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PROJECT REPORT

On

EXTRACTION AND CHARACTERISATION OF ALKALOIDS AND CARBOHYDRATES FROM MORINDA CITRIFOLIA

Submitted by

NIMI JOSE (AM22CHE010)

SONA SAJEEVKUMAR (AM22CHE015)

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

2023-2024

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Submitted to the Examination of Master's degree in Chemistry

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CERTIFICATE

This is to certify that the project work "**EXTRACTION AND CHARACTERISATION OF ALKALOIDS AND CARBOHYDRATES FROM MORINDA CITRIFOLIA**" is the work done by **Nimi Jose** and **Sona Sajeevkumar** under the guidance of **Dr. Elizabeth Kuruvilla, Assistant Professor,** 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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DEPARTMENT OF CHEMISTRY
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CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)

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CERTIFICATE

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Dr. Elizabeth Kuruvilla

Project Guide

DECLARATION

We hereby declare that the project work entitled "**EXTRACTION AND CHARACTERISATION OF ALKALOIDS AND CARBOHYDRATES FROM MORINDA CITRIFOLIA**" 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 of **DR. ELIZABETH KURUVILLA**, ASSISTANT PROFESSOR, Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam .This project work is submitted in the partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry.

NIMI JOSE
SONA SAJEEVKUMAR

Acknowledgements

We are very grateful to God Almighty for his continuous blessings for the successful completion of my project.

We would like to express our profound sense of gratitude to Dr. Elizabeth Kuruvilla, our guide and Assistant Professor and Research guide, the Department of Chemistry, St. Teresa's College Ernakulam for her valuable guidance, personal attention, meaningful suggestions, help, and encouragement.

We thank Dr. Saritha Chandran, HOD of the Chemistry Department, for providing the opportunity to do the project and giving all the support and guidance that helped me complete the project duly.

We would like to express my heartfelt gratitude to all the teachers and nonteaching staff for their wholehearted help throughout my project.

We extend our sincere gratitude to Sr. Vinitha C.S.S.T provincial superior and manager and to Dr. Alphonsa Vijaya Joseph, Principal of St. Teresa's College Ernakulam for being the pillars of support and providing good infrastructure for the study and development of students.

We heartily thank STIC (CUSAT) and NIT Calicut for providing all the spectroscopic assistance needed for the characterization of the sample within the time limit.

Acknowledgements

We thank the Teresian Instrumentation and Consultancy Center (TICC) for the support and guidance for my project.

Last not but least, we are grateful to our loving families and friends for the care, support, and concern they provide to follow our passion.

Nimi Jose

Sona Sajeevkumar

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Chapter 1

Introduction

1.1 MEDICINAL PLANTS

Healing with medicinal plants is as old as humanity itself, and the relationship between humans and the search for medicine in nature goes back far into the distant past, with evidence coming from a variety of sources. There are 4,444 original documents, preserved monuments, and even plants. Awareness of the use of medicinal plants is the result of years of fighting disease, through which humans learned to find medicine from the bark, seeds, fruiting bodies, and other parts of plants. Modern science has recognized its positive effects and has incorporated it into modern drug therapy. Many drugs of plant origin known from ancient civilizations are used for thousands of years. The development of knowledge and awareness regarding the development of ideas related to the use of medicinal plants has improved the ability of pharmacists and physicians to meet the challenges that arise with the proliferation of professional services to facilitate human life. About half of the world's flowering plant species (125,000 species) live in tropical forests. Tropical rainforests continue to contain a vast reservoir of potential drug species. They continue to provide natural product chemists with valuable compounds as starting points for new drug development. Since only about 1% of tropical species have been studied for their pharmaceutical potential, the potential for finding additional compounds is enormous.[1]

The therapeutic plant utilized for the present project is

Morinda citrifolia plant (Noni)

MEDICINAL PLANT	PHYTOCHEMICALS PRESENT	MEDICINAL USES	IMAGE
MORINDA CITRIFOLIA (NONI)	Alkaloids,flavonoids, steroids, carbohydrates, carboxylic acids	it boosts immune system, used for the treatment of diabetes, wound healing, physical indurance, blood pressure .	

Table 1: Noni fruit, medicinal use and phytochemicals present

1.1.1 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 meters 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 ready, smells and tastes bad.[2]An evergreen bush called Morinda citrifolia produces a natural product when it is ready that tastes and smells unequivocally of butyric acid. Beta-carotene, ascorbic acid, terpenoids,

alkaloids, beta-sitosterol, carotene, polyphenols such flavonoids, flavone glycosides, rutin, and others are among the cancer prevention agents found in Morinda citrifolia L. natural product. Moreover, it may boost resistant framework action. M. citrifolia is used as a nourishment supplement in different countries. It is utilized to remedy an assortment of sicknesses in people's medication, including joint pain, cancer, diabetes, liver disarranges, intestinal sickness, hypertension, TB, contamination, and cardiovascular illnesses[3].

1.2 PHYTOCHEMICALS

Phytochemicals are organically dynamic, actually, chemical compounds found in plants that have appeared to have well-being points of interest for people past those related to macronutrients and micronutrients [4]. Phytochemicals are chemical substances that plants make, more often than not to help them stand up to illnesses from parasites, microbes, and plant infections. They are moreover devoured by creepy crawlies and other creatures [5]. They develop diverse plant parts, checking the roots stems, clears, blooms, common items, and seeds. They are not essential for human survival and are not considered essential supplements, but they do have basic qualities that can offer help in expecting or treating many common tribulations.

Phytochemicals in food can appear in a combination of nourishments, checking characteristic items, vegetables, vegetables, aggregate grains, nuts, seeds, living beings, herbs, and flavours [6]. Phytochemicals are developed in a combination of plant parts, tallying the roots, stems, takes off, sprouts, characteristic items, and seeds [7]. The outside layers of the

diverse plant tissues routinely contain high concentrations of phytochemicals, especially colour compounds[8].

1.2.1 Classification of Phytochemicals

Phytochemicals are divided into two categories, essential metabolites and accessory metabolites, depending on how they contribute to the plant's digestive system. Essential metabolites such as lipids, purines, pyrimidines, nucleic acids, carbohydrates, amino acids, and proteins are fundamental to plant life. On the other hand, the remaining plant materials are produced by cells through auxiliary metabolic processes that are separate from the basic metabolic pathway[9] .The organic effects of these chemicals delivered by the plant's digestive system include antioxidant activity, antibacterial activity, the balance of detoxifying chemicals, promotion of a safe system, reduction of platelet accumulation, hormonal digestive system balance, anti-inflammatory properties and anti-cancer activity. There are approximately 1,000 known and unknown phytochemicals. It is generally known that plants produce these substances for self-sufficiency, but later it was thought that certain secondary plant substances could also protect humans from disease [10]. Phytochemicals are regularly classified into six broad categories based on their chemical and cosmetic properties. These classifications include lipids, phenols, terpenoids, alkaloids, and carbohydrates. [11]

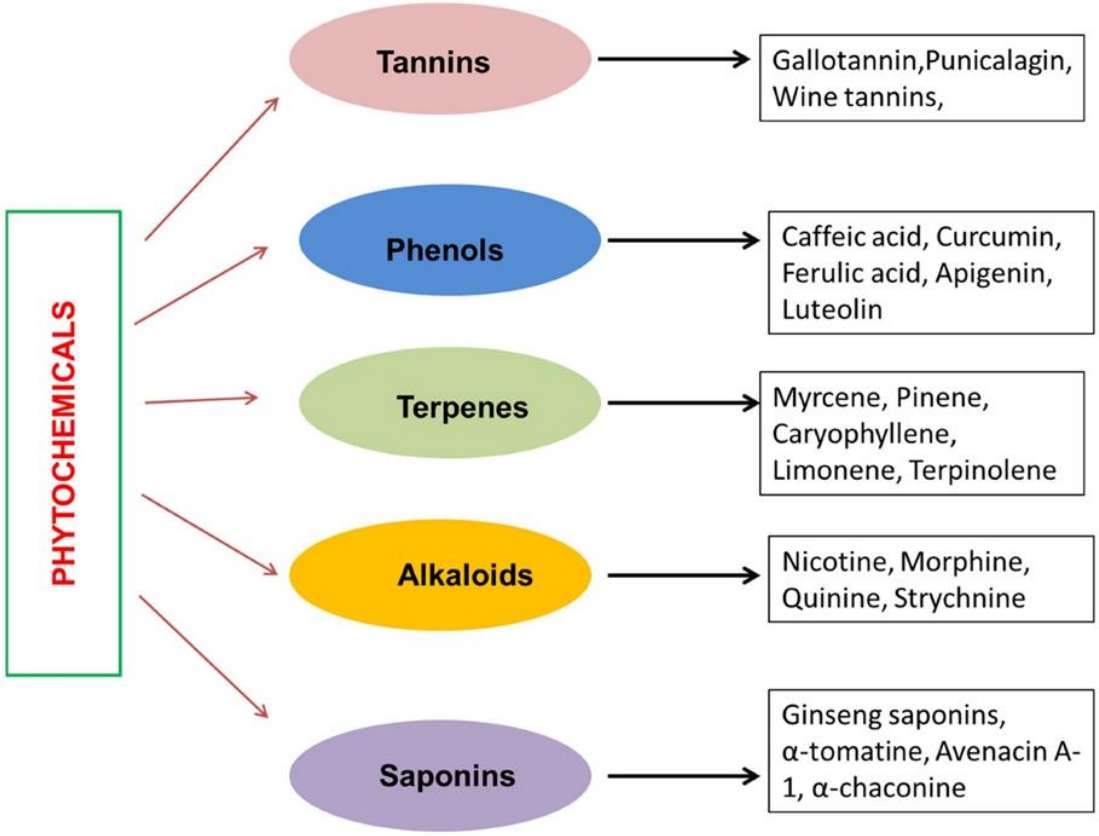

Fig 1: Classification of Phytochemicals [5]

A FEW OF THE PHYTOCHEMICALS ARE:

1.2.1 (a) ALKALOIDS

Alkaloids are vital for plant security and survival since they protect plants from microorganisms (antibacterial and antifungal exercises), creepy crawlies, and herbivores (nourishing obstacles), as well as from other plants, utilizing chemicals that are allelopathically dynamic [12]. Plants that

contain alkaloids have been utilized as colours, flavours, solutions, and harms for all intents and purposes since the daybreak of civilization. Alkaloids have immense pharmacological impacts, counting antihypertensive impacts (different indole alkaloids), antiarrhythmic impacts (quinidine, spareien), antimalarial activity(quinine) and anticancer activities (dimeric indoles, vincristine and vinblastine) [13]. As with caffeine and nicotine, a few alkaloids have stimulant properties. Morphine is utilized as a pain reliever, and quinine is an antimalarial medicine [14].

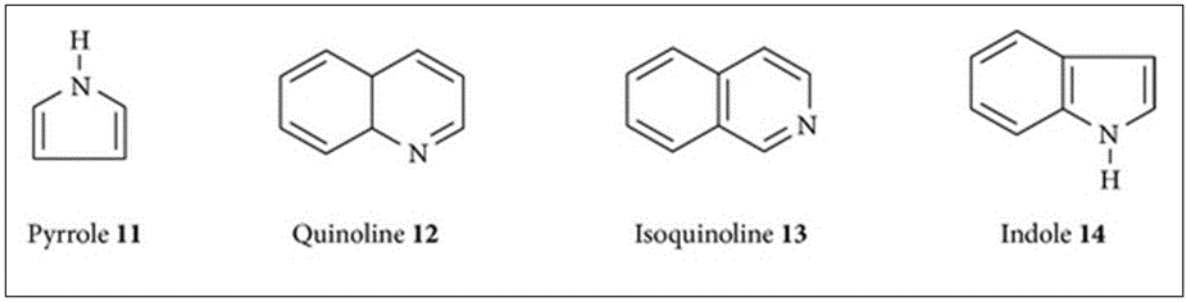

Fig 2: Typical basic structure of Alkaloids[12]

1.2.1 (b) FLAVONOIDS

Plants contain a bunch of auxiliary polyphenolic compounds known as flavonoids, which are habitually included in human diets. Polyphenolic substances called flavonoids are broadly shown in nature. More than 4000 flavonoids have been distinguished, numerous of which can be found in natural products, vegetables, tea, coffee, and fruit-flavoured drinks [15]. Due to their broad organic and pharmacological activities, flavonoids are gaining much importance . In spite of the fact that flavonoids appear to have

an assortment of natural properties, including antimicrobial, cytotoxic and antitumor impacts, their capacity to operate as powerful cancer prevention agents that can shield the human body from free radicals and receptive oxygen species are some of their properties . The flavonoids' capacity to operate as cancer prevention agents depends on their atomic makeup. The location of hydroxyl bunches and other characteristics within the chemical structure of flavonoids play a key part in the antioxidant and free radical rummaging capacities of these compounds. In differentiation, flavonoids like luteolin and catechins are more viable cancer-prevention agents than minerals like vitamin C, vitamin E, and beta-carotene[16].An assortment of compounds known as flavonoids play a critical part in guarding natural frameworks against the harmful impacts of oxidative forms on macromolecules like DNA, lipids, proteins, and carbohydrates [17].

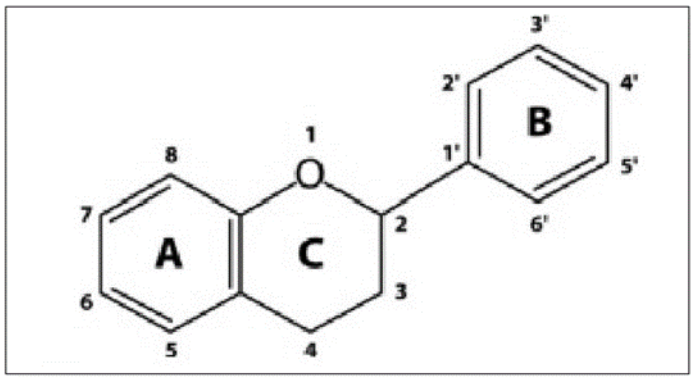

Fig 3: Basic structure of flavonoid[15]

1.2.1 (c) TANNINS

Tannins are a heterogenous course of high-molecular-weight polyphenolic chemicals that can shape reversible and irreversible complexes with an assortment of substances counting proteins (fundamentally), polysaccharides (cellulose, hemicellulose, pectin, etc) alkaloids, nucleic acids, minerals and others [18]. Hence can be classified into four fundamental groups based on their basic properties: Gallotannins, Ellagitannins, Complicated tannins, and Condensed tannins [19]

(1) Galloyl units or their meta-depsides subordinates coupled to different polyol, catechin-, or triterpenoid units constitute galloyl units in tannins

(2) Ellagitannins are tannins without a glycosidically connected catechin unit but with at slightest two galloyl units that are C-C joined to one another

(3) Complex tannins are tannins with a glycosidic bond interfacing a catechin unit with a gallotannin or ellagitannin unit.

(4) Condensed tannins are all oligomeric and polymeric proanthocyanidins made when the C-4 of one catechin is connected to the C-8 or C-6 of the taking after monomeric catechin.

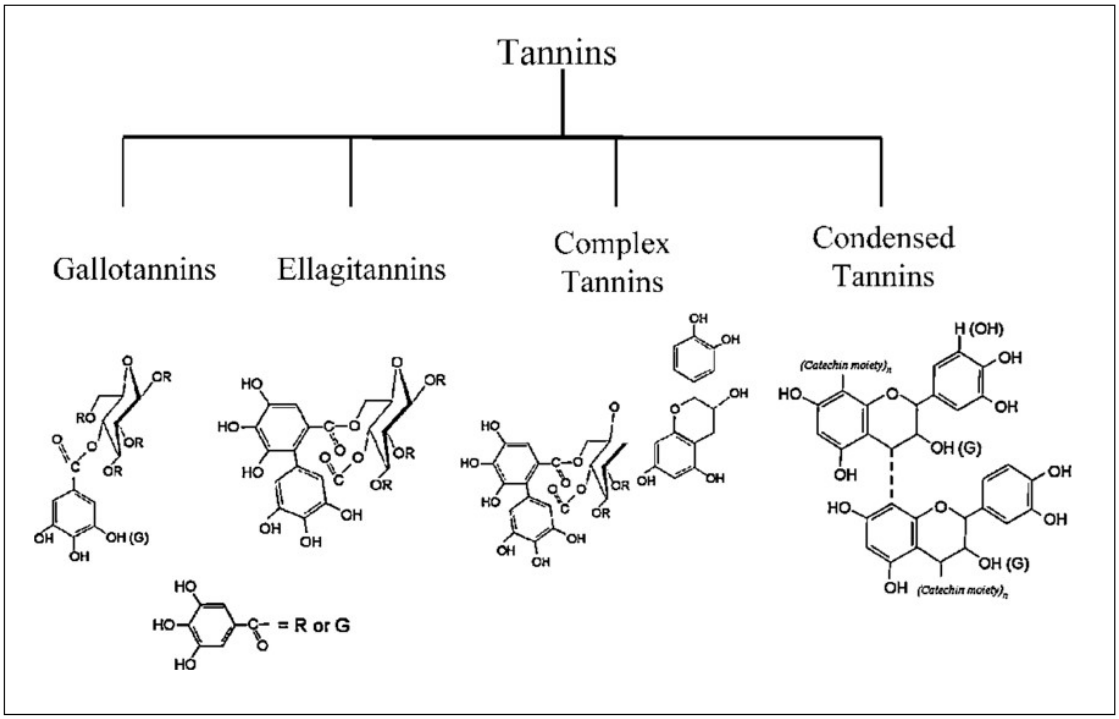

Fig 4: Classification of Tannins[18]

Tannin-containing plant extricates are utilized as astringents, and diuretics, against stomach and duodenal tumors [20], and as anti-inflammatory, clean, antioxidant, and haemostatic pharmaceuticals [21]. Tannins are utilized as caustics for cationic colors (tannin colors) within the dyestuff business, as well as within the fabricating of inks (press gallate ink). Tannins are utilized within the nourishment trade to clear wine, lager, and fruit juices. Tannins are too utilized in material colors, as cancer prevention agents within the natural product juice, brew, and wine businesses, and as coagulants in an elastic generation. As of late, tannins have provoked the intrigue of researchers, owing to the rising prevalence of lethal infections and various cancers [22].

1.2.1 (d) PHENOLIC COMPOUND

The biggest and most predominant course of phytochemicals within the plant kingdom is phenolic phytochemicals. Flavonoids, phenolic acids, and polyphenols are the three primary categories of dietary phenolics [23]. They are auxiliary metabolites from plants, and they play a noteworthy protective compound part. Phenolics have numerous qualities, and their antioxidant characteristics are significant in building up their work as preventative measures against malady forms caused by free radicals. The foremost inquired about and biggest course of plant phenols are flavonoids [24]. Hydroxybenzoic and hydroxycinnamic acids, which are both commonly utilized, are inside the wide gather of phenolic acids. Tannins, too alluded to as phenolic polymers, are high molecular-weight atoms that drop into two categories: Hydrolyzable tannins and Condensed tannins.

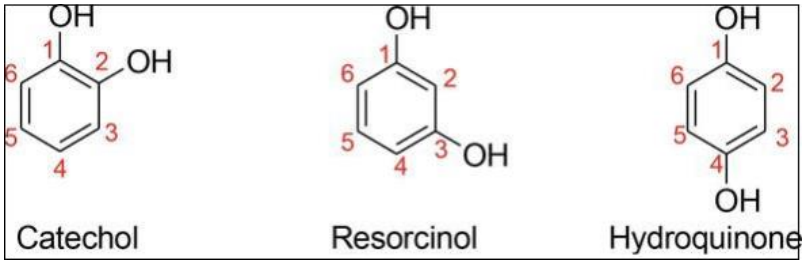

Fig 5: Basic structure of phenolic compound [23]

1.2.1 (e) STEROIDS

A steroid is a natural particle having four rings sorted out in a certain chemical setup that's physiologically dynamic. The two primary organic parts of steroids are flagging atoms and as basic components of cell films

that influence layer ease. Various steroid species can be found in parasites, creatures, and plants. The sterols lanosterol (found in opisthokonts) or cycloartenol (found in plants) are utilized to create all steroids in cells. Squalene, a triterpene, is cyclized to create lanosterol and cycloartenol.[25] The normal steroid center structure comprises four "combined" rings made up of three six-membered cyclohexane rings (rings A, B, and C within the to begin with figure) and one five-membered cyclopentane ring (the D ring), totaling seventeen carbon particles. The useful bunches that are joined to this four-ring center and the oxidation state of the rings decide how diverse steroids are. With a third hydroxy gather and a skeleton inferred from cholestane, sterols are a sort of steroid. Moreover, steroids can experience more exceptional adjustments, such as ring basic changes, such as the expulsion of one of the rings. Vitamin D_3 is one of the secosteroids created by cutting Ring B.

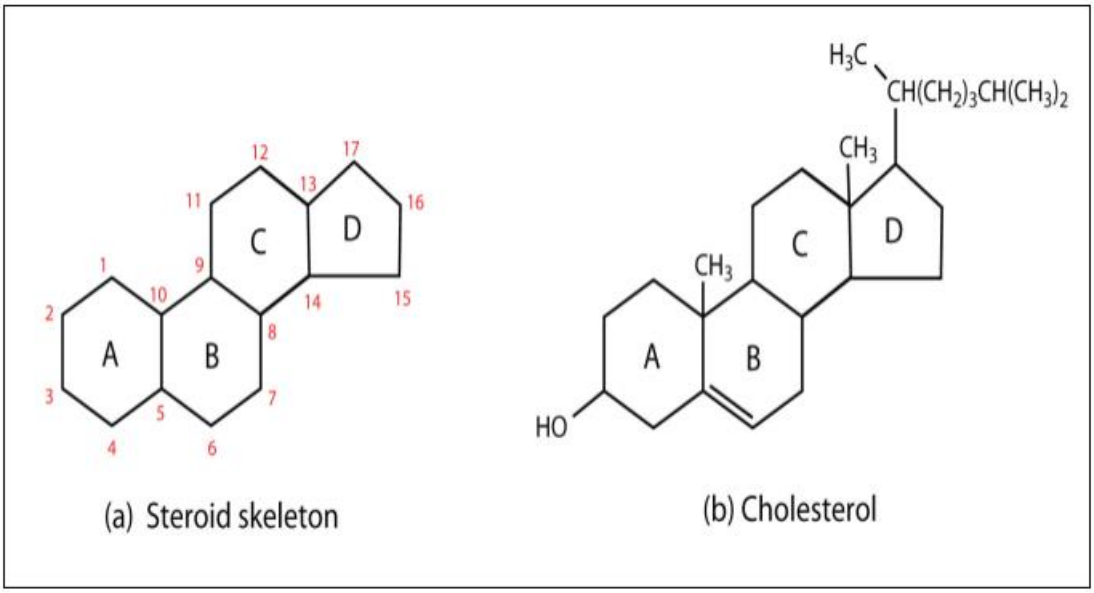

Fig 6: Basic structure of steroids [25]

1.2.1 (f) SAPONINS

A type of secondary metabolite 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, are the two most common forms of steroid aglycones [26]. The carbohydrate component is made up of one or more sugar moieties which 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 position [27].

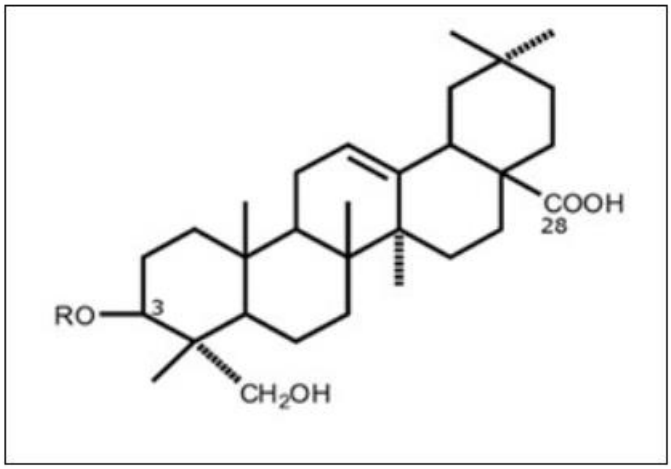

Fig 7: Basic structure of Saponins [27]

1.2.1(g) 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 clutter. Quinones improve general well-being through their antioxidant movement. Numerous of the anti-cancer medicines that have been or are as of now experiencing clinical trials are quinone-related substances. [28] Quinones are photoproducts of the discussed contamination, and their nearness has toxicological impacts as well. Quinones are speedy redox cycling compounds with the capacity to associate with hydroxyl, thiol, and amine bunches. the Audit summarizes the current information with respect to the oxido-reductive and electrophilic properties of quinones as well as the explanatory instruments utilized for their investigation. It incorporates a common presentation around the physiological, and therapeutical capacities of quinones. A number of ponders are detailed to cover the chemical reactivity in an endeavor to get it quinones as organically dynamic compounds. Information extending from typical explanatory strategies to think about quinones determined from plant or organic networks to the utilization of labelled compounds are displayed. The illustrations show how chemical, organic, and analytical knowledge can be coordinated to have a distant and much better understanding of the mode of activity of the quinones. [29]

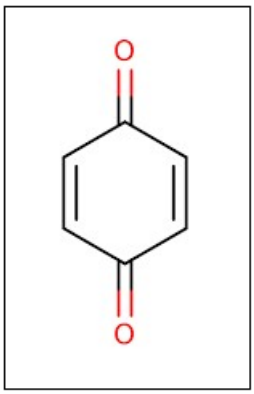

Fig 8: Basic structure of Quinones[28]

1.2.1 (h) ANTHRAQUINONES

A fragrant chemical particle having the equation $C_{14}H_8O_2$, anthraquinone (also known as anthracenedione or dioxoanthracene) incorporates a number of other names. Diverse quinone subsidiaries are included in isomers. The isomer 9,10-anthraquinone (IUPAC:9,10-dioxoanthracene), in which the keto groups are arranged on the central ring, is the one to which the term "anthraquinone" alludes. It could be a component of various colours and is utilized to dye paper pulp. Some plants contain natural substances called anthraquinones. They are straightforward anthrones or bianthrones chemically talking. Anthraquinones are used as shades, colours, and pharmaceuticals.[30]

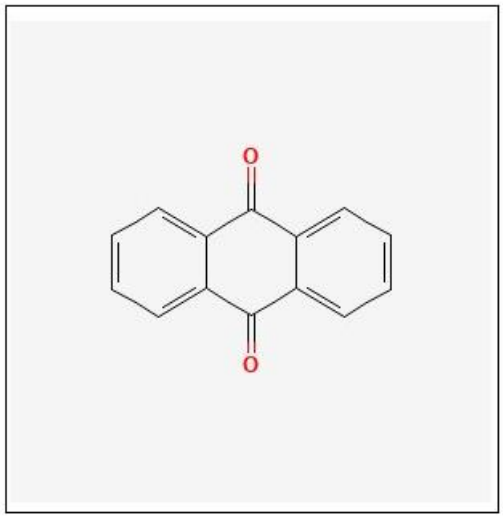

Fig 9: Basic structure of Anthraquinones[30]

1.3 POLYSACCHARIDES

Glycans, or polysaccharides, are big molecules made up of over twenty monosaccharides or their derivatives connected in a linear or branching pattern by O-glycosidic linkages (poly-numerous; saccharide-sugar). They may form branching structures, which sets them apart from linear polymers like proteins and nucleic acids. This is so that other monosaccharides can react with one another to produce branched chain structures. Each monosaccharide consists of one keto (aldehyde or ketone) group and several –OH groups.[31] Since they have a wide range of biological and pharmacological activities, including anti-tumor, immunomodulatory, antimicrobial, antioxidant, anticoagulant, antidiabetic, antiviral, and hypoglycemia activities, polysaccharides—essential macromolecules that are practically present in all living forms and have important biological functions—are garnering more attention. This makes them one of the most promising candidates in the biomedical and pharmaceutical fields. There are

numerous sources of polysaccharides, including animals, plants, microbes, and algae. The fundamental idea behind their wide range of uses in the biomedical and pharmaceutical industries is that because of their physicochemical characteristics, they are amenable to physical and chemical changes that result in improved attributes. [32]

In general, polysaccharides differ from proteins, nucleic acids, and even most glycoproteins and glycolipids in that they have repetitive structural features. These recurring features form the basis for classifying polysaccharides according to structure. Homoglycans are polysaccharides that contain a type of sugar. Polysaccharides based on a given sugar are distinguished based on differences in one or more of the following characteristics: ring size, anomeric configuration, type of linkage, and absence or presence of branching. almost an infinite number of structures are possible for polysaccharides. However, in reality, even for D-glucans, which form by far the most abundant and widespread group of homoglycans, only a small proportion of possible structural organization types are known. In heteroglycans, the main homoglycan chain carries other sugar moieties attached as side chains. Different heteropolysaccharides will carry different side chains attached to similar homoglycan chains. The difficulty in classifying polysaccharides lies in the lack of consistency and clarity in using structure as a classification tool without reference to the biosynthetic process.[33]

Most living forms contain polysaccharides, which are vital polymers with crucial biological roles. As one of the most promising candidates in the biomedical and pharmaceutical fields, they are gaining attention due to their diverse biological and pharmacological activities, including anticancer, immunomodulatory, antibacterial, antioxidant, anticoagulant, and

antidiabetic effects. There are many different sources of polysaccharides, including animals, plants, microbes, and algae. A fundamental idea for their numerous uses in the biological and medical fields is that they can undergo physical and chemical modifications to increase their properties because of their physicochemical characteristics.

It should be noted that gel is the most typical state of polysaccharides, both in biological and artificial systems. Polymer chains often form an interconnected network that creates characteristic textures and properties. Polysaccharide gels have many different biological functions, mainly in young plant cell walls, in animal fluids and connective tissues and in bacterial envelopes. They also have wide commercial applications, including in food products, cosmetics, paper and textiles.[34] The gel state is considered characteristic of polysaccharides in that polypeptides and globular proteins may characteristically appear as compact particles with a high degree of internal order, or nucleic acids may appear as pairs of complementary and highly ordered linked chains. Polysaccharides also make a unique contribution to natural polymer chemistry by representing, in terms of molecular structure, the interactions of molecules in three-dimensional chains to provide the physical and biological properties of gels.[35]

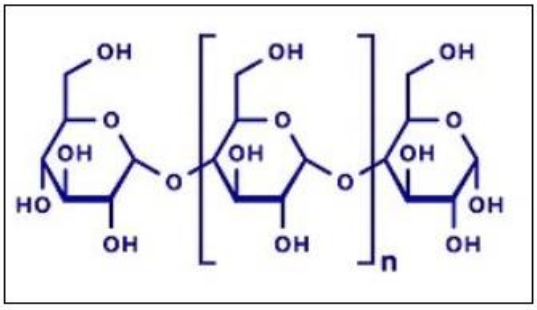

Fig 10: Basic structure of polysaccharides[31]

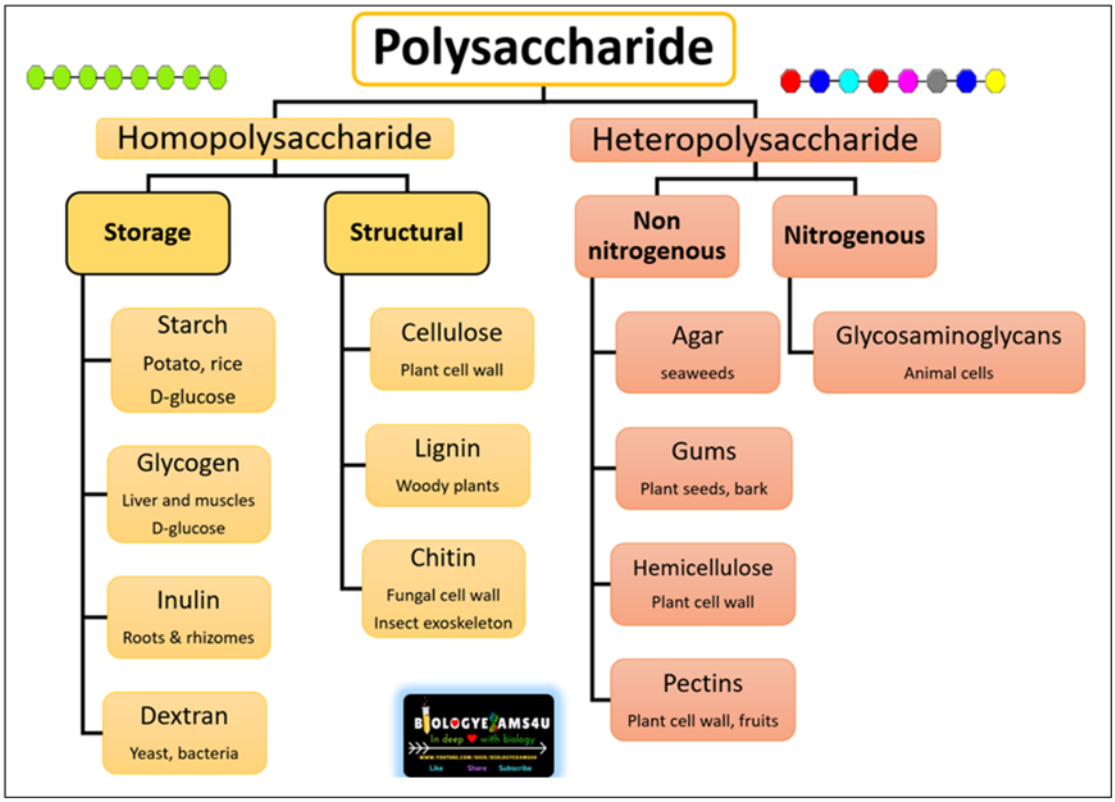

Fig11: Classification of polysaccharides[33]

Bioactive polysaccharides are widely classified based on their origin, structure, applications, solubility and chemical composition. Based on their chemical composition, they exhibit properties of homopolysaccharides (homoglycans) and homopolysaccharides (heteroglycans). Homoglycans are composed of a single type of monosaccharide, such as glycogen and cellulose, which are made up of glucose molecules, while homoglycans are composed of a different type of monosaccharide, for example heparin and chondroitin sulphate (CS).

Depending on the glycosides linked to the glycan, polysaccharides can be classified into proteoglycans and glycoproteins, and into glycolipids and glycoconjugates.[36] According to groups based on origin, biologically active polysaccharides are often classified into those of animal origin (chondroitin sulphate, heparin and hyaluronan), of plant origin (pectin, inulin, ginseng polysaccharides, xylans and arabinans), bacteria (exopolysaccharides, capsule form),polysaccharides and peptidoglycan, lichens, fungi and algae. In the section below, the bioactive properties associated with polysaccharides are examined based on their natural sources to understand their functions. Herbs occupy an important place in the traditional medicines (Ayurveda of India, ancient Chinese medicine, plant medicine in Western countries and Kamp medicine of Japan) of many countries due to Their associated medicinal benefits for many diseases.

The results of recent pharmacological studies show that the main components of medicinal plants often include tannins, polysaccharides, high molar mass proteins and low molar mass components such as terpenoids (quassinoids, sesquiterpenes and Rabdosia diterpenes), saponins, alkaloids (protoberberine alkaloids, phenanthridine, etc.) and flavonoids (Scutellaria flavones) [37]. Among the above-mentioned compounds in medicinal plants, polysaccharides are considered to be the main bioactive compounds responsible for various pharmacological potentials such as anti-cancer, antioxidant, hepatoprotective, anti-virus, radiation protection, immune stimulation and anti-fatigue. Innate polysaccharides found in many herbs are known to stimulate the human immune system, inhibit viral replication, search free radicals, and inhibit lipid peroxidation. Recent advances related to the application of polysaccharides found in medicinal plants for disease prevention and treatment are recorded in Table 2.

Polysaccharides	Biological Activities	Mechanism of Action
β-glucan	Anti-obesity activity	• Reduced energy intake • Increase in fullness and satiety • Decreased hunger and increased satiety and fullness.
Pectin, Algal Fucoidan	Anti-microbial activity	• Inhibitory effects on the entry of enveloped viruses including herpes and HIV into cells • Inhibit the formation of syncytium formation
Fucoidan, Polygonum multiflorum Thunb Polysaccharides	Anti-oxidant activity	• Inhibited ERK and p38-MAPK signaling pathways scavenging free radicals (e.g., superoxide anion radical, hydroxyl radical, and hydroxyl peroxide), • Prevents lipid oxidation and protein glycation • Inhibits the formation of ROS and RNS
Red algae sulfated polysaccharides, Carrageenan, Fucoidan, Chondroitin sulfate	Anti-inflammatory activity	• Lowered the expression of inducible nitric oxide synthase (iNOS) • Inhibited the expressions of TNF-a, interleukin-1b (IL-1b) and interferon-c (IFN-c) • Repressed pro-inflammatory cytokines • Suppresses the activity of COX-2
β-glucan	Anti-diabetic activity	• Suppressed the formation of AGE • Reduced postprandial glucose and insulin responses • Increases the level of antioxidant enzymes in the body
Konjac glucomannan	Hypo-cholesterolemic activity	• Reduced the plasma cholesterol • Significantly lowered Serum total, HDL-C, and LDL-C • Reduces the concentration of serum MDA
Pectin, Ginseng polysaccharides, Heparan sulfate	Anti-tumour activity	• Constrains the production of prostaglandin E2 • Prevents from the oxidation of DNA • Stimulates macrophages to produce helper types 1 and 2 (Th1 and Th2) cytokines • Promotes the formation of ternary complexes • Displaces growth factors
Ginseng polysaccharides	Immune modulatory activity	• Downregulates the secretion of inflammation related mediator nitric oxide (NO) and cytokines (TNF-a, IL-6, and IL-1b) • Reduces the activation of neutrophils
β-glucan, Pectin, Gums, Konjac glucomannan	Gastro-protective activity	• Supplement increased faecal bulk • Alter postprandial lipid and lipoprotein composition
Acanthopanax polysaccharides	Neuro-protective activity	• Increase SOD and GSH-Px activities and IL-10 levels • Reduces the levels of MDA, IL-1, and TNF-α • Prevents the formation of inflammatory cytokines

Table 2: biological activities of bioactive polysaccharides[37]

Polysaccharides and their derivatives are more popular in medicine than synthetic polymers due to their ability to their biodegradability, non-toxic properties and biocompatibility and low processing costs. The mentioned benefits associated with polysaccharides isolated from natural sources make it a valuable ingredient in the pharmaceutical, nutritional, food and cosmetic industries. Currently, polysaccharides are used in health care and disease

control, and 4,444 new fields have also been discovered, including cancer diagnosis, inhibition and treatment; in the provision of drugs; from an antibacterial and antiviral perspective; and tissue engineering. Various clinical studies have confirmed that oral administration of pectin to infants and young children significantly reduces diarrhea and other intestinal infections. This may be due to a decrease in the concentration of pathogenic bacteria such as Citrobacter, Salmonella, Enterobacter, Shigella, Proteus and Klebsiella. A linear relationship was observed between probiotic concentrations and gut health. The bioactive potential of Fucoidans – a sulphated polysaccharide derived from brown algae – has demonstrated significant antiviral potential against cytomegalovirus, HIV and HSV (herpes simplex virus). Fucoidan is composed of large amounts of L-fucose and sulphate groups as well as galaturonic acid, xylose, mannose and galactose. This sulphated polysaccharide is also known to prevent UV-B-induced matrix metalloproteinase-1 (MMP-1) expression by inhibiting the ERK (extracellular signal-regulated kinase) pathway. Therefore, it can be used as a functional ingredient in skin ointments to prevent skin aging. Some other algal fractions have antiviral properties and enzyme inhibitory activity that inhibits syncytium formation. The sulphate group present is essential for the anti-HIV activity , and its potency increases with the degree of sulfation. Monosaccharides. disaccharides, oligosaccharides, and polysaccharides are the four chemical classification of saccharides. Sugars are the humblest (slightest nuclear weight) carbohydrates, which are monosaccharides and disaccharides. [39]Though carbohydrate classification is complex, the names of monosaccharides and disaccharides as often as possible conclusion inside the postfix -ose, which was deduced from the word glucose and is by and utilized for about all sugars, such as fructose , sucrose , ribose, lactose , and so on.

1.4 GENERAL STRATEGIES OF EXTRACTION

1.4.1 Infusion

It is the strategy of extricating Flavors or chemical compounds from plant matter by clearing out the fabric to remain suspended in a dissolvable (hot or cold) for an extended period of time, such as water, oil, or liquor. This strategy works well for extricating effortlessly dissolvable bioactive components.[40]

1.4.2 Decoction

It is the nonstop hot extraction employing water as a solvent. Plant matter that has been dried, ground, and powdered is put into a spotless holder. After that, water is added and mixed. The extraction is at that point animated by utilizing warm all throughout the strategy. The procedure takes a few minutes, customarily around 15 minutes. It is utilized to remove plant texture that's both hot and water-soluble.[40]

1.4.3 Maceration

In a holder with a plug, combine the sample that has been coarsely ground into a powder with the solvent. After that, it is always mixed for atleast three days at room temperature to break down its soluble components. To attain exhaustive extraction, the substance is intermittently blended. After extraction, the micelle and Marc are isolated utilizing filtration or decantation. The micelle is at that point vanished in a stove or on the beat

of a water shower to partition it from the menstruum. This approach is good for utilizing thermolabile plant material.[40]

1.4.4 Soxhlet Extraction

The dried sample is placed in a filter paper and sealed. It is kept in the thimble. The solvent in the flask is refluxed, the condensed solvent covers the thimble and then siphoned into the flask bringing the extracted materials into the flask. The process is repeated. [40]

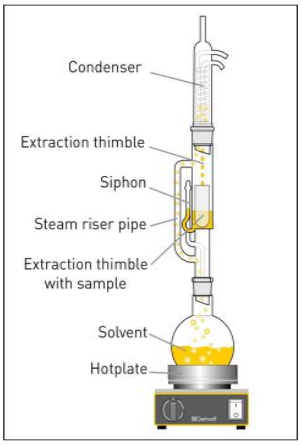

Fig 12: Soxhlet Extraction[40]

1.4.5 Supercritical liquid extraction

It is the method of utilizing supercritical liquids as the extricating dissolvable to isolate one component (the extractant) from another (the framework). A CO_2 pump, a weight cell to hold the test, a way to keep the weight within the framework, and a collecting vessel are all required parts of the framework. In a warming zone, the fluid is pumped and warmed to supercritical temperatures. The material to be extricated is along these lines

broken down because it rapidly diffuses into the strong framework interior of the extraction vessel. The extricated fabric settles out when the broken-down fabric is cleared from the extraction cell into a separator at a lower weight. After that, the CO_2 can be cooled, compressed once more, reused, or discharged into the atmosphere.[41]

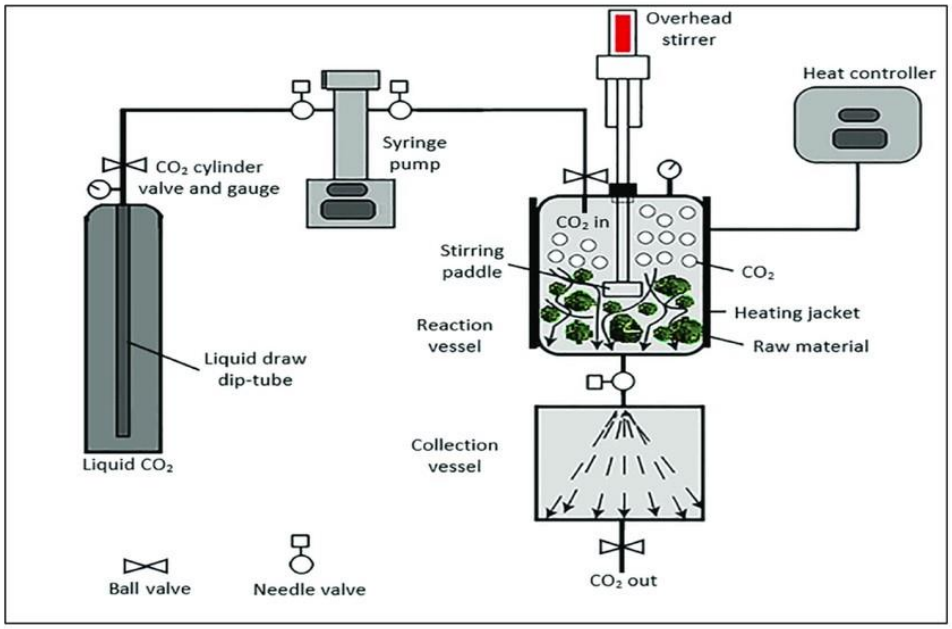

Fig 13: Supercritical fluid extraction[41]

1.4.6 solvent extraction:

Solvent extraction is the process of moving a material from one solvent to another as a result of the solubility or distribution coefficient difference between these two immiscible (or barely soluble) solvents. In comparison to the ion exchange approach, it offers a higher degree of selectivity, faster mass transfer, and superior separation performance over chemical precipitation. Compared to distillation, solvent extraction has a number of

benefits, such as low energy consumption, large production capacity, fast action, straightforward continuous operation, and ease of automation. [42]

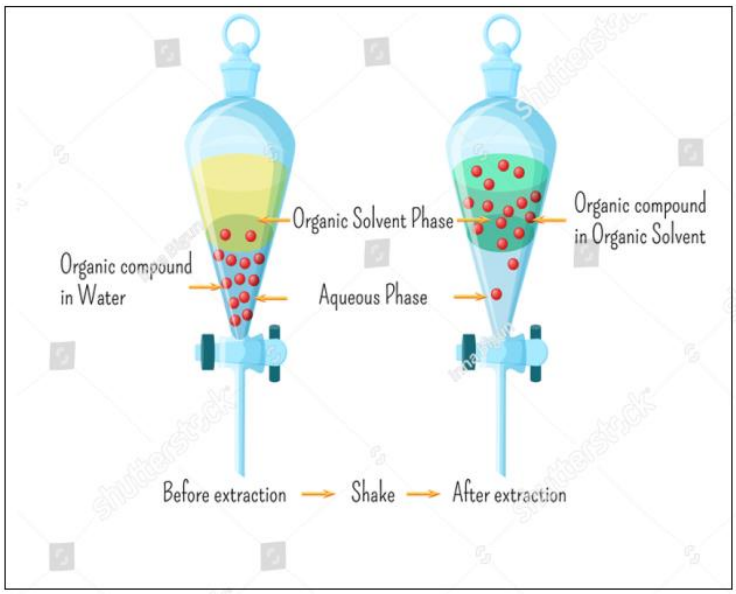

Fig 14: solvent extraction[42]

1.4.7 Ultrasound- assisted extraction

This process uses very high frequency acoustic energy above 20 kHz to damage the plant cell wall and increase the surface area of the drug for solvent penetration. This results in the release of secondary metabolites. The plant material must first be dried before being ground into a fine powder and properly sieved. After the prepared sample is mixed with the appropriate extraction solvent, it is loaded into an ultrasonic extractor. The high acoustic energy used reduces the heat required and accelerates extraction.[43]

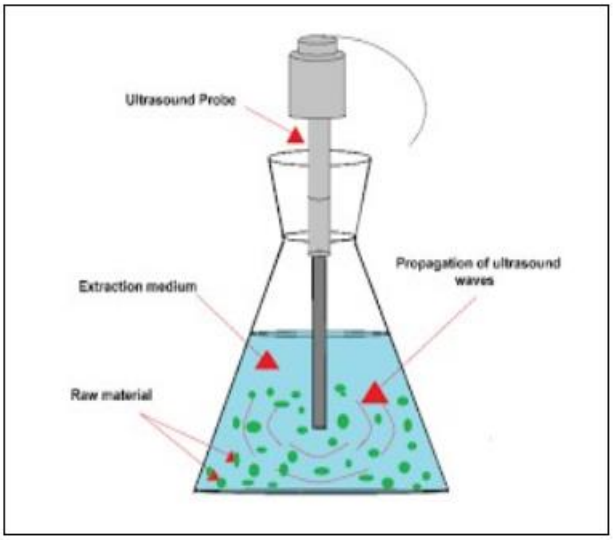

Fig 15: Ultrasound- assisted Extraction[43]

1.5 ANTIBACTERIAL ASSAY

1.5.1 Nutrient media preparation

Nutrient broth was prepared by dissolving 1.3 g of nutrient broth in 100 mL of distilled water. Test tube was filled with 5 ml of nutrient broth and sterilized using an autoclave. Nutrient agar medium was prepared by mixing 1.3 g of nutrient broth and 2 g of agar in 100 mL of distilled water. The medium was autoclaved and 20 ml each was poured into sterile Petri dishes under aseptic conditions.[44]

1.5.2 Preparation of microbial cultures

Test microorganisms E. coli and Staphylococcus were inoculated into 5 ml of sterile nutrient broth and stored at 37 °C for overnight incubation.[44]

1.5.3 Well diffusion method

Lawn cultures of each bacterium were prepared using sterile cotton swabs. Sterilized cotton swab is dipped into the bacterial suspension and moved it back and forth from top to bottom to avoid covering the space. Rotate the plate 90 degrees and repeat the process to coat the entire plate with bacteria. After preparing the lawn, 6 mm diameter wells were cut into the agar plates using a sterile borer cutter. The wells were labelled and 20 µL of samples (noni alkaloid + DMSO and noni polysaccharide + DMSO) were loaded into the corresponding wells. The antibacterial activity of the samples was compared with available standard antibiotics. The incubation of plate was carried out for 24 hrs at a temperature of 37 degree Celsius. The radius of each zone was measured in centimetres using a standard ruler. If a compound is effective against bacteria at a certain concentration, colonies will not grow. This is the inhibition zone and a measure of the compound's effectiveness. The larger the free area around the recess, the more effective the connection.[44]

1.5.4 Disc diffusion method

Filter paper discs with a diameter of 0.6 cm were punched out and sterilized in an autoclave. It was then dipped into the sample and used to test for antimicrobial susceptibility. The method used for antimicrobial sensitivity was the Kirby-Bauer disk diffusion method. The lawn cultures of each bacterium were prepared using sterile cotton swabs. A sterile cotton swab was dipped into the bacterial suspension and moved back and forth from top to bottom, leaving no empty spaces. Rotate the plate 90 degrees and repeat the same process until the entire plate is covered with bacteria. Once

the lawn is prepared, place a sterile filter paper impregnated with the sample to be tested on the plate. The antibacterial activity of the sample (specify name) was compared to available standard antibiotics. The incubation of the plate was carried out at 37 degrees Celsius for 24 hours. The radius of each zone was measured in centimetres using a standard ruler. If a compound is effective against bacteria at a particular concentration, no colonies will grow. This is the inhibition zone and a measure of the compound's effectiveness. The larger the free area around the filter paper, the more effective the connection will be.[44]

1.5.5 Sterilization and Disposal

After the experiment, autoclave the plate for 20 minutes to kill any bacteria. All glassware used in the experiments were also autoclaved to remove any bacteria that may be present.[44]

1.6 SCOPE AND POSSIBILITIES

The Plant chosen for the extraction and characterization strategies is exceptionally wealthy in restorative properties and is utilised as antihypertensive, anti-cancer, and antimicrobial property. In numerous places, this plant is utilized as a domestic cure for numerous ailments.

1.7 OBJECTIVES

- Phytochemical screening of Morinda citrifolia. This plant was chosen due to its tall restorative substance and neighbourhood accessibility.

- Extraction of alkaloids and polysaccharides from these plants.
- Characterization of the plant extraction utilizing FT-IR Spectroscopy, ^{1}H NMR ,^{13}C NMR Spectroscopy and COSY.
- Antibacterial study of alkaloid and carbohydrate extracted.

Chapter 2

Literature Survey

1. Novie, Hendro, Suharyo and Kristina analyzed the anti-tuberculosis effects of noni extracts and components. This study reveals the truth that unpurified extracts from Morinda citrifolia lin (noni) natural products and their compounds, such as flavonoids, scopoletin, anthraquinones, and alkaloids, have antituberculous activity against Mycobacterium tuberculosis (H37RV). Crude extracts of non-natural products were the most important dynamic compounds compared to other groups for M. Tuberculosis (H37RV). The minimum inhibitory dose of noni natural products against Mycobacterium tuberculosis (H37Rv) microorganism is 40 mg/ml. Based on the obtained results, noni natural products may be a potential drug source as an adjunct therapy to antituberculous drugs. [45]

2. New medicinal plants of the tropics were discussed by Yashaswini, Venugopal, Hegde and Mokashi. Noni (Morinda citrifolia L.) may be a tropical plant in the Rubiaceae family. It grows as a small evergreen tree or shrub, reaching 3-6 meters in height. The natural product is different, oval in shape, 5-7 cm long, soft and watery. Natural products contain more than 150 compounds with nutraceutical properties. The most important compounds are scopoletin, octanoic acid, vitamin C, terpenoids, alkaloids, anthraquinones, β-sitosterol, carotenes, vitamin A, amino acids, accubins, etc. Considering all of the beneficial properties of noni, Xeronine could be a miracle cure. It states that it exists in noni as its precursor prexeronine. Noni is the most important herbal and nutraceutical product traded around

the world. Fruits are presented in completely different forms. Aged and pasteurized Noni juice, Noni powder, Noni capsules, etc. Noni has the potential to be a successful medicinal plant, becoming one of the most important active ingredients in nature's medicine cabinet and a permanent nutritional supplement to meet our nation's health needs. Noni has been studied in detail based on its ethnobotany, phytochemistry, production, handling, and added value as a nutritious medicinal plant. [46]

3. Phytochemical screening of noni leaf ethanolic extracts was carried out in Anisole of Wang, Winda and Setia in Pejagan villages. Noni, a natural product, is one of the most widespread medicinal plants in Indonesia and is highly effective. In any case, the utilization rate of Noni Clearer has not yet reached its maximum. This suggested using phytochemical screening to test the presence of auxiliary metabolites in ethanolic extracts of noni plants. The ethanolic extract of Noni from Pejagan City was found to mainly contain alkaloids, terpenoids, steroids, flavonoids, tannins, and saponins.[47]

4. Sarkar, Bhattacharya, Yen, and Jyothi described and studied the therapeutic effects of ripe noni fruit (2022). In this study, methanol extracts of fresh ripe noni (NF) fruit (Morinda citrifolia) were analyzed using GC-MS, FTIR, and XRD methods. Comprehensive evaluation was performed using proximity analysis (PA), gross heating value (HHV), bulk density (BD), and swelling index (SI). Qualitative analysis of NF extracts incubated in various solvents such as distilled water, chloroform, dimethyl sulfoxide (DMSO), dimethylformamide, and methanol was found to be positive for starch, terpenoids, saponins, and cardiac glycosides. The ratios in PA of volatile content, ash content, and fixed carbon were 78.799 ± 0.592, 7.18 ± 0.044, and 14.02 ± 0.553,

respectively. AP is designed to harness energy from biomass burned in gaseous, essential (volatile), solid (solid carbon) and inorganic waste (ash) states. When estimating the energy recovery capacity of fruit biomass, it is important to consider the PCS 17.185 ± 0.103 MJ/kg. Compositional analysis (CA) was used to determine the proportions of extractives (4.497 ± 0.346), cellulose (33.114 ± 0.261), lignin (9.569 ± 0.399), and hemicellulose (17.89 ± 0.608). All of these elements have important antimicrobial properties. In our study, we investigated BD (0.312 ± 0.001 g/cm3) and SI (1.535 ± 0.022%), which increase the susceptibility of biomass to microbial activities. FTIR and XRD show CO, OH, NH, O=C=O, CH and OH bonds with constant lattice spacing. This makes it possible to determine how the material will interact with living tissue after implantation. However, no research articles regarding noni fruit oil as a topical analgesic were found in the literature. [48]

5. Ly, Nguyen, Oanh Nguyen, Duy, and Ke conducted a photochemical analysis of the wound healing activity of noni leaf extract. The botanical components were recognized and the antioxidant, anti-inflammatory, and wound-healing activities of Morinda citrifolia (Noni) leaf extract were evaluated. The results showed that noni contained alkaloids, tannins, saponins, coumarins and flavonoids The total flavonoid content (NLE) of noni leaf extract was 2.649 mg RU g-1 per dry matter. NLE acted dynamically against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus, exhibiting antioxidant and anti-inflammatory effects with IC50 values of 133.99 and 70.21 μg mL-1, respectively. NLE also had wound-healing effects in mice, reducing wound area and leading to histological recovery after 11 days of treatment. Extraction of noni leaves caused mild skin lesions on the skin of rabbits.[49]

6. Hashim Ibrahim and his colleagues carried out a chemical analysis of Noni seeds and characterized the oil extracted from Noni seeds. The approximate composition, mineral antinutritional properties and amino corrosive profile of noni seeds (Morinda citrofolia) were studied, and the physicochemical properties and fatty acid content of the oil extract were studied. The values of moisture, burnt residue, unrefined protein, unrefined fat, and crude fiber are 5.46 ± 0.01, 1.83 ± 0.01, 1.72 ± 0.02, 5.40 ± 0.07, and 4.67 ± 0.06%, respectively. The calorific value of the seeds was determined to be 1585.90 KJ. This seed has been found to be a potential source of minerals such as sodium, potassium, magnesium, calcium, phosphorus, iron, and zinc. Antinutritional components such as alkaloids, cyanides, flavonoids, oxalates, and saponins were measured. The basic amino acids glutamic acid, leucine, and aspartic acid were individually reported at concentrations greater than 6.33, 6., and 5.11 mg/100 g of crude protein. After measuring the physical and chemical properties of the seed oil, the saponification value of the oil was found to be 32. 26 mg KOH/g [50].

7.A review of the properties and uses of Morinda citrifolia was prepared by Almeida, Olveria, Hotza. Morinda citrifolia, commonly known as noni, is a perennial herb found in Southeast Asia and cultivated for over 2000 years. Noni has attracted attention from pharmaceutical and nutritional industry analysts for its ability to flexibly adapt and leverage its asset structure to meet specific recovery goals. Currently, chemical and health analyses of M. citrifolia have shown that it has bioactive properties such as acids, alcohols, phenols, sugars, anthraquinones, carotenoids, esters, triterpenoids, flavonoids, glycosides, lactones, iridoids, and ketones. It has been. The presence of more than 10,000 secondary plant substances has

been demonstrated, including lactones, lignans, nucleosides, triterpenides, sterols, and fragrances. The high health-promoting activity of M. citrifolia can produce therapeutic effects such as antibacterial and antioxidant properties. The most mechanical products of this factory are soft drinks (fruit juice drinks), powders (from dried natural products), oils (from seeds), and leaf powders. Organic and phytotherapeutic applications of M. citrifolia are promising but require more extensive research. Therefore, in this study, we will discuss the traditional uses of Morinda citrifolia, its biochemical, phytotherapeutic and toxicological properties, as well as its subsequent production and production of products derived from noni natural products. We have collected the most up-to-date and comprehensive data. It was pointed out that there is a need to investigate progress in standardization [51].

8. Variations in the biological activities along with the physiochemical properties of polysaccharides at different stages of maturity of Noni were analyzed by Cai, Ling, Li, and Mansoor. The work points to examining the maturation-related changes in polysaccharides of Morinda citrifolia L. (Noni) at five stages of development. The work demonstrated the critical impact of natural product maturities on the extraction yields, atomic weights, uronic corrosive substance, sugar levels, monosaccharide concentrations and extents, antioxidant capacities, and DNA defensive impacts of Noni polysaccharides. Be that as it may, no natural product development arrangement had a conspicuous effect on the sulfuric radical substance and preparatory structure characteristics. Noni polysaccharides extricated at organize 5 (N5) had the biggest extraction surrender, the most elevated sugar substance, and the foremost strong rummaging impact on DPPH and ABTS radicals. The more grounded DPPH and ABTS radical

scavenging activities of N5 may well be contributed by its higher substance of fucose and rhamnose and smaller atomic weight. [52]

9. A study by Yang, Mo, Zheng, Li, Tang, Wu et al. (2020) highlights the ameliorative effects of noni polysaccharides on oxidative stress and hepatitis in mice fed with a high-fat diet and its possible mechanism. Nonalcoholic fatty liver disease is associated with gut microbiota, increased oxidative stress, and worsening of the condition. We demonstrate that noni-polysaccharide (NFP) reduces oxidative stress and liver injury in mice fed with high-fat diet (HFD) by regulating short-chain fatty acids (SCFA), intestinal obstruction and balance of intestinal microflora. We aimed to investigate possible contributing factors. In mice, 4 weeks of HFD-mediated enrichment followed by 5 weeks of NFP treatment (100 mg/kg body weight) induced hepatic oxidative burst, inflammation, and dysbiosis has been shown to reduce weight gain and improve lipid digestion, growth, and stimulate liver oxidation in HFD-fed mice. In addition to these beneficial effects, NFP also significantly affects SCFA production and HFD-induced altered intestinal dysbiosis, as evidenced by microbiota differences in material and the improvement of its composition. Treatment was performed using NFW (10 ml/kg body weight) and NFP (50, 100 and 200 mg/kg body weight) for 4 weeks. They showed that NFW and NFP reduced weight gain, relative liver weight, and relative abdominal fat weight in mice fed a low-calorie versus high-fat diet. Furthermore, NFW and NFP reduced hepatic malondialdehyde concentrations and increased the contribution of Trolox to liver antioxidant capacity. NFP significantly increased the mobility of superoxide dismutase and glutathione peroxidase in the liver, and administration of NFP at 100 and 200 mg/kg body weight resulted in an

increase in calculated values relative to whole numbers. red blood cells in the liver. It was a success, demonstrating the progress of the antioxidant movement. NFW and NFP control the extent of tumor damage and calculate liver and serum alpha, interleutin-6, and nitric oxide concentrations. All NFP measurements significantly reduced hepatic kappa B concentrations, and 100 and 200 mg NFP/kg body weight showed a potent anti-inflammatory effect. These results demonstrate that NFW and NFP reduce oxidative stress and worsen symptoms in mice fed a high-fat diet, with 100 mg NFP/kg body weight having an effect. much greater than an equivalent dose of NFW polysaccharides. This shows that PFN plays an important role. It plays an important role in NFW [53].

10. Li, Niu, Zhang, and Zdng studied the activity of polysaccharides extracted from Morinda Citrifolia using different extraction methods to highlight their antioxidant and antiproliferative properties. Three extraction strategies were connected for noni polysaccharide (NP) extraction, hot water integrated extraction (HWE), electric field assisted extraction (PEFAE) and ultrasound-assisted extraction (EAU). The extraction efficiency, sweetener, uronic corrosivity, atomic weight, monosaccharide content, antioxidant activity and antiproliferative capacity of the three NP tests were completely different. In all cases, their basic preparation characteristics and types of monosaccharides are similar. Specifically, NP-EAU exhibited the highest extraction efficiency, lowest atomic weight, and best antioxidant activity. In particular, NP-EAU was found to have excellent anti-proliferation capabilities. Compared with HWE-NP and PEFAE-NP, the estimated IC50 of UAE-NP on HepG2 cells decreased by 45. 45% and 33. 14% respectively. The most notable antioxidant activity of NP-EAU can be attributed to its lower atomic

weight, lower Lady, and higher Fuc. The most clearly demonstrated antiproliferative effect of NP-EAU may be related to moo atomic weight. Overall, UAE could be a potential process for high-quality NP extraction due to its high reconstitution, high proficiency, and outstanding biological activity. [54]

11. Alwala, Kumar Mandala, Bojja, Chitamuru and Rudra synthesized silver nanoparticles and tested the antibacterial properties of the ethanolic extract of Morinda Citrifolia. Noni is beneficial as a unique dietary supplement that will be added to the list of drugs that can treat cancer, heart disease (hypertension), arthritis, allergies, irregular menstruation, stomach ulcer thickening, diabetes, immune system and eye inflammation. a very important part is that it will improve nutrition, etc. The main objective of this study was to evaluate the extractability of aqueous and ethanolic extracts evaluated by ecological methods and also to screen for use in ethnomedicine, by screening the phytochemicals of Morinda citrifolia L extract. [55]

12. Olivier Potera and Matthias Hamburger studied the phytochemistry, pharmacology and safety of Morinda citrifolia (Noni) fruit. Since the 1990s, products derived from Noni (Morinda citrifolia) have been sold in the United States and spread around the world. Noni will have many different effects. Noni natural product juice was certified as a new food by the European Commission in 2003. This article summarizes the latest information on phytochemistry, pharmacology, safety aspects safety, health claims and benefits of noni natural products and non-native products. Information regarding the chemical composition of natural noni products has expanded significantly over time. Many in vitro and partly in vivo studies have shown many potential positive effects. In all cases,

clinical information was missing. The potential clinical significance of the results from the pharmacological review will not be revealed during the exposure. Based on toxicological assessment, noni juice is considered safe. Subsequent reports of cases of hepatotoxicity have led to a reassessment of the safety issue in Europe. [56]

13. Thin-layer chromatography for rapid identification of fruit and leaves of Morinda citrifolia L. (Noni) is reviewed by Brett J West and Shixin Deng. Morinda citrifolia L., commonly known as Noni, is a rapidly growing global product. Therefore, there is a need for a rapid and cost-effective identification test for noni fruit and leaf products. Thin layer chromatography (TLC) was developed to measure deacetylasperulosidic acid in noni fruit and leaves. A TLC method was developed for the determination of scopoletin in noni products and rutin in noni leaf products. TLC results were confirmed by high-performance liquid chromatography (HPLC) analysis. Concentrations of marker compounds detected by HPLC show that these TLC methods have very good sensitivity and utility for the determination of noni fruit and leaf components from a variety of commercial sources and products worldwide. These methods do not require expensive equipment or specialized laboratories and can be easily transferred to other laboratory settings.[57]

14. Hermilasari, Hardyanty Subair, and Irianto studied the effectiveness of noni fruit extract (Morinda citrifolia) in reducing blood pressure in vitro and silico. Hypertension is a leading cause of stroke. Controlling high blood pressure can prevent stroke. Noni fruit has been shown to reduce blood pressure. Noni fruit contains the compounds scopoletin and xeronine, which act as antihypertensive drugs. The aim of this study was

to determine the effectiveness of noni fruit extract in the management of hypertension. The research method used included assigning a pre-and post-test control group. Six Wistar rats were divided into three groups, one treatment group and two control groups. Group P1 was induced with ketamine 0; 05ml+adrenaline 0. 2 ml + noni fruit extract 6 ml, K (-) group produced with ketamine 0. 05 ml + epinephrine 0. 2 ml without extract. [58]

15. Studies on the physicochemical properties of noni fruit (Morinda citrifolia) have been studied by T Anitha, KR Vijayalatha, and G Sandeep. Morinda citrifolia L. is reported to have many therapeutic effects, including antibacterial, antiviral, antifungal, anticancer, antihelminthic, analgesic, hypotensive, anti-inflammatory and immune-enhancing. In the present study, the shelf life of fruit juice from M. citrifolia grown in Tamil Nadu was evaluated for their ability to (a) decompose chemical compounds (b) bacterial growth and (c) sensory properties of M. citrifolia juice at early and early stages. after 6 months. Degradability of chemical compound(s) in M. citrifolia juice was assessed using (a) thin layer chromatography (TLC) fingerprint profiles and (b) phytochemical classes. Microbiological analysis is performed by assessing the presence or absence of bacteria [59]

16. Exploiting the pharmaceutical potential of Noni fruit extracts, specifically the antibacterial and antifungal effects of H. against antibiotic-resistant microorganisms and oral pathogens, has been investigated by Ahmet Kati. The growing threat posed by antibiotic-resistant microorganisms requires innovative treatments. This study examines the pharmaceutical potential of noni fruit extract (NFE) from Morinda citrifolia as a promising solution against antibiotic-resistant strains and

oral pathogens. NFE exhibits strong antibacterial activity and competes with common antibiotics. This study focuses on Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis), Gram-negative bacteria (Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae) and antibiotic-resistant strains (resistant Enterococci). vancomycin (VRE).[60]

Chapter 3

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 plant chosen for the extraction of alkaloids is Morinda Citrifolia (Noni). The fruit was dried and powdered. 10 grams of the plant powdered sample was used for further studies.

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, 15 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

The plant material was put in a round-bottom flask with an air condenser and 300 ml of ethyl acetate was added in order to extract the plant material. After that, it is boiled for roughly six hours to extract all of the plant material. After filtering the material, any liquid that remains is removed by pressing the solids that remain. The resultant liquid is used to determine the different phytochemical elements after being purified by filtration or decantation.

3.2.2 Detection of Alkaloids

HAGER'S method

The presence of alkaloids can be detected utilizing Hager's method. Each test tube had 0.2 g of the chosen plant sample for the phytochemical investigation.3 ml of hexane, was added and shaken well. 5 ml of 2% HCl was put into a test tube containing a blend of hexane and plant extract. Few drops of picric acid were added and shaken well. Formation of yellow coloured precipitate shows the presence of alkaloids.

3.2.3 Detection of Flavonoids

LEAD ACETATE TEST

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

3.2.4 Detection of carbohydrates

MOLISCH'S TEST

2ml of the extract was added to 2 drops of alcoholic alpha naphthol and 1ml of concentrated sulfuric acid was added along the side of the test tube. The occurrence of the violet ring demonstrated the presence of carbohydrates.

3.2.5 Detection of Phenolic Compound

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

3.2.6 Detection of Steroids

The plant extract was mixed with a few drops of sulphuric acid which turned the solution red in colour.

3.2.7 Detection of Carboxylic Acids

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

3.3 Solvent Extraction Technique

3.3.1 Alkaloids Extraction

About 10 gm of Morinda Citrifolia plant was taken after proper drying and powdering in an RB flask. It was wetted with about 25 ml of NH4OH (25 % at room temperature). The entire mixture was dissolved in 300ml of ethyl acetate and was kept for stirring in a magnetic stirrer for about 72 hours. After 72 hours, the solution was filtered using a filter paper into a beaker. The solution in the beaker was kept for evaporation in a water bath or until the ethyl acetate was completely evaporated. After the evaporation of ethyl acetate, the contents in the beaker were dissolved in 100ml of distilled water and were acidified to a pH of 3-4 using Conc H2SO4. The entire content was stirred for about 1 hour in a magnetic stirrer. the contents in the beaker were extracted using diethyl ether in a separating funnel in order to remove all the impurities in it followed by basification of the solution using NH4OH to a pH of 9-10. The entire contents were then again extracted with chloroform solution in small quantities in order to extract the alkaloid from the solution. The extract obtained was evaporated to remove the chloroform and washed with Na2SO4 to remove all the water contents which gives the crude alkaloids.

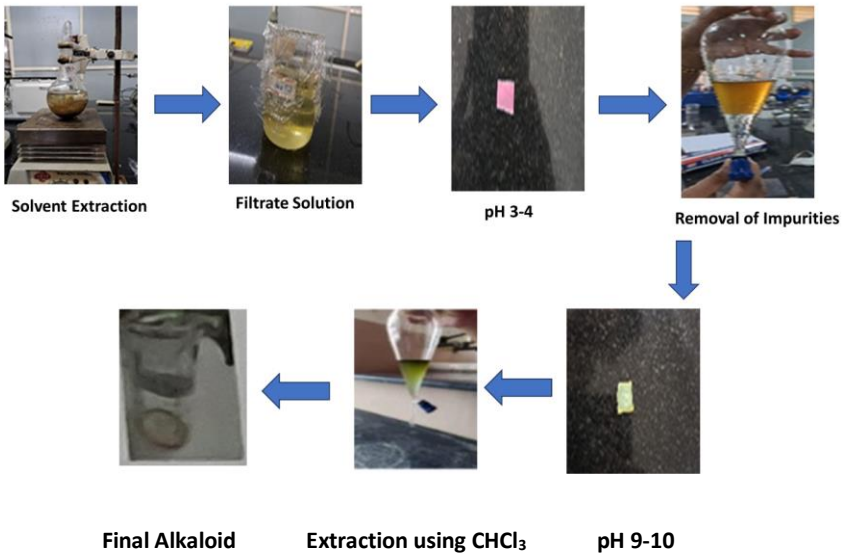

Fig 16: Different stages of alkaloid extraction

3.3.2 Polysaccharide Extraction

Dried powder is suspended in organic solvent (1.3, v/m) and soaked overnight at room temperature to remove lipids. Pigments are also removed this way. The residue is extracted three times with water at 75°C-85°C in a water bath for 2-4 hours. Suspension is precipitated using propyl alcohol. The precipitate is collected, concentrated, centrifuged, or filtrated to separate the supernatant. The precipitate is dried using an IR lamp.

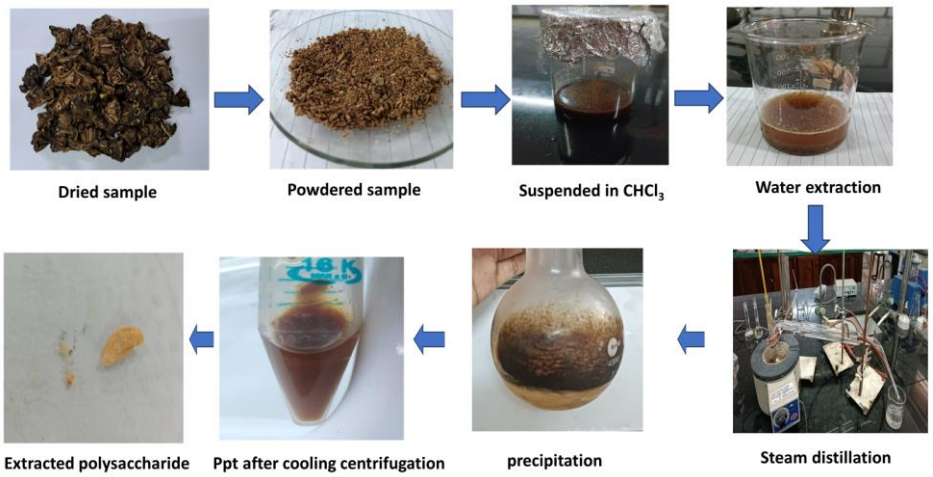

Fig 17: Different stages of polysaccharide extraction

3.3.3 Determination of Antibacterial Activity

3.3.3 (a) Nutrient media preparation

The nutrient broth was prepared by dissolving 1.3 g of nutrient broth in 100 mL of distilled water. The test tube was filled with 5 ml of nutrient broth and sterilized using an autoclave. Nutrient agar medium was prepared by mixing 1.3 g of nutrient broth and 2 g of agar in 100 mL of distilled water. The medium was autoclaved and 20 ml each was poured into sterile Petri dishes under aseptic conditions.

3.3.3 (b) Preparation of microbial cultures

Test microorganisms E. coli and Staphylococcus were inoculated into 5 ml of sterile nutrient broth and stored at 37 °C for overnight incubation.

3.3.3 (c) Well diffusion method

Lawn cultures of each bacterium were prepared using sterile cotton swabs. Sterilized cotton swab is dipped into the bacterial suspension and moved it back and forth from top to bottom leaving no space uncovered. Rotate the plate 90 degrees and repeat the process to coat the entire plate with bacteria. After preparing the lawn, 6 mm diameter wells were cut into the agar plates using a sterile borer cutter. The wells were labeled and 20 µL of samples (noni alkaloid + DMSO and noni polysaccharide + DMSO) were loaded into the corresponding wells. The antibacterial activity of the samples was compared with available standard antibiotics. The incubation of the plate was carried out for 24 hrs at a temperature of 37 degrees Celsius. The radius of each zone was measured in centimeters using a standard ruler. If a compound is effective against bacteria at a certain concentration, colonies will not grow. This is the inhibition zone and a measure of the compound's effectiveness. The larger the free area around the recess, the more effective the connection.

3.3.3 (d) Disc diffusion method

Filter paper discs with a diameter of 0.6 cm were punched out and sterilized in an autoclave. It was then dipped into the sample and used to

test for antimicrobial susceptibility. The method used for antimicrobial sensitivity was the Kirby-Bauer disk diffusion method. Her lawn cultures of each bacterium were prepared using sterile cotton swabs. A sterile cotton swab was dipped into the bacterial suspension and moved back and forth from top to bottom, leaving no empty spaces. Rotate the plate 90 degrees and repeat the same process until the entire plate is covered with bacteria. Once the lawn is prepared, place a sterile filter paper impregnated with the sample to be tested on the plate. The antibacterial activity of the sample (DMSO+Noni alkaloid and DMSO+Noni polysaccharide) was compared to available standard antibiotics. The incubation of the plate was carried out at 37 degrees Celsius for 24 hours. The radius of each zone was measured in centimeters using a standard ruler. If a compound is effective against bacteria at a particular concentration, no colonies will grow. This is the inhibition zone and a measure of the compound's effectiveness. The larger the free area around the filter paper, the more effective the connection will be.

3.3.3 (e) Sterilization and Disposal

After the experiment, autoclave the plate for 20 minutes to kill any bacteria. All the glassware used in the experiments was also autoclaved to remove any bacteria that may be present.

3.4 Characterization Techniques

3.4.1 FT-IR Spectroscopy

The term "Fourier transfer infrared" (FTIR) alludes to the foremost well-known kind of infrared spectroscopy. All infrared spectroscopies work beneath the preface that a few IR vitality is retained when it passes through a fabric. It is famous which radiation enters the test. It yields Critical data approximately the functional gathered shown within the test. Here the IR spectra were recorded on a JASCO FT-IR -5300 Spectrometer within the extend 4000-400cm- I utilizing KBr pellets at the Office of Connected Chemistry CUSAT.

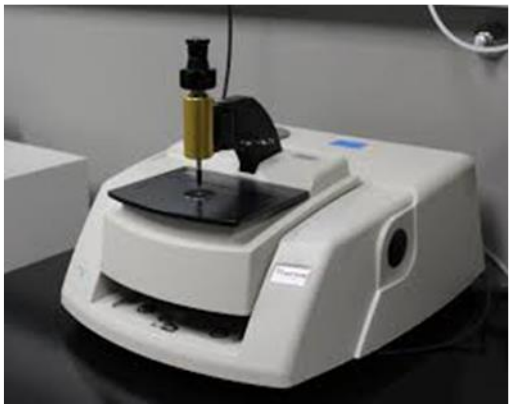

Fig 18: FT-IR Instrument[44]

3.4.2 ^{1}H NMR Spectroscopy

1H NMR is the go-to strategy to assist in distinguishing or affirming the structure of natural compounds or those that contain protons. A solution-state proton range is moderately quick to procure, compared with other

cores, and a part of data around the structure of a compound can be concluded from it. NMR spectra were identified utilizing $CDCl_3$ on a Bruker Progress DRX 300NFTNMR spectrometer at the National Institute of Technology (NIT), Calicut with TMS as the standard.

Fig:19 NMR Spectrometer[44]

3.4.3 ^{13}C NMR Spectroscopy

It is an analytical technique used to identify and work out the structure of molecules. The number of signals indicates how many different carbons or sets of equivalent carbons are present . The splitting of a signal indicates a number of hydrogens attached to each carbon. NMR spectra of samples were recorded in $CDCl_3$ using TMS as an internal standard in Bruker Advance III 400MHz FT-NMR Spectrometer at the National Institute of Technology (NIT), Calicut.

3.4.4 2D Correlation Spectroscopy

This technique involves a simple pulse sequence in which a (π/2)x pulse is first introduced into the 1H channel to create an evolutionary stage. After some time, a second (π/2) y pulse is introduced to create the acquisition phase. The 1H-1H COSY pulse sequence includes different relaxation delay times (t1) and acquisition times (t2). The experiment is repeated with different values of t1 and t2. Therefore, the value of t1 increases at regular intervals, producing a series of different FID data during t2. COSY offers a 3-bond coupling (3JH-H).

Chapter 4

Results and discussion

4.1 Phytochemical Screening

Phytochemical screening of Morinda Citrifolia was conducted. Morinda Citrifolia showed the presence of Alkaloids, Flavonoids, phenol, saponins, and tannins. The figure indicates the results of the phytochemical screening. The results are summarized below

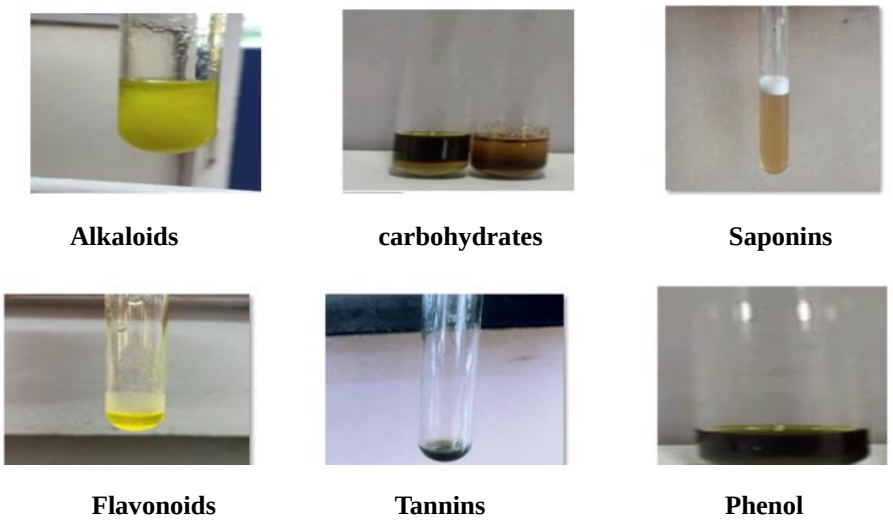

Fig 20: Screening test of Phytochemicals in Morinda Citrifolia

4.2 Spectroscopic Analysis

Alkaloids extracted from Morinda Citrifolia was characterized by Infrared Spectroscopy and NMR Spectroscopy. The polysaccharides extracted was characterised using FT-IR.

4.2.1 Morinda Citrifolia Alkaloids

4.2.1(a) FT-IR Spectroscopy

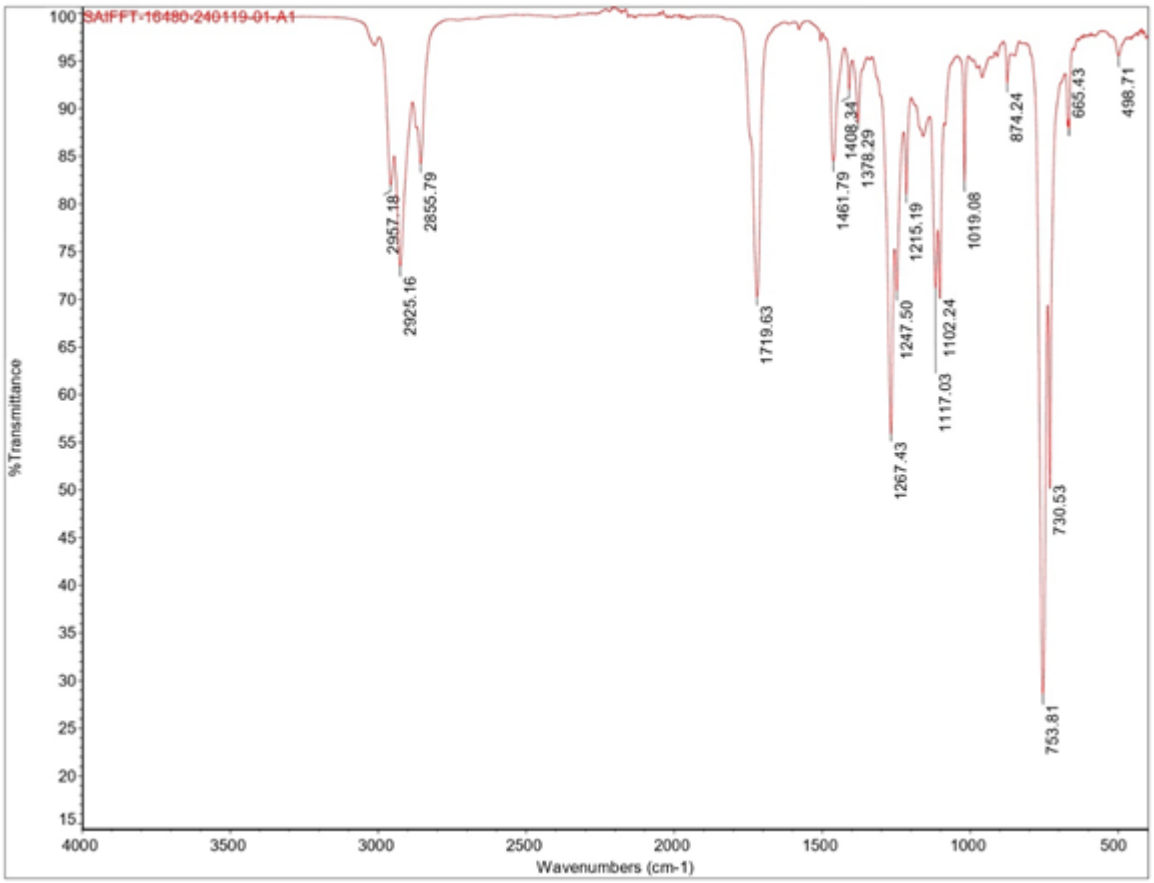

Fig 21: IR Spectrum of Morinda Citrifolia Alkaloids

A peak is found at 1247cm-1 indicating the presence of a C-N stretch of aliphatic tertiary amines. C-C-C stretch was also characterized due to a peak at 1102-1117 cm-1. The peak between 1370-1460 cm-1 indicates C-H bending and the peak at 1719 cm-1 indicates C=O bending. C-H stretch is indicated by a peak at 2855-2957 cm-1.

4.2.1(b) ^{13}C NMR Spectroscopy

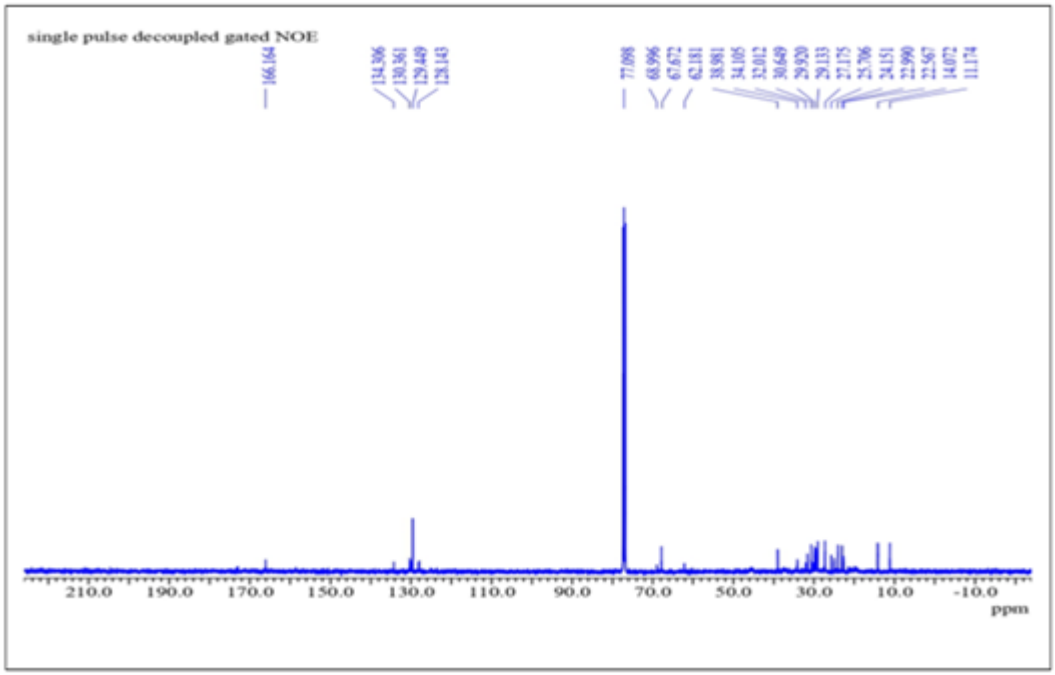

Fig 22: ^{13}C NMR Spectrum of Morinda Citrifolia Alkaloids

A peak is found at 11-38 ppm indicating the presence of Aliphatic protons. C-N carbons were also characterized due to a peak at 60-77 ppm. The peak at 130-134 ppm indicates double bond and the peak at 166 ppm indicates carbonyl carbon and ketone group.

4.2.1(c) ^{1}H NMR Spectroscopy

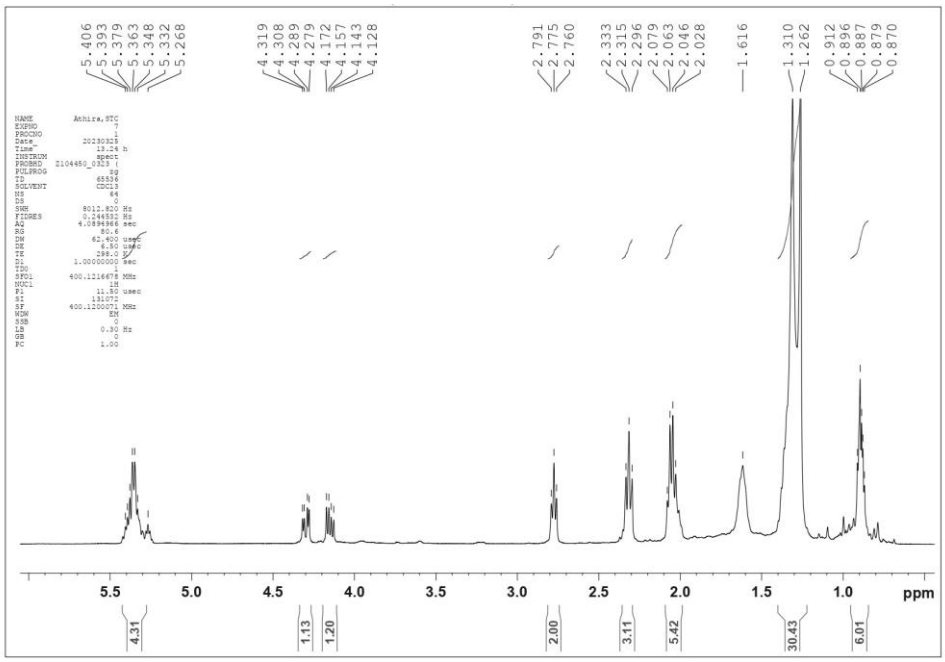

Fig 23: ^{1}H NMR Spectrum of Morinda Citrifolia Alkaloids

A peak is found at 0.82-0.93 ppm indicating the presence of Aliphatic methyl protons. R-CH_2 was also characterized due to a peak at 1-1.5 ppm. the peak at 1.7-2 ppm indicates C-H protons and the peak at 1.57 ppm indicates Aliphatic protons. vinylic cis protons are indicated by a peak at 4.136-5.325 ppm. the presence of residual proton (solvent) in $CDCl_3$ is shown by a peak of chemical shift value 7.247 ppm.

4.2.1(d) COSY Analysis

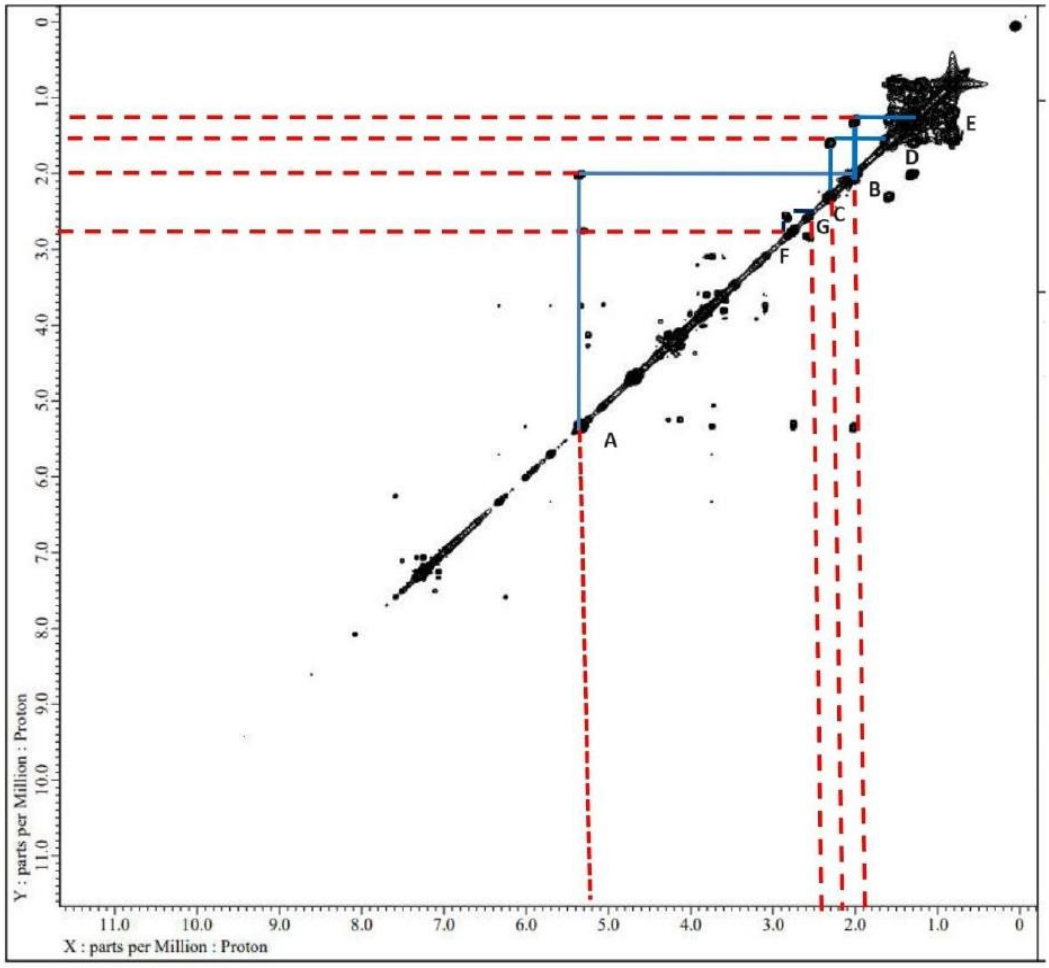

Fig 24: COSY Spectrum of Morinda Citrifolia Alkaloids

A and B shows the coupling between 5.2 and 2, C and D shows the coupling between 2.3 and 1.5 , B and E shows the coupling between 1.9 and 1.25 and F and G shows the coupling between 2.4 and 2.8 .

4.2.2 Morinda Citrifolia Polysaccharides

4.2.2 (a) FT-IR Spectroscopy

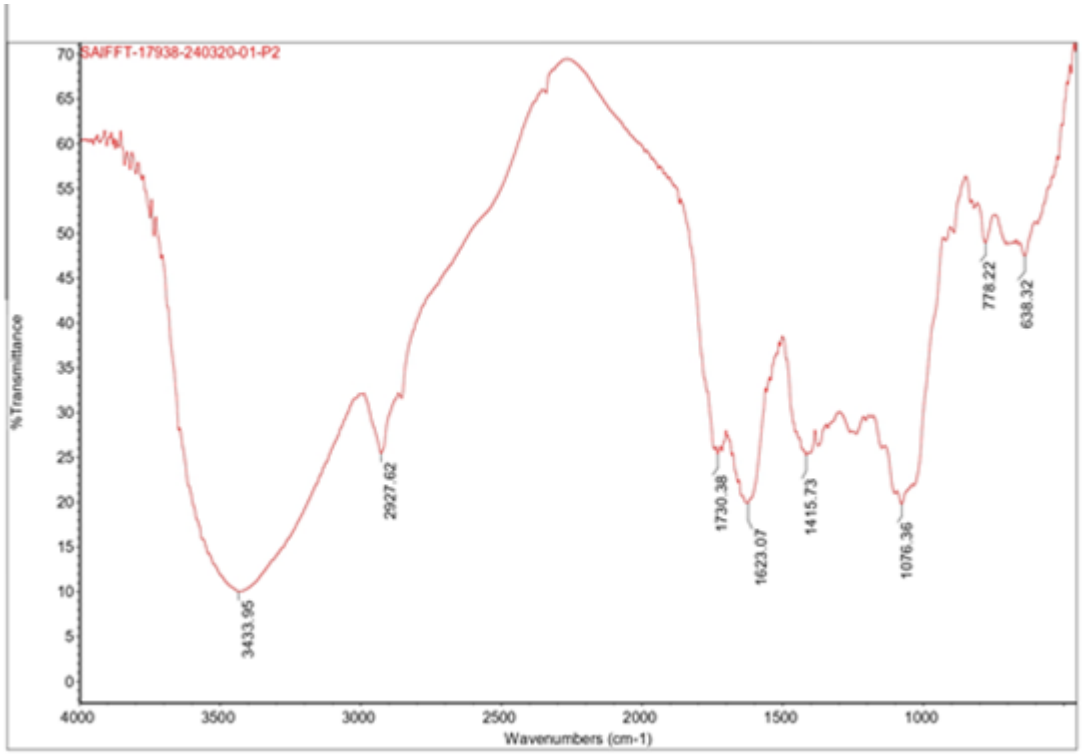

Fig 25 : IR Spectrum of Morinda Citrifolia Polysaccharides

A peak is found at 3410 cm-1 indicating the presence of O-H stretching whch indicates the presence of polysaccharides. C=C stretching of a conjugated alkene is indicated by a peak in the range 1600-1650 cm-1 .

4.2.3(a) Antibacterial Assay

The alkaloid and the polysaccharide extracted from the dried sample of Morinda Citrifolia fruit was mixed with DMSO and its antibacterial activity against E- coli and Staphylococcus were observed after a period of 24 hours.

Sample	E-coli	Staphylococcus
	24 hours	24 hours
DMSO + Noni Alkaloid	NIL	NIL
DMSO + Noni polysaccharide	0.7 cm	1 cm

Table :3 Study of Anti-bacterial properties of Morinda Citrifolia

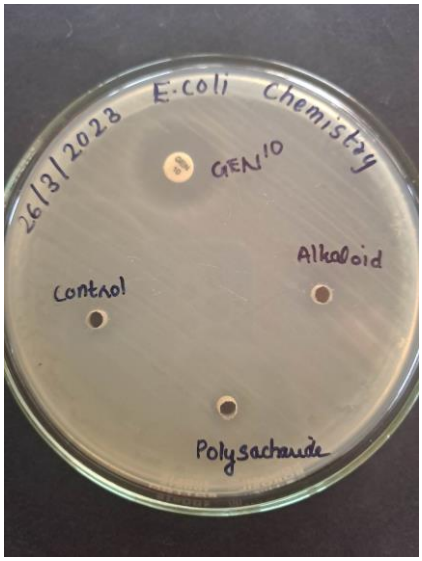

Fig 26:Antibacterial Activity of Alkaloid & Polysaccharide on E coli

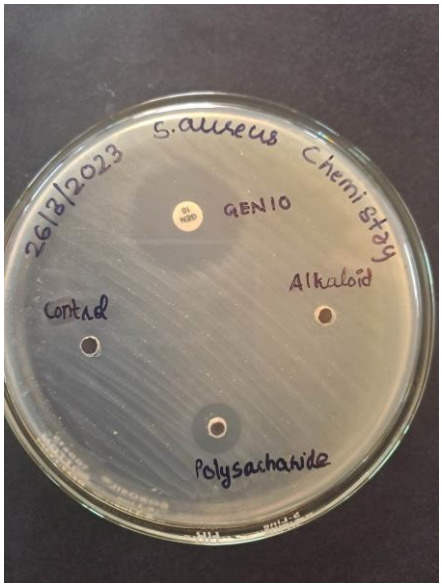

Fig27: Antibacterial Activity of Alkaloid and Polysaccharide on S.aureus

4.2.3(b) Comparative Study of Antibacterial Activity

For comparison, the powdered fruit of Morinda Citrifolia was also studied for antibacterial activity. The sample was mixed with methanol, Ethyl acetate and water and its antibacterial activity against E-coli and staphylococcus were observed after incubation of 24 hours and 48 hours. The sample showed maximum activity against staphylococcus.

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

Table 4: Antibacterial activity of Morinda citrifolia in different solvents after 24 and 48 hours

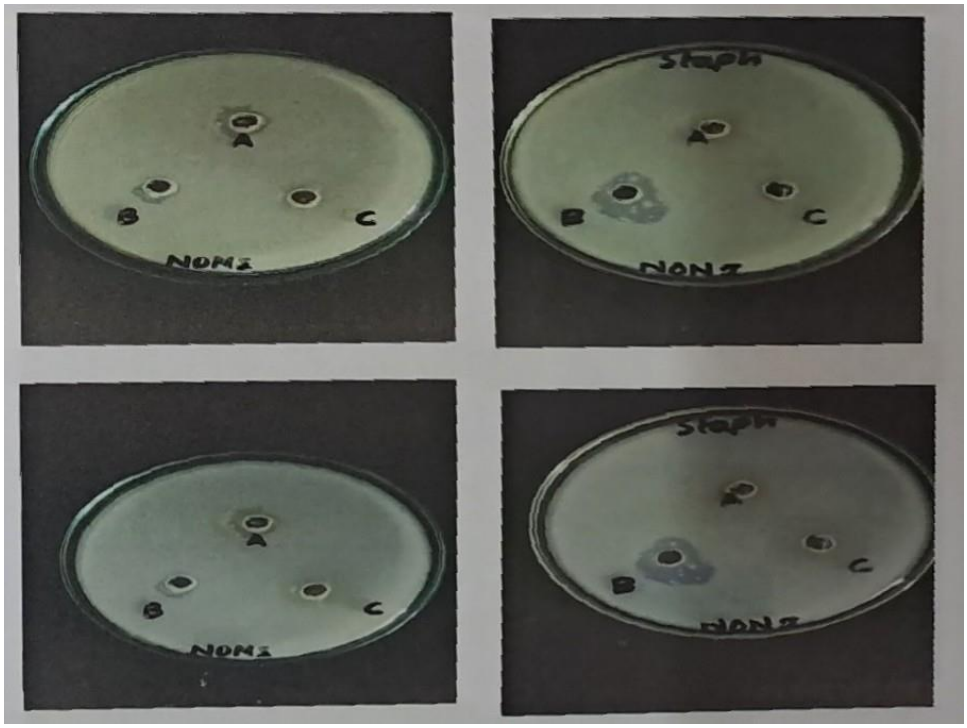

Fig 28: Antibacterial activity of Morinda citrifolia on different solvents after 24 hrs and 48 hrs of incubation period

Chapter 5

Conclusions

The screening of Phytochemicals confirmed the presence of alkaloids, Flavonoids, carbohydrates, tannins, saponins and phenol in Morinda citrifolia. The extraction of alkaloids and polysaccharides was extracted from Morinda citrifolia. The extracted alkaloids were characterized using IR, NMR and COSY spectroscopy. While the polysaccharide extracted was characterized using IR spectroscopy.

The anti-bacterial property of the extracted polysaccharides and the alkaloids from Morinda citrifolia using Gentamicin as a standard indicated that the polysaccharides extracted had greater activity towards staphylococcus than E coli. The alkaloid extracted showed no activity against both the bacteria which suggested that the anti-bacterial property of Noni powder dissolved in various solvents such as methanol, ethyl acetate and water that appears in the work done earlier is probably due to the presence of another chemicals .

References

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

[2] Sridevi Nagalingam, Changam Sheela Sasikumar and KotturathuMammenCheria Asian J Pharm Clin Res, Vol 5, Suppl 2, 179-181(2912)

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

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

[5] Mamta Saxena, Jyothi Saxena, Rajeev Nema, Dharmendra Singh, Abhishek Gupta Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry. 1(6): 168-182. (2013).

[6] 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

[7] Costa MA, Zia ZQ, Davin LB, Lewis 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 ;

[8] 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 ;

[9] Russell J, Stephen T, Dale R, Kip, Phytochemicals : The good, the bad and the ugly, American Cancer Society Phytochemicals. October 2007

[10] 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

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

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

[13] Wink M, Schmeller T, Latz-Briining B. Modes of action of allelochemical alkaloids: Interaction with neuroreceptors, DNA and other molecular targets. Journal of Chemical Ecology, 24: 1888-1937. (1998)

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

[15] Pridham JB. In: Phenolicsin Plants in Health and Disease, Pergamon Press, New York; 34-35. 1960

[16] Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as Nutraceuticals: A Review.Tropical Journal of Pharmaceutical Research; 7: 1089-1099. 2008

[17] 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

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

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

[20] 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

[21]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

[22] Blytt HJ, Guscar TK, Butler LG. Antinutritional effects 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

[23] 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.

[24]Flitsch St. Ulijn RV. "Sugars tied to the spot". Nature. 421 (6920). 249-220. Bibcode:2003 Natur. 421..219F. doi:10.1038/421219a. PMID 12529622. S2CID 4421938. (January 2003)

[25] Walton NJ, Mayer MJ, Narbad A. Molecules of Interest: Vanillin,

Phytochemistry, 63:505-515. 2003

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

[27] "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

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

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

[30] 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

[31]-[37] Samee Ullah, 2ORCID AAK, Khalil AA, Scholar S orgGoogl., Song FS 1ORCID andYuanda. Sources, Extraction and Biomedical Properties of Polysaccharides.

[38] 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

[39] Flitsch St, Ulijin R.V." Sugars tied to the spot ". Nature. 421 (6920): 249-220. Bibcode:2003Natur. 421..219F. doi:10.1038/421219a. PMID 12529622. S2CID4421938. (January 2003)

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

[41]G N Sapkale, S M Patil, U S Surwase, P K Bhatbhage. Supercritical Fluid Extraction. Int.J.Chem. Sci.:8(2):729-743,2010

[42] Rostagno MA, Prado JM, editors. Natural product extraction: principles and applications. Royal Society of Chemistry; 2013.

[43] 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

[44] Santos DG, Cunha AP, Ribeiro AC, Brito DH, Alenca LM, Farias DF, Carvalho AF, Sousa JA, Leal LK, Lopes N, Linhares RE. Characterization and Biological Activity of Native and Sulfated Noni (Morinda citrifolia Linn.) Pectin. Journal of the Brazilian Chemical Society. 2024 Mar 1;35: e-20230156.

[45] Novie E, Hendro W, Suharyo Hadi S, Tri NK. Anti-tubercular activity of extract and compounds of noni (Morinda citrifolia Linn). International Journal of Pharmacy and Pharmaceutical Sciences. 2017;9.

[46] Yashaswini S, Venugopal CK, Hegde RV, Mokashi AN. Noni: a new medicinal plant for the tropics. African journal of plant science. 2014;8(5):243-7.

[47] Mubarokah FA, Yuliasari W, Wibowo TS, Bangkalan AFYH, Surabaya SM. Phytochemical Screening of Noni (Morinda citrifolia L) Leaf Ethanol Extract in Pejagan Village, Bangkalan Regency. Indones J Interdiscip Res Sci Technol. 2023;1(7):661–8.

[48] Sarkar B, Bhattacharya P, Chen CY, Maity JP, Biswas T. A comprehensive characterization and therapeutic properties in ripened Noni fruits (Morinda citrifolia L.). International Journal of Experimental Research and Review. 2022; 29:10-32.

[49] Ly HT, Pham Nguyen MT, Nguyen TK, Bui TP, Ke X, Le VM. Phytochemical analysis and wound-healing activity of noni (Morinda citrifolia) leaf extract. Journal of Herbs, Spices & Medicinal Plants. 2020 Oct 1;26(4):37993.

[50] Etsuyankpa MB, Ndamitso MM, Oluwatoyin IV, Ibrahim H, Ogah SP. Chemical analysis of Noni (Morinda citrofolia) seeds and the characterization of the seeds oil. American Journal of Applied Chemistry. 2017;5(4):57-61.

[51] Almeida ÉS, de Oliveira D, Hotza D. Properties and Applications of Morinda citrifolia (Noni): A Review. Compr Rev Food Sci Food Saf. 2019 Jul;18(4):883-909. doi: 10.1111/1541-4337.12456. Epub 2019 Jun 10. PMID: 33336991

[52] Cai J, Liang Z, Li J, Manzoor MF, Liu H, Han Z and Zeng X Variation in physicochemical properties and bioactivities of Morinda

citrifolia L. (Noni) polysaccharides at different stages of maturity. Front. Nutr. 9:1094906. doi: 10.3389/fnut.2022.1094906 (2023)

[53] Yang X, Mo W, Zheng C, Li W, Tang J, Wu X. Alleviating effects of noni fruit polysaccharide on hepatic oxidative stress and inflammation in rats under a high-fat diet and its possible mechanisms. Food & function. 2020;11(4):2953-68.

[54] Li J, Niu D, Zhang Y, Zeng XA. Physicochemical properties, antioxidant and antiproliferative activities of polysaccharides from Morinda citrifolia L. (Noni) based on different extraction methods. Int J Biol Macromol. 2020 May 1; 150:114-121. doi: 10.1016/j.ijbiomac.2019.12.157. Epub 2020 Jan 30. PMID: 32006573.

[55] Alwala J, DR M, Mandla VK, Bojja S, Chittamuru S, Nalvothula R, Rudra MP. Interpretative In Vitro Phytochemical, TLC, Synthesis of Silver Nanoparticles and Their Antibacterial Screening of Aqueous and Ethanolic Extract of Morinda Citrifolia L. (Noni) Fruit and Their Comparative Study. World Journal of Pharmaceutical Research. 2014 Jun 16;3(6):989-1007.

[56] Potterat O, Hamburger M. Morinda citrifolia (Noni) fruit--phytochemistry, pharmacology, safety. Planta Med. 2007 Mar;73(3):191-9. doi: 10.1055/s-2007-967115. Epub 2007 Feb 7. PMID: 17286240.

[57] West BJ, Deng S. Thin layer chromatography methods for rapid identity testing of Morinda citrifolia L. (Noni) fruit and leaf. Adv. J. Food Sci. Technol. 2010;2(5):298-302.

[58] Hijriansyah, L. O. A. H., Hermilasari, H., Subair, H., Irianto, I., Armyn, A. A. U.., & Hakim, S. Study in vitro and in silico on

effectiveness noni fruit extract (Morinda Citrifolia) to reducing hypertension. Canrea Journal: Food Technology, Nutrition, and Culinary Journal, (2020). 3(2), 57–64.

[59] Anitha T, Vijayalatha KR, Sandeep G, Kanchana R. Studies on physico-chemical properties of noni fruit (Morinda citrifolia). International Journal of Chemical Studies. 2019;7(1):1301-2.

[60] Kati A. Harnessing the pharmaceutical potential of noni fruit extract: antibacterial and antifungal effects against antibiotic-resistant microorganisms and oral pathogens. Journal of Research in Pharmacy. 2023 Sep 1;27(5).