PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *Ipomoea muricata* (L.) Jacq. LEAF EXTRACT

SILDA VAS

REGISTER NUMBER: AM23BOT009

DEPARTMENT OF BOTANY AND CENTER FOR RESEARCH

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SILDA VAS

AM23BOT009

DEPARTMENT OF BOTANY

ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

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ST.TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM

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Author Name	SILDA VAS
Course of Study	M.Sc. Botany
Name of Guide	Ms. Rishika P.S.
Department	Department of Botany & Centre for Research
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This is to certify that the dissertation entitled "Phytochemical Analysis and Antibacterial Activity of *Ipomoea muricata* (L.) Jacq. Leaf extract" submitted in partial fulfillment of the requirements for the award of the M.Sc. Degree of Mahatma Gandhi University, Kottayam is an authentic work carried out by Silda Vas (Reg No: AM23BOT009) under the supervision and guidance of Miss. Rishika P.S. I, further certify that no part of the work embodied in the dissertation has been submitted for the award of any other degree or diploma.

Miss Rishika P.S.
(Supervising Teacher)
Guest Faculty
Dept. of Botany
St. Teresa's College (Autonomous),
Ernakulam.

Smt. I.K. Nishitha
Head of the Department of Botany
St. Teresa's College (Autonomous),
Ernakulam.

St. Teresa's College (Autonomous) DEPARTMENT OF BOTANY Ernakulam

Place: Ernakulam
Date: 2/5/25

Examiners
1.
2. 05/05/25

PRINCY MOL. A. P
Assistant Professor
Department of Botany
Sacred Heart College
Thevara, Kochi - 682 013

DECLARATION

I hereby declare that the work which is being presented in the dissertation, entitled **"PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *Ipomoea muricata* (L.) Jacq. LEAF EXTRACT"** in fulfilment of the requirements for the award of the degree of Master of Science in Botany and submitted to St. Teresa's College (Autonomous), Ernakulam is an authentic record of my own work carried out during M. Sc. Period under the supervision of Miss. Rishika P.S.

The matter embodied in this dissertation has not been submitted by me for the award of any other degree of this or any other University/institute.

Silda Vas

Place: Ernakulam

Date: 2/5/25

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Place: Ernakulam

Date: Silda Vas

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ABSTRACT

Ipomoea muricata (L.) Jacq. is a vigorous annual climbing or trailing plant and grows primarily in the seasonally dry tropical biome.This plant belongs to the morning glory family Convolvulaceae. The plant is acknowledged for its potential therapeutic qualities and has numerous applications in traditional and folk medicine.The present study is focused on the qualitative and quantitative analysis of phyochemicals and also the antibacterial activity of *Ipomoea muricata* leaf extract.The leaves was collected, dried,made into powder,extracted with ethanol using soxhlet apparatus. Only carbohydrates were detected in the extract during the qualitative screening of phenols, flavonoids, tannins, alkaloids, and carbohydrates.The quantitative analysis of the same phytochemicals showed varying levels of bioactivity ,phenolic content was 0.413 mg/mL, and activity was observed, indicating the presence of phenolic compounds with potential antioxidant properties.The flavonoid content was 0.322 mg/mL,without any significant activity. Tannins were present at 0.223 mg/mL, with activity observed, implying possible antioxidant role. The alkaloid content was 0.149 mg/mL, but no activity was detected. The carbohydrate content was high at 1.409 mg/mL, with observed activity, suggesting the presence of reducing sugars that may influence overall bioactivity. In summary, the leaf extract exhibited notable activity for phenolics, tannins, and carbohydrates, while flavonoids and alkaloids showed little or no bioactivity.The anti-bacterial study of extract screened against *Staphylococcus aureus* and *Escherichia coli* using disc diffusion method does not exhibit antibacterial property.

INTRODUCTION

Convolvulaceae are a family of upright herbs, trees, shrubs, and twining vines that belong to the morning glory family. They have regular pentamerous blooms with plaited corollas and alternating leaves. Although 90% of the Convolvulaceae species are found in the tropics, the family has a cosmopolitan distribution (Yadav et al .,2018).Except for the higher montane zone, the Indian subcontinent is home to Convolvulaceae species (JRI Wood et al.,2022). *Ipomoea muricata* (L.) Jacq often known as lavender moonvine, is a climbing vine in the genus Ipomoea. People in its native and wider area utilize its many components as food, medicine, and poison. Clove bean (*I.muricata*) is one of the most important underexploited vegetables cultivated mainly for its fruits and thickened pedicels. The clove bean is seen growing from sea level as in western coasts of Kerala to an elevation of 1700 m in the Himalayas (SJ Salikutty et al.,2008).

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Solanales
Family	Convolvulaceae
Genus	*Ipomoea*
Species	*Ipomoea muricata*

In the Morning Glory family (convolvulaceae), the lavender moonvine is a perennial herbaceous vine that was imported. It has long twining vines with alternating heart-shaped leaves. The vines can reach a maximum length of fifteen feet. The stem is coated in tubercules, and the sap is milky. At night, the pale lavender blooms open, and shortly after sunrise, they close. Salverform flowers have a long, thin tube with a flat, enlarged limb. With a trellis or arbor to climb on, it thrives in full

sun or partial shade.The ideal temperature range for *Ipomoea muricata* seeds is between 70°F and 85°F (21°C and 29°C). *Ipomoea muricata* can live for two to five years in ideal circumstances. It is a plant that grows quickly; it usually reaches maturity in 3–4 years and averages 3–6 feet in height. It has a fibrous root structure that can extend widely but is usually shallow. The poisonous components of *Ipomoea muricata* are mostly found in its leaves and seeds. *I. muricata* is a decorative plant that is utilized in landscaping and gardens.It is drought tolerant and can be used to attract butterflies and bees.containers, as a border plant, and as a ground cover.The leaves are petiolate, measuring 7-18 cm 6-17 cm, and can be ovate or 3-lobed, cordate with rounded auricles, with a glabrous apex and length of petiole 3-15 cm.1-2 inflorescences (–5) -flowered, pedunculate cymes; peduncles, which range in length from 2.5 to 20 cm, are typically long, but occasionally have soft spines; bracteoles caducous; robust, greatly swelled ascending pedicels, 1.5 to 4.5 cm;The fruit's sepals are uneven, glabrous, accrescent, white with a green midrib, narrowly oval on the outside (10–14 mm) and attenuate into a point up to 7 mm long, while the inner 7–12 mm are broadly ovate and suddenly narrow to an awn that is 3–4 mm long.The glabrous, dark lilac corolla is 5–6 cm long and narrowly cylindrical below, but it widens to 10 mm below the limb, which is about 4 cm in diameter, spreading, and unlobed. Ovoid, glabrous, rostrate capsule that is 1.5–2 cm long and wide, with a persistent style that is about 3 mm long.seeds are glabrous, 8-10 mm long. Rarely plentiful but dispersed throughout the tropics. Conservation Least Concern (LC). This plant blooms between April and May, toward the end of the wet season, just as other annuals (Wood et al.2015).Parts of the plant are consumed as vegetables in China and India. In the Indian state of Kerala, the plant is known as nithya vazhutana, or clove bean, and the inflated peduncles are eaten, usually as a thoran or pan-fried .It has aphrodisiac, purgative, analgesic, febrifuge, antiseptic, antibacterial, and antifungal properties. Seeds have antimicrobial, hypotensive, psychoactive, cardiac depressive, spasmolytic, and intestinal-stimulating qualities. The plant sap is used as an insecticide in the Philippines, and the seeds are used as vulnery and are thought to be a very effective antidotal treatment for poisoning. This plant is used to kill bedbugs using its juice. Cuts, blisters from burns, and chronic and gangrenous lesions are among the skin conditions that the seeds, stems, and leaves are claimed to be useful in treating. It is employed in the treatment of the gastrointestinal tract (Dwivedi et al., 2006). A recent study claims that *I.muricata* can also be used as a cardiac depressant to treat Alzheimer's disease (Ohja.G et al.,2016).Seeds of *I. muricata* are used as a traditional carminative and laxative remedy (Ono et al.,2024).The crude medication of

I. muricata is used to treat pharyngitis with glycerol preparations and otitis externa with an otic preparation(Ysrael,2003).Although the pharmacological components of *I.muricata* are still unknown, it exhibits anti-inflammatory, analgesic, hemostatic, and anti-oxidant properties (Peng J.et al.,2025). Ipalbidine has been given analgesic qualities (Honda et al., 2003). The seeds of *I. muricata* were also found to contain the indolizidine alkaloid E-ipomine (Dawidar et al., 1977). The seeds yielded the imidazolidine alkaloidal ipomine, ipalbidine, ipalbinium, and ipalbine. In addition to these alkaloids, muricatins have antimutagenic properties. (Tewari & Misra, 1953). Madhya Pradesh's rural residents utilize the immature flower pedicels as a digestive aid and appetizer. Resin glycosides have the ability to reverse multidrug resistance (MDR) and are a significant chemotaxonomic marker of the Convolvulaceae family (Wang WQ, 2018). One of its main alkaloids, lysergol, has uterine and intestinal stimulatory, psychotropic, and hypotensive effects (Patil S, 2013). The synthesis of the contemporary anti-Parkinson medication "Cabergoline" also uses lysergol as a starting material (Ashford et al.,2002).*Ipomoea muricata* seeds' crude ethanolic extract was tested for antibacterial efficacy against 45 clinically isolated microorganisms. Strong antibacterial action against *Staphylococcus aureus* isolated from wounds, abscesses, and wound discharge was demonstrated by a 30% hydroalcoholic extract of seeds. The extract shown high action against S. saprophyticus isolated from urine and wounds, as well as activity against *B. subtilis* from corneal scraping, peritoneal fluid, urine, and wound discharge. Gram-negative clinical isolates and *S. aureus* from seminal fluid, endotracheal sputum, throat swab, blood, placental swab, and urine were resistant to the crude extract (Carmelita C. et al.,2012). *Ipomoea's* rich phytochemical makeup and varied pharmacological characteristics make them promising for use in future treatments. Potential applications in fields such as anti-inflammatory, anti-fungal, hepatoprotective, anti-diabetic, and anti-cancer treatment are suggested by both traditional uses and modern research.

REVIEW OF LITERATURE

Plants are a major source of synthetic substances that are used in medicine to treat a variety of ailments (Qin-Feng ZH & Qin-Shi ZH.,2019). Plant-based chemicals form the foundation of contemporary medicine and have been utilized for medicinal purposes since the dawn of humanity (Solowey E. et al.,2014).Due to their high chemical diversity,natural products like plant extracts offer a wealth of chances to find new medications, either as pure chemicals or as standardized extracts (Cos P. et al.,2006). Numerous plant species have shown notable pharmacological properties in both human and animal research, including cytotoxic, antioxidant,antimicrobial, hepatoprotective, immunomodulatory, analgesic, antiproliferative, anti-inflammatory, hypoglycemic, hypotensive, hypolipidemic, diuretic, etc (Nugroho et al. 2020; Putra et al. 2020; Ray and Saini 2021).Furthermore, these plants are rich in anthraquinone, flavonoids, alkaloids, phenols, saponins, tannins, cardiac glycosides, steroids, and other compounds.Many plant parts, such as leaves, bark, seeds, seed coats, roots, flowers, and pulps, can be used to make phytochemical.Phytochemicals derived from medicinal plants have therapeutic significance and may be a source of medicinal medicines. In order to find new biomolecules that can be employed directly or as lead molecules to create more potent molecules, the pharmaceutical industry relies heavily on the qualitative and quantitative evaluation of phytochemical extraction from medicinal plants (Ingle KP et al.,2017). To identify phytochemicals, clarify their structural formulas, and unravel their biosynthetic processes, modern analytical techniques like electrophoresis, chromatography, enzymology, and isotope techniques have been employed (Hussein et al., 2018; Okada et al., 2010).Plants make a significant contribution to a variety of businesses,including fine chemicals, cosmetics, medicines, medications, and industrial raw materials.Medicinal herbs have demonstrated their ability to treat a variety of fatal illnesses, such as cancer and illnesses linked to viral infections, such as AIDS and hepatitis (R.A Dar et al.,2017). Dietary fibers, polysaccharides, carotenoids, polyphenols, isoprenoids, phytosterols, saponins, and other phytochemicals have been linked in recent research to a number of health advantages, including the prevention of diabetes, obesity, cancer, and cardiovascular disorders. Because of this, phytochemicals have become more and more popular.These days, a variety of disorders are treated or prevented with the help of foods that include phytochemicals as a component (functional foods) and phytochemicals in concentrated

form (nutraceuticals). These phytochemicals' structural stability and purity determine their potential health benefits. The phytochemical's matrix, extraction technique, solvent, temperature, and extraction time all affect the yield, purity, and structural stability of the recovered phytochemicals (Kumar A.et al.,2023). Because of the usage of the "more powerful and potent synthetic drug," phytomedicine nearly became extinct in the first half of the twenty-first century. Due to the many negative consequences of modern medications, however, the benefits of medicinal plants are once again being recognized, as some of them have been shown to be just as effective as synthetic medications with fewer or no negative effects and contraindications (Mamta S.et al.,2016).

The Morning Glory family, Convolvulaceae, includes roughly 59 genera and 1,600 species. It includes herbaceous to woody vines, less common herbs, shrubs, and infrequently trees. It also has regular pentamerous flowers with plaited corollas and alternating leaves (Yadav et al., 2018). The two main genera, Ipomoea and Convolvulus, include over one-third of the species .Typically, the family produces funnel-shaped blooms having five fused petals and five sepals, with the milky sap-filled stems(Sahid & Rao 2016).One of the most obvious anatomical features of the Convolvulaceae is the presence of cells in the plant roots and foliar tissues that release resin glycosides. According to Wagner (1973), these glycoresins are a significant chemotaxonomic marker of this family.Convolvulaceae have a wide range of phytotherapeutic and medicinal uses, which is indicative of their phytochemical diversity and variety of alkaloids (Eich, 2008). Numerous plants in the Convolvulaceae family exhibit chemicals with antidiabetic and wound-healing properties.When combined with antimicrobial therapy, these chemicals can help diabetic patients to heal their wounds and lessen the risk of adverse responses and drug resistance (P Ambika , 2019).

Among the Convolvulaceae, the genus *Ipomoea* has the most species, with roughly 500–600 species (Austin & Huáman, 1996).It has a pantropical distribution and includes herbs, shrubs, vines, lianas and trees (Wood & et al., 2020).This genus has been utilized for agricultural, ceremonial, medical, and nutritional purposes.Numerous illnesses, including exhaustion, inflammation, diabetes, hypertension, constipation, arthritis, rheumatism, meningitis, and hydrocephaly, are treated with these species. Several of these species exhibited hypoglycemic, analgesic, anticoagulant, antibacterial, and anticancer properties (Srivastava D.2017).Several

Ipomoea species are used to treat illnesses, but the most popular application is as a purgative to relieve constipation (Pereda-Miranda and Bah 2003).*I.batatas* is the most well-known nutritious species in this genus and is grown and eaten practically everywhere in the world (Zhao et al., 2005; Bovell-Benjamin, 2007). One of the richest sources of bio-elements including calcium, magnesium, iron, zinc, and copper, *I.aquatica* is consumed in many nations, including India (Rao et al.,1990).The ergot-type alkaloids found in some *Ipomoe*a species make them useful hallucinogens. In pre-Columbian periods, several of them were utilized by ancient people to achieve a mental state suitable for divination during religious rituals and magical healing techniques (Daló & Moussatché, 1978; Taber et al., 1963). Furthermore,they have biological activities or therapeutic effects including antibacterial, analgesic, spasmolitic, spasmogenic, hypotensive, psychotomimetic, and anticancer..Benzenoids, flavonoids, anthocyanins, glycolipids, lignans, phenolic compounds, coumarins, norisoprenoids, diterpenes, isocoumarins, ergoline alkaloids, indolizidine alkaloids, nortropane alkaloids, and triterpenes are among the bioactive substances present in plants of this genus (Meira et.al.,2012).

Ipomoea muricata is a yearly vine that grows up to several meters tall and is supported by trellises. Many tiny spiculate protuberances that sprout from the epidermis give stems their grassy and rough appearance. Simple, smooth, soft, and whole, the leaves have a cordate base, rounded lobes, and an apex that gradually narrows into a brief caudex. The blades measure between 4 to 9 cm in width and 6 to 10 cm in length with light green palmately netted veins; the petioles can grow up to 10 cm long.The axillary inflorescence has one to several flowers. The peduncle that holds each beautiful blossom is a little less than the length of the petiole.The actinomorphic, pink-purple, campanulate, funnel-shaped corolla opens at night. The fruit is a capsule with thin walls that dehisces using valves. ovoid, 0.8–1.5 cm in diameter.Each capsule contains 2-4 fonned glabrous,beige colored seeds (Rosalinda C. Solevilla, B.Q. Guevara, et al.,1991).

Since ancient times, the Dominicans of the Philippines have utilized *Ipomoea muricata*, also called "Tonkin," for medicinal purposes. The seeds, stems, and leaves of this plant are believed to be useful in treating a variety of skin conditions, including gangrenous and chronic wounds, cuts, and blisters from burns.Analgesic and antiseptic qualities were demonstrated by *I.muricata* seeds.Additionally, antimicrobial and antifungal substances were found (Ysrael, 2003).The

bioactive alkaloids lysergol and chanoclavine in *Ipomoea muricata* seeds were quantitatively estimated using a reverse-phase high-performance liquid chromatographic approach that is quick, easy, sensitive, gradient, and repeatable.Lysergol,clavine alkaloid, is a bioenhancer for the nutrients and medications (Maurya et al.,2011).By blocking ATPase-dependent efflux pumps, chanoclavine, a tricyclic ergot alkaloid obtained from *Ipomoea muricata*, works in concert with tetracycline to fight MDRE (Multi Drug Resistent Enterobacteriaceae) (Dwivedi G.R,2019). Acetylcholinesterase (AChE) inhibition has been seen as a fortunate treatment approach for a number of neurological conditions, including Alzheimer's disease (AD). In contrast to donepezil, which has demonstrated a potent but transient effect, crude methanolic seed extract of *I. muricata* has been demonstrated to induce modest but long-lasting inhibition of AChE (Santiago L.A. et al.,2015).The seeds yielded the indolizidine alkaloidal ipomine, ipalbidine, ipalbinium, and ipalbine (Exconde et al., 2004).According to ethnomedical data from old books written by Dominican friars, Ipomoea muricata seeds, also called "Pepitas deI maravillosas" or "Pepitas deI Tonkin," were traditionally used to treat a variety of skin conditions, including ulcers, wounds, lesions, boils, burns, insect and snake bites, etc. In order to treat duodenal and stomach ulcers, the powdered seeds were also eaten orally. Additionally, it was said to have anti-inflammatory and analgesic effect (Rosalinda C. Solevilla, B.Q. Guevara, et al.,1991).Researchers isolated two hexahydroindolizine alkaloids from the basic portion of Ipomoea muricata Jacq. seeds cultivated in Senegal: ipalbidine, which was previously identified, and ipomine,C30H35NO8 a novel alkaloid (Dawidar et al.,1977).D-galactose and D-mannose have been found to make up the galactomannan from Ipomoea muricata seeds in a 1:1·8 ratio (S.N. Khanna & P.C. Gupta,1967). The seeds of I. *muricata* are used to make the herbal remedy "Tianqiezi," which is used in China and the Philippines as a snakebite medication and purgative (Wang et al., 2002). Prior research on *I. muricata* has documented the presence of resin glycosides and resin glycosidic acids (Noda et al., 1988a, Noda et al., 1988b, Ono et al., 2016).

secondary metabolites and phytochemical analysis

As a natural defense mechanism against environmental stressors and microbial attacks, different plant sections create phytochemicals known as plant secondary metabolites (PSMs). These substances serve a variety of well-known medicinal purposes in addition to offering protection since they are connected to numerous metabolic processes both inside and outside of plants.

Since ancient times, plant secondary metabolites have been widely used as a component of medications and for medicinal and other culinary applications due to their remarkable biological activity.They are found in extremely small amounts in plant cells, but problems with purity have led to the production of their chemical counterparts and their use in industry. The production and concentration of these PSM are influenced by environmental, morphogenetic, genetic, and processing variables (Kumar.S et al.,2022).Plants produce a vast array of chemical molecules that are divided into primary and secondary metabolites according to their functional groups, chemical class, and biosynthetic origin. Plant secondary metabolism results in the synthesis of secondary metabolites (Geetha.N et al.,2014). Due to their immense therapeutic qualities, they serve as the foundation for the development of numerous pharmaceutical industries.In the past, the only way to identify crude medications or plant extracts was to compare them to the standard descriptions found in the literature. However, as pharmacognosy has advanced, a variety of methods have been used recently to standardize crude drugs (Geetha.N. et al.,2014). Since the plants that produce them might not need them much, plant compounds are considered secondary metabolites. According to Solomon Charles et al. (2013), they are generated in every part of the plant body, including the bark, leaves, stem, root, flower, fruits, seeds, and so on.Alkaloids, flavonoids, terpenoids, tannins, coumarins, quinones, carotenoids, and steroids are examples of secondary metabolites. Even while some natural compounds have unique anticancer properties based on their physicochemical characteristics, every year a number of new secondary metabolites are isolated from plants, offering a source of opportunities to research against malignant diseases. Plant secondary metabolites are often excellent starting points for new therapeutics. Changes in these chemicals' molecular structures, however, are reducing their toxicity and adverse effects while increasing their anticancer efficacy and selectivity as well as their capacities for absorption, distribution, metabolism, and excretion (Bhatti,2022).For the synthesis of compounds with particular activities to treat a variety of health conditions and chronic diseases, it is desirable to know the correlation between the phytoconstituents and the bioactivity of plants (Pandey et al., 2013). . Finding novel sources of molecules that are useful in medicine and industry, such as alkaloids, flavanoids, phenolic compounds, saponins, steroids, tannins, terpenoids, etc., requires the use of phytochemical screening (Akindele and Adeyemi, 2007). Every year, a number of novel cytotoxic secondary metabolites are extracted from plants, opening up new avenues for research into potential treatments for cancer. Secondary metabolites

found in plants can frequently provide as great drug development leads. One calculated method to improve these more promising chemicals' anticancer efficacy and selectivity, as well as their absorption, distribution, metabolism, and excretion qualities, while also reducing their toxicity and adverse effects, is to change their chemical structure (Guo, Z.2017, Yao,H.et al 2017) Particularly, phenolic compounds are regarded as one of the most significant types of naturally occurring antioxidants. One or more hydroxyl groups joined with one or more aromatic rings form their molecules. The chemical classification of polyphenols includes phenolic acids (hydroxybenzoic acids, hydroxycinnamic acids), flavonoids (flavones, flavonols, flavanones, flavanonols, flavanols, anthocyanins), isoflavonoids (isoflavones, coumestans), stilbenes, lignans, and phenolic polymers (proanthocyanidins—condensed tannins and hydrolyzable tannins)(Machu L.et al., 2015).Flavonoids are polyphenolic substances having 15 carbons and two aromatic rings joined by a bridge of three carbons. They are present in all parts of the plant world and are the most abundant phenolic (Harborne 1993). They are found in large concentrations in the fruit's skin and the leaf epidermis. According to Koes et al. (1994) and Pierpoint (2000), flavonoids play a variety of roles in plants, including UV protection, pigmentation, nitrogen-fixing nodule stimulation, and disease resistance. Alkaloids are naturally occurring organic compounds that frequently have a heterocyclic ring with at least one nitrogen atom. According to Kokate et al. (2005), these chemicals are often colorless, although several colored alkaloids have been recorded. For example, betanidin is red, sanguinarine salt is copper-red, and berberine is yellow. Alkaloids can be used to create medications because of their antiproliferative, antibacterial, and antioxidant properties (Qiu et al., 2014). Plant components like leaves, roots, and fruits contain tannin, a bitter, astringent polyphenolic substance made up of a wide variety of oligomers and polymers. In addition to precipitating proteins, they also form complexes with minerals, cellulose, and starch. The molecular weights of tannins vary from 500 to more than 3000. Shapeless, yellowish, light brown aggregates that resemble powder, flakes, or sponges are known as tannins (Minocha S.et al.,2015).

The study of plants, or phytochemical analysis, is a fast-growing field of chemistry with a number of objectives, including classifying substances, isolating bioactive molecules, analyzing their structural makeup, and conducting qualitative and/or quantitative assessments. Optimizing extraction processes is the first step in plant analysis since they are crucial to the extraction results, such as the separation of bioactive chemicals from natural products and the choice of the

tests that will be utilized later in the analyses. Because it is essential to remove the required elements of the intricate natural matrix without harming them, the extraction process is crucial (Mosić et al.,2020). Additionally, a number of variables that impact extraction efficiency include the solvent's characteristics, the raw materials' particle size, the solid/liquid ratio, temperature, and extraction duration (Zhang et al.,2018). Phytochemical extraction solvents can be classified as either organic (acetone, chloroform, butanol, methanol, ethyl acetate, methyl acetate, benzene, hexane, cyclohexane, etc.) or green (water, ethanol, glycerol, fatty oils, ionic liquids, acetic acid, isopropanol, supercritical CO2, deep eutectic solvents, natural deep eutectic solvents, etc.). Water is the most widely used and adaptable of the green solvents. Triterpenes and polyphenols are extracted using ethanol, which has a selective activity. Chloroform and methanol are poisonous by nature. Methanol is used to extract tannins, flavonoids, and saponins, among other substances. Alkaloids and anthocyanins are extracted using chloroform in combination with other solvents including ethanol and fatty acids (A.kumar et al.,2023, Laboukhi-Khorsi et al.,2017, Gopalasatheeskumar et al.,2017). In Soxhlet extraction, a tiny quantity of dry material is put into a thimble, which is subsequently put in a distillation flask that contains solvents such petroleum ether, toluene, and hexane, among others (López-Bascón et al.,2020). It is an automatic continuous extraction method with great efficiency that uses less solvent and time than percolation or maceration. Thermal degradation is more likely to occur at high temperatures and over extended extraction times. Reflux and percolation are both advantages of the Soxhlet extraction technique, which uses siphoning and refluxing principles to continuously extract with new solvent. The extraction process takes around 24 hours at 65–100 °C (Shahab et al., 2020). This extraction is influenced by a number of variables, including temperature, solvent selection, drying rate, sample size reduction, and plant material selection. Since fresh plant materials include active enzymes and too much water might deteriorate the quality of phytochemicals, drying is essential for extraction. Since a lower particle size increases surface area and extraction rate, soxhlet extraction also necessitates size reduction and grinding. For extraction, an inert solvent that is simple to remove should be employed. To select solvents with increasing polarity, for instance, the order of acetone, petroleum ether, ethyl acetate, chloroform, methanol, ethanol, and water is utilized. This procedure is also costly due to the lengthy extraction procedure and the large amount of solvents used. Environmental issues are also raised by the solvent's post-use disposal. The target molecule may potentially undergo thermal degradation as a result of the high

extraction temperatures (Zhang et al., 2018, Patel et al., 2019). Although it is not appropriate for samples with a high moisture content, the Soxhlet extraction method is however a dependable technique for the extraction of fat-soluble phytochemicals (Kumar A. et al.,2023). Conventional Soxhlet has the following most notable benefits: the sample is frequently exposed to new solvent components, which aids in shifting the transfer equilibrium. The system's temperature stays comparatively high because part of the heat from the distillation flask enters the extraction cavity. Following the leaching phase, no filtration is needed. Since the fundamental equipment is cheap, simultaneous extraction in parallel can boost sample throughput. Compared to most of the most recent techniques (microwave extraction, supercritical fluids, etc.), this straightforward technology requires little specialized training, is non-matrix dependent, and has the potential to extract more sample mass (M.D Luque de Castro & L.E García-Ayuso,1998). The main disadvantages of Soxhlet extraction over other traditional methods for preparing solid samples are the lengthy extraction process and the substantial solvent waste, which can lead to further environmental issues in addition to being costly to dispose of. Samples are typically extracted for extended periods of time at the solvent's boiling point, and when thermolabile analytes are present, the potential for thermal breakdown of the target molecules cannot be disregarded. Agitation, which would speed up the process, is not possible with the traditional Soxhlet apparatus. An evaporation/concentration step following the extraction is required due to the significant volume of solvent utilized. The method is difficult to automate and limited to solvent selectivity (M.D Luque de Castro & L.E García-Ayuso,1998).

Phytochemicals can be analyzed both qualitatively and quantitatively by Gas Chromatography-Mass Spectroscopy (GCMS). Gaseous, liquid, and solid materials can all be subjected to GCMS. Prior to examination based on the mass to charge ratio, the samples are first transformed into a gaseous condition. High Performance Liquid Chromatography can be used with substances that dissolve in solvents. Phytochemical separation, detection, and qualitative and quantitative analysis can all be accomplished with high performance thin layer chromatography. Phytochemicals are detected via spectroscopy. The following are commonly employed in phytochemical research. UV- To find out whether the system is conjugated(the coloured compounds such as β- carotene, crocetin are in system of extensively conjugated pi-electrons). IR spectroscopy to determine which functional groups are contained in the chemical. Mass spectroscopy to ascertain the compound's molecular weight and detect the existence of Cl

and Br isotope trends. C-Nuclear Magnetic Resonanse Spectroscopy (NMR) to determine the number of different kinds of carbon atoms in the chemical. H-NMR To determine the number of different kinds of hydrogen atoms in the chemical and the connections between them.

Antibacterial properties of secondary metabolites

When bacteria become resistant to antibiotic medications that were once proven to be successful in treating the infection they cause, this is known as antibacterial resistance. This implies that patients are at a higher risk of worse clinical outcomes and even death when antibiotics are unable to combat resistant bacteria, allowing infections to persist (R Barbieri et al.,2017). Worldwide, bacterial infections are regarded as a serious public health issue. Multi-drug resistance can also result in bacterial infection, which raises the risk of death and morbidity (Kumar P. et AL.,2009).Antibiotic resistance has so gained international attention. The effectiveness of various medications is at risk due to the rise in bacterial multidrug resistance. Numerous researchers have investigated the antibacterial properties of plants' leaves, flowers, stems, roots, and fruits using various solvent systems (C Jain et al,2019).Thus, new antibacterial medications that are less expensive and have minimal toxicity are required to treat a variety of illnesses. Because they are abundant in natural chemicals, secondary metabolites from plants are currently being studied for the development of novel medications (MZ Bhatti,2022). Numerous studies conducted in recent years have demonstrated that phytochemicals have antibacterial properties through a variety of mechanisms, including bacterial membrane damage, virulence factor suppression (including the inhibition of enzyme and toxin activity), and antibiotic biofilm formation. The research of phytochemicals' antibacterial properties has received a lot of attention in the last 10 years, particularly with regard to multidrug-resistant Gram-positive and Gram-negative bacteria (Borges et al., 2015b).

From the acidic portion of the *Ipomoea muricata* seeds, two substances that are effective against Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 6633, and Streptococcus pyogenes ATCC 19165 were separated using chromatographic techniques (M Nonato et al,1986) Using the agar cup method, the antibacterial activity of Ipomoea aquatica leaf and flower extracts against the gram-positive *Staphylococcus aureus* bacteria was examined. Methanol extracts of *I. aquatica* leaves and flowers both included flavonoids and anthocyanins, according to the results of the initial phytochemical screening. The antibacterial activity of the methanol extracts of the

leaves and flowers was demonstrated; this could be because the extracts contain flavonoids (Manvar, M. N. 2018). Artificially grown sweet potato *Ipomoea batatas* (L.) Lam. leaves were tested for their antibacterial effectiveness against *Escherichia coli* O157:H7, *Bacillus cereus*, and *Staphylococcus aureus*, three Gram-positive and Gram-negative bacteria. Five different bifidobacteria that are beneficial to human health, however, were unaffected by the antibacterial extract of the leaves. Protein accounted for 70% of the antibacterial extract, whereas polysaccharides made up 30%. Natural sugars were present in the polysaccharide fraction in the following order: xylose > galactose > arabinose > glucose > rhamnose > mannose > fucose. According to these findings, the antibacterial component might be a substance that resembles pectin. Therefore, there is great promise for using sweet potato leaves in a practical way to stop the growth of bacteria that cause food poisoning (S Islam,2008).

Antibacterial activity

Many standard procedures for antimicrobial testing are available today, as confirmed by esteemed organizations such as the British Society for Antimicrobial Chemotherapy, the National Committee for Clinical Laboratory Science, and the European Committee for Antimicrobial Susceptibility Testing.Diffusion, dilution, and bioautographic methods are the three categories of screening techniques now accessible for identifying the antimicrobial activity of natural compounds. The diffusion and bioautographic techniques are referred to as qualitative procedures since they merely provide an indication of the presence or lack of compounds having antibacterial action. However, once dilution approaches identify the least inhibitory concentration, they are regarded as quantitative assays. The official technique for regular antibiotic susceptibility testing in many clinical microbiology labs is agar disk-diffusion testing, which was created in 1940 (N.G. Heatley,1944). In this widely used method, a standardized inoculum of the test microorganism is applied to agar plates. The test substance at the desired concentration is then put on the agar surface in the form of filter paper discs, which have a diameter of roughly 6 mm. The right conditions are used for the Petri dishes' incubation. Measurements are made of the widths of the inhibitory growth zones when the antimicrobial drug diffuses into the agar and stops the test microorganism from germinating and growing

(M Balouiri et al.,2016).Furthermore, because it is hard to measure the quantity of the antimicrobial agent that diffuses into the agar medium, the agar disk-diffusion method is

inappropriate for determining the minimum inhibitory concentration (MIC). However, by comparing the inhibitory zones with stored algorithms, an estimated MIC can be determined for certain bacteria and drugs (A. Nijs et al.,2003). However, the disk-diffusion assay has a lot of advantages over other techniques, including convenience of use, affordability, the capacity to test a large number of bacteria and antimicrobial drugs, and the results' ease of interpretation.

AIMS AND OBJECTIVES

1. To conduct the phytochemicals analysis of leaves of *Ipomoea muricata* (L.) Jacq.

2. To identify the quantity of phytochemicals present in the leaves of *I.muricata* (L.) Jacq.

3. To analyse antibacterial property of *I.muricata* leaf extract against *S.aureus* and *E. coli.*

MATERIALS AND METHODS

1.Collection of Plant Material

The plant material was collected from Udayamperoor,Ernakulam,kerala. It was identified by evaluating the morphological characters and the identity was confirmed as *Ipomoea muricata*.(Fig 1)

Fig 1:*Ipomoea muricata*-Habit

Fig 2:*Ipomoea muricata* –shade dried

2.Preparation of Plant Extract

The leaves were collected without petiole,cleaned and shade-dried (Fig 2) for 1 week.The dried leaves were made into fine powder using a grinder.The leaf extract was prepared using Soxhlet apparatus (Fig 3).20g of the powdered leaf was extracted using 300 ml of 95% ethanol at 50°C for two days.The solvent was collected (Fig 4) and allowed to evaporate.The residue was taken and stored in a glass bottle.

Fig 3: Extraction using Soxhlet apparatus

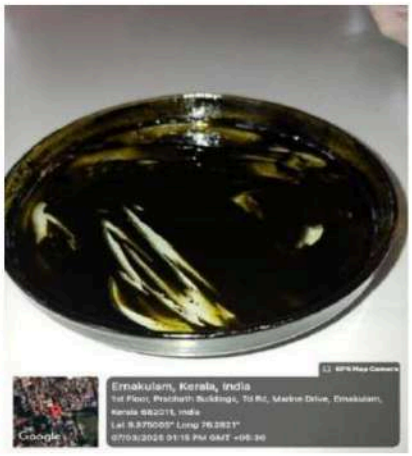

Fig 4:Collected extract

3.Qualitative Phytochemical screening

Phytochemical analysis were done for the detection of Phenols,Flavanoids,Alkaloids, Tannins, Carbohydrates .

To detect the presence of phenols ;Ferric chloride Test (Mac,1963), flavanoids; Shinoda Test (Sofowora,1993), alkaloids;Dragendorff's test (Ciulci,1994), tannins;Braymer's test,and carbohydrates; Molishc's test (Ramakrishnan et al.,1994) were done.

Preparation of stock solution

The stock solution for phytochemical analysis of the exract was prepared by mixing 500 mg of the extract in 25 ml of Dimethyl sulfoxide.

A) Test for phenols

Ferric chloride Test (Mac,1963)

The extract was dissolved in distilled water and a few drops of neutral 5% ferric chloride solution were added. Phenolic compounds were indicated with the presence of a dark green color.

B) Test for Flavonoids

Shinoda Test (Sofowora,1993)

4 ml of the extract was taken in separate test tubes, a piece of magnesium ribbon and concentrated hydrochloric acid were added drop wise. A colour ranging from crimson to magenta indicates the presence of flavonoids.

C) Test for Alkaloids

Dragendorff's test (Ciulci,1994)

1 ml of extract was taken in a test tube (the solvent used is distilled water), and 2 to 3 drops of Dragendorff's reagent was added. An orange-red precipitate or turbidity with Dragendorff's indicated the presence of alkaloids.

D) Test for Tannins

Braymer's test

A portion of the extract was mixed with 2 mL of distilled water followed by the addition of a few drops of 10% ferric chloride solution. The formation of bluish black or greenish-black coloration was taken as positive for the presence of tannins.

E) Test for Carbohydrates

Molisch's Test

1mL of the extract was measured out from the stock solution and transferred into a test tube. To this, two drops of Molisch's reagent were added and mixed well. Then 1mL of concentrated Sulphuric acid was added along the sides of the test tube without shaking. At the junction of the two liquids, a violet-colored ring appears indicating the presence of carbohydrates.

4.Quantitative Phytochemical Analysis

Sample preparation

The sample stock solution was prepared by dissolving 10 mg of the sample in 1 mL of ethanol, resulting in a concentration of 10 mg/mL.This stock solution was used for subsequent assays.

A. TOTAL PHENOLIC CONTENT

The amount of phenol in the aqueous extract was determined by the Folin-Ciocalteu reagent method with some modifications. 2 .5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% solution of Na2CO3 were added to 1ml of plant extract. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765nm. A set of reference standard solutions (S1-S5) of tannic acid (0.1,0.5,1.0,1.5 and 2 mg/ ml) were prepared in the same manner as described earlier.The total phenol content was determined from the standard curve.

B. FLAVONOID QUANTITATIVE ANALYSIS

The total flavonoid content was determined using the method described by Park et al. (2008). In a 10 ml test tube, 0.3 ml of plant extracts, 3.4 ml of 30% methanol, 0.15 ml of NaNO2 (0.5 M)

and 0.15 ml of AlCl3.6H2O (0.3M) were mixed.After 5 min, 1 ml of NaOH (1 M) was added. The solution was mixed well and the absorbance was measured at 506 nm. A standard curve for total flavonoids was prepared using rutin standard solutions ranging from 0.5 to 20mg/ml (S1-S5), following the same procedure. The total flavonoid content was determined from the standard curve.

C. TANNIN QUANTITATIVE ANALYSIS

The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions (S1-S5) of tannic acid (0.1,0.5,1.0,1.5 and 2 mg/ ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were

measured against the blank at 700 nm with an UV/ Visible spectrophotometer. The total tannin content was determined from the standard curve.

D. ALKALOID QUANTITATIVE ANALYSIS

Sample preparation

Due to colour interference samples are diluted two times and this diluted sample is taken for the assay.

For the assay, add 1 ml sample to 5 mL of 60% sulfuric acid and allowed to react for 5 minutes. Subsequently, 5 mL of 0.5% formaldehyde in 60% sulfuric acid was added to the mixture, which was then allowed to stand for 3 hours at room temperature. The absorbance of the final solution was measured at 565 nm using a spectrophotometer. A set of reference standard solutions (S1-S5) of atropine (0.1,0.5,1.0,1.5 and 2 mg/ ml) were prepared in the same manner as described earlier. The total alkaloid content was determined from the standard curve.

E. CARBOHYDRATE ESTIMATION (DNSA METHOD)

Sample preparation

Due to colour interference samples are diluted two times and this diluted sample is taken for

the assay.

The estimation of carbohydrates was carried out using the DNSA (3,5-dinitrosalicylicacid) method, which is based on the reduction of DNSA by reducing sugars to form a colored complex measurable at 540 nm. Glucose standards were prepared at concentrations of 0, 0.4, 0.8, 1.2, 1.6, and 2.0 mg/mL. From each standard solution, 200 µL was transferred into separate labeled test tubes (S1–S5). Similarly, 200 µL of test sample was transferred into separate tube. To all tubes, including the standards, samples, and blank, 0.5 mL of DNSA reagent was added and mixed thoroughly. The tubes were then placed in a boiling water bath for 15 minutes to allow for color development. After heating, 0.5 mL of 40% potassium sodium tartrate solution (Rochelle's Salt) was added to each tube and mixed well to stabilize the color. The tubes were allowed to cool to room temperature, and the absorbance of each solution was measured at 540 nm using a

spectrophotometer. The glucose standard readings were used to construct a calibration curve, from which the carbohydrate content in the test samples was determined.

5.Antibacterial Analysis

The anti-bacterial activities of *Ipomoea muricata* were examined by Disk diffusion method using two bacterial strains: Gram positive *Staphylococcus aureus* and Gram negative *Escherchia coli.*

Preparation of the bacterial culture

To study the effect of plant extract on the growth of the bacteria, the bacteria was sub cultured from the stock in Nutrient broth medium.To prepare nutrient broth medium weigh 1.3g nutrient broth and dissolve it in 100ml distilled water. Then it is transferred into five test tube and autoclave for 15 minutes at 120°C and 15 psi pressure. Then the bacteria are inoculated to it and incubate at 37°C for 24hrs.

Preparation of agar plates

To prepare the agar plates weigh 1.3g nutrient broth and 2g agar agar and dissolved in 100ml of distilled water. Then it is autoclaved at 120°C and 15 psi pressure for 15. minutes. The prepared agar is poured into the sterile petriplate and kept for setting. The sub cultured bacteria was inoculates into the agar plate by carpet culturing. The well was created using the well cutter. 20µl sample was loaded in the well. The antibacterial disc was used as positive control and Dimethyl sulfoxide was used as negative control, Incubate it in sterile condition for 24 hrs.

RESULTS

1.Qualitative phytochemical analysis

The phytochemical analysis of *Ipomoea muricata* leaf extract revealed the presence of Carbohydrate .

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Sl no.	Phytochemical	Outcome
1	Phenols	–
2	Flavanoids	–
3	Alkaloids	–
4	Tannin	–
5	Carbohydrate	+

Table 1:Results of phytochemical analysis of leaf extract of *I.muricata*

Fig 5: Phenols- Ferric chloride Test

Fig 6:Flavonoids-Shinoda test

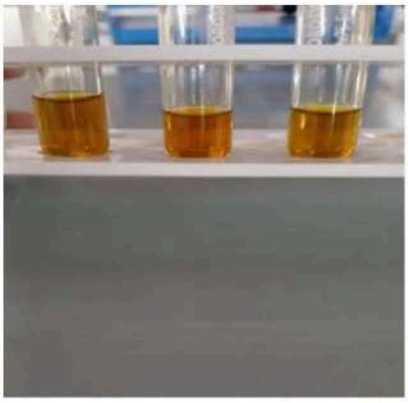


Fig 7:Alkaloids-Dragendorff's test

Fig 8: Tannins-Braymer's test

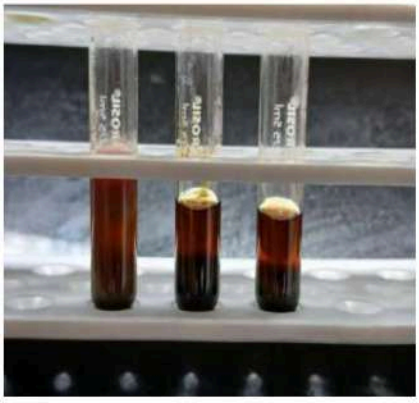


Fig 9:Carbohydrates-Molisch's Test

Qauntitative Phytochemical analysis

A) TOTAL PHENOLIC CONTENT

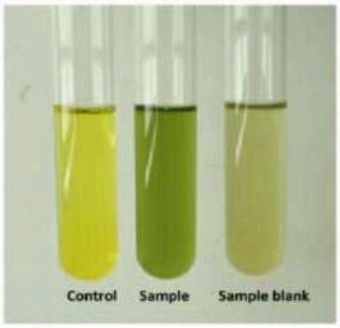
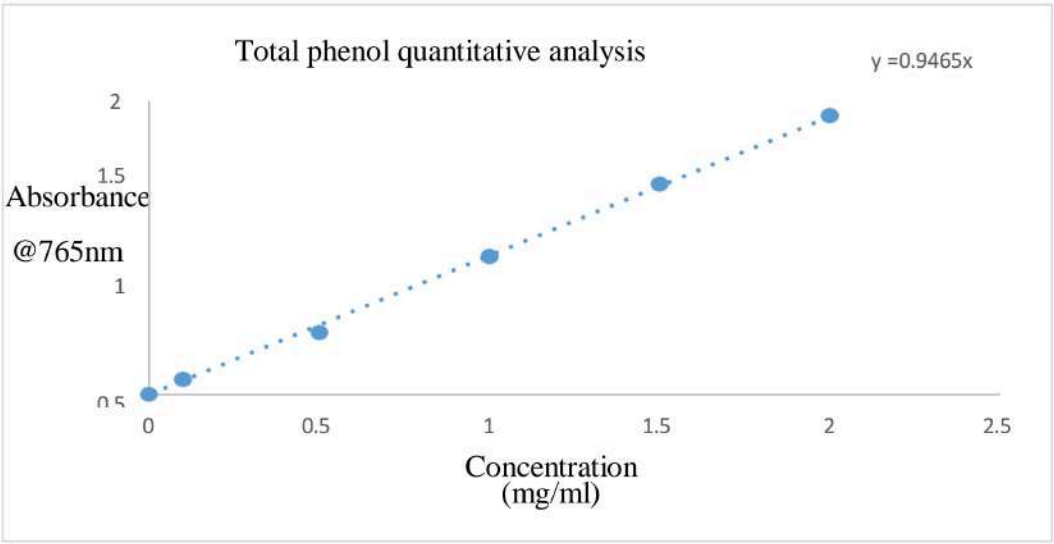


Figure 1: Total phenol quantitative analysis of control and sample



Graph1:Standard graph of Total phenol quantitative analysis

Table 2 :Concentration of Total phenol from graph		
Sample name	Corrected Absorbance	Concentration of Phenol from graph (mg/ml)
Leaf extract	0.391	0.413

B. FLAVONOID QUANTITATIVE ANALYSIS

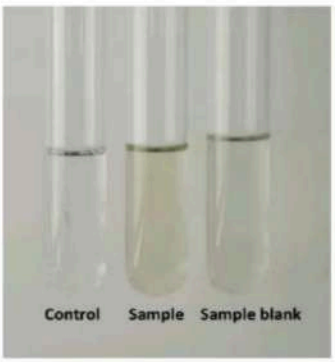
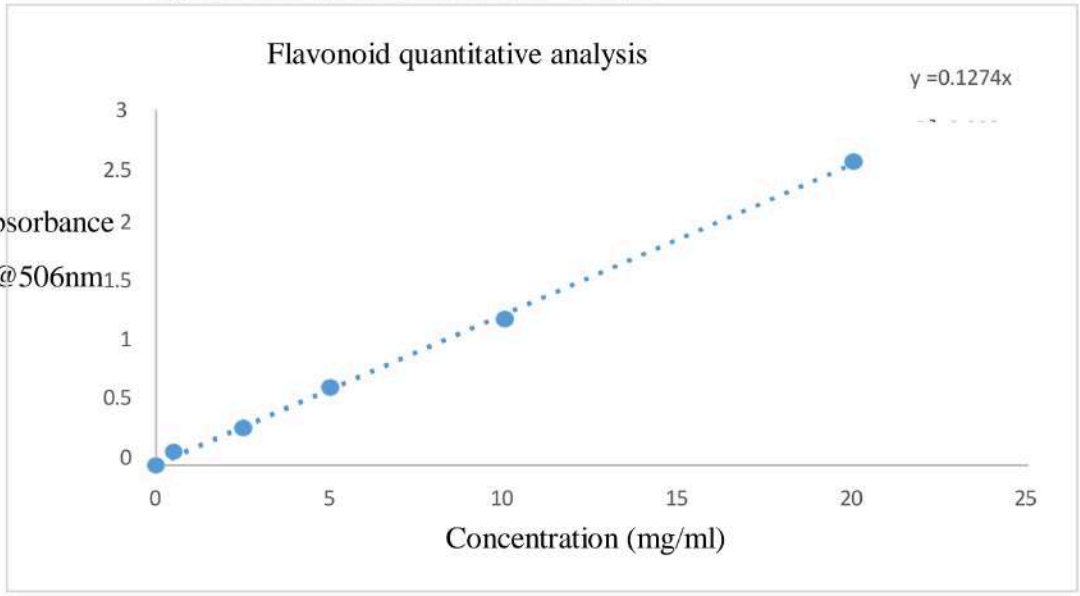


Figure 2: Flavonoid quantitative analysis of control and sample

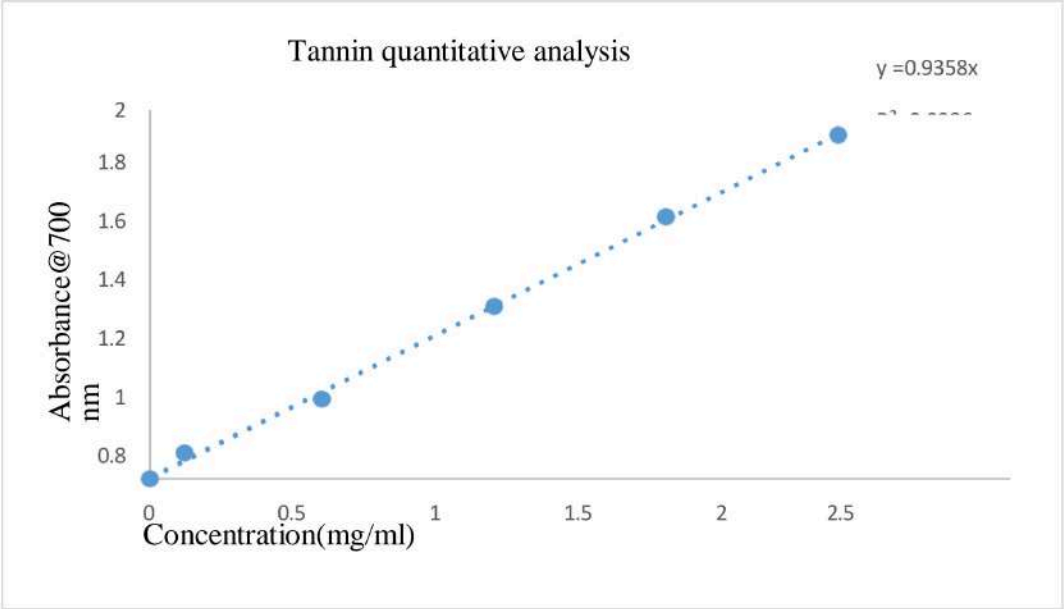


Graph2:Standard graph of Flavonoid quantitative analysis

Table 3:Concentration of flavonoid from graph		
Sample name	Corrected Absorbance	Concentration of Flavonoid from graph (mg/ml)
Leaf extract	0.041	0.322

C. TANNIN QUANTITATIVE ANALYSIS

Figure 3: Tannin quantitative analysis of Control and Sample

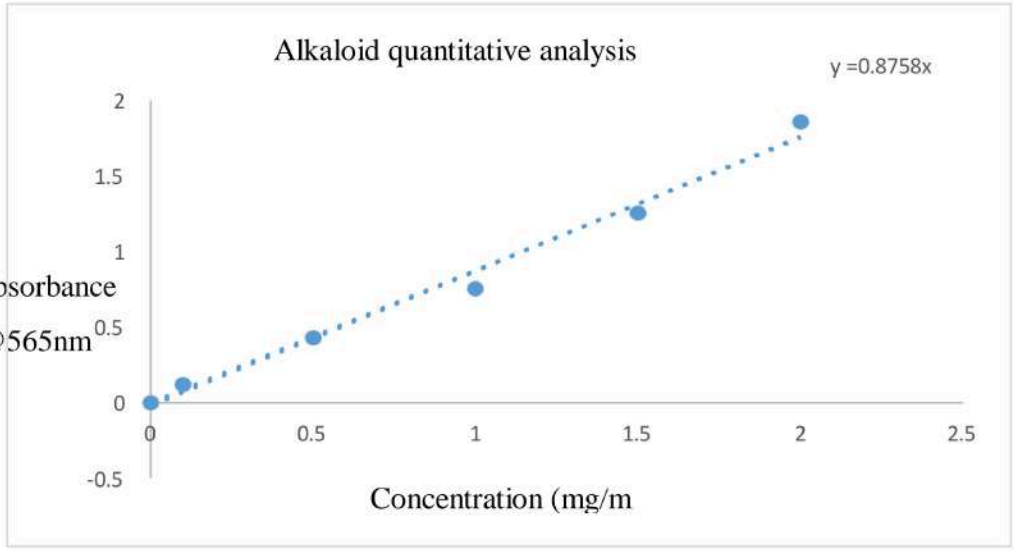


Graph3:Standard graph of Tannin quantitative analysis

Table 4 :Concentration of Tannin from graph		
Samples	Absorbance@700nm	Concentration of Tannin From graph (mg/ml)
Leaf extract	0.208	0.223

D. ALKALOID QUANTITATIVE ANALYSIS

Figure 4: Alkaloid quantitative analysis of Control and Sample



Graph4:Standard graph of Alkaloid quantitative analysis

Table 5 :Concentration of Alkaloid from graph		
Samples G	Absorbance@565nm	Concentration of Alkaloid from graph (mg/ml)
Leaf extract	0.131	0.149

E. CARBOHYDRATE ESTIMATION (DNSA METHOD)

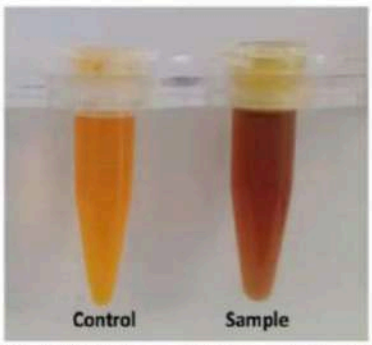
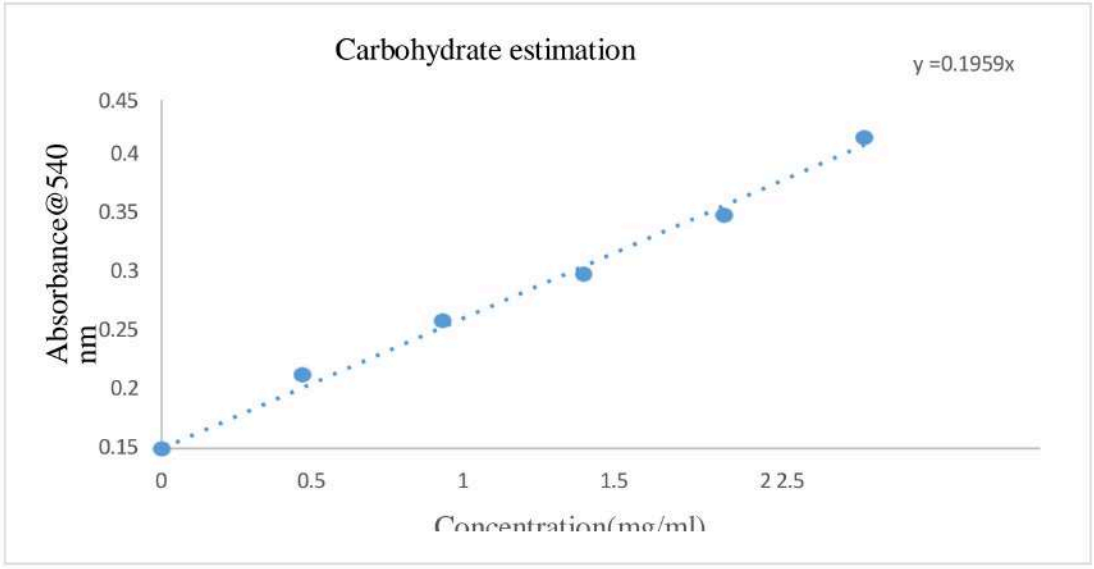


Figure 5: Carbohydrate quantitative analysis of Control and Sample



Graph5:Standard graph of Carbohydrate estimation

Table 6 :Concentration of Carbohydrate from graph		
Samples	Absorbance@540nm	Concentration of Carbohydrate from graph (mg/ml)
Leaf extract	0.276	1.409

Antibacterial analysis

The antibacterial analysis of *Ipomoea muricata* leaf extract does not exhibit any antibacterial property against *S.aures* (Fig 10) and *E. coli* (Fig 11).

Fig 10:*S.aures*

Fig 11: *E. coli*.

DISCUSSION

The plant kingdom contains a wealth of possible medications, and in recent years, people have become more conscious of the significance of medicinal plants. Plant-based medications are reasonably priced, readily accessible, safe, effective, and rarely cause adverse effects. The World Health Organization (WHO) claims that the best way to get a range of medications is through medicinal plants. Traditional remedies are used by over 80% of people in wealthy nations, which contains substances that are taken from medicinal plants (Yadav & Munin Agarwala,2011).

In addition to their antibacterial, analgesic, spasmolitic, spasmogenic, hypoglycemic, hypotensive, anticoagulant, anti-inflammatory, psychotomimetic, and anticancer properties, *Ipomoea* species. Alkaloids, phenolic chemicals, and glycolipids are the most prevalent components of these plant extracts that exhibit biological activity (Meira et al.,2012).The phytochemical screening of *Ipomoea batatas* (L.) Lam crude leaf extract revealed positive results for phenolic acids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, and triterpenes/steroids.while in the present study the qualitative screening of phytochemicals indicated the presence of carbohydrate.The phytochemical analysis of the leaf extract showed varying levels of bioactivity compared to the standard curves. The total phenolic content was 0.413 mg/mL, and activity was observed, indicating the presence of phenolic compounds with potential antioxidant properties. The flavonoid content was 0.322 mg/mL, but no significant activity was observed, suggesting limited contribution to bioactivity. Tannins were present at 0.223 mg/mL, with activity observed, implying possible antioxidant role. The alkaloid content was 0.149 mg/mL, but no activity was detected, indicating minimal contribution to the extract's bioactivity. The carbohydrate content was high at 1.409 mg/mL, with observed activity, suggesting the presence of reducing sugars that may influence overall bioactivity. In summary, the leaf extract exhibited notable activity for phenolics, tannins, and carbohydrates, while flavonoids and alkaloids showed little or no bioactivity.

I. aquatica leaf extracts in both methanol and water demonstrated antimicrobial efficacy against both Gram-positive and Gram-negative bacteria. In the agar disc diffusion method, the methanolic leaf extract of *I. aquatica* had a larger zone of inhibition (15–25 mm) than the aqueous leaf extract (08–19 mm) (Manvar 2013).while in the anti-bacterial study of the *Ipomoea muricata* leaf extract showed that the leaf extract of *Ipomoea muricata* does not exhibit any antibacterial activity. This could be because the leaf extract made by ethanol extraction using a Soxhlet equipment lacked a number of phytochemicals. Numerous phytochemicals may be absent because of various ecological circumstances, climatic conditions, or extraction techniques.

CONCLUSION

In the present study the qualitative ,quantitative analysis of phytochemicals and antibacterial activity of *Ipomoea muricata* leaf extract were done . Qualitative analysis give positive result for molish's test indicating the presence of carbohydrates .There were no desired results for phenols, flavanoids, alkaloids,and tannins.The quantitative phytochemical analysis of leaf extract showed the following amount of phytochemicals , total phenolic content was 0.413 mg/mL,indicating the presence of phenolic compounds with potential antioxidant properties. The flavonoid content was 0.322 mg/mL,Tannins were present at 0.223 mg/mL, implying possible antioxidant role. the alkaloid content was 0.149 mg/mL, the carbohydrate content was high at 1.409 mg/mL,Overall they exhibited notable activity for phenolics, tannins, and carbohydrates, while flavonoids and alkaloids showed little or no bioactivity.In the test of anti-bacterial acitivity of the extract by disc diffusion method showed that the leaf extract of *I. muricata* does not exhibit any antibacterial activity. Additional research is necessary to separate the plant's bioactive components and determine the mechanisms of action that could aid in the creation of many medications to treat different illnesses.

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