

DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH
ST. TERESA'S COLLEGE (AUTONOMOUS)

PROJECT REPORT

On

ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS FROM MORINDA CITRIFOLIA

Submitted by

AJILU GEEVER
(AB23CHE027)

In partial fulfillment for the award of the

Bachelor's Degree in Chemistry



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ERNAKULAM



Name : AJILU GEEVER
Register Number : AB23CHE027
Year of Work : 2025-2026

This is to certify that the project **“ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS FROM MORINDA CITRIFOLIA”** is the work done by **AJILU GEEVER**.

Dr. Saritha Chandran A.
Head of the Department

Dr. Elizabeth Kuruvilla
Staff-member in charge

Submitted to the Examination of Bachelor's Degree in Chemistry

Date: 10/11/26

Examiners: SENTIN DEVASUKUTS7, Nangun.
Lansado Ruffan.



DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

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ERNAKULAM



CERTIFICATE

This is to certify that the project work entitled **“ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS FROM MORINDA CITRIFOLIA”** is the work done by **AJILU GEEVER** under my guidance in the partial fulfilment of the award of the Degree of Bachelor of Science in Chemistry at St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam.


Dr. ELIZABETH KURUVILA

DECLARATION

I hereby declare that the project work entitled "**ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS FROM MORINDA CITRIFOLIA**" submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, is a record of an original work done by me under the guidance of **Dr. ELIZABETH KURUVILA**, Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam and this project work is submitted in the partial fulfilment of the requirements for the award of the Degree of Bachelor of Science in Chemistry.



AJILU GEEVER

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Chapter 1

Introduction

1.1 MEDICINAL PLANTS

Nature contains different types of natural products. There are many ways in which human diseases can be treated using natural products obtained from plants, animals and minerals. Nowadays applications of medicinal plants are growing in faster rate and have wide range of acceptance. Undoubtedly, plants play vital role in ecosystem by providing essential services to the ecosystem. Animals and other living organisms cannot survive in the ecosystem without plants, however herbal plants especially medicinal plants act as a good indicator of ecosystem health, humans have considered medicinal plants since ancient times. It was said that early humans were aware of the properties of plants more or less before they recognized and exploited the plants around them for different purposes like food, fuel, shelter etc. Plants were frequently used as medicine, drugs and disinfectants in countries like ancient Persia. In fact, in earlier times human used medicinal plants for the treatment of different diseases and such plants were only source for them for treating diseases. Over 50,000 plant species are used for making of pharmaceutical and cosmetic products [1].

Plant species especially, the medicinal plants have made substantial contribution to the emergence of many traditional herbal therapies. The largest countries in Asia, which have a wide range of relatively well-known medicinal plants, are in India and China. In India, out of 17,000 species of higher plants, 7500 species have been recognized for medicinal uses. About 8000 species of angiosperms, 44 species of gymnosperms and 600 species of pteridophytes is recognized in the Indian Himalaya and out of these 1784 species is accepted as medicinal plants. The advantages of

these medicinal plants are they are readily available, have long shelf life and can be transported easily. The most important advantage is that the herbal medicines have very less side effects [2].

Many plants have antimicrobial, anti-inflammatory and wound healing properties, due to the phytochemicals in the plants. Plants contain wide variety of secondary metabolites. These include tannins, alkaloids, phenolic components and flavonoids. These secondary metabolites found in plants are responsible for their antimicrobial properties. The medicinal plants were used for the treatment of diseases such as respiratory disorders, cutaneous infections, gastrointestinal disorders etc. As stated by World Health Organization (WHO), the excellent source to acquire a wide variety of drugs is medicinal plants [3].

1.1.1 MORINDA CITRIFOLIA (Noni)



Figure 1.1: Morinda citrifolia

The therapeutic plant selected for the present project is *Morinda citrifolia*. *Morinda citrifolia* (Noni) is a small tree belonging to the coffee family, Rubiaceae. It has got many other names like Indian mulberry, awl tree, cheese fruit, nino, nona etc. Polynesians and Tahitians identified this plant

as a medicinal plant over 2000 years ago or more. Almost all part of this plant has many uses. The fruits of this plant were used as medicine. Other parts such as leaves stem and roots were also used as medicine. This plant had an extended application, as it was used to cure cough, cold, pain, liver diseases, malaria and blood pressure in Polynesia and Southeast Asia. Noni is a small tree or shrub, which is about 3-10 m in height. Its flowers are perfect with about 75-90 inch in size from ovoid to globose. It contains unifoliate and glossy leaves, which are arranged in opposite manner. Noni fruit is yellowish white in colour and fleshy. It is about 5-10 cm long and 3-4 cm in diameter. It is pulpy and putrid when ripened. Seeds have air chamber and it allow it to retain its efficacy even after floating in water for months. It has lateral root system and taproot system similar to that of citrus and coffee plants. Wood of noni is yellowish in colour and it has a characteristic fetor odour when ripened. Noni has the ability to withstand even in harsh environments. It is found on coral atolls or mafic lava flows. It can also withstand wide range of conditions like seasonal waterlogging. Propagation of noni can occur from seed, root or stem. However, the best way of propagation is by seeds and stem cutting. About 160 phytochemical compounds have been detected and isolated from its different parts. The chemical components are varied depending on the different parts of the body. The different chemical constituent includes, flavonoids, alkaloids, phenolic components, organic acids, alcohols, phenols, esters, iridoids, lactones, lignans, triterpenoids etc.

1.1.2 USES OF NONI FRUIT

The Noni fruit and its products are used in multiple forms, including juices, capsules, powders, teas, and extracts. In traditional Polynesian culture, almost every part of the Noni plant—leaves, fruit, bark, roots, and seeds—

is used for therapeutic or nutritional purposes. The ripe fruit is often consumed as a juice to promote general wellness and boost the immune system. Noni juice is popular in many countries as a health tonic that supports digestion, increases energy, and detoxifies the body. Apart from medicinal use, Noni is also utilized in cosmetics and skincare products because of its antioxidant and moisturizing properties. Noni oil, extracted from its seeds, is used for treating dry skin, acne, and scalp conditions. In agriculture, parts of the plant are used as a natural pesticide. In many Pacific islands, Noni is also valued as a source of natural dye, and its wood is used for carving small tools and crafts. Noni fruit also plays an important role in nutrition and food industries. It can be blended into smoothies, herbal drinks, and functional foods to enhance nutritional value. Researchers have explored its use in developing natural food preservatives and supplements due to its antimicrobial activity. In recent years, Noni-based beverages have become popular health drinks in many countries, marketed for their ability to enhance vitality and strengthen immunity.

1.2 PHYTHOCHEMICALS PRESENT IN NONI

The medicinal importance of Noni lies mainly in its rich phytochemical composition. The fruit contains a variety of biologically active compounds such as alkaloids, flavonoids, phenolic acids, terpenoids, saponins, anthraquinones, and iridoids. One of the most studied phytochemicals in Noni is scopoletin, a compound known for its anti-inflammatory and vasodilatory effects. Damnacanthal, another important component, is believed to have anti-cancer properties due to its ability to inhibit abnormal cell growth. Morindone, morindin, and alizarin are anthraquinones that possess antibacterial and antioxidant activities. Noni fruit also contains vitamin C, beta-carotene, and minerals like potassium,

calcium, and magnesium that support general health. The polysaccharides and glycosides found in Noni contribute to immune system modulation and cellular repair. These phytochemicals work together to protect the body from oxidative stress, promote tissue regeneration, and enhance the body's natural healing processes. Studies have also shown that the seeds and leaves of Noni contain essential fatty acids and plant sterols that can help lower cholesterol and reduce inflammation in the body. About 160 phytochemical compounds have been already identified in the noni plant, and the major micronutrients are phenolic compounds, organic acids and alkaloids of the phenolic compounds, the most important reported are anthraquinones and also aucubin, asperuloside, and scopoletin. The main organic acids are caproic and caprylic acids.

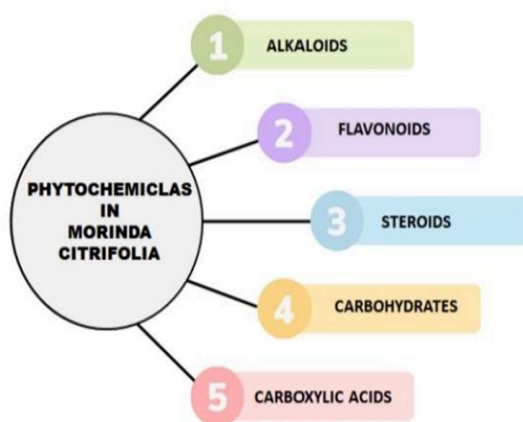


Figure 1.2: Phytochemicals in noni

chemical composition differs largely according to the part of the plant. Physico-chemical composition of the fruit has not yet been reported and only partial information is available on noni juice. The fruit contains 90%

of water and the main components of the dry matter appear to be soluble solids, dietary fibres and proteins. The fruit protein content is surprisingly high, representing 11.3% of the juice dry matter, and the main amino acids are aspartic acid, glutamic acid and isoleucine. Minerals account for 8.4% of the dry matter, and are mainly potassium, sulfur, calcium and phosphorus; traces of selenium have been reported in the juice. Vitamins have been reported in the fruit, mainly ascorbic acid. Phenolic compounds have been found to be the major group of functional micronutrients in noni juice: Damnacanthal is an anthraquinone that has been characterized recently and has some important functional properties.

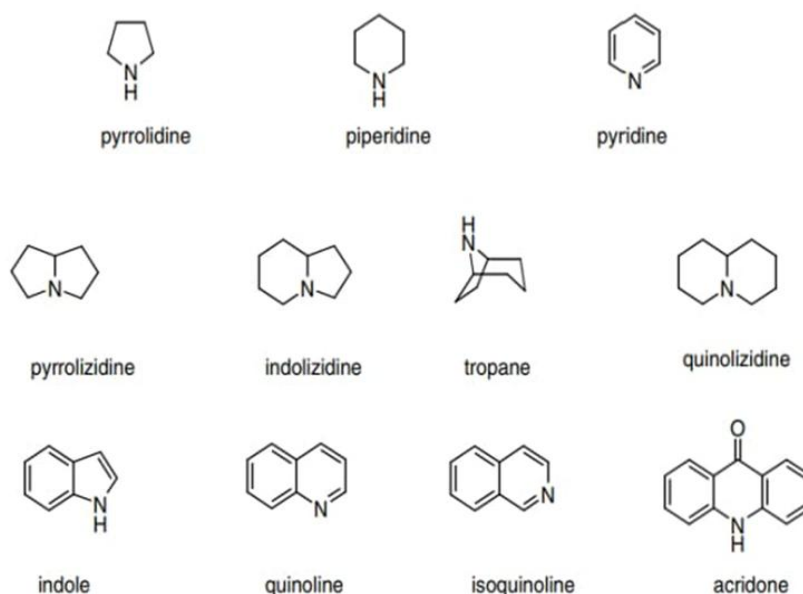


Figure 1.3: Basic structural unit of alkaloids [4]

1.3 ANTI-OXIDANT PROPOERTIES

The anti-oxidant properties of ethanol and ethyl acetate extracts of noni fruit have been assessed using the ferric thiocyanate method (FTC) and thiobarbituric acid test (TBA). The authors found that ethyl acetate extract

exhibited strong inhibition of lipid oxidation comparable to the same weight of pure α -tocopherol and butylatedhydroxy toluene (BHT). Radical scavenging activity was also measured in vitro by the tetrazoliumnitroblue (TNB) assay on a commercial juice, by assessing the potential capacity of the juice to protect cells or lipids from oxidative alteration promoted by superoxide anion radicals (SAR). The SAR scavenging activity of noni juice was shown to be 2.8 times higher than that of vitamin C, 1.4 times that of pycnogenol (PYC) and almost of the same order as that of grape seed powder [5].

1.4 ANTI- BACTERIAL ACTIVITY

The fruit contains relatively large amounts of sugars that are not fermented when fruits are stored in closed containers at ambient temperature. This property is used to transport the fruit by boat from the scattered Pacific islands to processing plants without specific treatment. It has been reported that noni inhibits the growth of certain bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella*. The same author claims that the anti-microbial effect observed may be due to the presence of phenolic compounds such as acubin, L-asperuloside, alizarin, scopoletin and other anthraquinones. Another study showed that an acetonitrile extract of the dried fruit inhibited the growth of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, and *Streptococcus pyrogene*. It has also been found that ethanol and hexane extracts of noni have an antitubercular effect since they inhibit by 89–95% the growth of *Mycobacterium tuberculosis*[6],[7]. The major components identified in the hexane extract were E-phytol, cycloartenol, stigmasterol, β -sitosterol, campesta-5,7,22-trien-3- β -ol, and the ketosteroids, stigmasta-4-en-3-one and stigmasta-4-

22-dien-3-one. Other studies have reported a significant antimicrobial effect on different strains of Salmonella, Shigella, and E. coli. Furthermore, they showed that the anti-microbial effect is highly dependent on the stage of ripeness and on processing, being greater when the fruit is ripe, without drying [8].

1.5 SCOPE AND POSSIBILITIES

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

1.6 OBJECTIVES

The objective of the project is to conduct phytochemical analysis and more specifically isolate and characterize the phytochemicals in fruit of Morinda citrifolia using techniques such as UV-Visible spectroscopy and GC-MS. Furthermore, evaluation of the antibacterial and anti-oxidant activity of the extracted phytochemicals will also be carried out.

Chapter 2

LITERATURE STUDY

Morinda citrifolia L., commonly known as noni, has been traditionally used for centuries by medical practitioners in Hawaii and Polynesia to prevent and treat a wide range of illnesses. In recent decades, its popularity has increased globally as a dietary supplement, functional food ingredient, and natural health enhancer. The plant contains numerous bioactive phytochemicals exhibiting antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory, and immunomodulatory properties.

The widespread cultivation of *M. citrifolia* across tropical regions, including the USA, Brazil, Tahiti, Malaysia, and Australia has enhanced its therapeutic and commercial value due to environmental variations influencing its phytochemical composition. Different plant parts such as fruits, seeds, bark, leaves, and flowers are utilized for their nutritional and medicinal benefits, although the fruit is regarded as the richest source of bioactive compounds. This review focuses on the pharmacological activities, industrial applications, and isolated phytochemicals of noni fruit, seeds, leaves, and roots, supported by in vitro, in vivo, and clinical studies. Hamza T., Adejoke, Hitler Louis, Oluwatobi Amusan, and Gloria Apebende emphasized the critical role of natural products in the pharmaceutical industry, particularly in managing diseases such as cancer, malaria, and piles.[13] They highlighted that bioactive compounds including alkaloids, flavonoids, phenols, saponins, and tannins are essential plant metabolites with therapeutic potential. Their review

provides an overview of alkaloids, including extraction and purification techniques, pharmacological significance, and potential adverse effects associated with misuse.

Roy A. discussed the therapeutic applications of plant-derived alkaloids, noting their anti-tumor, antiviral, anti-inflammatory, and antimalarial activities.[14] Alkaloids, classified based on their biosynthetic origin and heterocyclic ring structure (e.g., indole, piperidine, tropane, purine, pyrrolizidine, imidazole, quinolizidine, isoquinoline, and pyrrolidine), are recognized for their antioxidant properties and protective effects against chronic diseases. The review highlighted alkaloid-based drugs and their broad therapeutic spectrum.

Antia G. Perera and colleagues examined the production, extraction, isolation, and purification methods of plant alkaloids.[15] Their review also detailed the chemical structures, plant sources, and therapeutic uses of different alkaloid groups, emphasizing their pharmaceutical importance.

Amy C. Brown investigated the potential anticancer and immunostimulatory properties of noni juice.[15] Findings suggested that certain concentrated components in noni juice, rather than the crude juice itself, may stimulate immune responses and exhibit limited anticancer activity in vitro and in vivo animal studies.

Mohammed Ali, Mruthanjaya Kenganora, and Santhapete Nanjundaiah Manjula reviewed the phytochemical and mineral composition of different parts of *Morinda citrifolia*. [16] They reported the presence of amino acids, anthraquinones, fatty acids, flavonoids, iridoids, lignans, polysaccharides, sterols, sugars, and terpenoids. These compounds are associated with therapeutic benefits in conditions such as colon, esophageal, breast, and colorectal cancers, cardiovascular diseases, diabetes, and arthritis.

M. Y. Wang and C. Su explored the cancer-preventive potential of noni during early carcinogenesis.[17] Their findings suggested that noni juice may inhibit carcinogen–DNA adduct formation and exert antioxidant activity, contributing to its protective effects.

Afa K. Palu and colleagues investigated the immunomodulatory mechanisms of noni, particularly its interaction with cannabinoid receptors.[18] In vitro studies showed activation of CB2 receptors and inhibition of CB1 receptors. In vivo studies in mice demonstrated modulation of cytokine production, indicating potential immune-regulating properties.

Peter E. Murray and co-researchers evaluated *Morinda citrifolia* juice as an alternative endodontic irrigant to sodium hypochlorite (NaOCl), identifying it as a potential natural substitute for intracanal disinfection.[19]

B. Shivananda Nayak, Steve Sandiford, and Anderson Maxwell assessed the wound-healing activity of ethanolic extracts of noni leaves using rat models. Results showed enhanced wound contraction, reduced epithelialization time, increased hydroxyproline content, and improved histological parameters, supporting its traditional topical use.[20]

Z. Mohd Zin, Abdul-Hamid A., and Osman A. examined the antioxidant activity of extracts from noni root, fruit, and leaves.[21] Their findings indicated that antioxidant activity in roots is attributed to both polar and non-polar compounds, whereas in fruits and leaves it is mainly associated with non-polar compounds.

Oliver Potterat and Matthias Hamburger reviewed the phytochemistry, pharmacology, and safety profile of *Morinda citrifolia*. Studies demonstrated antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Traditional uses of leaves, fruits, and roots were

supported by experimental evidence suggesting immune-enhancing and anti-inflammatory effects.[22]

Chafique Younos and colleagues investigated the analgesic and behavioral effects of noni extracts. Experimental studies revealed significant dose-dependent central analgesic activity without observable toxicity.[23]

Sarvananda Letchuman and co-authors analyzed the therapeutic potential and molecular mechanisms of plant-based alkaloids. Their review emphasized the comparatively lower toxicity of natural compounds and highlighted the need for improved extraction methods and research into personalized and combination therapies.[24]

Thi Cam Tu Phan and colleagues studied the effects of noni fruit extract on juvenile whiteleg shrimp. Results demonstrated improved growth performance, digestive enzyme activity, survival rates, and environmental stress tolerance, suggesting applications in aquaculture.[25]

T. P. Tim Cushnie and co-researchers addressed the urgent need for new antibacterial agents due to pandrug-resistant bacteria.[26] Their review explored natural, semi-synthetic, and synthetic alkaloids with direct antibacterial or antibiotic-enhancing activity, including effects on quorum sensing and bacterial virulence factors.

Ali Esmail Al-Snafi reviewed the therapeutic properties of alkaloid-containing medicinal plants, highlighting their broad pharmacological applications and long-standing clinical relevance.[27]

Shiyang Zhou and Gangliang Huang analyzed the chemical composition and pharmacological activities of *Morinda citrifolia*, identifying anthraquinones, phenylpropanoids, flavonoids, terpenoids, glycosides, steroids, and fatty acids as key constituents. Reported biological activities include antibacterial, antioxidant, anti-inflammatory, analgesic,

hypoglycemic, hepatoprotective, cardioprotective, and antitumor effects.[28]

Madhukar Lohani and colleagues examined the immunomodulatory actions and clinical applications of noni. Their review emphasized its bioactive polysaccharides and phytochemicals, suggesting potential prophylactic and therapeutic benefits, while recommending further toxicological and controlled clinical studies.[29]

Finally, Brett J. West evaluated the antioxidant activity of noni juice in vitro and in healthy human volunteers. The study reported increased antioxidant activity in plasma and erythrocytes following consumption, supporting its potential health benefits under normal dietary conditions.[30]

Overall, the literature indicates that *Morinda citrifolia* is a pharmacologically active medicinal plant with diverse therapeutic properties. However, further well-designed clinical studies are required to confirm its efficacy, safety, and mechanisms of action for broader medical application.

Chapter 3

3.1 Materials and Methods

The material and experimental methods are described in this chapter.

3.1 MATERIALS

Chloroform, 2 N hydrochloric acid, 2% v/v of a strong solution of ammonia, ethanol, anhydrous sodium sulfate, ethyl acetate, 25 g of fruit powder, pH paper.

3.2 METHOD OF PREPARATION OF PLANT EXTRACT

The fruit powder was refluxed with ethanol-chloroform mixture in the ratio 1:3 containing 2% v/v of a strong solution of ammonia for 6 hours. The resultant mixture was extracted three times with 2N hydrochloric acid. The acid extracts were combined and their pH adjusted to 8.0 by addition of strong ammonia solution. Chloroform was added and the alkaloid-rich component was extracted into the chloroform layer. The chloroform layer was evaporated to dryness to obtain plant extract.

3.2.1 SOLVENT EXTRACTION TECHNIQUE

25 g of fruit powder was accurately weighed and mixed with 30 mL ethanol and 70 mL chloroform. The round-bottom flask was washed with ethanol to prevent sample loss, and the fruit powder along with the solvent mixture was transferred into the flask. To enhance alkaloid liberation, 2.5 mL of ammonia was added. The reflux setup was assembled by connecting cooling water through the bottom inlet of the condenser, allowing the water to exit from the top outlet to maintain efficient condensation. After refluxing, the mixture was filtered through a funnel lined with filter paper. A 2N hydrochloric acid solution was prepared using 125 mL of water and 25 mL of concentrated HCl, calculated using the formula

$N_1 V_1 = N_2 V_2$. The filtrate was transferred into a separating funnel along with 20 mL of the prepared 2N HCl and shaken gently with intermittent venting to release built-up pressure. The aqueous and chloroform–ethanol layers were allowed to separate, and this extraction was repeated three times, resulting in the collection of approximately 60 mL of the acidic aqueous extract. The pH of this acidic extract was then adjusted to around 8 using strong ammonia added drop wise. Finally, the chloroform extract was allowed to evaporate naturally at room temperature to yield the crude alkaloid material. The dried residue was dissolved in chloroform and transferred into a sample bottle. The volume was gradually reduced and diluted with 1 mL of ethyl acetate before being transferred to a sample vial. The prepared sample was then sent for further analytical characterization.

3.3 DETECTION OF PHYTOCHEMICALS

3.3.1 Hager's Method

The presence of alkaloids was detected using Hager's method. 0.2 g of the chosen plant sample was placed in each test tube for phytochemical investigation. 3 mL of hexane was added to the test tube and shaken well. 5 mL of 2 % HCl was added to the test tube containing the hexane-plant extract mixture. A few drops of picric acid were added to the mixture and shaken well. Presence of alkaloid was confirmed by the formation of yellow colored precipitate.

3.3.2 Lead Acetate Test

A few drops of lead acetate solution were added to 2 mL of the aqueous solution of the extract. Formation of yellow color, showed the presence of flavonoids.

3.3.3 Braymer's Test

A few drops of 10% FeCl_3 solution was added to aqueous solution of the extract, resulting in a greenish black color, demonstrating the presence of tannins.

3.3.4 Molisch's Test

2 mL of the extract was added to 2 drops of alcoholic alpha-naphthol and then 1 mL of concentrated sulfuric acid was carefully added along the sides of the test tube resulting in the formation of a violet ring that demonstrated the presence of carbohydrates.

3.4 CHARACTERIZATION TECHNIQUES

3.4.1 UV- Visible spectroscopy

UV- Visible spectroscopy is an analytical technique that measures the absorption of light in the U-V and visible region by a sample. It gives information about the electronic transition taking place the sample. It is a powerful technique for qualitative and quantitative analysis. This analysis was done on JASCO V-770 and the spectrum was taken in the wavelength range between 200-600 nm.

3.4.2 GC-MS

Gas chromatography-mass spectrometry is an analytical technique that constitutes gas chromatography and mass spectrometry. In this technique, gas chromatography helps to separate different organic compounds in a sample and mass spectrometry helps to identify the separated compounds based on their m/z ratio. Thus, it is a powerful analytical technique for identification of different compounds in a sample. This analysis was done in Agilent 7010C Triple Quadrupole GC-MS system (GC/TQ, 8890GC) at Inter University Instrumentation Centre (IUIIC), MGU, Kottayam.

3.5 DETERMINATION OF ANTIBACTERIAL ACTIVITY

3.5.1 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 was poured into sterile petri dishes under aseptic conditions.

3.5.2 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.

3.5.3 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 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 labelled and 20 µL of sample (noni alkaloid + 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 h at a temperature of 37° C. 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.

3.5.4 STERILIZATION AND DISPOSAL

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

3.6 a) DETERMINATION OF ANTIOXIDANT ACTIVITIES

A mixture of 1.5 mL sample (alkaloid before column chromatography and after column chromatography) and 1.5 mL of 0.2 mM ethanolic DPPH solution was vortexed and incubated in darkness for 30 minutes. The absorbance was

measured at 517 nm with ethanol as the blank and DPPH solution without the sample as the control. The sample with lower absorbance expresses a more significant free radical scavenging activity (RSA).

3.6 b) ANTIOXIDANT STUDIES

3.6.1 Materials and methods

The free radical scavenging activity of the sample was evaluated by assessing its ability to scavenge DPPH radicals. A DPPH reagent solution was prepared by mixing 100 μ L of DPPH (1 mm) with 100 μ L of methanol (98%). Test solutions were prepared by adding varying volumes of the sample (T1=20 μ L, T2=40 μ L, T3=60 μ L, T4=80 μ L, T5=100 μ L) to the DPPH reagent solution, adjusting the final volume to 300 μ L. A control sample was prepared using solvent alone, and ascorbic acid (10 mm) served as the standard. After 30 minutes of incubation in the dark at room temperature, the absorbance was recorded at 515 nm. Antioxidant activity was indicated by a colour change from purple to yellow or colourless, depending on the level of radical scavenging. The IC₅₀ value was determined from the dose-response curve. The experiment was done in duplicate.

Chapter4

RESULTS AND DISCUSSION

4.1 Phytochemical screening

Phytochemical screening of *Morinda citrifolia* was conducted using standard tests. *Morinda citrifolia* showed the presence of alkaloids, flavonoids, phenol, saponins, and tannins. The table 4.1 shows the results of the phytochemical screening.

Table 4.1: Screening test of phytochemicals in *Morinda citrifolia*

TEST	OBSERVATION	INFERENCE	FIGURE
Hager's method 0.2 g of the chosen plant sample was placed in each test tube for phytochemical investigation. 3 mL of hexane was added to the test tube and shaken well.	Formation of yellow coloured precipitate	Presence of alkaloid	

5 mL of 2% HCl was added to the test tube containing the hexane-plant extract mixture. A few drops of picric acid were added to the mixture and shaken well.			
Mayer's test Add 1-2 mL of Mayer's reagent to the plant extract and mix well.	Formation of white precipitate	Presence of alkaloids	
Wagner's test Add 1-2 mL of Wagner's reagent to the plant extract and mix well.	Formation of brown or yellowish precipitate	Presence of alkaloid is confirmed	

Lead acetate test A few drops of lead acetate solution were added to 2 mL aqueous solution of extract.	Formation of yellow color	Presence of flavonoids	
Braymer's test A few drops of 10% FeCl_3 solution was added to aqueous solution of the extract.	Formation of greenish black color	Presence of tannins	
Aqueous solution of the extract was treated with distilled water.	Formation of a honey comb froth	Presence of saponins	

Molisch's test 2 mL of the extract was added to 2 drops of alcoholic alpha-naphthol and then 1ml of concentrated sulfuric acid was carefully added along the sides of the test tube.	Formation of a violet ring	Presence of carbohydrates	
A few mL of aqueous extract was mixed with a few drops of 5% ferric chloride solution.	Formation of a dark green color	Presence of phenolic compounds	

4.2 Spectroscopic analysis

Phytochemicals extracted from *Morinda citrifolia* were analysed using Gas Chromatography. The dried extract from the chloroform layer was redissolved in ethyl acetate and subjected to GC-MS analysis. The GC-MS analysis showed the presence of 68 phytochemicals. Figure 4.2.1 shows the chromatogram from the GC-MS analysis. The compounds obtained at different retention times with component percentage and match factors are given in table 4.2.1.

4.2.1 GC-MS analysis

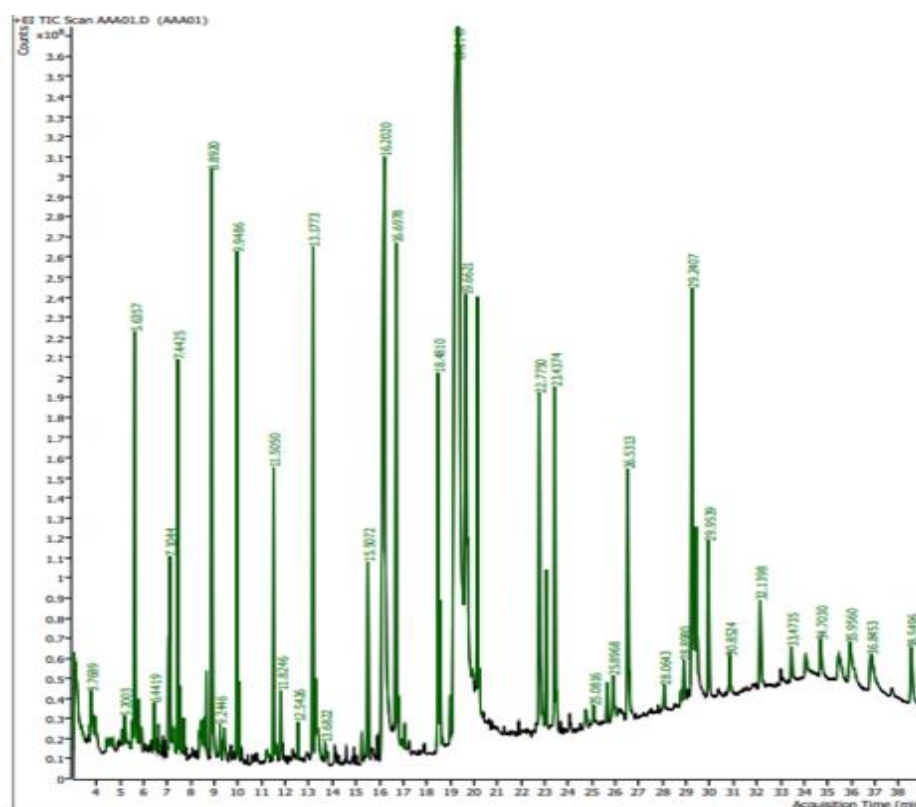


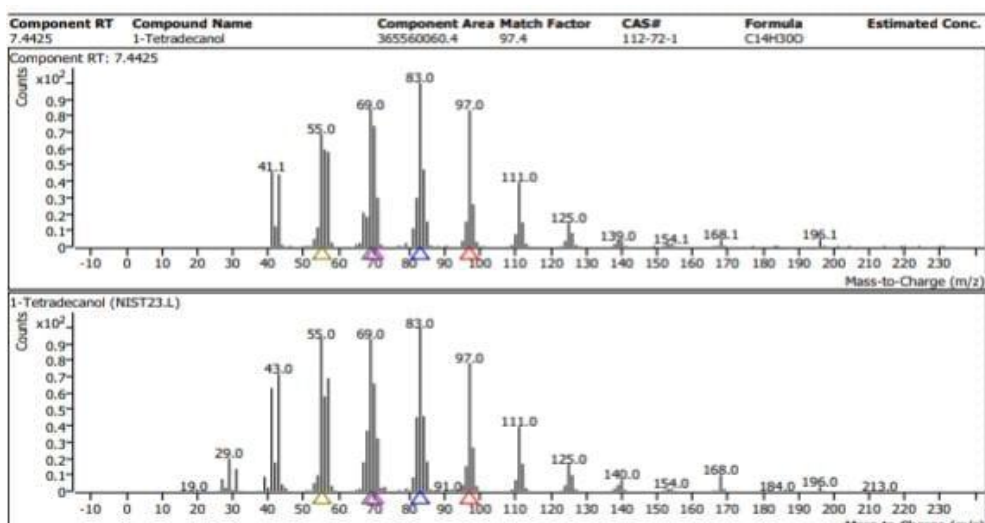
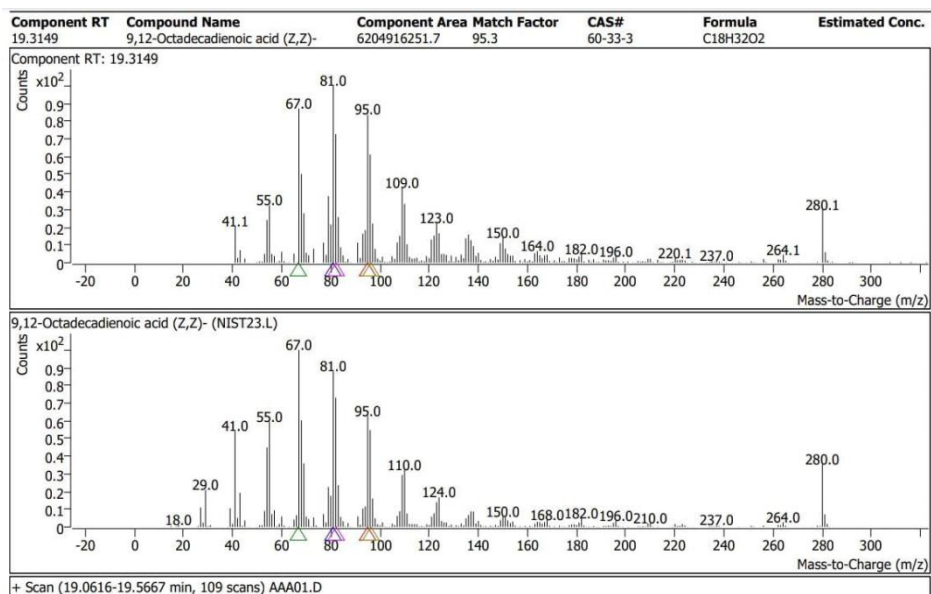
Figure:4.2.1 Spectrum from GC-MS analysis

Table no 4.2.1: List of compounds from GC-MS spectrum

Component RT	Compound Name	Component Area	Area %	CAS#	Formula	Library Molecular Weight	Match Fac
3.0510	Acetic acid, pentyl ester	705037006.7	2.815	628-63-7	C7H14O2	130.099	75.7
3.7689	p-Xylene	139823230.9	0.5582	106-42-3	C8H10	106.078	92.1
3.9403	Benzene, 1,3-dimethyl-	42664121.9	0.1703	108-38-3	C8H10	106.078	88.2
4.5935	.beta.-Hydroxyethyltheophylline tert-butyl dimethylsilyl ether	64297816.6	0.2567	1000442-93-8	C15H26N4O3S	338.177	59.5
5.0799	1,5-Hexanediol	34001273.9	0.1357	928-40-5	C6H14O2	118.099	60.9
5.2003	1,4-Pentadiene, 2,3,4-trimethyl-	84370703.7	0.3368	72014-90-5	C8H14	110.11	69.3
5.5107	Hexanamide	40111080.4	0.1601	628-02-4	C6H13NO	115.1	60.3
5.6357	Octanoic acid	688336700.9	2.748	124-07-2	C8H16O2	144.115	94.9
5.7794	Cyclododecane	97554579.5	0.3895	294-62-2	C12H24	168.188	92.0
5.9091	Naphthalene	35361095.5	0.1412	91-20-3	C10H8	128.063	76.1
6.4419	Pentadecane	46882788.7	0.1872	629-62-9	C15H32	212.25	87.1
6.6364	Methyl 1-methylpyrrole-2-carboxylate	43315872.0	0.1729	37619-24-2	C7H9NO2	139.063	71.7
7.1044	n-Decanoic acid	393791648.9	1.572	334-48-5	C10H20O2	172.146	92.7
7.2665	1-(2-Vinylphenyl)ethanone	71001102.7	0.2835	52095-40-6	C10H10O	146.073	74.9
7.4425	1-Tetradecanol	365560060.4	1.459	112-72-1	C14H30O	214.23	97.4
7.5166	Tetradecane	72200049.1	0.2882	629-59-4	C14H30	198.235	92.1
7.6881	Vanillin	39737761.4	0.1586	121-33-5	C8H8O3	152.047	92.3
8.4848	Villosine	139954696.5	0.5587	149182-83-2	C15H20O5	280.131	68.8
8.5867	11,13-Dimethyl-12-tetradecen-1-ol acetate	49539506.4	0.1978	1000130-81-0	C18H34O2	282.256	60.7
8.6654	Heptadecane	92821520.7	0.3706	629-78-7	C17H36	240.282	86.9
8.8920	2,4-Di-tert-butylphenol	987727925.8	3.943	96-76-4	C14H22O	206.167	95.8
9.2446	Hexadecane, 2,6,11,15-tetramethyl-	41763961.8	0.1667	504-44-9	C20H42	282.329	85.3
9.4206	Tridecanoic acid	66200072.3	0.2643	638-53-9	C13H26O2	214.193	69.6
9.9486	1-Hexadecanol	606814782.6	2.423	36653-82-4	C16H34O	242.261	96.9
10.0459	Hexadecane	79503483.4	0.3174	544-76-3	C16H34	226.266	94.9
11.2364	tert-Hexadecanethiol	34793484.1	0.1389	25360-09-2	C16H34S	258.238	64.8
11.5050	Dodecyl acrylate	347581404.0	1.388	2156-97-0	C15H28O2	240.209	97.8
11.6069	2,4,6,8-Tetrathiatricyclo[3.3.1.1(3,7)]decane, 1,10-dimethyl-	37302426.1	0.1489	57289-11-9	C8H12S4	235.982	57.4
11.8246	Henicosane	88739168.8	0.3543	629-94-7	C21H44	296.344	89.1
12.5426	Tetradecanoic acid	48850449.7	0.195	544-63-8	C14H28O2	228.209	78.1
13.1773	1-Octadecanol	737214971.2	2.943	112-92-5	C18H38O	270.292	95.9
13.2884	Octadecane	233292859.4	0.9314	593-45-3	C18H38	254.297	80.6
13.6822	Cyclo(propylvalyl)	48077945.6	0.1919	5654-87-5	C10H16N2O2	196.121	84.4
15.2571	Lidocaine	31974170.6	0.1277	137-58-6	C14H22N2O	234.173	79.4
15.5072	Hexadecanoic acid, methyl ester	314575273.0	1.256	112-39-0	C17H34O2	270.256	82.7
16.2020	n-Hexadecanoic acid (Z,Z)-, methyl ester	2465347208.2	9.842	57-10-3	C16H32O2	256.24	95.8
16.6978	Behenic alcohol	873685208.5	3.488	661-19-8	C22H46O	326.355	89.5
16.7996	Eicosane	135665406.6	0.5416	112-95-8	C20H42	282.329	89.4
17.0729	Propanoic acid, 3-mercapto-, dodecyl ester	45208652.4	0.1805	6380-71-8	C15H30O2S	274.197	81.7
18.4810	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	560402478.1	2.237	112-63-0	C19H34O2	294.256	97.8
18.5831	9-Octadecenoic acid (Z)-, methyl ester	230180605.7	0.9189	112-62-9	C19H36O2	296.272	97.4
19.0093	Methyl stearate	72797632.4	0.2906	112-61-8	C19H38O2	298.287	91.5
19.3149	9,12-Octadecadienoic acid (Z,Z)-	6204916251.7	24.77	60-33-3	C18H32O2	280.24	95.3
19.5621	Octadecanoic acid	1649400446.6	6.585	57-11-4	C18H36O2	284.272	84.6
20.1533	1-Heptacosanol	709772519.1	2.834	2004-39-9	C27H56O	396.433	90.2
20.2367	Docosane	89618837.9	0.3578	629-97-0	C22H46	310.36	85.8
22.7750	1,4-Pentadien-3-one, 1,5-diphenyl-	687684551.7	2.745	538-58-9	C17H14O	234.104	96.3
23.0577	3,3',5,5'-Tetra-tert-butylbiphenyl-2,2'-diol	258338488.4	1.031	6390-69-8	C28H42O2	410.318	95.8
23.4374	n-Tetracosanol-1	597876439.0	2.387	506-51-4	C24H50O	354.386	95.7
24.7342	1,2-Dehydro-as-hydrindacene	39823409.2	0.159	96508-75-7	C13H14	170.11	60.1
25.0816	Pentacosane	37169694.5	0.1484	629-99-2	C25H52	352.407	77.0
25.6421	Benzylidienyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate	59683350.7	0.2383	3734-33-6	C28H34N2O3	446.257	81.3
25.8968	Bis(2-ethylhexyl) phthalate	126271461.5	0.5041	117-81-7	C24H38O4	390.277	87.1
26.5313	Hexacosyl heptafluorobutyrate	482173057.4	1.925	1000351-83-3	C30H53F7O2	578.393	91.4
28.0643	Henatriacontane	41360232.6	0.1651	630-04-6	C31H64	436.501	80.2
28.8980	2-phenanthreneacetic acid, 1,2,3,9,10,10a-hexahydro-3-oxo-	143420855.7	0.5726	1000396-72-6	C16H16O3	256.11	62.8
29.2407	13-Docosenamide, (Z)-	859970937.3	3.433	112-84-5	C22H43NO	337.334	90.6
29.4259	Octacosanol	261522136.3	1.044	557-61-9	C28H58O	410.449	91.6
29.9539	Squalene	240025901.0	0.9583	111-02-4	C30H50	410.391	92.3
30.8524	Heptatriacontane	80603177.4	0.3218	7194-84-5	C37H76	520.595	84.5
32.1398	Octacosyl heptafluorobutyrate	191975204.8	0.7664	1010351-83-6	C32H57F7O2	606.425	87.3
33.4735	Tetracontane	60360994.7	0.241	4181-95-7	C40H82	562.642	82.2
34.0683	Vitamin E	80220952.8	0.3203	59-02-9	C29H50O2	430.381	81.3
34.7030	17-Pentatriacontene	92247252.9	0.3683	6971-40-0	C35H70	490.548	82.6
35.4790	Campesterol	124843092.5	0.4984	474-62-4	C28H48O	400.371	84.9
35.9560	Stigmasterol	210752966.5	0.8414	83-48-7	C29H48O	412.371	83.7
36.8453	.gamma.-Sitosterol	227243297.0	0.9072	83-47-6	C29H50O	414.386	90.0
38.5496	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	156924357.0	0.6265	31570-04-4	C42H63O3P	646.451	84.5

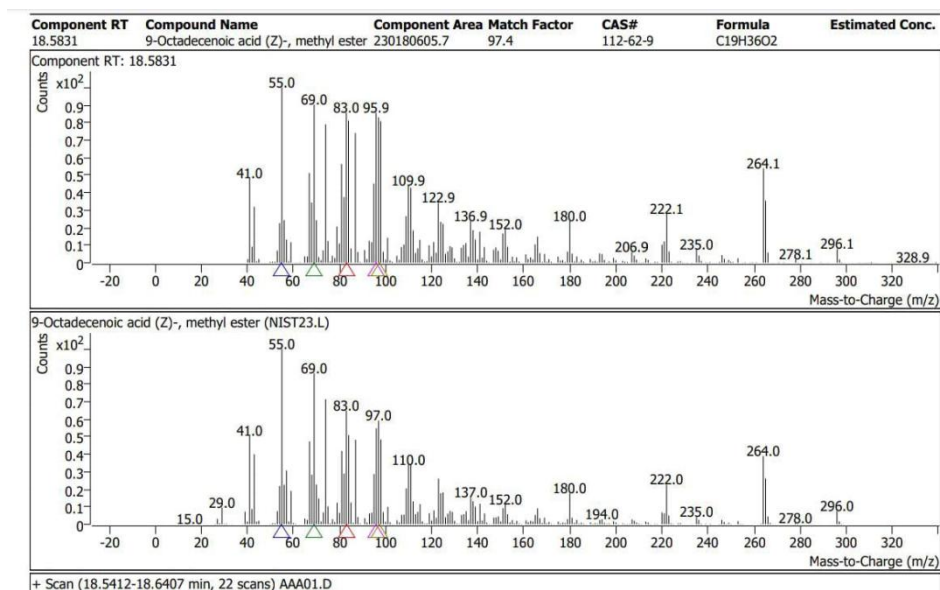
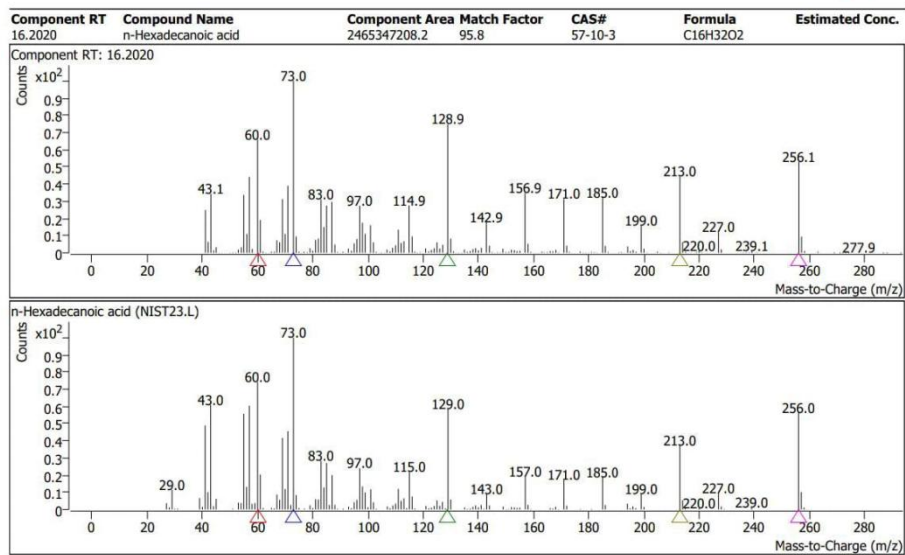
High match factors (given in bracket as percentage) were observed for the following compounds. Octanoic acid (94.9), 1-Tetradecanol (97.4), 2,4-Di-tert-butylphenol (95.8), 1-Hexadecanol (96.9), Dodecyl acrylate (97.8), 1-Octadecanol (95.9), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (97.8), 9-Octadecenoic acid (Z)-, methyl ester (97.4) , 9,12-Octadecadienoic acid (Z,Z)- (95.3) , 1,4-Pentadien-3-one, 1,5-diphenyl- (96.3) , 3,3',5,5'-Tetra-tert-butylbiphenyl-2,2'-diol (95.8) and n-Tetracosanol-1 (95.7).

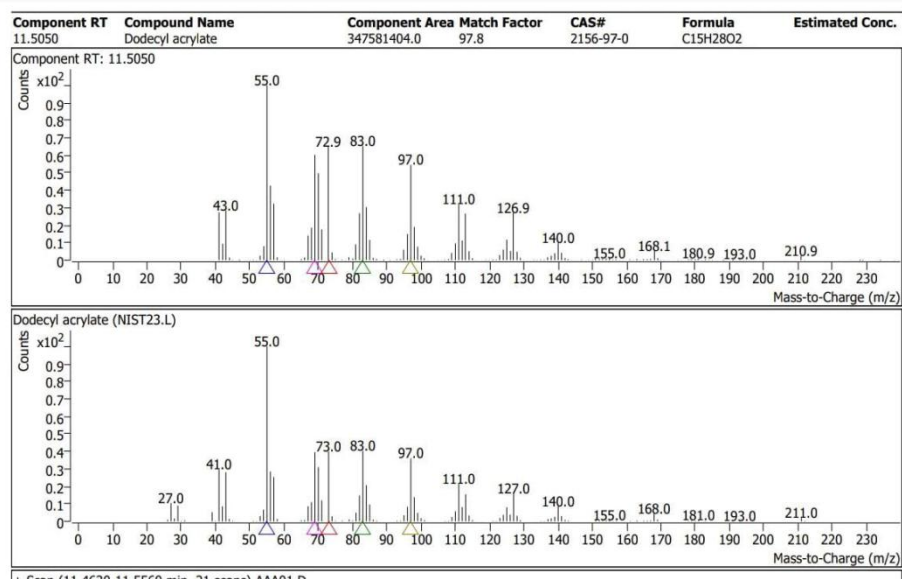
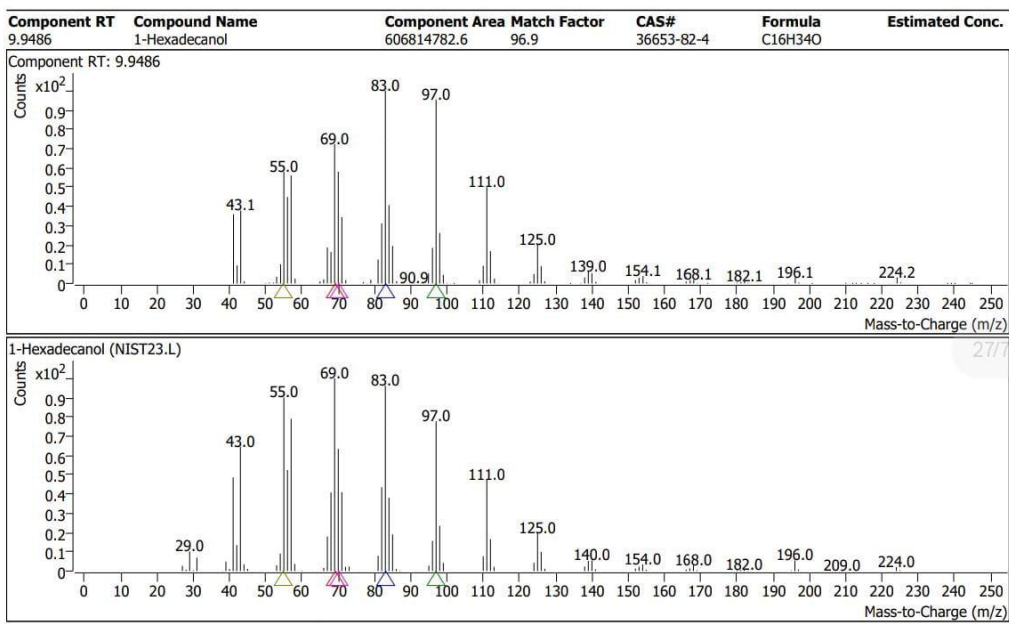
The mass spectral analysis of these components was compared with the mass spectra of library of compounds and the exact fragmentation patterns confirmed the presence of these components in the noni extract. The graph of each component which shows match factor above 90% with reference spectrum, library spectrum, name, match factor and structure are given in Figure 4.2.2.

**1-Tetradecanol**

+ Scan (19.0616-19.5667 min, 109 scans) AAA01.D

9,12-Octadecadienoic acid(Z,Z)

**9-Octadecenoic acid (z) -, methyl ester****n-Hexadecanoic acid**

**Dodecyl acrylate****1-Hexadecanol**

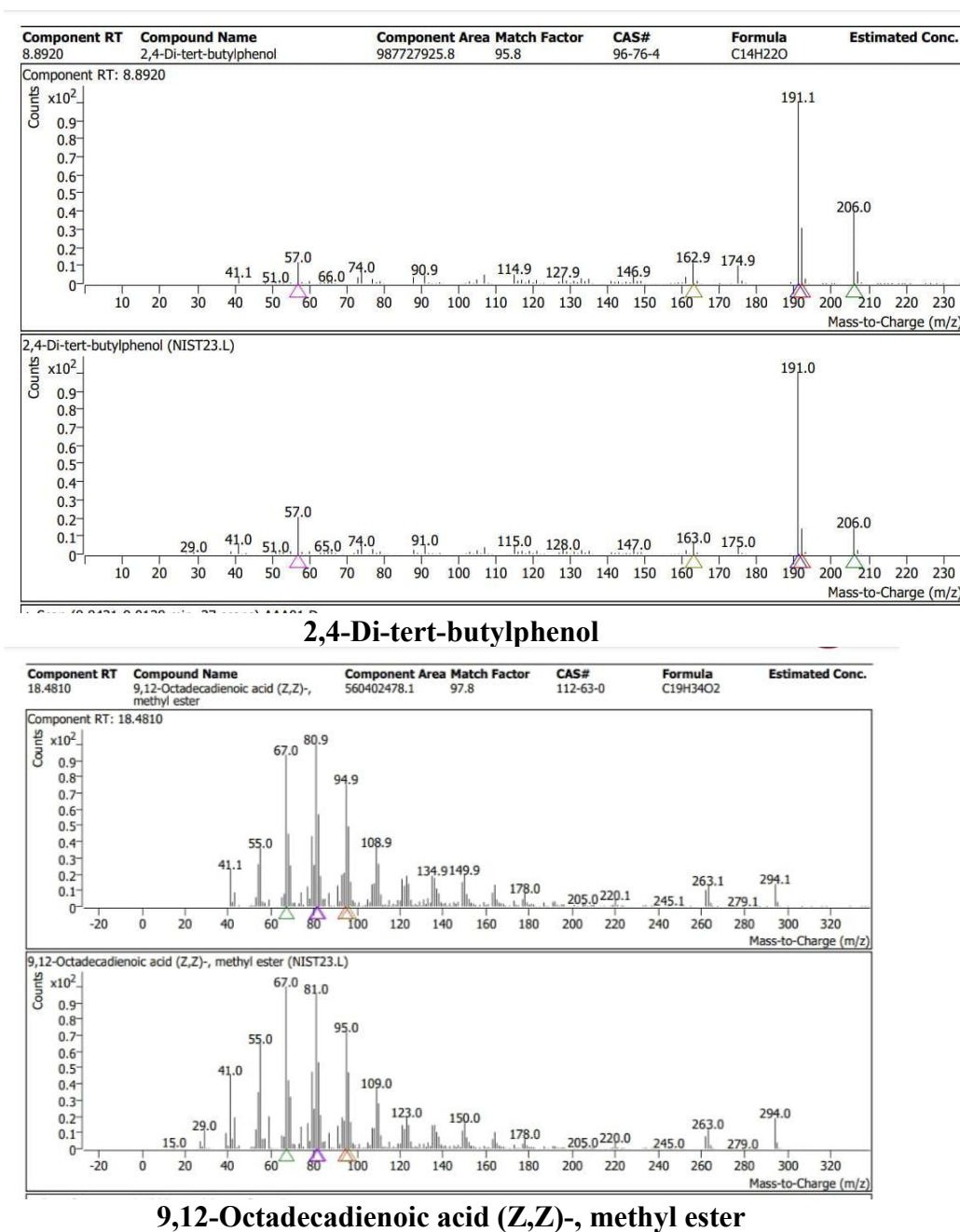


Figure 4.2.2 Mass spectrum of phytochemical components showing match factor above 95 % compared with library spectrum.

Alkaloids such as Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium

Benzoate, (Z)-13-Docosenamide, Lidocaine, Cyclo(propylvalyl), Methyl 1-methylpyrrole-2-carboxylate with 75 to 85% match with the standards.

4.3 UV-Visible spectroscopy

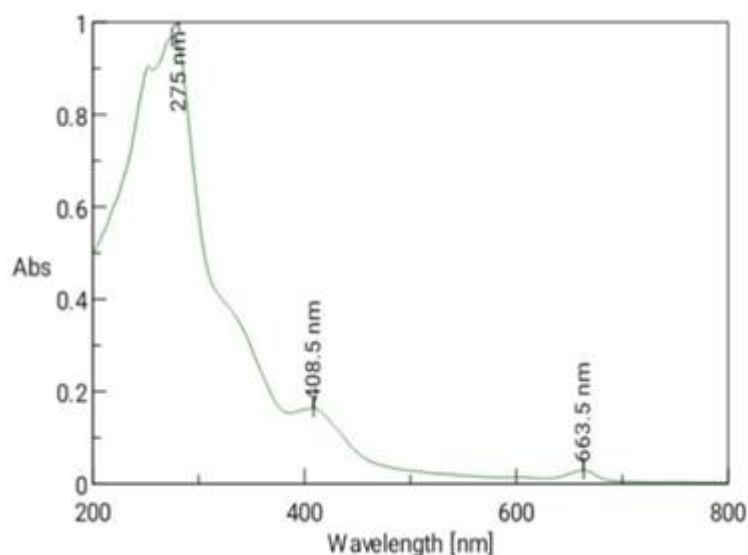


Figure 4.2.1: Visible spectrum of *Morinda citrifolia* phytochemical extract

The UV-Visible spectrum of the extract was recorded in ethanol over a wavelength range of 200-700 nm. The spectrum showed two absorption bands in the wavelength range 200-400 nm and a tail extending to 600 nm. The absorption spectrum of the extract supports the GC analysis showing the presence of organic molecules absorbing in the UV-Visible region.

4.3 Biological studies

4.3.1 Antioxidant activity

The free radical scavenging activity of the sample was evaluated by assessing its ability to scavenge DPPH radicals. A DPPH reagent solution was prepared by mixing 100 µl of DPPH (1 mM) with 100 µl of methanol (98%). Test solutions were prepared by adding varying volumes of the sample (T1=20 µl, T2=40 µl, T3=60 µl, T4=80 µl, T5=100 µl) to the DPPH reagent solution, adjusting the final volume to 300 µl. A control sample was prepared using solvent alone, and ascorbic acid (10mM) served as the standard. After 30 minutes of incubation in the dark at room temperature, the absorbance was recorded at 515 nm.

Antioxidant activity was indicated by a colour change from purple to yellow or colourless, depending on the level of radical scavenging. The IC₅₀ value was determined from the dose-response curve. The experiment was done in duplicate.

$$\text{Radical scavenging activity \%} = \left(\frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \right) \times 100$$

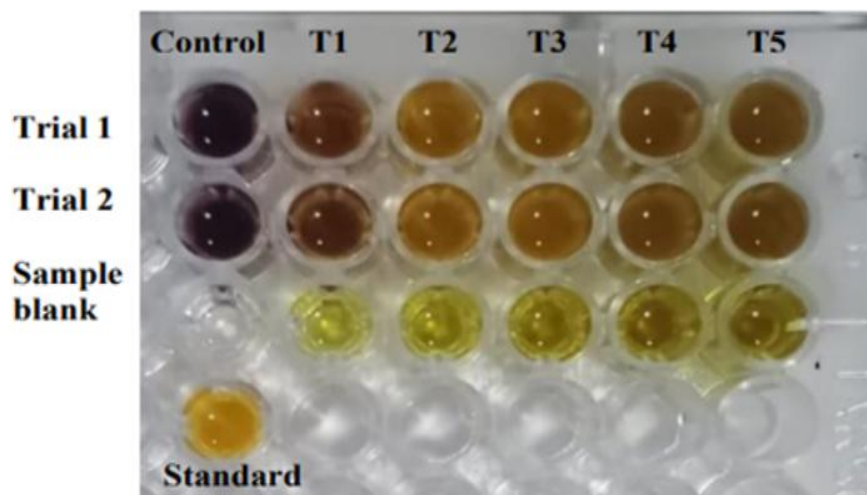


Figure 4.3.1: DPPH Free Radical Scavenging Assay of sample with standard and control.

The liquid sample demonstrated dose-dependent antioxidant activity in the DPPH assay, with radical scavenging increasing from 52.81% at 20 μ L to 78.55% at 100 μ L. This indicates a strong free radical scavenging potential. However, as all tested concentrations resulted in scavenging activity above 50%, the IC_{50} value could not be accurately determined.

Table 4.3.1: Percentage Scavenging activity of DPPH by sample.

Sample Name	Sample Dose (μ L)	Corrected absorbance		Average Radical scavenging activity %	Standard deviation
		Trial 1	Trial 2		
T1	20	0.527	0.531	52.810	0.252313
T2	40	0.458	0.456	59.233	0.126156
T3	60	0.447	0.442	60.348	0.315391
T4	80	0.338	0.339	69.804	0.063078
T5	100	0.245	0.236	78.546	0.567704
Control		1.121			
Standard=100 μ l (10mM ascorbic acid)		0.102			90.901

4.3.2 a) Antibacterial assay

The extract from the dried sample of *Morinda citrifolia* fruit was mixed with DMSO and its antibacterial activity against *Escherichia coli*, *Vibrio cholera* and *Staphylococcus aureus* were observed after a period of 24 hours.

Table 4.3.2: Study of antibacterial properties of Morinda citrifolia extract

Sample	E coli	Vibrio	Salmonella
	24 hrs	24 hrs	24 hrs
DMSO+NONI EXTRACT	Nil	Nil	Nil

**Figure 4.3.2: Antibacterial activity of Morinda citrifolia with different bacteria after 24 hours**

The results are summarised in table 4.3.2. The sample showed no activity in the antibacterial assay against E.coli, V.cholerae and S.aureus.

Chapter 5

Conclusion

The dried fruit extract of *Morinda citrifolia* was analyzed for various phytochemicals. Conventional tests, including Hager's test, Wagner's test, lead acetate test, Braymer's test, Molisch's test, and ferric chloride test, confirmed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds. GC-MS analysis of the ethyl acetate extract revealed a diverse array of phytochemicals, such as amide alkaloids, long-chain saturated and unsaturated fatty acids and esters, alcohols like behenic alcohol, phenols, sterols, and tocopherols (including vitamin E). The DMSO solution of the ethyl acetate extract showed no antibacterial activity against *E. coli*, *V. cholerae*, or *S. aureus*. Additional antibacterial testing is needed with various solvents and bacterial strains to assess the fruit's antibacterial potential. Antioxidant testing using the DPPH assay demonstrated excellent antioxidant activity in *Morinda citrifolia* fruit. These results suggest the fruit's potential benefits for both nutritional and cosmetic applications. However, further research is required to explore these possibilities.

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