PROJECT REPORT

On

"EXTRACTION AND CHARACHTERIZATION OF ALKALOIDS AND FLAVONOIDS FROM MEDICINAL PLANTS"

Submitted by

ATHIRA K

AM21CHE005

In partial fulfillment for the award of the Post graduate Degree in Chemistry

DEPARTMENT OF CHEMISTRY
AND
CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM

2021-2023

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CERTIFICATE

This is to certify that the project work entitled "**EXTRACTION AND CHARACHTERIZATION OF ALKALOIDS AND FLAVONOIDS FROM MEDICINAL PLANTS**" is the work done by **ATHIRAK** under the guidance of**Dr.Elizabeth Kuruvilla,** Department of Chemistry and Centre for Research, St. Teresa's College, Ernakulam in partial fulfilment of the award of the Degree of Master of Science in Chemistry at St. Teresa's College, Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

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DECLARATION

I hereby declare that the project work entitled "**EXTRACTION AND CHARACHTERIZATION OF ALKALOIDS AND FLAVONOIDS FROM MEDICINAL PLANTS**"submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, Kerala is a record of an original work done by me under the guidance ofDr.Elizabeth Kuruvilla, Assistant professor, Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam (Internal Guide) and. This project work is submitted in the partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry.

ATHIRA K

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ATHIRA K

CONTENTS

List of figures	

List of Tables

Chapter 1

INTRODUCTION

1.1 MEDICINAL PLANTS

Since ancient times, people have found and utilised therapeutic plants, often known as medicinal herbs, in traditional medical practises. Numerous chemical compounds are produced by plants for a variety of purposes, including defence and protection against herbivorous mammals, fungus, insects, and illnesses [1]. The phrase "medicinal plants" describes a variety of plant species used in herbalism, some of which have therapeutic characteristics. These healing herbs are recognised as a rich source of ingredients for the synthesis and production of drugs. Any plant that has substances that can be used therapeutically or that can be used as raw materials to create powerful medications is regarded as a medicinal plant. Medicinal plants are the main suppliers of various valuable substances and/or drugs. The therapeutic efficacy of essential oils and secondary metabolites in medicinal plants is widespread. Some of the main advantages mentioned for the therapeutic usage of medicinal plants in treating various disorders include their safety, economic viability, efficacy, and accessibility.

Medicinal plants used for phytochemicals studies are

- Clerodendrum Infortunatum plant (Periyalla)
- Morinda citrifolia plant (Noni)
- Calotropis Gigantea plant (Erukku)

MEDICINAL PLANTS	**PHYTOCHEMICALS PRESENTS**	**MEDICINAL USES**	**IMAGES**
Clerodendrum infortunatum (Periyalla)	Flavonoids,Tannis , Steriods ,Saponins.	Malaria,skin diseases,tunmours ,Antidrandruff	
Morinda citrifolia (Noni)	Alkaloids, Flavonoids,Tannins,Saponin, Steroids,Phenols, Carbohydrates, Terpinoides	Boots immune system,wound healing,diabetes,physical indurance	
Calotropis gigantea (Erukku)	Alkaloids,Flavonoids, Steroids,Tannis, Saponins,phenols, Carbohydrates.	Asthma,cancer,Skin disease,snake bite	

Table 1 : Medicinal plants, medicinal uses and the phytochemicals present

1.1.1 Clerodendrum infortunatum

A plant from the Verbenaceae family that has been used as medicine in India for centuries is called Clerodendruminfortunatum L. In Hindi and Malayalam, the plant is referred to as Peruvelam and Bhant, respectively. It is frequently observed in India's arid plains and lands. The therapeutic qualities of leaves, bark, roots, flowers, stems, and seeds are present. The

plant's many parts have been used to cure digestive issues, anaemia, malaria, inflammatory illnesses, tumours, snake bites, and more. The plant's leaf juice is used to treat vitiated illnesses like cough, fever, ascarides, and scorpion stings. The plant's root bark is used as an anthelmintic, as well as a treatment for inflammatory illnesses, indigestion, and abdominal pain [2].Phytochemicals evaluation of the plant revealed the presence of Flavonoids, Tannins, and Saponnins. The leaves and root are used to treat tumours, dandruff, malaria, scabies, skin conditions, ulcers, spasms, scorpion stings, snake bites, and as a laxative, vermifuge, anticonvulsant, and ascaricide. The leaves and root are frequently utilised as antihyperglycemic in several traditional practises.

Fig 1 : Picture of Clerodendrum infortunatum

1.1.2 Morinda citrifolia

One of the most significant traditional Polynesian medicinal plants is Morinda citrifolia Rubiaceae, also known commercially as Noni, Indian mulberry, Ba ji Tian, Nono or Nonu, Cheese fruit, and Nhau. Native to open coastal regions at sea level and in forested areas about 1,300 feet above sea level, it is a small tropical evergreen shrub or tree that is three to twelve metres tall and has a straight trunk. Due to the internal ovaries of numerous closely packed flowers coalescing, the fruit is roughly 12 cm in

size and, when ripe, smells and tastes bad.[3]An evergreen shrub called Morinda citrifolia produces fruit when it is ripe that tastes and smells strongly of butyric acid.Beta-carotene, ascorbic acid, terpenoids, alkaloids, beta-sitosterol, carotene, polyphenols such flavonoids, flavone glycosides, rutin, and others are among the antioxidants found in Morinda citrifolia L. fruit . Additionally, it may boost immune system activity. M. citrifolia is utilised as a food supplement in various nations. It is used to cure a variety of ailments in folk medicine, including arthritis, cancer, diabetes, liver disorders, malaria, hypertension, TB, infection, and cardiovascular diseases.[4]

Fig 2 : Picture of Morinda citrifolia

1.1.3 Calotropis Gigantea

is a big shrub that can reach a height of 4 m (13 ft). It bears clusters of waxy, either white or lavender-colored blooms. The five pointed petals that make up each flower are joined by a little "crown" that rises from the centre and contains the stamens. of calotropis, the aestivation is valvate, meaning that the sepals or petals of a whorl only touch at the margin and do not overlap. The plant has milky stems and round, light green leaves. Heart-related glycosides, fatty acids, and calcium oxalate are all present in

Calotropis gigantea latex. Calotropone is also present in the roots.[5]Calotropis gigantea has been utilised as a traditional medicine in India for many years and has been said to have a number of purposes due to the high bioactivity of calotropin. Indian practitioners of Ayurveda have utilised the root, leaf, and bark of C. procera to treat liver and spleen ailments as well as bacterial infections, swelling with redness, boils, and shortness of breath. The herb was used to cure fevers, elephantiasis, nausea, vomiting, and diarrhoea as well as cutaneous, digestive, respiratory, circulatory, and neurological diseases. Calotropis procera's milky juice has been used as a remedy for snake bites, cancer, and arthritis.[6]

Fig 3 : Picture of Calotropis gigantea

1.2 PHYTOCHEMICALS

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants that have been shown to have health advantages for humans beyond those associated with macronutrients and micronutrients [7]. Phytochemicals are chemical substances that plants create, usually to aid them in resisting diseases from fungi, bacteria, and

plant viruses. They are also consumed by insects and other animals [8]. They build up in various plant parts, including the roots, stems, leaves, flowers, fruits, and seeds. A large range of chemical substances that are not nutrients but are present in plant meals and may have health impacts are known as phytochemicals. They are not necessary for human survival and are not considered essential nutrients, but they do have vital qualities that can help prevent or treat some common ailments.

Phytochemicals in food can be present in a variety of foods, including fruits, vegetables, legumes, whole grains, nuts, seeds, fungus, herbs, and spices [9]. Phytochemicals build up in a variety of plant parts, including the roots, stems, leaves, flowers, fruits, and seeds [10]. The outer layers of the various plant tissues frequently contain high concentrations of phytochemicals, especially colour compounds. According to the variety, processing, cooking, and growing conditions of each plant, levels differ [11]. Supplemental phytochemicals are also offered, however there is insufficient proof that they offer the same health advantages as dietary phytochemicals [12].

1.2 CLASSIFICATION OF PHYTOCHEMICAL

Phytochemicals are divided into two categories, primary and secondary metabolites, according to how they contribute to plant metabolism. Primary metabolites, which include lipids, purines, pyrimidine of nucleic acids, and carbohydrates, amino acids, and proteins, are essential for plant life. The remaining plant compounds, on the other hand, are created by cells through secondary metabolic routes, which are descended from the basic metabolic pathways. The biological effects of these chemicals, which are produced by secondary plant metabolism,

include antioxidant activity, an antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, reduction of platelet aggregation, modulation of hormone metabolism, and an anticancer property. There are about a thousand phytochemicals, both known and undiscovered. Although it is well known that plants create these substances to protect themselves, recent studies have shown that certain phytochemicals can also shield humans from disease [13].

Based on their chemical makeup and properties, phytochemicals are often divided into six broad categories. These classifications include lipid, phenolic, terpenoids, alkaloids, and carbohydrates [14].

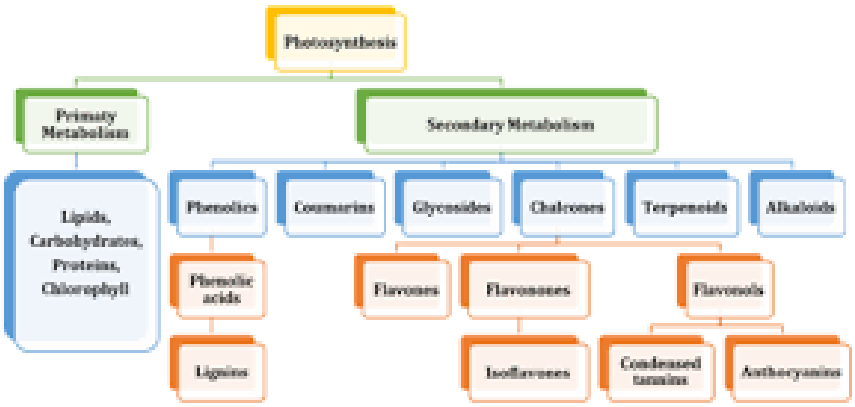


Fig 4 : Classification of phytochemicals

Some of the important phytochemicals are:

ALKALOIDS

Alkaloids are important for plant protection and survival because they protect plants from microorganisms (antibacterial and antifungal activities), insects, and herbivores (feeding deterrents), as well as from

other plants, using chemicals that are allelopathically active[15]. Plants that contain alkaloids have been used as colours, spices, medications, and poisons virtually since the dawn of civilization. Alkaloids have a wide range of pharmacological effects, including as antihypertensive effects (various indole alkaloids), antiarrhythmic effects (quinidine, spareien), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, and vinblastine). These are but a few instances demonstrating the enormous economic significance of this class of plant constituents[16]. As with caffeine and nicotine, several alkaloids have stimulant properties. Morphine is used as an analgesic, and quinine is an antimalarial medication[17].Typical basic structures of alkaloids are:

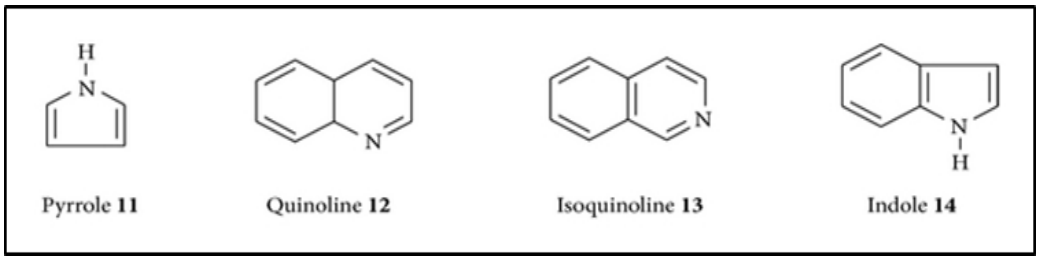


Fig 5: Typical basic structures of Alkaloids

FLAVONOID

Plants contain a group of secondary polyphenolic compounds known as flavonoids, which are frequently included in human diets. Polyphenolic substances called flavonoids are widely present in nature. More than 4,000 flavonoids have been identified, many of which can be found in fruits, vegetables, tea, coffee, and fruit-flavored drinks[18].Due to their extensive biological and pharmacological actions, flavonoids have attracted interest recently. Although flavonoids have been shown to

possess a variety of biological properties, including antimicrobial, cytotoxic, anti-inflammatory, and antitumor effects, their ability to function as potent antioxidants that can shield the human body from free radicals and reactive oxygen species is the one that almost every group of flavonoids is best known for. The flavonoids' ability to function as antioxidants depends depending on their molecular make-up. The location of hydroxyl groups and other characteristics in the chemical structure of flavonoids play a key role in the antioxidant and free radical scavenging abilities of these compounds. In contrast, flavonoids like luteolin and catechins are more effective antioxidants than minerals like vitamin C, vitamin E, and beta-carotene. According to research, flavonoids offer a wide range of beneficial effects, including anti-inflammatory, enzyme-inhibiting, antibacterial, oestrogenic, anti-allergic, antioxidant, vascular, and cytotoxic anticancer activity[19].

A variety of compounds known as flavonoids play a significant role in defending biological systems from the damaging effects of oxidative processes on macromolecules like DNA, lipids, proteins, and carbohydrates[20]. The flavonoid's basic structure is displayed:

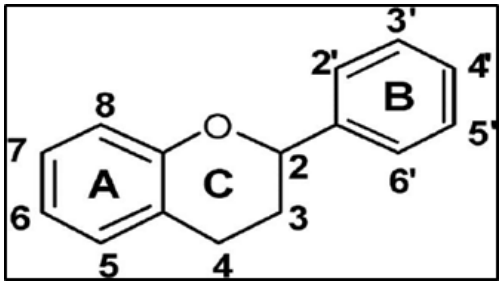


Fig 6: Basic structure of Flavonoid

TANNINS

Tannins are a heterogeneous class of high-molecular-weight polyphenolic chemicals that can form reversible and irreversible complexes with a variety of substances, including proteins (primarily), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids, minerals, and others [21]. Therefore, it is feasible to classify the tannins into four main groups based on their structural properties: gallotannins, ellagitannins, complicated tannins, and condensed tannins [22] (Figure 7).

(1) Galloyl units or their meta-depsidic derivatives coupled to various polyol-, catechin-, or triterpenoid units constitute galloyl units in tannins.

(2) Ellagitannins are tannins without a glycosidically linked catechin unit but with at least two galloyl units that are C-C attached to one another.

(3) Complex tannins are tannins with a glycosidic bond connecting a catechin unit to a gallotannin or ellagitannin unit. (4) Condensed tannins are all oligomeric and polymeric proanthocyanidins created when the C-4 of one catechin is linked to the C-8 or C-6 of the following monomeric catechin.

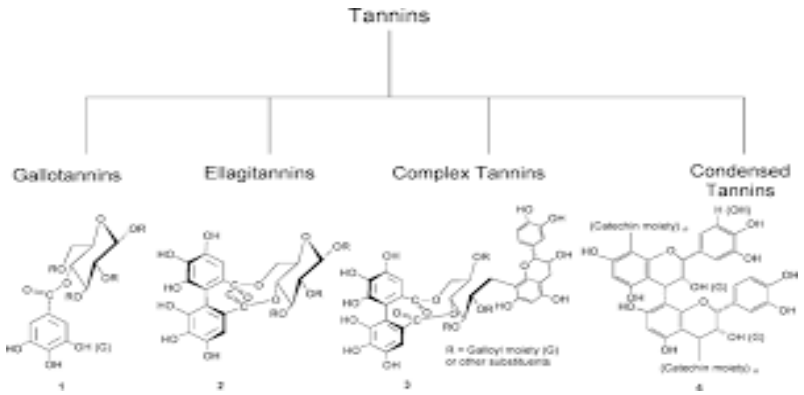


Fig 7 : Different types of Tannins

Tannin-containing plant extracts are employed as astringents, diuretics, against stomach and duodenal tumours [23], and as antinflammatory, antiseptic, antioxidant, and haemostatic pharmaceuticals [24]. Tannins are employed as caustics for cationic dyes (tannin dyes) in the dyestuff business, as well as in the manufacturing of inks (iron gallate ink). Tannins are used in the food business to clear wine, beer, and fruit juices. Tannins are also used in textile colours, as antioxidants in the fruit juice, beer, and wine industries, and as coagulants in rubber production. Recently, tannins have piqued the interest of scientists, owing to the rising prevalence of lethal diseases such as AIDS and numerous cancers [25].

CARBOHYDRATES

A carbohydrate is a bio molecule consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen-oxygen atom ratio of 2:1 (as in water) and thus with the empirical formula $C_n(H_2O)_n$ (where m may or may not be different from n), which does not mean the H has covalent bonds with O (for example with CH_2O, H has a covalent bond with C but not with O). The phrase is most commonly used in biochemistry as a synonym for saccharide sugar [26], which encompasses sugars, starch, and cellulose]. Monosaccharide's, disaccharides, oligosaccharides, and polysaccharides are the four chemical groups of saccharides. Sugars are the smallest (lowest molecular weight) carbohydrates, which are monosaccharides and disaccharides. [27] While carbohydrate nomenclature is complex, the names of monosaccharides and disaccharides frequently end in the suffix -ose, which was derived from the word glucose and is now used for almost all sugars, such as fructose (fruit sugar), sucrose (cane or beetroot sugar), ribose, lactose (milk sugar), and so on.

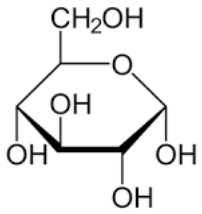


Fig 8:Basic structure of Carbohydrates

PHENOLIC COMPOUND

The largest and most prevalent class of phytochemicals in the plant kingdom are phenolic phytochemicals. Flavonoids, phenolic acids, and polyphenols are the three main categories of dietary phenolics. Phenolics include a hydroxyl group (-OH) a set of chemical compounds where an aromatic hydrocarbon group and the (-OH) are directly bound. The simplest class of these natural chemicals is thought to be phenol (C6H5OH). A significant and intricate set of chemical elements that are present in plants are called phenolic compounds[28]. They are secondary metabolites from plants, and they play a significant defensive compound role. Phenolics have many human-friendly qualities, and their antioxidant characteristics are crucial in establishing their function as preventative measures against disease processes caused by free radicals. The most researched and largest class of plant phenols are flavonoids[29]. Hydroxybenzoic and hydroxycinnamic acids, which are both commonly used, are within the broad group of phenolic acids. Tannins, also referred to as phenolic polymers, are high-molecular-weight molecules that fall into two categories: hydrolyzable tannins and condensed tannins.

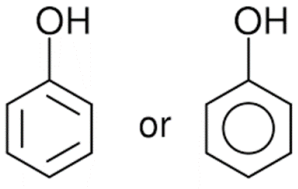


Fig 9: Basic structure of Phenols

STERIODS

A steroid is an organic molecule having four rings organised in a certain chemical configuration that is physiologically active. The two main biological roles of steroids are as signalling molecules and as critical elements of cell membranes that affect membrane fluidity. Numerous steroid species can be found in fungi, animals, and plants. The sterols lanosterol (found in opisthokonts) or cycloartenol (found in plants) are used to make all steroids in cells. Squalene, a triterpene, is cyclized to produce lanosterol and cycloartenol.[30] The typical steroid core structure consists of four "fused" rings made up of three six-membered cyclohexane rings (rings A, B, and C in the first figure) and one five-membered cyclopentane ring (the D ring), totaling seventeen carbon atoms. The functional groups that are joined to this four-ring core and the oxidation state of the rings determine how different steroids are. With a third hydroxy group and a skeleton derived from cholestane, sterols are a type of steroid. Additionally, steroids can undergo more drastic modifications, such as ring structural changes, such as the removal of one of the rings. Vitamin D3 is one among the secosteroids produced by cutting Ring B.

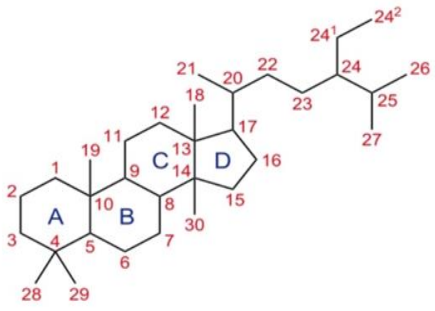


Fig 10 : Structure of 24-ethyl-lanostane, a hypothetical steroid with32 carbon atom .Its core ring system (ABCD)

SAPONIN

A type of secondary metabolites known as saponins can be found across the plant world. They create a stable foam in aqueous solutions, like soap, which gives them their "saponin" moniker. Chemically speaking, the saponins as a group consist of triterpenoids, steroid alkaloids, and glycosylated steroids. Spirostan and furostan derivatives, which are the two most common forms of steroid aglycones, are shown in Figures 8A and B, respectively. A derivative of oleanane makes up the primary triterpene aglycone (Figure 8C)[31]. The carbohydrate component is made up of one or more sugar moieties that are glycosidically connected to a sapogenin (aglycone) and comprise glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid. Monodesmoside saponins are those that have a single sugar molecule connected at the C-3 position, while bidesmoside saponins are those that contain at least two sugars, one attached to the C-3 and one at the C-22 positions[32].

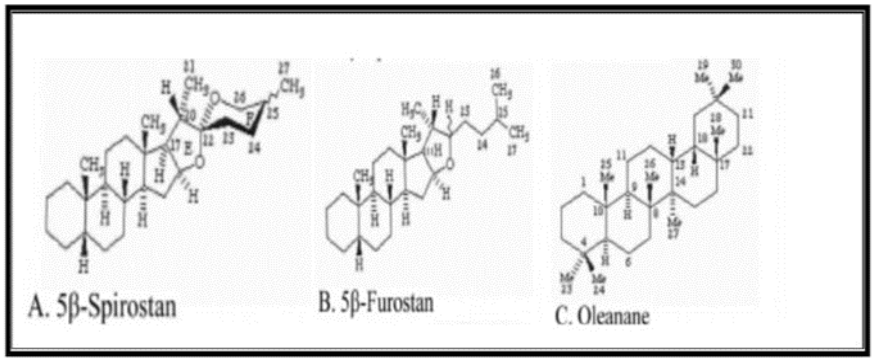


Fig 11: Different forms of Saponins

QUINONES

Quinones are a group of organic and synthetic chemicals with a number of advantageous properties. Quinones are electron transporters that are

essential for photosynthesis. They are a class of chemicals known as vitamins that can prevent and treat conditions including osteoporosis and cardiovascular disorders. Quinones enhance overall health through their antioxidant activity. Many of the anti-cancer medications that have been or are currently undergoing clinical trials are quinone-related substances. Quinones are photoproducts of air pollution, and their presence has toxicological effects as well. Quinones are quick redox cycling compounds with the ability to connect to hydroxyl, thiol, and amine groups. Review summarizes the current knowledge with respect to the oxido-reductive and electrophilic properties of quinones as well as to the analytical tools used for their analysis. It includes a general introduction about the physiological, and therapeutical functions of quinones. A number of studies are reported to cover the chemical reactivity in an attempt to understand quinones as biologically active compounds. Data ranging from normal analytical methods to study quinones derived from plant or biological matrices to the use of labeled compounds are presented. The examples illustrate how chemical, biological and analytical knowledge can be integrated to have a better understanding of the mode of action of the quinones.

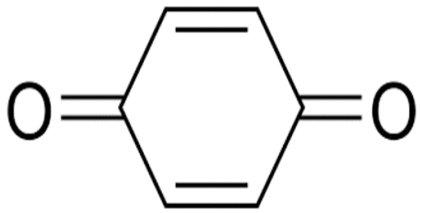


Fig12: Basic structure of Quinones

ATHRAQUINONES

An aromatic chemical molecule having the formula C 14H 8O 2, anthraquinone (also known as anthracenedione or dioxoanthracene) has a

number of other names. Different quinone derivatives are included in isomers. The isomer 9,10-anthraquinone (IUPAC: 9,10-dioxoanthracene), in which the keto groups are situated on the central ring, is the one to which the term "anthraquinone" refers. It is a component of numerous colours and is used to bleach paper pulp.Some plants contain organic substances called anthraquinones. They are simple anthrones or bianthrones chemically speaking. Anthraquinones are utilised as pigments, dyes, and pharmaceuticals.

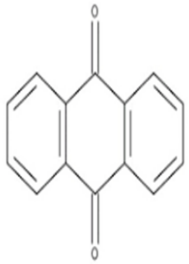


Fig 13 : Basic structure of Anthraquinones

1.3GENERAL METHODS OF EXTRACTION

1.3.1Infusion:

It is the procedure of extracting flavours or chemical compounds from plant matter by leaving the material to stay suspended in a solvent (hot or cold) for an extended period of time, such as water, oil, or alcohol. This technique works well for extracting easily soluble bioactive components.[33]

1.3.2Decoction:

Continuous hot extraction utilising a predetermined amount of water as a solvent is what this procedure entails. Plant matter that has been dried, ground, and powdered is put into a spotless container. After that, water is added and mixed. The extraction is then accelerated by using heat throughout the process. The procedure just takes a few minutes, typically around 15 of them. It is used to extract plant material that is both heat- and water-soluble.[33]

1.3.3 Maceration:

In a container with a stopper, combine the crude medication that has been coarsely ground into powder with an acceptable quantity of the solvent. After that, it is constantly stirred for at least three days at room temperature in order to dissolve its soluble components. To achieve thorough extraction, the substance is periodically mixed and, if placed inside a bottle, shaken. Following extraction, the micelle and Marc are separated using filtration or decantation. The micelle is then evaporated in an oven or on top of a water bath to separate it from the menstruum. This approach is practical and excellent for using with thermolabile plant material.[33]

1.3.4 Soxhlet Extraction:

It involves utilising a Soxhlet extractor to move the partially soluble parts of a solid into the liquid phase. The solid is put into a filter paper thimble, which is subsequently put into the Soxhlet extractor's main chamber. The partially soluble components are gradually transferred to the heated, refluxing solvent as it enters the main chamber.[33]

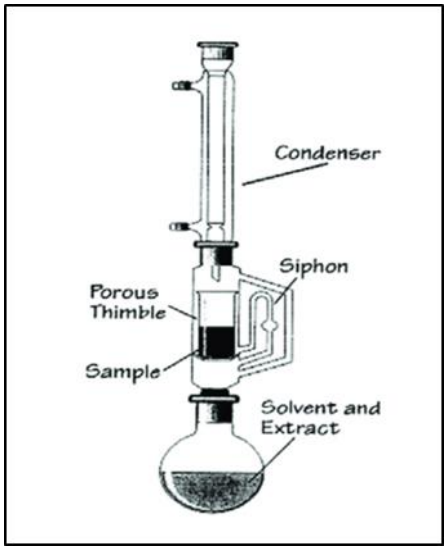


Fig 14 :Soxhlet Extraction

1.3.5 Supercritical fluid extraction:

It is the process of employing supercritical fluids as the extracting solvent to separate one component (the extractant) from another (the matrix). A CO2 pump, a pressure cell to hold the sample, a way to keep the pressure in the system, and a collecting vessel are all required parts of the system. In a heating zone, the liquid is pumped and heated to supercritical temperatures. The material to be extracted is subsequently dissolved as it quickly diffuses into the solid matrix inside the extraction vessel. The extracted material settles out when the dissolved material is swept from the extraction cell into a separator at lower pressure. After that, the CO2 can be cooled, compressed again, recycled, or released into the atmosphere.[34]

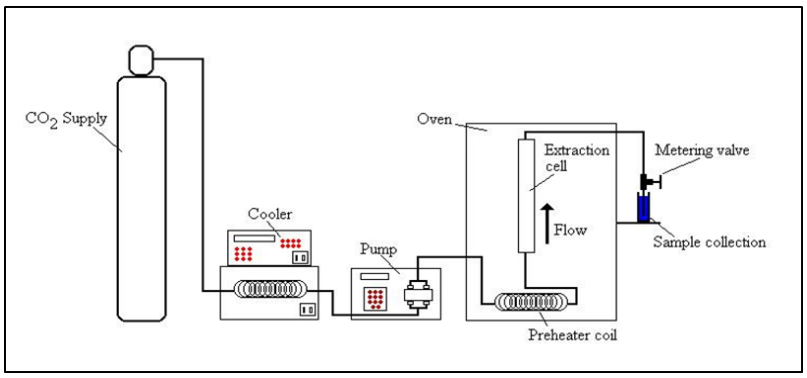


Fig 15 ; Supercritical fluid extraction

1.3.6 Ultrasound-assisted extraction:

In order to damage plant cell walls and enhance the drug surface area for solvent penetration, this procedure applies sound energy at very high frequencies, greater than 20 KHz. Secondary metabolites will consequently be released. Plant material must first be dried before being ground into a fine powder and properly sieved. After mixing the prepared sample with the proper extraction solvent, it is loaded into the ultrasonic extractor. The high sound energy applied speeds up extraction by lowering the need for heat.[35]

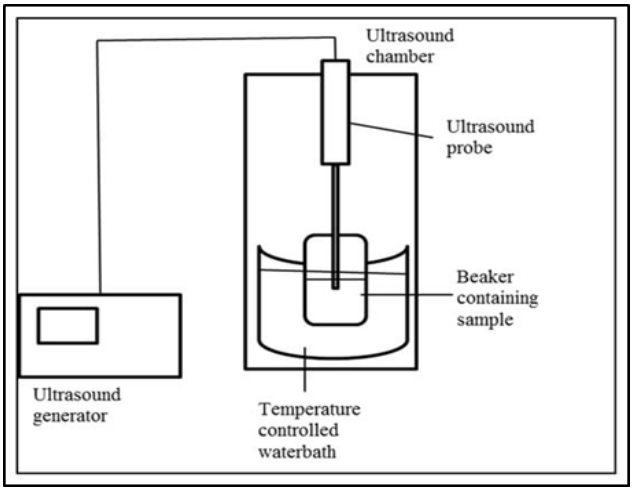


Fig 16 :Supercritical fluid extraction

1.4 Scope and Possibilities

The Plants selected for the extraction and characterization techniques are very much rich in medicinal properties and it is used to treat and cure many diseases. They are used as anti-inflammatory, anti-nociceptive, antihypertensive, anti-cancer, antimicrobial, Noni aids in weight management, reduce blood pressure and help to maintain oral hygiene, acts as immune booster. In many places these plants are used as the home remedy to cure many illnesses.

1.5 Objectives

- Phytochemical screening of *Clerodendrum infortunatum, Morinda citrifolia, Calotropis* gigantean. These plants were selected due to high medicinal content and local availability
- Extraction of alkaloids and Flavonoids from these plants
- Characterization of the plant extract using UV-Visible Spectroscopy, FT-IR Spectroscopy, and NMR Spectroscopy
- Antibacterial study of these plants in different solvents
- Comparison of the antibacterial activity with the standard antibiotic Tetracycline

Chapter2

Literature review

Soumen Saha and his coworkers (2018) goal of the study were to use chloroform extract of *Clerodendruminfortunatum* leaves to create an herbal fungicide that is safe for the environment. Following activity-guided flash chromatography purification, the extract produced eight fractions, numbered F3 through F10. The fractions' total phenol and flavonoid concentrations ranged from 0.12 to 48.25 mg GAE/g and 0.03 to 25.29 mg QE/g, respectively. Seven phenolic acids were identified by LC-MS/MS analysis across several fractions, with a total range of 0 to 2.17 mg/g. With the extract and fractions, an emulsifiable concentrate (20%) formulation was created and tested against Phomopsis vexans, which causes fruit rot disease in brinjal. F8 had the strongest antifungal activity of the different fractions (ED50 = 46.8 g/ml). Total phenol, total flavonoid, and total phenolic acids were linked with the antifungal activity of the leaf extract/fractions r = (0.60to-0.69). Tcinnamic acid and benzoic acid among the phenolic acids had the stronge st antifungal effects.here is also information about the connection between phenolic composition and activity.

LalRaisaHelen et al. (2018) worked on Traditional Indian medicine plantClerodendruminfortunatum L. to cure a number of illnesses. The plant's root bark is used to cure indigestion, bloating, and other digestive problems. The plant's root bark was chosen for this investigation based on its traditional uses. Aqueous acetone extract was discovered to have the highest antioxidant activity in our prior investigation on the screening of

several extracts for this property. Polyphenolics were discovered in the extract after a phytochemical analysis. In the current work, the total phenolic content was measured after the aqueous acetone extract had been purified using bioassay-guided fractionation. Total antioxidant assay and the DPPH radical scavenging assay were used to measure the antioxidant activity of the fractions.The phenolic-rich ethyl acetate fraction was discovered as having considerable radical scavenging and overall antioxidant activity. Therefore, the phenolic chemicals may be to blame for the therapeutic qualities displayed by the plant's root bark. For the creation of novel pharmaceuticals, the proportion needs to be further characterised.

Amjad IbraimOraibi et al. (2018) The goal of the investigation was to study the chemical components of Calotropis Procera. The aerial parts of C. Procera, including the leaves and stems, were macerated in 95% aqueous methanol for three days before being fractionated with petroleum ether, chloroform, ethyl acetate, and n-butanol. This was done because no phytochemical examination has previously been conducted in Iraq. High-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) were used to determine the flavonoid content of the ethyl acetate fraction. From this fraction, flavonoids were extracted and identified by mass spectrometry, IR, HPLC, and HPTLC.

Haziz Sinaet al. (2021) Studied that a variety of maladies are traditionally treated with morindacitrifolia, a plant with broad nutritional and therapeutic properties. The goal of this research is to examine the phytochemistry of M. citrifolia fruit juice and assess its antibacterial and antiradical activities. The extract of two different types of M. citrifolia

fruit juice was stained in tubes as part of the phytochemical analysis. The DPPH radical reduction method was used to assess the antioxidant activity of these drinks. Its antibacterial activity was examined using the well diffusion method while 10 reference bacterial strains were growing in vitro. Large groups of secondary metabolites were found in the fruit juices of M. citrifolia, according to qualitative phytochemistry.Fruit juices from M. citrifolia have stronger antioxidant activity than ascorbic acid and exhibit dose-dependent antioxidant activity. On the other hand, antimicrobial activity showed that fruit juices reduce growth inhibitory action of Streptococcus oralis, Enterococcus faecalis, Proteus mirabilis, S. epidermidis, Proteus vulgaris, and Pseudomonas aeruginosa. For each juice on the strains, the observed difference is significant (p 0.001). These findings support the use of M. citrifolia in conventional medicine and serve as the foundation for the creation of a novel medication to treat both dietary disorders and long-term illnesses brought on by oxidative stress.

Reem Abou Assi et al. (2017) studied Morindacitrifolia L. (Noni) has long been utilised by traditional healers in Hawaii and Polynesia to treat or prevent a wide range of ailments. M. citrifolia is becoming more and more well-liked as a dietary supplement, a food functional ingredient, or a natural health enhancer globally. M. citrifolia includes phytochemicals that have immune-stimulating, analgesic, hypotensive, anti-inflammatory, antiviral, antifungal, anticancer, and antitumor properties. Additionally, M. citrifolia's growing popularity has encouraged businesses to use it as a component of many different goods and for a variety of purposes, such as a green insecticide and a natural source for pharmaceuticals and chemical reagents.Due to the variety in harvest places, M. citrifolia's extensive

distribution in tropical climates across the world—from the USA to Brazil and on to Tahiti, Malaysia, and Australia—contributed to enriching its uses and potentials. Fruits, seeds, barks, leaves, flowers, and other M. citrifolia parts are all used separately for their nutritional and medicinal benefits, but the fruit is thought to contain the most useful chemical compounds. Through describing the completed in vitro and in vivo experiments as well as clinical data, this study discusses in depth the industrial uses and the pharmacological activity of M. citrifolia fruit, seed, leaf, and root, together with their separated phytochemical components.

Meenakshi Sharma et al. (2015) conducted the investigation to assess the ethanolic extract of Calotropis gigentica's in vitro antibacterial activity.Using the disc diffusion method, the inhibitory effect of the ethanolic extract was examined against Streptococcus mutans and Lactobacillus casei.At 1.25% concentration, the lowest inhibitory zones for S. mutans and lactobacilli in an ethanolic extract of Calotropis gigentica were 16 mm and 14 mm, respectively.S. mutans and lactobacilli were shown to be successfully combated by Calotropis gigentica.

P. O. Akindele et al. (2017) studied that in Ondo State Specialist Hospital, the phytochemical and antibacterial properties of Calotropis procera Leaf Organic Fractions were examined in relation to vancomycin- and methicillin-resistant bacteria isolated from wound patients. Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, and Streptococcus pyogenes are the bacteria isolates that were employed.The antibacterial properties of the extracts on resistant bacterial isolates were ascertained using the agar well diffusion method. While cold and hot water extracts recorded the lowest zones of inhibition values of 3.54 mm and 5.53 mm respectively against

Klebsiella pnuemoniae and Pseudomonas aeruginosa, ethanol extract had the highest zones of inhibition against Staphylococcus aureus and Escherichia coli with 16.03 mm and 12.05 mm. The nHexane extract, however, demonstrated no inhibitory impact on Proteus mirabilis and Streptococcus pyogenes.

Rafaela Figueiredo FONTES et al. (2023) studied the preparation of noni (Morindacitrifolia) freeze-dried powder and analysis of its bioactive and antioxidant content was the two main objectives of this work. Physical and chemical characteristics, as well as the presence of carotenoids, chlorophyll, sugars, ascorbic acid, phenolics, flavonoids, and antioxidants, were determined for seed, peel, pulp, and lyophilized powder. By using LC-MS, phenolic chemicals were identified. The lyophilized pulp powder had increased antioxidant activity for FRAP (10360.39 to 467970.40 mmol/100 g) as well as high amounts of phenolics and flavonoids (7486.38 g GAE/g and 385.57 g QE/g, respectively). The amount of ascorbic acid in the lyophilized powder is higher (336.62 mg/100 g). The main constituents were rutin, caffeic, artepillin C, quercetin-3-glucoside, kaempferol, vanillin, and vanillic. The antioxidant impact that was present in the fruit's various parts was prevalent, and lyophilization was used to better preserve this potential. As a result, the noni can be regarded as a good source of phenolic compounds with a high potential as free radical scavengers.

Thongchai Taechowisan et al. (2019) explained that there have been reports of using Morindacitrifolia L. as a medicinal plant to treat abscesses and microbiological infections. In order to analyse the main components

of the ethanolic extract of M. citrifoliafruit .Antibacterial, antioxidant, and cytotoxic capabilities, this study was conducted.Thin layer chromatography and silica gel 60 column chromatography were used as isolation techniques. Spectroscopic techniques were used to archive the identification of pure substances. Purified compounds' cytotoxicity, antioxidant, and antibacterial activities were investigated.It can be inferred that rutin and asperulosidic acid, which have antibacterial, antioxidant, and cytotoxic activities, were the main components identified from the crude extract of M. citrifolia fruits in NakornPathom, Thailand. They might offer encouraging advancements in the treatment of oxidative stress and infectious illnesses.

Suchita Singh et al. (2014) studied that Herbal plants are a reliable source of both conventional and contemporary medications that are helpful for primary healthcare. The richest source of bioactive organic compounds on the planet is plants. As an alternative to the inefficient modern medication, the active metabolites like phytochemicals from medicinal plants were being researched for the creation of innovative and biodegradable effective pharmaceuticals. The therapeutic value of Calotropis gigantea in treating conditions including scabies, asthma, dyspepsia, colds, and coughs is significant. Calotropis gigantea leaf extracts made from methanol and petroleum ether was used to study the phytochemical aspects of the leaf. The findings imply that the leaf's phytochemical qualities can be used to treat a variety of ailments.The plant Calotropis gigantea is a perennial shrub that can be found in tropical and subtropical regions up to 900 metres above sea level. It grows in all types of soils and environmental conditions and doesn't require any special cultivation techniques. According to the results of the experiment, methanol and petroleum ether might have formed a large number of active ingredients

that are in charge of a variety of biological functions. Further research is required to elute novel active chemicals from the medicinal plants, which may offer a new technique to treat many incurable diseases, so that it may be used for the development of conventional medicines.

Chapter3

Materials and Methods

This chapter provides a brief overview of the materials and experimental procedures used in the current study.

3.1 MATERIALS

3.1.1 POWDERED PLANT MATERIAL

The plants chosen for the extraction of alkaloids and flavonoids were Clerodendruminfortunatum (Periyalla ,Morinda citrifolia (Noni) and Calotropis gigantea (Erukku) . The leaves and fruit were dried and powdered. Five gram each of the three plant powdered samples were used for further studies.

(a) (b) (c)

Fig 17: Plants and powdered samples of (a) Clinfortunatumerodendrum (Periyalla) , (b) Morinda citrifolia (Noni) and (c) Calotropis gigantea (Erukku)

3.1.2 CHEMICALS

Ammonium hydroxide with a standard concentration value of 30% m/m is produced by Nice Chemicals (P) Ltd. in Kochi. To conduct the experiment, a 25%m/m mixture was needed. So, 7.5 ml of NH4OH with a 25% m/m concentration was collected. Nice Chemicals (P) Ltd. in

Kochi was contacted and utilised to supply ethyl acetate, concentrated sulfuric acid, diethyl ether, chloroform, sodium sulphate, lead acetate, and ferric chloride.

3.2 EXPERIMENTAL METHODS

3.2.1 PREPARATION OF PLANT EXTRACT FOR DETECTION OF PHYTOCHEMICALS

To obtain the plant extract, the plant material was placed in a round bottom flask fitted with an air condenser. 300 mL of ethyl acetate was added. It was then heated for about 6 hours until complete extraction of plant material occurs. After this, the material was strained and the remaining solid was squeezed to remove all the remaining liquid. The obtained liquid was clarified by decantation or filtration and used for the identification tests of various phytochemical constituents.

3.2.2 DETECTION OF ALKALOIDS

HAGER'S METHOD

The presence of alkaloids can be determined using Hager's technique. Each test tube had 0.2 g of the chosen plant samples added to it for the phytochemical analysis, along with 3 ml of hexane, which was mixed in, shaken well, and filtered. Then, 5 ml of 2% HCl was put into a test tube containing a mixture of hexane and plant extract. A few drops of picric acid were added to the heated, filtered, and then-filtered liquid. Alkaloids are present when a precipitate with a yellow colour forms.

3.2.3 DETECTION OF FLAVONOIDS

LEAD ACETATE TEST

A few drops of Lead acetate solution were combined with 2 ml of the extract's aqueous solution. The presence of flavonoids is indicated by the precipitate's yellow coloration.

3.2.4 DETECTION OF TANNINS

FERRIC CHLORIDE TEST

2 ml of aqueous solution of the extract was added to a few drops of 10% Ferric chloride solution. The occurrence of a greenish black colour indicated the presence of tannins.

3.2.5 DETECTION OF CARBOHYDRATES

MOLISCH'S TEST

2ml of the extract wasadded to 2 drop of alcoholic alpha naphthol and1ml of concentrated sulfuric acid along the side of the test tube.The occurrence of violet ring indicated presence of carbohydrates.

3.2.6 DETECTION OF PHENOLIC COMPOUND

A few ml aqueous extract solution was added to few drops of 5% ferric chloride solution. The occurrence of dark green colour indicated the presence of phenolic compound.

3.2.7 DETECTION OF SAPONINS

FROTHING TEST

3 ml of aqueous solution of extract was mixed with 10 ml distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 5 mins. It was allowed to stand for 15 mins and observed for honeycomb froth.

3.2.8 DETECTION OF QUINONES

The plant extract was mixed with few drops of concentrated hydrochloric acid which shows a green colour precipitate

3.2.9 DETECTION OF STEROIDS

The plant extract was mixed with few drops of sulphuric acid which turns the solution red colour

3.2.10DETECTION OF ANTHRAQUNONES

The plant extract was mixed with 10ml of 10% ammonium solution and shake vigorously for 30 secs the solution turns red colour.

3.2.11 DETECTION OF CARBOXYLIC ACID

The plant extract was mixed with 1ml sodium bicarbonate solution shows the appearance of effervescence.

3.3 SOLVENT EXTRACTION TECHNIQUE

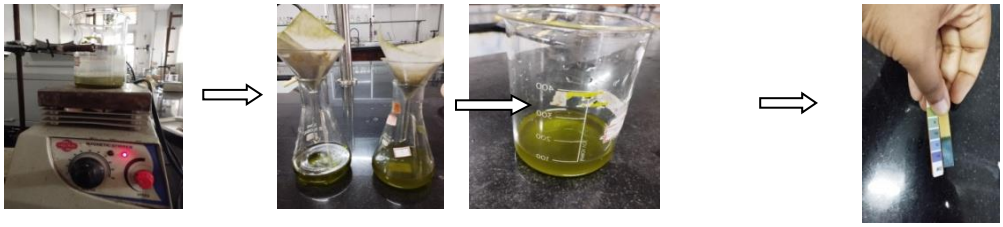
3.3.1 Alkaloids Extraction

For the extraction of the alkaloid, about 5 g of the given sample was wetted with 7.5ml of 25% NH_4OH. The entire substance was placed at room temperature conditions. To this 300ml of Ethyl acetate was added. This solvent mixture was then stirred for about 72 hours. The extract was filtered out. After the filtration, the residue formed was dissolved in water and acidified by adding about two drops of Conc.H_2SO_4 to pH 3-4. The solvent is further extracted using a separating funnel by addition of 50 ml of diethyl ether to remove all type of impurities generated. After basifying, the aqueous solution to pH 9-10 with 25% NH_4OH the alkaloid was then extracted with chloroform. The extract was treated with anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure to obtain crude alkaloids. Pictorial representation of the above method is given below. The extracted alkaloids were further characterized using UV-Visible, IR and NMR spectroscopy. The instrumentation facility at STIC CUSAT was used for this purpose.

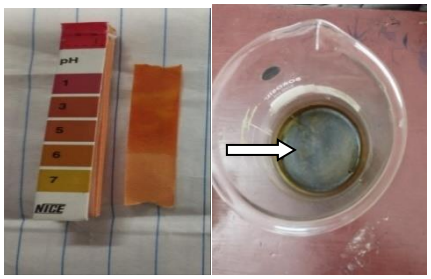
Fig 18: The methods of extraction of Alkaloids

3.3.2 Flavonoids extraction

Take 10 g of the powdered sample and 5g of the sodium hydroxide pellets in a beaker and add 90 ml of methanol and 10ml of water to the beaker and kept on the magnetic stirrer in room temperature for 2 to 3 hours. The solution is filteredandthe filtrate isacidifiedusing dilute hydrochloricacidandtheacidifiedsolutionisdistilledtoremoveimpuritiesandit isdissolvedinchloroform and evaporated to dryness. The flavonoids extracted were further characterized using IR and NMR Spectroscopy.



Solvent extraction Filteration Filterate solution Basic pH



Acidic pH Evaporated to dryness

Fig 19 The method of extraction of Flavonoids

3.4 CHARACTERIZATION TECHNIQUES

3.4.1 UV-Visible Spectroscopy

In the ultraviolet and the entire, nearby visible parts of the electromagnetic spectrum, absorption spectroscopy or reflectance spectroscopy is referred to as UV spectroscopy or UV-visible spectrophotometer (UV-Vis or UV/Vis). This methodology is frequently employed in a variety of practical and theoretical applications since it is reasonably affordable and simple to execute. The sample must only be able to absorb in the UV-Visible range, or it must be a chromophore. Fluorescence spectroscopy is enhanced by absorption spectroscopy. In addition to the measurement of

wavelength, variables of interest include absorbance (A), transmittance (%T), and reflectance (%R), as well as how they change over time. UV-Visible spectra using water as the solvent were taken from Botany Department St. Teresa's college using a Thermo electron Nicolet Evolution 300 UV Visible Spectrometer. [36]

3.4.2 FT-IR Spectroscopy

The term "Fourier transform infrared" (FTIR) refers to the most popular kind of infrared spectroscopy. All infrared spectroscopies operate under the premise that some IR energy is absorbed when it passes through a material. It is noted which radiation enters the sample. It yields Important information about the functional group present in the sample. Here the IR spectra were recorded on a JASCO FT-IR -5300 Spectrometer in the range 4000-400cm-1 using KBr pellets at the Department of Applied Chemistry CUSAT.

3.4.3 ^{1}H NMR Spectroscopy

^{1}H NMR is the go-to technique to help identify or confirm the structure of organic compounds or those that contain protons. A solution-state proton spectrum is relatively fast to acquire, compared with other nuclei, and a lot of information about the structure of a compound can be deduced from it. NMR spectra detected using CDCl3 on a Bruker Advance DRX 300NFTNMR spectrometer at SAIF, CUSAT with TMS as the standard.

3.4.4 ^{13}C NMR Spectroscopy

The ^{13}C NMR is directly about the carbon skeleton not just the proton attached to it. The number of signals tells us how many different carbons

or set of equivalent carbons. The splitting of a signal tells us how many hydrogens are attached to each carbon. NMR spectra of samples were recorded in CDCl3 using TMS as internal standard in Bruker Advance III, 400MHz FT-NMR Spectrometer at sophisticated Test and instrumentation centre (SAIF), cochin university of science and Technology, Kochi, India.

3.5 ANTIBACTERIAL ASSAY

The antibacterial activities of the 3 plants extract in methanol, ethyl acetate and water were assessed by agar well diffusion method. One ml of the fresh culture of E.Coli was inoculated in the sterile Petri dishes distinctly. Wells were made using a sterile cork borer into agar plates containing inoculums. Then, 100 µl of each test solution was added to respective wells. The test solutions were methanolic extract (A), Ethyl acetate extract (B), Water extract (C). Then, the plates were incubated at 37°C for 24 hours. Antimicrobial activity was detected by measuring the zone of inhibition (including the diameter of the wells) that appeared after the incubation period.. Tetracycline, an antibiotic was used as the standard.

Chapter4

Results and discussion

4.1 PHYTOCHEMICAL SCREENING

Phytochemical screening of Morinda citrifolia Clerodendrum infortunatum and Calotropis gigantea were conducted. Both Morinda citrifolia and Calotropis gigantea showed the presence of Alkaloids, flavonoids, phenol, saponins, tannins.Clerodendrum infortunatum showed the absence of Alkaloids, Phenols, and Carbohydrates. Figure shows the results of the phytochemical screening. The results are summarized in the table

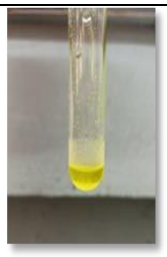
					
Alkaloids	Flavonoids	Saponins	Phenol	Tannins	Carbohydrates

Table 2 : Phytochemical screening of Morinda citrifolia

				
Alkaloids	Flavonoids	Saponins	Phenol	Tannins

Table 3: Phytochemical screening of Calotropis gigantea

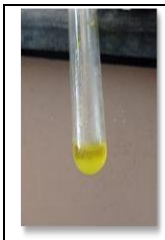
		
Flavonoids	Tannins	Saponins

Table 4 : Phytochemical screening of Clerodendrum infortunatum

4.2 SPECTROSCOPIC ANALYSIS

Alkaloids and flavonoids extracted from Morinda citrifolia and Calotropis gigantea were characterized by Infrared spectroscopy, NMR Spectroscopy.Flavonoids extracted from Clerodendrum infortunatum were characterized by IR spectroscopy.

4.2.1Morinda citrifolia(Alkaloids)

4.2.1(a) UV Visible Spectroscopy

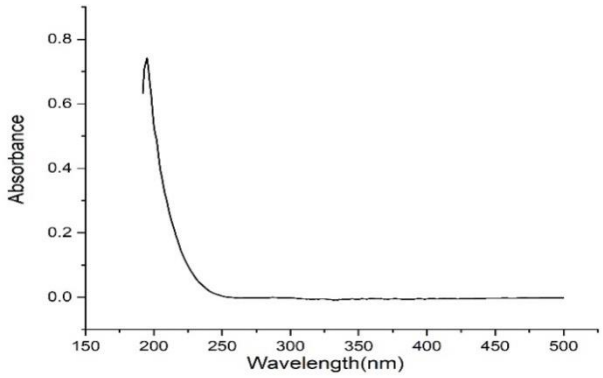


Fig 20: UV visible spectrum of the Alkaloids extracted from Morinda citrifolia

The absorption maximum is observed at 200 nm which shows the absence of any conjugated bonds in the molecule. However isolated carbonyl groups and double bonds may be present.

4.2.1(b) IR Spectroscopy

The IR Spectrum (KBr) shows a peak at 3429.38 cm^{-1} are due to N-H Stretching. Peaks at 2924.72, 2853.79 cm^{-1} which are due to =C-H stretching indicating the presence of an olefinic bonds. The characteristic peak at1745.18 cm^{-1} is to C=O (carbonyl) stretching which shows the presence of ester group while the peak at 1464.71cm^{-1} shows C-H bend.

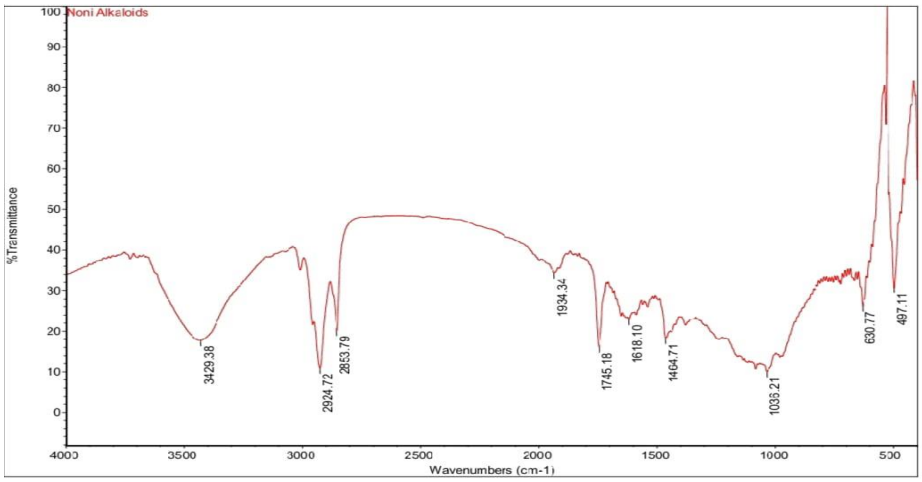


Fig 21: IR Spectrum of Morinda citrifolia Alkaloids

4.2.1(c) ^{1}H NMR Spectroscopy

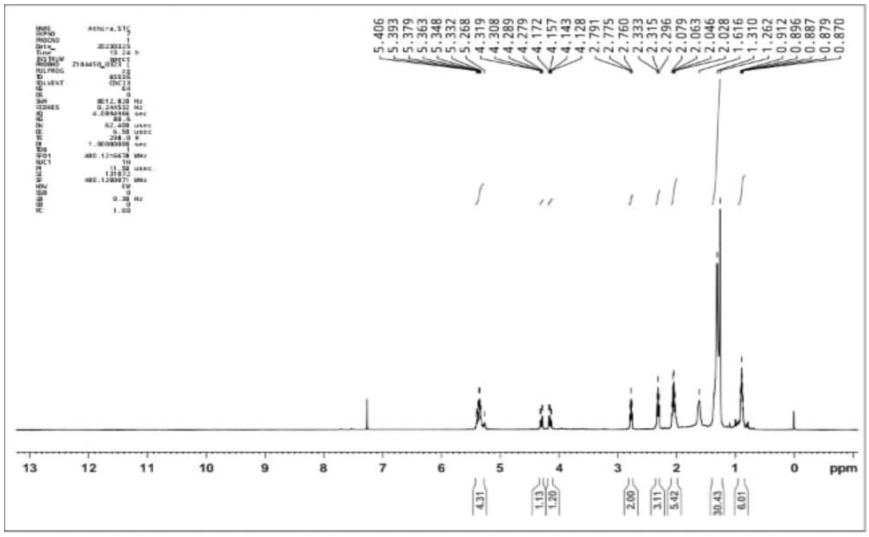


Fig 22:^{1}H NMR Spectrum of Morinda citrifolia alkaloids

The proton NMR spectrum was recorded in CDCl3 in 400MHz NMR spectrometer.The few multiplets in the range at 0.8-0.9 is due aliphatic methyl protons.From 1-1.5 to 2 we can see CH2 anCH protons . The broad single peak at 1.5 ppm may be due to weakly coupled protons that are bondedto heteroatom like nitrogen and indicates the presence of N-H protons. Multiplets in 4.128 to 4.319 ppm range gives the presnce of doublet of doublet which shows J value of 6 Hz indicating the presnce of vinylic cis protonds.presnce of vinylic geminal protons are also indicated from the doublets at 4.279-4.319 ppm. Multiplets at around 5 ppm and 2-3 to 2.9 ppm indicates the presnce of protons in the vicinity of ester or carbonnyl groups.

4.2.1 (d) ^{13}C NMR Spectroscopy

The peak at 180 ppm corresponds to the carbonyl carbon of ketone or ester group. The peak at 10-20 represnd CH3 groups.82 is the sovlent peak and the peaks in the ange 60-70are due C-N OR C-O bonds. Peaks near 130-140 can be the presnce of double bond.peak at 20 is CH3and peaks at 30-40 can be CH and CH2 groups.

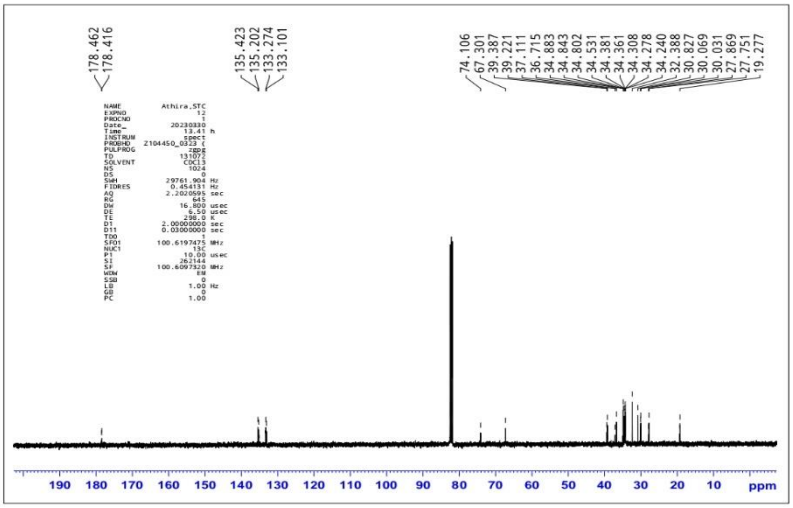


Fig 23:^{13}C NMR Spectrum of Morinda citrifolia Alkaloids

4.2.2 Morinda citrifola Flavovoids

4.2.2 (a) IR spectroscopy

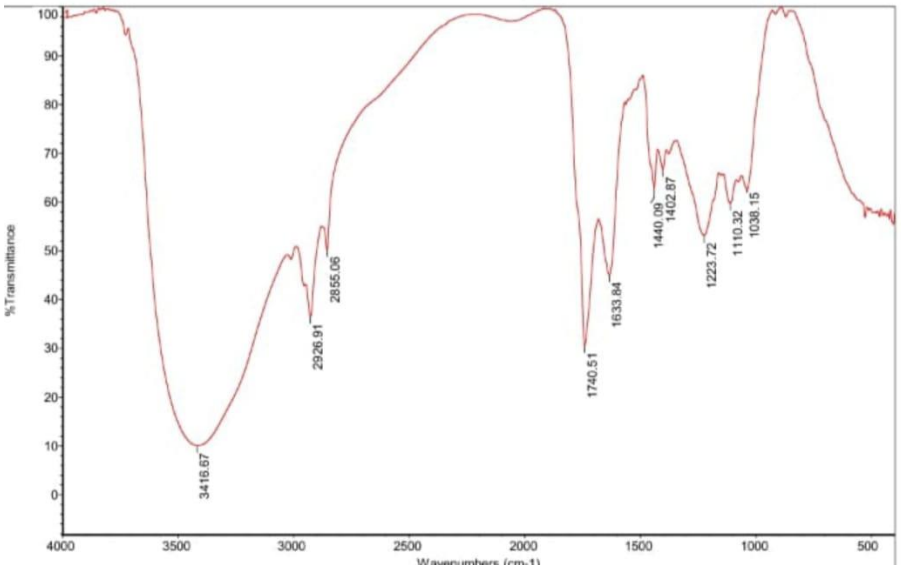


Fig 24: IR Spectrum of Morinda citrifolia Flavonoids

The IR Spectrum (KBr) shows the peak at 3416.67cm^{-1} shows the presnce of O-H stretching. The peaks at 2926,2855 are due to C-H aliphatic asymmetry strectching vibration. The characteristic peak at 17405.51 is due C=O due to the presence of carbonyl group. 1633cm^{-1} corresponds to 1110.32, 1038.15, 1223.72 corresponds to C-O streching.

4.2.2 (b) NMR Spectroscopy

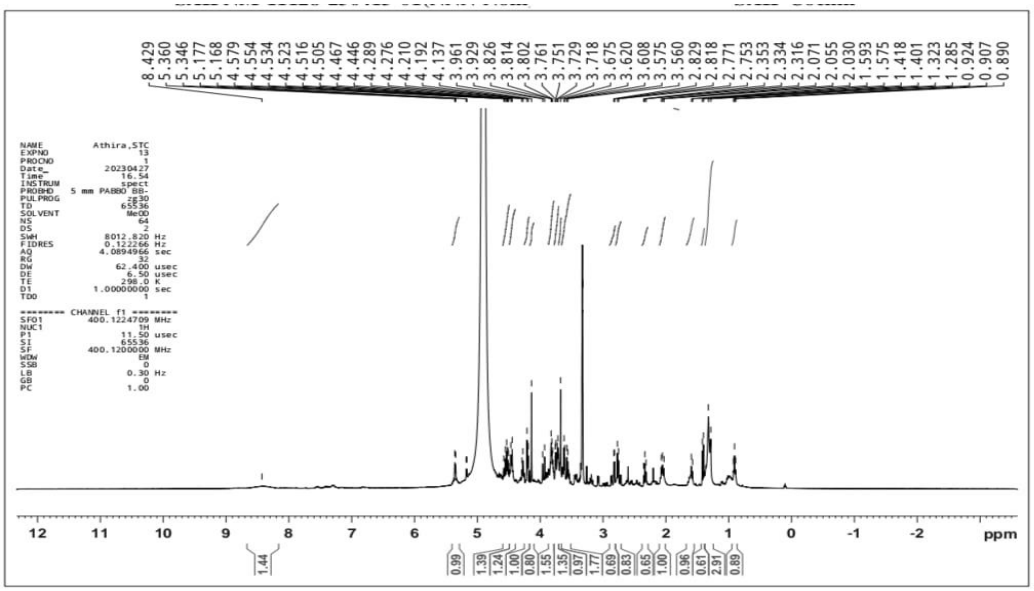


Fig 25 :NMR Spectruum of Morind Citrifolia Flavonoids

The few multiplets at 0.8-0.9 represent CH3 protons and the peaks in the range 1.5-2 represent CH2 and CH protons. Peaks at 3-3.5 is due OH protons.

4.2.3 Calotropis gigantea (Alkaloids)

4.2.3 (a) IR Spectroscopy

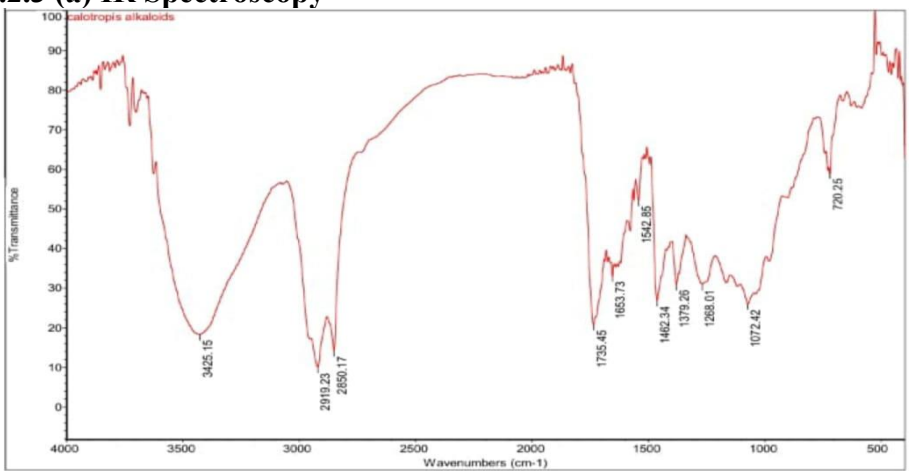


Fig 26: IR Spectrum of Calotropis gigantea Alkaloids

The IR spectrum (KBr) shows peak at 3425.15 which is due to N-H stretching. Peaks at 2919.28,2850.17 is due to C-H aliphatic asymmetric stretching.peak at 1735.45 is due to C=O stretching.1462.34cm^{-1} due to C-H bend and 1653.73cm^{-1} due to C=C stretching.1268.o due to C-N aliphatic stretching. And tha peaks at1072.42 due to C-O stretching and 720 due to C-H rock.

4.2.4 Calotropis gigantea Flavonoids

4.2.4(a) IR Spectrum

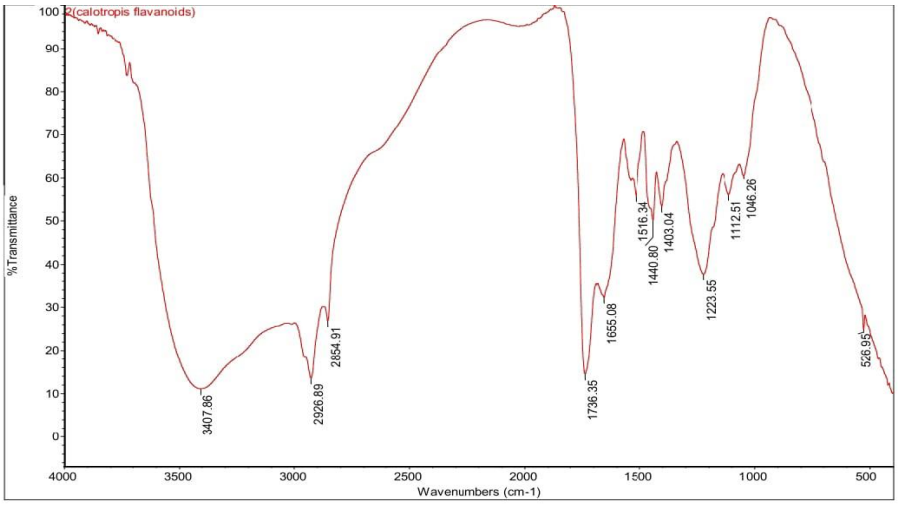


Fig 27: IR Spectrum of Calotropis gigantea Flavonoids

The IR spectrum (KBr) shows peak at 3407.86cm^{-1} due to OH stretching. And the peaks at 2926.89,2854.91 are due to C-H stretching. Peak at 1736.35 due to C=O stretching. And peak at 1440.86 due to C-Cstretch inn thr ring.peak at 1232.35 due to ether linkage.112.55,1046.26cm^{-1} due C-O stetching.

4.2.4 (b) NMR Spectroscopy

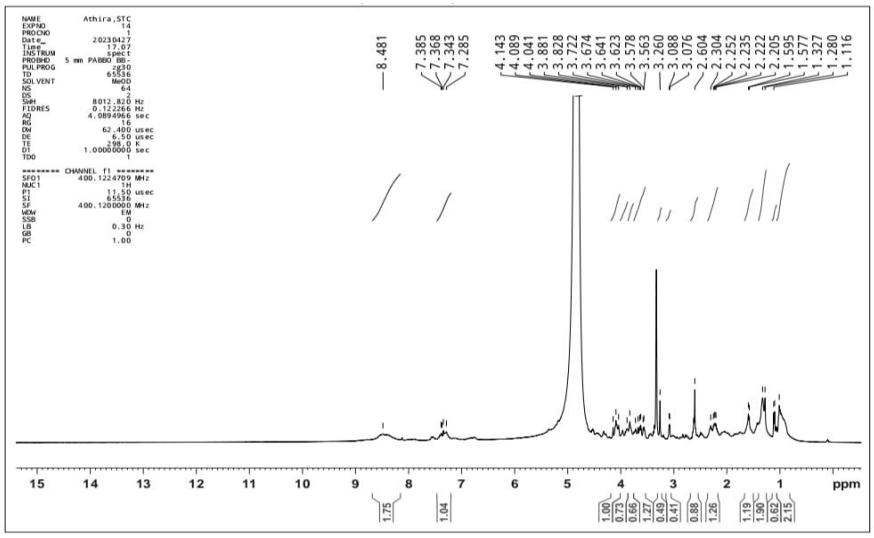


Fig 28: NMR Spectrum of calotropis gigantea Flavonoids

The few multiplets at 0.8-0.9 represent CH3 protons and the peaks in the range 1.5-2 represent CH2 and CH protons. Peaks ner 7 range represent the presnce of aromatic ring. Peak at 8.5 represent CO group. Peaks at 3-3.5 range can be carbon attatached to heteroatom.

4.2.5 Clerodendrum infortunatum (Flavonoids)

4.2.5 (a) IR Spectroscopy

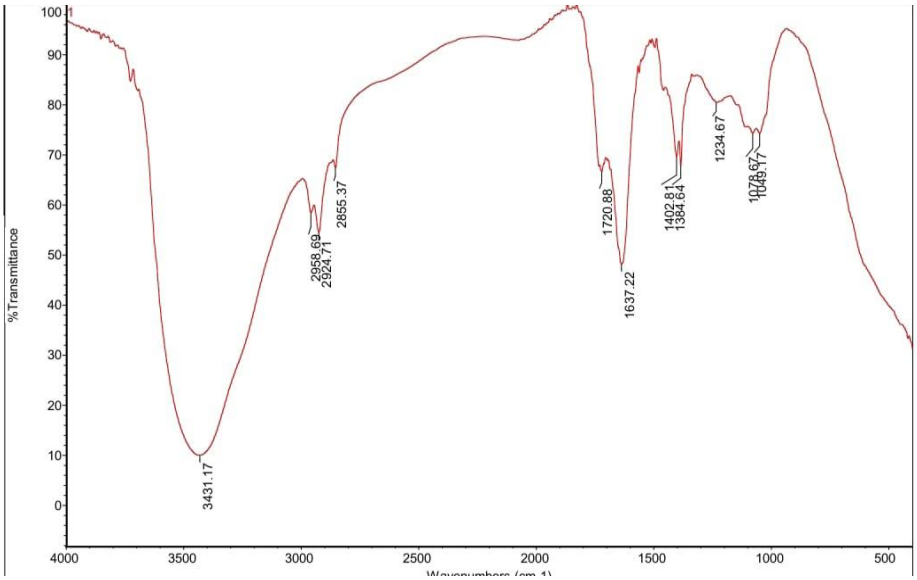


Fig 29: IR Spectrum of Clerodendrum infortunatum Flavonoids

The IR spectrum (KBr) shows peak at 3431.17 due to O-H stetching.peaks at 2958,2924.71 cm^{-1} due to C-H stretching.peak at 1720.88 due C=O stretching.and peaks at 1234,1078.67,1049.17cm^{-1} due to C-O stetching.

4.3 ANTIACTERIAL ASSAY

4.3.1 Solvents

Standard solventswere taken Methanol, Ethyl Acetate and water and its antibactetial activity against E-coli and Staphylococcus was observed.Following are the results obtained after 24 hours of incubation period.

Solvents	E-coli	Staphylococcus
Methanol	8mm	5mm
Ethyl Acetate	7mm	4mm
Water	Nil	Nil

Table 5: Antibacterial activity solvents

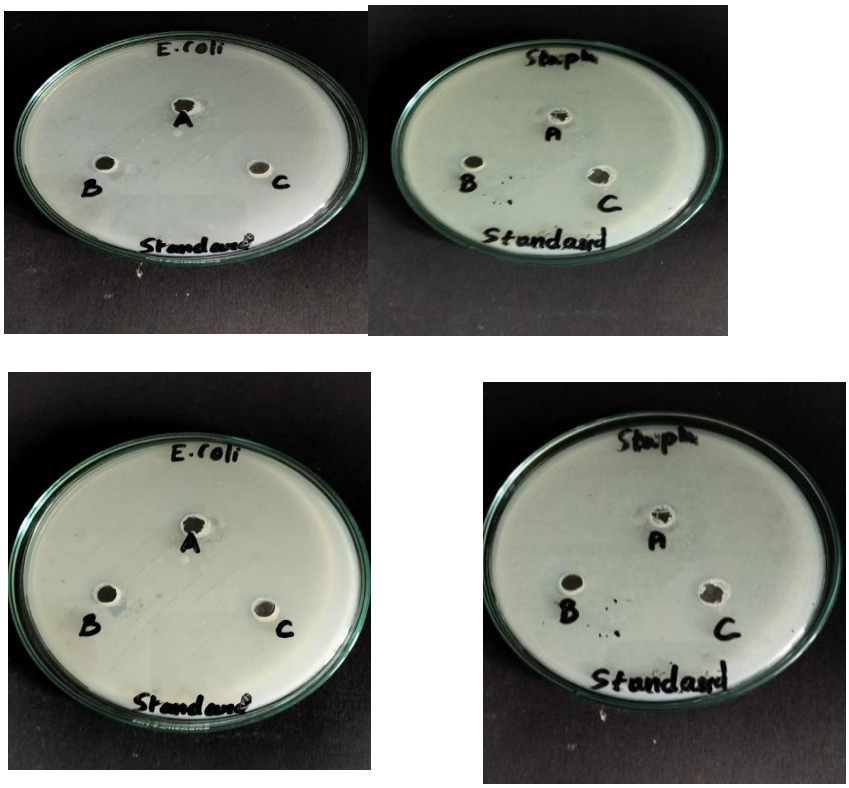


Fig 30:Antibacterial activity of the solvents after 24 and 48 hours of incubation period.

4.3.2Morinda citrifolia

The powdered fruit of morinda citrifolia is mixed with methanol,Ethyl acetate and water and its antibacterial activity against E-coli and staphyloccous were obsevered after incubation of 24 hours and 48hours

Sample	E-coli		Staphylococcus	
	24hrs	48hrs	24hs	48hrs
Methanol+Noni powder	8mm	10mm	11mm	16mm
Ethyl acetate+Noni powder	7mm	9mm	14mm	16mm
Water +Noni powder	Nil	Nil	Nil	Nil

Table 6: Antibacterial activity of Morinda citrifolia in different sovents after 24 and 48 hours.

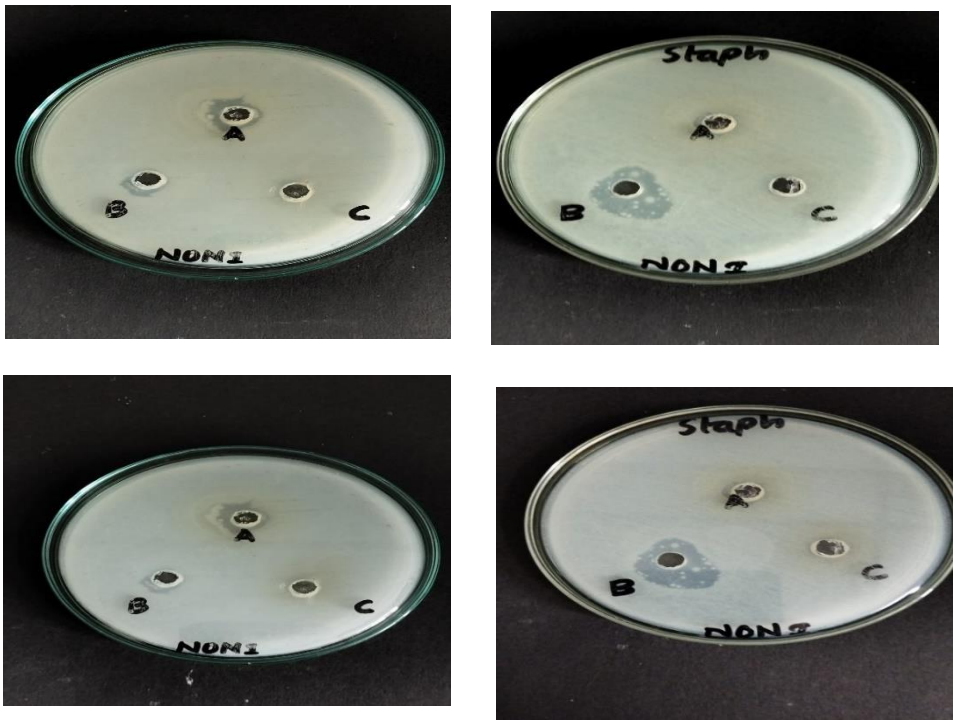


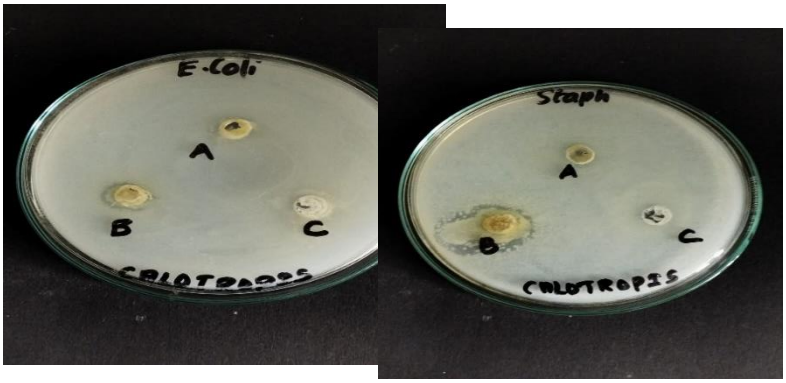
Fig 31:Antibacterial activity of the Morinda citrifoliaon different solvents after 24hrs and 48hrsof incubation period.

4.3.3Calotropis gigantea

The powdered sample is mixed with methanol ,Ethyl acetate,and water and antibacterial activity against E-coli,and staphylococcus were observed after incuabating for 24 and 48 hours

Sample	E-coli		Staphylococcus	
	24hrs	48hrs	24hrs	48hrs
Methanol+powdered sample	7mm	7mm	5mm	6mm
Ethyl acetate +powdered sample	7mm	8mm	18mm	21mm
Water+powdered sample	Nil	Nil	Nil	Nil

Table 7: Antibacterial activity of calotropis gigantea in different solvent after 24 and 48 hours of incubation.



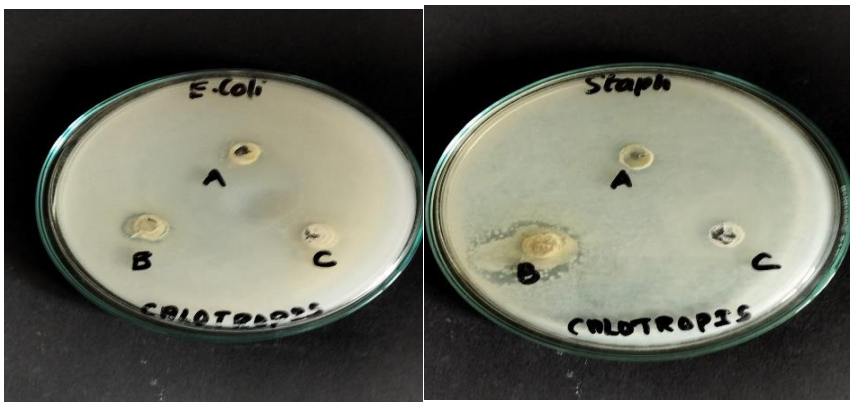


Fig 32:Antibacterial activity of the Calotropis gigantea in different solvents after 24 and 48hrours of incubation period.

4.3.4 Clerodendrum infortunatum

The powdered sample is mixed with methanol, Ethyl acetate and water and its antibacterial activity against E-coli and Staphylococcus were determined after incubating for 24 and 48 hours

Sample	E-coli		Staphylococcus	
	24hrs	48hrs	24hrs	48hrs
Methanol +powdered sample	6mm	11mm	11mm	13mm
Ethyl acetate+powdered sample	11mm	11mm	6mm	9mm
Water +powdered sample	Nil	Nil	Nil	Nil

Table 8: Antibacterial activity of Cleridendrum infortunatum in different solvents after 24 and 48 hours of incubation period.

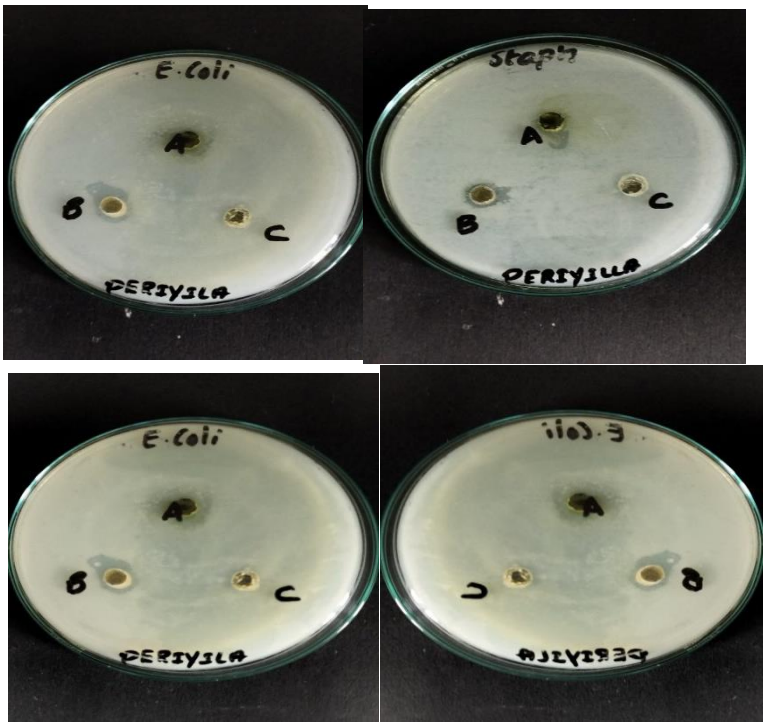


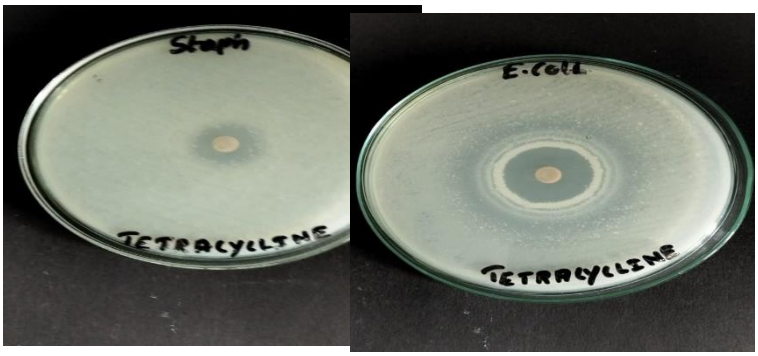
Fig 33:Antibacterial activity of the Clerodendrum infortunatum in different solvents after incubating for 24 and 48hrs.

4.3.5 Tetracyclin

Tetracyclin is taken as a stanadrard antibiotic to compare the antibacterila activity ecoli and staphalococcus in different plant extracts after incubation of 24 and 48 hours.

Anti biotic	Ecoli		Staphalococcus	
	24hours	48hrs	24hours	48hours
Tetracyclin	10mm	11mm	10mm	12mm

Table 9 Antibacterial activity aginst tetracyclin



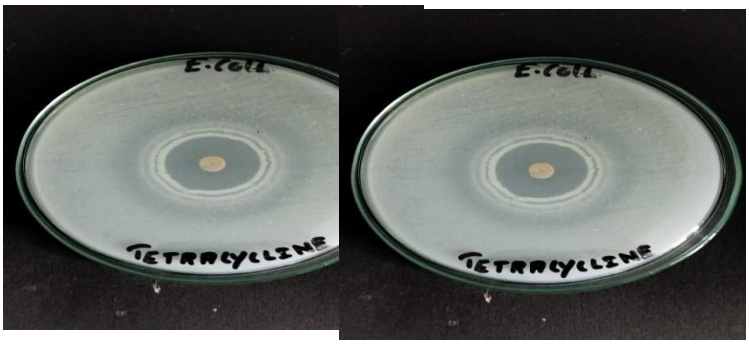


Fig 34 antibacterial activity of tetracyclin against E-coil and staphylococcus

Chapter5

Conclusions

Phytochemical screening of showed the presence ofAlkaloids, flavonoids, tannins, saponnin, phenols,and carbhohydrates in Morinda citrifolia. Calotropis gigantea showed the presence of Alkaloids, flavonoids, saponnins, steroids, and phenols. Clerodendrum infortunatum showed the presence of Flavonoids,tannins, and Saponnins. Alkaloids and Flavonoids were successfully extracted from Morinda citrifolia and Calotropis gigantean.Flavonoids were extracted from Clerodendrum infortunatum and characterized using IR and NMR spectroscopy. A further study using advanced NMR techniques is required to completely elucidate the structure of these phytochemicals.

Antibacterial studies, with tetracycline as the standard revealed that Morinda citrifolia and Calotropis gigantea has greater antibacterial activity towards Staphylococcus bacteria compared to E.coli. The ethyl acetate extract of Calotropis gigantea had 1.8 times greater activity than the tetracycline standard. Similarly Morinda citrifolia showed 1.3 times more antibacterial activity than the tetracycline standard, supporting their use in wound healing in ayurvedic medicines.

References

[1]Gershenzon J, Ullah C. "Plants protect themselves from herbivores by optimizing the distribution of chemical defenses". Proc Natl AcadSci USA. 119 (4)(January 2022)

[2] Das B, Pal D, Haldar A. A review on biological activities and medicinal properties of Clerodendruminfortunatum Linn. International Journal of Pharmacy and Pharmaceutical Sciences 6(10): 41-43.(2014)

[3] Sridevi Nagalingam, Changam Sheela Sasikumar* and KotturathuMammenCheria Asian J Pharm Clin Res, Vol 5, Suppl 2, 179-181(2012)

[4] Ali, M., M. Kenganora, and S.N. Manjula. Health benefits of *Morindacitrifolia* (Noni): A review. Pharmacogn. J. 8:321–334. 2016

[5]Sridevi Nagalingam, Changam Sheela Sasikumar* and KotturathuMammenCheria Asian J Pharm Clin Res, Vol 5, Suppl 2, 2012, 179-181(2012)

[6]Upadhyay, RaviKant "Ethnomedicinal, pharmaceutical and pesticidal uses of Calotropisprocera (Aiton) (Family: Asclepiadaceae)". International Journal of Green Pharmacy. 8 (3): 135–146.(2014).

[7] Hasler CM, Blumberg JB. Symposium on Phytochemicals: Biochemistry and Physiology. Journal of Nutrition; 129: 756S-757S.1999

[8] MamtaSaxena, JyothiSaxena, Rajeev Nema, Dharmendra Singh, Abhishek Gupta Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry. 1(6): 168-182.(2013).

[9] Mathai K.Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump, 271: 274-275.2000

[10]Costa MA, Zia ZQ, Davin LB, Lewis NG. Chapter Four: Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops. In Recent Advances in Phytochemistry, vol. 33, Phytochemicals in Human Health Protection, Nutrition, and Plant Defense, ed. JT Romeo, New York, 67-87.1999;

[11] Meagher E, Thomson C. Vitamin and Mineral Therapy. In Medical Nutrition and Disease, 2nd ed., G Morrison and L Hark, Malden, Massachusetts: Blackwell Science Inc, 33-58, 1999;

[12] American Cancer Society. Phytochemicals. Available at http://www.cancer.org/eprise/main/docroot/ETO/content/ETO_5_3X_Phytochemicals, June 2000.

[13] Narasinga Rao. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. Asia Pacific Journal of Clinical Nutrition,: 12 (1): 9-22.2003

[14] Yancui Huang, Di Xiao, Britt M. Burton-Freeman, IndikaEdirisinghe Chemical Changes of Bioactive Phytochemicals during Thermal Processing. Reference Module in Food science.(2016).

[15]Molyneux RJ, Nash RJ, Asano N. Alkaloids: Chemical and Biological Perspectives, Vol. 11, Pelletier SW, ed. Pergamon, Oxford,; 303.1996

[16] Wink M, Schmeller T, Latz-Briining B. Modes of action ofallelochemical alkaloids: Intraction with neuroreceptors, DNA and other molecular targets. Journal of chemical Ecology, ; 24: 1888-1937. (1998)

[17] Rao RVK, Ali N, Reddy MN. Occurrence of bothsapogenins and alkaloid lycorine in Curculigoorchioides. Indian Journal Pharma Science, ; 40: 104-105, 1978

[18]Pridham JB. In: Phenolicsin Plants in Health and Disease, Pergamon Press, New York,; 34-35.1960

[19]Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as Nutraceuticals: A Review.Tropical Journal of Pharmaceutical Research,; 7: 1089-1099.2008

[20]Atmani D, Nassima C, Dina A, Meriem B, Nadjet D, Hania B, Flavonoids in Human Health: From Structure to Biological Activity.Current Nutrition & Food Science, 5: 225-237.2009

[21]Schofield P, Mbugua DM, Pell AN. Analysis of condensed tannins: a review. Animal Feed Science Technology; 91: 21-40.

[22]Mangan JL.Nutritional effects of tannins in animal feeds. Nutrition Research and Reviews, 1988; 1: 209-231, 2001

[23] De Bruyne T, Pieters L, Deelstra H, Vlietinck A. Condensed vegetables tannins: biodiversity in structure and biological activities. Biochemical System Ecology; 27: 445–59, 1999

[24]Dolara P, Luceri C, De Filippo C, Femia AP, Giovannelli L, Carderni G, Cecchini C, Silvi S, Orpianesi C, Cresci A. Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. Mutation Research; 591: 237–46, 2005

[25]Blytt HJ, Guscar TK, Butler LG. Antinutritionaleffects and ecological significance of dietary condensed tannins may not be due to binding and inhibiting digestive enzymes. Journal of Chemical Ecology,; 14: 1455-1465.1988

[26]Avenas P (2012). "Etymology of main polysaccharide names" (PDF). In Navard P (ed.). The European Polysaccharide Network of Excellence (EPNOE). Wien: Springer-Verlag. Archived from the original (PDF) on February 9, 2018. Retrieved January 28, 2018.

[27]Flitsch SL, Ulijn RV. "Sugars tied to the spot". Nature. 421 (6920): 219–220. Bibcode:2003Natur.421..219F. doi:10.1038/421219a. PMID 12529622. S2CID 4421938.(January 2003)

[28] Walton NJ, Mayer MJ, Narbad A. Molecules of Interest:Vanillin. Phytochemistry, 63:505-515.2003

[29] Dai J, Mumper R. Plant phenolics: extraction,analysis and their antioxidant and anticancer properties. Molecules;15: 7313-7352.,2010

[30] "Lanosterol biosynthesis". Recommendations on Biochemical & Organic Nomenclature, Symbols & Terminology. International Union Of Biochemistry And Molecular Biology. Archived from the original on 2011-03-08. Retrieved -11-28.2006

[31] Bohlmann J, Meyer-Gauen G, Croteau R. Plant terpenoid synthases: Molecular biology and phylogenetic analysis. Proc Natl Acad Sci USA; 95: 4126–4133.,1998

[32] LasztityR, Hidvegi M, Bata A. Saponins in food. Food Review International; 14: 371-390., 1998

[33] Abdullahi R Abubakar, Mainul Haque. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci,; 12(1): 1-10.2020

[34] G N Sapkale, S M Patil, U S Surwase, P K Bhatbhage. Supercritical Fluid Extraction. Int. J. Chem. Sci,; 8(2): 729-743.2010

[35] Nur Amirah AsifaRaishaZahari, Gun Hean Chong, LuqmanChuah Abdullah, Bee Lin Chua. Ultrasonic- Assisted Extraction Process on

Thymol Concentration from PlectranthusAmboinicus Leaves: Kinetic Modelling and Optimization. MDPI, 8(3): 1-17.2020

[36] Skoog, Douglas A.; Holler, F. James; Crouch, Stanley R. Principles of Instrumental Analysis (6th ed.). Belmont, CA: Thomson Brooks/Cole. pp. 169–173. ISBN 978-0-495-01201-6.(2007)