Exploring Phytochemical Secrets of *Gymnacranthera canarica*: Conservation and Therapeutic insights

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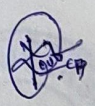

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COMPLETION CERTIFICATE

This is to certify that the dissertation report "Exploring Phytochemical Secrets of *Gymnacranthera canarica*: Conservation and Therapeutic insights" is a bonafide work carried out by Ms. Rachi Naik under my supervision in partial fulfillment of the requirements for the award of degree of M.Sc. in the Discipline of Botany at the School of Biological Sciences and Biotechnology, Goa University.

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PREFACE

In the pursuit of understanding the intricate relationship between nature's bounty and human health, this dissertation endeavours to explore the multifaceted properties of *Gymnacranthera canarica*, a plant species native to diverse ecosystems. The following pages encapsulate a meticulous investigation into the phytochemical composition, antioxidant potential, larvicidal activity, antimicrobial efficacy, and essential oil constituents of *Gymnacranthera canarica*.

At the heart of this research lies a profound curiosity about the hidden treasures within nature's pharmacopeia. *Gymnacranthera canarica*, with its rich botanical heritage, offers a promising avenue for uncovering novel bioactive compounds that may hold the key to addressing contemporary health challenges.

The journey embarked upon in this dissertation begins with a thorough exploration of phytochemical profile of *Gymnacranthera canarica*. Through various extraction methods and analytical techniques, the presence of alkaloids, phenolic compounds, flavonoids, glycosides, and other phytoconstituents is meticulously elucidated. The subsequent chapters delve into the antioxidant and larvicidal activities of *Gymnacranthera canarica* extracts, shedding light on their potential therapeutic applications.

Moreover, this dissertation meticulously investigates the essential oil composition of *Gymnacranthera canarica* leaves, employing advanced chromatographic techniques to identify and characterize its chemical constituents. By unravelling the complex interplay of volatile compounds within the plant's essential oil, we gain valuable insights into its pharmacological properties.

Furthermore, the antimicrobial potential of *Gymnacranthera canarica* extracts and essential oil is rigorously evaluated against pathogenic bacterial strains, offering a glimpse into their efficacy as natural antimicrobial agents. Through comprehensive analysis and discussion, this dissertation seeks to contribute to the growing body of knowledge surrounding medicinal plantsand their diverse therapeutic applications.

As the culmination of rigorous research endeavours, this dissertation stands as a testament to the interdisciplinary collaboration between botanical science, pharmacology, and public health. It is my sincere hope that the insights gleaned from this work will inspire further explorationand foster a deeper appreciation for the remarkable healing potential of nature's botanical treasures.

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Ms. Rachi Mahesh Naik

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ABBREVIATIONS USED

Entity	Abbrevation
Thin Layer Chromatography	TLC
Total Phenolic Content	TPC
Total Flavonoid Content	TFC
1,1-diphenyl-2-picrylhydrazyl	DPPH
Gas Chromatography Mass Spectrometry	GC-MS
Gas Chromatography Flame Ionization Detector	GC-FID
Lethal Concentration 30 (the concentration of a substance required to kill 30% of a population)	LC30
Lethal Concentration 50 (the concentration of a substance required to kill 50% of a population)	LC50
Meter	m
Centimeter	cm
Microgram	µg
Milligrams	mg
Milliliters	mL
Millimeter	mm
Degree Celsius	ºC
Grams	gm
hours	hrs
Number	No
Percent	%
Serial	Sr
Standard error	SE
Volume in volume	v/v
Weight in volume	w/v

ABSTRACT

The present dissertation explores the phytochemical composition and biological activities of Gymnacranthera *canarica*, a plant native to tropical regions. The study investigates various extracts of *G. canarica*, including leaf and bark extracts, along with its essential oil, to elucidate its phytochemical profile and potential bioactivities. ***Introduction:*** *G. canarica* is known for its medicinal properties in traditional medicine systems. However, comprehensive studies on its phytochemical constituents and biological activities are lacking. Therefore, this dissertation aims to fill this gap by conducting a detailed investigation. ***Methodology:*** The methodology involved the extraction of phytochemicals using different solvents like ethanol, methanol, and water. Various qualitative and quantitative analyses, including thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), and spectrophotometric assays, were employed to characterize the phytoconstituents. Antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, while antimicrobial activity was evaluated against selected bacterial strains using the disc diffusion method. Larvicidal activity against *Culex quinquefasciatus* larvae was also determined. ***Results:*** The phytochemical analysis revealed the presence of alkaloids, phenolic compounds, flavonoids, glycosides, and saponins in *G. canarica* extracts. GC-MS analysis identified various bioactive compounds in the essential oil, including Patchoulene, δ-Cadinene, and Aromadendrane. The ethanolic and methanolic extracts exhibited significant antioxidant and antimicrobial activities, with the methanolic extract showing higher phenolic and flavonoid contents. The larvicidal activity against *C. quinquefasciatus* larvae demonstrated promising results, especially with the pure leaf essential oil. ***Conclusion:*** *G. canarica* possesses diverse phytochemicals with significant biological activities. The findings suggest its potential applications in pharmaceutical and agrochemical industries.

CHAPTER 1: INTRODUCTION

1.1 General Overview

Myristica swamps, characterized by their freshwater swamp forest environment dominated by species of Myristica, have garnered significant attention in recent decades. Initially identified as a distinct evergreen forest type by Krishnamurthy in 1960, these swamps were later classified as the most endangered forest ecosystem in India by Rodger and Panwar in 1988. Located in the Western Ghats, these swamps represent a rare and unique ecosystem housing a plethora of rare, relic, threatened, and endemic flora and fauna (Ranganathan et al., 2021). Recognized as a global biodiversity hotspot, Myristica swamps serve as living museums, preserving invaluable biological diversity.

Despite their ecological importance, Myristica swamps face severe threats from various anthropogenic activities. Agricultural encroachment, including shifting cultivation and monoculture plantations, hydropower projects leading to submergence, and escalating mining operations, has resulted in the fragmentation and degradation of these swamps in the Western Ghats. Once part of an extensive network along watercourses within pristine forests, these swamps now exist as fragmented patches (Chandran et al., 2001). Decades ago, Myristica swamps were identified as a special habitat in the Travancore region of the South Indian Western Ghats (Chandran et al., 2001). However, the ongoing threats and degradation have significantly impacted these unique ecosystems, underscoring the urgent need for conservation efforts and further research to understand and mitigate the challenges faced by Myristica swamps. The nutmeg family or Myristicaceae, which was named after the genus Myristica, has its Malay indigenous names derived from the word ‘blood’ or ‘darah’ (pendarah, chendarah, penarah, darahan, chendarahan, pendarahan, penarahan) because of its characteristic red sap, which is found generally in all species worldwide (Sinclair, 1958). The Myristicaceae is a

pantropical family with approximately 370 species worldwide and 19 genera with each being restricted to a single continent. There are five genera in Asia, viz., *Endocomia, Gymnacranthera, Horsfieldia, Myristica, and Knema* (Utteridge & Miliken, 2014).

1.1.1 Study Plant (*Gymnacranthera canarica)*

There are seven species of the genus *Gymnacranthera* Warb. in the Indo-Malayan region. It is a member of the Myristicaceae family (Mabberly, 1987). The endemic and vulnerable *Gymnacranthera canarica* (King) Warb. tree species is only found in the Myristica swamp ecosystems of evergreen forests in the Western Ghats (WCMC 1998).

These trees have evolved knee roots, which have noticeable lenticels on the surface for breathing, in order to survive in the swamp. According to (Chandran et al., 2010), these remnant forests are frequently referred to as old forest patches. Tropical freshwater swamp forests are the classification given to this type of vegetation patch by (Champion & Seth 1968). Swamps form when there are non-biotic factors such as deeply forested hills with deep soil that allows water to be stored above the rock layer, gently sloping or flat-bottomed valleys between heavily forested hills, slow and continuous seepage of water into the valley, and an average of 3,000 mm of rain fall (Chandran et al., 1999; Varghese et al., 1999).

1.1.2. Classification of *Gymnacranthera canarica*

Common name: Kanara Nutmeg
Class: Magnoliopsida
Order: Magnoliales
Family: Myristicaceae
Genus: *Gymnacranthera*
Species: *canarica*

1.1.3. Phytochemical studies

Phytochemicals, naturally occurring compounds found in plants, have gained significant popularity in recent years due to their myriad medicinal benefits. These compounds play a crucial role in combating various diseases such as asthma, arthritis, and cancer. Unlike pharmaceutical chemicals, phytochemicals are known for their lack of side effects, making them "man-friendly medicines" (Banu & Cathrine, 2014).

Plants have long been utilized as a source of medicinal agents, with many modern drugs being derived from plant-based compounds, often based on their traditional uses in indigenous medicine (Cragg & Newman, 2013). The diverse array of bioactive molecules produced by plants makes them a valuable reservoir of different types of medicines (Singh & Srivastava, 2013).

Numerous plants are widely used by tribal communities worldwide, with many believed to possess antibacterial properties according to traditional knowledge. It is now widely acknowledged that nature has provided remedies for various illnesses through plants (Murugan & Saranraj, 2011). As a result, contemporary researchers are increasingly focusing on evaluating and characterizing various plants and their constituents based on their traditional claims in Ayurveda, with the aim of identifying potential treatments for a range of ailments.

Phytochemicals serve as integral components of a plant's defense system, aiding in protection against various diseases (Sabovljevic & Sabovljevic, 2008). These bioactive compounds, which include phenols, flavonoids, alkaloids, glycosides, lignins, and tannins, are predominantly sourced from plants and plant-based products. Among them, flavonoids and phenols are particularly noteworthy for their antioxidant properties, which contribute to the health benefits of fruits, vegetables, and medicinal plants (Scalbert et al., 2005).

Phenolic compounds, classified as secondary metabolites, are synthesized by plants through pathways such as the pentose phosphate, shikimate, and phenylpropanoid pathways. These compounds play essential roles in various physiological processes within plants, including growth, defense, pigmentation, and lignification. With over 8,000 different polyphenolic chemicals identified across numerous plant species, phenolic compounds exhibit diverse structural characteristics. They typically consist of aromatic rings with one or more hydroxyl substituents, and variations in the number of phenol rings and the structural components linking them differentiate different polyphenols. Stilbenes, lignans, flavonoids,and phenolic acids are among the four classes used to categorize phenolic compounds (Bravo, 1998; Salehi et al., 2021).

1.1.3a. Phenolic Acids

Phenolic acids, constituting a significant portion of the polyphenolic compounds in our diet, are ubiquitous in all plant materials. They are characterized by two distinct carbon frameworks: hydroxycinnamic and hydroxybenzoic structures. Among these,hydroxycinnamic acids, including p-coumaric, caffeic, ferulic, and Sinapic acids, are more prevalent than hydroxybenzoic acids (Han et al., 2007). The high redox potential of phenolic acids enables them to serve as both singly oxygen quenchers and reducing agents, imparting antioxidant properties to these compounds (Ignat, 2011).

1.1.3 b. Flavanoids

Flavonoids, constituting the largest class of phenolic compounds with over 3,000 structures, are characterized by an oxygenated heterocycle composed of two aromatic rings

connected by three carbon atoms (Tsao, 2012). Further subdivision into numerous sub-groups, such as anthocyanins, flavan-3-ols, flavones, flavanones, and flavonols, is possible (Merken & Beecher, 2000). Among these, flavones and flavonols are the most prevalent due to differences in the hydroxylation pattern and variations in the chromane ring (Ring C).

Individual variations within each sub-group arise from differences in the quantity and configuration of hydroxyl groups, as well as the degree of glycosylation and/or alkylation (Spencer, Mohsen, Minihane, & Mathers, 2008). Flavonoids contribute to the vibrant hues of flowers, fruits, and mid-leaf foliage, with over 4,000 different types identified. Common examples of flavonoids include epicatechin, myricetin, quercetin, and catechins. (Anusha et al., 2019)

1.1.4. Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC), discovered by (Tswett, 1906), is a chromatographic method used to separate mixtures. It involves using a sheet of glass, plastic, or aluminium foil coated with a thin layer of an adsorbent substance, such as cellulose, silica gel, or aluminium oxide (blotter paper). This layer of adsorbent serves as the stationary phase. Upon application of the sample onto the plate, a solvent or solvent combination (referred to as the mobile phase) is drawn up the plate by capillary action.

The separation occurs as different analytes ascend the TLC plate at varying speeds (Bele & Khale, 2010). TLC is a crucial technique in many investigations for isolating aparticular compound or determining the purity of a mixture. Its popularity stems from its affordability, simplicity, short development time, high sensitivity, and reproducibility, makingit one of the simplest and most versatile methods for separation.

TLC finds applications in various industries and research areas, including pharmaceutical production, clinical analysis, industrial chemistry, environmental toxicity assessment, food chemistry, water analysis, inorganic and pesticide analysis, assessment of dye purity, cosmetics, and analysis of plant materials and herbal products (Santiago & Strobel, 2013). Its wide range of applications underscores its importance as a fundamental tool in analytical chemistry.

1.1.5. Essential Oil

Essential oils, also known as aromatic plant essences, are volatile compounds produced by plants, exhibiting a wide range of colours and fragrances. These oils can be found in various plant organs, including buds, flowers, leaves, stems, seeds, fruits, roots, wood, and bark, where they are stored in specialized structures such as glandular trichomes, cavities, canals, and secretory cells (Patra, 2012). Extracting essential oils from aromatic plants can be achieved through methods like steam or water distillation, solvent extraction, expression under pressure, supercritical fluid, and subcritical water extraction.

Comprising complex mixtures of several substances, essential oils are primarily composed of monoterpenes and sesquiterpenes, which are hydrocarbons with the general formula $(C_5H_8)_n$. These hydrocarbons give rise to various oxygenated compounds, including oxides, alcohols, aldehydes, esters, ethers, ketones, and phenols, resulting in a diverse array of chemical constituents. In addition to monoterpenes and sesquiterpenes, essential oils may contain phenylpropenes and compounds with nitrogen or sulfur (Svoboda & Hampson, 1999).

Since ancient times, aromatic herbs have been valued for their therapeutic properties,

flavor-enhancing abilities, and preservative qualities. Essential oils serve as valuable sourcesof bioactive compounds for industries such as food, cosmetics, pharmaceuticals, and household products. Due to their diverse chemical composition, essential oils exhibit various biological activities, including antiviral, antibacterial, antiparasitic, anti-inflammatory, insecticidal, antifungal, anticarcinogenic, antioxidant, and antimutagenic properties (Hassan et al., 2016). As a result, essential oils have garnered significant attention as natural and safe therapeutic agents.

1.1.6. Volatile Oils as Plant Protection Agents and their Antimicrobial activity

Numerous plants are known to possess volatile oils with antibacterial properties (Deans et al., 1992). This process serves as a chemical defense mechanism against pathogens that infect plants. For instance, pathogens can easily enter wound sites caused by herbivores. When leaves with volatile oil glands are wounded, the glands rupture, causing the oil to flow over the wound. The antimicrobial activity of the oil is therefore beneficial to the plant's defense against pathogens. A majority of aromatic and medicinal plants do not succumb to many common diseases, suggesting the effectiveness of volatile oils in plant protection. Additionally, it is suggested that complex oils present a greater barrier to pathogen adaptation than simplermixtures of monoterpenes. The intricate mixtures of monoterpenes and sesquiterpenes in the whole oil represent the strongest barrier to fungal infection (Piccaglia et al., 1993). The growth of microbes is reduced in several ways by volatile oils, depending on their chemical composition and concentration (Tassou et al., 1995).

1.1.7. GC/MS (Gas chromatography mass spectrometry)

Gas chromatography (GC) and mass spectrometry (MS) complement each other. While GC can separate volatile or semivolatile substances with remarkable resolution, it cannot identify them. On the other hand, MS can provide extensive structural information to precisely identify compounds but struggles with their separation. Therefore, it is not surprising that the two techniques were combined soon after GC was developed in the mid-1950s (Hites, 1997).

Gas chromatographic mass spectrometry (GC-MS) is the most crucial instrument for identifying and quantifying volatile and semivolatile organic chemicals in complex mixtures. It is highly useful in these situations because it can determine the molecular weights and, occasionally, the elemental compositions of unknown organic compounds. GC-MS is commonly employed to quantify contaminants in drinking water and wastewater, making it a cornerstone of approved EPA procedures. Additionally, it is utilized in measuring pharmaceuticals and their metabolites in urine and blood (Hassan et al., 2016).

One of the key advantages of GC-MS is its ability to identify unknown organic substances by interpreting spectra a priori or by comparing spectra with reference spectra. This capability finds regular applications in identifying reaction products by synthetic organic chemists and conducting quality control analyses of industrial products. However, for the organic compounds to be analyzed, they must first be in solution to be injected into the gas chromatograph. Volatile organic solvents such as hexane or dichloromethane are commonly used for this purpose (Hites, 1997).

More than two billion people, primarily in tropical regions, are susceptible to diseases like dengue, filariasis, malaria, and hemorrhagic fever that are spread by mosquitoes (Murugan

et al., 2012). These infectious diseases primarily affect poor populations in tropical areas, transmitted through mosquito bites that draw blood. The three most significant mosquito genera responsible for transmitting diseases to humans are Culex, Aedes, and Anopheles (Azokou et al., 2013).

Despite proven methods to prevent mosquito-borne illnesses, mosquitoes continue to pose a serious threat to public health. This is partly due to global political and economic issues, especially those affecting countries like China and India (WHO, 1989). Mosquito larval habitats, often found in humid areas such as swamps, marshes, standing water around buildings, and irrigation canals, are frequently disturbed. Modifying these larval habitats can reduce the mosquito population and prevent disease spread. However, the World Health Organization advises against using carbamates, parathion, temephos, and organochlorines like DDT and endosulfan due to concerns about environmental impact and mosquito resistance (Ruikar et al., 2012; Chouaibou et al., 2012; Promsiri et al., 2006). In response to these challenges, there is a growing interest in exploring natural plant compounds as alternatives to synthetic pesticides. These compounds are environmentally friendly and biodegradable, offering a safer option for pest control. Plant-based repellents have been used since ancient times to deter mosquitoes(Pani et al., 2015).

1.1.8. Antimicrobial Property

Since the beginning of human civilization, plants have produced a wide variety of phytochemicals, many of which have been utilized by humans for their therapeutic benefits. Since the development of powerful and efficient synthetic antimicrobial agents, microbial infections can now be prevented and treated using them. However, the emergence of bacterial strains resistant to antibiotics, the decline in the effectiveness and safety of antimicrobials, and

the hunt for novel antimicrobials to combat the emergence of incurable diseases by conventional antimicrobial agents have rekindled interest in the investigation of phytochemicals as a synthetic antimicrobial compound substitute (Patra, 2012). Essential oils (EOs) have long been recognized for their antibacterial and antifungal properties, making them widely used in the food and medical industries. As interest in natural compounds grows, the scientific community is actively exploring new applications and uses for these substances. In vitro studies on EOs and their constituents have shown promising activity against various food- borne pathogens and spoilage microbes, indicating their potential as alternative antimicrobial agents (Bassole & Juliani, 2012).

1.2 AIMS AND OBJECTIVES

The dissertation aims to reveal an extensive repertoire of chemical constituents across leaves, bark, and seeds of *Gymnacranthera canarica*, uncovering variations and concentrations of diverse compounds, offering a comprehensive understanding of its chemical makeup. Bioactive fractions from the plant parts and essential oils are expected to yield potential therapeutic agents, shedding light on compounds with antimicrobial, antioxidant properties, contributing to pharmaceutical research.

- **Objective 1:** To unveil the hidden chemical repertoire of *Gymnacranthera canarica*, by meticulously analyzing its diverse chemical constituents across leaves, bark, and seeds, unveiling their variations and concentrations.

- **Objective 2**: To decipher the secrets within the essential oil, exploring its nuanced compounds and their potential industrial or medicinal applications.

- **Objective 3**: To isolate and characterize promising bioactive fractions, delving into their potential as agents against infections, or oxidative stress, using modern

analytical techniques to unravel its therapeutic potentials.

1.3 HYPOTHESIS

It is hypothesized that *Gymnacranthera canarica* possesses a diverse array of chemical constituents across its leaves, bark, and seeds, exhibiting variations in concentration. Theessential oil extracted from *Gymnacranthera canarica* is expected to contain nuanced compounds with potential industrial or medicinal applications. Additionally, bioactive fractions isolated from *Gymnacranthera canarica* are anticipated to demonstrate significant antimicrobial and antioxidant properties, presenting promising therapeutic interventions against infections and oxidative stress.

1.4 Scope

The scope of this dissertation encompasses a comprehensive investigation into the chemical constituents of *Gymnacranthera canarica*, focusing on its leaves, bark, and seeds. This exploration will involve the identification and quantification of diverse compounds present in different plant parts using advanced analytical techniques. Furthermore, the scope extends to the extraction and analysis of essential oils from *Gymnacranthera canarica*, aiming to uncover its potential applications in various industries, including pharmaceuticals and cosmetics.

Additionally, the dissertation will involve the isolation and characterization of bioactive fractions from *Gymnacranthera canarica*, with a specific focus on their antimicrobial and antioxidant properties. This investigation will contribute to understanding the therapeutic potential of *Gymnacranthera canarica* in combating infections and oxidative stress-related conditions. Furthermore, the scope includes the evaluation of the pharmacological activities of bioactive fractions, aiming to elucidate their efficacy as therapeutic agents. This dissertation

will utilize modern analytical techniques to provide a comprehensive understanding of the chemical composition and pharmacological properties of *Gymnacranthera canarica*, thereby contributing to pharmaceutical research and the development of novel therapeutic interventions.

CHAPTER 2: LITERATURE REVIEW

In the dominion of phytochemical research, Balamurugan *et al*. (2019) provided a seminal guide to the analysis of phytochemicals, offering detailed insights into the methodsand techniques employed for the collection, processing, and extraction of these compounds from a variety of plant sources. Their comprehensive paper also delved into both qualitativeand quantitative analyses, laying the groundwork for subsequent studies in the field.

Building upon the foundational work; Gangana & Bhat (2022) conducted a study focused on investigating the phytochemical composition and cytotoxic potential of *G. farquhariana* extracts. Employing aqueous and methanolic solvents, their research uncovered a rich array of phytoconstituents in both extracts, including proteins, carbohydrates, alkaloids, cardiac glycosides, flavonoids, saponins, tannins, anthraquinones, glycosides, phenolics, and phlobatannins. Additionally, Gangana & Bhat (2022) employed LCMS profiling to identify ten potential anti-breast cancer compounds present in the extracts of *G. farquhariana*. Their investigation unveiled differing anticancer activities between methanol seed extracts and aqueous leaf extracts. The former demonstrated greater efficacy against the MCF7 cell line while exhibiting lower cytotoxicity on HEK293 cells compared to the latter.

Rassak (2017) conducted a comprehensive review of recent advancements and emerging challenges in the development of antiangiogenic drugs for cancer treatment. Their analysis confirmed the pharmacological effect of *Myristica malabarica* extract, attributing its antiangiogenic properties to the plant's rich array of phytochemicals, thus validating its ethnomedical use. Furthermore, a qualitative phytochemical examination of mace by Thakur et al., (2014) revealed the presence of amino acids, proteins, carbohydrates, phenolics, flavonoids, terpenoids, and tannins. Their study also found that methanolic extracts exhibited

significant DPPH scavenging activity, along with a total phenolic content of 238.52 mg gallic acid equivalents per gram weight.

Tantry & Bhat (2016), investigated the biochemical characteristics of *Myristica fatua*. They found that while water extracts lacked phenolics, tannins, and resin, methanolic extracts contained a variety of phytochemical elements including carbohydrates, proteins, alkaloids, tannins, and phenolics. Moreover, they evaluated the antibacterial activity against five different bacteria and assessed antioxidant activity using the DPPH method.

Premkumar (2017) conducted an evaluation of Pharmacognostical, Phytochemical, Sedative, and Hypnotic activity on the seed aril of *Myristica malabarica* Lam, revealing significant sedative and hypnotic activity in the ethanolic extract compared to benzene and aqueous extracts.

Mendhekar et al., (2017) conducted a study on Pharmacognostic, Phytochemical, physicochemical, and TLC profile of *Myristica malabarica*. Their findings revealed the presence of alkaloids, saponin, tannin, and flavones glycosides in the extract. Additionally,they isolated chemical constituents such as Malabaricones, Malabaricanol, and Isoflavones. Their study screened the Mace (Aril) for phytochemical constituents, highlighting its potential as a source of medicinally active elements and suggesting further exploration for the isolation and synthesis of modern medicines.

Thomas & Krishnakumari (2014) conducted a phytochemical profiling of Myristica fragrance seed extracts using various organic solvents. Their qualitative analysis confirmed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, phenols, anthraquinones, cardiac glycosides, coumarins, anthocyanins, chalcones, emodins, and triterpenoids in the seed extracts. Crasta & Singh (2022) focused on studies concerning the phytochemistry and pharmacology of *Myristica malabarica* Lam. Their objective was to

provide an overview of current research on *M. malabarica*, assessing its pharmacological and phytochemical activities as well as potential ethnomedical applications.

In the study by Koperuncholan & John (2011), the antimicrobial and phytochemical screenings of Myristica dactyloides Gaertn were explored. The researchers investigated extracts from various parts of the plant using different solvents and evaluated their antimicrobial activity. The ethanol root bark extract showed significant activity against bacteria, while limited activity was observed against fungi. On the other hand, Misso *et al*. (2018) conducted a comprehensive analysis of phytochemicals and biological activities of S. ochocoa extracts. Their study revealed the presence of various bioactive compounds such as polyphenols, tannins, flavonoids, and sterols in the bark extract. Furthermore, the extracts exhibited promising antiangiogenic, anti-inflammatory, and antioxidant activities, suggesting their potential for therapeutic applications.

Subha et al., (2013) identified alkaloids, steroids, flavonoids, terpenoids, glycosides, and carbohydrates in methanol, acetone, and chloroform extracts. Utilizing spectroscopic methods, they elucidated the chemical structures of these phytochemical substances isolated through thin layer and column chromatography. Asgarpanah & Kazemivash (2012) delved into the phytochemistry and pharmacological properties of Myristica fragrans Hoyutt. Their exploration aimed to showcase recent advancements in utilizing M. fragrans for phytotherapy, suggesting the potential development of furanocoumarins as therapeutic remedies.

Yadav and Agarwal (2011) conducted a comprehensive study to determine the overall phenolic and flavonoid contents, as well as the presence of phytochemicals, in various medicinal plants. Their research validated the utilization of organic and crude aqueous solvent extracts as conventional medical treatments for a range of illnesses by demonstrating the presence of bioactive chemicals in these extracts.

Sabulal et al., (2006) isolated essential oils from the leaves of *Myristica malabarica* and *Gymnacranthera canarica* (Myristicaceae) via hydrodistillation and analyzed them using GC/FID and GC/MS. The major components identified in *M. malabarica* leaf oil were β-caryophyllene (27.3%), α−humulene (13.8%), and α−copaene (11.5%), while *G. canarica* leaf oil comprised β−caryophyllene (23.4%), linalool (13.4%), and α−humulene (11.3%). Sesquiterpene hydrocarbons constituted 77.3% and 58.1% in *M. malabarica* and *G. canarica* leaf oils, respectively. The extraction and wide-ranging uses of essential oil from *Myristica malabarica* leaves in sectors including pharmaceuticals, soap manufacturing, and fragrance were the main topics of study by Crasta & Singh (2022). The review's objective was to provide an overview of the most recent research on *Myristica malabarica*, assessing its pharmacological, phytochemical, and pharmacognocal properties as well as its ethnomedicinal applications.

Kaliyaperumal et al., (2022) worked on *Myristica fragrans* essential oil (MFEO), extracting it from leaf, mace, seed, and kernel through various methods. They identified primary chemical constituents such as sabinene, eugenol, myristicin, caryophyllene, β- myrcene, and α-pinene. Clinical and experimental investigations confirmed various activities of MFEO, including antioxidant, antimicrobial, anti-inflammatory, anticancer, antimalarial,anticonvulsant, hepatoprotective, antiparasitic, insecticidal, and nematocidal properties.

The chemical makeup of the essential oil from *Myristica fragrance* Houtt was assessed by Maya et al., (2004) through an evaluation of 65 nutmeg germplasm accessions from the Indian Institute of Spices Research, Experimental Farm (Kerala). They found important components in the essential oil, including sabinin, safrole, myristicin, and elemicin, and noted variation among accessions.

In addition to performing an antibacterial investigation and employing gas chromatography-mass spectrophotometry to analyze the oil, Adewole (2013) investigated *Monodora myristica* for secondary metabolites. They detected fatty acids and organic chemicals in the oil, such as oleic acid, stearic acid, palmitic acid, and eicosanoic acid. Throughantimicrobial screening, the oil's susceptibility to the tested isolates was determined.

Pal et al., (2011) were the first to report on the essential oil of *Myristica fragrans* Houtt (Nutmeg) collected from Andaman Nicobar Island. They analyzed it using GC and GC-MS, identifying twenty-eight compounds that accounted for 92.9% of the content. Major compounds included α-pinene (9.4%), sabinene (41.7%), β-pinene (7.3%), myrcene (2.5%), limonene (3.7%), terpine-4-ol (5.8%), safrole (1.4%), and myristicin (2.7%). The essential oil exhibited microbiological activity, except against *Pseudomonas aeruginosa* and *Candida albicans*.

Tambe et al., (2019) conducted gas chromatography analysis to estimate the relative concentrations of eugenol and terpineol in various samples against standard compounds. They tested the essential oils for antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus* spp.) and the fungus *Aspergillus niger*. Additionally, they subjected de-oiled clove and nutmeg meal to phytochemical and biomolecule detection for flavonoids, tannins, terpenoids, saponins, glycosides, steroids, anthraquinones, carbohydrates, fats, and proteins.

Anusha et al., (2019) screened the methanolic extracts of *Gymnacranthera canarica* seeds for antidiabetic, antimicrobial, anti-inflammatory, antioxidant activities, and total phenol and flavonoid quantification. They found that the methanolic extract exhibited promising antimicrobial activity against both bacteria and fungi, particularly sensitive against gram- positive bacteria. The extract also showed potential antidiabetic activity through α-amylase

inhibition. However, the phenol content (8.75 µg/mg) did not significantly correlate with antioxidant activities, while a high flavonoid content (103.3 µg/mg) might be responsible forthe anti-inflammatory effect of the seeds.

Bauri *et al*. (2022) investigated antiproliferative acyl phenols and arylnonanoids fromthe fruit rind of *Myristica malabarica* lam. They identified eight known compounds, including malabaricones A-D, promalabaricone-B and C, 1-(2,6-dihydroxyphenyl) tetradecan-1-one, and ericanone, confirming their structures using x-ray crystallography. *In vitro* assays indicated moderate antiproliferative activity against A2780 human ovarian cancer cells for the isolated phenols.

Anusha et al., (2022) evaluated the physiological and biochemical responses of *Gymnacranthera canarica* seeds to different desiccation treatments. Their results highlighted that *G. canarica* seeds are highly sensitive to desiccation, losing total viability within 15 days post-harvest, indicating active seed metabolism and the absence of metabolic arrest. Desiccation intensified malondialdehyde and electrolyte leakage while reducing formazan formation. It also increased protease activity, concurrent with viability loss. Desiccation led to decreased phenol and starch content but increased proline, fat, sucrose, and total soluble carbohydrates. The early loss of viability was linked to membrane integrity loss, ROS formation, and associated lipid peroxidation, implying the seeds' truly recalcitrant nature.

Tambat et al. (2006) focused on improving the germination of *Gymnacranthera canarica* seeds. Despite confirming seed viability at 98% via TZ test, initial germination stoodat only 40%, indicating dormancy issues. Seed coat removal alone increased germination by 20% (to 60%) over the control (40%), revealing partial seed coat permeability to water. GA3 treatments at 50, 100, and 250 ppm, combined with partial seed coat removal, significantly enhanced germination by 39%, 33%, and 30%, respectively, compared to the control. However,

a higher GA3 concentration (500 ppm) showed an inhibitory effect (10%). No consistent pattern was observed concerning IAA treatment.

The condition of the Myristica marshes in the Western Ghats and their dangers were examined by Chandran et al., (2001). All of the marshes they looked at showed a marked lack of regeneration, according to their findings. They emphasized the critical necessity for concentrated conservation efforts to save these exceptional environments and the equally exceptional plants that occupy them.

Keshavachandra & Krishnakumar (2016) conducted studies to assess the viability, germination, and storage behavior of *Gymnacranthera canarica* seeds. Their observations indicated an initiation of germination after two weeks, with maximum (90%) germination observed at an initial moisture content of 38.04 ± 1.75%. However, when the moisture content dropped to 14.26 ± 2.3% after 70 days of storage, germination significantly decreased to 3%. Seeds failed to germinate beyond this moisture level. Their desiccation study revealed the seeds' recalcitrant behavior and suggested they could be stored under lab conditions for up to two and a half months. *Gymnacranthera canarica* Warb., an endangered and endemic tree species of the Western Ghats, India, that has adapted to freshwater Myristica swamps, was the subject of an analysis by Tambat et al., (2010) on reproductive fitness. Their research showed that G. canarica's reproductive fitness was directly impacted by a reduction in swamp area, dispelling the myth that species that naturally occur in many tiny populations are less susceptible to the negative impacts of small population size.

Gymnacranthera farquhariana seeds obtained from marshes were examined for anomalies by Tambat *et al*., (2007), who discovered a 13% abnormality rate. When comparing aberrant and normal seeds, comparative analysis could not find any discernible changes in seed parameters. Furthermore, the mace content of the seeds in both categories stayed constant.

Bhat & Kaveriappa (2009) conducted ecological studies on Myristica swamp forests in Uttara Kannada district, Karnataka, India, focusing on floristic composition, structure, diversity, and edaphic factors. Using the transect method, they highlighted Myristicaceae dominance in the swamps, mainly represented by *G. farquhariana* and *M. fatua* var. magnifica, with high importance value indices. They reported high endemism, with 23 species being endemic and ten rare and threatened species in the Western Ghats. Soil analysis revealed slightly lower nitrogen, phosphorous, and potassium contents compared to other forest ecosystems in the region.

Thacker & Karthick (2022) investigated the response of diatoms to changing water quality in Myristica swamps across the central Western Ghats. Analyzing samples from 17 swamps and environmental parameters, they identified 44 diatom species with restricted distribution to this unique environment among 91 species. Swamps with less anthropogenic disturbance exhibited higher diatom species richness. This study suggested that even in harsh environments like swamps, diatoms can serve as valuable environmental indicators.

Jumaily and Barzanchi (2013) performed a chemical analysis of a naturally occurring lignan dimer extracted from nutmeg seeds. They also evaluated its ability to scavenge free radicals. Their findings indicated that compared to vitamin C, the partially purified dimer exhibited 45% activity, while the purified dimer showed 75% activity. In conclusion, the review of literature highlights the diverse phytochemical composition and pharmacological properties of various plant species, including Myristica, *Gymnacranthera,* and others. These studies provide valuable insights into the potential therapeutic applications and chemical constituents of these plants, laying the groundwork for further research in this field.

CHAPTER 3: METHODOLOGY

3.1. Plant Materials Collection and Identification

Gymnacranthera canarica specimens were collected from Nirankarachi Rai, Valpoi, Goa, and the Netravali Wildlife Sanctuary, Goa. The species identity was confirmed using the herbarium available at Goa University. Samples of leaves, bark, and seeds were collected from these locations and utilized for the intended studies.

3.2. Phytochemical Studies

3.1.1. Preparation of plant extracts

The plant samples (leaves, bark, seed) were collected, shade-dried, and powdered. Subsequently, 10g of powder was weighed using a weighing balance, and the sample was extracted with 10 ml of different solvents such as distilled water, methanol, and ethanol using a Soxhlet apparatus. The resulting extracts were then utilized to conduct various phytochemical tests.

1. Detection of Alkaloids (Evans 1997)

A. Wagner's test (Wagers, 1993)

To a few mL of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish-brown precipitate confirms the test as positive.

Wagner's reagent-

Iodine (1.27g) and potassium iodide (2g) is dissolved in 5 mL of water and made up to 100 mL with distilled water.

B. Mayers test (Evans, 1997)

To a few mL of filtrate, few drops of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate confirms the test as positive.

Mayer's reagent-

Mercuric chloride (1.358g) is dissolved in 60 mL of water and potassium iodide (5.0g) is dissolved in 10 mL of water. The two solutions are mixed and made up to 100 mL with with water.

2. Detection of Carbohydrates and Glycosides (Ramakrishnan *et al*.,1994)

A. Fehling's test

One mL of filtrate is boiled on water bath with 1mL each of Fehling solutions A and A red precipitate indicates the presence of sugar.

Fehling's Solution A-

Copper sulphate (34.66g) is dissolved in distilled water and made up to 500 mL using distilled water.

Fehling's Solution B-

Potassium sodium tartarate (173g) and sodium hydroxide (50g) is dissolved in water and made up to 500 mL.

B. Barfoed's test

To 1 mL of filtrate, 1 mL of Barfoed's reagent is added and heated on a boiling water bath for 2 min. Red precipitate indicates the presence of sugar.

Barfoed's reagent- Copper acetate, 30.5 g is dissolved in 1.8 mL of glacial acetic acid.

C. Borntagers's test (Evans, 1997)

To 2 mL of filtered hydrolysate, 3 mL of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides.

3. Detection of Saponins (Kokate, 1999)

The extract was diluted with water and made up ti 20 mL. The suspension was shaken in a graduated cylinder for 15 minutes. A two cm layer of a foam indicated the presence of saponins.

4. Detection of Proteins and Amino acids (Fisher, 1968; Ruthmann, 1970)

5mL of extract was dissolved in 10 mL of distilled water and filtered through Whatman No. 1 ffilter paper and the filtrate was subjected to test for proteins and amino acids.

A. Biuret test (Gahan, 1948)

An aliquot of 2 mL of filtrate was treated was treated with 1 drop of 2% copper sulphate solution. To this, 1 mL of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicated the presence of proteins.

5. Detection of Phenolic compounds and Tannins

A. Alkaline reagent test

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavonoids.

3.3. Thin layer chromatography (TLC) analysis

The TLC analysis involved profiling for various compounds including alkaloids, flavonoids, glycosides, essential oils, steroids, sterols, bitter principles, saponins, triterpenoids, coumarins, and tannins. Aluminium foil backed silica gel plates (Merk, Darmstadt, Germany) sized 10 cm × 5 cm were employed for the experiment. Extracts from different parts (leaves, bark, seed) of *Gymnacranthera canarica* were analyzed to identify their phytochemical compositions. Various solvent systems were utilized based on polarity to separate the compounds effectively. The methanolic plant extract was applied to a single TLC plate as concentrated bands, positioned 1.5 cm from the edge, and dried. Spot examination was conducted under visible, long UV, and short UV light before and after spraying with a derivatizing agent (Wagner and Bladt, 1996; Naik and Sellappan, 2020).

3.4. Determination of Total Phenolic Content

Total phenolic content was determined using the Folin Ciocalteu's method. A standard Gallic acid solution was prepared by dissolving 10 mg in 10 mL of distilled water to achieve a concentration of 1 mg/mL. The total phenolics in the plant extracts were expressed as milligrams of Gallic acid equivalents (GAE) per gram of extract. For the analysis, 1 mg/mL extracts were prepared in respective solvents (methanol and ethanol), and 200 µL of each sample was introduced into test tubes, with the final volume adjusted to 0.5 mL with distilled

water. Then, 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent was added and shaken. After 5 minutes, 2 mL of 7.5% sodium carbonate solution was added, and the test tubes were covered with aluminium foil and incubated for 1 hour at room temperature. Following incubation, the absorbance was measured at 760 nm spectrophotometrically. All determinations were performed in triplicate. The development of a blue colour upon reaction indicates the presence of reducing compounds, including polyphenols, which can be quantified spectrophotometrically (Savitree, et al., 2004).

3.5. Total Flavonoid Determination.

The total flavonoid content was measured using the aluminium chloride colorimetric assay described by Chang et al. (2002). In this method, a reaction mixture containing 1 mg of extract and 4 mL of distilled water was prepared in a 10 mL volumetric flask. To this flask, 0.30 mL of 5% sodium nitrite solution was added and allowed to react for 5 minutes, followed by the addition of 0.3 mL of 10% aluminium chloride. After another 5 minutes, 2 mL of 1M sodium hydroxide was added, and the volume was adjusted to 10 mL with distilled water. Additionally, a series of reference standard solutions of morin (20, 40, 60, 80, and 100 µg/mL) were prepared. The absorbance of both the test and standard solutions was measured against a reagent blank at 510 nm using a UV/Visible spectrophotometer.

3.6. Antioxidant Activity

3.6.1. DPPH radical scavenging assay

The free radical scavenging activity of methanolic and ethanolic extracts from the leaves and bark of Gymnacranthera canarica was assessed using the DPPH method as described by Ahmed et al. (2013) and Brand-Williams et al. (1995). This method determines the ability of

the plant extracts to donate hydrogen atoms by measuring the decolorization of an ethanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Initially, a stock solution of DPPH radical (8 mg/100 mL in ethanol) was prepared. Then, 2.0 mL of the DPPH solution was added to 1 mL of the diluted extract solution, and the mixture was incubated for 30 minutes at 37ºC. Following incubation, the absorbance was measured at 517 nm against ethanol using a double beam spectrophotometer. The reduction in the intensity of the violet/purple color of the DPPH solution indicates the antioxidant activity of the plant extracts, with stronger antioxidant activity resulting in greater decolorization and a shift towards shades of yellow.

To calculate the proportion of DPPH radical inhibition by the sample; following formula was used:-

$$\text{DPPH scavenging activity (\%)} = (A_0 - At_x)/(AT_0) \text{ X } 100$$

Where, AT_0 = absorbance value of the control reaction; AT_X = absorbance value of each extract.

3.7. Extraction of Essential Oil

The fresh leaves 400g were subjected to hydro-distillation in Clevager -type apparatus for 6 hours. The essential oil obtained was dried over anhydrous magnesium sulphate and stored at 4-6ºC. The resulting oil yield from the extraction process was measured at 0.5 mL.

3.8. Larvicidal Activity

3.8.1. Sample preparation

Methanolic extract of leaf, leaf essential oil and the liquid waste product received below oil accumulation during oil extraction were used for the larvicidal bioassays. Methanolic extract was prepared by 1mg/ml ratio. Leaf essential oil was obtained by hydro-distillation where 400 g of fresh leaves were ground in 5000 ml of distilled water and subjected to hydro-distillation.

3.8.2. Mosquito larvae

Mosquito larvae of *Culex quinquefasciatus* were screened for larvicidal activity. The mosquitoes were collected from different parts of Goa and are maintained by the National Institute of Malaria Research, Campal, Panaji, Goa. The female mosquitoes were blood fed for egg laying. Once the eggs hatched, the larvae were given baby food as a sourceof food.

3.8.3. Larval bioassay

Detection of susceptibility of larvae to extracts: For each plant sample, Methanolic extract of leaf (1mg/mL), leaf essential oil and the liquid waste product of essential oil; 3 sets of plastic bowls were maintained, containing 3 bowls in each set. Each bowl was filled with 100 mL of distilled water. 20 healthy *Culex quinquefasciatus* larvae were selectively added in each of the bowl containing distilled water and to this 25µL, 50µL, 75µL and 100µL dose of each plant sample were added separately. A control of methanol for methanolic extract and distilled water for waste product of oil and essential oil sample was maintained. Mortalitywas assessed by direct observation of larvae movements after 24 and 48 hrs. Three replicates of tests and control were maintained. Comparative studies were done to check which plant sample shows the highest mortality rate.

Mortality percentage was calculated using the following formula:

=Average of dead larvae/Total number of larvae X 100.

3.9. Gas Chromatography-Mass Spectroscopy Analysis

GC-MS analysis of leaf essential oil of *Gymnacranthera canarica* was carried out using 2 mg of methanol. The GC-MS analysis was done using a Shimadzu QP-2010 Gas Chromatography Mass Spectroscopy instrument equipped with a RTX-5 column (30 nm x 0.25mm ID X 0.25 µm) and a mass detector. Helium was the carrier gas at a flow rate of 1 mL/min and the injector was operated at 250ºC and the oven temperature was programmed as follows; 50ºC at 8ºC/min to200ºC (5 min) at 7ºC/min to 290°C (10 min).

3.10. Antimicrobial Activity

Antimicrobial studies were performed on methanolic and ethanolic leaf extract and essential oil of *Gymnacranthera canarica* using 4 different bacterial cultures namely; *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhi.* The antimicrobial test was conducted in Microbiology department at Ponda Education Society's college of Arts & Science in Farmagudi, Ponda, Goa.

3.10.1. Sterilization of glassware and culture vials

Sterilization of the required equipment's and media was carried out by Autoclave and dry heat sterilization methods. Necessary glassware's were washed in detergent solution and dried in a hot air oven. Petri plates, test tubes, forceps, L shaped spreader were washed, dried and wrapped separately in a paper and sterilized by Autoclaving at 15 Lbs. for 1 hour.

3.10.2. Preparation of Media

Malt extract media and agar were accurately weighed and dissolved in distilled water stirred well and digested by gentle heating in a microwave followed by autoclaving at 15 Lbs for 1 hour 30 minutes and cooled at room temperature.

3.10.3. Antimicrobial susceptibility test

The bacterial strains of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* and *Klebsiella pneumoneae* were collected from Microbiology department at Ponda Education Society's college of Arts & Science in Farmagudi, Ponda, Goa. All the four bacterial strained were subcultured using nutrient agar medium by following proper safety precautions.

For sub-culturing, from the test tube containing pure bacterial cultures, a loopful of the culture was taken and streaked on a previously pre-sterilised petriplates containing solidified media. The sub-cultured plates were incubated at room temperature for 24 hours to obtain fine isolated colonies.

3.10.4. Preparation of Microbial suspension

Microbial suspension was done in a Laminar air flow by following proper safety measures. For the preparation of microbial suspension, one loop of stock culture of particular microorganism was transferred to 5 mL of sterile distilled water in a conical flask and mixed well.

3.10.5. Disc Diffusion Method

Antimicrobial study was carried out by the disc diffusion method using subcultured strains. Sterile filter paper disc of 7 mm in diameter were used as a disc. A swab of bacterial suspension (100µL) was spread on petriplates containing nutrient agar medium. The paper discs were dipped in the plant sample and allowed to soak in the sample of which antimicrobial activity to be tested. The disc was then placed on the culture plates. For the methanolic leaf extract, methanol was used as a control and for the leaf essential oil, n-hexane was used a control. The plates were incubated for 24 hours at 37º C. Antimicrobial activity was noted by observing the presence or absence of clear inhibition zone around the discs.

CHAPTER 4: RESULTS

4.1. Phytochemical analysis of *Gymnacranthera canarica*

The preliminary phytochemical analysis of *Gymnacranthera canarica* extracts revealed diverse constituents across different solvent types. Methanolic extracts from the leaves and seeds exhibited the presence of alkaloids (via Wagner's test), carbohydrates (Fehling's test), glycosides (Borntrager's test), proteins (Biuret test), saponins, and phenolic compounds and tannins (alkaline reagent test). Ethanolic extracts showed similar results, with the addition of carbohydrates detected in bark samples. Distilled water extracts displayed alkaloids, carbohydrates (Barfoed's test in leaves), glycosides, proteins (in leaves), saponins, and phenolic compounds and tannins (across all samples). Notably, bark extracts lacked carbohydrates but displayed positive results for most other constituents. These findings highlight the rich phytochemical diversity of *Gymnacranthera canarica*, indicating its potential therapeutic value. Further investigations are warranted to isolate and characterize these bioactive compounds for potential pharmacological applications.

These results suggest that *Gymnacranthera canarica* possesses a diverse range of phytochemicals, including alkaloids, carbohydrates, glycosides, proteins, saponins, andphenolic compounds, which may contribute to its potential medicinal properties. However, further detailed analysis is required to isolate and characterize these phytochemical constituents and evaluate their pharmacological activities.

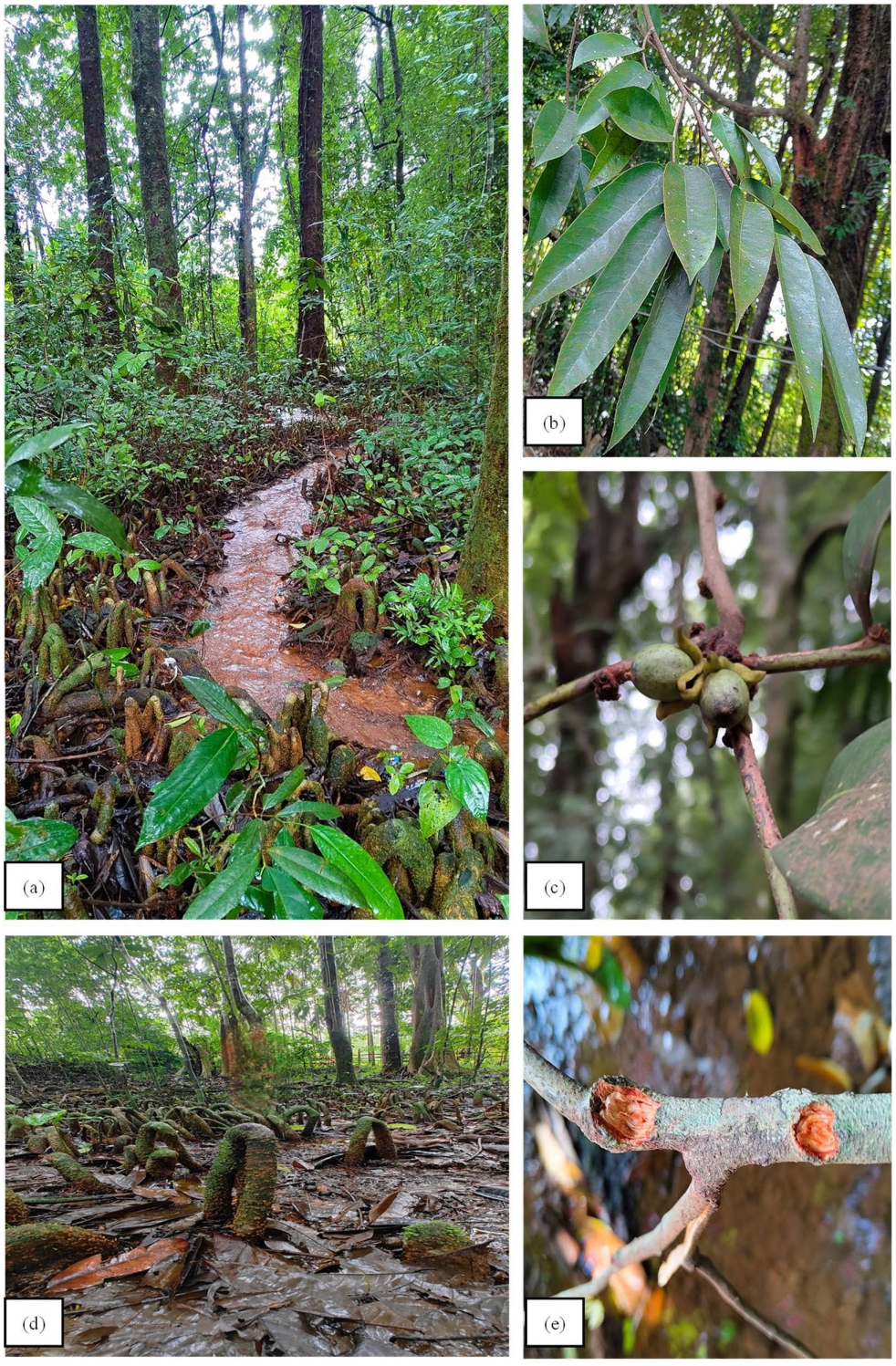


Plate 4.1: ***Gymcranthera canarica*** **(a) habit, (b) leaves, (c) fruit, (d) knee roots, (e) red colour latex.**

Table 4.1: Phytochemical analysis of methanolic extract of *Gymnacranthera canarica.*

Sr. No.	Phytochemical test	Leaf	Bark	Seed
1	**Detection of Alkaloids**			
a.	Mayer's test	+	-	+
b.	Wagner's test	+	+	+
2	**Detection of Carbohydrates**			
a.	Fehling's test	+	-	-
b.	Barfoed's test	-	-	+
3	**Detection of glycosides**			
a.	Borntrager's test	+	+	+
4	**Detection of proteins**			
a.	Biuret test	+	+	+
5	**Detection of Saponin**	+	-	-
6	**Detection of phenolic compound and tannins**			
a.	Alkaline reagent test	+		+

Table 4.2: Phytochemical analysis of ethanolic extract of *Gymnacranthera canarica.*

Sr. No.	Phytochemical test	Leaf	Bark	Seed
1	**Detection of Alkaloids**			
a.	Mayer's test	+	-	+
b.	Wagner's test	+	+	+
2	**Detection of Carbohydrates**			
a.	Fehling's test	+	+	-
b.	Barfoed's test	-	-	+
3	**Detection of glycosides**			
a.	Borntrager's test	+	+	-
4	**Detection of proteins**			
a.	Biuret test	+	+	+
5	**Detection of Saponin**	+	-	-
6	**Detection of phenolic compound and tannins**			
a.	Alkaline reagent test	+	-	+

Table 4.3: Phytochemical analysis of aqueous/water extract of *Gymnacranthera canarica.*

Sr. No.	Phytochemical test	Leaf	Bark	Seed
1	**Detection of Alkaloids**			
a.	Mayer's test	-	-	+
b.	Wagner's test	+	+	+
2	**Detection of Carbohydrates**			
a.	Fehling's test	-	-	-
b.	Barfoed's test	+	-	-
3	**Detection of glycosides**			
a.	Borntrager's test	+	+	+
4	**Detection of proteins**			
a.	Biuret test	+	+	-
5	**Detection of Saponin**	+	-	-
6	**Detection of phenolic compound and tannins**			
a.	Alkaline reagent test	+	+	+

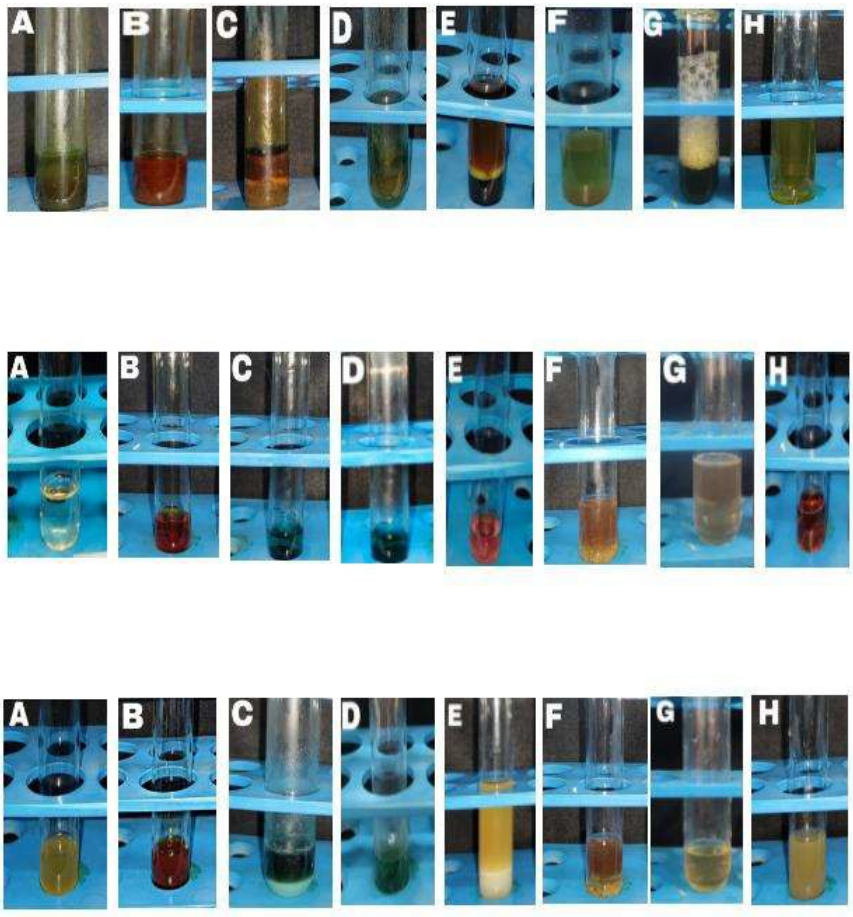


Plate 4.2: Detection of phytochemicals:- **Row 1:** phytochemical detection of leaf Methanolic extract; **Row 2:** phytochemical detection of bark ethanolic extract; **Row 3:** phytochemical detection of seed ethanolic extract where; **A.** Mayer's test for Alkaloids; **B.** Wagner's test for Alkaloids; **C.** Fehling's test for Carbohydrates; **D.** Barfoed's test for Carbohydrates; **E**. Borntrager's test for Glycosides, **F.** Biuret test for proteins; **G**. Test for Saponins; **H**. Alkaline reagent test for detection of flavonoids.

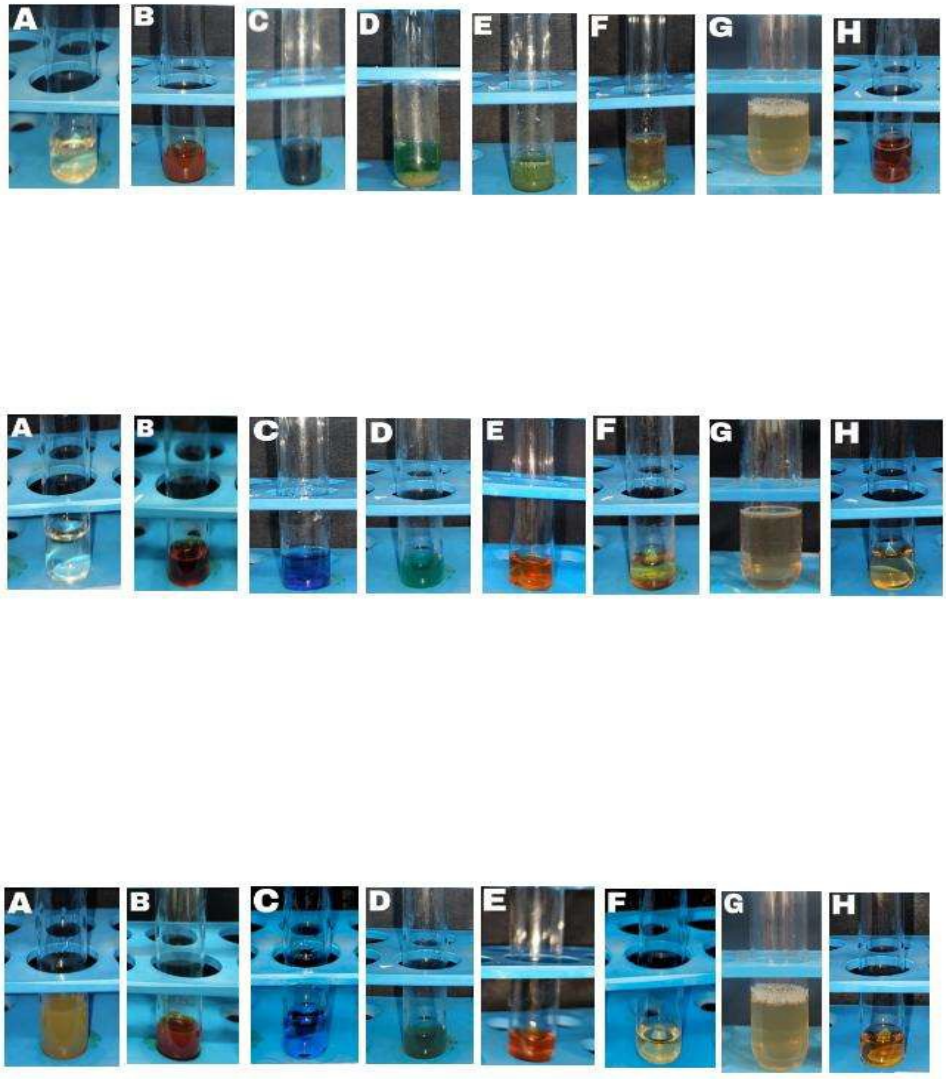


Plate 4.3: Detection of phytochemicals:- **Row 1:** phytochemical detection of leaf distilled water extract; **Row 2:** phytochemical detection of bark distilled water extract; **Row 3:** phytochemical detection of seed distilled water extract where; **A.** Mayer's test for Alkaloids; **B.** Wagner's test for Alkaloids; **C.** Fehling's test for Carbohydrates; **D.** Barfoed's test for Carbohydrates; **E**. Borntrager's test for Glycosides, **F.** Biuret test for proteins; **G.** Test for Saponins; **H.** Alkaline reagent test for detection of flavonoids.

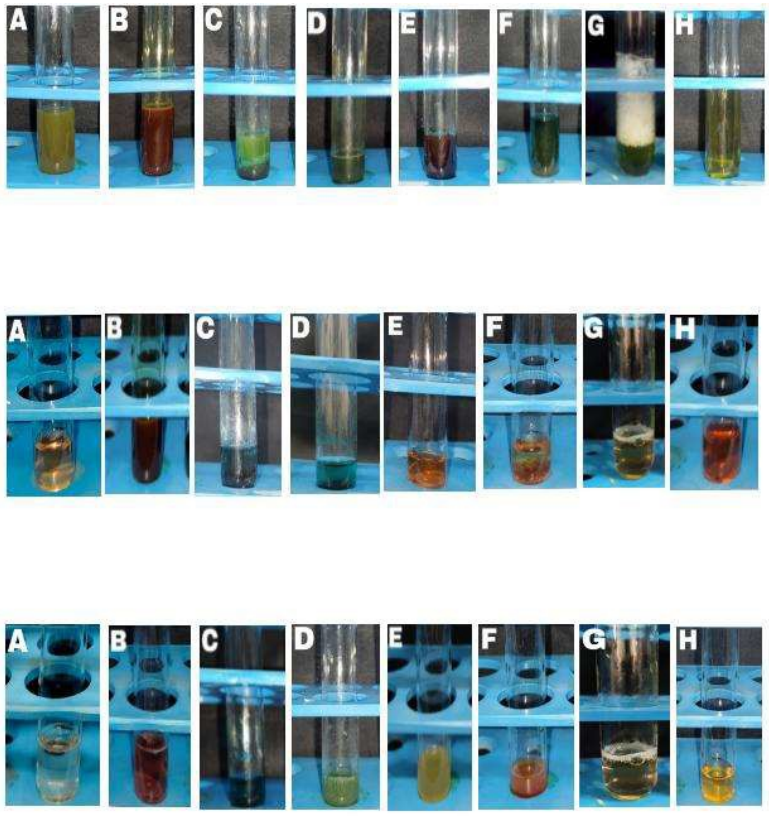


Plate 4.4: Detection of phytochemicals:- **Row 1:** phytochemical detection of leaf Ethanol extract; **Row 2:** phytochemical detection of bark Methanol extract; **Row 3:** phytochemical detection of seed Methanol extract where; **A.** Mayer's test for Alkaloids; **B.** Wagner's test for Alkaloids; **C.** Fehling's test for Carbohydrates; **D.** Barfoed's test for Carbohydrates; **E**. Borntrager's test for Glycosides, **F.** Biuret test for proteins; **G.** Test for Saponins; **H.** Alkaline reagent test for detection of flavonoids.

4.2. TLC (Thin layer chromatography) analysis for presence for bio-actives

4.2.1. TLC for Alkaloids

The thin-layer chromatography (TLC) analysis aimed to detect alkaloids in Gymnacranthera canarica extracts, employing a pre-coated TLC Silica Aluminium Plate. A solvent system comprising Toluene, Ethyl acetate, Methanol, and Ammonia 25% was utilized for the experiment. Methanolic extracts from the leaf, bark, and seed of Gymnacrantheracanarica were applied to the TLC plates. The results revealed a total of 9 distinct bands on the TLC plate, with the leaf extract exhibiting the highest number of bands, followed by the bark and seed extracts. Upon spraying with Dragendroff reagent, orange bands indicative of alkaloids was observed. In the leaf extract, seven distinct spots were detected with Rf values ranging from 0.12 to 0.82. Similarly, one distinct spot each was observed in the bark and seed extracts, with Rf values of 0.25 and 0.96, respectively. These findings suggest the presence of alkaloids in the *Gymnacranthera canarica* extracts, highlighting their potential pharmacological significance. Further characterization of these compounds is warranted to elucidate their specific chemical identities and therapeutic properties. (**Table 4.4**).

Table 4.4: Detection of Alkaloid

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
Toluene: Ethyl acetate: Diethyl amine (7:2:1)	Leaf extract	7	0.12	Orange	Alkaloid
			0.37	Orange	Alkaloid
			0.41	Black	Unknown
			0.50.	Brown	Unknown
			0.62	Brown	Unknown
			0.66	Green	Unknown
			0.82	Green	Unknown
	Bark extract	1	0.25	Orange	Alkaloid
	Seed extract	1	0.96	Orange	Alkaloid

4.2.2. TLC for Flavonoids

The thin-layer chromatography (TLC) analysis for flavonoids in *Gymnacranthera canarica* extracts utilized a pre-coated TLC Silica Aluminium Plate. A solvent system composed of Ethyl acetate, Formic acid, Glacial Acetic Acid, and Water was employed. Upon loading the extracts onto the TLC plates, a total of 5 bands were observed, with the leaf extract exhibiting the highest number of bands compared to the bark and seed extracts. After spraying with Methanolic Sulphuric acid, distinct fluorescent bands were obtained, indicating the presence of flavonoids. In the leaf extract, three distinct spots were detected with Rf values ranging from 0.54 to 0.86, displaying colors such as green and fluorescent. The bark extract showed two distinct bands with Rf values of 0.44, appearing brown in color, while the seed extract displayed one band with Rf value of 0.74, appearing bright brown, and another band with Rf value of 0.76, displaying fluorescent green. These results suggest the presence of flavonoids in the *Gymnacranthera canarica* extracts, with the leaf extract exhibiting the highestabundance of these compounds. Further characterization and isolation of these flavonoids are necessary to explore their potential pharmacological activities and applications in traditional medicine (**Table 4.5**).

Table 4.5: Detection of Flavonoid

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
Ethyl acetate: Formic Acid: Glacial Acetic Acid: water (10: 0.5: 0.5: 1.3)	Leaf extract	3	0.54	Green	Unknown
			0.69	Florescent	Flavonoid
			0.86	Florescent	Flavonoid
	Bark extract	2	0.44	Brown	Unknown
	seed extract	1	0.74	Bright Brown	Flavonoid
			0.76	Florescent Green	Flavonoid

4.2.3. TLC for Saponins

The thin-layer chromatography (TLC) analysis for saponins in *Gymnacranthera canarica* extracts utilized a solvent system comprising Chloroform, Acetic acid, Methanol, and Water. Upon application of this solvent system, distinct bands were observed in visible light, displaying colors such as blue, blue-violet, and yellow-brown. After derivatization, a blue- violet zone was visible under 366nm, confirming the presence of saponins in the leaf, bark, and seed extracts of *Gymnacranthera canarica*.

In the leaf extract, a total of 11 distinct spots or bands were detected, with Rf values ranging from 0.17 to 0.98. These bands exhibited colors such as violet-blue and blue, indicating the presence of saponins. Similarly, the bark extract displayed 2 bands with Rf values of 0.58 and 0.66, both appearing blue and violet, further confirming the presence of saponins. The seed extract exhibited 9 bands with Rf values ranging from 0.08 to 0.97, displaying colors such as violet-blue, yellow-brown, and pink, suggesting the presence of saponins as well.

These findings provide evidence for the presence of saponins in Gymnacranthera canarica extracts, with variations observed in the number of bands and their Rf values among the leaf, bark, and seed extracts. Further analysis and characterization of these saponins are warranted to explore their potential biological activities and therapeutic applications (**Table 4.6).**

Table 4.6: Detection of Saponin

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
1-Butanol: Water: Ethyl Acetate (20: 10: 5)	Leaf extract	11	0.17	Violet Blue	Saponin
			0.33	Green	Unknown
			0.4	Blue	Saponin
			0.53	Blue	Saponin
			0.61	Green	Unknown
			0.72	Blue	Saponin
			0.77	Green	Unknown
			0.82	Violet Blue	Saponin
			0.86	Blue	Saponin
			0.93	Green	Unknown
			0.98	Blue	Saponin
	Bark extract	2	0.58	Blue	Saponin
			0.66	Violet	Saponin
	Seed extract	9	0.08	Red	Unknown
			0.2	Violet Blue	Saponin
			0.30.	Yellow brown	Saponin
			0.4	Violet blue	Saponin
			0.64	Violet Blue	Saponin
			0.73	Violet Blue	Saponin
			0.8	Pink	Unknown
			0.93	Violet Blue	Saponin
			0.97	Blue	Saponin

4.2.4. TLC for Tannin

The TLC analysis for tannins in *Gymnacranthera canarica* extracts revealed the presence of tannin components solely in the leaf extract. Upon derivatization, dark blue colour zones were observed in visible light, confirming the presence of tannins. However, no bands indicating the presence of tannins were detected in the bark and seed extracts, suggesting their absence in these parts of the plant.

The solvent system utilized for this experiment consisted of Toluene, Ethyl acetate, and Formic Acid in a ratio of 6:4:0.3. In the leaf extract, a total of 4 distinct spots or bands were observed, with Rf values ranging from 0.21 to 0.50. Among these bands, those with Rf values of 0.34 and 0.41 appeared blue, indicative of the presence of tannins. Conversely, no bands indicating the presence of tannins were observed in the bark and seed extracts.

These results indicate that tannins are present in the leaf extract of *Gymnacranthera canarica* but are absent in the bark and seed extracts. Further investigation is necessary to elucidate the distribution and potential biological significance of tannins in *Gymnacranthera canarica*, as well as their pharmacological properties and applications (**Table 4.7**).

Table 4.7: Detection of Tannin

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
Toluene: Ethyl acetate: Formic acid (6: 4: 0.3)	Leaf extract	4	0.21	Green	Unknown
			0.34	Blue	Tannin
			0.41	Blue	Tannin
			0.50.	Yellow	Unknown

4.2.5. TLC for Triterpenoids

The TLC analysis for triterpenoids in *Gymnacranthera canarica* extracts indicated the presence of triterpenoid compounds solely in the leaf extract. Utilizing a solvent system of n-Hexane and Ethyl acetate in a 1:1 ratio, a total of 5 distinct bands were observed on the TLC plate. These bands appeared after spraying with Anisaldehyde Sulphuric Acid, with the blue band in the leaf extract confirming the presence of triterpenoids.

However, no bands indicative of triterpenoids was detected in the bark and seed extracts, suggesting their absence in these parts of the plant. The bands observed in the leaf extract had Rf values ranging from 0.69 to 0.97, exhibiting colours such as light green and dark green before transforming into a distinct blue band at Rf value of 0.97.

These findings highlight the presence of triterpenoids in the leaf extract of *Gymnacranthera canarica*, while they are absent in the bark and seed extracts. Further investigation is warranted to explore the specific types of triterpenoids present in *Gymnacranthera canarica* and their potential pharmacological and therapeutic applications (**Table 4.8**).

Table 4.8: Detection of Triterpenoids

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
n- Hexane: Ethyl acetate (1:1)	Leaf extract	5	0.69	Light green	Unknown
			0.86	Light green	Unknown
			0.89	Light green	Unknown
			0.94	Dark green	Unknown
			0.97	Blue	Triterpenoid
	Bark extract	1	0.64	Blue	Triterpenoid

4.2.6. TLC for Sterols and Steroids

The TLC analysis aimed at detecting sterols and steroids in the methanolic extracts of *Gymnacranthera canarica* revealed their absence in all plant parts.

For sterols detection, the TLC plate was run using chloroform: ethyl acetate (4:6) as the solvent system. However, after spraying with the suitable reagent, Anisaldehyde Sulphuric Acid, no desired bands were observed in the leaf, bark, and seed extracts of the plant, indicatingthe absence of sterols.

Similarly, to detect the presence of steroids, the TLC plate was run using n-butanol: methanol: water (3:1:1) as the solvent system. Again, after spraying with Anisaldehyde Sulphuric Acid, no desired bands were observed in any of the plant extracts, confirming the absence of steroids.

These results suggest that *Gymnacranthera canarica* does not contain sterols or steroids in detectable amounts in its leaf, bark, and seed extracts. Further investigation may be needed to explore other classes of compounds and their potential pharmacological activities within the plant (Table 4.9; Table 4.10).

Table 4.9: Detection of Sterols

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
Chloroform: ethyl acetate (4:6)	Leaf extract	0	–	No color change	Unknown
	Bark extract	0	–	No color change	Unknown
	Seed extract	0	–	No color change	Unknown

Table 4.10: Detection of Steroids

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
N-butanol: methanol: water (3:1:1)	Leaf extract	0	–	No color change	Unknown
	Bark extract	0	–	No color change	Unknown
	Seed extract	0	–	No color change	Unknown

4.2.7. TLC for Coumarins

The TLC analysis conducted to detect coumarins in the methanolic extracts of *Gymnacranthera canarica* revealed the presence of these compounds in all plant parts. Using chloroform as the solvent system, the TLC plate exhibited several colored bands. Under long UV light, fluorescent bands were observed, confirming the presence of coumarins in the plant extracts, particularly after spraying with vanillin sulphuric acid reagent.

In the leaf extract, a total of 14 distinct spots or bands were observed on the TLC plate. These bands displayed green fluorescence at 366 nm, indicative of coumarin compounds. TheRf values ranged from 0.13 to 0.96, with most bands exhibiting green fluorescence, confirming the presence of coumarins.

Similarly, the bark and seed extracts also showed the presence of coumarins, albeit with fewer bands compared to the leaf extract. In the bark extract, five distinct bands were observed, displaying green fluorescence at 366 nm, confirming the presence of coumarins. The Rf values ranged from 0.29 to 0.49. In the seed extract, five bands were also observed, with Rf values ranging from 0.2 to 0.66, all exhibiting green fluorescence at 366 nm, indicating the presence of coumarins.

These findings demonstrate the presence of coumarins in the leaf, bark, and seedextracts of Gymnacranthera canarica, suggesting the potential pharmacological significance of these compounds in the plant. Further research is warranted to elucidate the specific coumarin constituents present and their potential biological activities (**Table 4.11**).

Table 4.11: Detection of Coumarin

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
Chloroform	Leaf extract	14	0.13	Green Fluorescence at 366 nm	Coumarin
			0.26	Green Fluorescence at 366 nm	Coumarin
			0.29	Green Fluorescence at 366 nm	Coumarin
			0.33	Green Fluorescence at 366 nm	Coumarin
			0.37	Violet Blue	Unknown
			0.41	Green Fluorescence at 366 nm	Coumarin
			0.45	Green Fluorescence at 366 nm	Coumarin
			0.48	Violet Blue	Unknown
			0.53	Green Fluorescence at 366 nm	Coumarin
			0.66	Green Fluorescence at 366 nm	Coumarin
			0.72	Green Fluorescence at 366 nm	Coumarin
			0.74	Green Fluorescence at 366 nm	Coumarin
			0.85	Violet Blue	Unknown
			0.96	Violet Blue	Unknown
	Bark extract	5	0.29	Green Fluorescence at 366 nm	Coumarin
			0.33	Violet Blue	Unknown
			0.37	Blue	Coumarin
			0.44	Violet Blue	Unknown
			0.49	Violet Blue	Unknown
	Seed extract	5	0.2	Violet	Unknown
			0.33	Green Fluorescence at 366 nm	Coumarin
			0.42	Green Fluorescence at 366 nm	Coumarin
			0.58	Green Fluorescence at 366 nm	Coumarin
			0.66	Green Fluorescence at 366 nm	Coumarin

4.2.8. TLC for Essential oils

The TLC analysis conducted to detect essential oils in the methanolic extracts of *Gymnacranthera canarica* revealed the presence of essential oils in all plant parts.

Distinct colour bands were observed on the TLC plate, indicating the presence of essential oils in the leaf, bark, and seed extracts. The blue colour bands, observed under visible light,confirmed the presence of essential oils after spraying with vanillin sulphuric acid reagent.

The solvent system used for this analysis was toluene: ethyl acetate (9.3:0.7). In the leaf extract, six distinct bands were observed, with Rf value ranging from 0.05 to 0.44. Similarly, in the bark and seed extracts, multiple bands were observed with varying Rf values, indicating the presence of essential oils.

These results suggest that *Gymnacranthera canarica* contains essential oils in its leaf, bark, and seed extracts. Further studies may be warranted to characterize these essential oils and explore their potential applications in various fields (**Table 4.12**).

Table 4.12: Detection of Essential oil

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
Toluene: Ethyle acetate (9.3: 0.7)	Leaf extract	6	0.05	Light green	Unknown
			0.09	Dark green	Unknown
			0.17	Dark green	Unknown
			0.28	Purple	Unknown
			0.38	Dark green	Unknown
			0.44	Blue	Essential oil
	Bark extract	6	0.06	Green	Unknown
			0.12	Blue	Unknown
			0.16	violet	Unknown
			0.24	violet pink	Unknown
			0.32	Green	Unknown
	Seed extract	4	0.28	Violet	Unknown
			0.32	pink	Unknown
			0.38	pink	Unknown
			0.70	blue	Essential oil

4.2.9. TLC for Bitter Principles

The TLC analysis aimed to detect bitter principles in the methanolic extracts of *Gymnacranthera canarica*. Although prominent bands were not observed, significant vertically extensive violet-red zones were visible after derivatization, indicating the presenceof bitter principles. The solvent system used for this analysis was Ethyl acetate: Methanol: Water (7.7:1.5:0.8).

In the leaf extract, two distinct zones with a violet-red colour were observed, with Rf values of 0.2 and 0.6, suggesting the presence of bitter principles. Similarly, the bark extract exhibited two zones with a red-brown colour, indicating the presence of bitter principles, with Rf values of 0.26 and 0.66. In the seed extract, a single zone with a violet-pink colour was observed, confirming the presence of bitter principles, with Rf value of 0.93.

These findings suggest that *Gymnacranthera canarica* contains bitter principles, as evidenced by the distinctive zones observed in the TLC analysis (Table 4.13).

Table 4.13: Detection of Bitter Principle

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
Ethyl acetate: Methanol: Water (20: 2.8:2)	Leaf extract	2	0.2	Violet red	Bitter principle
			0.6	Violet red	Bitter principle
	Bark extract	2	0.26	Red brown	Bitter principle
			0.66	Red brown	Bitter principle
	Seed extract	1	0.93	Violet pink	Bitter principle

4.2.10. TLC for Glycosides

The TLC analysis aimed to detect glycosides in the methanolic extracts of *Gymnacranthera canarica* using the solvent system Ethyl acetate: Methanol: Water (20: 2.8:

2). A total of five bands were observed on the TLC plate after spraying with AlcoholicPotassium Hydroxide, and these bands were exclusively found in the leaf extract. However, no bands were observed in the bark and seed extracts.

In the leaf extract, eight distinct bands were observed, with Rf values ranging from 0.12 to 0.93. Among these bands, two bands appeared as dark blue, indicating the presence of glycosides. The other bands exhibited colours such as yellow and green, suggesting the presence of other unidentified compounds. These results suggest that *Gymnacranthera canarica* contains glycosides, as evidenced by the dark blue bands observed in the leaf extract during TLC analysis (**Table 4.14**).

Table 4.14: Detection of Glycoside

Solvent System	Samples	Number of distinct spots/bands	Rf Value	Colors with spraying reagents	Possible compounds
Ethyl acetate: Methanol: Water (20: 2.8: 2)	Leaf extract	8	0.12	Dark Blue	Glycoside
			0.18	Yellow	Unknown
			0.24	Yellow	Unknown
			0.32	Green	Unknown
			0.6	Dark Blue	Glycoside
			0.65	Dark Blue	Glycoside
			0.70.	Green	Unknown
			0.93	Yellow	Unknown

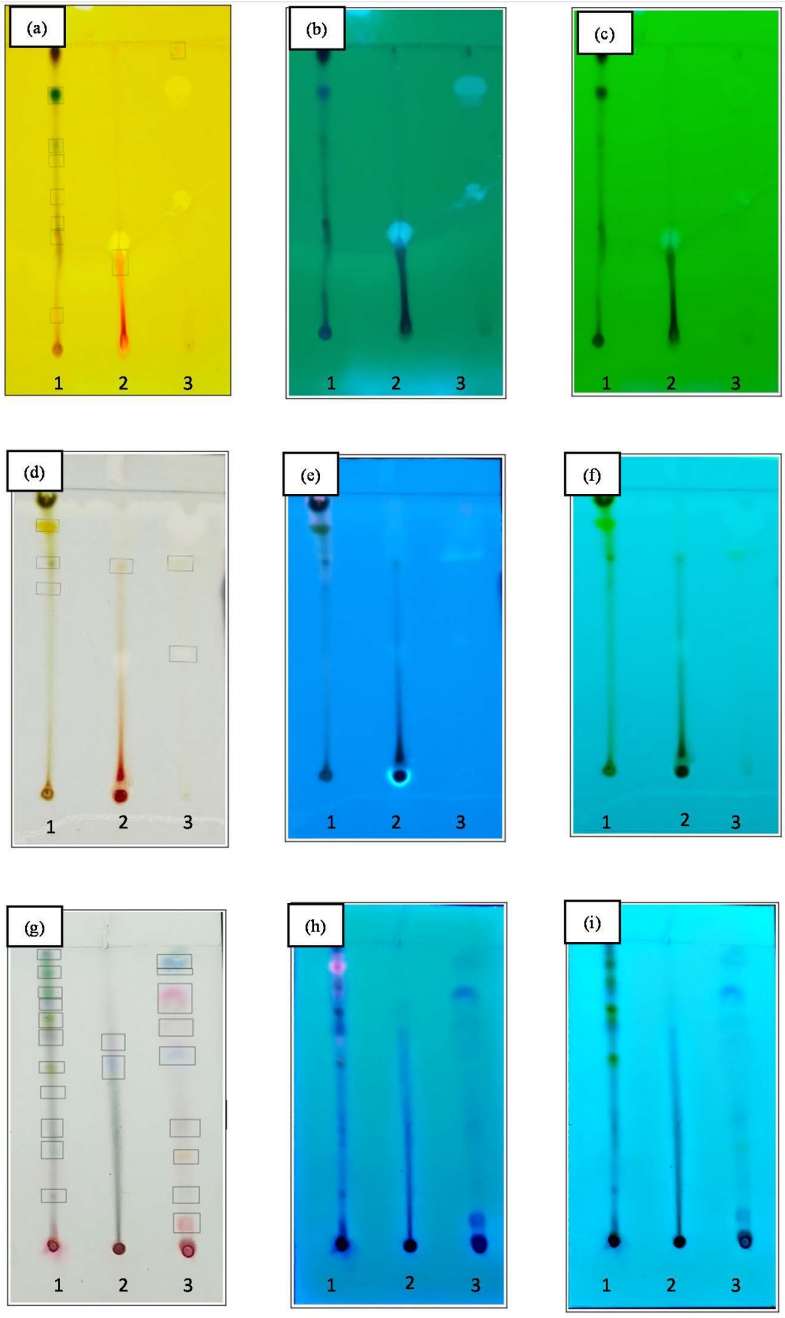


Plate 4.5: TLC isolation of alkaloids under (a) visible light, (b)long UV, & (c) short UV, TLC isolation of flavanoids under (d) visible light, (e) long UV, & (f) Short UV, TLC isolation of Saponins under (g) visible light, (h) Long UV, & (i) Short UV

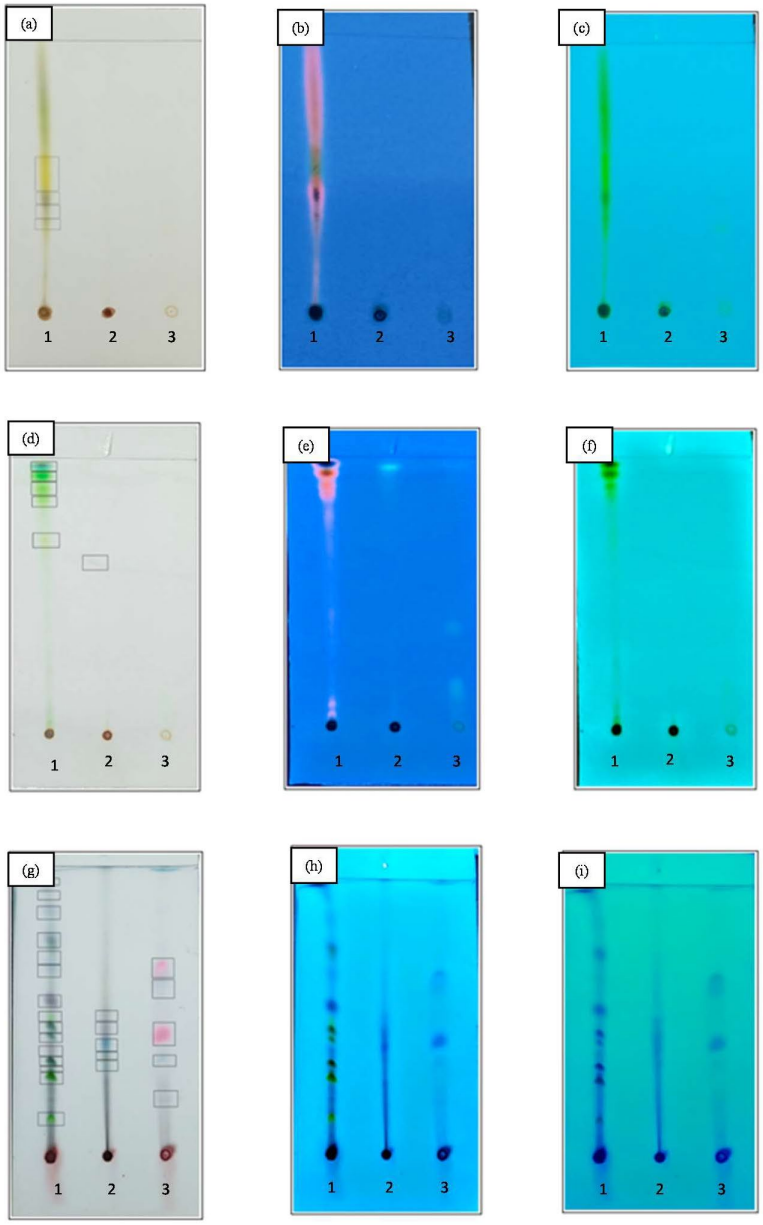


Plate 4.6: TLC isolation of Tannin under (a) visible light, (b)long UV, & (c) short UV, TLC isolation of Triterpinoid under (d) visible light, (e) long UV, & (f) Short UV, TLC isolation of Coumarin under (g) visible light, (h) Long UV, & (i) Short UV

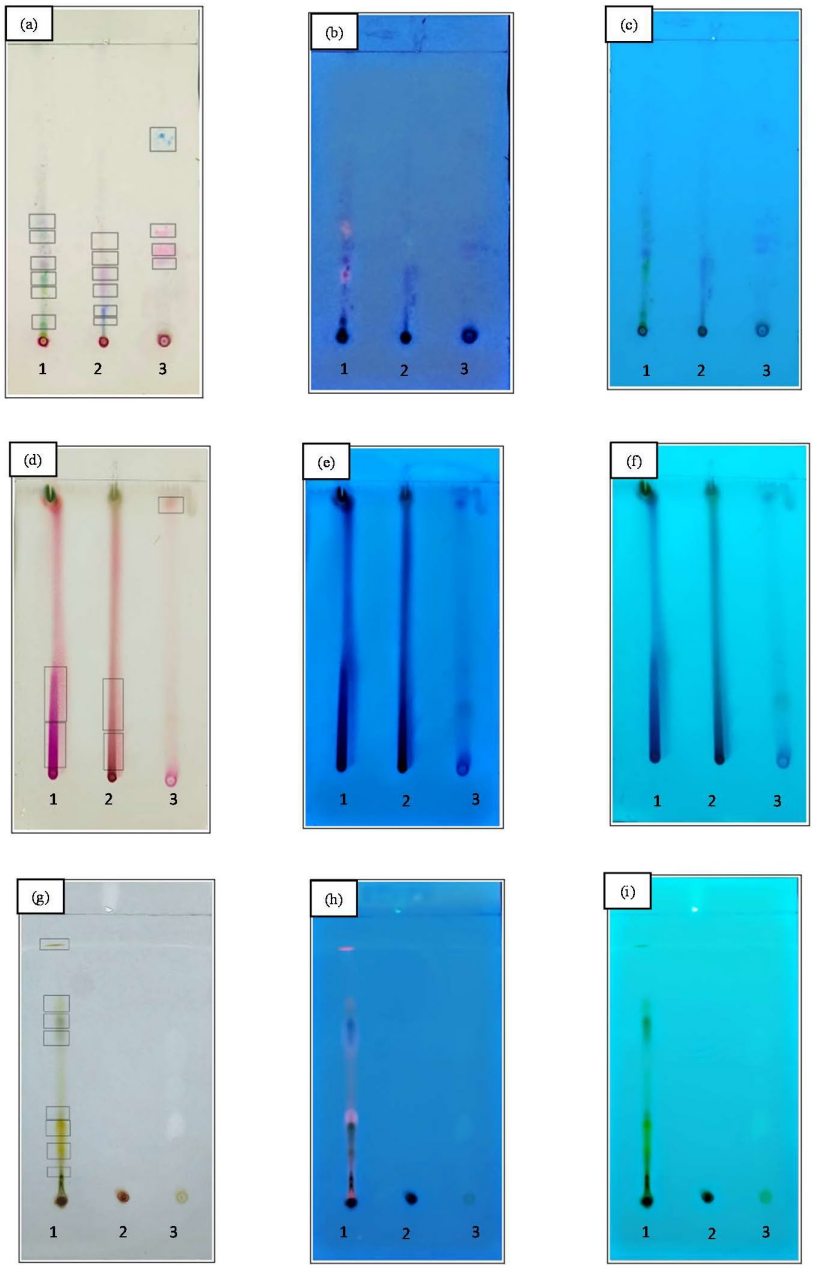


Plate 4.7: TLC isolation of Essential oil under (a) visible light, (b)long UV, & (c) short UV, TLC isolation of Bitter principles under (d) visible light, (e) long UV, & (f) Short UV, TLC isolation of Glycocides under (g) visible light, (h) Long UV, & (i) Short UV

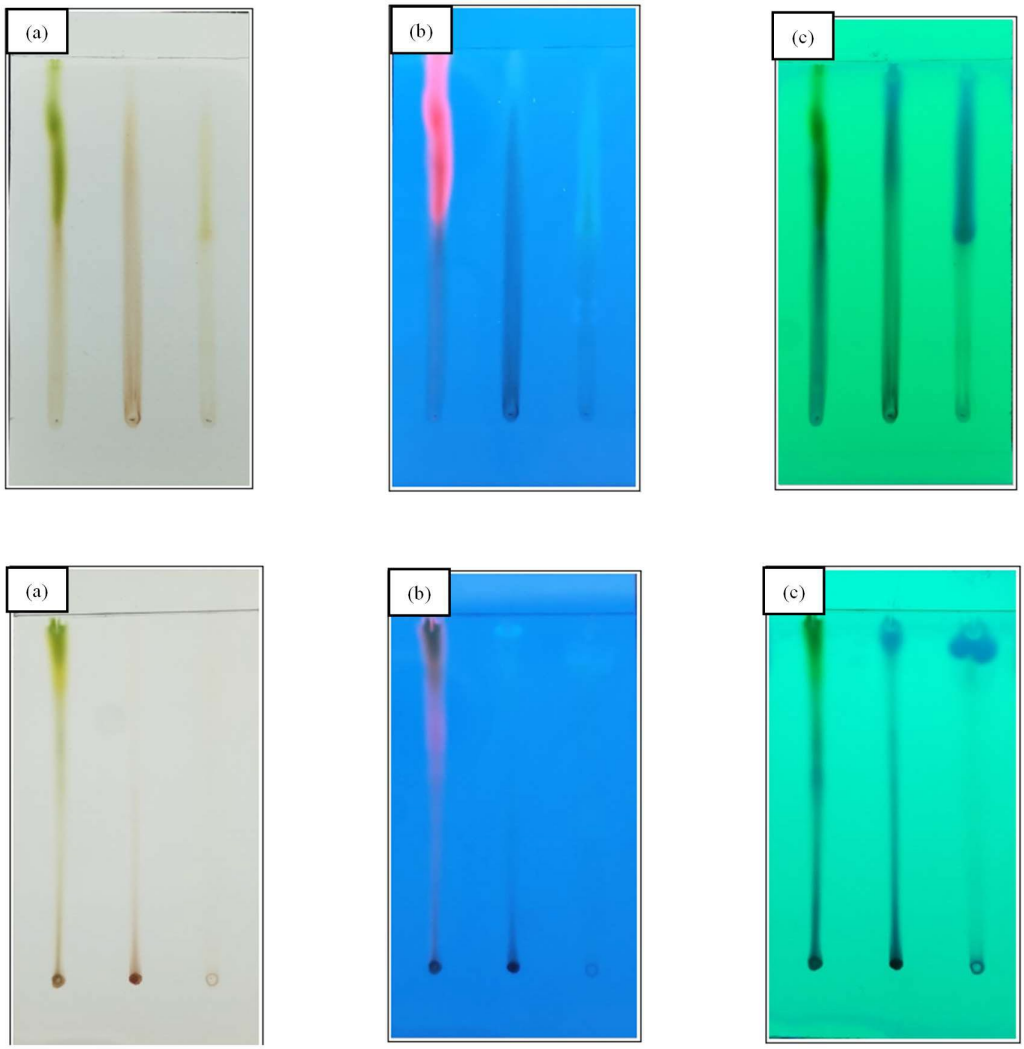


Plate 4.8: TLC isolation of Sterols under (a) visible light, (b)long UV, & (c) short UV, TLC isolation of steroids under (d) visible light, (e) long UV, & (f) Short UV.

4.3. Total Phenolic Content (TPC)

The estimation of total phenolic content (TPC) was a crucial aspect of our study, aiming to quantify the phenolic compounds present in the ethanolic and methanolic extracts of *Gymnacranthera canarica* leaf and bark. Phenolic compounds are renowned for their antioxidant properties and potential health benefits.

In our investigation, the methanolic extracts of both leaf and bark demonstrated substantially higher TPC levels compared to their ethanolic counterparts. Specifically, the methanolic extract of the leaf showcased a robust total phenolic content of 471.1 µg of gallic acid equivalents per 10 gram (µg GAE/g). This indicates a significant concentration of phenolic compounds within the leaf's methanolic extract, suggesting its potential as a rich source of antioxidants. Similarly, the methanolic extract derived from the bark exhibited a commendable total phenolic content of 347.6 µg of gallic acid equivalent per 10 gram (µg GAE/g), reinforcing the presence of valuable phenolic constituents within the bark.

Contrastingly, the ethanolic extracts yielded slightly lower TPC values. The ethanolic extract of the leaf displayed a total phenolic content of 230.4 µg of gallic acid equivalents per 10 gram µg GAE/g, while the methanolic extract of the bark demonstrated a total phenolic content of 123.7 µg of gallic acid equivalents per 10 gram µg GAE/g. Although these values were marginally lower than those obtained from the methanolic extracts, they still signify notable concentrations of phenolic compounds, albeit to a lesser extent.

Overall, these findings underscore the effectiveness of methanol as a solvent for extracting phenolic compounds from *Gymnacranthera canarica* leaf and bark. The substantially higher TPC levels in the methanolic extracts highlight the potential health- promoting properties of this plant species, warranting further investigation into its therapeutic applications.

Table 4.15: Total Phenolic Content (TPC) mg of gallic acid equivalent (GAE) in ethanolic and methanolic extract of bark and leaf ectract

Plant extract	Concentrations of TPC mg of Gallic Acid equivalents (GAE)
Methanolic leaf	471.16 ± 0100
Methanolic bark	347.63 ± 0516
Ethanolic leaf	230.43 ± 0935
Ethanolic bark	123.76 ± 0651

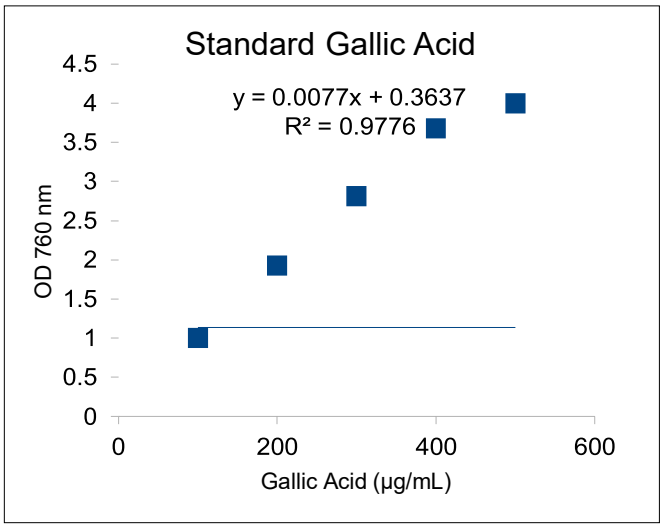


Fig. 4.1 Calibration curve for Gallic acid for determination of Total Phenolic Content (mg of Gallic Acid equivalents (GAE)/g of extracts) at varying concentrations.

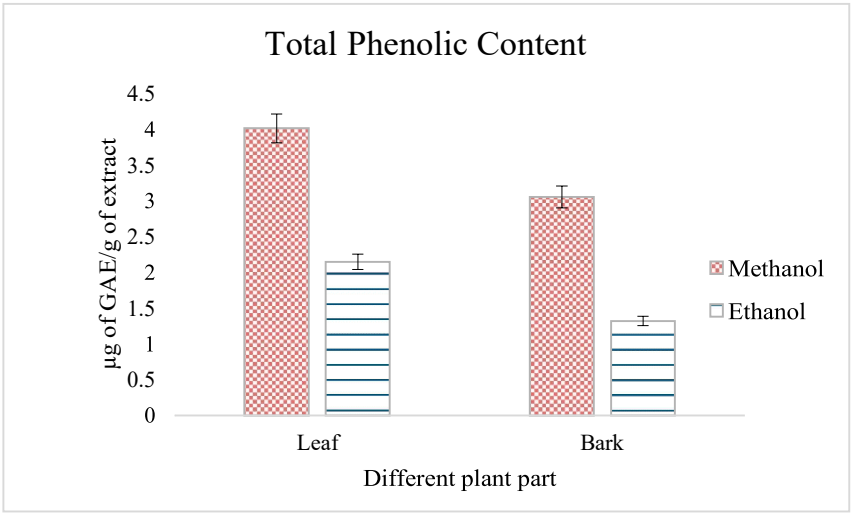


Fig. 4.2 Determination of Total Phenolic Content (TPC) in mg of Gallic Acid equivalent (GAE/g) of Methanolic and Ethanolic extract of leaf and bark of *Gymnacranthera canarica*

4.4. Total Flavonoid Content (TFC)

The total flavonoid content (TFC) in the ethanolic and methanolic extracts of *Gymnacranthera canarica* leaf and bark were determined, expressed as quercetin equivalence per 1 gram (mg QE/g). Flavonoids are a class of polyphenolic compounds known for their antioxidant and anti-inflammatory properties, making them valuable for potential health benefits.

Our analysis indicated that the methanolic extracts of both leaf and bark exhibited higher TFC values compared to their ethanolic counterparts. Specifically, the methanolic extract of leaf showed a TFC of 363 mg QE/g, while the methanolic extract of bark demonstrated a TFC of 356.3 mg QE/g. In contrast, the ethanolic extract of leaf displayed a TFC of 288.5 mg QE/g, and the ethanolic extract of bark showed a TFC of 117.4 mg QE/g. These findings suggest that methanol extraction was more efficient in extracting flavonoids from *Gymnacranthera canarica* compared to ethanol extraction.

Table 4.16: Total flavonoid content of methanolic and ethanolic extract of leaf and bark extract of Gymnacranthera canarica plant parts. Data represents mean values standard deviation.

Plant extract	Total Flavonoid content (mg of QE/g of extract)
Methanolic leaf	363.00 ± 0.001
Methanolic bark	356.33 ± 0.001
Ethanolic leaf	288.55 ± 0.004
Ethanolic bark	117.4 ± 0.003

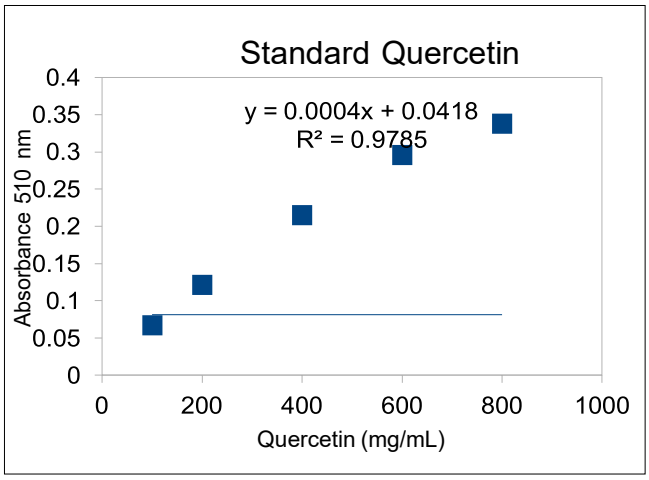


Fig. 4.3 Calibration curve for Quercetin for determination of Total Flavonoid Content (mg of Quercetin equivalent (QE)/g of extracts at varying concentrations.

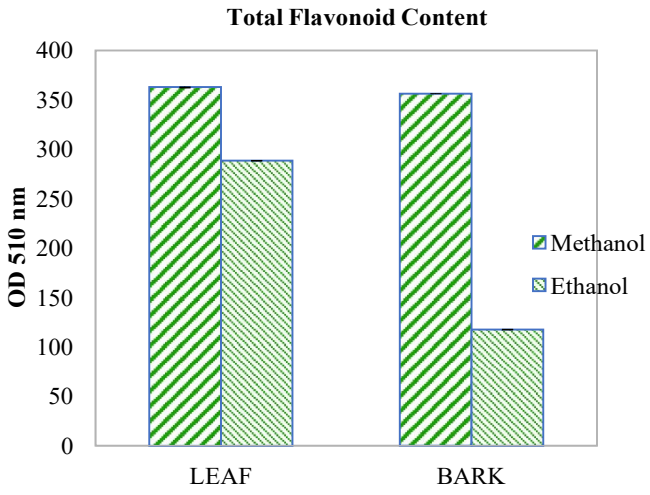


Fig. 4.4 Determination of Total Flavonoid Content (mg of Quercetin equivalent (QE)/g of Methanolic and Ethanolic extract of leaf and bark of *Gymnacranthera canarica*

4.5. Antioxidant studies in *Gymnacranthera canarica* using two different solvents

The antioxidant activity of ethanolic and methanolic extracts from the leaf and bark of *Gymnacranthera canarica* was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. This assay measures the decrease in absorption of the DPPH solution after the addition of an antioxidant, indicating its ability to scavenge free radicals. Ascorbic acid was utilized as a positive control in this study. Upon interaction with the antioxidants, DPPH is reduced to DPPH-H, resulting in a decrease in absorbance at 517nm.

All four plant extracts exhibited antioxidant activity. Among them, the ethanolic bark extract displayed the highest free radical scavenging activity, while the methanolic bark extract showed the lowest antioxidant activity. The scavenging potential of the antioxidant compounds present in the extracts was evidenced by the decrease in absorbance with increasing extract concentration, indicative of their hydrogen-donating ability.

The efficacy of an antioxidant in inhibiting a specific biochemical process can be quantified by determining its IC50 value, representing the concentration at which the substance exerts half of its

maximal inhibitory effect. The results presented in Table No. 3 and Table No. 3.1 depict the percentage scavenging activity of DPPH by both ascorbic acid and the methanolic and ethanolic extracts of the leaf and bark, at various concentrations ranging from 20 µg/mL to 100 µg/mL. Amongst all the studied plant extracts ethanolic leaf and bark showed least IC50 value indicating that methanolic extract of leaf and bark has highest anti-oxidant potential. (**Table 4.17**).

Table 4.17. IC_{50} value of L- Ascorbic Acid and methanolic and ethanolic extract of *Gymnacranthera canarica*.

Sr. No.	Sample	IC_{50} value (µg/mL)
1	Leaf methanol	40.5136017
2	Leaf ethanol	10.06333333
3	Bark methanol	109.6235012
4	Bark ethanol	70.95053004

Table 4.18: DPPH free radical scavenging assay: % scavenging activity of DPPH by ascorbic acid and methanolic and ethanolic leaf extract.

Sr. No.	Concentration (µg/mL)	L-Ascorbic acid	Plant extract	
			Methanolic Leaf	Ethanolic Leaf
1	20	0.311 ± 0.003	0.284± 0.005	0.176 ± 0.015
2	40	0.226 ± 0.001	0.184± 0.005	0.146 ± 0.02
3	60	0.196 ± 0.002	0.047± 0.003	0.116 ± 0.015
4	80	0.086 ± 0.002	0.033± 0.004	0.096 ± 0.001
5	100	0.011 ± 0.011	0.028± 0.001	0.093 ± 0.001

Table 4.19: DPPH free radical scavenging assay: % scavenging activity of DPPH by ascorbic acid and methanolic and ethanolic bark extract.

Sr. No.	Concentration (µg/mL)	L-Ascorbic acid	Plant extract	
			Methanolic Bark	Ethanolic Bark
1	20	0.311 ± 0.003	0.315 ± 0.004	0.315 ± 0.002
2	40	0.226 ± 0.001	0.278 ± 0.002	0.214 ± 0.003
3	60	0.196 ± 0.002	0.265 ± 0.003	0.176 ± 0.001
4	80	0.086 ± 0.002	0.219 ± 0.004	0.173 ± 0.001
5	100	0.011 ± 0.011	0.194 ± 0.004	0.132 ± 0.003

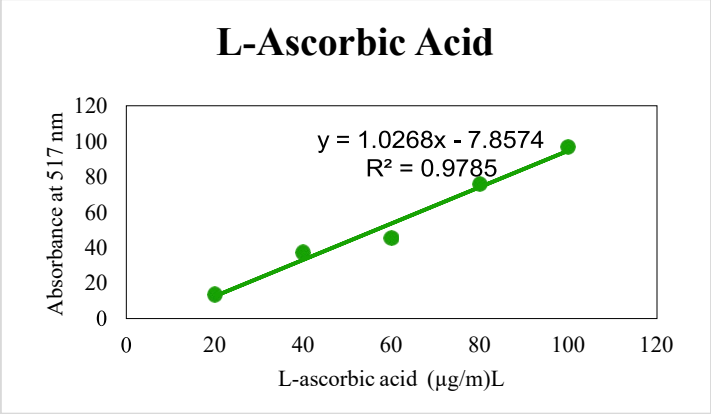


Fig. 4.5 Calibration curv for L-Ascorbic Acid to determine total DPPH radical scavenging activity

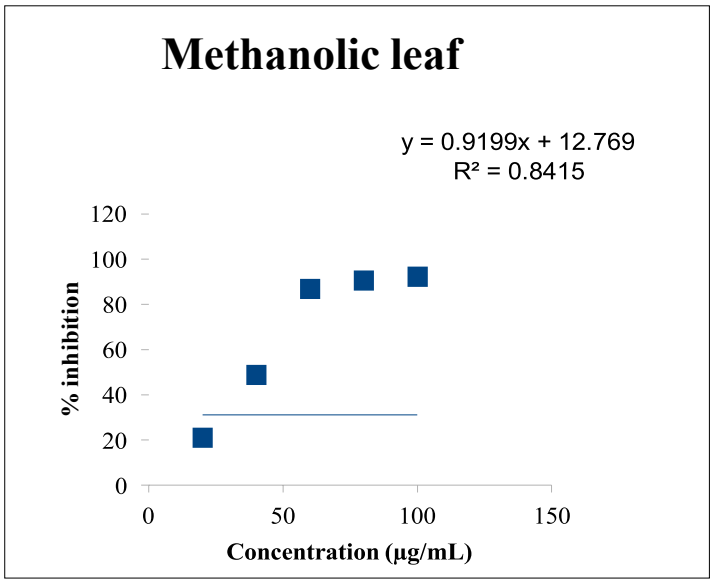


Fig. 4.6 DPPH radical scavenging activity of Methanolic leaf extract of *Gymnacranthera canarica*

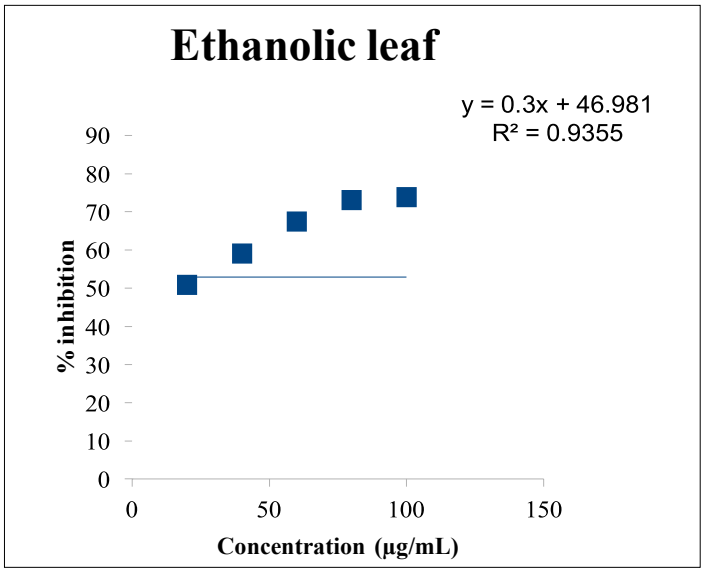


Fig. 4.7 DPPH radical scavenging activity of Ethanolic leaf extract of *Gymnacranthera canarica*

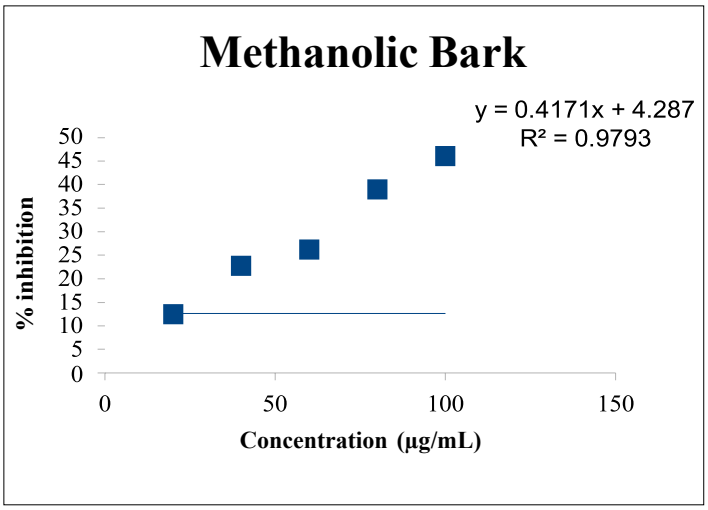


Fig. 4.8 DPPH radical scavenging activity of Methanolic bark extract of *Gymnacranthera canarica*

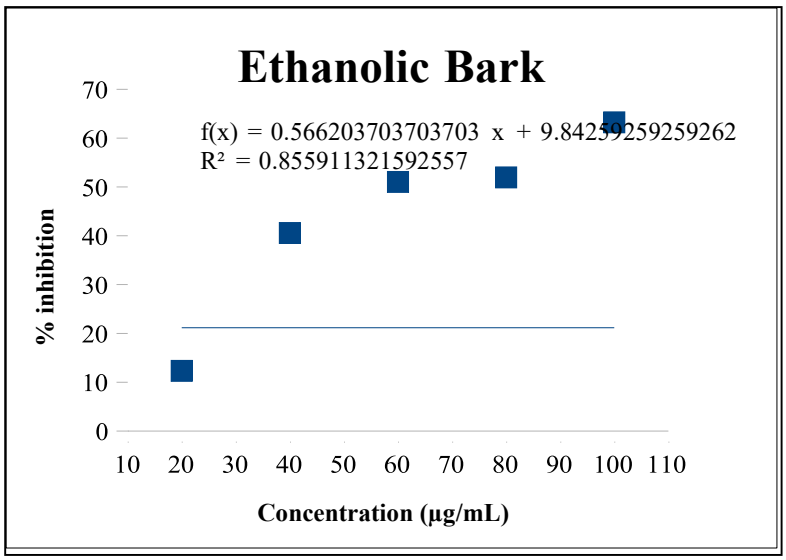


Fig. 4.9 DPPH radical scavenging activity of Ethanolic bark extract of *Gymnacranthera canarica*

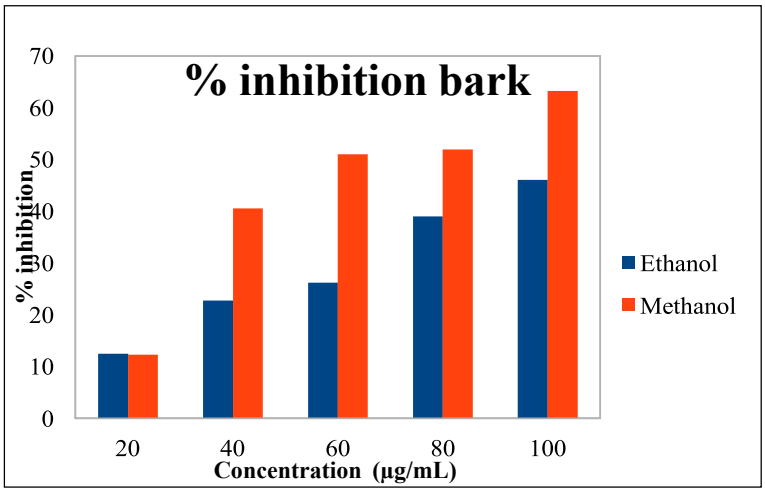


Fig. 4.10 DPPH radical scavenging activity of Ethanolic bark extract of *Gymnacranthera canarica*

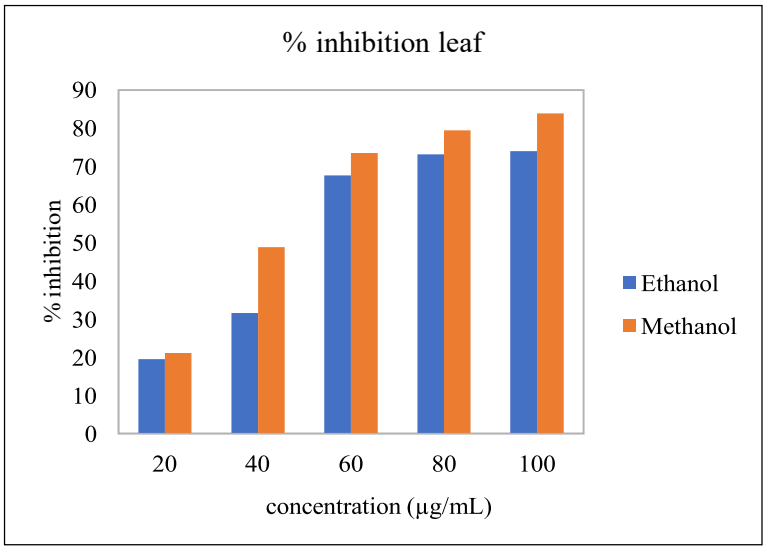


Fig. 4.11 DPPH radical scavenging activity of Ethanolic leaf extract and Methanolic bark extract of *Gymnacranthera canarica*

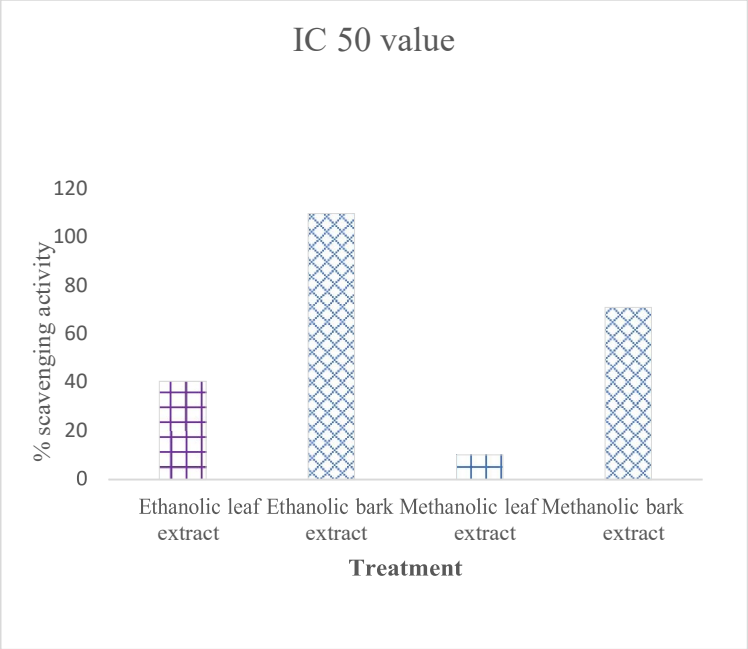


Fig. 4.12. IC_{50} value of Methanolic and Ethanolicleaf and bark extract of *Gymnacranthera canarica*

4.7. LARVICIDAL ACTIVITY

The larvicidal activity of the methanolic leaf extract, waste product of essential oil, and pure essential oil of *Gymnacranthera canarica* was evaluated against the III instar larvae of *Culex quinquefasciatus.*

Among the tested samples, the pure leaf essential oil of *Gymnacranthera canarica* exhibited higher toxicity against *Culex quinquefasciatus* larvae compared to the methanolic leaf extract and waste product of essential oil.

Different concentrations of each plant sample (25µl, 50µl, 75µl, 100µl) were assessed for larvicidal activity. At the lowest concentration of 25µl, none of the three plant samples showed larvicidal activity at 24 hours. Similarly, except for the pure oil sample, none of the samples displayed positive activity towards the larvae after 48 hours.

As the concentration of the samples increased, there was a corresponding increase in larvicidal activity observed. These findings highlight the potential of *Gymnacranthera canarica* extracts and essential oils as larvicidal agents against *Culex quinquefasciatus* larvae, with the pure leaf essential oil demonstrating the most promising activity.

Table 4.20: Effect of leaf sample of *Gymnacranthera canarica* against 3rd instar *Culex quinque fasciatus* larvae.

Sr. No.	Name of the Plant	*Gymnacranthera canarica*
1	Name of the sample used	Pure essential oil waste product of essential oil Methanolic leaf extract
2	Volume of water	250 mL
3	Number of larvae exposed in each bowl	25
4	Replicates	3
5	Concentration used	25 mL, 50 mL, 75 mL, 100 mL

Table 4.21: Larvicidal activity of leaf essential oil of *Gymnacranthera canarica* against 3rd instar of *Culex quinquefasciatus* larvae after 24 hours of exposure.

Sr. no.	Dose (µL)	Number of dead larvae			% mortality
		R1	R2	R3	
1	25 µl	0	0	0	0
2	50 µl	3	3	4	13.3%
3	75 µl	3	4	4	14.6%
4	100 µl	5	6	8	25.3%
5	Control	0	0	0	0

Table 4.22: Larvicidal activity of leaf essential oil of *Gymnacranthera canarica* again 3rd instar of *Culex quinquefaciatus* larvae after 48 hours of exposure.

Sr. no.	Dose (µL)	Number of dead larvae			% mortality
		R1	R2	R3	
1	25 µl	0	0	0	2.6%
2	50 µl	18	16	18	69.3%
3	75 µl	20	18	21	78.6%
4	100 µl	22	24	26	96%
5	Control	0	0	0	0

Table 4.23: Larvicidal activity of leaf waste product of essential oil of *Gymnacranthera canarica* against 3rd instar of *Culex quinquefaciatus* larvae after 24 hours of exposure.

Sr. no.	Dose (µL)	Number of dead larvae			% mortality
		R1	R2	R3	
1	25 µl	0	0	0	0
2	50 µl	1	1	2	5.3%
3	75 µl	2	2	2	8%
4	100 µl	2	2	4	10.6%
5	Control	0	0	0	0

Table 4.24: Larvicidal activity of leaf waste product of essential oil of *Gymnacranthera canarica* against 3rd instar of *Culex quinquefaciatus* larvae after 48 hours of exposure.

Sr. no.	Dose (µL)	Number of dead larvae			% mortality
		R1	R2	R3	
1	25 µl	0	0	0	0
2	50 µl	4	5	4	17.30
3	75 µl	8	7	7	29.30
4	100 µl	10	11	13	45.30
5	Control	0	0	0	0

Table 4.25: Larvicidal activity of methanolic leaf extract o*f Gymnacranthera canarica* against 3rd instar of *Culex quinquefaciatus* larvae after 24 hours of exposure.

Sr. no.	Dose (µL)	Number of dead larvae			% mortality
		R1	R2	R3	
1	25 µl	0	0	0	0
2	50 µl	0	0	0	0
3	75 µl	0	0	1	1.3%
4	100 µl	0	1	2	4%
5	Control	0	0	0	0

Table 4.26: Larvicidal activity of methanolic leaf extract of *Gymnacranthera canarica* against 3rd instar of *Culex quinquefaciatus* larvae after 48 hours of exposure.

Sr. no.	Dose (µL)	Number of dead larvae			% mortality
		R1	R2	R3	
1	25 µl	0	0	0	0
2	50 µl	0	1	1	2.6%
3	75 µl	1	2	1	5.3%
4	100 µl	3	3	4	13.3%
5	Control	0	0	0	0

Table 4.27: LC_{50} Value and LC_{30} Value

Sr. No.	Treatment	LC_{50} Value	LC_{30} Value
1	Essential oil (24 hrs)	50.77922078	30.77922078
2	Essential oil (48 hrs)	50.37024221	30.37024221
3	Waste liquid product (24 hrs)	50.95238095	30.95238095
4	Waste liquid product (48 hrs)	50.76470588	30.76470588
5	Leaf extract (24 hrs)	51.53846154	30.53846154
6	Leaf extract (48 hrs)	51.26190476	30. 26190476

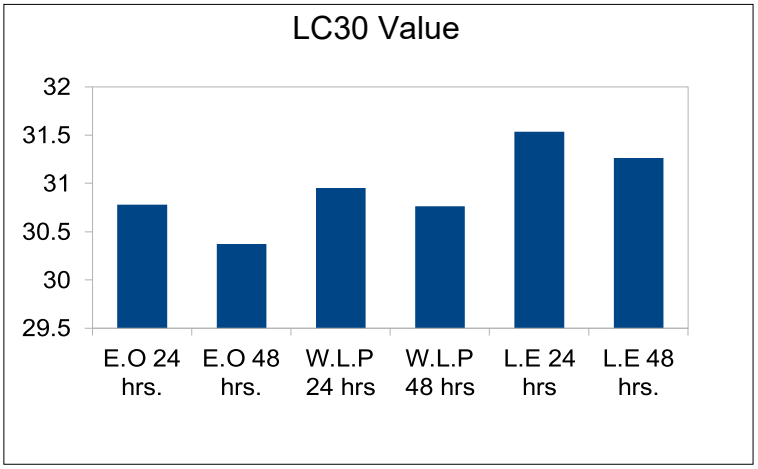


Fig. 4.13 Mosquito larvicidal activity (E.O 24)leaf essential oil after 24 hrs observation, (E.O 48) leaf essential oil after 48 hrs observation, (W.L.P 24) waste liquid remain product during E.O extraction after 24 hrs observation, (W.L.P 48) waste liquid remain product during E.O extraction after 48 hrs observation, (L.E 24) Leaf extract after 24 hrs observation, Leaf extract after 48 hrs observation.

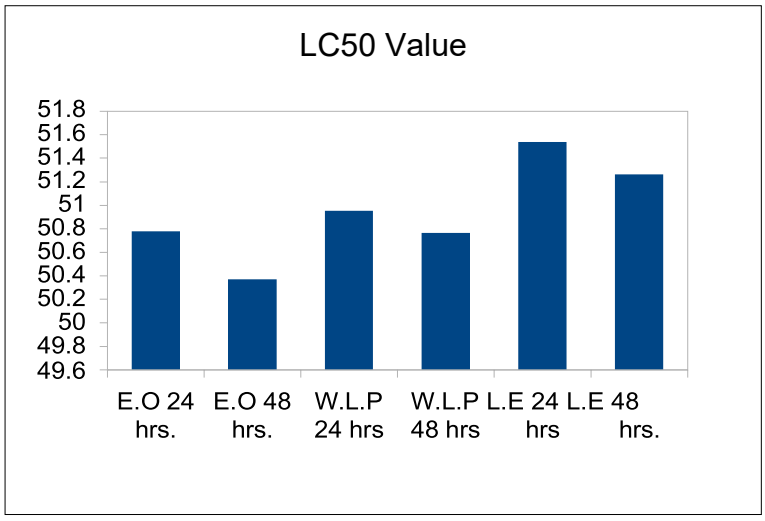


Fig. 4.14 Mosquito larvicidal activity (E.O 24)leaf essential oil after 24 hrs observation, (E.O 48) leaf essential oil after 48 hrs observation, (W.L.P 24) waste liquid remain product during E.O extraction after 24 hrs observation, (W.L.P 48) waste liquid remain product during E.O extraction after 48 hrs observation, (L.E 24) Leaf extract after 24 hrs observation, Leaf extract after 48 hrs observation.

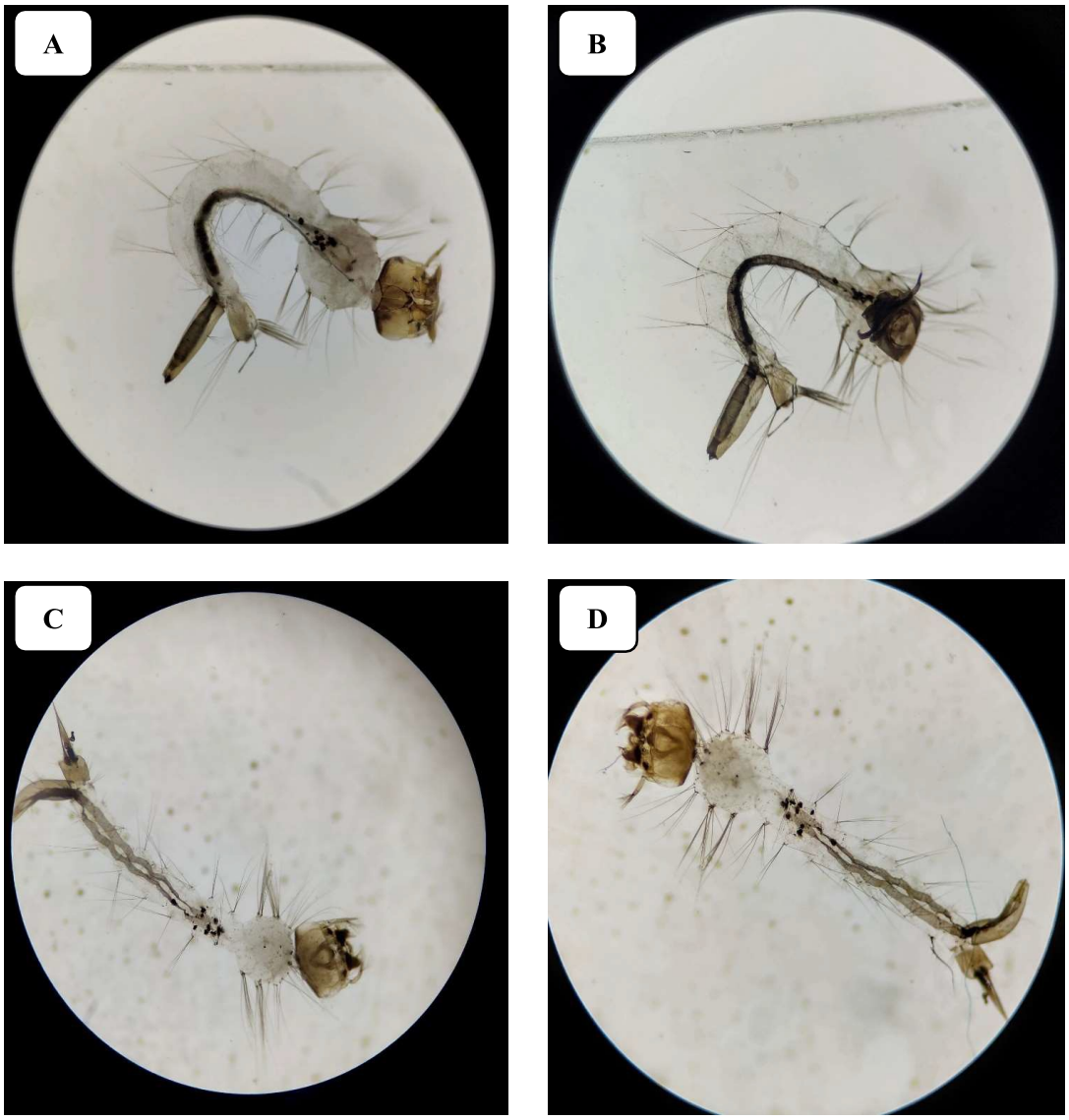


Plate 4.9: Larvicidal activity (a) 3rd instar *Culex quinquefasciatus* (untreated), (b) 3rd instar *Culex quinquefasciatus* exposed to methanolic leaf extract, (c) 3rd instar *Culex quinquefasciatus* exposed to essential oil, (d) 3rd instar *Culex quinquefasciatus* exposed to waste liquid product remain during essential oil.

4.8. Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS analysis of leaf essential oil was carried out in Jawaharlal Nehru Tropical Botanic Garden and Research Institute (TBGRI), Kerala, India. The oil extracted was moderately yellow in colour with a strong herbal aroma. Gas Chromatography Mass Spectrometry (GC-MS) analysis of the leaf essential oil from *Gymnacranthera canarica* revealed the presence of 53 compounds. Among these, six compounds exhibited the highest peak areas, namely Patchoulene, δ-Cadinene, Aromadendrane, Neryl isovalerate, γ-Cadinene, and Isocaryophyllene. Patchoulene demonstrated the highest peak, with a retention time of 32.65, an area percentage of 14.32, and a height percentage of 4.35.

1. **Patchoulene:** Identified with the molecular formula C15H24, Patchoulene is known for its various applications including skin conditions, antivirus properties, mood enhancement, and hair care.

2. **δ-Cadinene:** With the molecular formula C15H24, δ-Cadinene is associated with antimicrobial, insecticidal, anticancer, and aromatic properties.

3. **Aromadendrane:** This compound, with the molecular formula C15H24, is linked to antibiotic, anticancer, anti-inflammatory, and antidepressant activities.

4. **Neryl isovalerate:** With the molecular formula C15H26O2, Neryl isovalerate is recognized for its mosquiti repellency and use as a flavour and fragrance agent.

5. **γ-Cadinene:** Also with the molecular formula C15H24, γ-Cadinene exhibits antimicrobial, anticancer, and aromatic properties.

6. **Isocaryophyllene:** This compound, with the molecular formula C16H24, is attributed with various protective and enhancement properties including cardioprotective,

hepatoprotective, gastroprotective, neuroprotective, nephroprotective, cold tolerance enhancement, and antioxidant activities.

These findings highlight the diverse chemical composition of *Gymnacranthera canarica* leaf essential oil, along with the potential applications of its individual compounds in various fields such as medicine, aromatherapy, and pest control.

Fig: 4.14 Graph showing peaks of compounds present in the leaf essential oil of *G canarica*

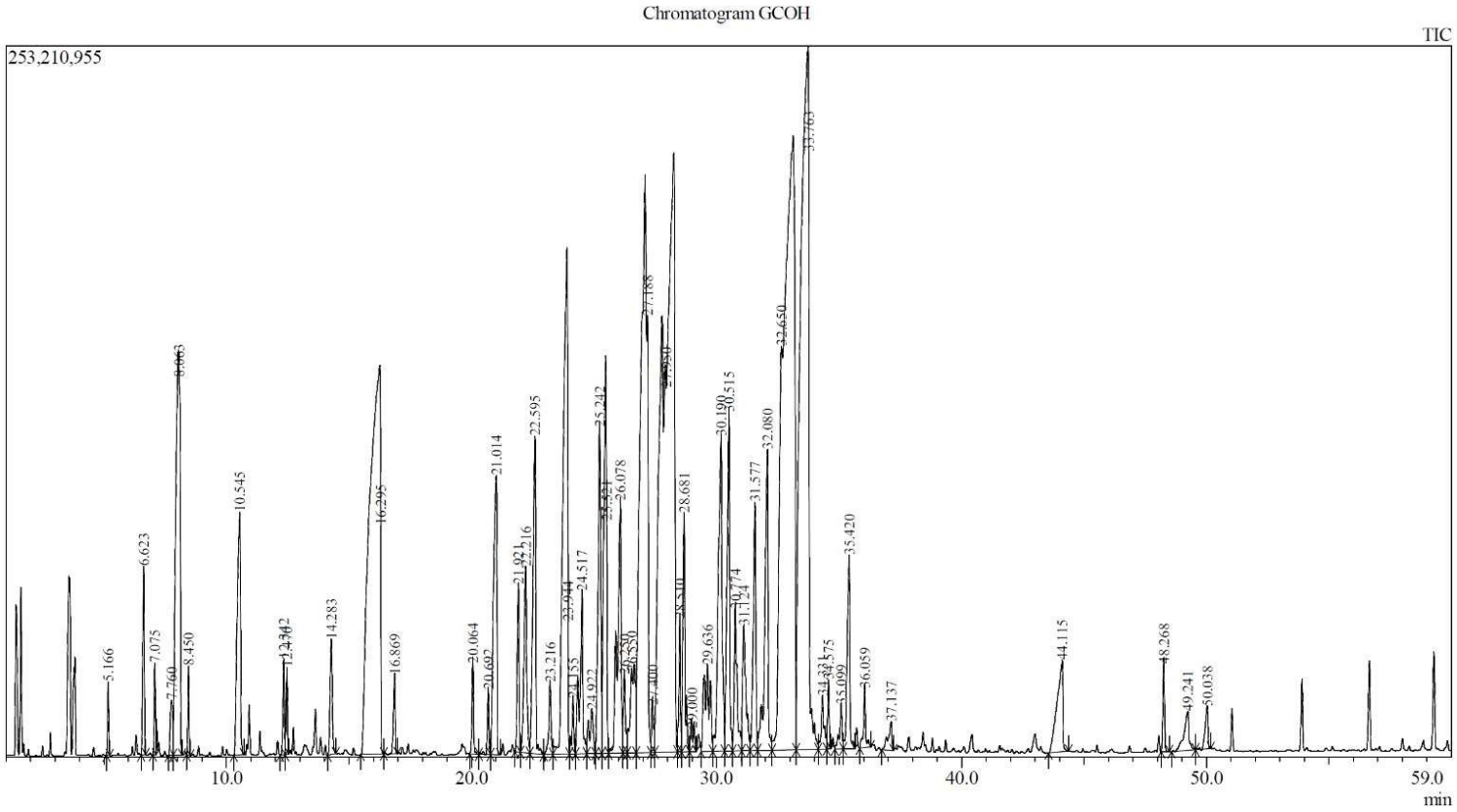


Table No. 4.28: Compounds shown in the leaf essential oil of *G canarica* by GC-MSanalysis.

Peak	R. Time	Area	Area%	Height	Height%	Compound
1	5.166	78287504	0.16	26082463	0.86	α-pinene
2	6.623	287069309	0.58	66955521	2.21	β-pinene
3	7.075	122543247	0.25	32698003	1.08	Cycloheptene
4	7.760	142301871	0.29	19472282	0.64	o-Cymene
5	8.063	1771389093	3.55	133428901	4.41	tetrahydro dicyclopentadiene
6	8.450	98988443	0.20	31794646	1.05	β-Ocimene
7	10.545	798051458	1.60	86100017	2.84	linalool
8	12.342	147621361	0.30	33683611	1.11	Isopulegol
9	12.470	123260039	0.25	30343484	1.00	Citronellal
10	14.283	256499017	0.51	40975034	1.35	α-Terpinyl propionate
11	16.295	4180805074	8.39	97032049	3.21	Geranyl isovalerate
12	16.869	146639755	0.29	27907307	0.92	guaniol
13	20.064	152526546	0.31	31059501	1.03	bicycloelemene
14	20.692	107599717	0.22	22285560	0.74	α-Cubebene
15	21.014	949622543	1.91	97660368	3.23	rhodinol
16	21.921	310213759	0.62	58857525	1.94	α-Yalangene
17	22.216	518922374	1.04	64459702	2.13	Geranyl acetate
18	22.595	895218081	1.80	111367962	3.68	levo-β-Elemene
19	23.216	168447864	0.34	24941304	0.82	α-Gurjunene
20	23.944	2285584197	4.59	73541754	2.43	Isocaryophyllene
21	24.155	116440327	0.23	19850391	0.66	Copaen
22	24.517	510865642	1.02	56096818	1.85	Aromandendrene
23	24.922	174120306	0.35	15772213	0.52	cubebene
24	25.242	884522434	1.77	114531541	3.78	humulene
25	25.521	1136492823	2.28	95862708	3.17	gamma-Muurolene
26	26.078	944557274	1.90	88574901	2.93	gamma-Muurolene
27	26.250	165307467	0.33	28581876	0.94	D-Germacrene
28	26.550	464863965	0.93	29565353	0.98	γ-Cadinene
29	27.188	3921917530	7.87	132528467	4.38	γ-Cadinene
30	27.400	105359527	0.21	18783956	0.62	Helminthogermacrene
31	27.950	6789355344	13.62	131734151	4.35	Amorphene
32	28.510	225328108	0.45	43483489	1.44	α-terpineol
33	28.681	534670763	1.07	80034563	2.64	α-Guajene
34	29.000	96819982	0.19	10234259	0.34	Elemol
35	29.636	490502457	0.98	29078858	0.96	Nerolidol
36	30.190	1204451231	2.42	109755339	3.63	Spathulenol

Peak	R. Time	Area	Area%	Height	Height%	Compound
37	30.515	895287281	1.80	117021150	3.87	α-guaiene
38	30.774	420136577	0.84	51621506	1.71	Viridiflorol
39	31.124	422348603	0.85	44374676	1.47	Ledol
40	31.577	629810616	1.26	85711783	2.83	Zizanene
41	32.080	1004599804	2.02	103260751	3.41	Amorphene
42	32.650	7138764366	14.32	131616608	4.35	Patchoulene
43	33.763	5867321557	11.77	131430898	4.34	Aromadendrane
44	34.331	155456368	0.31	18200121	0.60	Nerolidol
45	34.575	111726959	0.22	23325039	0.77	Shyobunol
46	35.099	113032853	0.23	15904826	0.53	Farnesal
47	35,420	525123770	1.05	67728879	2.24	methyl geranylgeranate
48	36.059	132866230	0.27	20225806	0.67	Farnesal
49	37.137	105677148	0.21	9720703	0.32	Myristic acid
50	44.115	543150122	1.09	32021784	1.06	Hexadecanoic acid
51	48.268	155024892	0.31	31858411	1.05	Phytol
52	49.241	200585028	0.40	13753844	0.45	Oleic acid
53	50.038	116488722	0.23	14464135	0.48	Stearic acid

Table 4.29: Compounds with the higher peak.

Peak No.	R. Time	Area %	Height%	Compound
42	32.65	14.32	4.35	Patchoulene
31	27.95	13.62	4.35	δ-Cadinene
43	33.763	11.77	4.34	Aromadendrane
11	16.295	8.39	3.21	Neryl isovalerate
29	27.188	7.87	4.38	γ-Cadinene
20	23.944	4.59	2.43	Isocaryophyllene

4.8.1. Compond name: Patchoulene

Molecular Formula: $C_{15}H_{24}$

IUPAC Name: 1,5,11,11-tetramethyltricyclo[6.2.1.02,6]undec-2(6)-ene

Uses:

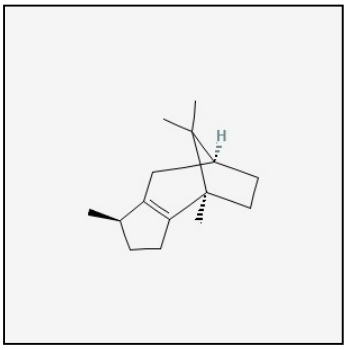
Skin conditions (Malhotra et al., 2021)

Antivirus agent (Isnaini et al., 2022)

Mood enhancement property (Astuti et al., 2022)

Hair care properties (Muhammad et al., 2022)

Structure:



4.8.2. Compound name: δ-Cadinene

Molecular Formula: $C_{15}H_{24}$

IUPAC Name: Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyle-1-(1-methylethyle)-(1S-Cis)

Uses:

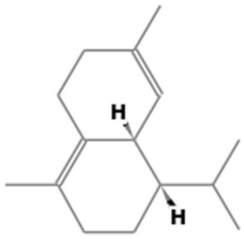
Atimicrobial property (Perez-Lopez et al., 2011)

Insecticidal property (Adjalian et al., 2015)

Anticancer and antiproliferative property (Ramadan et al., 2015)

Aromatic property (Kundu et al., 2013)

Structure:



4.8.3. Compound name: Aromadendrane

Molecular formula: $C_{15}H_{24}$

IUPAC Name: 1,1,7-trimethyl-4-methylidene-2,3,4a,5,6,7,7a,7b-octahydro-1aH cyclopropa[e]azulene

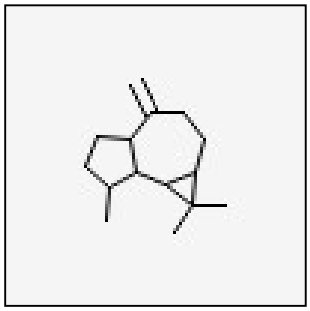
Uses:

Antibiotic property (Mulyaningsih et al., 2010)

Anticancer, anti-inflammatory property (Pavithra et al., 2018)

Antidepressant activity(Jalali et al., 2018)

Structure:



.8.4. Compound name: Neryl isovalerate

Molecular formula: $C_{15}H_{26}O_2$

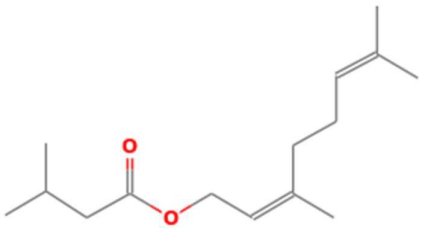
IUPAC Name: Butanoic acid, 3-methyl-, 3,7-dimethyl-2,6-octadienyl ester

Uses:

Mosquiti rep**4**ellency (Klimavicz et al., 2018)

Flavour and fragrance agent (Muller & Bachbauer 2011)

Structure:



4.8.5. Compound name: γ-Cadinene

Molecular formula: $C_{15}H_{24}$

IUPAC Name: Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4aβ,8aα)

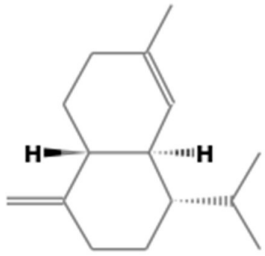
Uses:

Antimicrobial property (Perez-Lopez et al., 2011)

Anticancer and antiproliferative property (Ramadan et al., 2015)

Aromatic property (Kundu et al., 2013)

Structure:



4.8.6. Compound name: Isocaryophyllene

Molecular formula: $C_{16}H_{24}$

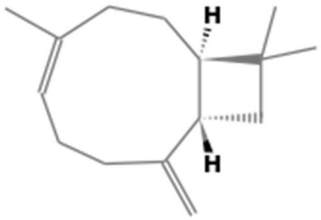
IUPAC Name: Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, (1R,4E,9S)

Uses:

Cardioprotective, hepatoprotective, gastroprotective, neuroprotective, nephroprotective, antioxidant, anti-inflammatory, antimicrobial and immune-modulator. (Sharma et al., 2017)

Cold Tolerance Enhancement. (Eeswara et al., 2023)

Structure:



4.9 Antimicrobial Activity

The antimicrobial activity of *Gymnacranthera canarica* leaf methanolic and ethanolic extract and leaf essential oil was evaluated against four pathogenic bacterial strains: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. After 24 hours of incubation, the leaf methanolic and ehanolic extract did not exhibit any zone of clearance against any of the bacterial cultures. However, the leaf essential oil displayed minimal zone of clearance, particularly notable against *Salmonella typhi*. Interestingly, a higher zone of clearance was observed in *Staphylococcus aureus* compared to *Salmonella typhi*, indicating a potential differential susceptibility among the tested bacterial strains. Notably, no antimicrobial activity was observed against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in both the leaf extract and essential oil samples.

The results from the disc diffusion method are summarized in (Table. 4.30). The leaf extract showed no zone of clearance against any of the tested bacterial strains, while the essential oil exhibited a small zone of clearance against *Salmonella typhi* and *Staphylococcus aureus*. In the control groups, which included the solvent or non-active substances, no zone of clearance was observed against any of the bacterial strains.

Table 4.30: Antimicrobial activity using disc diffusion method.

Sr. No.	Sample	Zone of clearance in (cm)			
		S. aureus	*S. typhi*	*P. aeruginosa*	*K. pneumoniae*
1	Ethanolic leaf extract	0	0	0	0
2	Methanolic leaf extract	0	0	0	0
3	Essential oil	1.1 cm	1.1 cm	0	0
4	Control + essential oil	2.4 cm	1.1 cm	0	0
5	Control	0	0	0	0

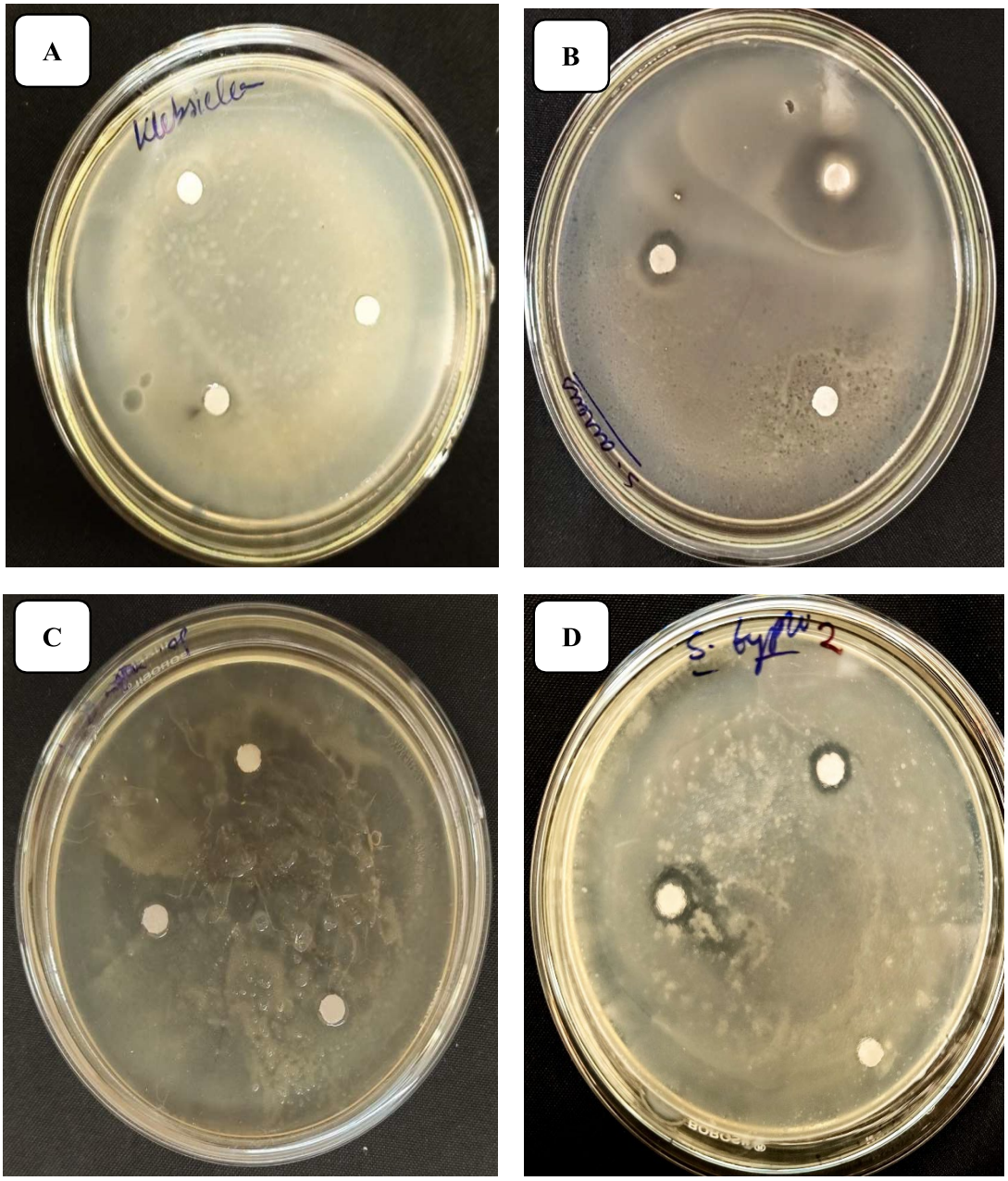


Plate 4.10: Antimicrobial tests using (a) leaf essential oil, (b) control + leaf essential oil, (e) control against; A. Klebsiella pneumoniae, B.*Staphylococcus aureus,* C. *Pseudomonas aeruginosa,* (D) *Salmonella typhi.*

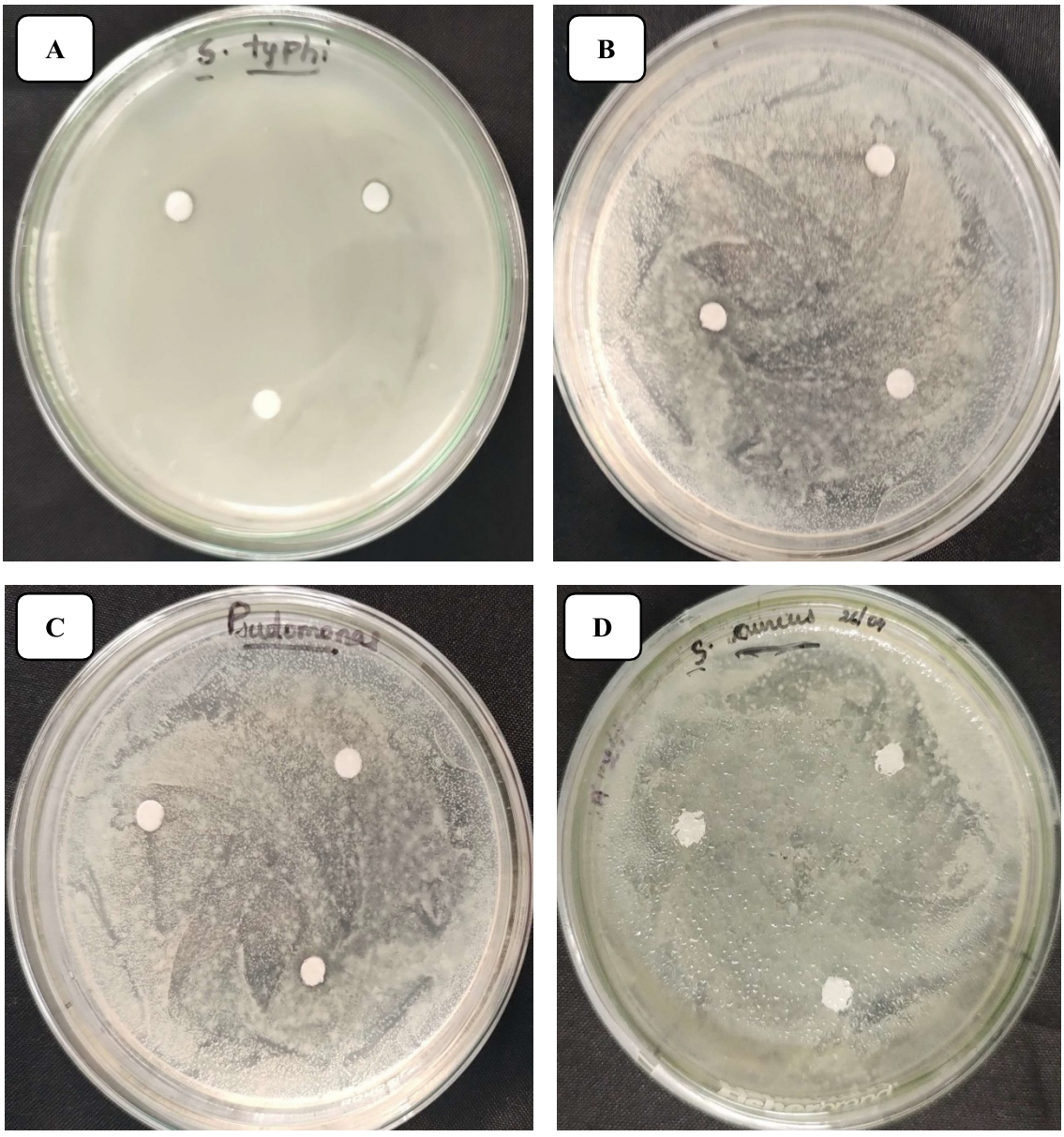


Plate 4.9: Antimicrobial tests using (a) ethanolic leaf extract (b)methanolic leaf extract, (c) control against; A. *Salmonella typh,* B. *Klebsiella pneumonia*e, C. *Pseudomonas aeruginosa,* (D) *Staphylococcus aureus.*

CHAPTER 5: DISCUSSION

The phytochemical analysis of *Gymnacranthera canarica* extracts revealed the presence of various bioactive compounds, including alkaloids, phenolic compounds, flavonoids, protein and amino acids, glycosides, saponins, and carbohydrates. These phytoconstituents were identified using distilled water, methanol, and ethanol extracts, along with a series of phytochemical tests and thin layer chromatography. Among these, phenolic compounds and flavonoids emerged as particularly noteworthy due to their recognized antioxidant properties and potential as phytomedicine sources (Baba and Malik, 2015).

Phenolic compounds play a crucial role in antioxidant activity by neutralizing free radicals through the donation of hydrogen atoms, thereby generating stable phenoxyl radicals. Consequently, determining the total phenolic content (TPC) is pivotal in assessing the antioxidant potential of plant extracts (Hatano *et al*., 1989). In this study, the TPC of *Gymnacranthera canarica* leaf and bark extracts, obtained using ethanol and methanol, respectively, was evaluated. Spectrophotometric analysis following standard procedures and calibration curves revealed that the methanolic extracts exhibited higher TPC compared to the ethanolic extracts.

These findings align with previous research by Anusha *et al*. (2019) and underscore the effectiveness of 100% methanol as an extraction solvent for TPC from *Gymnacranthera canarica* (Gagana & Bhat, 2022). The differential solubility of chemical constituents in solvents with varying polarities likely contributes to this observation. Additionally, the higher TPC in methanolic extracts may indicate a greater abundance of phenolic compounds, highlighting the potential health benefits associated with these extracts.

The results of this study emphasize the importance of solvent selection in phytochemical extraction and emphasize the antioxidant potential of *Gymnacranthera canarica* extracts, particularly those obtained with methanol. Further exploration into the specific phenolic compounds present in these extracts and their individual contributions to antioxidant activity could provide valuable insights for the development of novel antioxidant-rich pharmaceuticals or nutraceuticals.

Flavonoids, as noted by Harborne & Williams (2000), are highly diverse polyphenolic compounds with significant biological functions, including radical scavenging, which is crucial for plant growth, defence mechanisms, and response to stressors. The presence of flavonoids in *Gymnacranthera canarica* extracts, particularly in the methanolic extracts of leaves and bark, further supports their role as potent antioxidants.

Geetha *et al*. (2003) and Shimoi *et al*. (1989) have elucidated that flavonoids, including flavones, flavonols, and condensed tannins, exert antioxidant effects through their free hydroxyl (OH) groups, particularly at position 3. This property enables them to act as antioxidants both in vivo and in vitro. Consistent with these findings, the current study revealed higher total flavonoid content (TFC) in methanolic extracts compared to ethanolic extracts of *Gymnacranthera canarica*, corroborating similar observations made by Gagana & Bhat (2022).

Moreover, the association between phenolic content and antioxidant activity has been extensively explored in the literature. Kumar *et al*. (2008) demonstrated a significant correlation between DPPH radical scavenging activity and total phenolic content (TPC) in extracts of Kappaphycus alvarezii. Similarly, Katalinic *et al*. (2006) observed a linear relationship between antioxidant activity and polyphenol concentration, indicating the importance of phenolic compounds in antioxidant function. In line with these findings, the current study found that the methanolic extracts of Gymnacranthera canarica leaves and bark exhibited the highest antioxidant activity, as evidenced by DPPH assay results (Table 4.17 and Fig. 4.12).

The consistency between antioxidant activity and TPC values further strengthens the argument for the role of phenolic compounds in the antioxidant potential of *Gymnacranthera canarica* extracts. These findings align with previous research on other plant species, such as Myristica fragrans (Simamora *et al*., 2018), further supporting the notion of a direct relationship between total phenolics and antioxidant activity. Additionally, the negligible antioxidant activity observed in the methanolic seed extracts of *Gymnacranthera canarica*, as reported by Anusha *et al*. (2019), underscores the importance of phenolic compounds in determining antioxidant efficacy.

The investigation into the essential oil components of *Gymnacranthera canarica* leaf essential oil through gas chromatography revealed a diverse array of chemicals, totaling 53 different compounds. Among these, patchoulene and δ-cadinene emerged as the dominant compounds, followed by aromadendrane, neryl isovalerate, γ-cadinene, and isocaryophyllene. These findings align with previous research on essential oils, such as *Myristica malabarica*, which similarly showcased a rich composition of terpenoids, including δ-cadinene (Sabula *et al*., 2007). Discrepancies in chemical composition across different studies, as noted by Zachariah *et al*. (2008), underscore the influence of geographical factors on secondary metabolite production.

Antimicrobial activity of essential oils is often attributed to compounds like α-pinene and β-pinene, which possess notable antimicrobial properties (Sabulal *et al*., 2006). In the present study, the leaf essential oil of Gymnacranthera canarica exhibited significant antimicrobial activity against *Staphylococcus aureus*, and *Salmonela typhi* consistent with observations from similar studies on *Myristica fragrans* (Thileepan *et al*., 2017). Interestingly, no activity was observed against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* a result consistent with previous investigations into antibacterial activity of *Gymnacranthera canarica* (Anusha *et al*., 2019). This pattern of antimicrobial activity suggests that *Gymnacranthera canarica* shares some characteristics with other members of the Myristicaceae family, such as *Myristica fragrans*, highlighting the potential for further exploration of their pharmacological properties.

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