PROJECT REPORT

On

ISOLATION, CHARACTERIZATION AND BIOLOGICAL STUDIES OF THE ALKALOID COMPONENTS OF THE FRUIT OF *MORINDA CITRIFOLIA*

Submitted by

DAYA M K
(AM23CHE003)

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

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DEPARTMENT OF CHEMISTRY
AND
CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

M.Sc. CHEMISTRY PROJECT REPORT

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Register Number	**: AM23CHE003**
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This is to certify that the project "**ISOLATION, CHARACTERIZATION AND BIOLOGICAL STUDIES OF THE ALKALOID COMPONENTS OF THE FRUIT OF *MORINDA CITRIFOLIA***" is the work done by **DAYA M K.**

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Dr. Elizabeth Kuruvilla
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DEPARTMENT OF CHEMISTRY
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Dr. Elizabeth Kuruvilla
Project Guide

DECLARATION

I hereby declare that the project work entitled "**ISOLATION, CHARACTERIZATION AND BIOLOGICAL STUDIES OF THE ALKALOID COMPONENTS OF THE FRUIT OF *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.

DAYA M K

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Last, but not least, I am grateful to my loving families and friends for the care, support and concern they provide to follow my passion.

Daya M K

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Abbreviations

FT-IR	Fourier Transform Infrared spectroscopy
^{1}H NMR	^{1}H Nuclear Magnetic Resonance spectroscopy
^{13}C NMR	^{13}C Nuclear Magnetic Resonance spectroscopy
DEPT	Distortionless Enhancement by Polarization Transfer
COSY	Homonuclear Correlation Spectroscopy
UV-Visible	Ultraviolet-Visible spectroscopy
GC-MS	Gas Chromatography Mass Spectrometry
E-coli	*Escherichia coli*
S-aureus	*Staphylococcus aureus*

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 the 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 aromatic agents, drug 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 has 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)

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 used is cure cough, cold, pain, liver diseases, malaria and blood pressure in Polynesia and Southeast Asia.

Since it has many medicinal properties, National Medicinal Plant Board has included noni in the list of plants approved for cultivation [4].

Figure 1.1: ***Morinda citrifolia***

Noni is a small tree or shrub, which is about 3-10 m in height. Its flowers are prefect 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 color and fleshy. It is about 5-10 cm long and 3-4 cm in diameter. It is pulpy and putrid when ripe. 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 color and it has a characteristic fetor odor when ripe. Noni have 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 [5].

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 include, flavonoids, alkaloids, phenolic components, organic acids, alcohols, phenols, esters, iridoids, lactones, lignans, triterpenoids etc. [6]. Commonly found phytochemicals in noni is given in figure 1.2.

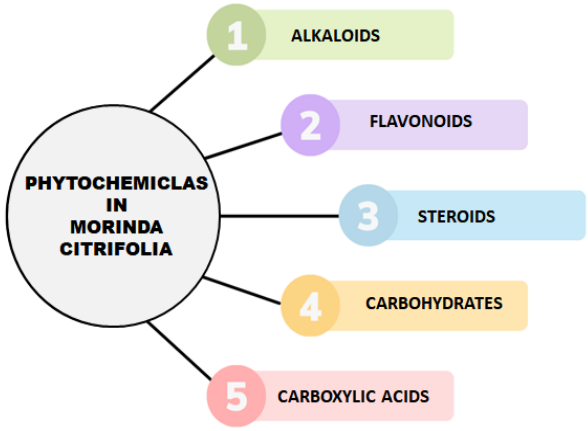


Figure 1.2: Phytochemicals in noni

1.2 Phytochemicals

Phytochemicals are certain chemical compounds that are extracted from plants, which exhibit various properties that have notable effect on the nutrition and health of humans. The word “phyto” means “plant” in Greek; they are non-nutritive and naturally occurring biochemical. Plants produce phytochemicals by their own in order to protect themselves from different environmental problems such as micro-organisms and water changes. The specific color, aroma flavor and texture in plants are due to these phytochemicals [7]. About 4,000 phytochemicals have been listed based on their different properties such as physical properties, chemical properties and their protective function. Out of these about 150

phytochemicals have been analyzed and researched in detail. Phytochemicals can be found in almost all parts of a plant such as in the stem, leaves, roots, flowers and seeds. There are wide variety of dietary phytochemicals found in whole grains, fruits, vegetables, nuts, spices and herbs [8]. These phytochemicals have much therapeutic effect in curing various diseases. Foods which contain phytochemicals that has nutritional value have beneficial effect on humans. They help to protect against numerous diseases such as diabetes, inflammation, high blood pressure, ulcers, parasitic infections etc. [9].

1.2.1 Classification of phytochemicals

Generally, phytochemicals have been classified into two major classes depending on their role in plant metabolism. They are primary metabolites and secondary metabolites. Examples of primary metabolites include amino acids, sugars, purines and pyrimidine of nucleic acids, proteins and chlorophylls. Secondary metabolites includes alkaloids, flavonoids, terpenes, saponins, phenolic components, curcumines and glucosides [10].

Phytochemicals can be divided into many major classes depending on their biological properties, structural properties and botanical origins. Mostly, phytochemicals were classified based on their structure such as alkaloids, phenolic components, limonids, saponins, terpenoids, secoiridoids etc. [11] . Some of them are explained below:

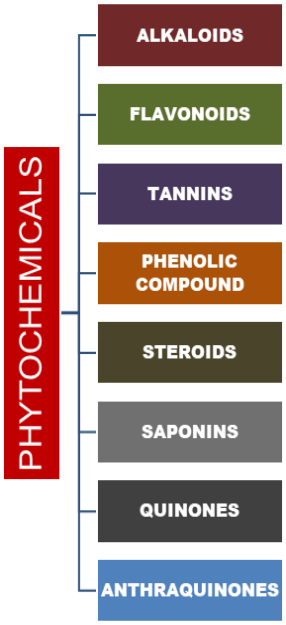


Figure 1.3: Classification of phytochemicals

1.2.1 a) Alkaloids

Alkaloids are one of the largest groups in secondary metabolites of plants. Some examples are neuroactive molecules like nicotine and caffeine and lifesaving medicines like emetine. Alkaloids are used for defense mechanism in plants against various predators and pathogens due to its toxic effects [12].

They are nitrogen-containing compounds and are widely distributed among different parts of plants. Most of the alkaloids are basic in nature and are optically active. These were derived from plants and are pharmacologically active. They are usually amino acid derivative which contain one or more heterocyclic nitrogen atoms. The word “alkaloid” was

coined from Arabic word *al-qali*; which means an early form of soda ash, from which the word "alkali" is derived [13].

A wide range of alkaloids is present in both plants and animals, which differ in the arrangement and combination of functional groups. This broad class of alkaloids has been further divided into many classes in plants based on their chemical structure, biosynthesis pathways and taxonomical groups. Some alkaloids have similar structure within a particular genus of plants but they may have large difference in their chemical and biological properties [14].

However, alkaloids are extremely toxic but they have some therapeutic properties in small quantities. These alkaloids help plants to fight against insects, herbivores and microorganisms. Alkaloids are colorless, crystalline or liquid at room temperature, optically active and are usually bitter in taste. They are used as medicinal agents all over the world due their properties like antispasmodic, analgesic and bactericidal effects [15].

Alkaloids like acetycholine, epinephrine, norepinephrine, dopamine, serotonin and gama aminobutyric acid affects the nervous system in humans. Some alkaloids like sanguinarine used in toothpaste and berberine found in opthamics have an antiseptic property due to its antibiotic activity [15].

Researchers have proposed many classifications for alkaloids. One of the common classification divides entire class of compounds into three classes:

True alkaloids - they are compounds derived from amino acids and contain heterocyclic nitrogen ring. Example include nicotine, atropine [15].

Proto alkaloids – they are compound derived from amino acids and contain nitrogen atom, which is not part of the heterocyclic ring. Example includes ephedrine [15].

Pseudo alkaloids – these are compounds, which are not the derivative of amino acids. Example includes caffeine, theobromine [15].

Another classification of alkaloids is based on their heterocyclic ring system and their biosynthetic precursor. This category includes; pyrrolizidine, quinolizidine, imidazole, indole, piperidine, pyrrolidine, purine, tropane and isoquinoline alkaloids. Different alkaloids have different properties and can be used for different medicinal purpose [16].

Indole alkaloid – contain serotonin, whose chemical name is 5-hydroxytryptamine. This category includes certain compounds like strychnine, vincamine, ajmaline, vincristine and ajmalicine. Among this vincristine and vinblastine are used as anticancer drug [16].

Tropane alkaloid- they contain 8-azobicylo [3.2.1] octane group and they are derivative of ornithine amino acid. The important members of these groups are hyoscyamine, atropine, scopolamine and cocaine. The tropane alkaloid possesses anticholinergic property [16].

Isoquinoline and quinoline- they are known as benzopyridines. They contain benzene ring fused with pyridine ring. An important member of quinoline alkaloid is quinine. Quinine are obtained from bark of *Cinchona officinalis* and *Cinchona ledgeriana*. Quinine is used against *Plasmodium vivax*, which is a protozoan that causes malaria. Other members of

quinoline alkaloids are cinchonidin, homocamptothecin, camptothecin and dihydroquinine. These compounds possess different properties like anti-inflammatory, anthelmintic, antimalarial, anti-bacterial and analgesic activity [16].

The structural isomer of quinoline alkaloid is the isoquinoline alkaloid. The subclasses of isoquinoline alkaloids include benzylisoquinolines, protoberberines morphine alkaloids and ipecac alkaloid. Narcotics, morphine, thebanine and codeine belong to this group. Alkaloids of this class exhibit properties like antioxidant, anti-diabetics, anti-inflammatory etc. [16].

Piperidine alkaloids- they contain saturated piperidine ring and they are known for their toxicity. They also exhibit properties like anticancer, bactericidal, depressant, herbicidal and fungal properties. Coniine, cynapine, lobeline are included in piperidine alkaloid [16].

Purine alkaloid- they are similar to piperidine, but they have unsaturated heterocyclic nitrogen ring. Nicotine, anabasin, anatabine are included in this group. They have antimicrobial properties [16].

Imidazole alkaloid- they contain imidazole ring and are derived from L-histine amino acid. They contain imidazole ring. An important member of this group includes pilocarpine. They are used for the treatment of glaucoma [16].

Pyrrolizidine alkaloid – it contain two five membered ring and share a common nitrogen at position 4. Important members are heliotrine, senecionine, clivorine etc. Plants use this alkaloid as a defense against herbivorous. They also used for the treatment of diseases like diabetes and cancer [16].

Pyrrolidine alkaloid – they are 5 membered ring containing nitrogen atom. They are derived from ornithine and lysine amino acid on addition of acetate or malonate. Important members are hygrine, cuscohygrine, and putrescine. They exhibit anti-tubercular, anti-bacterial and anti-fungal properties [16].

Quinolizidine – it consist of two 6 membered fused ring containing common nitrogen. Important members include lupinine, cytosine, sparteine and lupanine. They have anti-microbial properties [16].

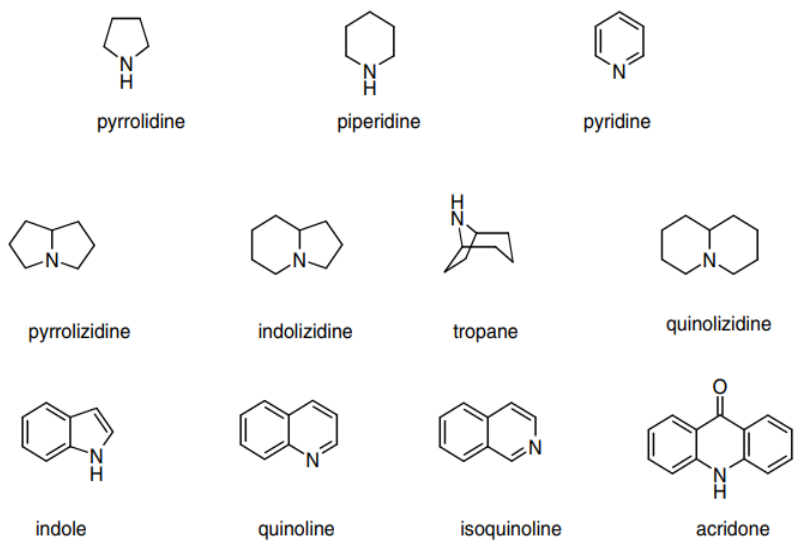
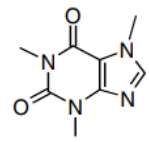
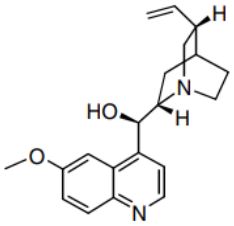


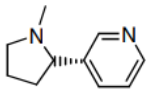
Figure 1.4 : Basic structural unit of alkaloids [13]



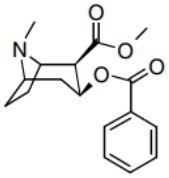
caffeine



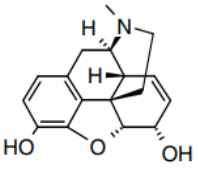
quinine



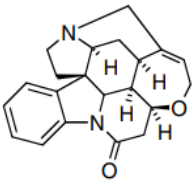
nicotine



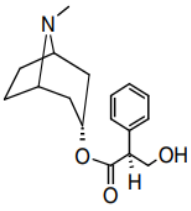
cocaine



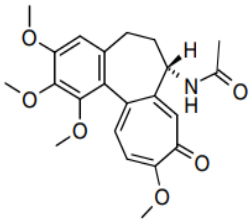
morphine



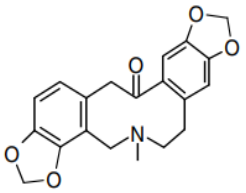
strychnine



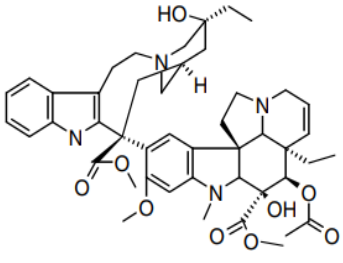
atropine



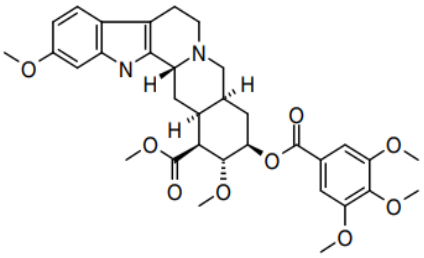
colchicine



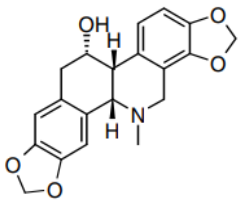
protopine



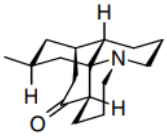
vinblastine



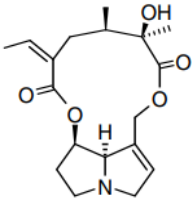
reserpine



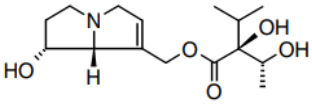
chelidonine



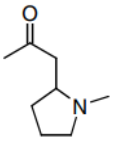
lycopodine



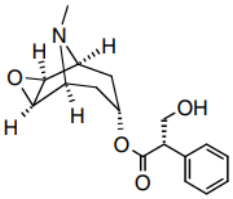
senecionine



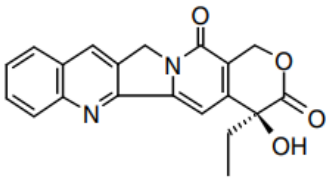
intermedine



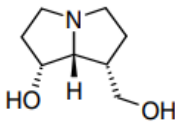
hygrine



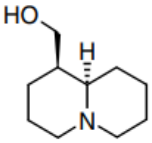
scopolamine



camptothecin



platynecine



lupinine

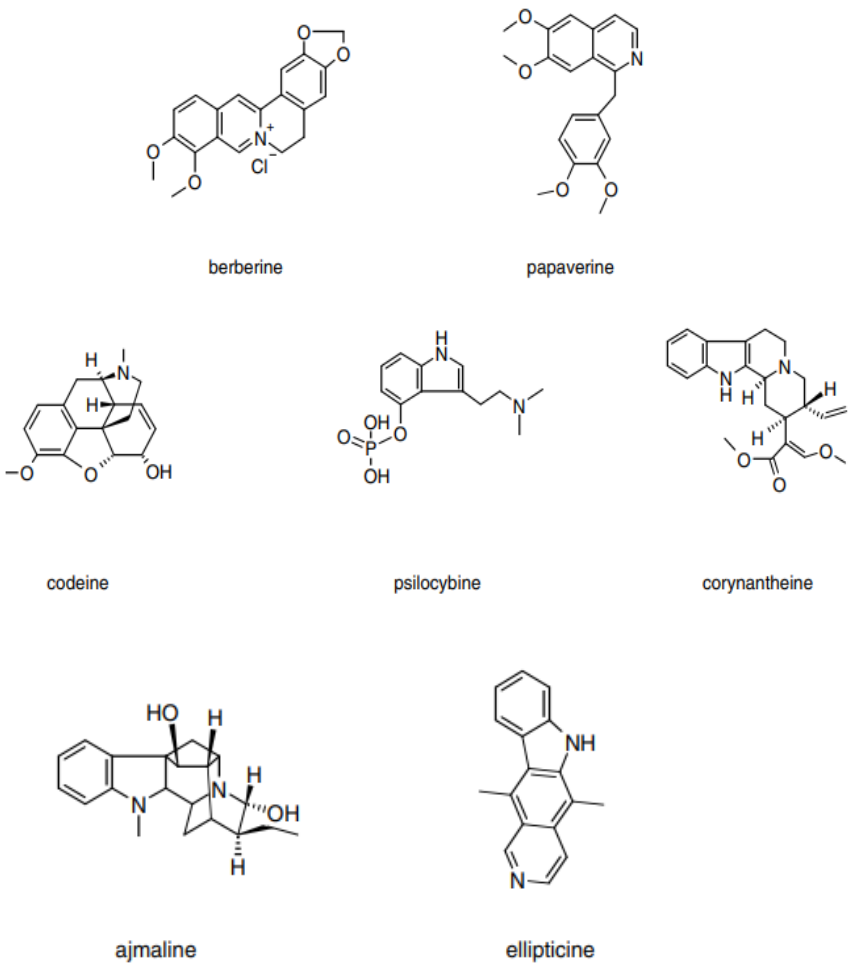


Figure 1.5 : Some examples of alkaloids [13]

1.2.1 b) Flavonoids

Flavonoids are phytochemical compound present in plants that are responsible for their color or flowers, fruits and sometimes leaves. It originated from a Latin word '*flavus*', which means yellow. Sometimes it act as co-pigment and impart color to plant. Animals are attracted by its color and thus help in pollination. It also protects the plant from UV damaging effect. The skeletal structure of flavonoids is 2-phenyl chromane or Ar-C_3-Ar skeleton [17].

Flavonoids fall under a large category of phenolic plant constituents. They are obtained from derivatives of 2-phenyl-benzo-γ-pyrone. The structure consists of two benzene ring denoted as A and B in figure 1.6. These two rings are joined by oxygen atom in pyrene ring C. All flavonoids contain a carbon skeleton of flavan system (C_6-C_3-C_6). Depending on the differences in the structure of flavonoid compounds, flavonoids are classified as flavanols, isoflavones, flavonols, flavanones, flavones and anthocyanins [18].

Flavonoids are used as prophylactic or therapeutic drug. Some isolated flavonoids have antimicrobiological properties [19]. It has different pharmacological effects like antioxidant, anti-inflammation, anti-platelet, anti-allergic, cytotoxicity and also reduces risk for heart disease or cancer etc. [20]. The intake of flavonoid containing foods helps in lowering the risk of some pathophysiologies associated with free radicle meditated diseases like ischemia-reperfusion injury and coronary heart diseases [21]. They can be used as adjuvants in photoprotective formulations as it has the potential to fight against UVA and UVB radiations [22].

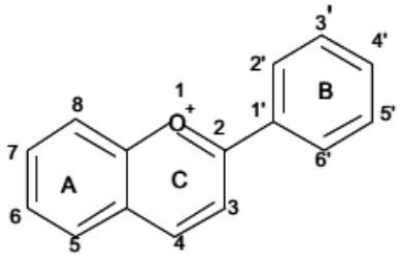


Figure 1.6 : The structure of flavylium cation [18]

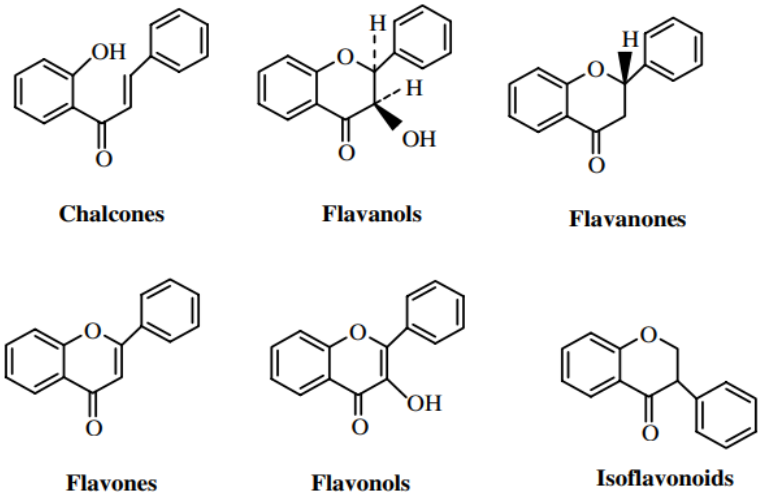


Figure 1.7: Basic structure of some flavonoids [17]

1.2.1 c) Tannins

These are phenolic compounds with high molecular weight. Their molecular weight ranges from 500 – 3000 Da. These compounds are found in leaves, fruit, bark, roots and wood of a plant and are mostly located in vacuoles. They act as defense mechanism against small insects, birds and mammalian herbivores. Based on difference in structure and properties tannins are classified into two main groups: hydrolysable and condensed

tannins. Hydrolysable tannins include gallotannins and ellagitannins [23]. Another group is called complex tannins, which has a catechin unit glycosidically bonded to a gallotannins or an ellagitannins unit. Condensed tannins are a group that contains oligomeric and polymeric proanthocyanidins. The linkage of C-4 of one catechin with C-8 or C-6 of neighboring monomeric catechin forms the condensed tannins [24].

Tannins are used as antimicrobial agents for preserving woods or for dental caries prevention. European oak, chestnut and some eucalypts are rich in tannins. Defense mechanism of tannins in plants is by different methods like increasing resistance against pathogens, preserving certain part such as wood against decay [25]. Tannins act as protein binding agents, antioxidants, prooxidants or toxin [26].

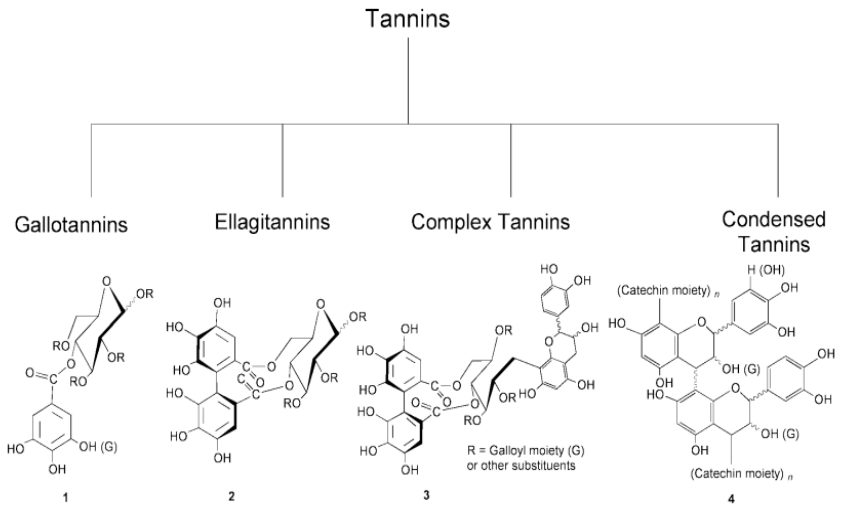


Figure 1.8: Classification of tannins [24]

1.2.1 d) Phenolic compounds

These are secondary metabolites and have vital role in plant resistance. Its structure contains at least one aromatic ring attached to one or more hydroxyl group and other substituents like carboxyl or methoxyl groups. These substituents give rise to polar nature to the compound and make it soluble in water. Usually they are prepared from phenylalanine amino acid, which is then converted into cinnamic acid. Phenolics are one of the biggest and broadest groups of plant active substance. It helps in regulation of germination of seeds and helps in plant growth. They play a vital role in defense mechanism [27].

Phenolic compounds are usually classified into two groups – simple phenols and complex phenol derivatives. Some of the compounds in simple phenols include – caffeic acid, p-hydroxybenzoic acid, vanillic acid, o-hydroxybenzoic acid, cinnamic acid, syringic acid [27].

Phenolic compounds helps in reducing the risk of degenerative diseases by lowering oxidative stress and by suppressing macromolecular oxidation in humans. They have anti carcinogenic properties. They act as free radical quencher and metal chelating agents [28]. Phenolic compounds have tremendous importance in plant-soil system. They are important area of study in medicinal and biological research [29].

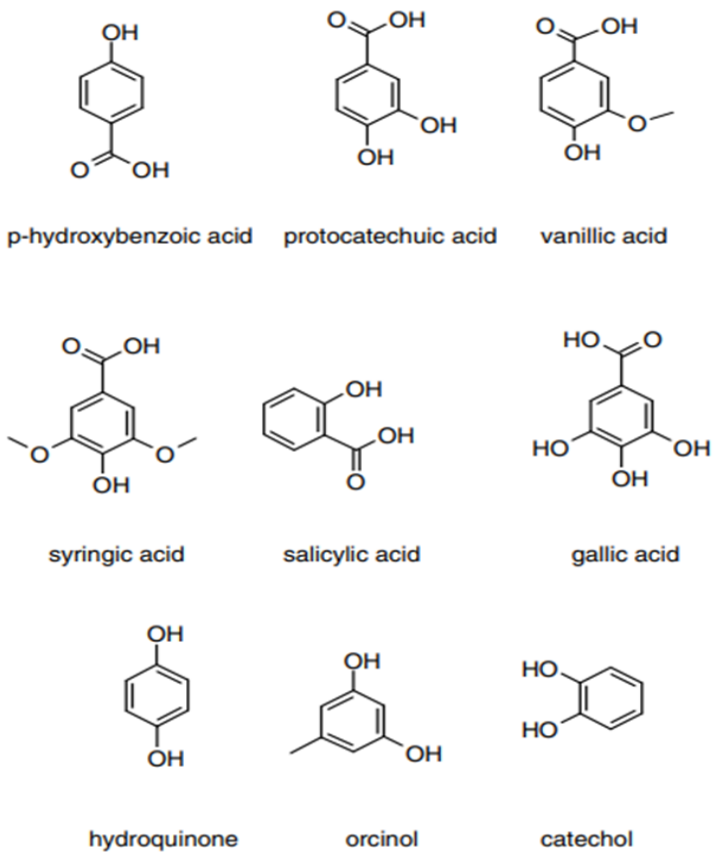


Figure 1.9: Simple phenols [13]

1.2.1 e) Steroids

Steroids are secondary metabolites and are found in all plants, which may be different from one another. These are one of the most important cell constituents. A typical example for plant sterols is cholesterol. These are class of steroids which having long side chain at C-17. Studies showed that, large quantities of sterols with 28 -29-carbon atoms have been isolated from the analysis of fungi or higher plants. Commonly isolated

compounds are ergosterol, stigmasterol and sitosterol. Among this ergosterol is used as a precursor for ergocalciferol (vitamin D) [30].

The first isolated steroid-hormone compound from plant is Brassinosteroids (BS). This helps in growth stimulation and adaptogenic processes. Naturally occurring source for BS is the pollen collected from flowers [31].

Plant steroids play an important role in physiological effect such as plant growth, reproduction and development. Plant steroids have chemical structure composed of four carbon rings known as the steroid nucleus. This nucleus consist of 5α or 5β-gonane tetracyclic carbon skeleton. This skeleton contains methyl substituents at C-10, C-13 carbon atoms and an alkyl side chain at C-17. The addition of different groups at different position in steroid nucleus gives rise to different types of steroids. Based on biological functions, taxonomy and structure these steroids are classified into seven groups. They are: brassinosteroids, cucurbitacins, bufadienolides, ecdysteroids, steroidal alkaloids, cardenolides, saponins and withanolides [32].

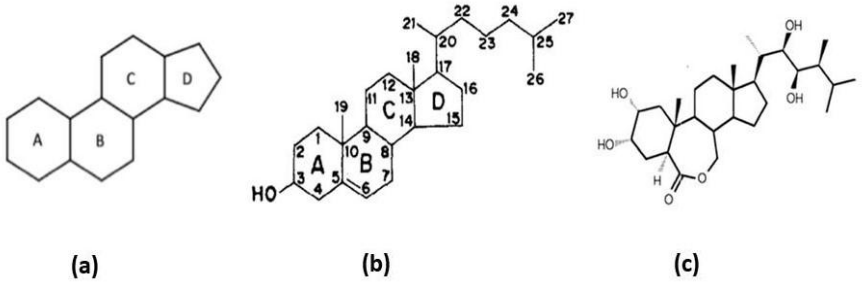


Figure 1.10: (a) Plant steroid backbone structure [32], (b) Cholesterol [30], (c) Brassinosteroid – Brassinolide [33]

1.2.1 f) Saponins

Saponins are wide range of secondary metabolites that are produced by plants [34]. They are naturally occurring bioorganic compounds. Their structure contains at least one glycosidic linkage (C-O sugar bond) between aglycone and a sugar chain at C-3 carbon. This aglycone nucleus is a water insoluble part and it has 27 to 30 carbon atom. Saponins contain one or two sugar moieties which are water soluble part and it contains 6 to 12 carbon atoms. The water insoluble part that is aglycone or sapogenin may be different in different saponins in plants. Based on the type of aglycone, saponins are classified into three groups: triterpenoid glycosides, steroid glycosides and alkaloid glycosides. The water soluble part has a variety of furanose ring or pyranose ring sugars [35].

They have antifungal activity and also help to protect the plant from the attack of pathogenic microbes [34]. They have anticarcinogenic properties and possess surface active properties due to its amphiphilic nature [36]. In humans, the consumption of saponins containing food helps to have hypo-cholesterolaemic diets [37].

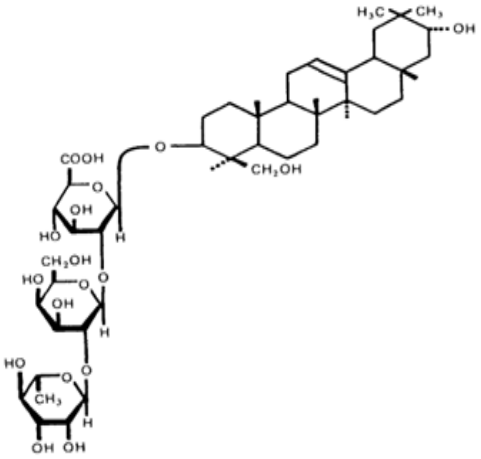


Figure 1.11: Structure of a typical saponin (from soya beans) [37]

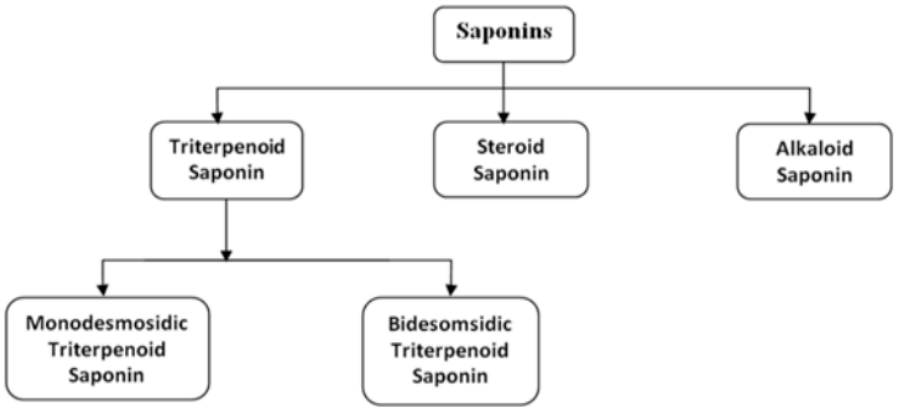


Figure 1.12: Classification of saponins [35]

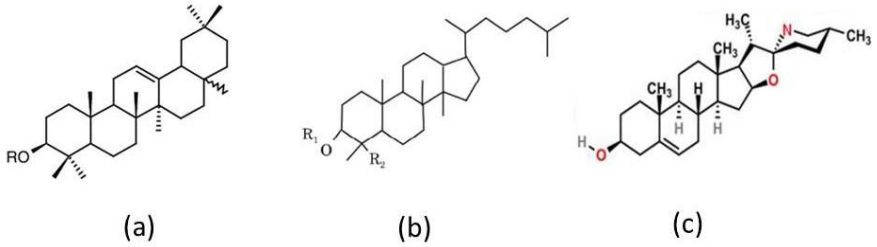


Figure 1.13: (a) Triterpenoid saponin R= sugar moiety [38], (b) steroid saponin [39], (c) alkaloid saponin [35]

1.2.1 g) Quinones

They are secondary metabolites found in plants [40]. Their structure consists of aromatic ring with di-one or di-ketone system. They are the derivative of hydroquinones on oxidation [41]. Based on number of benzene rings and fused rings in the structure, they are divided into four types [40]. They are benzoquinones, naphthoquinones, anthraquinones, and polyquinone [41].

Quinones constitute phenolic compounds having wide range of pharmacological activities. They have wide range of application in the field of medicine and pharmacy. They are found in bacteria, lichens, fungi, Angiosperms and Gymnosperms [42]. Quinones especially naphthoquinones possess antifungal, antibacterial and antitumor properties [41]. Quinones are used as dyes as they are coloured compounds. Chromophore present in it is benzoquinone, which have two carbonyl groups with two carbon- carbon double bond [43]. They possess antioxidant properties which help in improving health conditions in humans. Their biological effects are due to two properties. One is that it

can undergo oxido-reduction reaction reversibly and other one is that, they are electrophilic in nature [44].

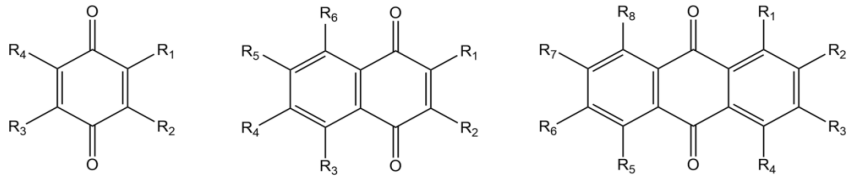


Figure 1.14:Chemical structure of benzoquinone, naphthoquinone and anthraquinone [43]

1.2.1 h) Anthraquinones

They are secondary metabolites found in plants. They play vital role in environmental and biological processes [45]. They have a rigid planar structure with three aromatic ring of anthracene having two keto functional group at 9 and 10 positions [46]. Different derivatives of 9, 10-anthraquinone can be prepared by chemical interconversion of side groups to methoxy, hydroxymethyl, hydroxyl, methyl radicle and glycosides [45].

These compounds possess anti-inflammation, anti-hyperlipidemia, immonoregulation and anticancer properties [47]. Anthraquinone derivatives obtained from bacteria, insects and fungi also show biological activities. Natural and synthetic anthraquinones have wide range of applications. Both are used in imaging devices, textile dying, pharmaceuticals, foods and cosmetics. They are also used for the treatment of malaria [46]. Naturally occurring anthraquinones exits as glycosides in plants. Different derivatives of anthraquinones, based on nature, number and position of substituents possess different inhibitory potential against

fungi and pathogenic protozoa [48]. Anthraquinones belongs to large group of quinone dyes, so they can be used as colouring agents. Some anthraquinones that occur naturally are colourless. The coloration in some natural anthraquinones are due to some synergic effect or due to some transformation process of reaction products [49]. Studies showed that anthraquinones can directly act on external coat of anthraquinone-sensitive virus and thus helps in the prohibition of virus adsorption and its successive multiplication [50].

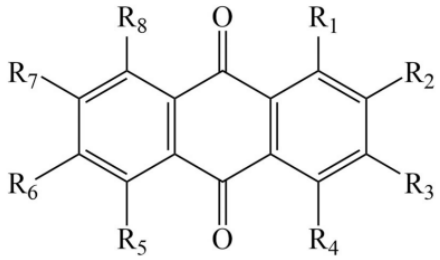


Figure 1.15: Structure of the parent nucleus of anthraquinones. R_1, R_2, R_3, R_4, R_5, R_6, R_7, and R_8, respectively represent substituent groups [45].

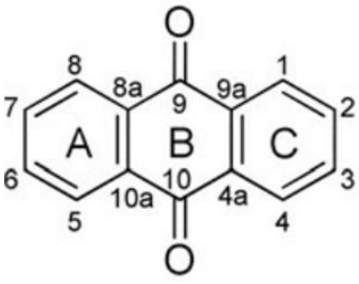


Figure 1.16: Anthraquinone [46]

1.3 General strategies of extraction

1.3.1 Infusion

It is an extraction method in which a solution is prepared using solid mixture of one or more constituents which is in contact with fluid solvent. This method is also known by different names like washing extraction, leaching, diffusion extraction etc. It is a method of extraction of infusible substance using a solvent and thus we obtain the required extract. This process can he accelerated by using crushed or grinded solid material and high temperature. Higher concentration of extract can be obtained if temperature if higher. Steep time or length of time, the solvent in contact with infusible matter is an important parameter in this process [51].

1.3.2 Decoction

It is a method used for the extraction of substance that is soluble in water. This method is usually used for the extraction of constituents that cannot be destroyed on application of heat. This method is used for the extraction of components from medicinal plants and it is a water based extraction technique. In this method, extraction is conducted by boiling the required plant part in water. Water-soluble chemicals from root, bark, fibrous plants are extracted by this method. It is an inexpensive method and easy to operate. However, it only suitable for the extraction of components that is heat stable [52].

1.3.3 Maceration

It is one of the oldest and simple method of extraction. In this method, the powdered sample is soaked in required solvent and kept it for long period [53]. During this process the solvent molecules diffuses through the cell wall of the sample and dissolves the components present in the plant

sample. After the required time, the extracts obtained are filtered out and solid residue is pressed to obtain maximum solvent. This method is a solid -liquid extraction technique. This method is cheaper but it is time-consuming method. This method is usually used for the extraction of active components from medicinal plants [52].

1.3.4 Soxhlet extraction

It is a conventional method of extraction. It is solid- liquid extraction method, when sample is a solid material. The apparatus consist of a heating mantle, R. B flask in which the solvent is placed, a thimble made from filter paper where sample is placed, a condenser and water inlet and outlet. In this method, the plant sample is placed in the thimble. Below this chamber, the R. B flask containing solvent is placed. It is heated and the solvent passes through the left arm to the condenser and then condensate drips into the thimble-containing sample. Sample interacts with hot solvent and extraction occurs. When this chamber is filled with desired extract, it is then return back to R. B flask through siphon tube and the cycle is repeated until the extraction is completed. It is a simple method of extraction [54].

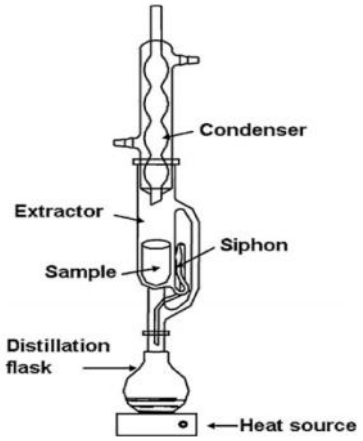


Figure 1.17: Soxhlet extractor [53]

1.3.5 Supercritical liquid extraction

It is a method of extraction of one substance from another substance using supercritical fluids as solvent for extraction. This method is usually used for the extraction from solid sample material but it can also be used for liquid sample. Commonly used supercritical fluid is CO_2 and sometimes it was modified using additional solvents like methanol or ethanol. Supercritical fluid extraction using CO_2 as fluid works under high pressure in order to obtain highly concentrated products from the sample, which is usually natural material. In this method, there is no waster of solvent as no solvent is left behind after the extraction process. The advantage of CO_2 as supercritical fluid is that it is inert, non-toxic and inexpensive and it has low critical temperature that is 31°C. So due these advantages this method is used for the extraction of essential oils and in the field of nutraceutical industries [55].

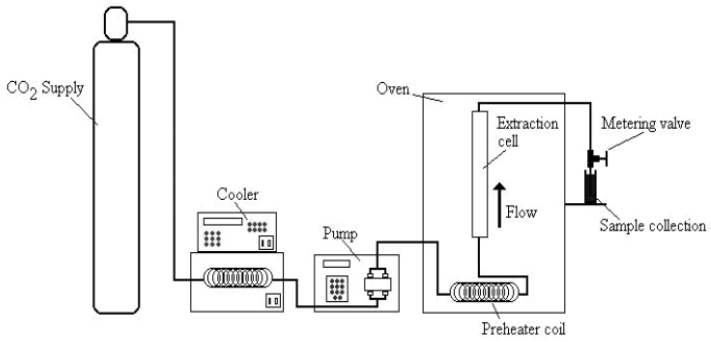


Figure 1.18: Schematic diagram of supercritical fluid extraction apparatus [55]

1.3.6 Solvent extraction

It is a liquid-liquid extraction method. The principle behind this technique is the partitioning of components based on their relative solubility between two immiscible liquids. Usually used immiscible liquids are water and an organic solvent. It is method of extraction of components present in one liquid phase into another liquid phase. This method is carried out using a separating funnel in chemical laboratories. This method could be used for the separation of particular component from a mixture by dissolving it in suitable solvent. It is method of separation of soluble substance from an insoluble substance [55]. This method is used for the separation of organic components, group of alkaloids and components in the rare earth metals [56].

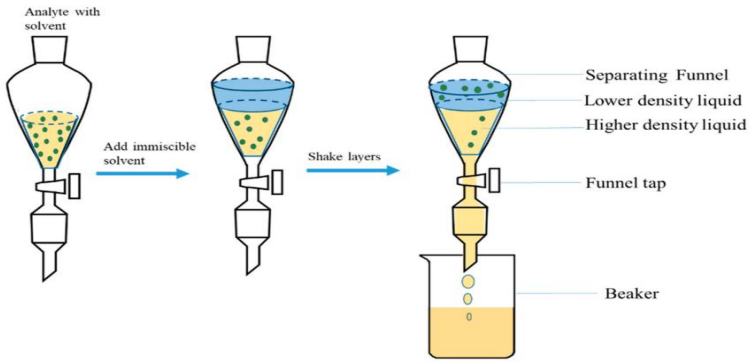


Figure 1.19: Diagrammatic illustration of liquid-liquid extraction [57]

1.3.7 Ultrasound- assisted extraction

Ultrasound assisted extraction is a method that is used to increase the efficiency of extraction through ultrasound induced (acoustic) cavitation and mechanical effects. Due to this ultrasound induced cavitation, the cell wall of the plant material is reputed and solvent molecules are penetrated into plant material. Thus, the intracellular products can be extracted. Mechanical effect caused by this method is agitation of solvent, which helps in the extraction by increasing the surface area of contact between required component and solvent. This allows the penetration of solvent into sample material. This method reduces the amount of solvent consumption and time for extraction. This method can be carried out at low temperature and thus thermal damage to the extract can be avoided [58].

1.4 Antibacterial and antioxidant activity of *Morinda citrifolia*

Morinda citrifolia possess antibacterial activity against different bacteria. Studies showed that noni fruit extract can be used as a natural sanitizer as it shows high antibacterial activity against *Listeria monocytogenes*. So it can be used to sanitize fresh-cut produce. The fruit extract also showed antibacterial activity against foodborne pathogens. Scopoletin, ursolic acid, asperuloside and rutin are the reported bioactive compounds in noni fruit extract obtained from HPLC analysis. Compounds that have antibacterial activity against foodborne pathogens are alizarin, acubin and scopoletin [59].

Noni fruit juice shows antibacterial activity against mycoplasmas. Mycoplasmas are mucosal pathogens present in extracellular epithelial surfaces [60]. Endophytic bacteria Enterobacter cloacae strain extracted from Noni fruit in ethyl acetate fraction showed antibacterial activity against *Staphylococcus aureus, Shigella dysenteriae, Streptococcus mutans and Escherichia coli.* Different bioactive compounds present in noni fruit can be used in the field of medicine and food industries [61].

Different types of natural antioxidants are found in plants. Studies showed that, root, leaf and fruit extract of noni showed antioxidant activities [62]. The antioxidant activity varies with the extraction technique used. Large numbers of antioxidants are present in noni fruit. This includes carotene, beta-carotene, alkaloids, flavonoids, terpenoids etc. Studies showed that *M.citrifolia* root possess higher antioxidant activities than corresponding leaf and fruit extracts. However higher radical scavenging activity is possessed by *M.citrifolia* juice. Groups like flavonoid, coumarin and phenolic components in noni fruit exhibit radical scavenging activity due to their antioxidant properties [63].

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 alkaloids in fruit of *Morinda citrifolia* using techniques such as FT-IR Spectroscopy, ^{1}H NMR, ^{13}C NMR Spectroscopy, DEPT, COSY, UV-Visible spectroscopy and GC-MS. Furthermore, evaluation of the antibacterial and anti-oxidant activity of the extracted alkaloids will also be carried out.

Chapter 2

LITERATURE SURVEY

Ji Yubin, Yu Miao, Wang Bing and Zheng Yao discuss the extraction, separation, and purification methods of alkaloids from natural medicine. Alkaloids in natural medicine are present in the form of free salt or salt alkaloids [64].

Hamza T, Adejoke, Hitler Louis, Oluwatobi Amusan, Gloria Apebende analyzed that the importance of natural products in the pharmaceutical industry cannot be understand, as they play a important role in the prevention and treatment of diseases such as cancer, malaria, and piles. Natural products, including alkaloids, flavonoids, phenols, saponins, and tannins, are bioactive compounds found in plants and are important for plant metabolic activities. These compounds could be served as alternatives medicines for treating various ailments because they are the compounds tested for their medical properties. This article provides an overview of the pharmacological importance named alkaloids, methods of extraction and purification of alkaloids in plants and the side effect caused by the abuse of alkaloids or alkaloids or alkaloid derivative drugs [65].

Roy A in his review paper on alkaloid discussed the therapeutic uses of plants encompass activities such as anti-tumor, anti-viral, anti-inflammatory and anti-malarial effects. Plants are viewed as valuable source of a diverse range of components that can aid in drug development. Alkaloids are secondary metabolized recognized for their therapeutic attributes. The alkaloid compounds have been categorized into several

groups, which include indole, piperidine, tropane, purine, pyrrolizidine, imidazole, quinolozidine, isoquinoline and pyrrolidine alkaloids based on their biosynthetic precursors and the structure of their heterocyclic rings. Through their ability to neutralize free radicals and inhibit oxidative reactions alkaloids show protective effects against chronic diseases. Pharmaceutical potential of alkaloids extracted from diverse plant species, were examined from some investigations, which reveals their broad spectrum of therapeutic applications. The main goal of this review is to provide an exhaustive overview of alkaloid based drugs sourced from various plants, highlighting their efficiency against a range of diseases [66].

Antia G. Perera, Lucia Cassani, Paula Garcia-Olivera, Paz Otero, Sepidar S. Mansoor, Javier Echave, Jianbo Xiao, J. Simal-Gandara, M.A Prieto discussed about the production, extraction, and potential therapeutic properties of plant alkaloids. Production of plant alkaloids, including extraction methods, isolation and purification, is presented in the review. A detailed analysis of different groups of alkaloids it's chemical structures, plant source, and uses, is also included [67].

The study conducted by Amy C. Brown probed the relationship between noni juice, and anticancer or immunostimulant properties. It has been suggested that a 'concentrated component' in noni juice, instead the pure noni juice, can activate the immune system to probably help the body in fighting cancer, and kill a small percentage of cancer cells, depending on the type among the in-vitro studies. In vitro and in vivo studies in animal proposed that unidentified substance in unpasteurized noni fruit juice may have slight amount of anticancer activity [68].

Mohammed Ali, Mruthanjaya Kenganora, Santhapete Nanjundaiah Manjula highlights the health benefits of *Morinda citrifolia*. Due to the potent antioxidant and proven health benefits of *Morinda Citrifolia,* it has been used in further countries for supplementary therapies. Phytochemical and mineral compositions of the different parts of the noni plant are the focuses on this review. Moreover, the pharmacological basis for the various health benefits, traditional, and medicinal applications of noni are confirmed. Amino acids, anthraquinones, fatty acids, flavonoids, iridoids, lignans, polysaccharides, sterols, sugars, terpenoids, etc., contains in the crude extracts of various parts of the plant and noni fruit juice have been stated they are clinically effective for a broad range of pathological conditions. In this literature they confirm noni pharmacologically active and used in different forms of cancer, such as colon, esophageal, breast and colorectal cancers and also in cardiovascular diseases, diabetes, arthritis and investigations [69].

M. Y Wang and C. SU conducted a study to investigate the idea that *Morinda citrifolia* contains a cancer preventive effect at the starting stage of carcinogenesis. The outcome propose that the prevention of carcinogen-DNA adduct formation and the antioxidant activity of noni juice may contribute to the cancer preventive effect of *Morinda citrifolia* [70].

An investigation conducted by Afa K. Palu, Anne Hirazumi Kim Brett J. West, Shixin Deng, Jarake Jensen, Leland White to study the mechanisms including cannabinoid in the immunomodulatory effects of *Morinda citrifolia L*. (noni) in vitro and in vivo in mice. In vitro, Tahitian Noni juice (TNJ) and Noni fruit juice concentrates (NFJC) (1, 5 mg/ml) were found to potently activate two (CB_2) receptors, by restricting cannabinoid (CB_1) receptors in a concentration dependent manner. In vivo, oral

administration of TNJ and libtum for 16 days exhibits a decrease in the production of IFN-gamma. These outcome shows that the immune system is regulated by noni through activation of the CB_2 receptors and inhibition of the IL-4, but increased production of IFN-gamma cytokines. Useful immunomodulation effects may also be useful in conditions involving insufficient immune responses [71].

Peter E. Murray, Franklin Garcia-Godoy, Kenneth N Namerow Sergio Kutler and Romi M. Farber did a study on the examination of *Morinda citrifolia* as an endodontic irrigant. *Morinda Citrifolia* juice has been identified as a secondary for the use of NaOCl as an intracanal irrigant, as the first fruit juice to be accepted for this purpose [72].

B.Shivananda Nayak, Steve Sandiford and Anderson Maxwell analysed the wound healing activity of ethanolic extract of *Morinda citrifolia* leaf. Topical treatment for wound healing is the basic traditional use of this plant found on leaves. Using excision and dead space wound models a study on the wound healing activity of the ethanol extract of noni leaves were conducted on rats. Enhanced wound contraction, decreased epithelialization time, increased hydroxyproline content and histological characteristics have been found to propose that noni leaf extract may have curative benefits in wound healing [73].

Z. Mohd Zin, Abdul-Hamid A and Osman A discussed antioxidative activity of extracts from Morinda citrifolia root, fruit, and leaf. A study was conducted to evaluate the antioxidative activity of extracts from different parts of *Morinda citrifolia*, including leaf, fruit, and root. The results conclude that many compounds offer antioxidative activity on different parts of *Morinda citrifolia*. It suggest that activity in the roots is

due to both polar and non-polar compounds, but in the fruit and leaf, only to non-polar compounds [62].

Oliver Potterat, Matthias Hamburger emphasizes the phytochemistry, Pharmacology, Safety of *Morinda citrifolia*. Investigations are done on the potential health benefits of *Morinda citrifolia*. To investigate its pharmacological properties research has been executed. Analysis has shown that *Morinda citrifolia* has antioxidant, anti-inflammatory, anti-inflammatory, and antimicrobial activities. Its extracts provide anticancer properties. For various purposes the leaves, fruits, and roots of *Morinda citrifolia* have been used in traditional medicine. The plant has been confirmed to have potential health benefits, including reducing inflammation and improving immune function. Generally, *Morinda citrifolia* was identified to have potential health benefits, and additional research is being performed to confirm its effectiveness [74].

Analgesic and behavioral effects of *Morinda citrifolia* was analyzed by Chafique Younos, Alain Rolland, Jaques Fleurentin, Marie-Claire Lanhers, Rene Misslin and Francois Mortier. It discusses the traditional therapeutic symptoms for the use of *Morinda citrifolia L.* The *Morinda Citrifolia* extract did not show any toxic effects but did show a significant, dose-related, central analgesic activity in the writhing and hotplate tests [75].

Sarvananda Letchuman, Hewa D. T. Madhuranga, M. B. L. N Kaushalya, Amal D. Premarthana, Muthupandian Saravanan analyses the novel therapeutic, properties, mechanisms, and plant-based innovations. Due to their lower toxicity compared to synthetic compounds, global focus shift towards natural therapeutic agents and an approach is taken in this review by evaluating the ecological and molecular factors depending on the

medicinal properties of alkaloids. The cooperate potential of alkaloids, when contrast with other phytochemicals, is emphasized in this review, providing new awareness into more potent, multi-compound therapeutic formulations. A novel perception illuminates the need for more research to improve alkaloid extraction methods and study their potential in personalized and combined therapies [76].

A study was conducted by Thi Cam Tu Phan, Thi Kim Lien Nguyen, Thi Phuong Troung, Thi Tuyet Ngan Pham, Truong Giang Huynh, Xuan Diep Doan on the effects of noni fruit extract on the growth performance, digestive enzymes, and stress tolerance of juvenile whiteleg shrimp. The results of this study shows that noni fruit extract can be used in shrimp feed to enhance growth, survival, and resistance to environmental stresses [77].

Reports have been made by T. P Tim Cushnie, Benjamart Cushnie, and Andrew J. Lamb on pandrug-resistant bacteria causing untreatable infections, making the use for new antibacterial therapies more pressing than ever. Other alkaloids with growth potential are emphasized in this review. Natural, semi-synthetic, and synthetic alkaloids of all classes reconsidered with a focus on those with direct antibacterial activity and those with antibiotic enhancing activity. The effects of alkaloids on toxicity gene regulatory systems, such as quorum sensing, and virulence factors like sortases, adhesins, and secretion systems, have been described. The review has been investigated with complications and limitations of the described research, and directions for future research [78].

Ali Esmail Al-Snafi investigated the curative properties of medicinal plants alkaloids. The clinical use of alkaloid containing plants has a extensive background, and various alkaloids are still being used in

medicine today. Alkaloids possess a large range of pharmacological and therapeutic effects. In the present thesis, the plant contents of alkaloids are evaluated for their medical, pharmaceutical, synthetic and plenty of other useful properties [79].

The Shiyang Zhou, Gangliang Huang, evaluated the chemical composition and pharmacological activities of *Morinda citrifolia*. It has been informed that the main chemical components of *Morinda citrifolia*, including anthraquinones, phenylpropanoids, flavonoids, terpenoids, glycosides, steroids, fatty acids, and their esters, are comprised. A large range of medicinal properties, which includes anti-bacterial, anti-oxidant, anti-inflammatory, and analgesic, hypoglycemic, hepatoprotective, protective cardiovascular, and anti-tumor effects, have been assign to *Morinda citrifolia*. Permission to reveal the nutritional and medicinal value of *Morinda citrifolia* and provide reference for more logical development and exhaustive utilization of *Morinda citrifolia* assets, a review of the chemical composition, pharmacological activity, and mechanism of *Morinda citrifolia* has been provided. The chemical composition, pharmacological activity, and mechanism of *Morinda citrifolia* are analyzed in this literature to give a complete knowledge of its nutritional understanding value [80].

Philip F. Uzor investigated the antimalarial activity of alkaloids from plants. In developing countries, malaria has been recognized as one of the main health problems. The condition kills a wide number of people every year, and the economic status of various countries is also affected. An critical search for original compounds, especially from environmental means such as therapeutic plants, has been justified. The first effective antimalarial drug, quinine, an alkaloid extracted from the cinchona tree,

has been noted. The alkaloids extracted and informed recently to exhibit antimalarial activity are discussed in the current work. Various classes of alkaloids, including terpenoidal, indole, bisindole, quinolone, and isoquinoline alkaloids, have been detected with hopeful antimalarial activity [81].

Bikash Adhikari presents a literature-based study of alkaloids from therpeutical plants in protective or cure methods to diabetes. Alkaloids, the main plentiful and varied group of secondary metabolites, show antidiabetic activity by different mechanisms. This analysis is evaluated helpful for the investigation of active alkaloids for the progress of a modern drug for people [82].

Babita Aryal, Bimal Kumar Raut, Salyan Bhattarai, Sobhika Bhandari including 13 reviewed the potential therapeutic applications of plant derived alkaloids against inflammatory and neurodegenerative diseases. Alkaloids, which arise from plants, have been identified as potential defensive agents against neurodegenerative disorders (NDDs) and chronic inflammations. Most of the alkaloids show anti-inflammatory action and neuroprotective interaction which it was discovered from the methodical study carried out to gather literature on activity [83].

Nasound Alasvand, Vahideh Assadollahi, Roberto Ambra, Ehsan Hedayati, Wesam Kooti, and Ilaria Peluso analyzed antiangiogenic effect of alkaloids. A review has been executed to define the impacts of alkaloids on angiogenesis, a method playing a crucial role in tumor growth and incursion whereby new vessels are formed. For the control and cure of cancers anti-angiogenic compounds, including herbal ingredients, non-herbal alkaloids and microRNAs, have been recognized as potential agents. Proof, which has been showed, suggests that alkaloid rich plants

have various engaging features that efficiently stop angiogenesis. In this review Important information on usually used alkaloid substances as potential angiogenic inhibitors are showed [84].

Petra Algenstaedt, Alexandra Stumpenhagen, and Johannes Westendorf studied the effect of *Morinda citrifolia L*. fruit juice on the blood sugar level and other serum parameters in patients with diabetes type 2. The potential of noni fruit juice to control rising blood sugar levels and other pathological parameters in patients with DT2 has been executed. Noni fruit juice has been an appropriate supplement to the diet of diabetic patients [85].

Janice S. Mani, Joel B. Johnson and Mani Naiker have studied the phytochemistry and anticarcinogenic effect of noni juice. A research gap has been shown in the comparative analysis on several marketed noni fruit juices accessible to decode their phytochemical composition and properties against carcinomas. The current analysis targets to fill this research gap and evaluate the juice's informal use as alternative medicine to control cancer. In this analysis, five marketing brands of noni were included. The noni juice samples include high levels of TP and antioxidant capacity and show some level of cytotoxic activity, which were divergent from the negative control [86].

Smita Nayak and Sushma Mengi carry out the immunostimulant activity of on T and B-lymphocytes. In this analysis, the stimulatory effects of the extracts and fractions of *Morinda citrifolia* fruits on vital components of the adaptive immune system, such as T lymphocytes and B-lymphocytes, were evaluated. The report of this analysis verify the cellular and humoral immunostimulant properties of *Morinda citrifolia* fruits and explain its application in classic medicine [87].

Haziz Sina, Gado Dramane, and Philippe Tchekounou including nine of them studied the phytochemical composition and in vitro biological activities of *Morinda citrifolia* fruit juice. The phytochemical analyses of the fruit juice of *Morinda citrifolia* and to examine its antiradical and antibacterial activity was the aim of this work. A phytochemical analysis was executed by tube staining tests of the extract of two types of fruit juice of *Morinda citrifolia*. Qualitative phytochemistry of *Morinda citrifolia* fruit juices shows the existence of large group of secondary metabolites, containing polyphenols, reducing compounds, mucilage, and terpenoids. The antioxidant activity of *Morinda citrifolia* is dose dependent and higher than that of ascorbic acid. Antimicrobial activity shows that the fruit juices stops the growth of *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, S. epidermidis, Proteus vulgaris, Streptococcus oralis, Enterococcus faecalis,* and *Escherichia coli*. An important difference was seen for each juice on the strains. These reports assist the use of *Morinda citrifolia* in classic medicine and are regarded as the starting points for the growth of a new drug to combat both dietary conditions and chronic conditions connected with oxidative stress [88].

Yanie Chan- Blanco, Fabrice Valillant, Ana M Perez, Marie-Pierre Belleville, Cira Zuniga and Pierre Brat have investigated the antioxidant activity of noni fruit. Antioxidant activity has also been revealed to stay fairly high [89].

Shah Faisal, Syed Lal Badshah, Bibi Kubra, Abdul-Hamid Emwas and Mariusz Jaremko did a comprehensive review of antiviral activity of alkaloids. In this review, a wide overview of the some naturally occurring alkaloids along with artificially produced alkaloid compounds having notable anti-viral properties is given in this review. Various antiviral

alkaloids have been explained in the literature in different experimental environments have been reviewed. These alkaloid compounds have important antiviral properties against various contagious viruses. Several significant stages of viral infection have been suppressed and targeted by these alkaloids at non-toxic doses. Generally, a high degree of accuracy has been shown by the anti-viral effects of alkaloids, implying that they could act as efficient and safe antiviral medicines if further investigated in clinical and pharmacological investigations [90].

Madhukar Lohani, Mohammed Majrashi, Manoj Govindarajulu, Mansi Patel including 13 of them studied the immunomodulatory actions of noni and its clinical applications. Noni has been found to be rich in bioactive substances and has clearly shown pro-oxidant and immunomodulatory effects. The pharmacological basis related to the phytochemicals, polysaccharides in noni, and its potential therapeutic effects are emphasized in this review. Reports show that noni is beneficial for several diseases, with its crude extracts showing medicinal benefit for a broad range of pathological diseases. It is believed that pharmacological and toxicological studies, in addition to well-designed controlled clinical trials, can confirm noni as an efficient and novel natural product for prophylactic and therapeutic use of several diseases [91].

Brett J.West has investigated antioxidant activity of *Morinda Citrifolia* juice. Research has discovered that *Morinda Citrifolia* fruit juice gives some potential health benefits. To analyze TNJ's immediate antioxidant effects, under less extreme conditions, in vitro tests and a trial in healthy young adult men were done. Mean antioxidant activity in plasma and erythrocytes of healthy volunteers was increased by TNJ. No increases

were seen in the water group. The results of this analyze show that TNJ show antioxidant benefits after ingestion under everyday conditions [92].

Chapter 3

MATERIALS AND METHODS

The material and experimental methods are described in this chapter.

3.1 Materials

3.1.1 Powdered plant material

Morinda citrifolia (Noni) was chosen as the plant for alkaloid extraction. Drying and powdering were performed on the fruit of *Morinda citrifolia*. 50 grams of the powdered plant sample was utilized for further studies.

3.1.2 Chemicals

Ammonium hydroxide with a standard concentration value of 30% m/m, ethyl acetate, 2M sulfuric acid, diethyl ether, chloroform, anhydrous sodium sulphate, lead acetate and ferric chloride were obtained from Nice chemicals (P) Ltd.

3.2 Methods

3.2.1 Preparation of plant extract for detection of phytochemicals

The plant material was placed in a round bottom flask with an air condenser and 150 mL of ethyl acetate was added for extraction. The mixture was then stirred for 72 hours. The extract was filtered out and any remaining liquid was removed by pressing the solids. The resultant liquid

was purified by filtration or decantation for determination of the different phytochemicals.

3.2.2 Detection of alkaloids

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 coloured precipitate [93].

Mayer's Test

Mayer's test is a chemical test used to detect the presence of alkaloids in plant extracts. Added 1-2 mL of Mayer's reagent to the plant extract and mixed well. White precipitate indicated the presence of alkaloids [93].

Wagner's Test

Wagner's test is a chemical method used to detect the presence of alkaloids in plant extracts.1-2 mL of Wagner's reagent was added to the plant extract and mixed. Formation of brown or yellowish precipitate indicated the presence of alkaloids [93].

3.2.3 Detection of flavonoids

Lead acetate test

A few drops of lead acetate solution were added to 2 mL of the extract's aqueous solution. Formation of yellow color, showed the presence of flavonoids [93].

3.2.4 Detection of tannins

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 [93].

3.2.5 Detection of saponins

Aqueous solution of the extract was treated with distilled water; a honey comb froth is formed, demonstrating the presence of saponins [94].

3.2.6 Detection of carbohydrates

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 [93].

3.2.7 Detection of phenolic compound

A few drops of aqueous extract was mixed with a few drops of 5% ferric chloride solution resulting in the formation of a dark green color that indicated the presence of phenolic compounds [93].

3.3 Solvent extraction technique

3.3.1 Alkaloid extraction

Approximately 50 gm of powdered *Morinda citrifolia* fruit was added into a 500 mL conical flask. The powder was wetted with approximately 75 mL of NH_4OH. The mixture was dissolved in 150 mL of ethyl acetate and stirred in a magnetic stirrer for approximately 72 hours. After 72 hours, the solution was filtered using a filter paper into the beaker. The solution was evaporated and the residue was dissolved in 50 mL of distilled water and acidified to a pH of 2-3 using 2 M H_2SO_4. The solution was stirred for approximately 1 hour in a magnetic stirrer. Impurities were removed through extraction with diethyl ether in a separating funnel, followed by basification of the solution to a pH of 8-9 using NH_4OH. It was then extracted with chloroform and treated with anhydrous Na_2SO_4. It was then filtered using filter paper. Chloroform is distilled out and the residue was subjected to column chromatography. TLC analysis was conducted for the residue before subjecting to column chromatography. Solvent systems used for TLC are ethyl acetate, chloroform and 1:1 ethyl acetate – chloroform mixture. Two components were observed in TLC analysis with 1:1 ethyl acetate – chloroform mixture as solvent. Component 1 had an R_f value 0.3 and component 2 had a R_f value 0.5 in 1:1 solvent mixture. Using column chromatography, these two fractions are separated. The

major fraction was eluted with ethyl acetate and used for further characterization.

^{1}H NMR ($CDCl_3$): δ 0.066, δ 0.845, δ 0.901, δ 1.235, δ 1.326, δ 1.643, δ 2.003, δ 2.225, δ 2.294, δ 2.769, δ 3.583, δ 3.961, δ 4.185, δ 4.673, δ 5.325, δ 5.448, δ 5.747, δ 6.265, δ 6.832, δ 6.904, δ 7.249, δ 7.575, δ 7.687, δ 8.101 ppm.

^{13}C NMR ($CDCl_3$): δ 13.942, δ 22.476, δ 25.202, δ 27.061, δ 29.701, δ 31.287, δ 34.258, δ 36.011, δ 07.498, δ 128.111, δ 130.320, δ 143.678, δ 164.530, δ 176.202, δ 209.282 ppm.

IR: 1278.34 cm^{-1}, 1415.01 cm^{-1}, 1465.21 cm^{-1}, 1663.32 cm^{-1}, 2857.2 cm^{-1}, 2927.84 cm^{-1}, 2956.81 cm^{-1}, 3363.63 cm^{-1}.

2D NMR: (0.902-1.235), (1.235-2.022), (2.022-2.312), (2.752-5.322), (6.265-7.599).

DEPT: DEPT 90 - δ 13.525, δ 25.173, δ 31.560, δ 34.152, δ 77.476, δ 128.662. The positive phase peaks in DEPT 135 are δ 14.172, δ 21.110 and the negative phase peaks are δ 25.025, δ 29.797, δ 31.143, δ 34.37, δ 36.054.

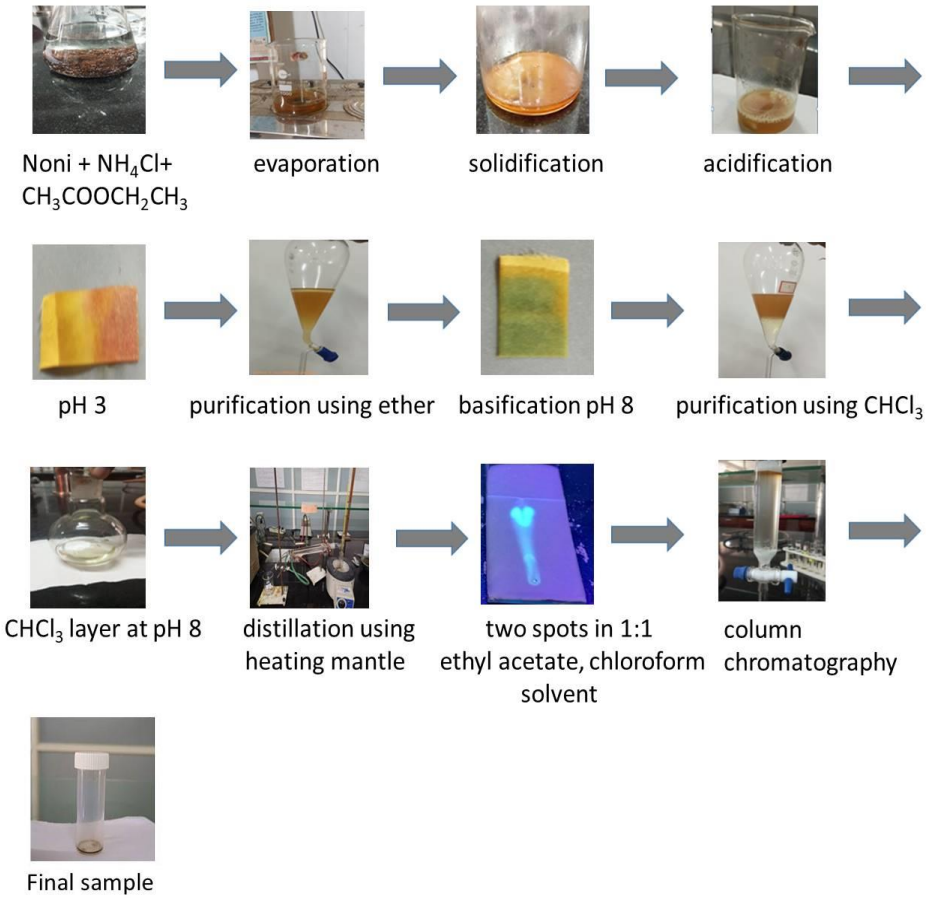


Figure 3.1: Different stages of alkaloid extraction

3.4 Characterization techniques

3.4.1 FT-IR Spectroscopy

The IR spectra were recorded on a Thermo Scientific Nicolet 912A0712 iS5 FT-IR Spectrometer. The spectra were taken in the wavelength ranging from 500-4000 cm^{-1} with a resolution of 4 cm^{-1} using KBr pellets.

3.4.2 ^{1}H NMR and ^{13}C NMR Spectroscopy

NMR spectra were recorded on JEOL (JEM –ECZ400S) NMR spectrometer at the Centralised Common Instrumentation Facility (CCIF), NMR lab, Thycaud, Thiruvananthapuram.

3.4.3 2D Correlation Spectroscopy

This technique involves a simple pulse sequence in which a (π/2) x pulse is first introduced into the ^{1}H channel to create an evolutionary stage. After some time, a second (π/2) y pulse is introduced to create the acquisition phase. The ^{1}H-^{1}H COSY pulse sequence includes different relaxation delay times (t_1) and acquisition times (t_2). The experiment is repeated with different values of t_1 and t_2. Therefore, the value of t_1 increases at regular intervals, producing a series of different FID data during t_2. COSY offers a 3-bond coupling (^{3}JH-H).This technique was done in JEOL (JEM –ECZ400S) NMR spectrometer at the Centralised Common Instrumentation Facility (CCIF), NMR lab, Thycaud, Thiruvananthapuram.

3.4.4 DEPT

It is Distortionless Enhancement by Polarization Transfer. It requires an FT-pulsed spectrometer. In the DEPT technique, the sample is irradiated with a complex sequence of pulses in both the ^{13}C and ^{1}H channels. The result of these pulse sequences is that the ^{13}C signals for the carbon atoms in the molecule will exhibit different phases, depending on the number of hydrogens attached to each carbon. Each type of carbon will behave slightly differently, depending on the duration of the complex pulses. These differences can be detected, and spectra produced in each experiment can be plotted. The phases of these carbon signals will also depend on the duration of the delays that are programmed into the pulse sequence. In one experiment, called a DEPT-45, only carbon atoms that bear any number of attached hydrogens will produce a peak. With a slightly different delay, a second experiment (called a DEPT-90) shows peaks only for those carbon atoms that are part of a methine (CH) group. With an even longer delay, a DEPT-135 spectrum is obtained. In a DEPT-135 spectrum, methine and methyl carbons give rise to positive peaks, whereas methylene carbons appear as inverse peaks. Quaternary carbons, which bear no hydrogen atom, appear in the ^{13}C NMR spectrum but are missing in the DEPT spectrum. This technique was done in JEOL (JEM – ECZ400S) NMR spectrometer at the Centralised Common Instrumentation Facility (CCIF), NMR lab, Thycaud, Thiruvananthapuram.

3.4.5 UV – Visible spectroscopy

UV- Visible spectroscopy is an analytical technique that measures the absorption of light in the UV 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 – 600nm.

3.4.6 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, 8890 GC) at Inter University Instrumentation Centre (IUIC), MGU, Kottayam.

3.5 (a) 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 each 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 hrs at a temperature of 37 ℃. 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 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 disc diffusion method. Here 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) was compared to available standard antibiotics. The incubation of the plate was carried out at 37 ℃ 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 compound will be.

3.5.5 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.

3.5 (b) 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) [95].

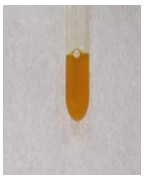
Chapter 4

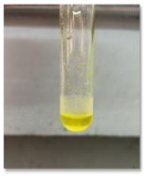
RESULTS AND DISCUSSION

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:

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	

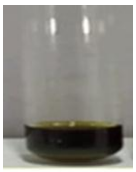
Molisch's test 2 mL of the extract was added to 2 drops of alcoholic alpha-naphthol and then 1ml of concentrated sulfuric acid was carefully added along the sides of the test tube.	Formation of a violet ring	Presence of carbohydrates	
A few mL of aqueous extract was mixed with a few drops of 5% ferric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

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

4.2 Spectroscopic analysis

Alkaloids extracted from Morinda Citrifolia were characterized by different spectroscopic techniques.

4.2.1 FT-IR Spectroscopy

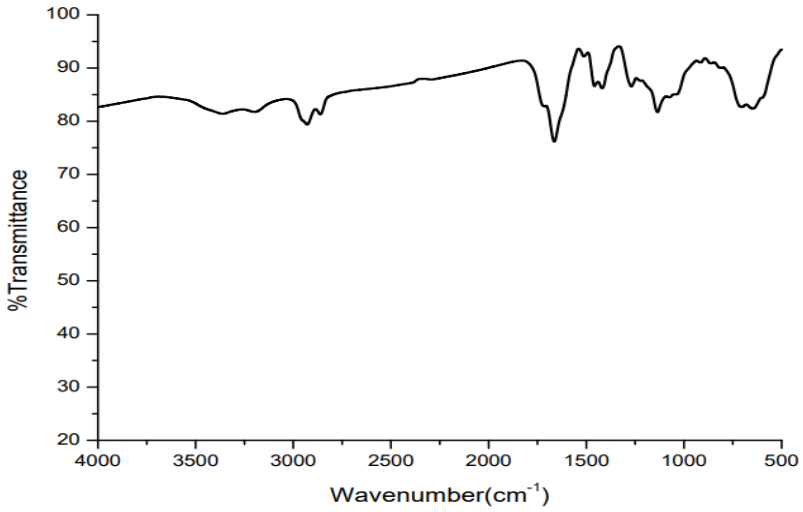


Figure 4.1: FT-IR spectrum of *Morinda citrifolia* alkaloid extract

A peak is found at 1278 cm^{-1} indicates C-O stretching frequency. C-N vibrations correspond to peak at 1415 cm^{-1}. Peak at 1465 cm^{-1} indicates the scissoring mode of a CH_2 group. A peak found at 1663 cm^{-1} indicated the presence of conjugated carbonyl stretching. Peaks from 2850 - 2960 cm^{-1} corresponds to symmetric and asymmetric stretching of CH groups in

conjugation. Weak bands at 3200 -3300 cm^{-1} may be due to =CH stretch in aromatic compound.

4.2.2 ^{1}H NMR Spectroscopy

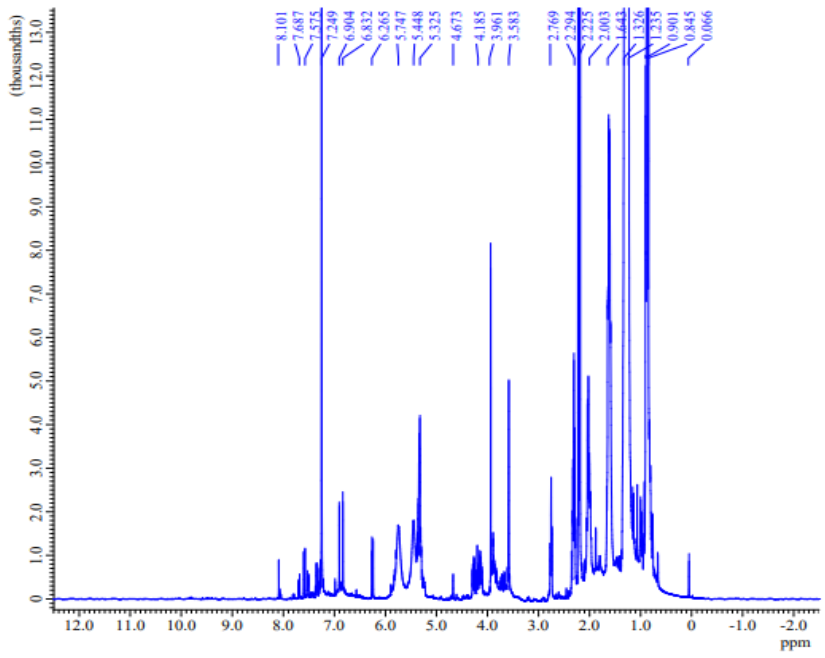


Figure 4.2: ^{1}H NMR spectrum of *Morinda citrifolia* alkaloid extract

Peaks found at 0.8-0.9 ppm indicate the presence of aliphatic methyl protons. R-CH_2 was also characterized due to a peak at 1.2-1.6 ppm. The peaks at 1.6- 2.6 ppm indicates C-H proton next to C=C and the peak at 2.2-2.7 ppm indicates benzyl or amide or carbonyl protons. Peaks from 3.5-3.9 indicate the presence of proton attached to an alcoholic group.

Vinylic cis protons are indicated by peaks at 4.1 – 5.7 ppm. The presence of residual proton (solvent) in $CDCl_3$ is shown by a peak of chemical shift value 7.249 ppm. Aromatic C-H protons are indicated by the presence of peaks ranging from 6.8-8.1 ppm. This second order spectra indicates the presence of chiral centres. In order to resolve this COSY and DEPT were analysed.

4.2.3 ^{13}C NMR Spectroscopy

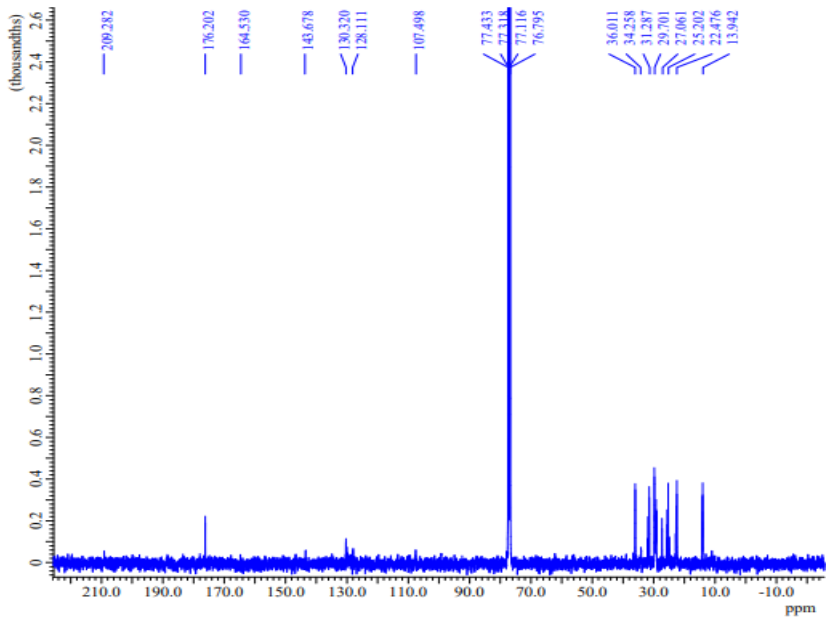


Figure 4.3: ^{13}C NMR spectrum of *Morinda citrifolia* alkaloid extract

The peaks found between 13-38 ppm indicate the presence of aliphatic carbon. A peak at 107.4 ppm indicate the presence of C=C. The peaks ranging from 128-176 ppm indicates aromatic carbon atoms and a peak at 209.2 ppm is due to carbonyl carbon.

4.2.4 DEPT analysis

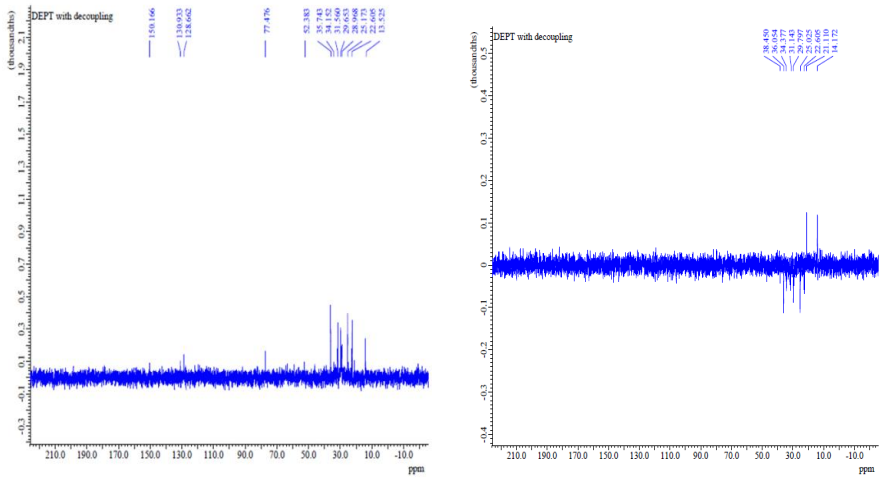


Figure 4.4: DEPT 90 and DEPT 135 of *Morinda citrifolia* alkaloid extract

The peaks obtained for DEPT 90 are δ 13.525, δ 25.173, δ 31.560, δ 34.152, δ 77.476, and δ 128.662. This indicated the presence of C-H groups. The positive phase peaks in DEPT 135 are δ 14.172, δ 21.110 and the negative phase peaks are δ 25.025, δ 29.797, δ 31.143, δ 34.37 and

δ 36.054. Positive phase indicates C-H and CH_3 peaks and negative peaks indicate CH_2 peaks.

4.2.5 COSY analysis

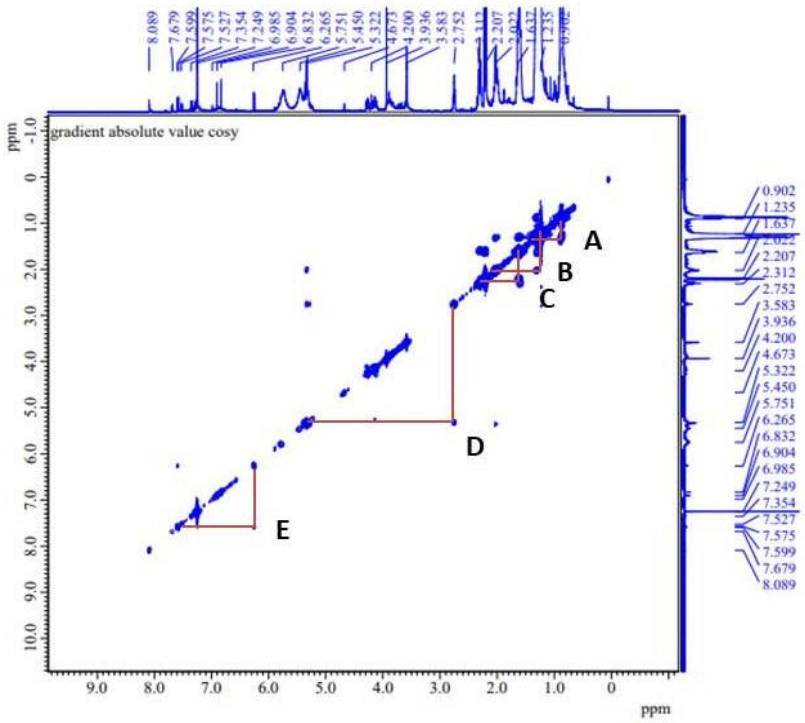


Figure 4.5: COSY spectrum of *Morinda citrifolia* alkaloid extract

Here signal A is due to the correlation between protons at 0.902 and 1.235 ppm. Signal B is due to the correlation between protons at 1.235 and 2.022 ppm. Signal at C is due to the correlation between protons at 1.637 and

2.312 ppm. Signal at D is due to the correlation between protons at 2.752 and 5.322 ppm. Signal E is due to the correlation between protons at 6.265 and 7.599 ppm.

4.2.6 UV-Visible spectroscopy

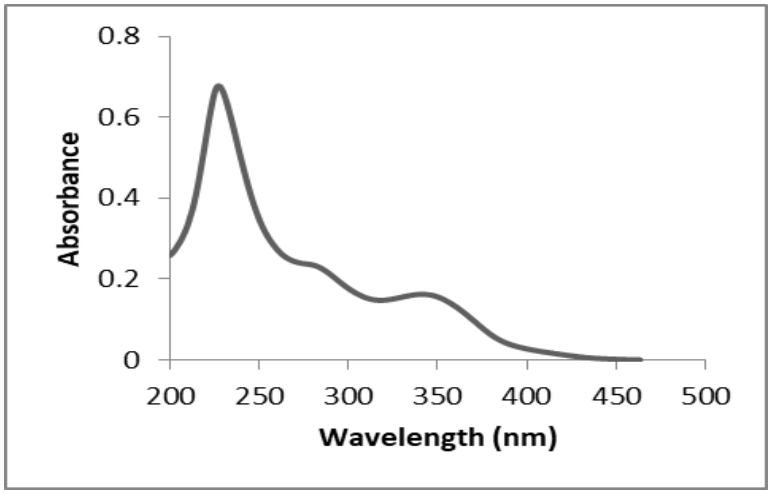


Figure 4.6: UV-Visible spectrum of *Morinda citrifolia* alkaloid extract

The UV-Visible spectrum of the sample was recorded in ethanol over a wavelength range of 200-600 nm. The spectrum showed three absorption bands in the wavelength range 200-400 nm and a tail extending to 400 nm. Spectrum indicates that the sample may contain coumarin derivatives.

4.2.7 GC-MS analysis

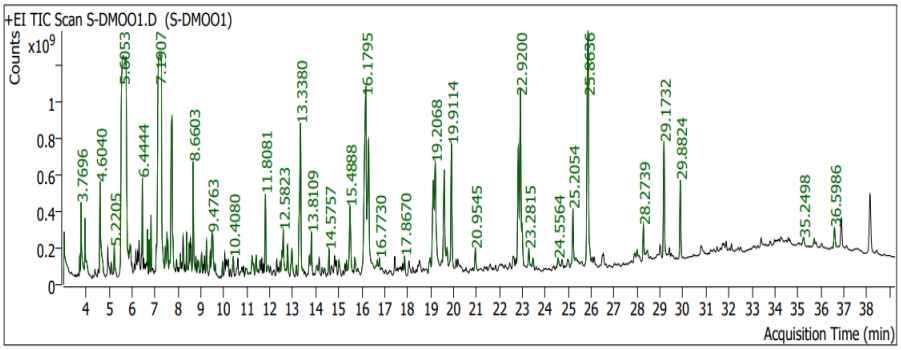


Figure 4.7: GC-MS spectrum of *Morinda citrifolia* alkaloid extract

RT	Compound Name	Component Area	Area %	CAS#	Formula	Library Molecular Weight	Match Factor
3.0371	Acetic acid, butyl ester	1466905713.4	1.28	123-86-4	C6H12O2	116.084	87.1
3.7186	Ethylbenzene	150119094.5	0.13	100-41-4	C8H10	106.078	92.7
3.7696	o-Xylene	1168950292.9	1.02	95-47-6	C8H10	106.078	98.7
3.9458	p-Xylene	988322554.8	0.86	106-42-3	C8H10	106.078	98.2
4.6040	2-Methylbutyramide	1967297388.6	1.71	1113-57-1	C5H11NO	101.084	93.5
5.0861	Butyric acid, 2-phenyl-, dodec-2-en-1-yl ester	234677697.6	0.20	1000406-86-7	C22H34O2	330.256	71.9
5.2205	Benzene, nitro-	486547372.9	0.42	98-95-3	C6H5NO2	123.032	86.0
5.6053	Hexanamide	16815203686.7	14.64	628-02-4	C6H13NO	115.1	95.9
5.9113	Naphthalene	290579671.9	0.25	91-20-3	C10H8	128.063	78.4
6.4444	Dodecane, 2,7,10-trimethyl-	716984016.1	0.62	74645-98-0	C15H32	212.25	94.2
6.6716	3-Eicosene, (E)-	321868619.1	0.28	74685-33-9	C20H40	280.313	89.4
6.7457	11-Methyldodecanol	248174159.9	0.22	85763-57-1	C13H28O	200.214	89.0
6.8245	1-Octanol, 2-butyl-	475547899.1	0.41	3913-02-8	C12H26O	186.198	92.6
7.1907	Octanamide	13590538088.7	11.84	629-01-6	C8H17NO	143.131	94.2
7.4457	1-Tetradecene	382003467.6	0.33	1120-36-1	C14H28	196.219	91.2
7.5199	Tetradecane	393091901.2	0.34	629-59-4	C14H30	198.235	93.3
7.5570	Biphenyl	339855705.1	0.30	92-52-4	C12H10	154.078	96.7
7.7377	Benzeneacetamide	4122403282.8	3.59	103-81-1	C8H9NO	135.068	94.2
8.1040	Pentadecane, 2,6,10-trimethyl-	228715359.5	0.20	3892-00-0	C18H38	254.297	91.9
8.3729	1-Decanol, 2-methyl-	416818073.1	0.36	18675-24-6	C11H24O	172.183	92.1
8.4888	Hexanoic acid, pentyl ester	328770999.6	0.29	540-07-8	C11H22O2	186.162	69.0
8.6603	Dodecane, 4,6-dimethyl-	1472646959.3	1.28	61141-72-8	C14H30	198.235	93.2
8.8550	Eicosane, 10-methyl-	133959215.6	0.12	54833-23-7	C21H44	296.344	74.7
8.9060	2,4-Di-tert-butylphenol	102691372.9	0.09	96-76-4	C14H22O	206.167	91.9
9.0266	Oxalic acid, hexadecyl 2-phenylethyl ester	278923613.6	0.24	1010309-67-0	C26H42O4	418.308	71.5

9.2398	Dodecane, 2,6,11-trimethyl-	411556990.6	0.36	31295-56-4	C15H32	212.25	92.6
9.4068	5-Tetradecene, (Z)-	256425503.6	0.22	41446-62-2	C14H28	196.219	85.3
9.4763	Dodecanoic acid	1034008521.7	0.90	143-07-7	C12H24O2	200.178	93.8
9.6246	Pentadecane, 3-methyl-	95546055.3	0.08	2882-96-4	C16H34	226.266	86.6
9.9352	Cetene	171792587.3	0.15	629-73-2	C16H32	224.25	93.9
10.1902	Cyclotetradecane	198436918.4	0.17	295-17-0	C14H28	196.219	87.2
10.4080	1-Tetradecanol	327414328.1	0.29	112-72-1	C14H30O	214.23	83.6
10.9505	Tetradecane, 2,6,10-trimethyl-	165826118.8	0.14	14905-56-7	C17H36	240.282	82.5
11.2286	Carbonic acid, eicosyl vinyl ester	474847685.4	0.41	1000382-54-3	C23H44O3	368.329	89.4
11.4048	Hexadecane	390993264.8	0.34	544-76-3	C16H34	226.266	92.8
11.8081	Heptacosane	1197825323.3	1.04	593-49-7	C27H56	380.438	92.3
12.4479	Octyl tetradecyl ether	166450893.1	0.14	1000406-38-5	C22H46O	326.355	90.7
12.5823	Tetradecanoic acid	1059024551.4	0.92	544-63-8	C14H28O2	228.209	95.7
12.7770	1-Decanol, 2-hexyl-	471128308.0	0.41	2425-77-6	C16H34O	242.261	91.8
12.9624	1,3-Benzodioxole-5-ethanamine, 7-methoxy-	483732487.7	0.42	23693-38-1	C10H13NO3	195.09	75.4
13.1526	E-15-Heptadecenal	107164230.4	0.09	1000130-97-9	C17H32O	252.245	92.9
13.3380	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	3604727973.3	3.14	7070-24-8	C13H18O3	222.126	82.4
13.7227	Isopropyl myristate	263010916.2	0.23	110-27-0	C17H34O2	270.256	89.3
13.8109	1,2-Dichlorododecane	534398165.9	0.47	75121-23-2	C12H24Cl2	238.126	93.4
14.0519	Nerolidyl propionate	96821677.6	0.08	1000132-16-4	C18H30O2	278.225	66.4
14.2790	Pentadecanoic acid	90245055.8	0.08	1002-84-2	C15H30O2	242.225	82.4
14.5757	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	270077022.9	0.24	84-69-5	C16H22O4	278.152	96.1
15.0114	Heptadecane, 2,6,10,15-tetramethyl-	192618158.9	0.17	54833-48-6	C21H44	296.344	91.6
15.3451	Heneicosane	138158384.2	0.12	629-94-7	C21H44	296.344	92.1
15.4888	Disulfide, di-tert-dodecyl	1455865369.2	1.27	27458-90-8	C24H50S2	402.335	83.5
15.7067	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	245928738.7	0.21	5654-86-4	C11H18N2O2	210.137	80.1
16.1795	n-Hexadecanoic acid	5696602268.0	4.96	57-10-3	C16H32O2	256.24	97.6
16.3047	Scopoletin	3908438081.8	3.40	92-61-5	C10H8O4	192.042	97.3
16.6803	Hexadecanoic acid, ethyl ester	305479993.1	0.27	628-97-7	C18H36O2	284.272	82.8
16.7730	Eicosane	196230950.0	0.17	112-95-8	C20H42	282.329	90.8
17.8670	Scopoletin, O-acetyl-	219500588.1	0.19	56795-51-8	C12H10O5	234.053	73.6
18.9795	3-Methyloctadecanoic acid	319077055.5	0.28	52304-07-1	C19H38O2	298.287	77.2
19.1232	9,12-Octadecadienoic acid (Z,Z)-	2256667968.8	1.97	60-33-3	C18H32O2	280.24	95.3
19.2068	cis-13-Octadecenoic acid	3089066255.0	2.69	13126-39-1	C18H34O2	282.256	95.8
19.5961	Octadecanoic acid	2312584092.0	2.01	57-11-4	C18H36O2	284.272	95.0

19.7213	Eicosane, 1-iodo-	508997164.0	0.44	1000406-31-8	C20H41I	408.225	82.8
19.9114	Hexadecanamide	2426446012.7	2.11	629-54-9	C16H33NO	255.256	93.8
20.9545	tert-Hexadecanethiol	424692260.3	0.37	25360-09-2	C16H34S	258.238	88.3
22.8273	Linoleamide	2395444827.4	2.09	3999-01-7	C18H33NO	279.256	97.6
22.9200	9-Octadecenamide, (Z)-	4464974545.8	3.89	301-02-0	C18H35NO	281.272	95.9
23.2815	Octadecanamide	459501182.9	0.40	124-26-5	C18H37NO	283.288	88.9
23.4649	Hexanedioic acid, dioctyl ester	191385441.0	0.17	123-79-5	C22H42O4	370.308	79.1
24.5564	cis-1-Chloro-9-octadecene	150533236.0	0.13	16507-61-2	C18H35Cl	286.243	83.1
24.9736	17-Pentatriacontene	166256887.7	0.14	6971-40-0	C35H70	490.548	79.0
25.2054	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1143952595.2	1.00	23470-00-0	C19H38O4	330.277	91.6
25.8636	Bis(2-ethylhexyl) phthalate	6454002284.8	5.62	117-81-7	C24H38O4	390.277	95.7
28.0004	Tetracosane	451384069.1	0.39	646-31-1	C24H50	338.391	80.6
28.2739	Octadecanoic acid, 2,3-dihydroxypropyl ester	764042427.5	0.67	123-94-4	C21H42O4	358.308	89.7
29.1732	13-Docosenamide, (Z)-	2116100399.0	1.84	112-84-5	C22H43NO	337.334	89.1
29.8824	Squalene	1171027200.2	1.02	111-02-4	C30H50	410.391	96.2
35.2498	Campesterol	210869350.6	0.18	474-62-4	C28H48O	400.371	81.5
35.7179	Stigmasterol	243450010.2	0.21	83-48-7	C29H48O	412.371	86.1
36.5986	.gamma.-Sitosterol	460704377.8	0.40	83-47-6	C29H50O	414.386	87.7

Table 4.2: GC-MS analysis

From GC-MS analysis the compounds that have match factor above 95% are o-xylene (98.7%), p-xylene (98.2%), hexanamide (95.9%), biphenyl (96.7%), tetradecanoic acid (95.7%), 1,2- benzenedicarboxylic acid, bis(2-methylpoyl)ester (96.1%), n-hexadecanoic acid (97.6%), scopoletin (97.3%), (z,z)-9,10-octadecadienoic acid (95.3%), cis-13-octadecanoic acid (95.8%), octadecanoic acid (95.0%), linoleamide (97.6%), (z)-9-octadecenamide (95.9%), bis(2-ethylhexyl)phthalate (95.7%) and squalene (96.2%).

GC-MS analysis indicated the presence of amide alkaloids, amine alkaloids and pyrrolo pyrazine alkaloids. Considering match factor and mass spectral analysis and comparison with NIST library amide alkaloids such as linoleamide, (z) - 9-octadecenamide, 13-docosenamide (z) is present.

For pyrrolo [1, 2-a] pyrazine-1, 4-dione, hexahydro-3-(2-methylpropy) though the peak intensities is less, the mass spectral analysis shows its

peak. Besides alkaloids, scopoletin, squalene, steroids such as sitosterol is also present.

The mass spectrums of compounds discussed are given below:

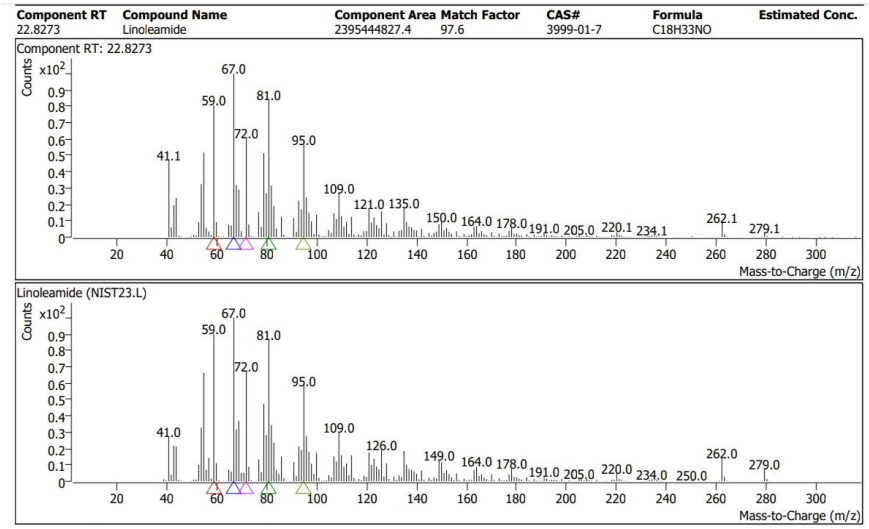


Figure 4.8: Mass spectrum of linoleamide

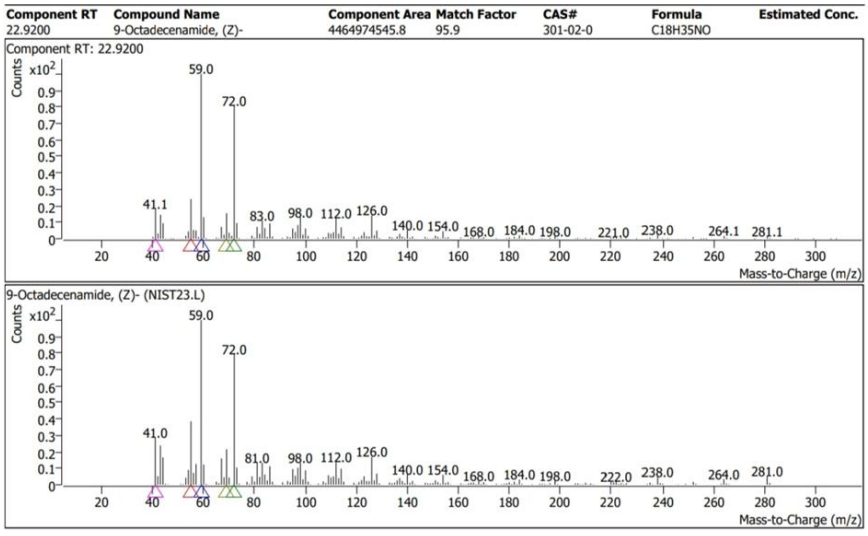


Figure 4.9: Mass spectrum of (z)-9-octadecenamide

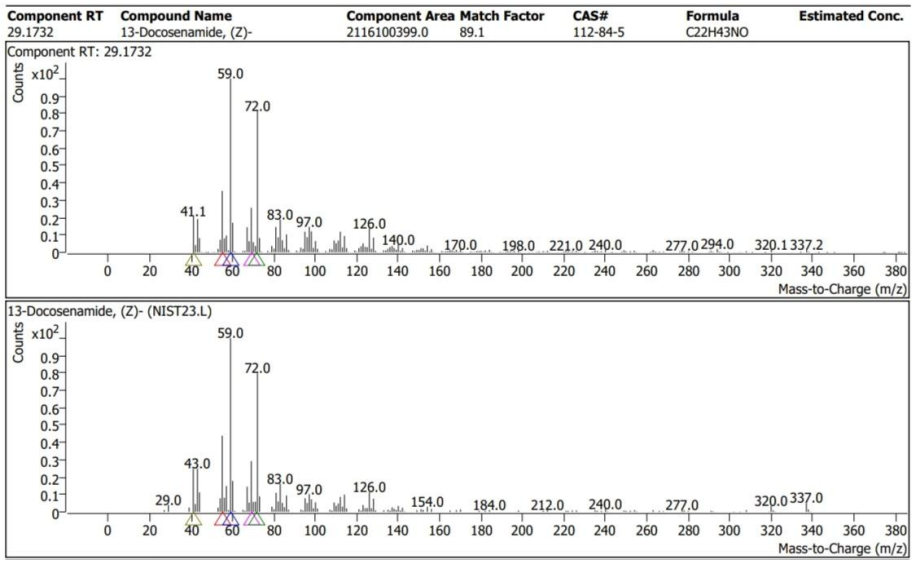


Figure 4.10: Mass spectrum of (z)-13-docosenamide

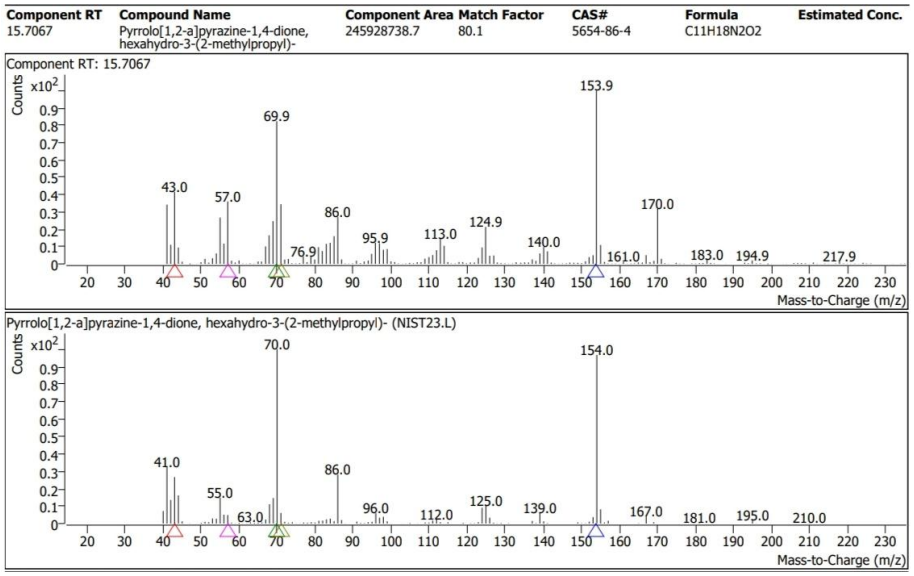


Figure 4.11: Mass spectrum of pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-

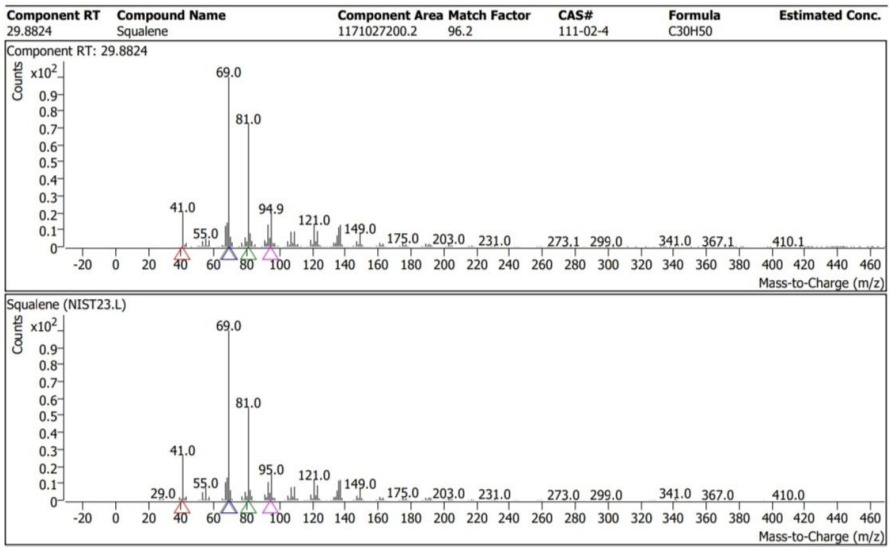


Figure 4.12: Mass spectrum of squalene

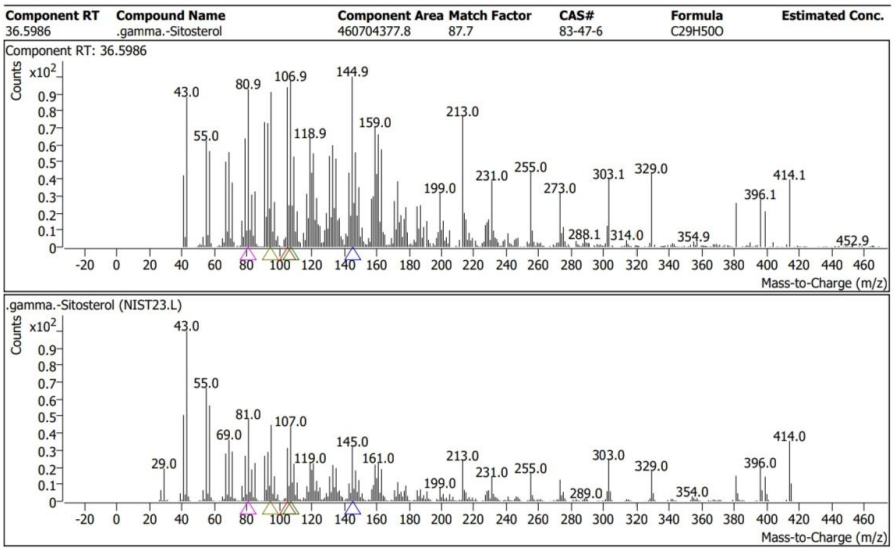


Figure 4.13: Mass spectrum of gamma-sitosterol

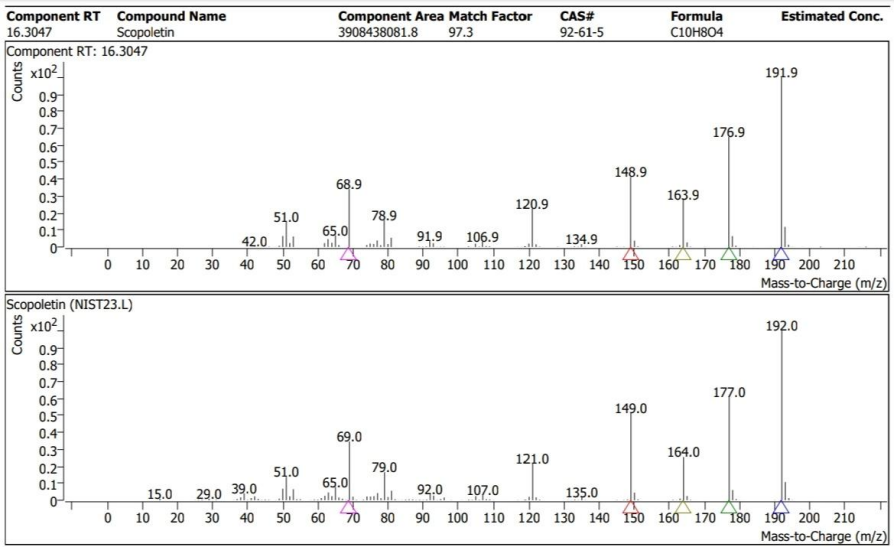


Figure 4.14: Mass spectrum of scopoletin

4.3 Biological studies

4.3.1 Antibacterial assay

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

Sample	*E-coli*	*S-aureus*
	24 hrs	24 hrs
DMSO + noni alkaloid	Nil	Nil

Table 4.3: Study of antibacterial properties of *Morinda citrifolia* alkaloid extract

4.3.2 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 *Escherichia coli* and *Staphylococcus aureus* were observed after incubation of 24 hours and 48 hours. The sample showed maximum activity against *Staphylococcus aureus.*

The extracted alkaloids showed no activity in the antibacterial assay. However, the noni powder extract showed significant antibacterial

activity. This may be due to synergic effect of different phytochemicals in the noni powder extract.

Sample	*E-coli*		*S-aureus*	
	24 hrs	48 hrs	24 hrs	48 hrs
Methanol + noni powder	0.8 cm	1 cm	1.1 cm	1.6 cm
Ethyl acetate + noni powder	0.7 cm	0.9 cm	1.4 cm	1.6 cm
Water + noni powder	Nil	Nil	Nil	Nil

Table 4.4: Antibacterial activity of *Morinda citrifolia* in different solvents after 24 and 48 hours

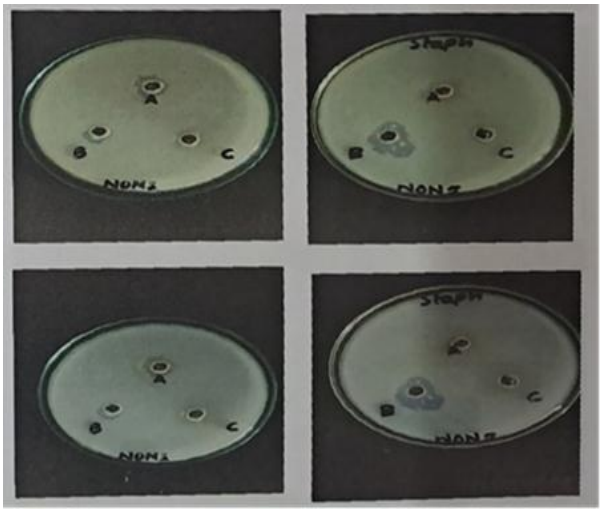


Figure 4.15: Antibacterial activity of *Morinda citrifolia* in different solvents after 24 hrs and 48 hrs of incubation period

4.3.3 Antioxidant activity

Percentage of DPPH scavenging activity =

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

SAMPLE 1 (alkaloid separated by column chromatography)

Absorbance of control (DPPH) = 2.5187

Absorbance of sample 1a = 0.9613

Absorbance of sample 1 b = 0.9740

Average absorbance = (0.9613 + 09740) /2 = 1.9353/2

= 0.9677

$$\text{Percentage of DPPH scavenging activity} = \frac{2.5187 - 0.9677}{2.5187} \times 100$$

$$= \frac{1.551}{2.5187} \times 100$$

$$= 0.6158 \times 100$$

Percentage of DPPH scavenging activity = 61.58 %

SAMPLE 2 (alkaloid before column chromatography)

Absorbance of control (DPPH) = 2.5187

Absorbance of sample 1a = 0.7900

Absorbance of sample 1 b = 0.8015

Average absorbance = (0.7900 + 0.8015) /2 = 1.5915/2

= 0.7958

$$\text{Percentage of DPPH scavenging activity} = \frac{2.5187 - 0.7958}{2.5187} \times 100$$

$$= \frac{1.7229}{2.5187} \times 100$$

$$= 0.6840 \times 100$$

Percentage of DPPH scavenging activity = 68.40 %

For studying antioxidant activity, alkaloid after (sample 1) and before (sample 2) subjecting to column chromatography was chosen. Ethanol was used as blank and DPPH was used as control. Absorbance was measured at 517nm. Alkaloid obtained after subjecting to column chromatography showed 61.58% of DPPH scavenging activity and alkaloid before subjecting to column chromatography showed 68.40%.

Chapter 5

CONCLUSIONS

This study was about extraction of alkaloid from *Morinda citrifolia* fruit using solvent extraction method and characterization of alkaloids using different spectroscopic studies. The screening of phytochemicals confirmed the presence of alkaloids, flavonoids, carbohydrates, tannins, saponins and phenolic compounds in *Morinda citrifolia*. Alkaloids were extracted from *Morinda citrifolia* fruit using solvent extraction method and column chromatography. The alkaloids separated after column chromatography were characterized using IR, ^{1}H NMR ^{13}C NMR, DEPT, COSY, UV-Visible spectroscopy and GC-MS. IR spectroscopy contains the peaks corresponding to C-O stretching frequency, C-N vibrations, CH_2 scissoring mode vibration, aromatic stretching, carbonyl stretching and stretching corresponding to symmetric and asymmetric modes of CH_2 in conjugation.^{1}HNMR and ^{13}C NMR indicated the peaks corresponding to aliphatic groups, alkene, aromatic groups and carbonyl groups. ^{1}H NMR obtained was a second order spectrum. It indicated the presence of chiral centres. In order to resolve this other spectroscopic analysis were carried out. In DEPT positive phase indicates C-H and CH_3 peaks and negative peaks indicate CH_2 peaks. In order to understand the correlation between different protons obtained from ^{1}H NMR, COSY was analyzed. In COSY correlated protons are (0.902 - 1.235), (1.23- 2.022), (1.637-2.312), (2.752-5.322) and (6.265 -7.599). UV-Visible spectrum showed three absorption bands in the wavelength range 200-400 nm and a tail extending

to 400 nm. Spectrum indicates that the sample may contain coumarin derivative which is confirmed by GC-MS analysis. GC-MS analysis showed that the sample contains chiefly amide alkaloids and pyrrolo [1, 2-a] pyrazine-1, 4-dione. Further spectroscopic studies are required to arrive at the structure of the alkaloid.

Antibacterial studies indicated that the alkaloid extract didn't show any antibacterial activity against *E.coli* and *S.aureus*. However the fruit powder extract showed maximum activity against *S.aureus*. Antioxidant studies using DPPH showed 68.40% DPPH scavenging activity.

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