiprotozoal, antiviral, antidiarrheal, analgesic, antimalarial, antioxidant, anti-inflammatory and anticancer activities. This is due to the high content of secondary metabolites such as polyphenols and triterpenoids. Other constituents include amino acids, fructose, resin, tannins, flavonoids, sterols, and fixed oils.

However, it has been identified that human beings are destroying these species due to construction of roads and vegetation purpose. *T. elliptica* being state tree of Goa and endemic to western ghat along with other species needs to be protected. In this study we proved that selected species of *Terminalia* have antioxidant and antimicrobial properties.

Objectives of the present investigation:

1. Gathering the ethnobotanical knowledge from the local people.
2. Collection and taxonomic identification of selected *Terminalia* species.
3. To study the anatomical characteristics of leaf and petiole of three selected *Terminalia* species (*Terminalia bellirica*, *Terminalia elliptica*, *Terminalia paniculata*) from the Western Ghats region of Goa.
4. To analyse the phytochemical profile of the chosen medicinal species by preliminary phytochemical analysis.
5. Screening and comparing antioxidant activity of selected plant parts using DPPH method.
6. Evaluation of antimicrobial activities of selected *Terminalia* species plant parts against microbial species *Aspergillus niger*, *Aspergillus* species and *E. coli*.
7. Evaluation of natural dye prepared from *T. paniculata* fruit for biological staining.

MATERIALS AND METHODS

3.1. Ethnobotanical Data Collection

Ethnobotanical data were collected from 1st July 2022 to 20th August 2022. The standard data collection method has been followed to document indigenous knowledge of the local community of Sanguem taluka on health uses, conservation, and threats of medicinal plants. Data were collected through basic oral interview. Information was carefully noted down and presented in **Table 1**.

3.2. Study Area and Plant Collection

The selected *Terminalia* species (*Terminalia elliptica*, *Terminalia paniculata*, *Terminalia bellirica*) were collected from the Western Ghats regions of Goa, namely Sanguem and Taleigao of Goa. Mature and healthy leaves and fruits were collected and appropriately cleaned with distilled water and dried for 14 days under shade at room temperature.

3.3. Comparative Anatomical Studies

Mature leaves, and petioles were collected for anatomical studies of all 3 species. Free-hand sections were taken from fresh leaf (middle portion of the leaf with midrib), and petiole (centre of the petiole). Sections were stained with 0.1% safranin for 2-3 min, rinsed with distilled water, mounted on a slide with 10% glycerine, and examined under a bright field microscope. The desirable portions were photographed by using a digital compact camera attached to the microscope and images were captured using TC-capture software.

3.4. Preliminary qualitative phytochemical analysis

3.4.1. Preparation of plant extract

Absolute methanol, ethyl acetate, chloroform and distilled water were used for maceration method. 10g of dried powder sample was mixed with respective solvents and kept at room temperature for 3 days. The extract was stirred after every 4 to 5 hours. The solution was filtered using Whatman filter paper Grade A. The extracts were evaporated using rotary evaporator. The crude extracts were stored in glass vials and kept in freezer for future use.

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

3.4.2. Test for Alkaloids

50mg of extract was stirred with a 1mL of dilute hydrochloric acid and filtrate. The filtrate is tested with alkaloidal reagents.

Precipitate reveals the presence of alkaloids.

1. **Mayer's Test**

One drop of Mayer's reagent was added to 2mL of filtrate. A white or creamy precipitate reveals the presence of alkaloids.

2. **Wagner's Test**

One drop of Wagner's reagent was added to 2mL of filtrate. A reddish-brown precipitate reveals the presence of alkaloids.

3. **Hager's Test**

1mL of Hager's reagent was added to 2mL of filtrate. A prominent yellow precipitate reveals the presence of alkaloids.

3.4.3. Test for Carbohydrates

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

1. Molish's Test

2 drops of alcoholic solution of α-naphthol were added in 2 mL of filtrate, the mixture was shaken well and 1 mL of concentrated sulphuric acid is added slowly along the sides of the test tube. A violet ring reveals the presence of carbohydrates.

2. Barfoed's Test

1mL of barfoed's reagent was added to 1ml filtrate and heated on a boiling water bath for 2 minutes. Red precipitate reveals the presence of sugars.

3. Benedict's Test

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

3.4.4. Test for Glycosides

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

1. Borntrager's Test

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

2. Legal's Test

2mL of extract was dissolved in pyridine and sodium nitroprusside solution then 10% sodium hydroxide was added till it became alkaline. Pink color reveals the presence of glycoside.

3.4.5. Test for Saponins

50mg of extract was diluted with 20mL of distilled water, this suspension was shaken in a graduated cylinder for 15 minutes. A 2cm layer of foam reveals the presence of saponins.

3.4.6. Test for Proteins

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

1. Biuret Test

One drop of 2% Copper sulphate solution, 1mL 95% Ethanol and 5-6 pellets of Potassium hydroxide were added to 2mL of aqueous filtrate. The pink color reveals the presence of proteins.

2. Ninhydrin Test

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

3.4.7. Test for Fats & Oils

4-5 drops of 0.5N Alcoholic potassium hydroxide solution were added to a small quantity of extract and a drop of phenolphthalein. This mixture was heated in a water bath for 2 hours. Soap formation reveals the presence of saponins.

3.4.8. Test for Phenolic compounds and Tannins

1. Ferric chloride Test

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

2. Gelatin Test

50mg of extract was dissolved in 1% solution Gelatin containing 10% Sodium chloride. White precipitate reveals the presence of phenolic compounds.

3. Lead acetate Test

50mg of extract was dissolved in 3mL of 10% Lead acetate solution. A bulky white precipitate reveals the presence of phenolic compounds.

3.4.9. Test for Terpenoids

2mL of Chloroform and 3mL of Sulphuric acid were added in 2mL of extract. A reddish-brown colouration at interface reveals the presence of terpenoids.

3.4.10. Test for Cardiac glycosides

0.5gm of plant extract was dissolved in 2mL of Glacial acetic acid and a drop of Ferric solution. Formation of brown ring at interface reveals the presence of cardiac glycoside.

3.4.11. Test for Resins

1mL of extract was dissolved in acetone & the solution was poured in distilled water. Turbidity indicated the presence of resins.

3.5. Antioxidant studies

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

A. **Preparation of DPPH:** Stock solution was prepared by dissolving 24mg of DPPH in 100mL of methanol in the dark and stored in an Amber-coloured bottle. The working solution was prepared by adding 10mL of Stock solution to 45mL of methanol.
B. **Preparation of L-ascorbic acid solution:** 10mg of ascorbic acid was dissolved in 10mL of distilled water. Serial dilution was performed to prepare solution with different concentration (12.5µg/mL- 200µg/mL).
C. **Preparation of Test solution:** 10mg of methanolic extract of leaf was dissolved in 10mL of methanol, and then serial dilution was performed to prepare the required concentrations (12.5µg/mL- 200µg/mL).
D. **Preparation of control:** 3mL DPPH was used as a negative control.

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

$$\textbf{Inhibition \%} = (A_0 - A_1)\, A_0 \times 100$$

Here, $\mathbf{A_0}$ is the absorbance of the control, $\mathbf{A_1}$ is the absorbance of the extract.

The antioxidant assay was performed in triplicate and the mean value was expressed.

3.6. Antimicrobial activity

Anti-fungal and antibacterial studies were done on 3 species of *Terminalia* namely *Terminalia paniculata*, *Terminalia elliptica* and *Terminalia bellirica.*

3.6.1. Sterilization of glassware and culture vials

Sterilization of Equipment's and Media: Dry Heat Sterilization –Required glassware was washed in Teepol and kept in oven for complete drying. Petriplates, pipettes, test tubes were also washed and were wrapped separately in paper and sterilized by autoclaving at 15lbs for 1 hour.

3.6.2. Preparation of media

Malt extract media and agar were accurately weighed and were then dissolved in distilled water. The media was digested in microwave by gentle heating and constant stirring; autoclaved at 15 lbs pressure for 1 hour 30 minutes and cooled at room temperature.

3.6.3. Antimicrobial susceptibility test

The fungal strains *Aspergillus niger* and *Aspergillus* sp. were collected from Goa University Fungus Culture Collection (GUFCC) Mycological laboratory, Botany Discipline, SBSB, Goa University. The bacterial strain *Escherichia coli* was collected from Microbiology Department, PES College, Farmagudi. Fungal strain subcultures were done using Malt Extract medium whereas bacterial strain subculture was done using Nutrient agar medium.

Media was poured in the pre-sterilised petriplates and kept for solidification. Loopful of pure culture from the plate was taken and streaked on the plate to obtain fine isolated colonies. The fungal petriplates were incubated at 37^{o}C for 24 hours and bacterial petriplates were incubated at room temperature for 24 hours.

3.6.4. Preparation of Microbial suspension

In laminar air flow the microbial suspensions were prepared by transferring one loop full of stock culture of respective microorganisms to 5 mL of sterile distilled water in conical flask. The suspension was incubated at low temperature in refrigerator for 24 hours.

Using subculture strains, antimicrobial study was carried out by following two different methods:

1) **Disc Diffusion Method**

   A swab of fungal suspension (200µl) was spread on petriplates containing Malt Extract Agar medium (MEA). Sterile filter paper disc (7mm in diameter) impregnated with (300µl) of plant extract were placed on the culture plates. The antibiotic tetracycline hydroxide (300µl) for fungi discs were used

as positive control. The plates were incubated at 37º C for 24 hours. Antifungal activity was noted by the presence of clear inhibition zone around the discs.

2) **Agar Well Diffusion Method**

Spreading of fungal suspension. The cooled Malt Extract Agar media was poured in sterile petriplates and allowed to solidify. The plates with media were seeded with the respective microbial (200µl) suspension using sterile swab and with the help of sterile glass spreader the suspension was spread on the plates uniformly under aseptic conditions. The plates were dried for 1 hour.

3) **Preparation of Wells**

Wells were made with the help of a sterile cork borer (7mm diameter) at four corners.

4) **Addition of extract on Wells**

The extract with different concentrations (100, 200 and 300µl) were added in respective wells using micropipettes in 3 wells of each petri dish. The antibiotic Tetracyclin hydroxide was poured in the 4^{th} well as a positive control. Later the plates were kept for incubation in an incubator at 37º C for 24 hours. After incubation period, antifungal activity was determined by measuring the zone of inhibition around each well and measured (in mm) (Jayprakash, 2013). Same procedure was carried out for bacterial stain on nutrient agar and ampicillin is used as positive control.

3.7. Preparation of stain

1. Preparation of 1% *T. paniculata* fruit stain from crude extract**:** Take 0.25g of crude extract and dissolve completely in 25 mL of distilled water.

2. Preparation of 5% *T. paniculata* fruit stain from crude extract**:** Take 1.25g of crude extract and dissolve completely in 25 mL distilled water.

3. Preparation of *T. paniculata* fruit stain from dried powder**:** Add 10g of dried fruit powder in 250 mL of water. Boil for 3-4 hours and filter.

Take thin section of dicot and monocot stem. Stain the section in safranin, 1%, 5% crude extract stain and 4% dried powder stain. Keep section in stain for different time interval such as 30 minutes and 1 hour. Wash off the excess stain and mount on clean slide with glycerol, put coverslip and observer under microscope.

RESULTS AND DISCUSSION

4.1. Ethnobotanical survey data

Table 1. List of documented medicinal plant species, family, local names, botanical names, parts used and medicinal uses.

Sr. No	Botanical name	Family	Local name	Parts used	Medicinal uses
1.	*Adhatoda vasica* Nees	Acanthaceae	Adulsa	Leaves	Fever, cough
2.	*Aegle marmelos* (L.) Corrêa	Rutaceae	Bel	Leaves, fruit	Menstrual disorder, diabetes
3.	*Alstonia scholaris* (L.) R.Br.	Apocynaceae	Saton	Leaves	Digestive disorder
4.	*Andrographis paniculata* (Burm.f.) Wall. ex Nees	Acanthaceae	Kirayte	Leaves	Deworming
5.	*Annona reticulata* Sieber ex A.DC.	Annonaceae	Aatin	Leaves	Fever
6.	*Annona squamosa* L.	Annonaceae	Sitafal	Leaves, fruit pulp	To cure diabetic
7.	*Bauhinia racemosa* Vahl	Caesalpiniaceae	Aapto	Bark, leaves	Wound
8.	*Boerhavia diffusa* L.	Nyctaginacea	Punanavo	Whole plant	Kidney stone
9.	*Bridelia retusa* (L.) Spreng.	Euphorbiaceae	Fatarphal	Fruit	Kidney stone

10.	*Careya arborea* Roxb.	Lecythidaceae	Kumyo	Bark, leaves	Digestive disorder
11.	*Cassia tora* L.	Fabaceae	Talkilo	Leaves	Skin related problem
12.	*Catharanthus roseus* (L.) G.Don	Apocynaceae	Sadafuli	Whole plant	Diabetes
13.	*Celastrus paniculatus* Willd.	Celastraceae	Kanglin	Roots	Jaundice
14.	*Centella asiatica* (L.) Urb.	Apiaceae	Gundari	Whole plant	Hair fall
15.	*Curcuma longa* L.	Zingiberaceae	Halad	Rhizome	Skin, wound
16.	*Cymbopogon citratus* (hort. ex DC.) Stapf	Poaceae	Ganjan	Leaves	Cough and cold
17.	*Cynodon dactylon* (L.) Pers.	Poaceae	Haryali	Whole plant	Cough, urinary disorder
18.	*Datura inoxia* Mill.	Solanaceae	Dhutro	Fruit, leaves	Tonsils, menstrual disorder
19.	*Eclipta prostrata* (L.) L.	Asteraceae	Meko	Leaves	Hair fall
20.	*Ficus hispida* Roxb. ex Wall.	Moraceae	Kharvat	Bark	Digestive disorder
21.	*Ficus racemosa* Willd.	Moraceae	Rumad	Latex	Scorpion bite
22.	*Holarrhena antidysentrica* (L.) Wall.	Apocynaceae	Nagalkudo	Latex, root, seed	Infection

23.	*Ixora coccinea* Comm. ex Lam.	Rubiaceae	Pitkoli	Leaves, roots	Digestive disorder
24.	*Leucas aspera* (Willd.) Link	Lamiaceae	Tumo	Leaves	Skin disorder
25.	*Mangifera indica* L.	Anacardiaceae	Ambo	Leaves	Deworming
26.	*Microcos paniculata* L.	Malvaceae	Hasoli	Leaves	Jaundice
27.	*Mimosa pudica* L.	Fabaceae	Laje zad	Leaves	Kidney stone
28.	*Naregamia alata* Wight & Arn.	Meliaceae	Pintmado	Leaves	Pint
29.	*Nerium odoratum* Lam.	Apocynaceae	Kaner	Root, leaves	Swelling around wound
30.	*Ocimum tenuiflorum* L.	Lamiaceae	Tulsi	Leaves	Cough
31.	*Piper nigrum* L.	Piperaceae	Mirya	Seed	Menstrual pain
32.	*Psidium guajava* L.	Myrtaceae	Per	Leaves	Digestive disorder
33.	*Sapindus emarginatus* Vahl	Sapindaceae	Rito	Fruit, bark	Hair fall
34.	*Syzygium cumini* (L.) Skeels	Myrtaceae	Zamla	Bark, seed	Jaundice
35.	*Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.	Apocynaceae	Anant	Roots	Menstrual pain

36.	*Tamarindus indica* L.	Fabaceae	Chinch	Bark	Skin related problem
37.	*Tectona grandis* L.f.	Lamiaceae	Sail	Root, bark	Urine stone
38.	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.	Combretaceae	Arjun	Bark	Diarrheal
39.	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Baheda	Leaves	Cough, piles
40.	*Terminalia elliptica* Willd.	Combretaceae	Katra, matti	Leaves, bark	Wound
41.	*Terminalia paniculata* Roth	Combretaceae	Kinal	Stem, leaves	Eye related problem
42.	*Tinospora cordifolia* Miers	Menispermaceae	Amrut vel	Stem	Cough
43.	*Ziziphus rugosa* Lam.	Rhamnaceae	Chunna	Leaves	Digestive disorder

4.1.1. Ethnobotanical survey data analysis

In the present survey all together 43 plant species were documented from the study area, which were used by local people for treating different types of diseases. Mode of preparation and administration varies depending upon disease. It is observed that almost all parts of the plant were utilized for medicinal purpose.

4.2. Collection and identification of selected *Terminalia* species.

Terminalia species are known to posses various secondary metabolies, hence based on their phytochemical, antioxidant and antimicrobial properties the following species were collected from Western Ghats region of Goa **(Table 2 and Plates 1, 2, 3)**. Taxomonic identification was done using herbarium sheets from herbarium of Botany Discipline, SBSB, Goa University. The collected specimens were dried and stored for further use.

Table 2. Names of the selected plant species and their location.

Sr. No.	Botanical Names	Local names	Place of collection
1	*Terminalia bellirica*	Baheda	Lat 15.460001°, Long 73.829115°
2	*Terminalia elliptica*	Matti	Lat 15.241236°, Long 74.162729°
3	*Terminalia paniculata*	Kindal	Lat 15.459024°, Long 73.83047°

4.3. Comparative leaf and petiole anatomy of *Terminalia* species

The finding of anatomical characterization of leaf and petiole of *Terminalia* species (*T. elliptica*, *T. paniculata*, *T. bellirica*) have been summarized in **Tables 3, 4** and **Plates 4, 5 and 6**.

4.3.1. Leaf anatomy

On the abaxial surface of epidermis, trichomes were observed in 2 out of 3 species of *Terminalia*. Unicellular trichome was observed *T. bellirica* **(Plate 4 a)** and multicellular trichome was observed in *T. paniculata* **(Plate 6 d)**. However, trichome were absent in *T. elliptica* leaf. The cell wall of epidermal layer is thickly cuticularized in all the species. Transverse section of leaves showed the presence of uniseriate layer of rectangular epidermal cells on both adaxial and abaxial side.

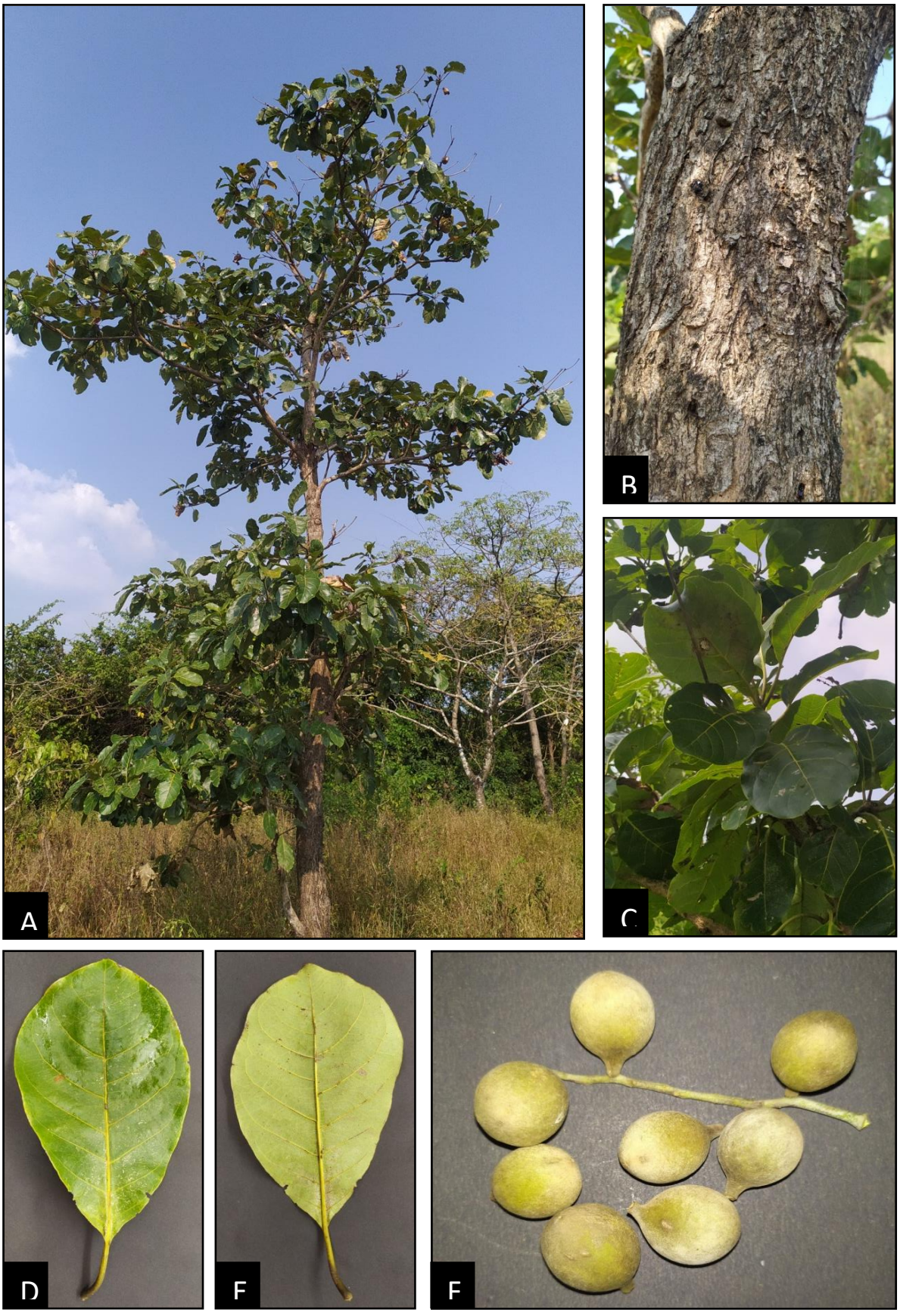


Plate 1. ***Terminalia bellirica*****: (A) Habit, (B) Bark, (C) Twig, (D) Abaxial leaf surface, (E) Adaxial leaf surface, (F) Fruits.**

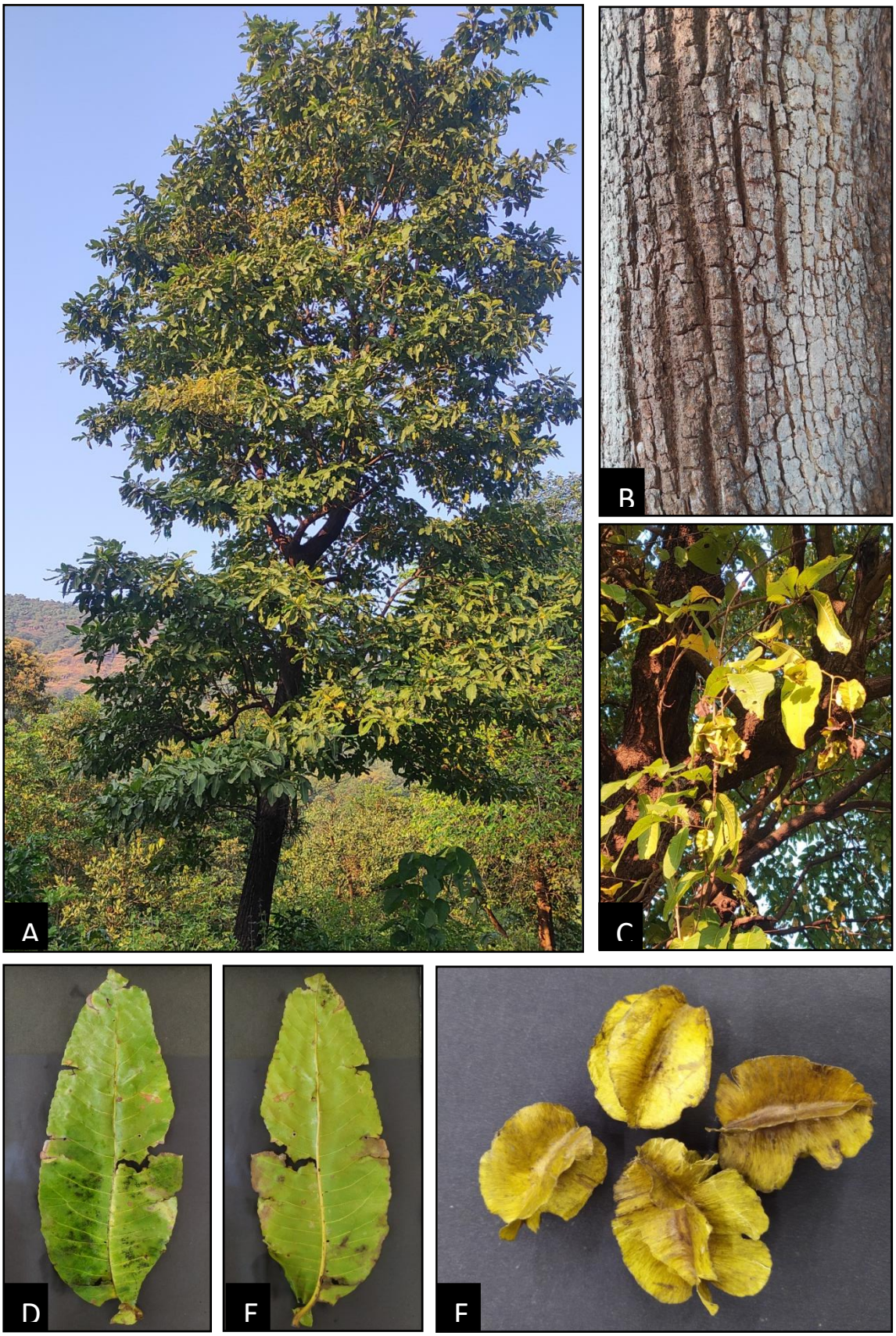


Plate 2. ***Terminalia elliptica*****: (A) Habit, (B) Bark, (C) Twig with fruits, (D) Abaxial leaf surface, (E) Adaxial leaf surface, (F) Fruits.**

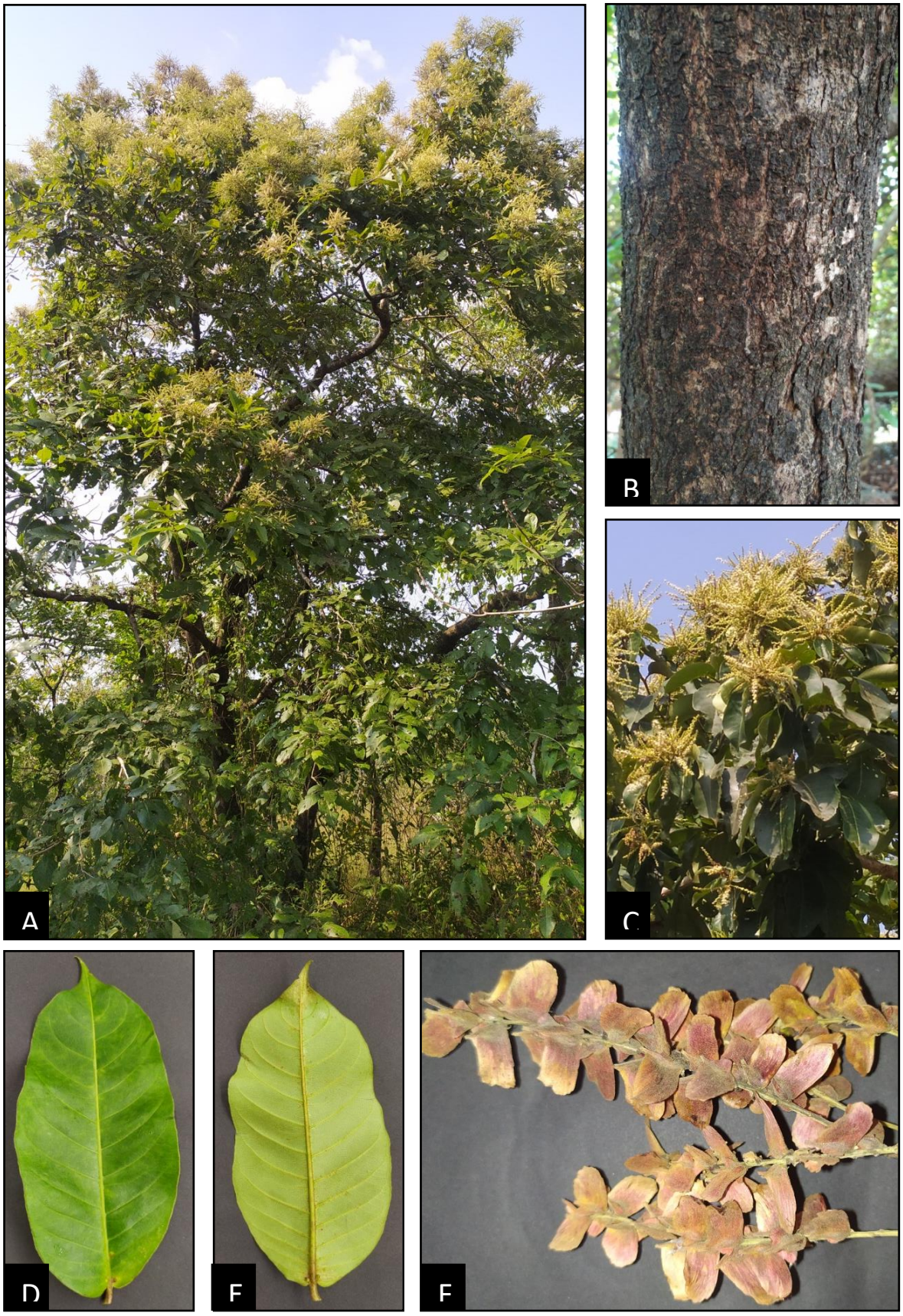


Plate 3. *Terminalia paniculata*: (A) Habit, (B) Bark, (C) Twig with flowers, (D) Abaxial leaf surface, (E) Adaxial leaf surface, (F) Fruits.

The mesophyll tissue was well developed, present in two layers; compactly arranged in cylindrical palisade parenchyma on the adaxial surface and loosely arranged irregular spongy parenchyma with intercellular spaces on the abaxial surface. The cells in the mesophyll layer were filled with chloroplast; high content of chloroplast was present in palisade cells as compared to spongy parenchyma cells.

Based on number of palisade parenchyma layer, the following two groups could be recognised:

1. Palisade parenchyma single layer: *T. paniculata*, *T. bellirica* **(Plates 6 & 4)**
2. Palisade parenchyma three layers: *T. elliptica* **(Plate 5)**

The vasculature was almost circular in shape; three strands; two small at each corner of upper layer and one large at the middle of midrib. The vascular system was conjoint, collateral, and continuous. Surrounding the phloem there were 3-4 layers of sclerenchyma patches. Globular secretory canals varying in size and number were present in all the three species, located in adaxial palisade layer. In all three species crystals were present which are usually in the form of druses. These were found to be embedded in palisade tissue, spongy tissue and near vascular bundles. The druses varied in number and size. More druses were observed in *T. paniculata*, *T. elliptica* as compared to *T. bellirica*.

Table 3. Anatomical characterization of leaf of *Terminalia* species

Name of species	Trichome	Cuticle	Epidermis	Palisade layer	Secretory canals	Druses	Vasculature
Terminalia bellirica	Present, Unicellular	Thick	1 layer	2 layers	Present	Present	Both distal ends fused
Terminalia elliptica	Absent	Thick	1 layer	2 layers	Present	Present	Both distal ends fused
Terminalia paniculata	Present, Multicellular	Thick	1 layer	2 layers	Present	Present	Both distal ends fused

4.3.2. Petiole anatomy

Among the different *Terminalia* species studied the shape, size of petiole varied. The outline was wavy with slightly winged at the corner in all three species. 1-3 layers of brownish periderm was observed in *T. paniculata* **(Plate 6)** whereas 1-2 layers of periderm was observed only at winged corner in *T. elliptica* **(Plate 5)**. Periderm layer was absent in *T. bellirica* **(Plate 4)**. Epidermis was single layer with rectangular shaped cells which are compactly arranged.

The cells of cortical region were irregular in size, compactly arranged. Cells in cortical region varied from 10- 18 layers in *T. bellirica*, 15- 20 layers in *T. paniculata* and 13- 22 layers in *T. elliptica*. Secretory canals present in cortical region varied in size. *T. elliptica* showed presence of 6 secretory canals, 5 secretory canals were noted in *T. paniculata* and 2 in *T. bellirica*. Many druses were observed in cortex region which were evenly distributed.

Three conjoint and collateral vascular bundles were present; one large at centre and two small placed at both end of adaxial region. Based on shape of vascular bundle following two groups are recognised:

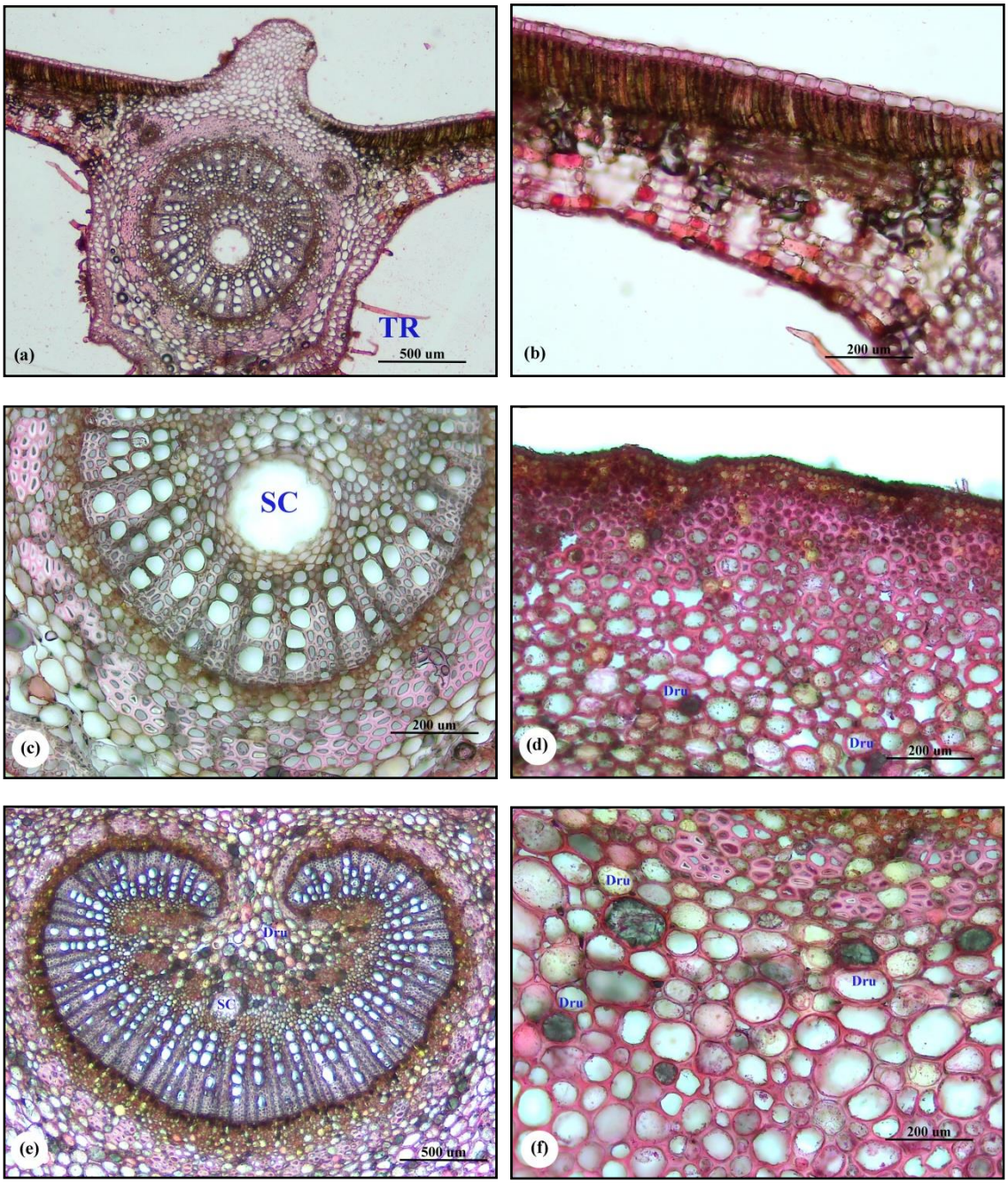


Plate 4. Anatomy of leaf and petiole of *T. bellirica*: (a) Overview of leaf (4X), (b) T.S of leaf showing epidermal cells, mesophyll layers (10X), (c) Section showing vasculature, secretory canals and druses (10X), (d) T.S of petiole showing epidermal layers, cortex and druses (4X), (e) T.S showing vasculature and druses (10X), (f) Section showing druses (10X). SC= secretory canals, Dru= druses, TR= trichome.

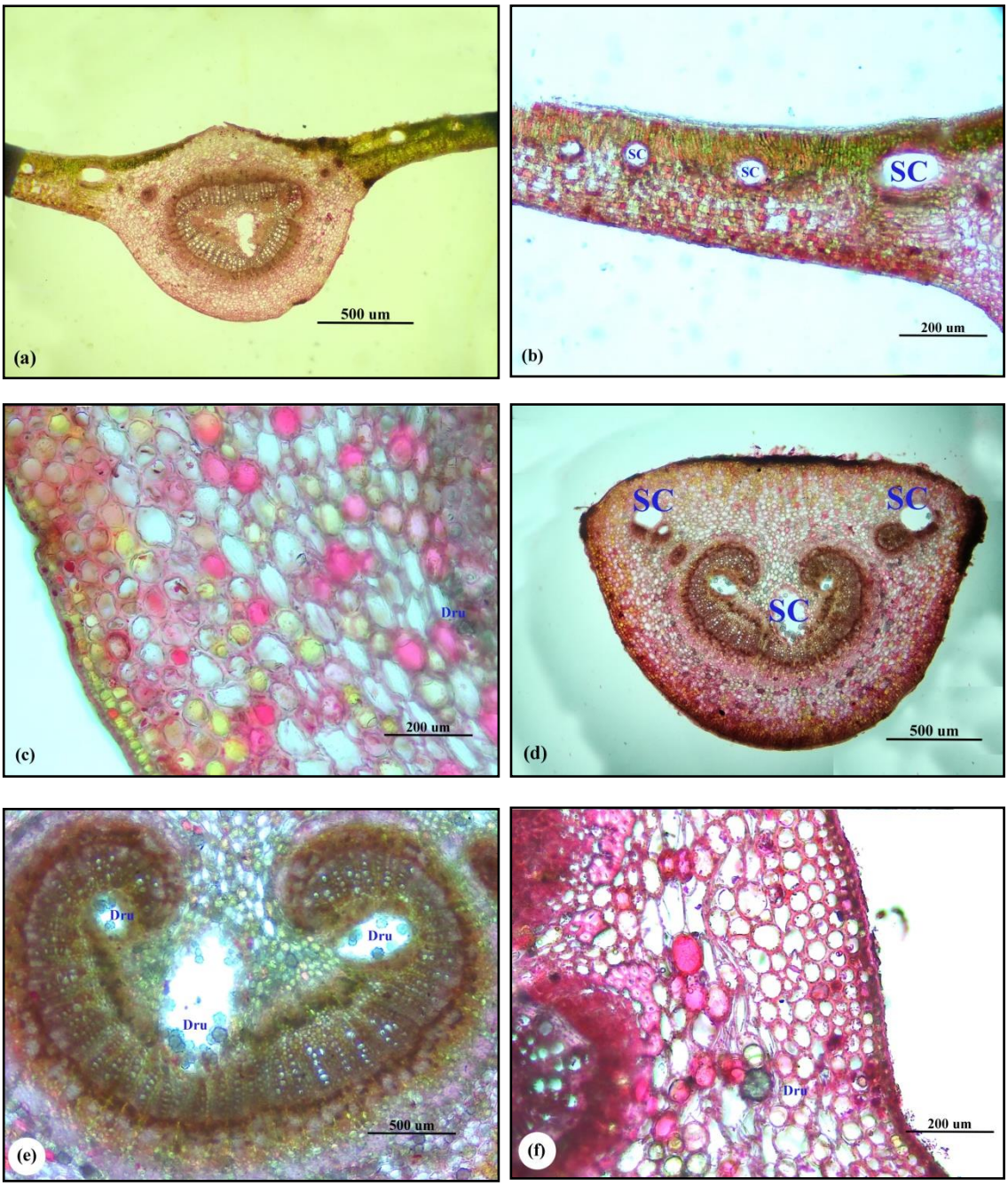


Plate 5. Anatomy of leaf and petiole of *T. elliptica*: (a) Overview of leaf (4X), (b) T.S of leaf showing mesophyll cells and secretory canals (10X), (c) Section showing epidermal cells, cortex and druses (10X), (d) Overview of petiole (4X), (e) T.S of petiole showing vasculature and druses (10X), (f) Section showing epidermis, cortex and druses (10X). SC= secretory canals, Dru= druses.

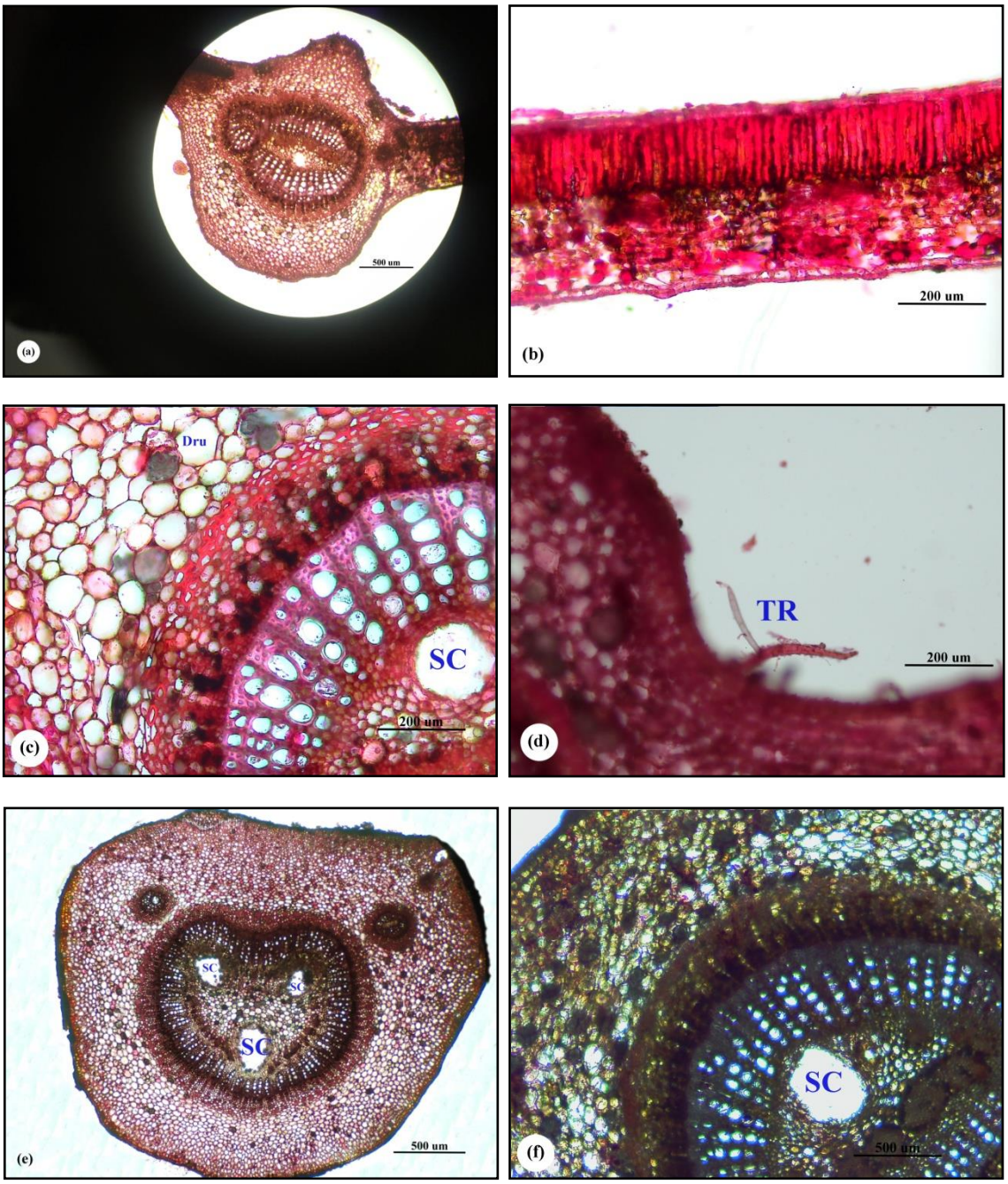


Plate 6. Anatomy of leaf and petiole of *T. paniculata*: (a) Overview of leaf (4X), (b) T.S of leaf showing epidermal cells, mesophyll layers (10X), (c) Section showing cortex, vasculature and druses (10X), (d) Section showing leaf trichome (10X), (e) T.S showing overview of petiole (4X), (f) Section showing epidermis, cortex, secretory canals and druses (10X). SC= secretory canals, Dru= druses, TR= trichome.

1. Arc-shaped in *T. elliptica* and *T. bellirica* **(Plates 4 & 5)**
2. Heart shaped in *T. paniculata* **(Plate 6)**

Surrounding the phloem there were 3-4 layers, sclerenchyma patches were present in all three species.

Table 4. Anatomical characterisation of petiole of *Terminalia* species

Name of the species	**Outline**	**Cuticle**	**Epidermis**	**Vasculature**	**Secretory canals**	**Druses**
Terminalia bellirica	Circular, slightly winged at corner, Wavy	Thick	1 layer	Arc-shape	Present	Present abundantly
Terminalia elliptica	Circular, slightly winged at corner, Wavy	Thick	1 layer	Heart shape	Present	Present abundantly
Terminalia paniculata	Circular, slightly winged at corner, Wavy	Thick	1 layer	Arc-shape	Present	Present abundantly

4.4. Phytochemical Analysis

Phytochemical analysis of *T. paniculata*, *T. elliptica* and *T. bellirica* was carried out to understand different secondary metabolites present in both leaves and fruits. Crude extracts were prepared using the solvents such as methanol, ethyl acetate, chloroform and distilled water from leaves and fruits of each species and phytochemical tests were carried out.

In *Terminalia bellirica* leaves alkaloids, carbohydrates and saponin were present. Tannins and phenolic compounds were found in all solvents, very intense in methanol solvent. Fats and oil as well as terpenoid were found only in distilled water. Proteins showed their presence in all the solvents. Resin was observed in methanol. Glycosides were absent in all the solvents **(Table no. 5, Plate 7)**.

In *Terminalia elliptica* leaves alkaloids, carbohydrates, saponin and proteins were present in all the four solvents. Presence of phenolic and tannins were observed in all four solvents, especially in methanol solvent it was found very intense. Fats and oil were present only in distilled water. Resin was observed in methanol. Leaves of *T. elliptica* showed the presence of Terpenoids only in distilled water however cardiac glycosides were absent in all four solvents **(Table no.6, Plate 8)**.

The leaves of *Terminalia paniculata* showed the presence of alkaloids, carbohydrates and saponin in all solvents. Phenolic and tannins compounds were present in all solvents except in chloroform whereas fats & oils were found only in water. Present of protein was observed in methanol and chloroform solvents however presence of terpenoid was found in ethyl acetate and water. Resin was observed in methanol. Glycosides were absent in all solvents **(Table no.7, Plate 9)**.

Table 5. Phytochemical analysis of *Terminalia bellirica* (leaves)

Sr. No.	Phytochemical Test	Methanol	Ethyl acetate	Chloroform	Distilled Water
1	**Detection of Alkaloids**				
a.	Mayer's test	+	-	+	-
b.	Wagner's test	+	+	-	-
c.	Hager's test	+	+	+	+
2	**Detection of Carbohydrates**				
a.	Molish's Test	++	++	++	++
b.	Barfoed's Test	++	++	+	+
c.	Benedict's Test	++	+	+	+
3	**Detection of Glycosides**				
a.	Borntrager's Test	-	-	-	-
b.	Legal's Test	-	-	-	-
4	**Detection of Saponin**	++	+	+	++
5	**Detection of Proteins**				
a.	Biuret Test	+	+	+	+
b.	Ninhydrin Test	-	-	-	-
6	**Detection of Fats & Oil**				
a.	Saponification	-	-	-	+
7	**Detection of Phenolic & Tannins**				
a.	Ferric chloride Test	++	+	+	++
b.	Gelatin Test	++	++	-	+
c.	Lead acetate Test	++	++	+	+
8	**Terpenoids**	-	-	-	+
9	**Cardiac glycoside**	-	-	-	-
10	**Resins**	+	-	-	-

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

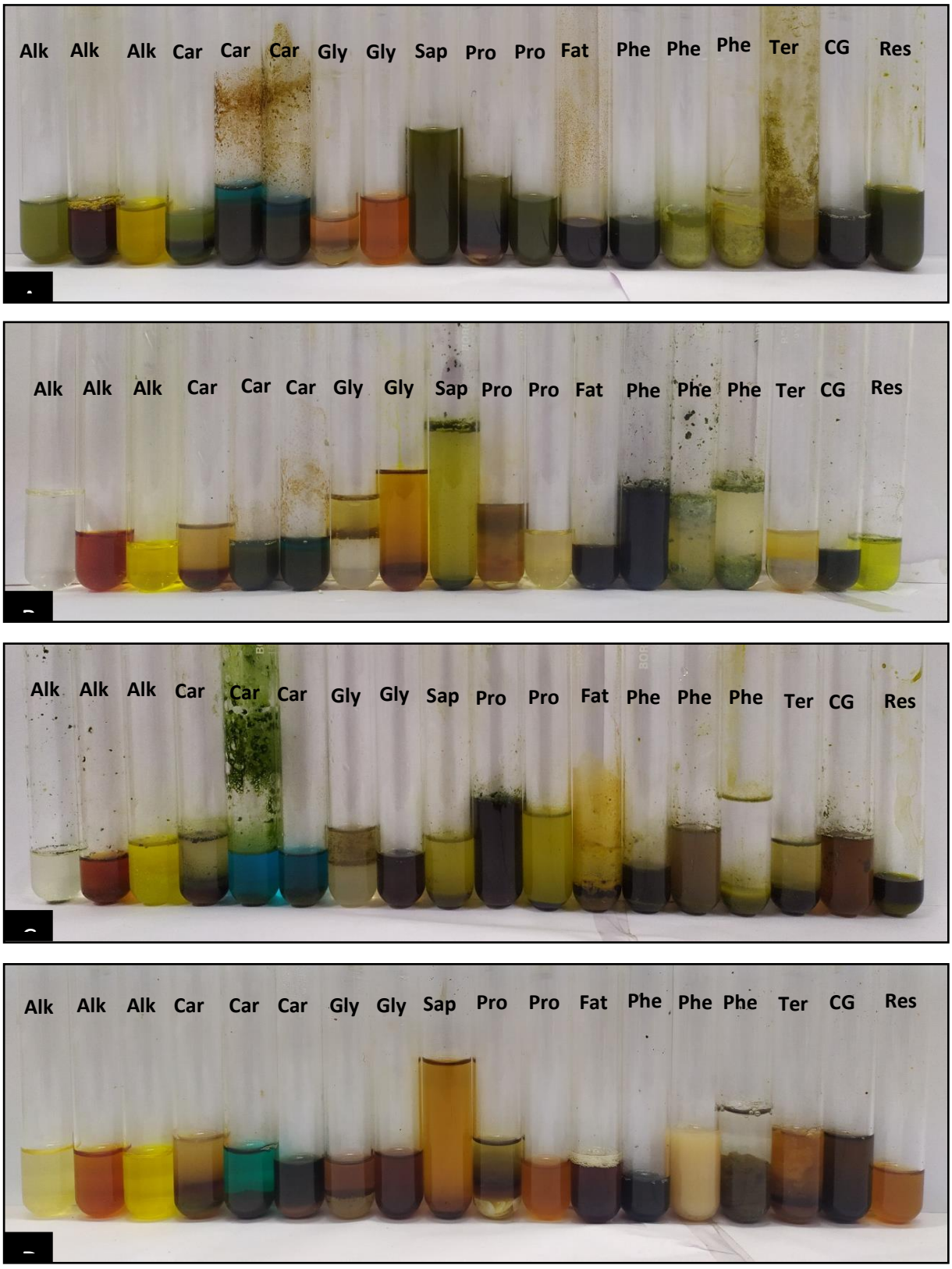


Plate 7. Preliminary qualitative phytochemical analysis of *Terminalia bellirica* leaves with: A) Methanol, B) Ethyl acetate, C) Chloroform, D) Distilled water. Alk=Alkaloids, Car=Carbohydrates, Gly=Glycoside, Sap=Saponin, Pro=Proteins, Fat=Fats & oils, Phe=Phenolics & tannins, Ter=Terpenoids, CG=Cardiac glycosides, Res=Resins.

Table 6. Phytochemical analysis of *Terminalia elliptica* (leaves)

Sr. No.	Phytochemical Test	Methanol	Ethyl acetate	Chloroform	Distilled Water
1	**Detection of Alkaloids**				
a.	**Mayer's test**	+	-	+	-
b.	**Wagner's test**	+	+	+	+
c.	**Hager's test**	++	+	++	+
2	**Detection of Carbohydrates**				
a.	Molish's Test	++	+	++	++
b.	Barfoed's Test	+	+	+	+
c.	Benedict's Test	+	+	+	+
3	**Detection of Glycosides**				
a.	Borntrager's Test	-	-	-	-
b.	Legal's Test	-	-	-	-
4	**Detection of Saponin**	++	+	+	++
5	**Detection of Proteins**				
a.	**Biuret Test**	+	+	+	+
b.	**Ninhydrin Test**	-	-	-	-
6	**Detection of Fats & Oil**				
a.	Saponification	-	-	-	+
7	**Detection of Phenolic & Tannins**				
a.	Ferric chloride Test	++	+	+	+
b.	Gelatin Test	++	+	+	+
c.	Lead acetate Test	++	+	+	+
8	**Terpenoids**	-	-	-	+
9	**Cardiac glycoside**	-	-	-	-
10	**Resins**	+	-	-	-

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

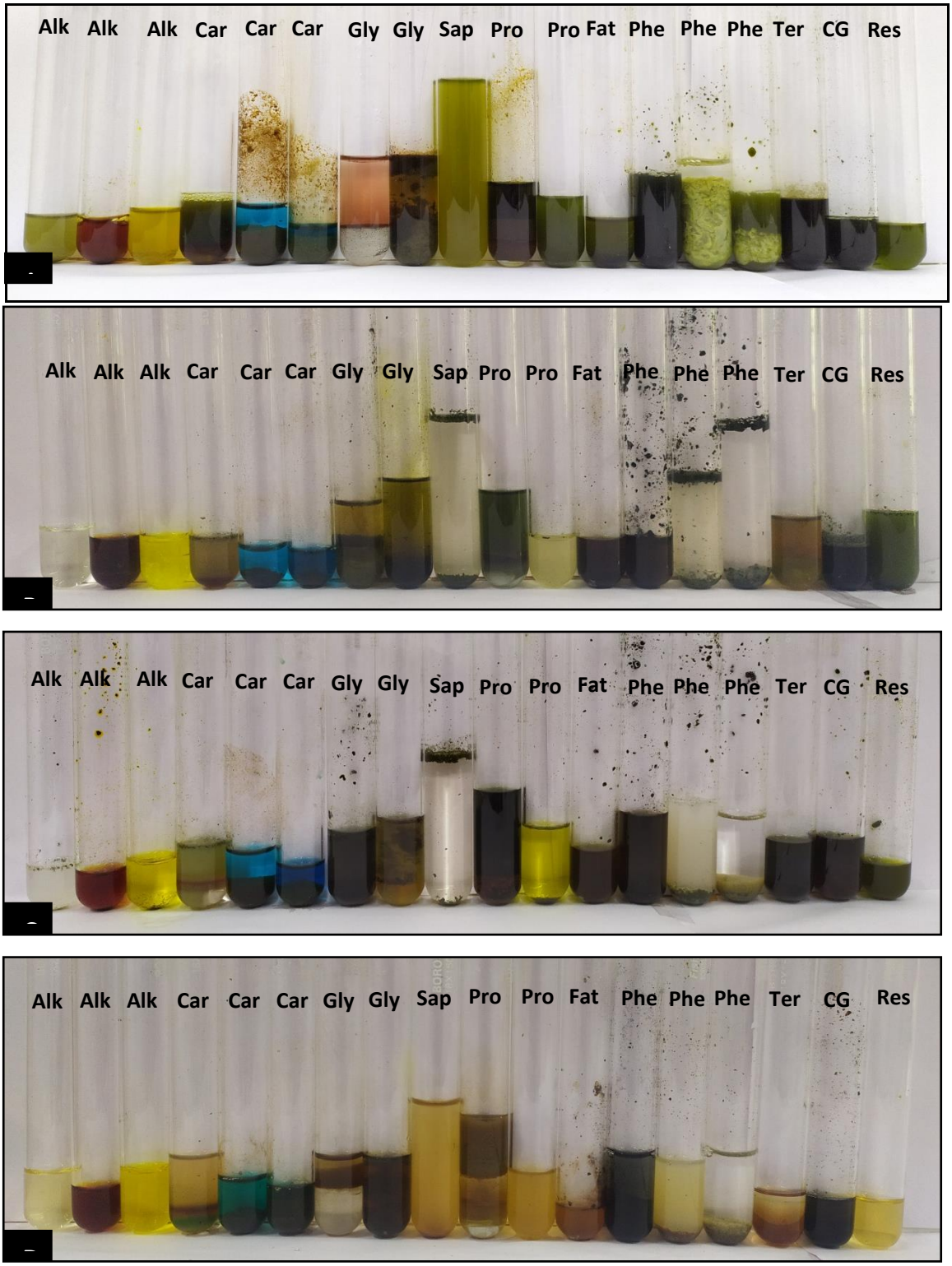


Plate 8. Preliminary qualitative phytochemical analysis of *Terminalia elliptica* leaves with: A) Methanol, B) Ethyl acetate, C) Chloroform, D) Distilled water. Alk=Alkaloids, Car=Carbohydrates, Gly=Glycoside, Sap=Saponin, Pro=Proteins, Fat=Fats & oils, Phe=Phenolics & tannins, Ter=Terpenoids, CG=Cardiac glycosides, Res=Resins.

Table 7. Phytochemical analysis of *Terminalia paniculata* (leaves)

Sr. No.	Phytochemical Test	Methanol	Ethyl acetate	Chloroform	Distilled Water
1	**Detection of Alkaloids**				
a.	Mayer's test	+	+	-	-
b.	Wagner's test	+	+	-	-
c.	Hager's test	+	+	+	+
2	**Detection of Carbohydrates**				
a.	Molish's Test	++	++	+	++
b.	Barfoed's Test	++	+	-	+
c.	Benedict's Test	++	-	-	+
3	**Detection of Glycosides**				
a.	Borntrager's Test	-	-	-	-
b.	Legal's Test	-	-	-	-
4	**Detection of Saponin**	++	++	+	++
5	**Detection of Proteins**				
a.	Biuret Test	+	-	+	-
b.	Ninhydrin Test	-	-	-	-
6	**Detection of Fats & Oil**				
a.	Saponification	-	-	-	+
7	**Detection of Phenolic & Tannins**				
a.	Ferric chloride Test	++	++	-	++
b.	Gelatin Test	++	-	-	++
c.	Lead acetate Test	++	+	-	++
8	**Terpenoids**	-	+	-	+
9	**Cardiac glycoside**	-	-	-	-
10	**Resins**	+	-	-	-

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

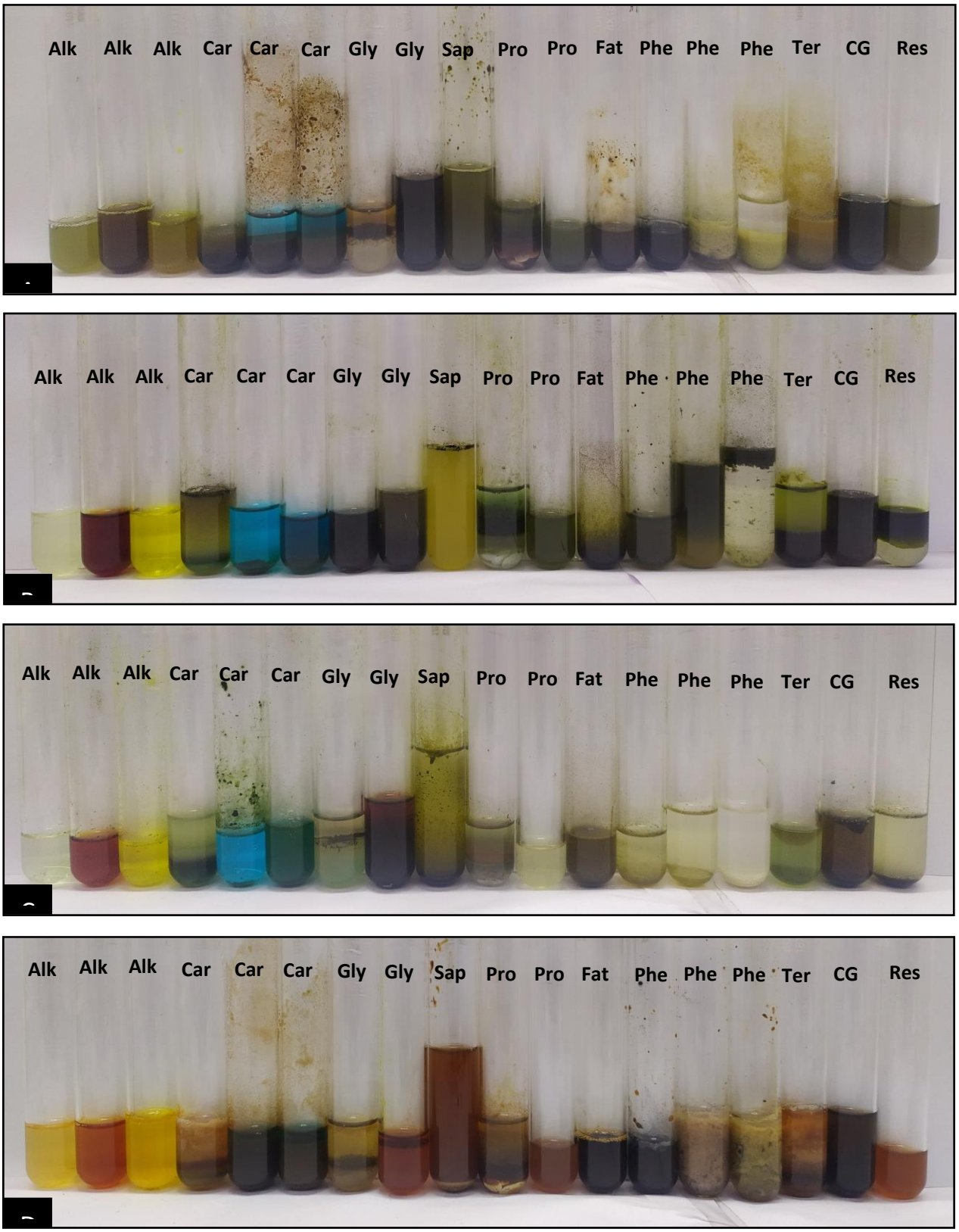


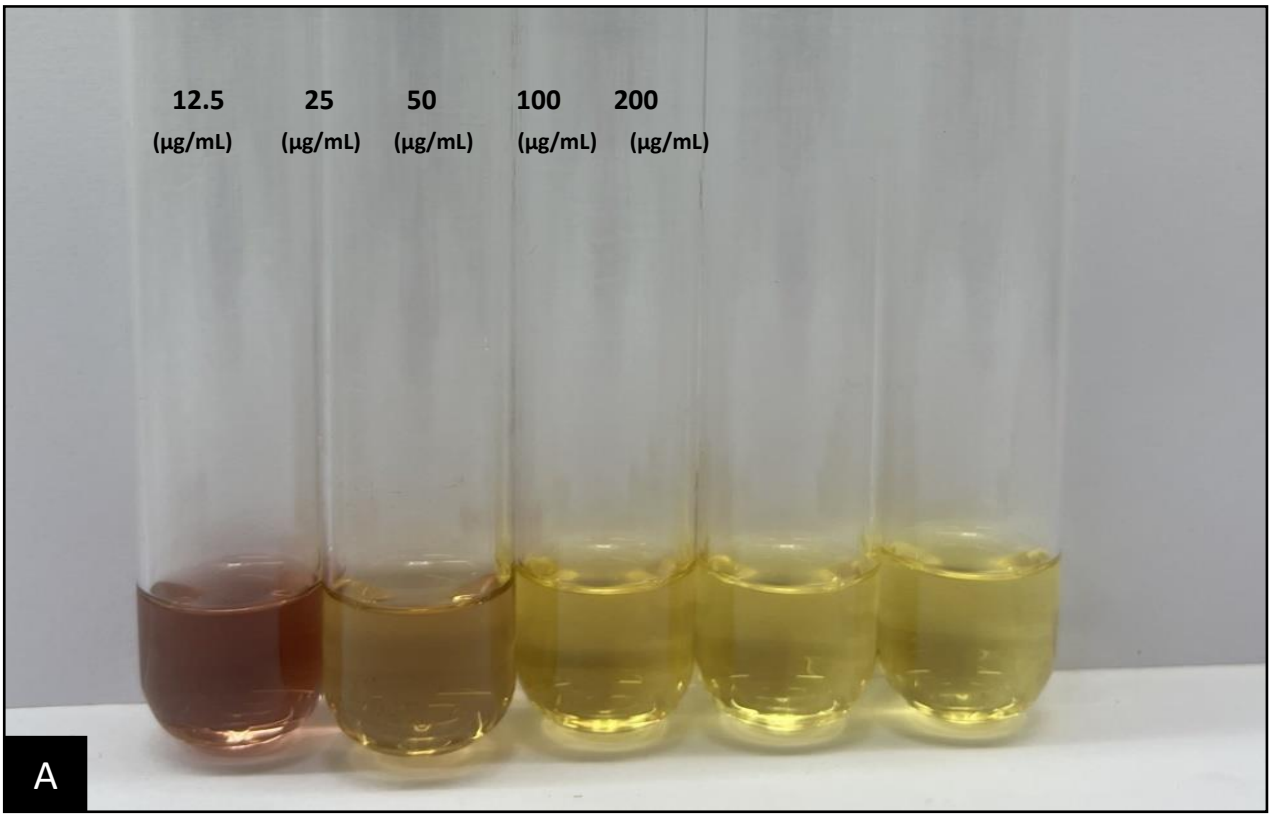
Plate 9. Preliminary qualitative phytochemical analysis of *Terminalia paniculata* leaves with: A) Methanol, B) Ethyl acetate, C) Chloroform, D) Distilled water. Alk=Alkaloids, Car=Carbohydrates, Gly=Glycoside, Sap=Saponin, Pro=Proteins, Fat=Fats & oils, Phe=Phenolics & tannins, Ter=Terpenoids, CG=Cardiac glycosides, Res=Resins.

4.5. Antioxidant studies in selected medicinal plants

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

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

The result showed that all three plants exhibited antioxidant activity. The highest free radical scavenging activity was shown by *T. elliptica* fruits extract with an IC_{50} value of 7.66µg/mL, while the *T. paniculata* fruits extract exhibited the lowest antioxidant activity with IC_{50} value of 66.13µg/mL. L-ascorbic acid was used as a standard to compare the radical scavenging activity of the extract, and its IC_{50} value was 56.6 µg/mL. IC_{50} value represents the concentration at which a substance exerts half of its maximal inhibitory effect. Thus, IC_{50} value is used to represent the effectiveness of an antagonist in inhibiting a specific biochemical process **(Tables 8, 9, 10 and Figures 1, 2, 3 and 4)**.



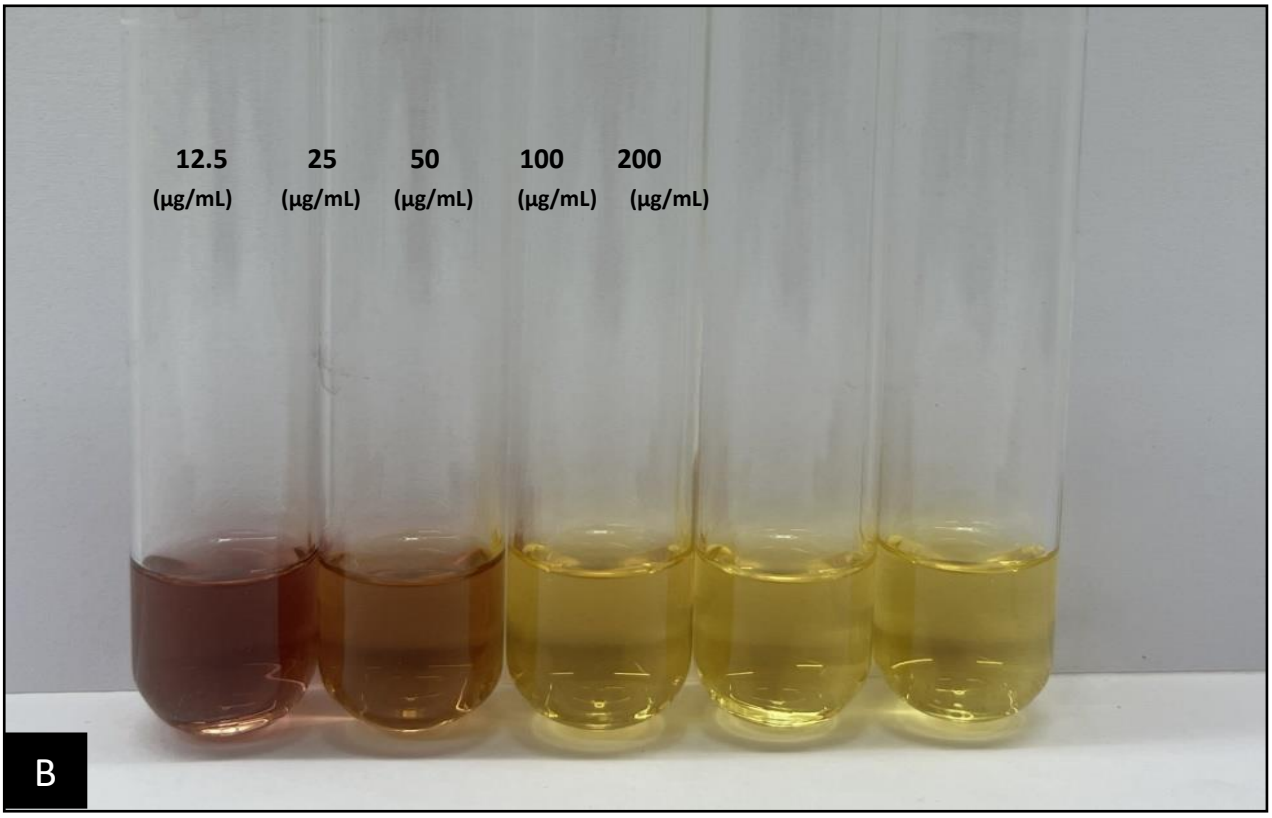


Plate 10. DPPH Radical scavenging activity assay of: (A) Standard L-ascorbic acid, (B) Different dilutions of *T. bellirica* fruit extract.

Table 8: DPPH free radical scavenging assay: % scavenging activity of DPPH by ascorbic acid and methanolic extract of *Terminalia paniculata, Terminalia elliptica, Terminalia bellirica* (leaves)

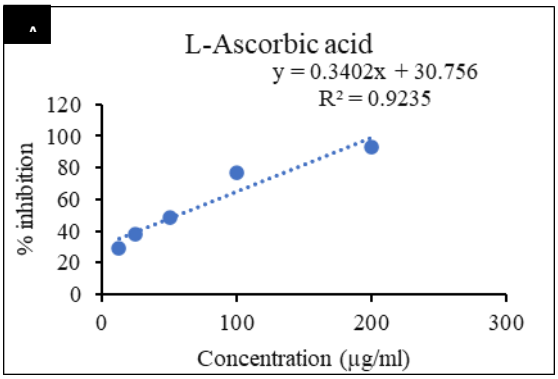
Sr. No.	Concentration (µg/mL)	L-Ascorbic acid	Methanolic extract		
			Terminalia elliptica	***Terminalia paniculata***	***Terminalia bellirica***
1	12.5	29 ± 0.11	30.4 ± 0.01	18.8 ± 0.02	30.4 ± 0.01
2	25	38.2 ± 0.12	45 ± 0.02	27.8 ± 0.11	45 ± 0.02
3	50	48.4 ± 0.01	72.5 ± 0.01	63.1 ± 0.05	72.5 ± 0.02
4	100	77.2 ± 0.08	80.6 ± 0.003	83.7 ± 0.08	80.6 ± 0.01
5	200	93.1 ± 0.002	83.5 ± 0.01	86.3 ± 0.05	83.5 ± 0.002

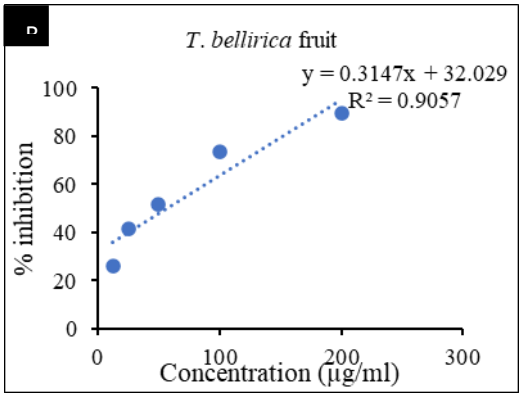
Results are reported as % Inhibition ± standard deviation of triplicate measurement

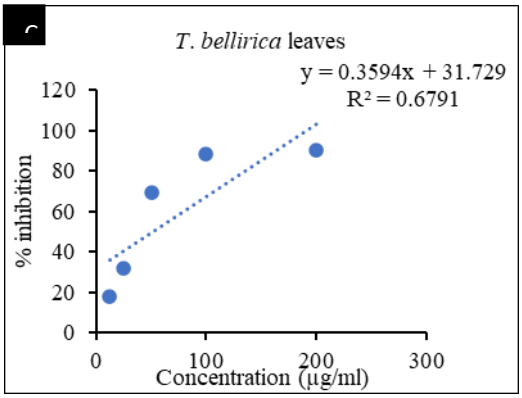
Table 9: DPPH free radical scavenging assay: % scavenging activity of DPPH by ascorbic acid and methanolic extract of *Terminalia paniculata, Terminalia elliptica, Terminalia bellirica* (fruits)

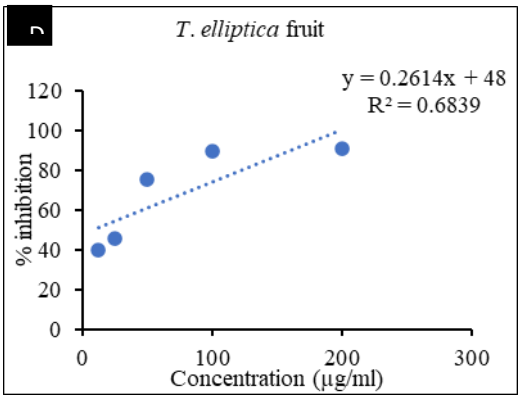
Sr. No.	Concentration (µg/mL)	L-Ascorbic acid	Methanolic extract		
			Terminalia elliptica	***Terminalia paniculata***	***Terminalia bellirica***
1	12.5	29 ± 0.11	39.9 ± 0.004	24.4 ± 0.02	26.2 ± 0.006
2	25	38.2 ± 0.12	45.8 ± 0.01	31.4 ± 0.01	41.5 ± 0.02
3	50	48.4 ± 0.01	75.1 ± 0.009	51.3 ± 0.01	51.5 ± 0.007
4	100	77.2 ± 0.08	89.7 ± 0.006	78.6 ± 0.01	73.4 ± 0.03
5	200	93.1 ± 0.002	90.8 ± 0.003	81.9 ± 0.003	89.5 ± 0.004

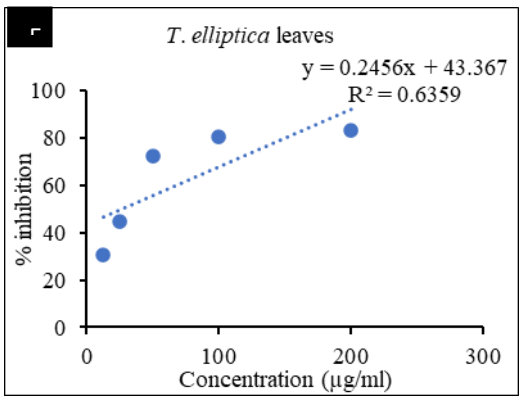
Results are reported as % Inhibition ± standard deviation of triplicate measurement

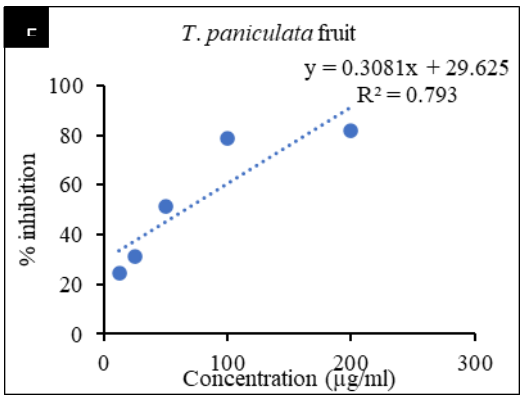












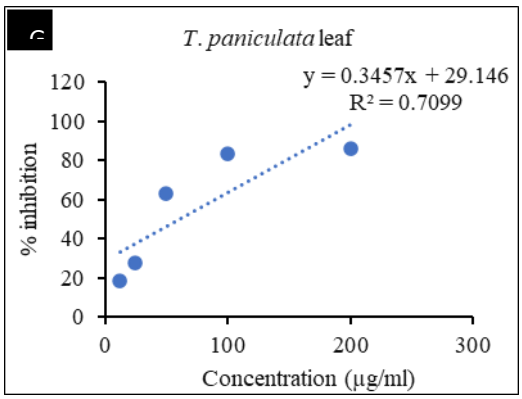


Figure 1. DPPH radical activity of: (A) L-Ascorbic acid, (B) *T. bellirica* fruit, (C) *T. bellirica* leaves, (D) *T. elliptica* fruit, (E) *T. elliptica* leaves, (F) *T. paniculata* fruit, (G) *T. paniculata* leaves.

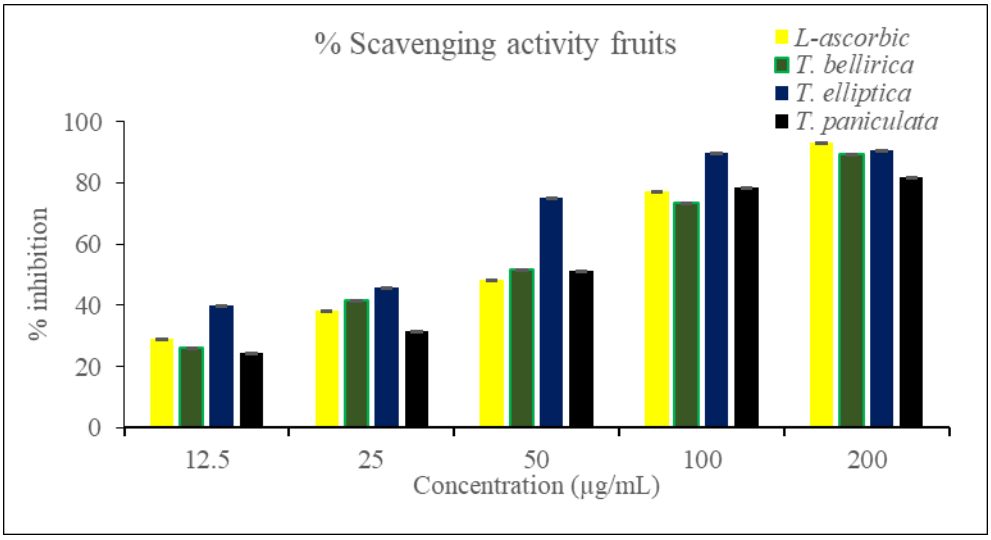


Figure 2. % scavenging activity of L- Ascorbic acid (standard) with fruit extract of *T. bellirica*, *T. elliptica* and *T. paniculata*.

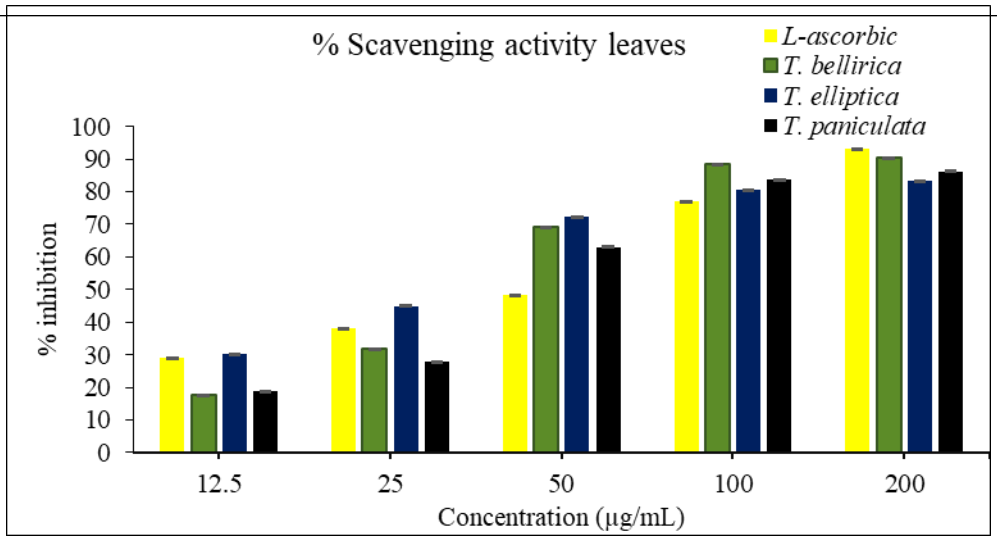


Figure 3. % scavenging activity of L- Ascorbic acid (standard) with leaves extract of *T. bellirica*, *T. elliptica* and *T. paniculata*.

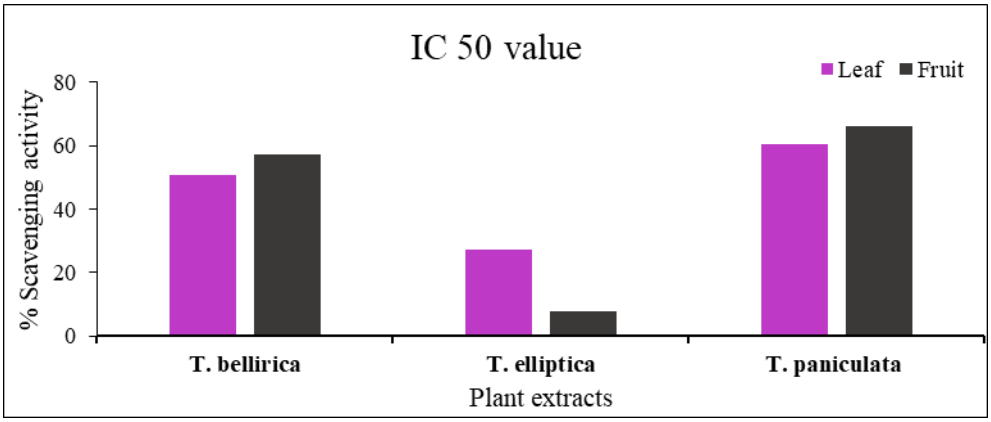


Figure 4. IC 50 value of *T. bellirica*, *T. elliptica* and *T. paniculata* fruits and leaf extracts.

Table 10: IC_{50} values of L-ascorbic acid, *Terminalia paniculata*, *Terminalia elliptica* and *Terminalia bellirica* (leaves and fruits)

Sr. No	Sample	IC_{50} values (µg/mL)
1	L-ascorbic acid	56.6
2	*Terminalia elliptica* (leaves)	27.01
3	*Terminalia elliptica* (fruits)	7.66
4	*Terminalia paniculata* (leaves)	60.32
5	*Terminalia paniculata* (fruits)	66.13
6	*Terminalia bellirica* (leaves)	50.84
7	*Terminalia bellirica* (fruits)	57.1

4.6. Antimicrobial Activity

The antimicrobial studies of crude leaves and fruits extract of *Terminalia paniculata*, *Terminalia elliptica* and *Terminalia bellirica* were evaluated against fungal species such as *Aspergillus niger*, *Aspergillus* sp., *E. coli* using following two methods:

1. Disc diffusion method
2. Agar well diffusion method

In the present study, normal method of disc diffusion where fungus was spread plated and disc soaked in extract were placed on malt extract medium. No - very small zone of inhibition were observed on the plates as well as contaminations were observed, even after complete autoclaving **(Plate 12)**. After, studying all possible methods to carry out the anti-fungal studies, we tested the Agar Well Diffusion method and carried out using malt extract medium in which it showed good antifungal

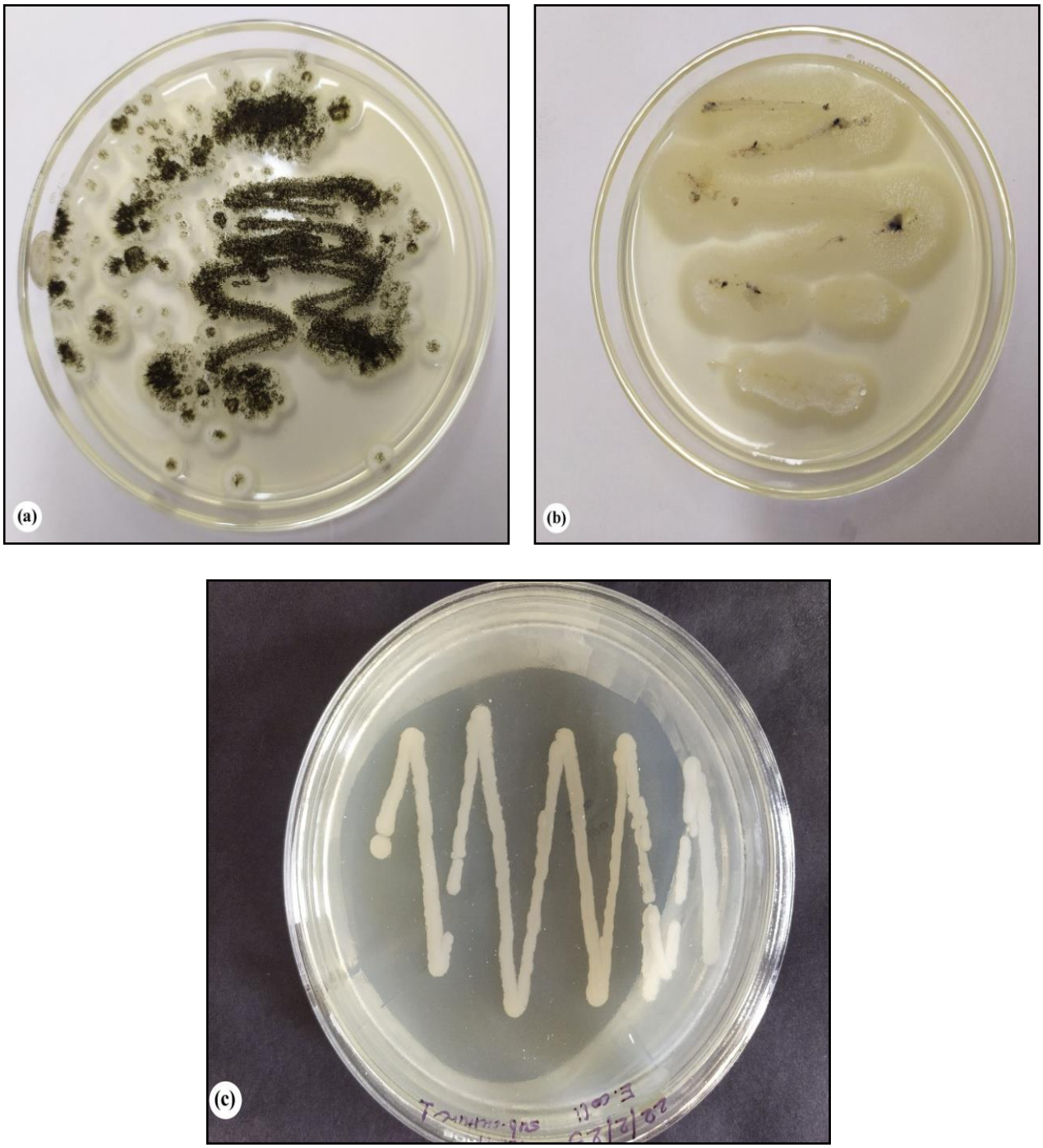


Plate 11. Image of microbial strains used: (a) *Aspergillus niger*, (b) *Aspergillus* sp. (c) *E. coli*.

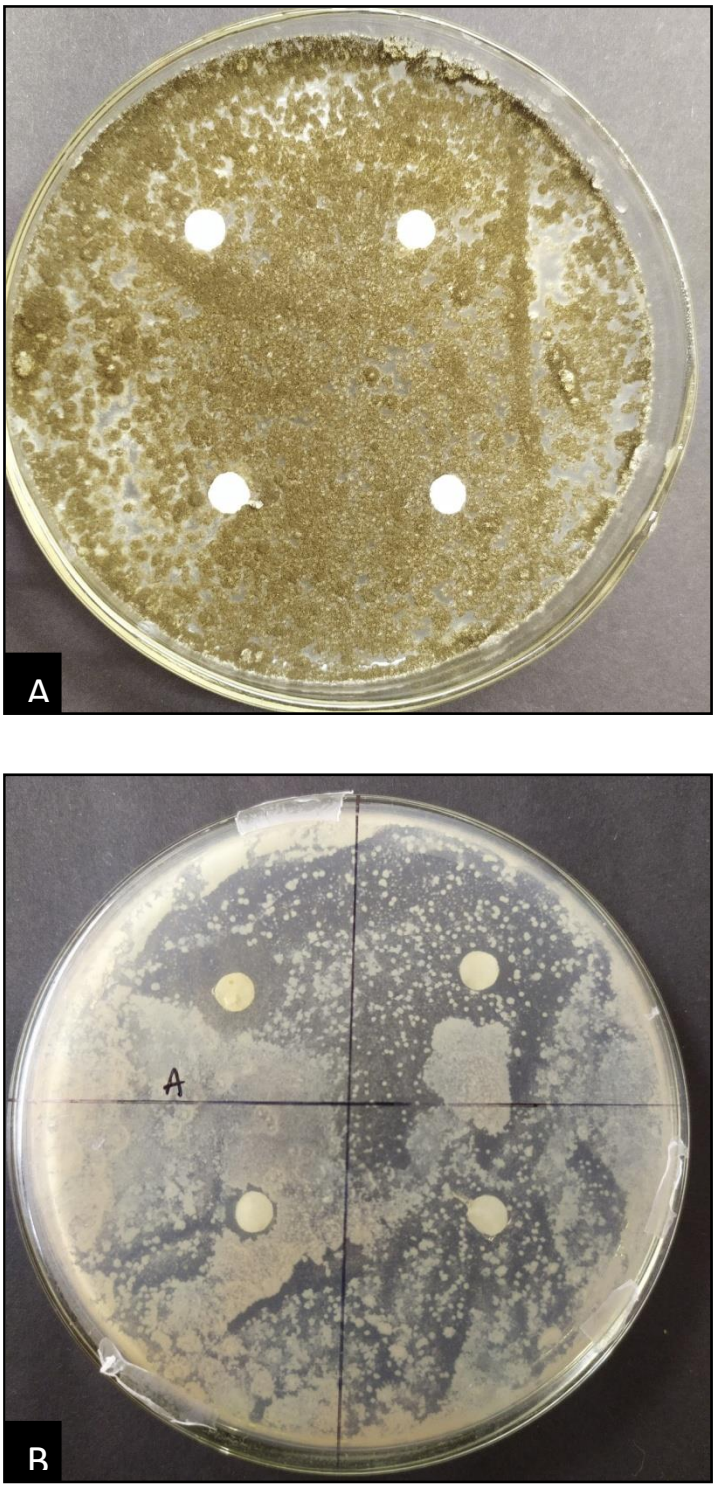


Plate 12. Antifungal activity by Disc diffusion method using leaf extracts of *T. bellirica*, *T. elliptica*, *T. paniculata*: (A) Image showing Negative results against *Aspergillus niger*, (B) Image showing positive result but contamination against *Aspergillus* sp.

activity against fungus **(Plate 13)**. The present work shows that Agar well diffusion method is an excellent method to carry out antifungal activity. It also shows that malt extract agar medium is a suitable medium for fungus and nutrient agar medium is best for bacteria.

Table 11. Antimicrobial activity using agar well diffusion method (leaves)

Sr. No	**Methanol plant extract / positive n negative control**	**Antimicrobial activity**		
		Aspergillus niger	***Aspergillus* sp.**	***E. coli***
1	*T. bellirica*	+	+	+
2	*T. elliptica*	+	+	+
3	*T. paniculata*	+	+	+
4	Positive control	+	+	++
5	Negative control (Methanol)	-	-	-

No inhibition (-), inhibition (+)

The Antimicrobial activity of methanolic extract of *T. elliptica, T. paniculata, T. bellirica* leaves on microbes *Aspergillus niger*, *Aspergillus* sp., *E. coli* strain are shown in Table no.11, Table no.12 and Plate 13. It has been observed that all leaves and fruits samples of methanolic extract of all three plants showed zone of inhibition. No zone of inhibition was observed on negative control plates whereas very good zone of inhibition noticed on positive control plates on all three stains of microbes. Further, three different concentrations (100µl, 200µl, 300µl) were prepared and zone of inhibition was measured in mm. showed in **Table no. 13 and Table no. 14, Plate 14, 15, 16 and Figure 5, 6, 7**.

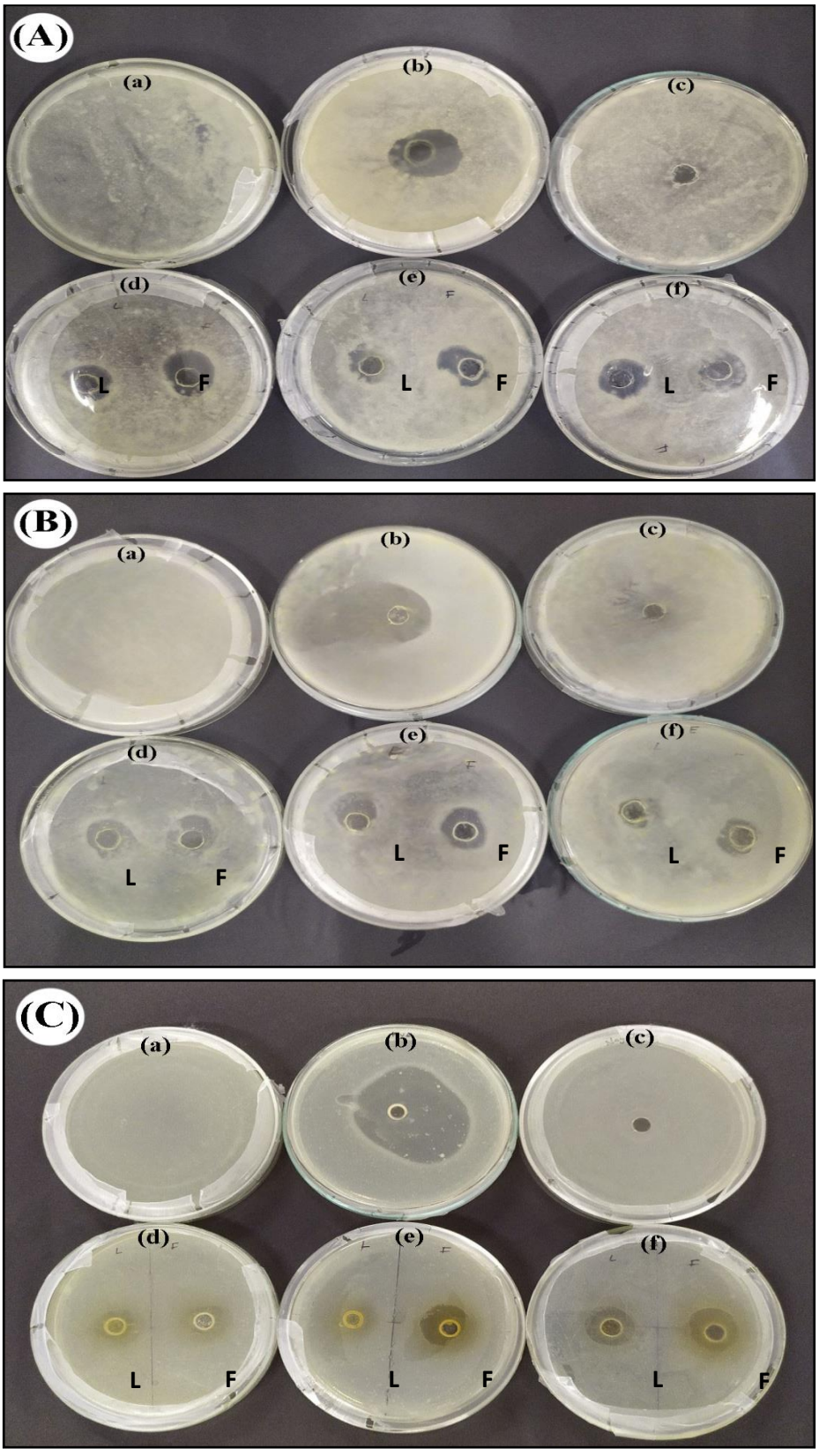


Plate 13. Image showing inhibition zone of antimicrobial activity of 3 *Terminalia* species (leaves and fruit) against: (A) *Aspergillus niger,* (B) *Aspergillus* sp., (C) *E. coli*.

(a) Microbial strain, (b) Positive control, (c) Negative control, (d) *Terminalia bellirica*, (e) *Terminalia elliptica*, (f) *Terminalia paniculata*. L= Leaves, F= Fruit.

Table 12. Antimicrobial activity using Agar well diffusion method (fruits)

Sr. No	Methanol plant extract / positive n negative control	Antimicrobial activity		
		Aspergillus niger	***Aspergillus* sp.**	***E. coli***
1	*T. bellirica*	+	+	+
2	*T. elliptica*	+	+	+
3	*T. paniculata*	+	+	+
4	Positive control	+	+	++
5	Negative control (Methanol)	-	-	-

No inhibition (-), inhibition (+)

Table 13. Antimicrobial activity of *T. elliptica, T. paniculata* and *T. bellirica* leaves using methanol solvent

Sr. No	Microbe	Concentration (µl)	*T. elliptica*	*T. paniculata*	*T. bellirica*
			Zone of inhibition (in mm)		
1.	*Aspergillus niger*	100 µl	0	1	1
		200 µl	3	2	2
		300 µl	6	4	4
		+ve control	5	6	5
2.	*Aspergillus* sp.	100 µl	0	1	0
		200 µl	4	3	2
		300 µl	7	6	5
		+ve control	8	8	8

3.	*E. coli*	100 µl	0	1	1
		200 µl	3	3	2
		300 µl	5	6	5
		+ve control	13	15	13

Table 14. Antimicrobial activity of *T. elliptica, T. paniculata* and *T. bellirica* fruits using methanol solvent

Sr. No	Microbe	Concentration (µl)	*T. elliptica*	*T. paniculata*	*T. bellirica*
			Zone of inhibition (in mm)		
1.	*Aspergillus niger*	100 µl	1	1	1
		200 µl	2	1	2
		300 µl	4	2	5
		+ve control	6	5	6
2.	*Aspergillus* sp.	100 µl	1	1	1
		200 µl	2	3	2
		300 µl	6	4	4
		+ve control	8	9	7
3.	*E. coli*	100 µl	0	0	0
		200 µl	3	3	2
		300 µl	6	6	5
		+ve control	15	13	14

The Antimicrobial activity of crude leaves and fruits extracts of *T. elliptica*, *T. paniculata* and *T. bellirica* were studies using different concentration (100µl, 200µl, 300µl). The leaves and fruits extract of *T. elliptica*, *T. paniculata* and *T. bellirica* was

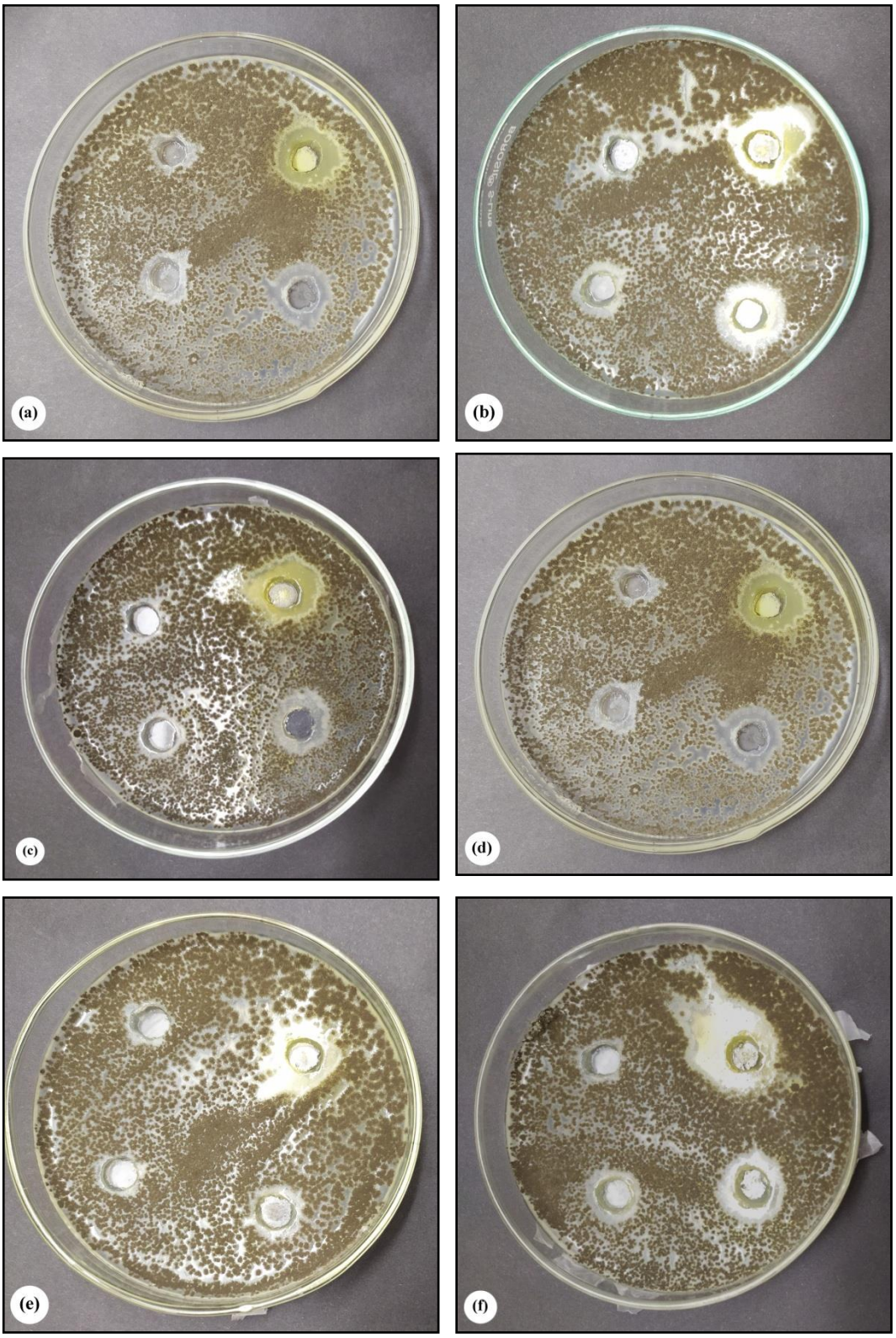


Plate 14. Image showing zone of inhibition with different concentrations (100 µl, 200 µl, 300 µl) on *Aspergillus niger*: (a) *T. bellirica* fruit extracts, (b) *T. bellirica* leaf extracts, (C) *T. elliptica* fruit extracts, (d) *T. elliptica* leaf extracts, (e) *T. paniculata* fruit extracts, (f) *T. paniculata* leaf extracts.

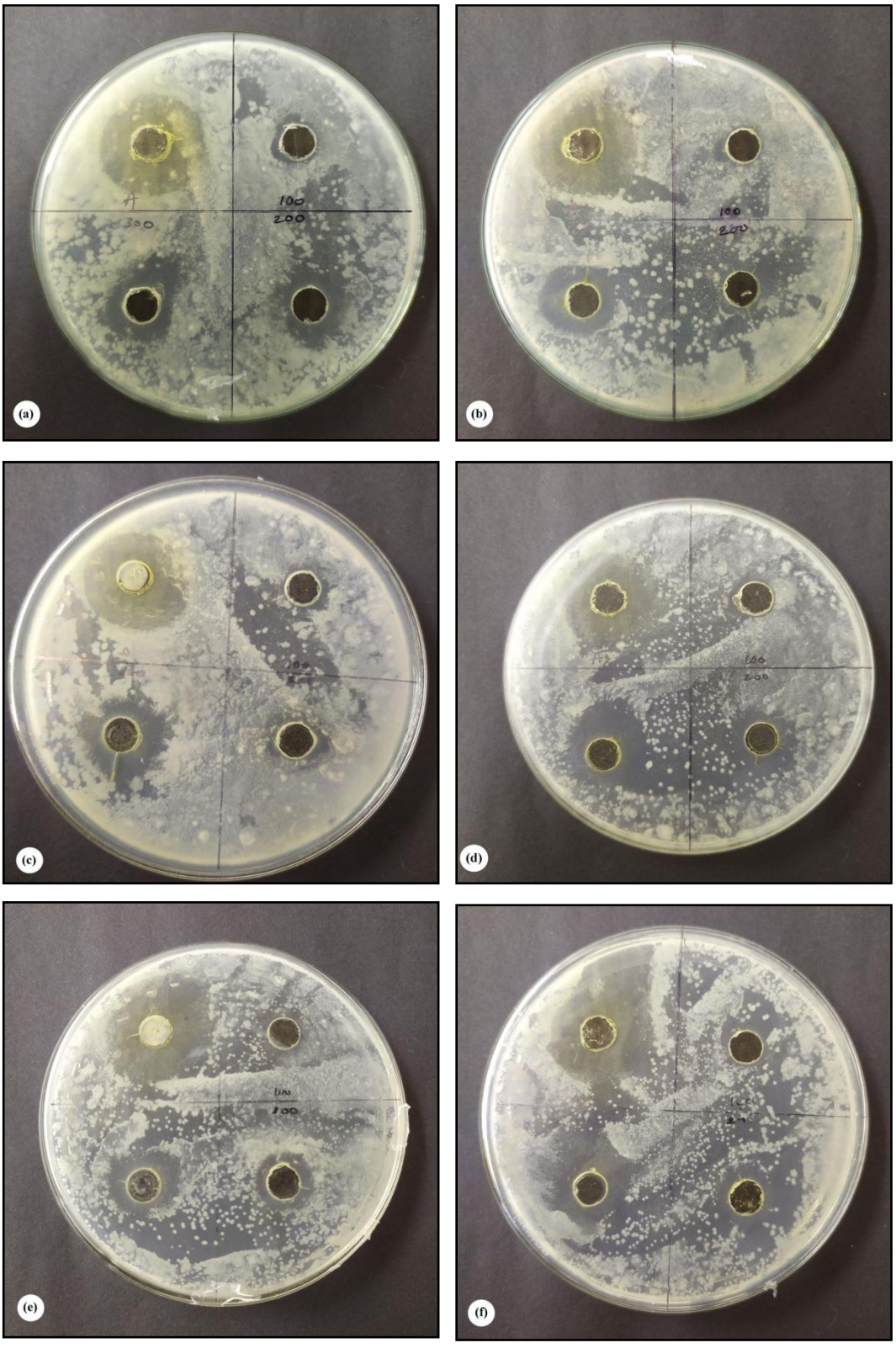


Plate 15. Image showing zone of inhibition with different concentrations (100 µl, 200 µl, 300 µl) on *Aspergillus* sp.: (a) *T. bellirica* fruit extracts, (b) *T. bellirica* leaf extracts, (C) *T. elliptica* fruit extracts, (d) *T. elliptica* leaf extracts, (e) *T. paniculata* fruit extracts, (f) *T. paniculata* leaf extracts.

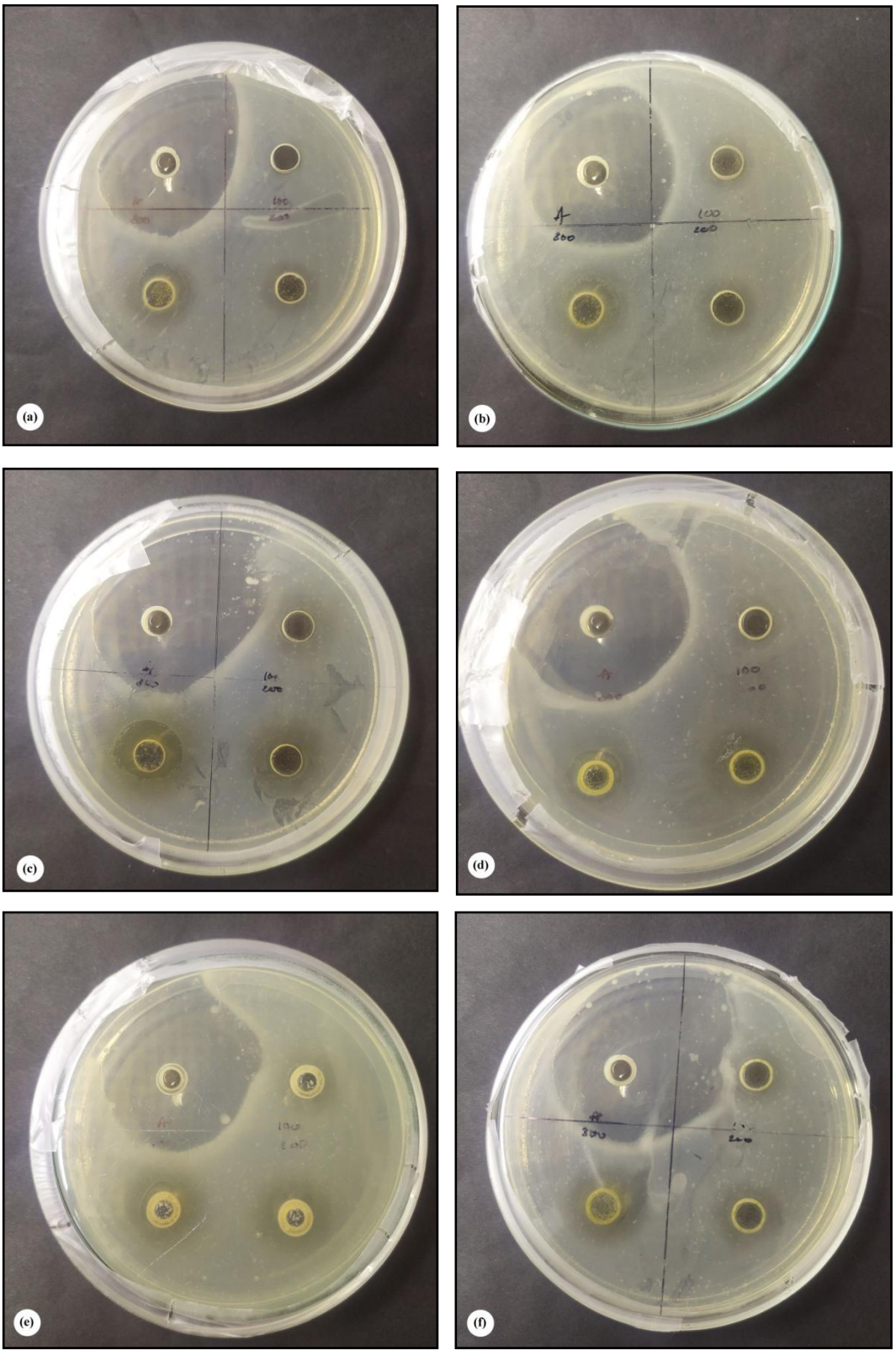


Plate 16. Image showing zone of inhibition with different concentrations (100 µl, 200 µl, 300 µl) on *E. coli*: (a) *T. bellirica* fruit extracts, (b) *T. bellirica* leaf extracts, (C) *T. elliptica* fruit extracts, (d) *T. elliptica* leaf extracts, (e) *T. paniculata* fruit extracts, (f) *T. paniculata* leaf extracts.

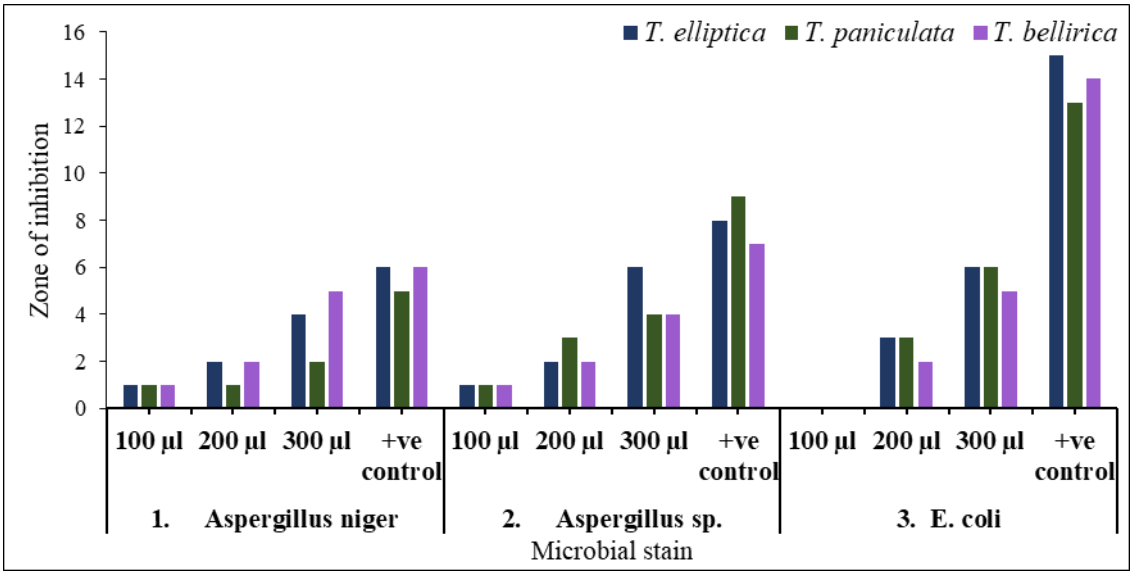


Figure 5. Antimicrobial activity of fruit extracts of *T. bellirica*, *T. elliptica* and *T. paniculata* in comparison with different concentrations on different microbial strains.

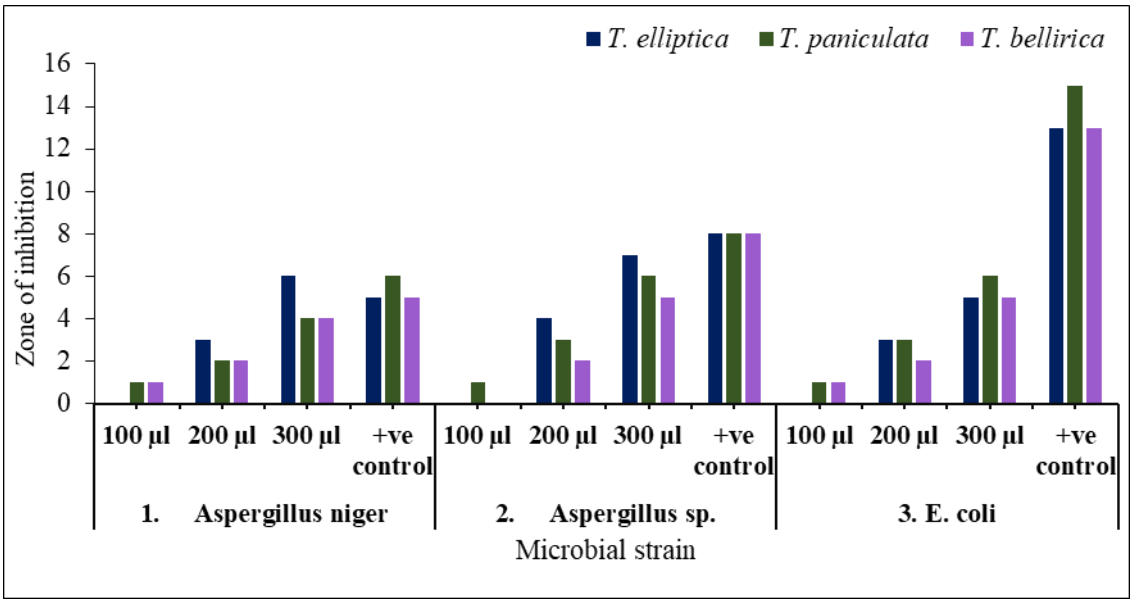
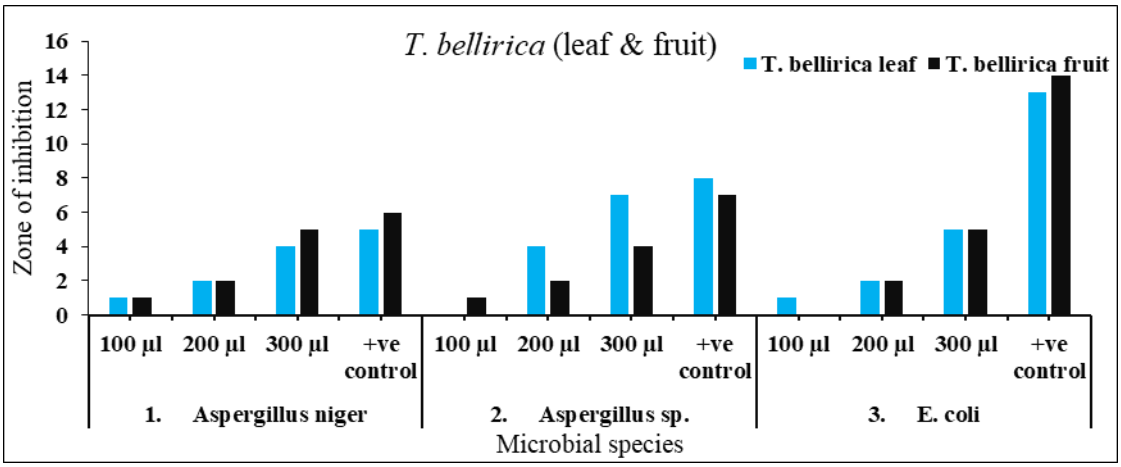
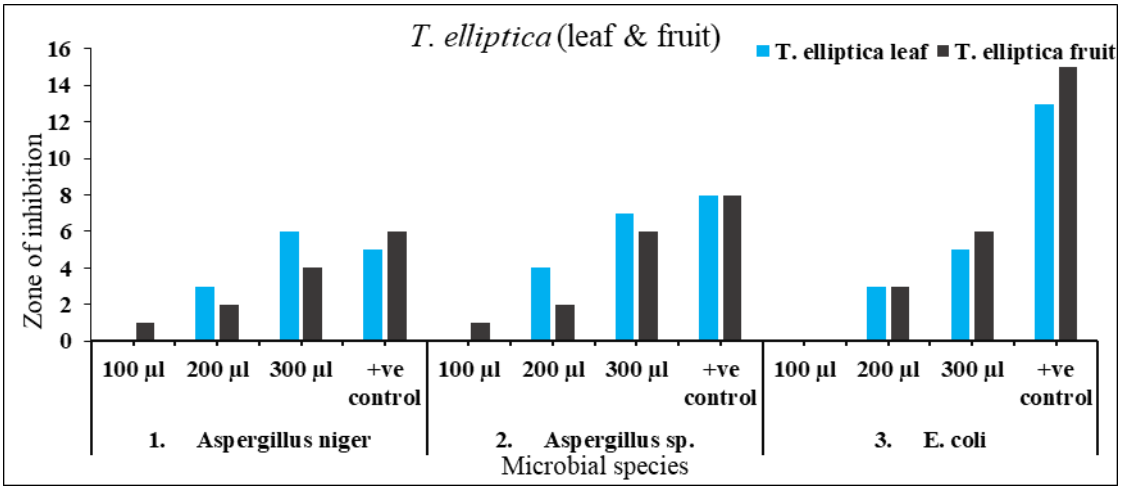


Figure 6. Antimicrobial activity of leaf extracts of *T. bellirica*, *T. elliptica* and *T. paniculata* in comparison with different concentrations on different microbial strains.





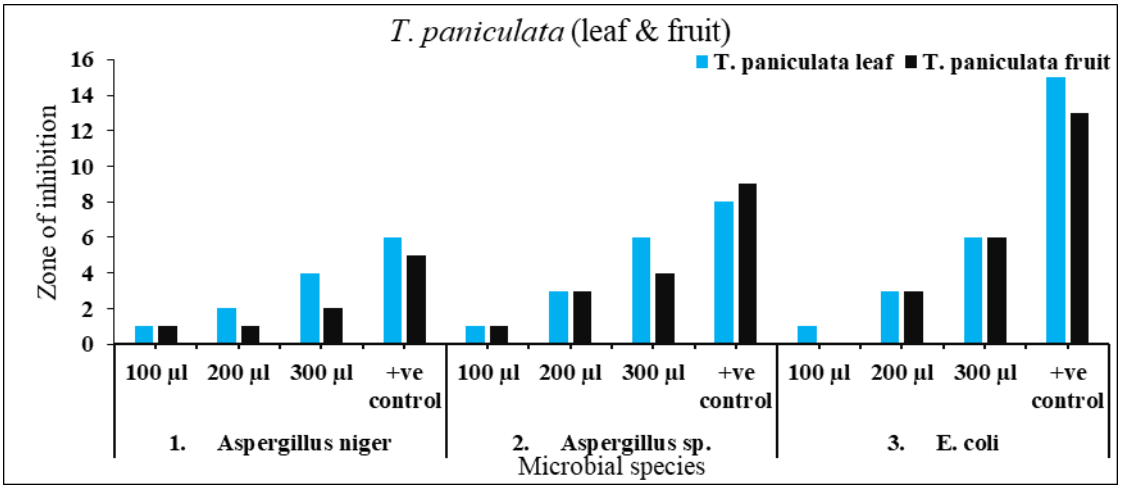


Figure 7. Antimicrobial activity: Bar graph showing comparison of (A) *T. bellirica*, (B) *T. elliptica*, (C) *T. paniculata*, leaf and fruit extracts on different microbial strain at different concentrations.

extracted using methanol and their antimicrobial activity were studied on *Aspergillus niger, Aspergillus* sp., *E. coli.* the result showed that methanol extract of plant species showed higher antimicrobial activity at 300µl concentration as compared to other two concentrations **(Figure 5, 6, 7)**.

4.7. Effect of prepared stain from *Terminalia paniculata* fruits extract on monocot and dicot stem

Prepared stains **(Plate 17)** could stain many regions of the transverse section of both monocot **(Plate 18)** and dicot stem **(Plate 19)** such as cuticle, epidermis, sclerenchyma cells and vasculature region. Section stained with 5% concentration were darker than 1% concentration. Also, as time duration was increased, cells were stained intensely. Stain prepared by crude extract method gave pinkish colour to the section whereas stain prepared directly by dried fruits powder gave brownish colour. It has been noticed that section stained with 5% concentration for 1 hour, showed staining intensity comparable to that of safranine.

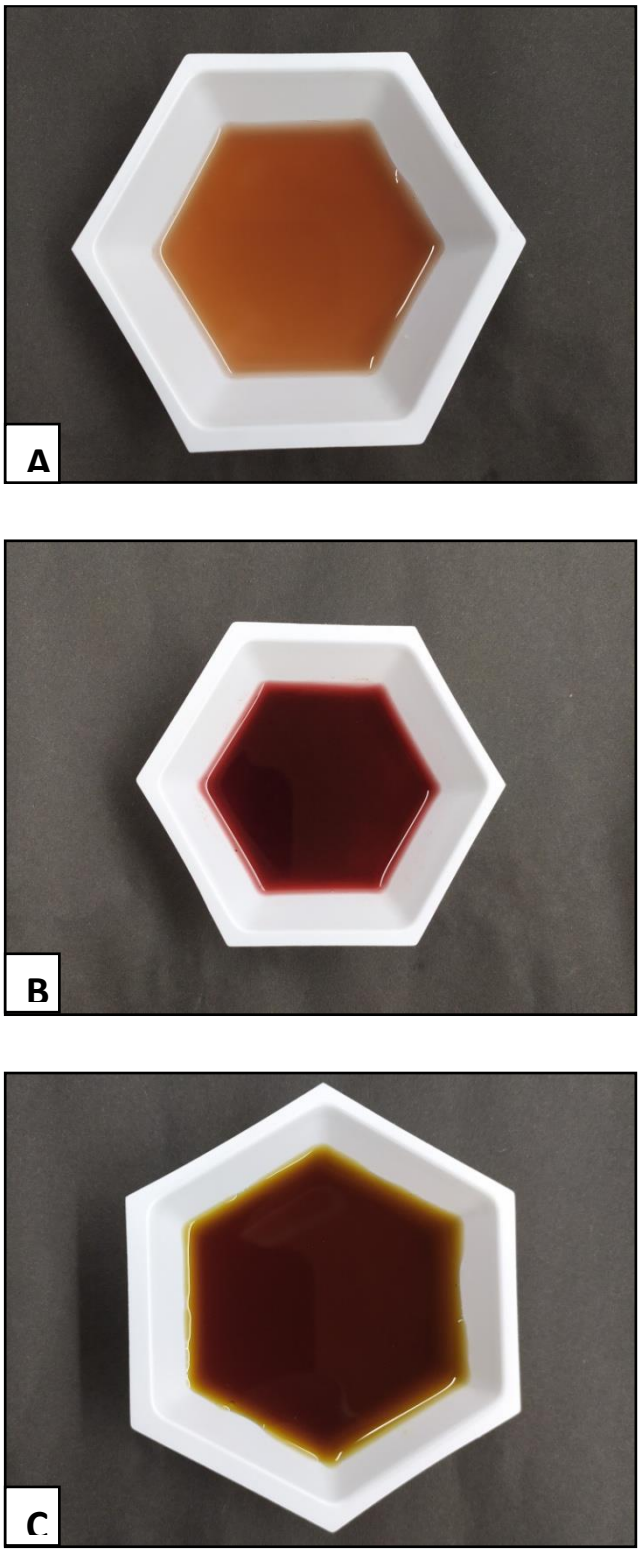


Plate 17. Image showing prepared stain from *T. paniculata* fruit extract: (A) 1% stain prepared from crude extract, (B) 5% stain prepared from crude extract, (C) stain prepared from dried powder.

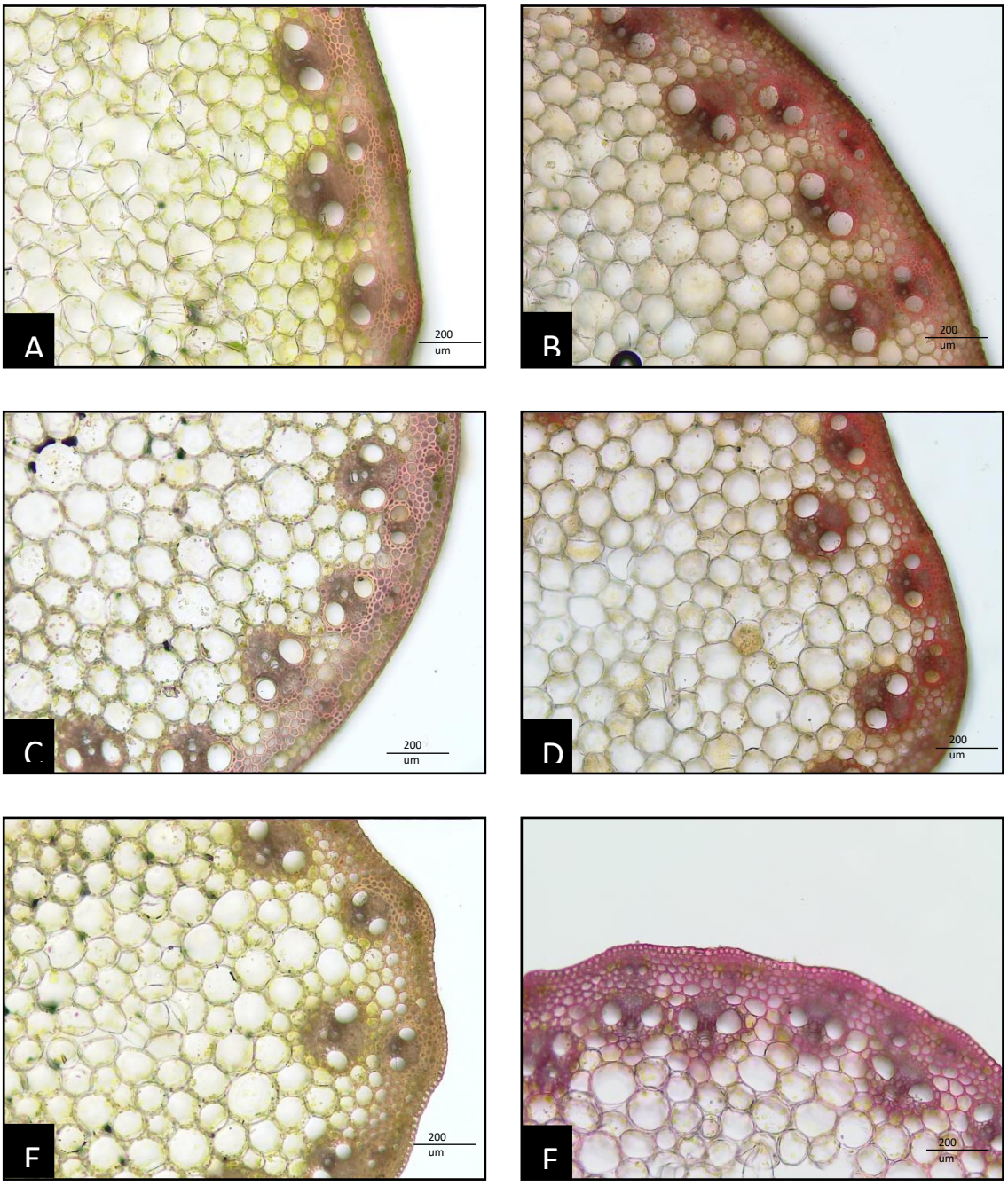


Plate 18. Natural dye staining effect based on time duration on monocot stem. Sections stained with: (A)1% crude extract stained for 30 minutes, (B) 1% crude extract stained for 1 hour, (C) 5% crude extract stained for 30 minutes, (D) 5% crude extract stained for 1 hour, (E) Dried powder extract stained for 1 hour, (F) safranin stained for 10 minutes. Sections showing stained cuticle, epidermal layer, sclerenchyma region and vasculature region.

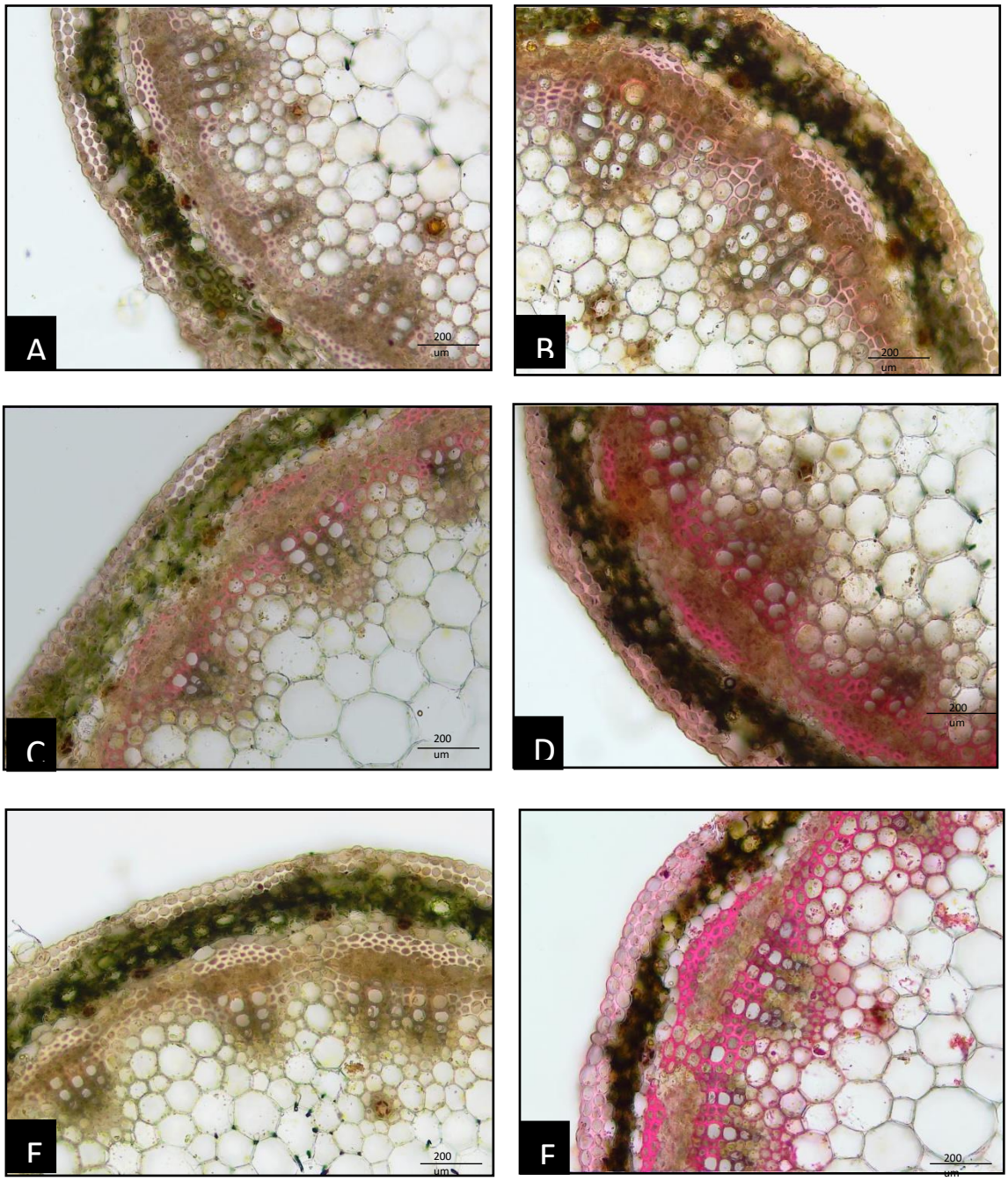


Plate 19. Natural dye staining effect based on time duration on dicot stem. Sections stained with: (A)1% crude extract stained for 30 minutes, (B) 1% crude extract stained for 1 hour, (C) 5% crude extract stained for 30 minutes, (D) 5% crude extract stained for 1 hour, (E) Dried powder extract stained for 1 hour, (F) safranin stained for 10 minutes. Sections showing stained cuticle, epidermal layer, sclerenchyma region and vasculature region.

CONCLUSION

Study on the anatomical characterization of leaf and petiole of the three medicinal plants viz, *Terminalia bellirica*, *Terminalia elliptica* and *Terminalia paniculata* showed presence of anatomical features such as cuticle, epidermis, palisade cells, parenchymatous cortex and vasculature surrounded by sclerenchyma patches apart from unique characters like presence of unicellular and multicellular trichomes, secretory canals and druses. The leaf anatomy of all three species revealed a dorsiventral structure. Trichomes were observed in T. *bellirica* and *T. paniculata*. Palisade region was divided into two parts namely compactly arranged palisade parenchyma on adaxial region and spongy parenchyma in abaxial region. In *T. paniculata* and *T. bellirica* single layer of palisade parenchyma was observed whereas three layers of palisade parenchyma were seen in *T. elliptica*. Cortical region of petiole ranged from 10-18 layers in *T. bellirica*, 13- 22 layers in *T. paniculata* and 15- 20 layers in *T. elliptica*. Petiole was circular with slightly winged at corners and wavy in nature. Arc-shaped vasculature was observed in petioles of *T. elliptica* and *T. bellirica* whereas vasculature was heart shaped in *T. paniculata*. Druses were evenly distributed in cortex and palisade region.

The present study on the preliminary qualitative phytochemical analysis of selected three species revealed the presences primary and secondary metabolites in leaves. Phytochemical tests done using maceration method revealed the presence of alkaloids, carbohydrates, saponin, proteins, oils, phenols, tannins and terpenoids. Three standard tests were carried out to detect the alkaloids namely Mayer's test, Wagner's test and Hager's test. For Mayer's test *T. bellirica* and *T. elliptica* gave positive result in methanol and chloroform whereas negative result in ethyl acetate and distilled water; *T. paniculata* showed positive result in methanol and ethyl acetate and

negative result in the other two solvents. Wagner's test showed positive result for *T. elliptica* in all solvents. For Wagner's test *T. bellirica* and *T. paniculata* showed presence of alkaloid in methanol and ethyl acetate on other hand showed negative result in chloroform and distilled water extract. It was observed that all extracts of three species showed presence of alkaloids with Hager's test.

Carbohydrates, saponin, tannins and phenolic compounds were intensely present in all three species whereas glucosides were absent in all three species. Detection of protein was performed by two tests, wherein Biuret test gave positive result in three plants and Ninhydrin test showed no result. All three species showed presence of terpenoids and oil in distilled water extract. Methanol showed presence of resins in all plants. Overall, methanol being a polar solvent, gave better results for the phytochemical analysis.

IC_{50} value of Free radical scavenging activity exhibited by *T. elliptica* fruits extract was 7.66 µg/mL, while leaf extract was 27.01 µg/mL. It revealed that *T. elliptica* leaves have higher antioxidant as compared to fruits. IC_{50} value of Free radical scavenging activity shown by *T. paniculata* fruits and leaf extract were 66.13 µg/mL and 60.32 µg/mL respectively. Values obtained delineates that *T. paniculata* leaves have higher antioxidant activity as compared to fruits. *Terminalia bellirica* IC_{50} value for fruits and leaves were 57.1 µg/mL and 50.84 µg/mL respectively, which showed that leaf extracts yield higher antioxidant activity as compared to fruits. L-ascorbic acid with IC_{50} value 56.6 µg/mL, was used as a standard to compare the radical scavenging activity of the extract. Overall, data shows that *T. elliptica* fruits exhibited highest antioxidant activity. Thus, it can be concluded that among the three plants studied, *T. elliptica* possesses more medicinal properties than *T. paniculata* and *T. bellirica*.

Antibiotic tetracycline hydroxide was used as positive control for fungus and ampicillin for bacteria. Absolute methanol was used as negative control. Positive control and plant extract showed zone of inhibition over microbes whereas no zone of inhibition was observed in negative control. The antimicrobial activity shows that Agar well diffusion method is an excellent method to carry out antifungal activity. It was observed that malt extract agar is a suitable medium for fungal growth and nutrient agar medium is best for bacterial growth. The study shows that antimicrobial activity is possessed by all three species of *Terminalia*. Higher zone of inhibition was observed in 300 µl concentration as compared to 100 µl, 200 µl concentration which means higher the concentration of sample more zone of inhibition.

Study on comparative antimicrobial activity with leaves and fruits showed variation in zone of inhibition. *T. paniculata* and *T. elliptica* leaf extracts showed higher zone of inhibition for fungal strain as compared to fruit extract. In *T. bellirica,* leaf extracts showed higher zone of inhibition on *Aspergillus niger* whereas fruit extract showed higher zone of inhibition for *Aspergillus* sp. For bacteria *T. elliptica* showed higher zone of inhibition for fruit extract as compared to leaves whereas in *T. paniculata* and *T. bellirica* both leaves and fruit extract showed equal zone of inhibition.

The study shows that *T. paniculata* fruits extract can be used as natural stain for simple anatomical characterisation. It is observed that prepared stain is viable only for two weeks and it is recommended to use freshly prepared stain. Tests performed in this study supports the literature review wherein antioxidant and antimicrobial properties were reported in all three species. *T. elliptica* being endemic to western ghats need to be protected and conserved. Further future study can be done towards

isolation of bioactive compounds from different parts of plant for pharmaceutical industry and micropropagation of these species.

SUMMARY

Genus *Terminalia* belonging to the family Combretaceae, comprises around 300 species distributed in tropical regions of the world. Bark, leaves and fruits are reported to possess medicinal properties and are used in preparation of many drugs. Also, fruits of some species are edible.

The present study aimed at comparative characterization of anatomy, qualitative phytochemistry, antioxidant as well as antimicrobial activity of leaves and fruits and preparation of a stain from one fruit. In this study leaves and fruit of three species were used namely *Terminalia bellirica*, *Terminalia paniculata* and *Terminalia elliptica*. The specimens were collected from different parts of Western Ghats of Goa. For anatomical study leaves and petiole of all three species were used. For phytochemistry, crude extract of leaves was used with four different solvents (methanol, ethyl acetate, chloroform and distilled water). Antioxidant and antimicrobial analysis were done using methanol extracts of leaves and fruits of all three plants, wherein two fungal strain (*Aspergillus niger* and *Aspergillus* sp.) and one bacterial strain (*E. coli*) were used. During the study it was observed that crude extract of *Terminalia paniculata* fruit yields a maroon-red colour, therefore stains of 1% and 5% concentrations were prepared from crude extract to study their staining properties on monocot and dicot stem.

Anatomical characters for leaves and petiole were observed by taking free hand sections, stained with 1% safranin, mounted with glycerol and observed under bright field microscope. Anatomical characters like trichome, cuticle thickness, palisade layers, number of druses and secretory canals varied among different species. In *Terminalia bellirica* unicellular trichome was observed whereas in *Terminalia*

paniculata multicellular trichome was seen however trichomes were absent in *Terminalia elliptica* leaves. Features including single layered epidermis, thick cuticle and two palisade layers were common in all three species. The transverse section of petiole in all three species studied observed circular outline with slightly winged at corner and wavy in nature. Ach-shaped vasculature was seen in *T. bellirica* and *T. paniculata* whereas heart shaped vasculature was seen in *T. elliptica*. In all parts examined presence of large number of druses and secretory cavities embedded in different tissues was noted. *T. elliptica* showed the highest number of druses and secretory cavities.

Phytochemical analysis indicated the presence of different primary and secondary metabolites in all four solvents. The result showed the presence of alkaloids, carbohydrates, saponins, proteins, tannins, phenols, oils, terpenoids, resins in all the species. It was observed that methanol extract has better extractive yield and all metabolites showed intense results in the same, followed by ethyl acetate and water. Least extractive yield was observed in chloroform extract. The phytochemical analysis revealed that the plant exhibits secondary metabolites, which means selected species have medicinal properties. Hence it shows that selected species has drug sources capability and can aid in treatment of various ailments.

The antioxidant studies were carried out for the leaves and fruit methanolic extract of all three species using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Both plant parts of all three species exhibited antioxidant activity. L-ascorbic acid was used as a standard to compare the radical scavenging activity of the extract, and its IC_{50} value was 56.6µg/mL. The highest free radical scavenging activity was exhibited by *T. elliptica* fruit extract with an IC_{50} value of 7.66µg/mL, while *T. paniculata* fruit extract exhibited the lowest antioxidant activity with IC_{50} value of 66.13µg/mL.

In antifungal studies, two methods were tried viz. disc diffusion method and agar well diffusion method wherein agar well diffusion method was found to be more effective and the same was adopted for evaluation of antifungal activity of three species of *Terminalia* using two plant parts. Further the three concentrations of extract were prepared (100µL, 200µL, 300µL) to evaluate zone of inhibition of all species with different concentrations of extract on selected microbes. 300µl concentration showed more zone of inhibition as compared to other two concentrations. Overall result indicated that both parts of all three species shows same zone of inhibition. The antifungal activity of all three species at 300µl was found to be equivalent to that of standard drug tetracycline hydroxide. This indicates that selected *Terminalia* species have high potential to inhibit fungi. Natural stain prepared during this study showed staining properties comparable to that of artificial stain. Prepared stain is capable for simple anatomical characterisation of various plant tissue.

REFERENCES

Acharyya, S., & Prasenjit Bhuniya, A. S. (2019). Evaluation of antimicrobial and anthelmintic activity of roots of *Terminalia paniculata*. Pharma. Innovation, 8, 1065-1068.

Akinsulire, O. P., Oladipo, O. T., Illoh, H. C., & Akinloye, A. J. (2018). Structure, distribution and taxonomic significance of leaf and petiole anatomical characters in five species of *Terminalia* (L.) (Combretaceae: Magnoliopsida). *Brazilian Journal of Biological Sciences*, *5*(10), 515-528.

Chauhan, N.S. 1999. Medicinal and Aromatic Plants of Himachal Pradesh. Indus Publishing Company, New Delhi

Cock, I. E. (2015). The medicinal properties and phytochemistry of plants of the genus *Terminalia* (Combretaceae). *Inflammo pharmacology*, *23*(5), 203-229.

CSIR, D. K. (1948). The wealth of India. *Council of Scientific and Industrial Research, New Delhi*.

Deb, A., Barua, S., & Das, B. (2016). Pharmacological activities of Baheda (*Terminalia bellirica*): a review. *Journal of pharmacognosy and phytochemistry*, *5*(1), 194.

Duraipandiyan, V., Ayyanar, M., & Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC complementary and alternative medicine*, *6*, 1-7.

Fahmy, N. M., Al-Sayed, E., & Singab, A. N. (2015). Genus *Terminalia*: A phytochemical and biological review. *Montin.) species. Med Aromat Plants*, *4*(5), 1-22.

Fahmy, N. M., Al-Sayed, E., & Singab, A. N. (2015). Genus *Terminalia*: A phytochemical and biological review. *Montin.) species. Med Aromat Plants*, *4*(5), 1-22.

Gangwar, A. K., & Ghosh, A. K. (2016). PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF '*TERMINALIA PANICULATA*'BARK.

Gershenzon J, Ullah C. Plants protect themselves from herbivores by optimizing the distribution of chemical defences. Proc Natl Acad Sci U S A. 2022 Jan 25;119(4):e2120277119. doi: 10.1073/pnas.2120277119. Epub 2022 Jan 20. PMID: 35084361; PMCID: PMC8794845.

Gupta, A., Kumar, R., Kumar, S., & Pandey, A. K. (2017). Pharmacological aspects of *Terminalia bellirica*. *Molecular Biology and Pharmacognosy of Beneficial Plants (Eds. AA Mahdi, M. Abid, MMAA Khan, MI Ansari, RK Maheshwari), Lenin Media Private Limited: Delhi, India*, 52-64.

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine*, *27*(1), 1-93.

Halliwell B (1995). How to characterize an antioxidant: an update. Biochem. Soc. Symp. 61: 73-101.

Jeyaprakash, K., Ayyanar, M., Geetha, K. N., & Sekar, T. (2011). Traditional uses of medicinal plants among the tribal people in Theni District (Western Ghats), Southern India. *Asian Pacific Journal of Tropical Biomedicine*, *1*(1), S20-S25.

Jigna, P., Rathish, N., & Sumitra, C. (2005). Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. *Indian journal of pharmacology*, *37*(6), 408-409.

Joshi, A. B., Aswathi, M., & Bhobe, M. (2013). *Terminalia tomentosa* Roxb (ex DC) Wight & Arn.: Phytochemical investigation. *Amer J Adv Drug Deliv*, *1*, 224-231.

Joshi, A. B., Bhobe, M., & Babu, A. (2013). Physicochemical and Phytochemical Investigation of Stem Bark of *Terminalia tomentosa* roxb (ex dc) Wight and

Arn. *International Journal of Advances in pharmacy, Biology and Chemistry, IJAPBC*, *2*(3).

Kadian, R., Parle, M., & Yadav, M. (2014). Therapeutic potential and phytopharmacology of *Terminalia bellirica*. *World journal of pharmacy and pharmaceutical sciences*, *3*(10), 804-819.

Kala, C.P., Dhyani, P.P. and Sajwan, B.S. 2006. Developing the medicinal plant sector in north India: challenges and opportunities. J. ethnobiology and ethnomedicine.

Kannan, P., Ramadevi, S. R., & Waheeta, H. (2009). Antibacterial activity of *Terminalia chebula* fruit extract. African Journal of Microbiology Research, 3(4), 180-184.

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of food science and technology*, *48*, 412-422.

Kumar, S., & Mathur, A. (2009). Localization of metabolites and enzymes in stem galls of *Terminalia arjuna*. *Asian Journal of Experimental Science*, *23*, 207-213.

Machumi, F., Midiwo, J. O., Jacob, M. R., Khan, S. I., Tekwani, B. L., Zhang, J., ... & Muhammad, I. (2013). Phytochemical, antimicrobial and antiplasmodial investigations of *Terminalia brownii*. *Natural product communications*, *8*(6), 1934578X1300800619.

Maridass, M. (2010). Survey of phytochemical diversity of secondary metabolism in selected wild medicinal plants. *Ethnobotanical leaflets*, *2010*(5), 8.

Mathew S, Abraham TE (2006). In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. Food Chem. Toxicol. 44: 198-206.

Moralita Chatri, Civil E. M., Des M. 2019. Characteristics of Leaves Anatomy of some *Syzigium* (Myrtaceae). Advances in Biological Sciences Research.

Pandey, B. P. (2001). *Plant anatomy*. S. Chand Publishing.

Parekh, J., & Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, *10*(2).

Pattanaik, C., & Sudhakar Reddy, C. (2008). Medicinal plant wealth of local communities in Kuldiha Wildlife Sanctuary, Orissa, India. *Journal of herbs, spices & medicinal plants*, *14*(3-4), 175-184.

Pfundstein, B., El Desouky, S. K., Hull, W. E., Haubner, R., Erben, G., & Owen, R. W. (2010). Polyphenolic compounds in the fruits of Egyptian medicinal plants (*Terminalia bellirica*, *Terminalia chebula* and *Terminalia horrida*): characterization, quantitation, and determination of antioxidant capacities. *Phytochemistry*, *71*(10), 1132-1148.

Prior, R. L., & Cao, G. (2000). Antioxidant phytochemicals in fruits and vegetables: diet and health implications. *HortScience*, *35*(4), 588-592.

Rice-Evans, C. (2004). Flavonoids and isoflavones: absorption, metabolism, and bioactivity. *Free Radical Biology and Medicine*, *36*(7), 827-828.

Sabnis, S. (2014). Antimicrobial efficacy of *Terminalia bellirica* against virulence factors of respiratory pathogens. *Int. J Curr. Microbiol & Appl Sci*, *3*, 215-21.

Saxena, V. A. S. U. N. D. H. A. R. A., Mishra, G., Saxena, A., & Vishwakarma, K. R. (2013). A comparative study on quantitative estimation of tannins in *Terminalia chebula*, *Terminalia bellirica*, *Terminalia arjuna* and *Saraca indica* using spectrophotometer. *Asian Journal of Pharmaceutical and Clinical Research*, *6*(3), 148-149.

Scartezzini, P., & Speroni, E. (2000). Review on some plants of Indian traditional medicine with antioxidant activity. *Journal of ethnopharmacology*, *71*(1-2), 23-43.

Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. *International Journal of Herbal Medicine*, *4*(4), 59-64.

Sharma, H., & Kumar, A. (2011). Ethnobotanical studies on medicinal plants of Rajasthan (India): A review. *Journal of Medicinal plants research*, *5*(7), 1107-1112.

Sharma, P., Kumar, P., Sharma, R., Gupta, G., & Chaudhary, A. (2017). Immunomodulators: Role of medicinal plants in immune system. *National Journal of Physiology, Pharmacy and Pharmacology*, *7*(6), 552.

Srinivasan, R., Dhanalekshmi, U. M., Gowri, T., & Duarah, S. (2016). *Terminalia paniculata* bark extract for antidiabetic activity. *International Journal of Pharmaceutical Sciences and Research*, *7*(3), 1331.

Sumathi, P., & Parvathi, A. (2010). Antimicrobial activity of some traditional medicinal plants. *Journal of Medicinal plants research*, *4*(4), 316-321.

Vashist, H., & Jindal, A. (2012). Antimicrobial activities of medicinal plants–Review. *Int J Res Pharm Biomed Sci*, *3*(1), 222-230.

Wright, M. H., Courtney, R., Greene, A. C., & Cock, I. E. (2016). Growth Inhibitory Activity of Indian *Terminalia* sp. against the Zoonotic Bacterium *Bacillus anthracis*. *Pharmacognosy Communications*, *6*(1).

Wright, M. H., Jean Arnold, M. S., Lee, C. J., Courtney, R., Greene, A. C., & Cock, I. E. (2016). Qualitative phytochemical analysis and antibacterial activity evaluation of Indian *Terminalia* sp. against the pharyngitis causing pathogen *Streptococcus pyogenes*. *Pharmacognosy Communications*, *6*(2).

Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of phytology*, *3*(12).

Poster Presented for "National Conference on Recent Trends in Plant Science & Biotechnology" held at Goa University, Goa during 3-4th November 2022

Qualitative Phytochemical Screening of genus *Terminalia* – a medicinal plant

Prachi P. Gaunkar and Krishnan Sellappan*

Botany Discipline, School of Biological Sciences and Biotechnology,
Goa University, Goa-403206, India
*Corresponding author: Krishnan Sellappan; E-mail: skrish@unigoa.ac.in

Genus *Terminalia* is a large deciduous tree belonging to the family Combretaceae. *Terminalia bellerica* (Gaertn.) Roxb, *Terminalia elliptica* (Pancher ex Guillaumin), and *Terminalia paniculata* (Roth) are distributed widely throughout the Indian subcontinent in the semi-evergreen and moist deciduous forest as a medicinal plant. Medicinal plants have been considered valuable and cheap sources of phytoconstituents used extensively in developing various drugs. From ancient times, plant and plant parts are used in traditional medicines like Ayurveda. Traditionally, *T. bellerica* is known for its effect in hoarseness of voice, asthma, bloating, piles, cough, and common cold. Seed oil is beneficial for skin disorders, whereas seed kernels are used to prevent vomiting. *T. paniculata* is used as a remedy for cholera, for treatment of inflamed parotid glands, and the menstrual disorder. *T. elliptica* is useful in pitta, ulcers, vata, wounds, dysentery, etc. The plant has been known to possess various pharmacological activities, such as antifungal, antibacterial, antioxidant, antidiabetic, antiulcer, anticancer, antiviral, and wound healing properties. The present study was undertaken to carry out the phytochemical investigation of the leaves of three *Terminalia* species using four solvents: methanol, ethyl acetate, chloroform, and distilled water using the maceration method. The solvents were chosen based on the polarity indices. Out of all the solvents, methanol showed a positive result and showed the maximum number of phytochemicals. Whereas chloroform being a non-polar solvent, showed a minor effect and least number of phytochemicals. The preliminary phytochemical screening of leaf extract revealed the presence of alkaloids, carbohydrates, protein, phenols, flavonoids, tannins, saponin, cardiac glycoside, and terpenoids. Further analysis of biological activity of the selected species may prove beneficial as alternative medicines in the traditional healthcare system.