IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Phytochemistry, Phytochemical Screening Of Medicinal Plants And Exraction Methods Of Phytochemical

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Abstract:

Phytochemistry plays a pivotal role in understanding the chemical constituents of medicinal plants and their biological activities. Phytochemical screening provides insights into bioactive compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides. These compounds contribute significantly to therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. This review highlights the importance of phytochemistry, methods of phytochemical screening, and the relevance of medicinal plants in drug discovery and development.

Keyword: Phytochemistry, Phytochemical Screening Phytochemicals Medicine, Extraction Methods.

1.Introduction

It will be recalled that in the food chain, plants are referred to as the producers because they had the ability to trap energy from sunlight, harness and assemble some basic units which they transform through some chemical process into complex high energy-yielding compounds that are readily available to organisms. Their generosity became overwhelmingly and practically complex to comprehend at a glance. A field has to emerge – "phytochemistry."[1]

Medicinal plants have been integral to traditional medicine systems for centuries. The therapeutic properties of these plants stem from their diverse phytochemicals[2]. The study of phytochemicals has been instrumental in the discovery of new plant natural products which are of commercial values in various industries such as the traditional and complementary medicine systems, pharmaceutical industries, nutraceuticals, and dietary supplement industries[3]. Phytochemicals (from the Greek word phyto,meaning plant) are biologically active,

naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients[4] .Phytochemicals have been in existence since time immemorial and are known to be responsible for the organoleptic properties (color, taste, flavor, aroma, and odor) of plants, such as the smell of garlic, ginger, and the deep purple color of blueberries[1]

The growing interest in natural remedies has propelled research into their chemical profiles and pharmacological potential. This review focuses on the role of phytochemistry in exploring medicinal plants and the methods employed in phytochemical screening[5]

Phytochemistry: Definition and Importance

Phytochemistry is the branch of science that focuses on the chemical composition of plants. It deals with the study of plant-derived compounds, particularly secondary metabolites, which have ecological and medicinal significance. These compounds are responsible for plants' therapeutic, aromatic, and protective properties[6].

Phytochemistry is important because phytochemicals are bioactive substances found in plants that have many health benefits for humans[7]

Phytochemicals protect cells from damage caused by free radicals, which are molecules that steal electrons from other compounds to stabilize themselves. This can help reduce the risk of many diseases, including cardiovascular disease, diabetes, and inflammatory diseases, Strengthening the immune system,Reducing inflammation,,Preventing DNA damage and helping DNA repair,Slowing cancer cell growth, Regulating hormones,Preventing damaged cells from reproducing[8]

Phytochemistry involves the extraction, isolation, characterization, and quantification of bioactive compounds in plants.

2 Classification of Phytochemicals is described as under;

Phytochemicals are broadly categorized into primary and secondary metabolites:

2.1.Primary Metabolites: Prinary constituents are essential for plant growth and development.
Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc.[9]

2.2 Secondary Metabolites: Responsible for ecological interactions and therapeutic properties.

Secondary constituentsare the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides.[10]

2.2.1 Phenolics

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. They are plant secondary metabolites, and they have an important role as defence compounds. phenolics exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes.
Phenolic acid compounds have been studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation and cancer. Indeed, tumour cells, including leukaemia cells, typically have higher levels of reactive oxygen species (ROS) than normal cells so that they are particularly sensitive to oxidative stress[11].

2.2.2 Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognised, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks[12]. The flavonoids appear to have played a major role in successful medical treatments of ancient times, and their use has persisted up to now. More than 4000 flavonoids have been described so far within the parts of plants normally consumed by humans and approximately 650 flavones and 1030 flavanols are known[13]. Flavonoids have gained recent attention because of their broad biological and pharmacological activities in these order Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, antiinflammatoryas well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. On the other hand flavonoids such as luteolin and cathechins, are better antioxidants than the nutrients antioxidants such as vitamin C, vitamin E and β-carotene. Flavonoids have been stated to possess many useful properties, containing anti-inflammatoryactivity, enzyme inhibition, antimicrobial activity, oestrogenic activity, anti-allergic activity, antioxidant activity, vascular activity and cytotoxic antitumor activity[14]. Flavonoids constitute a wide range of substances that play important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA[15,84] .

2.2.3 Tannins

From a chemical point of view it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers[16,17]. It might be said that the tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins

(mainly), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals, etc[18,19,20]. On the basis of their structural characteristics it is therefore possible to divide the tannins into four major groups: *Gallotannins, ellagitannins, complex tannins, and condensed tannins*[21,22,23]

Tannins are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink). In the food industry tannins are used to clarify wine, beer, and fruit juices. Other industrial uses of tannins include textile dyes, as antioxidants in the fruit juice, beer, and wine industries, and as coagulants in rubber Production[24].

In medicine, especially in Asian (Japanese and Chinese) natural healing, the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours[25], and as antiinflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals[26,85,86].

2.2.4. Alkaloids

Alkaloids are natural product that contains heterocyclic nitrogen atoms, are basic in character. The name of alkaloids derives from the “alkaline” and it was used to describe any nitrogen-containing base[27]. Alkaloids are naturally synthesis by a large numbers of organisms, including animals, plants, bacteria and fungi. Some of the fires natural products to be isolated from medicinal plants were alkaloids when they first obtained from the plants materials in the early years of 19th century, it was found that they were nitrogen containing bases which formed salts with acid. Hence they were known as the vegetable alkalis or alkaloids and these alkaloids are used as the local anesthetic and stimulant as cocaine[28]. Almost all the alkaloids have a bitter taste. The alkaloid quinine for example is one of the bitterest tasting substances known and is significantly bitter at a molar concentration[29].

Alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrens) and also against other plants by means of allelopathically active chemicals[30]. The useof alkaloids containing plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization. Alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine), andanticancer actions (dimeric indoles, vincristine, vinblastine). Some alkaloids have stimulant property as caffeine and nicotine, morphine are used as the analgesic and quinine as the antimalarial drug[26].

2.2.5 Terpenoids

The terpenoids are a class of natural products which have been derived from five-carbon isoprene units. Most of the terpenoids have multi cyclic structures that differ from on by their functional groups and basic carbon skeletons. These types of natural lipids can be found in every class of living things, and therefore considered as the largest group of natural products[31]. Many of the terpenoids are commercially interesting because of their use as flavours and fragrances in foods and cosmetics examples menthol and sclareol or because they are important for the quality of agricultural products, such as the flavour of fruits and the fragrance of flowers like linalool[32]. Terpene hydrocarbons therefore have molecular formula (C5H8) n and they are classified according to the number of isoprene units sush as **Hemiterpenoids, Monoterpenoids**, **Sesquiterpenes Diterpenes ,Triterpenes** ,**Tetraterpenoids.** Terpenoids can have medicinal properties such as anticarcinogenic (e.g. perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol.[33,34].

2.2.6 Saponins

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom They form a stable foam in aqueous solutions such as soap, hence the name “saponin”. Chemically, saponins asa group include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids. Many saponins are known to be antimicrobial, to inhibit mould, and to protect plants from insect attack. Saponins may be considered a part of plants’defence systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants[35]. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral[36,37].

3. COMMONLY USED EXTRACTION METHODS FOR PHYTOCHEMICAL

Extraction is the separation of medicinally active portions of plant using selective and standard procedures. It is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization [38].

There are several extractions methods for the separation of natural product from plants present. These methods can be called conventional (long been used) and modern (developed more recently).Conventional techniques are the one using organic solvents or water and are carried out generally at atmospheric pressure while modern techniques using pressure and / or elevated temperatures [39].

The extraction of natural products progresses through the following stages: [40] the solvent penetrates into the solid matrix; [41] the solute dissolves in the solvents; the solute is diffused out of the solid matrix; the extracted solutes are collected [42]. For the extraction procedures, solvents such as water, ethanol, chloroform, dichloromethane, hexane, ethyl acetate, methanol etc. are most commonly used. The conventional extraction methods generally use organic solvents and require a large volume of solvents and long extraction time [43,44]. Several of the commonly used extraction methods (conventional and modern) from medicinal plants are discussed below:

I Maceration

In this routinely used extraction method, an airtight vessel (preferably amber in color) is used. Then the vessel is packed with plant material such as barks, flowers, leaves, roots, seeds, and tubers which are coarsely powdered[45]. Menstruum, which is the solvent used in extraction, is subsequently poured over the plant material until being fully covered. Then the vessel is tightly sealed and stored for 3-7 days with continuous agitation regularly. The days kept for maceration depend on the type of plant material being used and the method of evaporation chosen. After the extraction process, the mixture is filtered using filter paper or a muslin cloth before evaporation. Then, the micelle can be evaporated with the help of an oven or a water bath to isolate it from the extraction solvent. This method is considered very suitable for heat-stable plant materials. Based on the requirement, the sample-to-solvent ratio can vary from 1:4, 1:5, and 1:10 [46,47].

II Infusion

This process is more similar to maceration. Here, finely powdered plant material is used for the extraction[48]. Hot or cold menstruum is then added over the finely powdered plant material and the mixture is stored for a short time. Infusion is considered more appropriate for the extraction of readily soluble biologically active compounds[49] Also, it is found to be a very suitable technique to obtain fresh extracts before use. Here, the sample-to-solvent ratio is generally 1:4 or 1:16 which depends on the requirement [50].

III Digestion

Digestion is a type of maceration where low heat is applied during the extraction procedure. This slightly warm temperature does not change the plant material's active elements. This results in more effective usage of the menstruum. In this method, we introduced the plant parts to be extracted into a container where the pre-heated menstruum is present. The sample-solvent mixture is kept for 30 minutes to 24 hours with intermittent shaking. A temperature between 35-40 is used commonly. Sometimes, this can go to a maximum of 50 . This extraction method is very useful to extract poorly soluble components present in tougher parts of plants [45].

IV Decoction

This is a continuous hot extraction technique where the powdered crude plant material is boiled with a specific amount of water for a period of specific time (about 15 minutes). Heat is applied throughout the procedure to speed up the extraction. This method is ideal when extracting water-soluble and thermally stable compounds from plant materials. In this process, the most commonly used sample-to-solvent ratios are 1:4 or 1:16 [51,52].

V Percolation

A percolator is a narrow cone-shaped container made of glass that has openings on both sides[53] The finely powdered plant material will be added to a clean vessel followed by adding a higher quantity of menstruum to soak the powder. This mixture is then stored for a certain time (4 hours). This mixture is then introduced to the percolator (during this procedure, the lower end has to be closed). Now, the system will be kept standing for 24 hours. After 24 hours, the solvent used for extraction is added from the upper end to flow down until the plant material is saturated with the solvent. The stop cork is opened at the lower end while adding menstruum from the top. The liquid is then collected from the bottom. This procedure is carried out with the help of gravitational force which helps to push down the solvent through the plant material. The pouring of the menstruum should end when the amount of menstruum reaches around 75% of the total preparation. Finally, the collected micelle will be filtered and concentrated [54]

VI Soxhlet Extraction

The Soxhlet extraction method is a liquid-solid extraction method. It is a very effective extraction technique in instances where the substances to be extracted have a restricted solubility in the menstruum, while having insoluble impurities[55] Continuous heat is applied in this procedure. Here, a glass instrument known as the Soxhlet extractor is utilized. This instrument comes with a solvent flask, a siphon tube, a condenser tube, and many other parts. The plant material which needs to be extracted is introduced to a part known as the thimble, which is a porous bag. First, the solvent flask should be filled with the menstruum[56]Then heat is applied from the bottom of the solvent flask. This results in the production of solvent vapor. This vapor will go up the distillation tube, into the main chamber, and up into the condenser where it will condense and drip down. As the solvent fills the main chamber, some of the desired compounds in the solid sample will be dissolved. Eventually, the chamber will be almost full of the solvent. When this happens, the siphon tube will empty the main chamber while transferring the solvent back to the solvent flask, promoting the process to start over again. With each subsequent extraction, more of the target components gets dissolved, leaving the insoluble contaminants in the thimble. This is how compounds of interest are removed from a sample . It can be utilized for the extraction of large numbers of drugs with low volumes of solvents. This is valid for plant material that can withstand high heat. It does not require filtration. The disadvantages of this method are,Requires a large amount of heat. Impossibility of regular shaking and not being suitable for thermolabile materials.

VII Microwave-Assisted Extraction

Microwave-assisted extraction is an advanced extraction technique used in the extraction of herbal medicines[57] It involves the mechanism of dipole rotation and ionic transfer to displace charged ions from the solvent and drug materials. This technique employs electromagnetic radiation having frequencies ranging from 300 MHz to 300 GHz and wavelengths ranging from 1 cm to 1 m. This approach uses microwave radiation to bombard a substance that absorbs electromagnetic energy and converts it into thermal energy. Due to the heat generated, it facilitates the solvent to penetrate the plant materia This method requires a minimum quantity of solvents and time for extraction. It also can give a higher output. However, being suitable only for flavonoids

and phenolic compounds and the possibility of degrading tannins and anthocyanins due to the high heat can be some downsides of this method[57]

VIII Sonication (Ultrasound-Assisted Extraction)

In this extraction technique, ultrasound frequencies are used that are ranging from 20 KHz to 2000 KHz[58] They can disturb the plant cell walls and thereby increase the surface area of plant material to facilitate the penetration of solvents. Subsequently, this facilitates the release of biologically active compounds.The advantages of this method are, This method can be used for small sample amounts. It gives us a high yield while reducing extraction time and the quantity of menstruum. Even though this method is useful in some incidents such as the extraction of rauwolfia roots, involving this process on a large scale can be a disadvantage because of the high cost. Also, the high ultrasound energy can sometimes degrade the phytochemicals due to the production of free radicals[59]

IX Reflux Extraction Method

The reflux method is a popular extraction method in Sri Lanka, and studies show that it can increase extraction yield whilst lowering the amount of solvent utilized[60]. Reflux extracting refers to a solid-liquid extraction method at a uniform temperature with replicated solvent evaporation as well as condensation for a set period of time without loss of solvent. The technique is commonly utilized in the herbal industry since it is effective, simple to operate, and affordable [61].

4. Biological Activities of Phytochemicals

The phytochemicals present in plants areresponsible for preventing disease and promotinghealth have been studied extensively to establish their efficacy and to understand the underlying mechanism of their action. Such studies have included identification and isolation of the chemical components, establishment of their biological potency both by *in vitro* and *in vivo* studies in experimental animals and through epidemiological and clinical-case control studies in man. Study findings suggest that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of lowdensity lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol normalizing blood pressure and clotting, and improving arterial elasticity. Phytochemicals have also been promoted for the prevention and treatment of diabetes, high blood pressure, and macular degeneration[62]

Applications of Phytochemicals in Medicine

4.1 Antioxidant Activity Phytochemicals like flavonoids, tannins, and phenolic acids neutralize free radicals. Prevent oxidative stress-related diseases such as cancer, diabetes, and cardiovascular disorders.Neutralizes free radicals and prevents oxidative stress. Phytochemicals like flavonoids and phenolics scave free radicals, reducing oxidative stress and preventing chronic diseases like cancer and cardiovascular disorders[87].

4.2 Anti-inflammatory Properties

Alkaloids, flavonoids, and terpenoids inhibit pro-inflammatory cytokines. Beneficial in conditions like arthritis, asthma, and inflammatory bowel disease. Anti-inflammatory Effects: Reduces inflammation in chronic diseases. Flavonoids and saponins modulate inflammatory pathways, aiding in the management of arthritis, asthma, and other inflammatory diseases.

4.3 Antimicrobial Activity

Tannins, alkaloids, and saponins are effective against bacterial, viral, and fungal infections. Examples: Berberine (antibacterial), Curcumin (antiviral). Effective against bacteria, fungi, and viruses. Compounds such as alkaloids, terpenoids, and tannins exhibit activity against pathogens like E. coli, S. aureus, and Candida albicans[82].

4.4 Anticancer Potential

Phytochemicals like terpenoids and alkaloids induce apoptosis, inhibit angiogenesis, and reduce tumor proliferation. Examples include taxol from Taxus brevifolia and vincristine from Catharanthus roseus.

4.5 Cardioprotective Effects

Flavonoids and glycosides improve heart health by reducing LDL cholesterol, improving vascular function, and reducing inflammation[62]

5. MATERIALS AND METHODS

5.1 Materials

Beakers, conical flask, measuring cylinders (different size), glass funnels, glass stirrer, cotton wool, spatula, bunsen burner, top mettler weighing balance, test tubes, stainless steel tray, thermostat water bath, oven, aluminum foil paper, hand gloves, mortar and pestle, analytical weighing balance, test-tube holder, refrigerator, meter rule, bottles, cabinet tripod stand, wire gauze, capillary tubes, filter paper, autoclave, UV box with UV lamp, and TLC paper[63,64]

5.2 Collection of plant materials

Fresh leaves of plants free from diseases were collected .Taxonomic identification of plants was carried out by the department of Botany[64]

5.3 Preparation of Plant extracts

The method of Alade and Irobi, (1993) was adopted for preparation of plant extracts with little modifications. Briefly three 20 g portions of the powdered plant material were soaked sepa- rately in 100 ml of water, hexane, and alcohol for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each extract was passed through Whatman filter paper no. 1 (Whatman, England). The filtrate obtained were concentrated in vacuo using rotary evapora- tor at 30°C[65,83]

6. **Phytochemical Screening**

identify plant compounds.The confirmatory qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids) present in the extracts following standard protocols[66]

6.1 Qualitative Screening

These tests help detect the presence of specific phytochemicals:

6.1.1 Test for Alkaloids: The plant extract was dissolved in 100 mL of water, filtered, and cooked in steam with 2 mL of the filtrate and three drops of 1% HCl. Then, 1 mL of the heated mixture was combined with 6 mL of the Mayer-Wagner reagent. The appearance of a cream or brown-red colored precipitate indicated the presence of alkaloids[66]

6.1.2 Test for Flavonoids and Glycosides: 200 mg of the plant extract was mixed with 10 mL of ethanol and filtrated. Two mL of the filtrate, concentrated HCl, and magnesium ribbon were mixed. The formation of a pink or red color indicates the presence of flavonoids. Adding 1 mL of distilled water and NaOH to 0.5 mL of crude extract, the formation of a yellowish color indicated the presence of glycosides

6.1.3 Test for Tannins: About 200 mg of the plant extract was boiled with 10 mL of distilled water; and 0.1% Ferric chloride was added to the mixture; which was then observed for blue-black coloration indicating the presence of tannins.

6.1.4 Test for Saponins: About 0.5 milliliters of the extract and 5 mL of distilled water were combined and agitated. Then, the formation of foam confirmed the presence of saponins.

6.1.5 Test for Steroids: About 1 mL of the crude extract was combined with 10 mL of chloroform and 10 mL of sulfuric acid, and the formation of the bilayer (red top layer and greenish bottom layer) reveals the presence of steroids.

6.1.6Test for Terpenoids: The presence of terpenoids was determined by the formation of a reddish-brown color in the test for terpenoids, which included mixing of 0.5 mL of crude extract with 2 mL of chloroform and 3 mL of sulfuric acid.

6.1.6 Test for Phenols:

About 1 mL of the extract was combined with three drops of FeCl3, and 1 mL of K2Fe (CN6). The formation of greenish- blue forms confirmed the presence of phenols[67,74]

6.2 Quantitative Analysis

6.2.1 Thin Layer Chromatography (TLC) Test

Thin layer chromatography was performed on TLC plate (aluminum silica gel pre-coated with layer thickness of 0.2 mm) using hexane/ethyl acetate mixtures (8:2) as an eluent[68] Spots were applied using capillary tube 1.5 cm from the bottom marked by a line ruled using a pin. The sample spotted on the plate was allowed to dry before the plate was placed into the chromatographic tank which was covered immediately. When the solvent reaches the top of the plate, the plate was removed, marked and dried. The number of the spots was detected under UV at 254 and 366 nm wavelengths and spraying with spotting reagent, using iodine vapor[68,64].

6.2.2 Column Chromatography (CC)

Column chromatography is the most effective technique used in separation of crude plant extracts into its components in pure form. This is a preparative chromatographic method and the stationary phase (silica gel) is packed in a column and then the mobile phase (eluent) is passed through the column after loading the extracts on the top of the stationary phase. The mobile phase carries the natural products present in the mixture at different rate based on their affinities to the stationary and mobile phase. Separated compounds can be collected along with the mobile phase [69].

6.2.3 Gas Chromatography (GC)

It is an analytical technique for separating compounds based primarily on their volatilities. GC provides both qualitative and quantitative information for individual compounds present in a sample. The gas phase is flowing and the liquid phase is stationary. The rate of migration for the chemical species is determined through its distribution in the gas phase. For example, a species that distributes itself 100% into gas phase will migrate at the same rate as the flowing gas, whereas, a species that distributes itself 100% into stationary phase will not migrate at all. Species that distribute themselves partly in both phases will migrate at an intermediate rate [70]. Gas chromatography involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is then transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase, which is adsorbed onto the surface of an inert solid[71]

6.2.4 High Performance thin Layer Chromatography (HPTLC)

It is a planar chromatography where separation of natural compounds is achieved on high performance layers with detection and data acquisition.These high performance layers are pre-coated plates coated with a sorbent of particle size 5-7 microns and a layer thickness of 150-200 microns.The reduction in thickness of layer and particle size results in increasing the plate efficiency as well as nature of separation [75]. HPTLC plates are

substantially more expensive (4- to 6-times more) than normal plates but are an efficient alternative when high sensitivity, accuracy and precision are required in situations demanding high performance [72].

6.2.5 High Performance Liquid Chromatography (HPLC)

It is a versatile, robust, and widely used technique for the isolation of natural products. HPLC is an analytical technique for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial etc. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of medicinal plants. In order to identify any compound by HPLC, a detector must first be selected, The extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase. Modern HPLC uses a non-polar solid phase, like C18 and a polar liquid phase, generally a mixture of water and another solvent. High pressure up to 400 bars is required to elute the analyte through column before they pass through a diode array detector (DAD). A DAD measures the absorption spectra of the analytes to aid in their identification. HPLC is useful for compounds that cannot be vaporized or that decompose under high temperature and it provides a good complement to gas chromatography for detection of compounds [76,77]

6.2.6 UV-Visible Spectroscopy

UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of compounds in both pure and biological mixtures. Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range. Natural compounds can be determined by using UV-visible spectroscopy [31]. Moreover, spectro-scopic UV-Vis techniques were found to be less selective and give information on the composition of the total polyphenol content. This technique is not time-consuming, and presents reduced cost compared to other techniques [78,79].

6.2.7 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy gives physical, chemical and biological properties of matter. One dimensional technique is routinely used but the complicated structure of the molecules could be achieved through two dimensional NMR techniques. Solid state NMR spectroscopy is used for the determination of molecular structure of solids. Radiolabeled 13C NMR is used to identify the types of carbon are present in the compound. 1 H-NMR is used to find out types of hydrogen are present in the compound and to find out how the hydrogen atoms are connected [77,78,79,].

6.2.8 Mass Spectroscopy

Mass spectrometry is a powerful analytical technique for the identification of unknown compounds, quantification of known compounds andto elucidate the structure and chemical properties of molecules[80] Through MS spectrum, the molecular weight of sample can be determined. This method mostly employed for the structural elucidation of organic compounds, for peptide or oligonucleotide sequencing and for monitoring the existence of previously characterizes compounds in complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule simultaneously [78,80].

7. ROLE OF PHYTOCHEMICALS IN PLANT DISEASE MANAGEMENT

Plants synthesize a large number of secondary metabolites numbering above 200,000 that do not play a direct role in their growth but help them to survive in the environment especially by providing defense against diseases and pests. The wide variety of secondary compounds is synthesized mainly by the isoprenoid, phenylpropanoid, alkaloid or fatty acid, or polyketide pathways. Plant disease management involves the reduction in the economic loss of plants due to diseases caused by pathogens. Plants have evolved several mechanisms which have led to the production of tens of thousands of phytochemicals. Earlier, plant-based chemicals constitute a very small portion which was overlooked. Since the introduction of Food Quality Protection Act of 1996, there has been a vast market opportunity for agroallied- based chemicals used in plant disease management in the United States and most of North America (Isman, 2000)[81]. A lot of essential oils in plants have shown a high potential for getting rid of insects. A range of essential oils such as cinnamaldehyde, α-pinene, extracts from clove (*Syzygium aromaticum*, major oil being eugenol) and star anise (*Illicium verum*) has been shown to have fumigant and antifeedant effect on red flour beetle (*Tribolium castaneum*), and the maize weevil (*Sitophilus zeamais*) (Ho et al., 1995, 1997; Huang and Ho, 1998; Huang et al., 1998). Eugenol and oils from the holy basil (*Ocimum suave*) have also shown to be effective against *Sitophilus granarius* and *Prostephanus truncatus* (Obeng-Ofori and Reichmuth, 1997). Essential oils of cumin, star anise, oregano, and eucalyptus have been shown to be active against greenhouse pests such as cotton aphid (*Aphis gossypii*) and carmine spider mite (*Tetranychus cinnabarinus*) Plant-derived aldehydes and ketones play key roles against pathogenic fungi. Among aliphatic aldehydes and ketones, cinnamaldehyde has been shown to have the most potent activity against fungi especially two species of *Penicillium* that causes disease in humans (*P. cyclopium* and *P. frequentans*). The effects of perillaldehyde and citral were slightly weaker but potent enough. *Penicillium ulaiense*, an important pathogen causing molds in citrus, and other *Penicillium* spp. causing molds in apple and pear can be targeted using these aliphatic aldehydes that have one or more double bondsconjugated to their carbonyl group. Among aromatic aldehydes, cuminaldehyde had been shown to have fairly potent antifungal activity (Kurita et al., 1981). The essential oils of *Thymbra spicata* and *Satureja thymbra* plants used as spices in Mediterranean cuisine have been shown to inhibit phytopathogenic fungi such as *Fusarium moniliforme*, *Rhizoctonia solani*, and *Phytophthora capsici* at a concentration of 400–800 μg/mL. Thymol and carvacrol have been identified as the major constituents in the essential oils involved in the fungicidal property, followed by monoterpenes γ-terpenin and p-cymene (Muller et al., 1995).

Phenolic compounds play a significant role in plant defense against bacteria and fungi. One important phenolic compound is coumarin. Halogenated coumarin, often brominated, chlorinated, or iodinated, is more stable than coumarin. It has been shown to be particularly effective against plant pathogenic fungi such as *Macrophomina phaseolina* (charcoal rot), *Phytophthora* spp. (damping off and seedling rot), *Rhizoctonia* spp. (damping off and root rot), and *Pythium* spp. (seedling blight). These four fungi are from different families, showing the broad spectrum activity of halogenated coumarins. In addition, halogenated coumarins have polymer seed coating abilities and less phytotoxicity, making them good candidates for natural pesticide development. In another

study, 7-hydroxylated coumarin has been shown to be effective against parasitism of *Orobanche cernua* in sunflower (Serghini et al., 2001)[1]

8. Future Perspective

A chemist or chemical scientist is one that is involved in research activities related to chemical analysis, confirmation of elements, elucidationof the structure of chemical compounds for industrial purposes. But a phytochemist is a specialist who is interested in the study of chemical interactions in plants based on the knowledge of chemical science which is employed for a successful isolation of its components and the determination of its molecular structure through the study of its properties. The phytochemists have a good command over medicinal plants through the study of plant physiology, morphology, internal structure elucidation, and metabolic activities. A phytochemist is one who is knowledgeable about the identification, characterization of different natural products by using biochemical analysis to understudy the chemical composition of different plant products. The utilization of plants for medicinal purposes is not a new approach. However, periodically, plants are explored for extraction of chemical compounds which are beneficial to humans in several aspects. Once a plant-derived product is confirmed to exhibit curative potentials, the product will be recommended for drug designing, clinical approach, and finally to pharma industries.

Today, a huge sector of the population is relying on medicinal plants for their preventive and curative properties. WHO stated that those traditional medicine/ethnomedicine are still being used to treat different ailments. Nearly 70% of the populations rely on these medicinal practices. People from remote areas and semi-urban regions of the globe still depend on eithercrude or purified product from the plant's origin. Standardization and quality control is an important factor in ethnomedicinal formulations. At present, the phytochemists aim to apply modern techniques to preserve and maintain the standard and quality of these plant products. Findings by phytochemists through research investigations are supporting the ethnomedicinal formulations used by the tribal doctors. Several chemical compounds derived from plants are undergoing clinical trials and some of them are in preclinical treatment. Similarly, research is to be focused on the possibility of developing new products in combination with natural compounds from ethnomedicine. They identified the diversity of chemical compounds observed with phytochemicals which have led to the formulations of novel drugs against multidrug-resistant pathogens.

Phytochemists are seriously embarking on research activities involving the extraction of natural compounds present in plants. Some of these phytochemicals have the ability to suppress the activity of cancer cells by encouraging cell cycle inhibition and apoptosis. There is a lot of demand for natural products of plant origin and these by-products are to replace the synthetic products in view of their side effects on human health. Therefore, a lot of attention is needed toward natural products in which a phytochemist plays a key role in this context. Owing to the increasing demand for novel drugs, so many important and vital compounds regularly being manufactured by the industries generate employment opportunities for experts in this field. In addition, the increasing acceptance of the chemical diversity of natural products is well suited to provide the core

scaffolds for future drugs. There will be further developments in the use of novel natural products and chemical libraries based on natural products in drug discovery campaigns[1]

9. Conclusion

Phytochemistry has emerged as a critical field in understanding the chemical basis of medicinal plants and their applications in healthcare. The study and screening of phytochemicals have revealed a vast array of bioactive compounds with significant pharmacological properties, ranging from anti-inflammatory and antioxidant to anticancer and antimicrobial activities. These findings highlight the essential role of medicinal plants in drug discovery and development, particularly as sources of novel therapeutics. Advancements in phytochemical screening methods and extraction techniques have revolutionized the field. Traditional methods like maceration and Soxhlet extraction remain widely used for their simplicity, while modern methods such as ultrasound-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction offer higher efficiency, selectivity, and sustainability. These innovations have addressed key challenges such as low yields, degradation of sensitive compounds, and the environmental impact of conventional processes.Future research should aim to address these limitations by integrating cutting-edge technologies like metabolomics, nanotechnology, and machine learning to optimize screening and extraction processes. Furthermore, sustainable and ethical approaches should guide future work to prevent overexploitation of natural resources and to ensure equitable benefit-sharing with indigenous communities who have long relied on these plants for traditional medicine. Strengthening collaborations between academia, industry, and regulatory bodies will also be crucial to translating phytochemical research into clinically approved drugs and nutraceuticals.

References

1)Egbuna, Chukwuebuka, Jonathan Chinenye Ifemeje, Stanley Chidi Udedi, and Shashank Kumar, eds. *Phytochemistry: Volume 1: Fundamentals, Modern Techniques, and Applications*. CRC Press, 2018.

2)Karunamoorthi, Kaliyaperumal, Kaliyaperumal Jegajeevanram, Jegajeevanram Vijayalakshmi, and Embialle Mengistie. "Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings." *Journal of Evidence-Based Complementary & Alternative Medicine* 18, no. 1 (2013): 67-74.

3) David, Bruno, Jean-Luc Wolfender, and Daniel A. Dias. "The pharmaceutical industry and natural products: historical status and new trends." *Phytochemistry Reviews* 14 (2015): 299-315.

4) Jayakumari. "Phytochemicals and Pharmaceutical: Overview." *Advances in Pharmaceutical Biotechnology: Recent Progress and Future Applications* (2020): 163-173.

5)Phillipson, J. David. "Phytochemistry and medicinal plants." *Phytochemistry* 56, no. 3 (2001): 237-243.

6) Brielmann, Harry L., William N. Setzer, Peter B. Kaufman, Ara Kirakosyan, and Leland J. Cseke. "Phytochemicals: The chemical components of plants." *Natural products from plants* 2 (2006): 1-49.

7) Dillard, Cora J., and J. Bruce German. "Phytochemicals: nutraceuticals and human health." *Journal of the Science of Food and Agriculture* 80, no. 12 (2000): 1744-1756.

8) Kocyigit, Abdurrahim, Eray Metin Guler, and Murat Dikilitas. "Role of antioxidant phytochemicals in prevention, formation and treatment of cancer." *Reactive Oxygen Species (ROS) in Living Cells; InterchOpen: London, UK* (2018): 21-45.

9) Alamgir, A. N. M., and A. N. M. Alamgir. "Phytoconstituents—active and inert constituents, metabolic pathways, chemistry and application of phytoconstituents, primary metabolic products, and bioactive compounds of primary metabolic origin." *Therapeutic Use of Medicinal Plants and their Extracts: Volume 2: Phytochemistry and Bioactive Compounds* (2018): 25-164

10) Abdullahi, R., and A. B. Hamza. "Physiological Roles of Phenolic Compounds Isolated from Medicinal Plants of Tropical Origin." *International Journal Of Science for Global Sustainability* 6, no. 4 (2020): 133-143.

11) Mandal SM, Chakraborty D, Dey S. Phenolic acids act as signaling molecules in plant–microbe symbioses. Plant Signal Behav, 2010; 5: 359-368.

12) Pridham JB. In: Phenolics in Plants in Health and Disease, Pergamon Press, New York, 1960; 34-35.

13) Harborne JB, Baxter H. The handbook of natural flavonoids, Volume 1 and 2. Chichester, UK: John Wiley and Sons, 1999.

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

15) Atmani D, Nassima C, Dina A, Meriem B, Nadjet D, Hania B, Flavonoids in Human Health: From Structure to Biological Activity.Current Nutrition & Food Science, 2009; 5: 225-237.

16) Lawal AM, Lawan MM, Apampa SA. Phytochemical analysis and thin layer chromatography profiling of crude extracts from GuieraSenegalensis (Leaves). *J Biotechnol Biomed Sci*. 2019;3(3):7–12. overview. Methods Enzyme molecule, 1990; 186: 1-85

17) Harborne JB. An overview of antinutritional factors in higher plants. In: Secondary plants products. Antinutritional and beneficial actions inanimal feeding Caygill JC and Mueller-Harvey I, eds. Nottingham Univ Press, UK, 1999; 7-16.

18) Schofield P, Mbugua DM, Pell AN. Analysis of condensed tannins: a review. Animal Feed Science Technology, 2001; 91: 21-40.

19) Vansoest PJ. Nutritional ecology of the ruminant, 2nd ed. Cornell Univ Press. Ithaca, NY, 1994; 476.

20) Mueller-harvey I, Mcallan AB. Tannins: Their biochemistry and nutritional properties. Advances in plant cell biochemistry and biotechnology, Vol. 1 Morrison IM ed. JAI Press Ltd, London (UK), 1992; 151-217.

21) Mangan JL. Nutritional effects of tannins in animal feeds. Nutrition Research and Reviews, 1988; 1: 209-231.

22) Mc-Leod MN. Plant tannins: Their role in forage quality. Nutrition Abstract Review, 1974; 44: 803- 812.

23) Mole S, Waterman PG. Tannic acid and proteolytic enzymes: enzyme inhibition or substrate deprivation? Phytochemistry, 1987; 26: 99-102.

24.)JX Li and ZY. Yu (2006). Cimicifugaerhizoma: from origins, bioactive cons

PII S0378-8741(98)00055-5

25) De Bruyne T, Pieters L, Deelstra H, Vlietinck A. Condensed vegetables tannins: biodiversity in structure and biological activities. Biochemical

System Ecology, 1999; 27: 445–59.

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

27) Mueller-Harvey I, McAllan AB. Tannins. Their biochemistry and nutritional properties. In: Advances in plant cell biochemistry and bbiotechnology, Vol. 1 Morrison IM, ed. JAI Press Ltd, London (UK), 1992; 151-217.

28) Rao RVK, Ali N, Reddy MN. Occurrence of both sapogenins and alkaloid lycorine in Curculigo orchioides. Indian Journal Pharma Science, 1978; 40: 104-105.

29) Mishra SN. Analytical methods for analysis of total alkaloids in root of Withania spp. Proc. All India workshop on M&AP, Faizabad, 1989; 492- 95.

30)Yahaya, Khalid Ibrahim. "Plant as Antimicrobial." *Scholastic Microbiology* 1 (2023): 17-18.

31) Elbein AD, Molyneux RJ. Comprehensive Natural Products Chemistry, Vol. 3, Barton D and Nakanishi K, ed. Amsterdam, 1999; 129.

32) Harborne JB, Tomas-Barberan FA. Ecological Chemistry and Biochemistryof Plant Terpenoids, Clarendon, Oxford, 1991.

33) Langenheim JH. Higher plant terpenoids: A phytocentric overview of their ecological roles. Journal of Chemical Ecology, 1994; 20: 1223- 1280.

34) Dudareva N, Pichersky E, Gershenzon J. Biochemistry of plant volatiles. Plant Physiology, 2004; 135: 1893–1902.

35) Takechi M, Matsunami S, Nishizawa J, Uno C, Tanaka Y. Haemolytic and antifungal activities of saponins or anti-ATPase and antiviral activities of cardiac glycosides. Planta Medica, 1999; 65: 585– 586.

36) Traore F, Faure R, Ollivier E, Gasquet M, Azas N, Debrauwer L, Keita A, Timon-David P, Balansard G. Structure and antiprotozoal activity of triterpenoid saponins from Glinus oppositifolius. Planta Medica, 2000; 66: 368–371.

37) George F, Zohar K, Harinder PS, Makkar, Klaus B. The biological action of saponins in animal systems: a review. British Journal of Nutrition, 2002; 88: 587–605.

38) V.Bulugahapitiya,‖Plant Based Natural Products Extraction and Phytochemical analysis‖, self, 2013. https://www.researchgate.net/publication/324136585

39) N.I.M. Njila, E. Mahdi, M.D. Lembe, Z. Nde and D. Nyonseu. (2017, May). Review on Extraction and Isolation of Plant Secondary Metabolites, 7th Int'l Conference on Agricultural, Chemical, Biological and Environmental Sciences. pp. 67-72. https://doi.org/10.15242/IIE.C051720.

40) P. Tonthubthimthong, S. Chuaprasert, P. Douglas and W. Luewisutthichat.(2011, March). Supercritical CO2 extraction of nimbin from neem seeds an experimental study, Journal of Food Engineering. 47 (4), pp. 289-293. https://doi.org/10.1016/S0260-8774(00)00131-X.

41) V. Duraipandiyan, M. Ayyanarand S. Ignacimuthu. (2006, October). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complementary Altern. Med. 6 (35), pp. 35-41. http:// doi.org/10.1186/1472-6882-6-35]

42) J.J. Roy Burman, "Tribal Medicine" 1st ed. A Mittal Publication, 2003, New Delhi, pp. 12.

43) W.Q. Zhang, G.L. Lin and C.W. Ye. (2018, April). Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Medicine. 13(20), pp. 1-26. https://doi.org/10.1186/s13020-018-0177-x.

44) B.S. Rathi, S. L. Bodhankar and A.M. Baheti. (2006, November). Evaluation of aqueous leaves extract of Moringaoleifera Linn for wound healing in albino rats, Indian Journal of Experimental Biology. 44 , pp. 898-901.

45)Alamgir, A. N. M., and A. N. M. Alamgir. "Herbal drugs: their collection, preservation, and preparation; evaluation, quality control, and standardization of herbal drugs." *Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1: Pharmacognosy* (2017): 453-495.

46). Pandey A, Tripathi S. Concept of Standardization, Extraction, and Pre-Phytochemical Screening Strategies for Herbal Drug. Journal of Pharmacognosy and Phytochemistry. 2014;2(5):115– 119.

47). Azwanida N. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength, and Limitation. Medicinal & Aromatic Plants. 2015;04(03). DOI: https://doi.org/10.4172/2167-0412.1000196

48)Abubakar, Abdullahi R., and Mainul Haque. "Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes." *Journal of Pharmacy and Bioallied Sciences* 12, no. 1 (2020): 1-10.

49)Jha, Avinash Kumar, and Nandan Sit. "Extraction of bioactive compounds from plant materials using combination of various novel methods: A review." *Trends in Food Science & Technology* 119 (2022): 579-591.

50)Jurinjak Tušek, Ana, Dunja Šamec, and Anita Šalić. "Modern techniques for flavonoid extraction—to optimize or not to optimize?." *Applied Sciences* 12, no. 22 (2022): 11865.

51) D. S. H. S. Peiris at al Phytochemical Screening for Medicinal Plants: Guide for Extraction Methods *Asian Plant Research Journal Volume 11, Issue 4, Page 13-34, 2023; Article no.APRJ.101150 ISSN: 2581-9992*

52). Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC. Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. Journal of Pharmacognosy and Phytochemistry. 2017;6:32–36.

53)Handa, S. S. "An overview of extraction techniques for medicinal and aromatic plants." *Extraction technologies for medicinal and aromatic plants* 1, no. 1 (2008): 21-40.

54)Stéphane, Fongang Fotsing Yannick, Bankeu Kezetas Jean Jules, Gaber El-Saber Batiha, Iftikhar Ali, and Lenta Ndjakou Bruno. "Extraction of bioactive compounds from medicinal plants and herbs." *Natural medicinal plants* (2021): 1-39.

55)Ghenabzia, Imane, Hadia Hemmami, Ilham Ben Amor, Soumeia Zeghoud, Bachir Ben Seghir, and Rokaia Hammoudi. "Different methods of extraction of bioactive compounds and their effect on biological activity: A review." *International Journal of Secondary Metabolite* 10, no. 4 (2023): 469-494.

56)Rudraswamy, Sushma, B. Godhi, Hommergally Puttabuddi Jai Shankar, Mruthunjaya Kenganora, and M. N. Sumana. "Detailed understanding of different extraction methods for the research on medicinal plants." *Indian Journal of Oral Health and Research* 7, no. 1 (2021): 14-20.

57) Flora-Glad Chizoba, Da-Wen Sun, and Jun-Hu Cheng. "Acceleration of microwave-assisted extraction processes of food components by integrating technologies and applying emerging solvents: A review of latest developments." *Trends in Food Science & Technology* 67 (2017): 160-172.

58)Pingret, Daniella, Anne-Sylvie Fabiano-Tixier, and Farid Chemat. "Ultrasound-assisted extraction." (2013). Alothman, Mohammad, Rajeev Bhat, and A. A. Karim.

59)"Effects of radiation processing on phytochemicals and antioxidants in plant produce." *Trends in food science & technology* 20, no. 5 (2009): 201-212.

60)Shanmuganathan, E., L. D. A. M. Arawwawala, K. G. P. Wasana, and A. P. Attanayake. "Selection and optimisation of extraction technique for the preparation of phenolic-and flavonoid-rich extract of leafy vegetable, Coccinia grandis (Linn.) Voigt." *International food research journal* 29, no. 5 (2022): 1032-1042.

61)Mohammad Azmin, Siti Nuurul Huda, Zainuddin Abdul Manan, Sharifah Rafidah Wan Alwi, Lee Suan Chua, Azizul Azri Mustaffa, and Nor Alafiza Yunus. "Herbal processing and extraction technologies." *Separation & Purification Reviews* 45, no. 4 (2016): 305-320.

62)Saxena, Mamta, Jyoti Saxena, Rajeev Nema, Dharmendra Singh, and Abhishek Gupta. "Phytochemistry of medicinal plants." *Journal of pharmacognosy and phytochemistry* 1, no. 6 (2013): 168-182.

63)Hassan, Ismail Adamu, Idris Abdullahi Nasiru, Amina Muhammed Malut, and Audu Sani Ali. "Phytochemical studies and thin layer chromatography of leaves and flower extracts of Senna siamea lam for possible biomedical applications." *Journal of Pharmacognosy and Phytotherapy* 7, no. 3 (2015): 18-26.

64)Dubale, Sileshi, Dereje Kebebe, Ahmed Zeynudin, Negera Abdissa, and Sultan Suleman. "Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia." *Journal of experimental pharmacology* (2023): 51-62.

65)Nyaberi, M. O., C. A. Onyango, F. M. Mathooko, J. M. Maina, and M. Makobe. "Determining efficient extraction procedure of phytochemicals from the fruit paste of Ziziphus abyssinica and Tamarindus indica." *Journal of Applied Biosciences* 112 (2017): 11045-11051.

66)Alamgir, A. N. M., and A. N. M. Alamgir. "Methods of Qualitative and Quantitative Analysis of Plant Constituents." *Therapeutic Use of Medicinal Plants and their Extracts: Volume 2: Phytochemistry and Bioactive Compounds* (2018): 721-804.

67) Singh, Yogendra, Karan Kumar Das, Mathews T. Thelly, Bhupendra Kumar, and Ayushmaan Roy. "PHYTOCHEMICAL STUDY AND IN VITRO ANTI-DIABETIC ACTIVITY OF EUCALYPTUS AND LEMON OILS."

68) Kaushik, Sunil. "Quantitative estimation os solanesol in various parts of nicotina tabacum by hpltc method collected from different geographical sources." Master's thesis, Rajiv Gandhi University of Health Sciences (India), 2010.

69) V.Bulugahapitiya,‖Plant Based Natural Products Extraction and Phytochemical analysis‖, self, 2013. https://www.researchgate.net/publication/324136585

70) K.P. Ingle, A.G. Deshmukh, A.D. Padole, S. Mahendra, S.M. Dudhare, P.M. Moharil and C.V. Khelurkar. (2017). Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. Journal of Pharmacognosy and Phytochemistry. 6 (1), pp. 32-36.

71)Falaki, Foujan. "Sample preparation techniques for gas chromatography." In *Gas Chromatography-Derivatization, Sample Preparation, Application*. IntechOpen, 2019.

72) Solanki SL, Modi CM, Patel HB, Patel UD. Phytochemical screening and thin-layer chromatography of six medicinal plants from the surroundings of Junagadh, Gujarat, India. *J Pharmacogn Phytochem*. 2019;8(4):3122–3126.

73) Mohammed Golam Rasul Extraction, Isolation and Characterization of Natural Products from Medicinal Plants International Journal of Basic Sciences and Applied Computing (IJBSAC) ISSN: 2394-367X, Volume-2 Issue-6, December 2018

74) W.Q. Zhang, G.L. Lin and C.W. Ye. (2018, April). Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Medicine. 13(20), pp. 1-26. https://doi.org/10.1186/s13020-018-0177-x.

75)Wilde TJJD, Ben IO, Woode E, Abotsi WKM. Preliminary phytochemical screening and in vitro antioxidant properties of Trichilia monadelpha (Thonn.) JJ De Wilde (Meliaceae). *J Med Biomed Sci*. 2013;2:6–15.

76) P. Tonthubthimthong, S. Chuaprasert, P. Douglas and W. Luewisutthichat.(2011, March). Supercritical CO2 extraction of nimbin from neem seeds an experimental study, Journal of Food Engineering. 47 (4), pp. 289-293. https://doi.org/10.1016/S0260-8774(00)00131-X

77) Hassan IA, Nasiru IA, Malut AM, Abdulkadir S, Ali AS. Phytochemical studies and thin layer chromatography of leaves and flower extracts of S enna siamea lam for possible biomedical applications. *J Pharmacogn Phytother*. 2015;7:18–26. doi:10.5897/JPP2014.0337

78)W. Kemp.(1991). Energy and electromagnetic spectrum: In Organic Spectroscopy. 3rdedn. Macmillan Press, London .

79) T.L.Eberhardt, X. Li , T.F. Shupe and C.Y. Hse. (2007, April). Chinese Tallow Tree (SapiumSebiferum) utilization: Characterization of extractives and cell-wall chemistry. Wood Fiber Science. 39(2), pp. 319-324.

80)Lehotay, Steven J., Katerina Mastovska, Aviv Amirav, Alexander B. Fialkov, Perry A. Martos, André De Kok, and Amadeo R. Fernández-Alba. "Identification and confirmation of chemical residues in food by chromatography-mass spectrometry and other techniques." *TrAC Trends in Analytical Chemistry* 27, no. 11 (2008): 1070-1090.

81) Bhatla, Satish C., and Manju A. Lal. "Secondary metabolites." In *Plant physiology, development and metabolism*, pp. 765-808. Singapore: Springer Nature Singapore, 2023.

82) Iqbal Ahmad et.al, Screening of some Indian medicinal plants for their antimicrobial properties Journal of Ethnopharmacology 62 (1998) 183–193 0378-8741/98/$19.00 © 1998 Elsevier Science Ireland Ltd. All rights reserved.

83) B.S. Rathi, S. L. Bodhankar and A.M. Baheti. (2006, November). Evaluation of aqueous leaves extract of Moringaoleifera Linn for wound healing in albino rats, Indian Journal of Experimental Biology. 44 , pp. 898-901.

84) Pretorius JC. Flavonoids: A Review of Its Commercial Application Potential as Anti- Infective Agents. Current Medicinal Chemistry- Anti Infective Agents, 2003; 2: 335-353.

85) Giner-Chavez BI. Condensed tannins in tropical forages. Doctoral Thesis. Cornell University Ithaca, NY, 1996.

86) Serrano J, Puupponen-Pimia R, Dauer A, Aura A, Saura-Calixto F. Tannins: current knowledge of food sources, intake, bioavailability and biological effects. Molecular Nutrition Food Research, 2009; 53: S310–29.

87) Lacaille-Dubois MA, Wagner H. Bioactive saponins from plants: An update. In Studies in Natural Products Chemistry; Atta-Ur-Rahman, ed. Elsevier Science. Amsterdam, 2000; 21: 633-687.