

Medicinal plant, chemical constituents, biosynthesis, and extraction

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Abstract: Phytochemicals obtained from various plants part like leaves, seed, seed, flowers, barks, roots, and pulps are normally utilised as a lead compound in various diseases treatment. Since ancient times, secondary metabolites are well known and utilized in treatment of diseases like analgesic, diabetes, asthma, anti-inflammatory, cancer, and also serve as alternatives for hormone therapy worldwide. The current review objective is to highlight the medicinal potency of bioactive constituents, chemical diversity, and utilization of medicinal plant products, isolation, identification, characterisation which requires vast analytical examination involving expert utilizing cutting edge analytical procedure, and instrumentations.

Keywords: Metabolites, Extraction, Biosynthesis, Medical plant, Structural elucidation.

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Introduction

The upsurge in utilization of plants for medicinal purpose is traced back to early man (Phillipson, 2001). Thus, plants still remain a major source, and reservoir of different phytoconstituents widely used in treatment of different diseases. Medicinal plants are also regarded as store house for rich dietary compounds like vitamins, biomolecules, and minerals which are known for maintenance of healthy body (Arvind, 2016). Globally, nearly 20,000 species of plants are well known for their medicinal relevance (Manash *et al.*, 2016). Medicinal plants distribution across the globe is in non-uniform pattern. Developing countries globally still depend on plants as a major lead in healthcare (Sakina *et al.*, 2014). Statistics from World Health Organization (WHO) stipulates that over 4 billion people consisting of 80% of the population in the world depend on medicinal plants in different diseases treatment (Fabricant and Farnsworth, 2001).

Medicinal plants are well accepted by World Health Organization (WHO) as a major constituent of primary health care. Approximately, 11% out of 252 pharmaceutical products available in the market are obtained from medicinal plants (Arvind, 2016). Medicinal value of plant strictly depends on the active principles also known as secondary metabolite, which produce different physiological actions in human body (Edeoga *et al.*, 2005). Plants are known to synthesize myriad of lead chemical constituents known as secondary metabolite with great potency in the discovery, and new pharmaceutical lead products development (McChesney *et al.*, 2007).

Plants possess a wide range spectrum of effects because of the availability of different phytoconstituents like alkaloid, flavonoids, glycosides, terpenes, tannins, steroids, saponins, and anthraquinones. Medicinal plant derived drugs are of high demand

due to safe, cheap, and readily available nature unlike synthetic drugs (Dey and De, 2015). Pilocarpine, vinblastine, curcumin, taxol, gitoxigenin, podophyllotoxin, allicin, vincristine, aspirin, digitoxigenin, digoxigenin, camptothecin, tubocurarine, morphine, atropine, capscicine, codeine, artemesinin, and ephedrine among others are well known plant derived drugs (Joy *et al.*, 1998).

Over 400 million years ago, plants have evolved high diversity of phytoconstituents which are toxic to microorganisms and animals. Most phytoconstituents are biologically active because of this high evolutionary origin. Plant used these secondary metabolites for defence against herbivore, and microbial prey. Medicinal plants therapeutic potential like anti-diabetic, anti-microbial, hypoglycemic, anti-malarial, anti-oxidant, anti-carcinogenic, anti-inflammatory, anti-cholinergic, and anti-leprosy activities are usually attributed to bioactive principles (Negi *et al.*, 2011). The herbal preparations promote excretion of toxic substances which aid in regaining normal strength, and increase energy of metabolism. Medicinal plants effectiveness and their pharmacotherapeutic potency depend on their complex diversity of chemical constituents.

Generally, phytochemicals are simply the non-nutritive naturally occurring chemical compounds available in medicinal plants part like stem, roots and leaves with defence mechanism potency. These chemical compounds aid in protection of plant against different diseases. Phytoconstituents are usually classified into primary and secondary metabolite. Proteins, sugar, chlorophyll are classified as primary metabolites and secondary metabolites include terpenoids, steroids, phenolic compounds, and alkaloids. Medicinal plants synthesised, and preserved variety of biochemical products known as metabolites in various part of plant which are extractable (Joy *et al.*, 1998).

Metabolites

Metabolite is simply the metabolism intermediates or products of metabolism, usually limited to molecules that are small. Metabolisms are chemical reactions that usually take place in cells of living beings, which complex chemical substances are, synthesize from simpler compounds, or degrading complexes compounds to simple compounds. Plants are autotrophic in nature with basically two metabolic processes, namely primary and secondary metabolism. Numerous medicinal plants with pharmacological effects are as a result metabolites availability. These metabolites from various plants are usually organic compounds which are grouped into primary metabolites and secondary metabolites (Arvind, 2016). Organic compounds like nucleic acid, polysaccharide, protein, starch, lipids, and glucose which are involved in normal growth and development are grouped as primary metabolites (Arvind, 2016). Alkaloids, steroids, terpenes, saponins, flavonoids, phenolics, and glycosides which are not part of normal growth development in plant are classified as secondary metabolites (Mamta *et al.*, 2013).

Primary metabolite

This class of compounds are involved in normal life process like storage, respiration, reproduction, cell division, and growth. These processes are involved in photosynthesis, glycolysis, citric acid or Krebs cycle, and associated pathways. Small molecules like nucleic acids, sugars, tricarboxylic acids, or Krebs cycle intermediates, amino acids, proteins, polysaccharides which are similar to living cells constitute of primary metabolites (Seigler, 1995).

Secondary metabolite

Secondary metabolite is organic molecules not associated with typical growth, development, and improvement of organism. These classes of compound are non-dietary chemicals produced by organism for development, fitness, behaviour, and population of members of other species (David and Emma, 2011). Secondary metabolite was first introduced by A. Kossel in 1891 (Durairaj *et al.*, 2018) and has no vital significance role in organism's life, photosynthesis, nutrient assimilation, solute transport, translocation, respiration, and differentiation (Mazid *et al.*, 2011). Distribution of this class of compounds is restricted whereas primary metabolites are found throughout plant kingdom. Secondary metabolite is usually present only in some species of plant or species that are related taxonomically.

Secondary metabolite otherwise known as phytoconstituent are plant chemical that are totally attributed to medicinal plant characteristics (Justin *et al.*, 2014). Absence of secondary metabolite does not curb plant life immediately which is contrary to primary metabolite. Organism survival will be slowed to a larger extent. Plant usually synthesised secondary metabolites for specific functions, but primary metabolites are basically shared for biological reasons in all plant species. Secondary metabolites classification is strictly based on function, biosynthetic pathway, composition, solubility in various solvents, and chemical structure.

Globally, more than 2,140,000 secondary metabolites are well known. These phytoconstituents are basically grouped into three main groups namely terpenoids, alkaloids and phenolics. Each of this class of compound is further grouped into subclasses based on complexity of the nature of their structure. The synthesis of secondary metabolites take place in different organs of the plant

like seeds, shoots, leaves, fruit, roots, and flowers. Specific compartments of the plant stored most metabolite. This process could be either takes place in the whole organ of the plant or some specialized kind of cells. Toxic phytoconstituents concentration could be extremely high within these compartments such that the metabolites is able to exert efficient defence against any herbivores (Herwig and Jutta, 2014) such as nematodes, insects, vertebrates, microbes, and other competing plants. Secondary metabolite is often manufactured by some synthetic pathways from primary metabolite which are modified, or share primary metabolite origin substrates (Justin *et al.*, 2014).

The phytoconstituents plays a myriad of vital roles in the plant interaction with their immediate environment, and plant development. Generally, metabolites could also have vital roles played in symbionts attraction like insects pollination (Herwig and Jutta, 2014). Animals associated with dispersal of seed usually use colour, and aromatic property of the secondary metabolites to be attracted to plant. Some vertebrates and insects have well developed special mechanisms that facilitate coping with these secondary metabolites toxic effects.

These could lead to ecological niche generation to recognize a specific plant host for feeding or other things. Some metabolites are excellent photo-oxidation and ultraviolet (UV) light protectors which are light induced oxidation reactions (Herwig and Jutta, 2014). Excellent knowledge of their biosynthesis and genes regulation which encode enzymes associated with syntheses under changing conditions will explain the complexity of these secondary metabolites.

Alkaloid

Alkaloid is a group organic compound containing nitrogen, commonly distributed in nature. Over 18,000 of various alkaloids have been isolated from different plants. Alkaloid is obtained from the word "alkaline" describing base containing nitrogen. Alkaloids are base that contain nitrogen in their structure forming salts on reaction with acid (Mamta *et al.*, 2013). The nitrogen atom in alkaloid is always protonated. Alkaloids are positively charged and are soluble in water. They are known as vegetable alkalis. Alkaloids are usually used in local anaesthetic and stimulant with much diversity in structure. The availability one or more atoms of nitrogen in their structure serve as a common feature within these molecules (Cushnie and Cushnie, 2014).

Alkaloid is synthesis naturally by a large numbers of organisms consisting of plants, bacteria, animals, and fungi found in the entire plant or some specific plant part. Although there is dearth of information on some alkaloids functions, thee class of compound basically play vital roles in plants as protection from pathogens, inhibitors of competitors, and deterrents for herbivores (Cushnie and Cushnie, 2014). There are differences between other nitrogenous containing natural compounds like proteins, peptides, amino acids, nucleic acid, nucleotides, and amines which are not classified as alkaloids, and alkaloids (Mamta *et al.*, 2013). This is a vast class of secondary metabolites with rational classification been difficult due to variety of molecular structure.

An excellent method of classification is merging this group into various families which is a function of the nature of molecule heterocyclic ring system (Krishnan *et al.*, 1983) Systematized nomenclature of alkaloids is not possible due to complexities in their structure. Alkaloid is usually grouped base on heterocyclic

ring system they possess, and biosynthetic pathway from amino acids. Alkaloid is grouped into three basic group namely protoalkaloids, true alkaloids, and pseudoalkaloids. These are amino acid derived compounds, and made up of heterocyclic ring structure containing atoms of nitrogen indicating true alkaloids.

Compounds containing nitrogen atom derived from amino acid constitute of protoalkaloids. These class of compound consist of carbon skeletons usually not obtained from amino acids constitute pseudoalkaloids (Amirkia and Heinrich, 2014). The individual members names are basically obtained from the plant name or physiological activity characteristic (Mamta *et al.*, 2013). The various classes of alkaloids include quinoline alkaloids, pyrrolidine, isoquinoline, pyrrolidine-pyridine, pyridine-piperidine, and pyridine.

Oxygen (O), chlorine (Cl), phosphate (P), and sulphur (S) can also be found in their structure (Hussain *et al.*, 2017). Generally, alkaloid is basically obtained through metabolism of amino acid with ability to act as a drug in malaria, and cancer treatment. This class of secondary metabolite is a major bioactive constituent of plant which plays a significant role in plants, and animals immune systems (Aniszewski, 2007). The presence of nitrogen atom in their basic skeletal structure is a significant characteristic of alkaloid. Alkaloid is also seen as 'heterocyclic structure, consisting of a nitrogen atom within the ring structure'.

The existence of cyclic hydrocarbon structure in two or more forms such as five carbons, six carbons, or seven carbons ring structure with nitrogen atom within the cyclic skeleton justify alkaloid. Structural foible with relatively high bioactive potential than other classes of phytochemicals from plant makes it alkaloid. The alkaloid originates biosynthetically from tyrosine mediated pathway originally known from plant divergent metabolites is known as benzyloisoquinoline alkaloids. This class of metabolite is widely used in traditional medicine because of their high pharmacological potency, and availability in plants, (Hagel *et al.*, 2015).

Flavonoid

Flavonoid is polyphenolic phytoconstituents with ubiquitous nature. The molecular frameworks of flavonoids are usually known by variety of phenolic structures commonly found in plants (Xia *et al.*, 2013). Furthermore, over 4,000 flavonoids exist in fruits, beverages, and vegetables, like coffee, tea, fruit drinks and vegetables. This class of compound also exist as methylated derivatives, aglycone, and glycosides. It consists of 6 membered ring connected to benzene ring either as pyrone (flavonols and flavones) or dihydroderivative like flavan-3-ols and flavanones. Flavonoids common basic building blocks constitute of two rings of benzene linked to three carbons of propene unit ($C_6-C_3-C_6$). This is usually built from a cinnamoyl Co-A starting moiety followed by extension chain utilizing three malonyl Co-A molecules (Dewick, 2009).

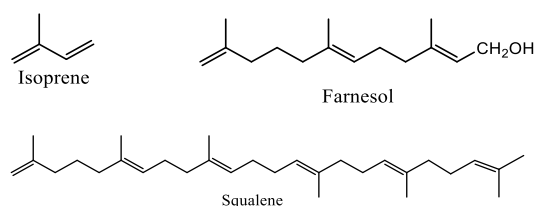
The various classes of flavonoid are usually differentiated by extra oxygen's available as moiety of the heterocyclic ring, and hydroxyl functional groups as well as aryl moieties positions (C_6) (Harborne and Williams, 2000). Flavonoids have two main group namely isoflavone (3- position) and flavone (2-position) by position of benzenoid substituent. Most flavonoids occur naturally with moiety of sugar in conjugated type. It could be group as monoglycosidic, diglycosidic with glycosidic linkage bonded at three or seven

position. The unit of carbohydrate could be galactose, L-rhamnose, glucorhamnose, D-glucose, or arabinose (Pretorius, 2003). As a result of availability of conjugated double bonds, this class of compound is usually brightly coloured (Harborne and Williams, 2000).

Terpenoids

Terpenoids are natural products form from isoprene units consisting of five carbons atoms as basic skeleton. This class of compound consist of many cyclic structures with differences only in basic carbon skeletons, and functional groups. This class of compound is synthesised through isoprenoid pathway. Terpenoids consist of the largest group of natural product and are present in all classes of living things (Elbein and Molyneux, 1999). Terpenoid like menthol are used as fragrances, flavours in foods, and cosmetics industries (Langenheim, 1994).

Terpene is commonly present as essential oils constituents, usually obtained from aromatic plant. Their building block constitute of isoprene unit, which is an hydrocarbon, $CH_2 = C(CH_3)-CH=CH_2$. These hydrocarbons with molecular formula $(C_5H_8)_n$ is grouped based on isoprene units number (Degenhardt *et al.*, 2003). This class of organic compound is largest and commonly widespread group of secondary metabolites typically of lower invertebrates and plants. This class of secondary metabolite are classified according to isoprene unit's number available in their molecule into hemi-, mono-, sesqui-, di-, sester-, tri, and tetraterpenoids (Heras *et al.*, 2003).



The farnesol is a sesquiterpenoid, consisting of three isoprene units bonded head-to-tail. The head of isoprene unit is the branched end while the 'tail' is the unbranched end. Head-to-tail technique is commonly and frequently used technique of bonding isoprene units to form mono-, sesqui-, di-, and sesterterpenoids (Heras *et al.*, 2003). The tail-to-tail method also occurs and is available at the tri- and tetraterpene molecules centre such as squalene and consists of 2 farnesyl residues bonded tail-to-tail.

This compound is classified based on the number of isoprene units in their structure: hemiterpenes 5 carbon atoms (one isoprene unit), monoterpenes ten carbon atoms (two isoprene units), sesquiterpenes fifteen carbon atoms (three isoprene units), diterpenes twenty carbon atoms (four isoprene units), triterpenes thirty carbon atoms (six isoprene units) (Mohammad *et al.*, 2010) tetraterpenes forty carbon atoms (eight isoprene units), and polyterpenes (five carbon atoms)_n where 'n' could be from nine to thirty thousand (McGarvey and Croteau 1995).

Saponins

Saponins are simply bioorganic compounds exist naturally with at least a glycosidic linkage consisting of carbon-oxygen sugar bond at carbon three linking the aglycone and sugar chain. Saponin hydrolysis gives rise to aglycone and glycone the sugar moiety (El Aziz *et al.*, 2019). Saponins are secondary metabolites that exist as glycosides in nature. This class of phytoconstituents is commonly found in plant kingdom, and also available in some animal like

marine invertebrates (Ozlem *et al.*, 2007). This class of compound consist of class of diverse organic molecules usually made up structure of triterpenoid or steroidal aglycone with one or two chains of sugar (Irma *et al.*, 2010).

The saponins structural diversity is seen in their unique biological and physicochemical properties (Bruneton1995; Rao; Francis *et al.*, 2002). Saponin usually foam usually used in fish poison, traditional soaps, molluscicides, and industrial utilizations. A wide range of biological spectrum is usually exhibited by saponins like anti-inflammatory, antiviral, antifungal, insecticidal, anticancer, antibacterial, and cytotoxic. This compound exhibit low cholesterol action in animals and human (Sparg *et al.*, 2004; Ellen *et al.*, 2007; Oboh and Omofoma, 2008; Cheng *et al.*, 2011; and Armelle *et al.*, 2018). The broad spectrum of saponins is attributed to the varying degree of hydroxylation on the aglycone.

Glycosides

Glycoside is classified as secondary metabolites with two molecules, a sugar moiety (glycone) bonded by ether bond to a non-carbohydrate nucleus. Glycosides is group based on structure of aglycone (nucleus). The aglycone nature in glycosides determines the method of classification which could be terpenes, phenols, and steroids (Justin *et al.*, 2014). Glycosides could be phenol, alcohol or sulphur base compounds usually characterized by bitter taste (Tian *et al.*, 2014; Ahmanov *et al.*, 2015). The sugar molecules are basically D-glucose, L-fructose and L-rhamnose.

Aglycone could be any secondary metabolite like terpene or flavonoid (Egamberdieva *et al.*, 2016). Aglycone moiety is released during an enzymatic action when damage occurs on plant tissue through freezing, trampling, mastication, and wilting. The enzymes responsible for hydrolysis are separated from glycosides in plant tissue. Thus, intimate contact of glycosides and enzymes only when plant tissue is disrupted. Some vital glycosides include glycoalkaloids, saponins, glucosinolates, cardiac glycosides, and cyanogenic glycosides.

Tannins

Tannin is categorised as phenolic compounds which can precipitate proteins and consist of different oligomers, and polymers group. This class of metabolite constitute of polyphenolic compounds with heterogeneous moiety are of high molecular weight which form irreversible and reversible complex with cellulose, starch, proteins, and minerals (Justin *et al.*, 2014). Exception of few high molecular weight structures, tannin is a water soluble compound. This class of phytoconstituents are usually synthesized in nature through phenylpropanoid pathway also known shikimic acid pathway. Other phenolics compounds like isoflavones, coumarin, aromatic amino acids, and lignin are also form from this pathway.

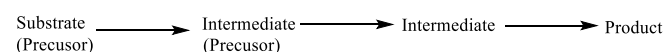
Tannin is group into four major categories based on characteristics of their structure namely condensed tannins, ellagitannins, gallotannins, and complex tannins. Gallotannins is a class of tannins in which their meta-depsidic derivatives or galloyl units are bonded to diverse catechin, polyol, or triterpenoid unit. Ellagitannin is a special class of tannin with at least two galloyl units carbon-carbon linked together without a glycosidic bond linking the catechin unit. Complex tannins are those classes of

organic compounds consisting of glycosidic bond between a catechin unit and ellagitannin unit or gallotannin.

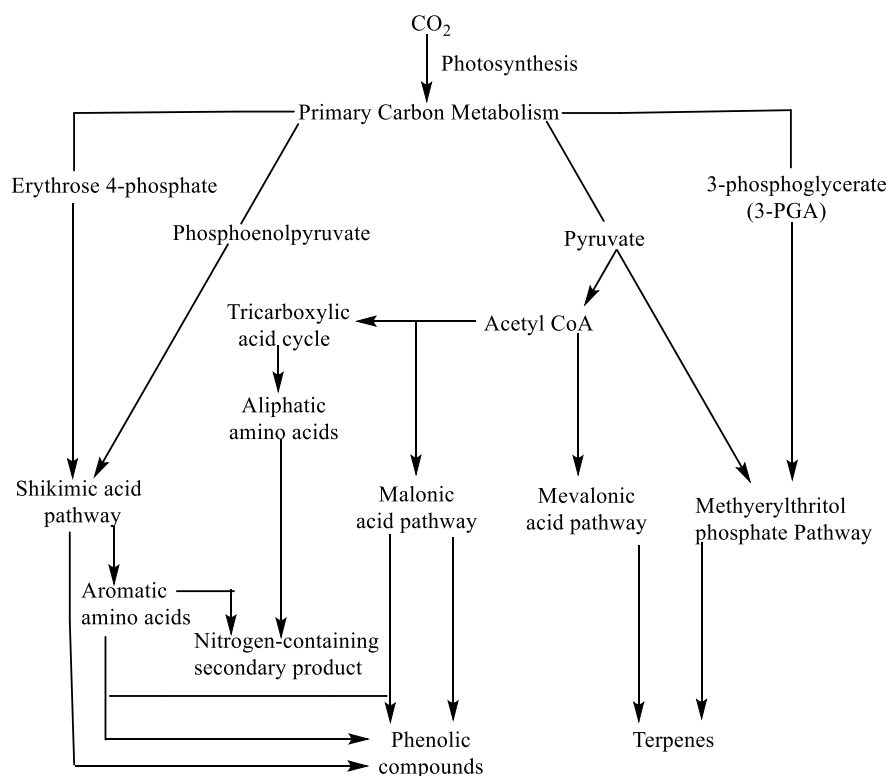
Condensed tannin consist of all polymeric proanthocyanidins and oligomeric compounds formed through the linkage of carbon four of one catechin with carbon eight or carbon six of the next monomeric catechin molecule. Medicinal plant extracts rich in tannins are commonly used as in the treatment of aliment such as diarrhoea, astringents, diuretics, duodenal and stomach tumours, antiseptic, anti-inflammatory, antioxidant, and haemostatic pharmaceuticals. Tannin is used in the production of cationic dyes commonly known as tannin dyes, and inks from gallate ink. This class of compound is use to clarify beer, wine, and fruit juice in the food industry. Tannins are utilised in textile industry, as coagulants in production of rubber, as antioxidants in the fruit juice, beer, and wine industries.

Biosynthesis

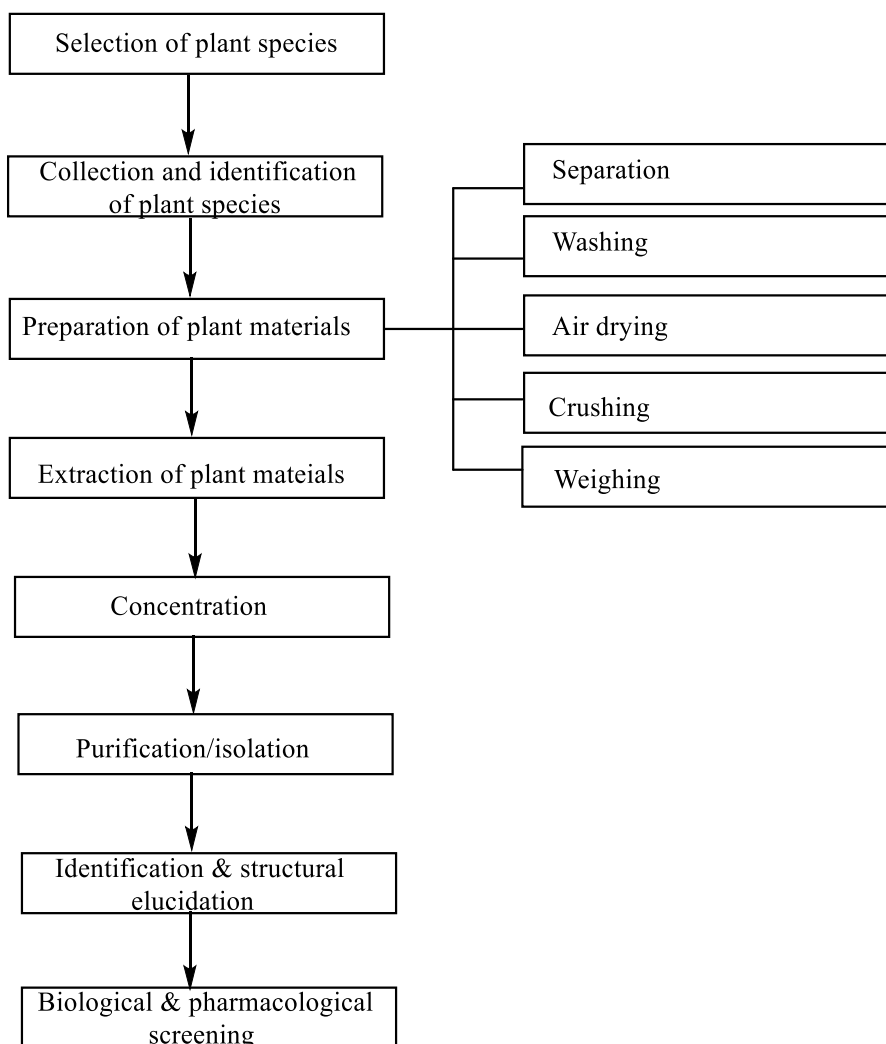
Primary and secondary metabolites are usually obtained through biosynthetic pathways. Basically, three main pathways exist leading to secondary metabolites synthesis in plant such as include shikimate, isoprenoid and polyketide pathways (Mohi, 2019). The biosynthetic pathways are usually obtained from various precursors or starting materials of primary metabolism. Precursor may be defined as a molecule used by biosynthetic enzyme as a substrate or starting materials, and converted into product (Herwig and Jutta, 2014). These products may be an intermediate along the pathway which serves as a precursor for the next biosynthetic enzyme. It could be the final product of the reaction chain. This indicates that an intermediate can simultaneously be utilised as a precursor at different stages in the pathway. Shikimic acid is a known amino acid metabolism intermediate. It also functions as a precursor or starting materials for the biosynthesis of aromatic secondary metabolites.



Biosynthetic pathways are usually an energy consuming processes normally energized by energy released through glycolysis of citric acid cycle, and carbohydrates (Justin, 2014). The process of amino acids, glucose oxidation, and fatty acids leads to generation of adenosene triphosphaphate (ATP) (Mohi, 2019). This molecule with high-energy generated by catabolism process (the production of energy through the conversion of complex molecules into simple ones) of primary compounds. Adenosene triphosphaphate (ATP) are reused in fuelling the anabolic reactions which constitute the intermediate molecule along biosynthetic pathways. The process of catabolism involves starting molecules oxidation and biosynthesis (Justin, 2014). Anabolism involves reduction reaction which utilises a reducing agent or hydrogen donor nicotinamide adenine dunucleotide phosphate (NADP). Coenzymes function as a catalyst, and adenosine diphosphate and pantetheine phosphate (ADP) is a very common type (Michal and Schomburg, 2013). The common biosynthesis pathway is through pentose for polysaccharides, glycosides while phenols utilizes shikimic acid pathway (Justin, 2014), aromatic alkaloids, tannins, phenols uses acetate-malonate, and alkaloids and mevalonic acid for steroids, terpenes, and alkaloids (Wallingford *et al.*, 1999).



Profiling of plant bioactive molecule



Selection of plant species

Plant selection is simply the evaluation and validation of ethnomedicinal information existing already, chemotaxonomical information regarding the choice plant. Information obtained from various historical documents, knowledge on the traditional use of plant from traditional medicine practitioner. Plant collection is usually done based on criteria like

- Documented ethnopharmacological evidence from the native people.
- The various ailments different plants can treat.
- Plant availability in the natural habitat.
- Sustainable usage of various plant parts like leaves, stem, root, bark, or whole plant (Vander Watt and Pretorius, 2001).

Plant species identification involves voucher specimens preparation. This is the vital segment of medicinal plant research. Standard procedures developed for plant materials pre-treatment (Heinrich *et al.*, 2004; Fabricant and Farnsworth, 2001). Traditional healer preparation mode and method of administration should also be considered.

Collection and identification of plant species

Traditional healer collects the medicinal plants and used it immediately or dries and stores for future use. Moreover, this information and art of traditional medicine practise has been preserved in some families. The information remains in the family from one generation to another (Heinrich *et al.*, 2004). Information availability, usually provided by local people provide primary basis of medicinal plants collection. However, especially from people with wide experience in the field and a wealth of empirical knowledge of local plants (Sarker *et al.*, 2006).

Selection of plants can equally be navigated with the help of information gathered from literature establishing plant as a significant source of lead compounds utilized in infectious diseases treatment. This makes medicinal plants collection challenging and plants with same genus are primary focus of many researchers. Thus, this establishes a promising and efficient way of renewable natural resources utilization (Jones and Kingborn, 2005).

Preparation of plant material

Processing of medicinal plant materials is always significant to avoid degradation of plant bioactive principles (Chikezie *et al.*, 2015). Unwanted materials separation from medicinal plant materials is the first stage. Washing of plant materials is vital leading to removal dirty to ascertaining the purity of plant materials. The next stage simply involves air drying of the plant materials in controlled atmosphere area. This is usually done in absence of well-ventilated area, humidity, and constant temperature. The air dried plant materials are pulverised with aid of mechanical techniques. This process reduces plant sample size dimensions ensuring homogenisation of plant materials for good extraction yield (Chikezie *et al.*, 2015; Sarker *et al.*, 2006; Kaufmann and Christen, 2002).

Extraction

Extraction is simply the separating of medicinally active constituents of plant materials utilizing some selective solvent in standard procedure. Medicinal plants analysis is usually the first step, since extraction of desired chemical constituents from plant materials need to be extracted before separation, and characterization (Mohammed, 2018). Extraction of plant material

is a significant process in natural product isolation and purification. The plant matrices naturally are complex which consist of large range of secondary metabolites or active principles with different physical and chemical properties. Examples of extraction processes are pressing, distillation method, sublimation, and solvent extraction (Zhang *et al.*, 2018).

Extraction of secondary metabolite proceeds through various stages such as solvent penetration into solid matrix, solute dissolves in solvents, solute diffuses out of solid matrix, and collection of solutes extracted. The properties of extraction solvent, raw materials solvent-to-solid ration, particle size, extraction temperature, and extraction duration definitely affect extraction efficiency (Du *et al.*, 2011; Yi *et al.*, 2012; Zhou *et al.*, 2012; Li *et al.*, 2014). Factors like time, plant matrix, temperature, and pressure usually affect the extraction processes (Hernández *et al.*, 2009).

Menstruum is simply the solvent utilized in medicinal plants extraction. The choice of menstruum basically relies on the nature of plant type, type of bioactive compounds extracted, and solvent availability. Solvent which is polar like methanol, water, and ethanol are widely employed in polar compound extraction. Solvents like hexane, ethyl acetate, dichloromethane which with less polarity are utilized in nonpolar or less polar compounds extraction (Sasidharan *et al.*, 2011; Pandey and Tripathi, 2014; Altemimi *et al.*, 2017)

Factors to consider in selecting extraction solvents

Myriad factors stipulated below are usually considered in choosing extraction solvent (Eloff, 1998; Das *et al.*, 2010).

- Selectivity is the inert nature of the chosen solvent in the process in extraction of active phytoconstituents.
- Safety deals with deal nature extraction solvent been nontoxic and non-ammable.
- Cost of the extraction been cheap as possible.
- Reactivity of the extraction solvent with the extract.
- Quickly recovery of the solvent extraction from the extract.
- Low viscosity of the extraction solvent will not allow ease of penetration.
- Boiling solvent temperature should be as low as possible to stop heat degrading the plant materials (Eloff, 1998).

Like dissolves like the law of intermiscibility and similarity. Solvents with polarity value close to solute polarity will certainly perform excellently. Solvents like ethanol and methanol are regarded as universal solvents for medicinal plant extraction for phytochemical profile (Sasidharan *et al.*, 2011). Excellent extraction is usually achieved from plant materials with fine particle size. Increase in surface area increases solvents penetration, diffusion and efficiency of extraction is enhanced which reduces particle size of the plant sample. Extremely reduced size of particle will leads to much solute absorbing into solid making filtration challenging (Zhang *et al.*, 2018).

Solubility increase with high temperatures, diffusion lead to solvents lost causing extracts unwanted impurities, and decomposition of components that are thermolabile. Extraction duration increases in a certain range of time could lead to increases

extraction efficiency. As solute equilibrium is reached outside and inside the solid material, increasing extraction time cannot affect the process of extraction (Sasidharan *et al.*, 2011; Azmir *et al.*, 2013; Chua, 2013). High extraction yield is obtained with greater solvent to solid ratio. High ratio of solvent- solid leads to excessive solvent of extraction, and taking a longer concentration time (Zhang *et al.*, 2018).

Bioactive molecules purification and characterisation is often possible after the process of extraction and concentration. Different extraction techniques can be adopted with various conditions establishing extraction selectivity. These techniques are quite the same over hundreds of years (Sasidharan *et al.*, 2011). Each extraction techniques have some objectives like extraction of target bioactive molecules from plant sample that are complex, increasing selectivity of analytical technique, increases bioassay sensitivity thereby increasing the concentration of target compounds, bioactive compound conversion into quite a suitable state for easy detection, good separation, provision of strong, and technique that are easily reproducible independent sample matrix variation (Smith, 2003).

Efficiency of extraction in any conventional technique basically relies on the solvents choice (Cowan, 1999). The target compound polarity is a vital factor for choice of solvent. Molecular affinity of solvent and solute, environmental safety, human toxicity, mass transfer, co-solvent, and financial feasibility are vital in choosing solvent for extraction of bioactive compound.

Features to consider in the choice extraction method

(a) Stability to heat

Plant samples that are stable to heat may be extracted with soxhlet extractor or microwave assisted extraction. Maceration or percolation could be used for plant sample that cannot with stand heat (Azwanida, 2015).

(b) Nature of solvent

Maceration is the most suitable method when water is utilised as solvent. Percolation and Soxhlet extraction is more appropriate for volatile solvent (Majekodunmi, 2015).

(c) Cost of the drug

Maceration is best method for extraction of cheap drugs, whereas percolation is prefer for costly drugs (Azwanida, 2015).

(d) Extraction time

Maceration method is used for plant sample that need long time exposure to menstruum. Extraction techniques such as ultrasound-assisted or microwave are preferred for a limited duration (Azwanida, 2015).

(e) Final volume required

Tinctures which are larger product is prepared through maceration. Soxhlet or percolation or extraction is reliable method for concentrated products (Majekodunmi, 2015).

(f) Intended use

Maceration method is recommended for extracts meant for human consumption, whereas any other methods in addition to maceration can be adopted for products for experimental testing (Majekodunmi, 2015).

Conventional extraction method

Bioactive molecules obtained from medicinal plant samples are usually extracted through different classical extraction methods. These methods strictly based on extracting power of various solvents utilize and application of heat and mixing. The classical method of obtaining bioactive molecules from plants include: maceration, soxhlet extraction, and hydrodistillation (Azmir *et al.*, 2013; Chua, 2013).

Soxhlet extraction

This extraction technique is designed basically for lipid extraction although not limited to lipid. Soxhlet extraction technique is commonly utilized in bioactive constituent's extraction from different medicinal plant (Grigonis *et al.*, 2005). The method serves as a model to compare with the new extraction technique. Generally, a little amount of dry sample is kept in thimble, and distillation flask containing solvent of interest. On getting to the level of overflow, the solution in the thimble holder aspirated through siphon process. Siphoning process unloads the solution into distillation flask. The extracted solution usually carries the solutes into bulk liquid (Rasul, 2011). The distillation flask retained the solute, and passes the solvent into solid plant bed. The extraction process is repeated until is completed.

Maceration

Maceration process utilised a whole or coarsely grounded plant sample, kept in a container with stopper containing organic solvent. The process continues and kept at ambient temperature for a minimum of three days with regular agitation for the soluble matter to dissolve. Maceration is quite a simple technique of extraction with a longer time of extraction, and lower extraction efficiency as demerit. It can be utilized for thermolabile constituent extraction (Zhang *et al.*, 2018). Maceration involves steps and the process is widely used for preparation of tonic at home. The method is inexpensive for essential oils, and bioactive constituent's extraction. The first stage involves grinding of plant samples into smaller particle sizes thereby increasing the surface area for good mixing with solvent.

Addition of good menstruum in a vessel with lid is the second stage (Azmir *et al.*, 2013). The third stage involves straining of liquid off. The marc which is the solid residue of the process of extraction is pressed to recover much quantity of occluded solutions. The strained obtained, mixing of the press out liquid, and impurities separated by processes of filtration. Maceration process involves occasionally shaking to facilitate increase diffusion and removal of solution concentrated from plant materials surface, introducing new solvent to extraction solvent or menstruum for greater yield in the extraction process (Azmir *et al.*, 2013).

Hydrodistillation

Hydrodistillation technique is widely known as traditional method of bioactive constituents, and essential oils extraction from plants source. This process can be done before plant samples dehydration. Generally, basically there are three methods of hydrodistillation process namely steam distillation, direct steam distillation, and water distillation (Vankar, 2004). Hydrodistillation technique simply entails the packing of plant samples into compartment with large amount of water and boil. Direct steam can be injection into plant materials alternatively (Azmir *et al.*, 2013).

Steam and hot water serve as a major influential factor to release the bioactive molecule from tissue of plant. Cooling indirectly with water condenses the vapour mixture of oil and water. The condensed mixture flows through the condenser to separator, where phytoconstituents and oil are automatically separate from water (Silva *et al.*, 2005). The main three physicochemical processes of hydrodistillation process include hydrodiffusion, hydrolysis, and decomposition by heat. Some volatile constituents will be lost when the extraction temperature is high. This limits the use of thermo labile compound extraction.

Non-conventional extraction techniques

Extraction process takes a longer time which is a major challenge in conventional extraction. The requirement is expensive with high purity solvent, low extraction selectivity, during evaporation large amount of solvent, and thermo labile constituent's thermal decomposition (Luque and Garcia, 1998). New and promising extraction methods are used to get rid of limitations of these extraction techniques. The promising methods are pulsed electric field assisted extraction, pressurized liquid extraction, microwave assisted extraction, ultrasound assisted extraction, and enzyme-assisted extraction, and supercritical fluid extraction (Selvamuthukumaran and John, 2017).

This method is well known as nonconventional extraction techniques and known as green techniques. These techniques are less toxic, hazardous, design to stop degradation, designing safer chemicals, catalysis, and design for efficiency of energy, safe solvents auxiliaries, and utilized of feedstock that are renewable, reduce derivatives, time analysis for pollution prevention, atom economy, and inherently safer chemistry for accident prevention.

Ultrasonic-assisted extraction

Ultrasound-assisted extraction (UAE) is an extraction method involving the utilization of maceration setup with the aid of ultrasound waves in the process of extraction (Chemat *et al.*, 2017). Ultrasound technique reduces time to minutes in the extraction process, reduction of solvent volume, and less energy consumption compared to other techniques of extraction. The extraction systems widely used Erlenmeyer flask connected to ultrasound bath and ultrasound reactor, constant stirring with ultrasound probe. The bioactive constituent's extraction from plant samples, ultrasound bath is quite the best, although the ultrasonic probes must be of high power as preferred for some applications.

Transducers are used as ultrasound wave's source in both systems (Chemat *et al.*, 2017). Different mechanisms are identified that help ultrasound effectiveness leading to extraction efficiency increase: fragmentation, local shear stress, sonoporation, erosion, sonocapillary effect, detexturation, and destruction structures of plant (Chemat *et al.*, 2017). This method of extraction is used with immiscible solvents mixtures of like hexane with water/methanol. This process will create heat that decomposes heat labile constituents (Chemat *et al.*, 2017). The container of extraction is usually kept in ice bath for temperature reduction in such condition.

Microwave-assisted extraction (MAE)

Microwave extraction method combines traditional solvent extraction and microwave. Extraction kinetics increases with solvent heating and plant tissue with microwave (Delazar *et al.*, 2012). Heating of well dried plant sample is in minute and

microscopic traces of moisture occur in the cells of plant. Evaporation and generation of large quantity of pressure on cell wall, moisture heating inside the cell of the plant is due to microwave effect leading to pushing and cell wall ruptures of cell wall. The exudation of active components in the ruptured cells occurs, leading to increase in the phytoconstituents yield (Krishnananda *et al.*, 2017)



Supercritical fluid extraction (SFE)

This extraction technique involves the use of supercritical fluid as extracting solvent in chemical extraction process (Herrero *et al.*, 2010). Any substance with pressure and temperature above its critical point is a supercritical fluid. The technique has no special gas phase or liquid instated a state between these two extremes, capable of effusing through solids such as gas and dissolve molecules such as liquid. Carbon dioxide commonly utilised as supercritical fluid during extraction. The technique is usually adopted for large-scale coffee beans decaffeination (Capuzzo *et al.*, 2013).

The equipment withdraws liquid carbon dioxide from a carbon dioxide tank first, heat and pressurizes beyond critical pressure (74 bar), temperature (31°C), and either supercritical flows carbon dioxide with biomass for extraction or filling the vessel containing biomass during static extraction (Krishnananda *et al.*, 2017). Separation of the extract from carbon dioxide after a predetermined contact period is usually done by the instrument. The gas condenses back to liquid state, and returns to tank for storage. Alternatively, carbon dioxide usage could be vented, leaving the extract in collection vessel. Due to hydrophobicity, carbon dioxide is a poor solvent for molecules that are polar (Fornari *et al.*, 2012).

The process can be circumvented by cosolvents pumping like methanol with biomass and supercritical carbon dioxide, leading to increase in the range of compounds extracted. These limitations create supercritical fluid extraction a niche in hydrophobic constituent's extraction, and analysis like essential oils (Pourmortazavi and Haji mirsadeghi, 2007). Cost is another limiting factor which arises primarily from pressurizing of carbon dioxide. When extraction speed and selectivity are considered supercritical fluid extraction is often utilised. Since various cosolvents may be utilized,

characteristics of supercritical fluid could be adjusted due to pressure, and temperature change, extractions can be carried out selectively (Stenholm *et al.*, 2013).

This could be done in a relative short time because of enhanced properties of diffusion in supercritical fluid compared to liquids. They are largely circumventing thermally degrading and oxidizing extracted constituents. The process involve less cost to dispose of example carbon dioxide could be free into air, and contaminant free, inexpensive, nontoxic, and eco-friendly (Patil and Shettigar 2010; McCloud, 2010).



Solvent extraction method

This is a universal extraction method currently utilised in extraction. The dried pulverised samples kept in a glass thimble during extraction with different solvents (Krishnananda *et al.*, 2017). The process takes place for 10 cycles for each plant extract. Adjustment of temperature is usually done below the solvent boiling point. The resulting solvent in the extract is filtered, vacuum concentrated, and kept for phytoconstituents analysis (Harborne, 1973).



Enzyme assisted extraction (EAE)

This extraction employs solvents with different selected enzymes depending on best performance environments, and catalyses organic compounds pathway needed. Some enzymes basically utilised in this method of extraction technique are protease, lipase, and phospholipase with effective reduction in solvents (Ncube *et al.*, 2008). Moreover, essential oils employed pectinase and α -amylase as appropriate enzymes. This extraction technique could not degrade constituents but setup is expensive. It is unavoidable because of nutrients, oxygen and temperature required in optimization of the process.

Purification/Isolation methods

Current chromatographic method of purification and spectroscopic method of bioactive constituent's analysis is simple but extraction methods adopted are the determining factor, input parameters and plant parts (Azmir *et al.*, 2013). Characteristics such as solubility, nature of extraction solvent, stability, and molecular weight, targeted bioactive compounds dipole moment are vital for efficiency in isolation process (Kaufmann and Christen, 2002; Sarker *et al.*, 2006; Sasidharan *et al.*, 2011). Generally, various chromatographic methods like flash chromatography, thin layer chromatography (TLC), column chromatography and high performance liquid chromatography (HPLC) are usually adopted for isolation of plant metabolite (Kaufmann and Christen, 2002) gas chromatography (GC), (Sasidharan *et al.*, 2011).

A biological plant material with dearth of information needs an appropriate isolation procedure to be developed starting from phytochemical evaluation, and bioassay (Sarker *et al.*, 2006). Isolation of natural products is widely classified into three groups such as activity guide isolation. This type of isolation involves the origin of activity, and structure guide isolation. This isolation technique searched for new or novel structure and, chemotaxonomical study. The isolation technique provides the information between relationship between histo, and chemo type. Each of this groups constitute of different idea involving a distinct strategy of isolation. Particularly, bioactivity guided and new compound isolation methods are different in quantity and methodology of fractionation adopted. The fractions of interest in these two techniques are basically different.

Identification and structural elucidation

This process is usually the first but quite challenging step in chemistry of natural product. Bioactive components identification and structure elucidation utilizes advanced knowledge and cutting-edge technology (Sarker *et al.*, 2006; Neda *et al.*, 2012; Chikezie *et al.*, 2015). Profiling of plant bioactive constituents strictly based on different techniques of spectroscopy, and higher chromatographic techniques like hyphenated techniques. A total morphostructural characterisation procedure is done with polarimetry, electronic microscopy, and x-ray crystallographic techniques (Segneanu *et al.*, 2012; Guo *et al.*, 2012; Ibekwe and Ameh, 2015). Optimal strategy employed a high technology which provides highly and fast efficient information on structure of the target compounds (Segneanu *et al.*, 2012; Sarker *et al.*, 2006).

Spectroscopic methods	UV-Visble spectroscopy
Infra-red	FT- Infra-red
Mass spectrometry: Nuclear Magnetic Resonance (NMR) spectroscopy:	(i) EIMS (ii) ESIMS (iii) CIMS (iv) FABMS (v) ESIMS (vi) One-dimensional techniques: ^1H NMR, ^{13}C J mod, ^{13}C DEPT, ^{13}C NMR, ^{13}C PENDANT, (ii) Two-dimensional techniques: ^1H - ^{13}C HMQC, ^1H - ^1H COSY, ^1H - ^{13}C HSQC, ^1H - ^1H COSY-Ir, ^1H - ^1H NOESY, ^1H - ^1H ROESY, ^1H - ^1H DQF-COSY, ^1H - ^1H TOCSY, ^1H - ^{13}C HMBC, and HSQCTOCSY
Chromatography techniques	Thin layer chromatography, High pressure liquid chromatography, and Column chromatography. Gas-chromatography: GC, GC-TOF-MS; GC-MS, GC-MS/MS, two-dimensional GC coupled with mass spectrometry GC-FTIR, (GC×GC-MS), GC-NMR Liquid chromatography: LC/NMR, LC/UV; LC/UV/MS; LC/MS; LC/MS-MS; LC- HPLC-NMR and UV-DAD,
Other analytic techniques	TEM; XRD; polarimetry

Conclusion

Medicinal plants are rich in phytoconstituents, and widely utilized in herbal medicine to treat different health hazards. Phytoconstituents with good biological potency are considered as an synthetic drugs alternative due to their availability, cheap, and safe. Although only a small portion of the existing medicinal plant species have been scientifically tested for their bioactivities. Natural products form medicinal plant, and traditional medicines have already made fruitful insight in modern medicine. The extraction and separation of natural product always creates vast challenges for characterization and identification process.

References

- Ahmanov, G., K. Alipieva, P. Denev, D. Todorov, A. Hinkov, S. Shishkov, S. Simova, and M.I. Georgiev. (2015). Flavonoid glycosides profiling in dwarf elder fruits (*Sambucus ebulus* L.) and evaluation of their antioxidant and anti-herpes simplex activities. *Industrial Crops Products* 63:58-64.
- Altemimi, A., Lakhssassi N., Baharlouei, A., Watson, D. G., Lightfoot, D. A. (2017) Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*: 6:42.
- Amirkia V, and Heinrich M. (2014) Alkaloids as drug leads – A predictive structural and biodiversity-based analysis. *Phytochem Lett*; 10:48-53.
- Aniszewski T. 2007 Alkaloids – secrets of life: Aklaloid Chemistry, Biological Significance, Applications and Ecological Role. 1st Ed. Elsevier Science Press: 334.
- Armelle T Mbaveng, Ndontsa BL, Kuete V, A (2018) Naturally occurring triterpene saponin ardisiacrispin B displayed cytotoxic effects in multi- factorial drug resistant cancer cells viaferroptotic and apoptotic cell death. *Phytomedicine*: 43(1):78–85.
- Arvind, K. S. (2016) Medicinal plants: Future source of new drugs *International Journal of Herbal Medicine* 4(4): 59-64.
- Azmir, J., Zaidul, I.S.M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A. Ghafoor, K., Norulaini, N. A. N., (2013) Techniques for extraction of bioactive compounds from plant materials: A review *Journal of Food Engineering* 117: 426–436
- Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif K.M. and Mohamed, A. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *J. Food Eng.*, 117: 426-436.
- Azwanida, N. N. (2015). A review on the extraction methods used in medicinal plants, principle, strength, and limitation *Med Aromatic Plants*: 4:196.
- Bruneton J. (1995). Pharmacognosie, Phytochimie, Plantes Me´dicinales; E´ditions Technique & Documentation, Paris, p 1120
- Capuzzo A., Maffei M. E., and Occhipinti, A. 2013 Supercritical fluid extraction of plant flavors and fragrances. *Molecules* 18:7194–7238
- Cheng T. C, Lu J. F, Wang J. S. (2011) Antiproliferation effect and apoptosis mechanism of prostate cancer cell PC–3 by flavonoids and saponins prepared from *Gynostemma pentaphyllum*. *J Agric Food Chem.* 59(20):11319–11329.
- Chikezie P C., Ibegbulem, C. O., Mbagwu, F. N. (2015) Bioactive principles from medicinal plants. *Research Journal of Phytochemistry* 9(3):88–115
- Chikezie, Paul C., Chiedozi O. Ibegbulem and Ferdinand N. Mbagwu (2015) Bioactive Principles from Medicinal Plants *Research Journal of Phytochemistry* 9 (3): 88-115

15. Chua, L. S., (2013). A review on plant-based rutin extraction methods and its pharmacological activities. *J. Ethnopharmacol.*, 150: 805-817.
16. Cowan, M. M., (1999) Plant products as antimicrobial agents *Clinical Microbiology Reviews* 12 (4), 564–582.
17. Cushnie, T. P. T., B. Cushnie, and A. J. Lamb (2014). Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *Int. J. Antim. Agents* 44(5):377-386.
18. Das, K., Tiwari R. K., Shrivastava D. K. (2010) Techniques for evaluation of medicinal plant products as antimicrobial agents: Current methods and future trends. *J Med Plants Res* 4:104-11.
19. Degenhardt J, Gershenzon J, Baldwin I. T, Kessler A. (2003). Attracting friends to feast on foes: Engineering terpene emission to make crop plants more attractive to herbivore enemies. *Current Opinion Biotechnology* 14: 169–176.
20. Delazar A., Nahar L., Hamedeyazdan S., Sarker S. D. (2012) Microwave-assisted extraction in natural products isolation. *Methods Molecular Biology*. 864:89-115.
21. Dewick, P. M. (2009) Medicinal natural product, a biosynthetic approach, 3rd edition. John Wiley and Sons: West Sussex.
22. Dey, A. and De, J. N. (2015) Neuroprotective therapeutics from botanicals and phytochemicals against Huntington's disease and related neurodegenerative disorders. *J. Herbal Med.*, 5: 1-19
23. Du, G., Zhao, H. Y., Song, Y. L., Zhang, Q. W., Wang, Y. T. (2011) Rapid simultaneous determination of isoflavones in *Radix puerariae* using high-performance liquid chromatography-triple quadrupole mass spectrometry with novel shell-type column. *J Sep Sci*. 34(19):2576–85
24. Durairaj Thirumurugan, Alagappan Cholarajan, Suresh S.S. Raja and Ramasamy Vijayakumar (2018) An Introductory Chapter: Secondary Metabolites. Interchopen 2-9
25. Edeoga, H.O., D.E. Okwu, and B.O. Mbaebie, (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4(7):685-688.
26. Egamberdieva, Dilfuza., Nazim Mamedov, Elisa Ovidi, Antonio Tiezzi, Lyle Craker (2016) Phytochemical and Pharmacological Properties of Medicinal Plants from Uzbekistan: A Review *Journal of Medicinally Active Plants* Vol. 5, Iss. 2
27. El Aziz MMA, Ashour A. S, Melad ASG. (2019) A review on saponins from medicinal plants: chemistry, isolation, and determination. *J Nanomed Res*. 8(1):6–12.
28. Elbein A. D, Molyneux R. J. (1999). *Comprehensive Natural Products Chemistry*, Vol. 3, Barton D and Nakanishi K, ed. Amsterdam 129
29. Ellen De Geyter, Ellen Lambert, Danny Geelen., (2007) Novel advances with plant saponins as natural insecticides to control pest insects. *Pest Technology*. 1(2):96–105.
30. Eloff, J. N. (1998) Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal Ethnopharmacol* 60:1-8.
31. Fabricant D. S, and Farnsworth, N. R. 2001 The value of plants used in traditional medicine for drug discovery. *Environ Health Perspective*: 109:69-75.
32. Fornari T., Vicente G., Vazquez E., GarciaRisco M. R., Reglero G. (2012) Isolation of essential oil from different plants and herbs by supercritical fluid extraction. *J. Chromatogr A* 1250:34–48
33. Grigonis, D., Sivik, P., Sandahl, M. and Eskilsson, C. (2005) Comparison of different extraction techniques for isolation of antioxidants from sweet grass (*Hierochloa odorata*). *Journal of Supercritical Fluids* 33(15), pp. 223–233.
34. Guo, X., Lankmayr E. (2012) Hyphenated techniques in gas chromatography. In: Mohd MA, editor. *Advanced Gas Chromatography—Progress in Agricultural, Biomedical and Industrial Applications*. InTech; Croatia. pp.5–26. ISBN: 978-953-51-0298-4
35. Hagel, J. M., Morris, J. S., Lee, E. J., Penix, I. D., Bross, C. D., Chang L, (2015) Transcriptome analysis of 20 taxonomically related benzyloisoquinoline alkaloid-producing plants. *BMC Plant Biol*; 15:1–16.
36. Harborne, J. B. (1973). *Phytochemical methods*, Chapman and Hall Ltd., London.49-88.
37. Harborne, J. B., Williams, C. A. (2000) *Advances Research in Flavonoids since 1992. Phytochemistry*: 55:481-504
38. Heinrich, M. J., Barnes, S., Gibbons and Williamson, E. M., (2004). *A Text Book of Fundamentals of Pharmacognosy and Phytotherapy*. 1st Edn., Elsevier, USA., pages: 309.
39. Heras, B. de las., Rodríguez, B., Boscá, L., and Villar, A. M. (2003) Terpenoids: Sources, structure elucidation and therapeutic potential in inflammation. *Current topics in medicinal chemistry* 3:171-185
40. Hernández, Y., Lobo, M. G., González, M., (2009) Factors affecting sample extraction in the liquid chromatographic determination of organic acids in papaya and pineapple. *Food Chemistry* 114 (2), 734–741.
41. Herrero, M., Mendiola J. A, Cifuentes A, and Ibanez E (2010) Supercritical fluid extraction: recent advances and applications. *J Chromatogr A* 1217:2495–2511
42. Herwig, O. G. and Jutta, L. (2014) *Plant Natural Products: Synthesis, Biological Functions and Practical Applications*, First Edition. Wiley-VCH Verlag GmbH & Co. KGaA. Published 2014 by Wiley-VCH Verlag GmbH & Co. KGaA.
43. Hussain, F., Rana Z, ShaH, Malik A, Hussain Z. (2017) Phytopharmacological potential of different species of *Morus alba* and their bioactive phytochemicals. A review: 7: 950–956.
44. Ibekwe N. N, Ameh S. J. (2015) Hyphenated techniques in liquid chromatography as current trends in natural products

- analysis. *International Research Journal of Pure &Applied Chemistry* 7(3):132-149.
45. Irma P., Agnieszka, G. and Danuta, S. (2010) Saponins as cytotoxic agents: a review *Phytochem Rev* 9:425–474
46. Jones, W. P. and Kinghorn, A. D. (2012). Extraction of plant secondary metabolites. *Methods Molecular Biology* 864:341–366
47. Joy, P. P., Thomas, J., Samuel, M. B., Skaria, P. (1998) medicinal plants kerala agricultural university Aromatic and Medicinal Plants Research Station DS. Plant Secondary Metabolism. New York: Springer Science: Business Media; 1995. ISBN: 978-1-4613-7228-8; ISBN: 978-1-4615-4913-0 (eBook). DOI: 10.1007/978-1-4615-4913-0
48. Justin N. Kabera¹, Edmond Semana¹, Ally R. Mussa¹ and Xin He (2014) Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties. *Journal of Pharmacy and Pharmacology* 2: 377-392
49. Kaufmann, B. and Christen P. (2002) Recent extraction techniques for natural products: Microwave- assisted extraction and pressurized solvent extraction. *Phytochemical Analysis* 13(2):105–113.
50. Krishnan R, Chandravada, M. V., Ramachander, P. R., Bharathkumar, H. (1983) Inter-relationships between growth and alkaloid production in *Catharanthus roseus* G. Don. *Herba Hungarica* 22: 47-54
51. Krishnananda, P., Ingle, A. G., Deshmukh, D. A., Padole, M. S., Dudhare, M. P., Moharil and Vaibhav C. K. (2017) Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry* 6(1): 32-36
52. Langenheim J. H. (1994) Higher plant terpenoids: A phytocentric overview of their ecological roles. *Journal of Chemical Ecology*, 1994; 20: 1223-1280.
53. Li, P., Yin, Z. Q., Li, S. L., Huang, X. J., Ye, W. C., Zhang, Q. W. (2014) Simultaneous deter- mination of eight favonoids and pogostone in *Pogostemon cablin* by high performance liquid chromatography. *Journal Liquid Chromatography Related Technology*.37(12):1771–84.
54. Luque-de-Castro, M. D., Garcia-Ayuso, L. E. (1998). Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *Analytica Chimica Acta*, 369: 1–10.
55. Majekodunmi, S. O. (2015) Review of extraction of medicinal plants for pharmaceutical research. *MRJMMS*: 3:521-7
56. Mamta Saxena, Jyoti Saxena, Rajeev Nema, Dharmendra Singh and Abhishek Gupta (2013) Phytochemistry of Medicinal Plants *Journal of Pharmacognosy and Phytochemistry* Vol. 1 No. 6 Page 168
57. Mamta, S, Jyoti, S., Rajeev, N., Dharmendra, S. and Abhishek, G. (2013) Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry* Vol. 1:168
58. Mazid M, Khan TA , Mohammad F. Role of secondary metabolites in defense mechanisms of plants *Biology and Medicine*, 3 (2) Special Issue: 232-249, 2011
59. McChesney, J. D., S. K. Venkataraman, and J.T. Henri. (2007). Plant natural products: Back to the future or into extinction? *Phytochem.* 68:2015- 2022.
60. McCloud T. G (2010) High throughput extraction of plant, marine and fungal specimens for preservation of biologically active molecules *Molecules* 15:4526–4563
61. Michal, G., and Schomburg, D. (2013). Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology. 2nd ed. Wiley, 416.
62. Mohamed Ashour, Michael Wink and Jonathan, Gershenzon (2010) biochemistry of terpenoids: monoterpenes, sesquiterpenes and diterpenes *Annual plant reviews* 40, 258–303.
63. Mohammed Golam Rasul (2018) Extraction, Isolation and Characterization of Natural Products from Medicinal Plants *International Journal of Basic Sciences and Applied Computing* 2(6)1-6
64. Mohi Uddin. (2019) Environmental Factors on Secondary Metabolism of Medicinal Plants. *Acta Scientific Pharmaceutical Sciences* 3.8: 34-46
65. Ncube, N. S., Afolayan, A. J. and Okoh, A. I. (2008) " Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends," *African Journal of Biotechnology.*, vol. 7, pp. 797-1806.
66. Neda I, Vlazan P, Pop R. O, Sfarloaga P, Grozescu I, Segneanu, A. E. (2012) Peptide and amino acids separation and identification from natural products. In: Krull IS, editors. *Analytical Chemistry*. Croatia: Intech;. pp.135–146. ISBN 978-953-51-0837-5
67. Negi, J. S., Singh, P. and Rawat, B. (2011). Chemical constituents and biological importance of swertia: A review. *Curr. Res. Chem.*, 3: 1-15
68. Oboh, H. A. and Omofoma, C. O., (2008) The effects of heat treated lima beans (*Phaseolus lunatus*) on plasma lipids in hypercholesterolemic rats. *Pak J Nutr.* 7(5):636–639.
69. Ozlem G " Uc" , Lu- " Ust " Unda " G" and Giuseppe Mazza (2007) Saponins: Properties, Applications and Processing *Critical Reviews in Food Science and Nutrition*, 47:231–258
70. Pandey, A., and Tripathi S., (2014) Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *Journal Pharmacogn Phytochem*: 2:115-9
71. Patil, P. S. and Shettigar, R. (2010). An advancement of analytical techniques in herbal research *Journal of Advanced Scientific Research*, 1(1) pp.8-14.
72. Phillipson J. David (2001) Phytochemistry and medicinal plants *Phytochemistry* 56 237-243

73. Pourmortazavi S. M., Hajimirsadeghi, S. S. (2007) Supercritical fluid extraction in plant essential and volatile oil analysis. *J. Chromatogr A* 1163:2–24
74. Pretorius, J. C. (2003). Flavonoids: A Review of Its Commercial Application Potential as Anti-Infective Agents. *Current Medicinal Chemistry Anti Infective Agents* 2: 335-353.
75. Rasul, M. G. (2011.) Extraction, Isolation and Characterization of Natural Products from Medicinal Plants. *International Journal of Basic Sciences and Applied Computing* 2(6), pp. 1 -6
76. Sarker S. D. and Nahar L (2012) An introduction to natural products isolation. *Methods Molecular Biology* 864:1–25
77. Sarker, S. D., Latif, Z. and Gray, I. (2006). Natural Product Isolation. 2nd Edn., Humana Press Inc., Totowa, New Jersey.
78. Sasidharan, S., Chen Y., Saravanan D., Sundram, K. M., and Yoga, L. L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines* 8(1):1–10
79. Segneanu, A. E., Gozescu, I., Dabici, A., Sfirloaga, P., Szabadai, Z. (2012) Organic compounds FT-IR spectroscopy. In: Uddin J, editor. Macro to Nano Spectroscopy. Croatia: InTech; pp.145–164. ISBN 978-953-51-0664-7
80. Seigler D. S. (1995) Plant Secondary Metabolism. New York: Springer Science: Business Media; ISBN: 978-1-4613-7228-8; ISBN: 978-1-4615-4913-0 (eBook). DOI: 10.1007/978-1-4615-4913-0
81. Selvamuthukumar, M. and John Shi (2017) Recent advances in extraction of antioxidants from plant by-products processing industries. *Food Quality and Safety* 1, 61–81
82. Smith, R. M., (2003) Before the injection modern methods of sample preparation for separation techniques *Journal of Chromatography A* 1000 (1–2), 3–27.
83. Sparg S. G., Light M. E., van Staden J. (2004) Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology*. 94(2–3):219–243.
84. Stenholm A, Goransson U, and Bohlin L. (2013) Bioassay-guided supercritical fluid extraction of cyclooxygenase-2 inhibiting substances in *Plantago major* L. *Phytochem Anal* 24:176–183
85. Tian, XR., G.T. Feng, Z.Q. Ma, N. Xie, J. Zhang, X. Zhang, and H.F. Tang. (2014). Three new glycosides from the whole plant of *Clematis lasiantha* Maxim and their cytotoxicity. *Phytochemistry Letters* 10:168-172.
86. Vankar, P. S., (2004) Essential oils and fragrances from natural sources. *Resonance* 9 (4), 30–41.
87. Wallingford, U. K., Benzie, I. F. F., and Strain, J. J. (1999). “Ferric Reducing/Antioxidant Power Assay: Direct Measure of Total Antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration.” *Methods in Enzymology* 299: 15-27.
88. Yi, Y., Zhang, Q. W., Li, S. L., Wang, Y., Ye, W. C., Zhao, J., and Wang, Y. T. (2012) Simultaneous quantification of major flavonoids in “Bawanghua”, the edible flower of *Hylocereus undatus* using pressurised liquid extraction and high performance liquid chromatography. *Food Chemistry* 135(2):528–33.
89. Zhang, L. L. and Wen-Cai Y. (2018) Techniques for extraction and isolation of natural products: a comprehensive review Qing-Wen *Chinese Medicine* 13:20 <https://doi.org/10.1186/s13020-018-0177->
90. Zhou, Y. Q., Zhang, Q. W., Li, S. L., Yin, Z. Q., Zhang, X. Q., Ye, W. C. (2012) Quality evaluation of semen oroxyli through simultaneous quantification of 13 components by high performance liquid chromatography. *Curr Pharm Anal.* 8(2):206–13.