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General Extraction, Isolation and Characterization Techniques in Drug Discovery: A Review

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Abstract

Extraction, isolation and characterization techniques of compounds from plant tissues are the basic foundations of drug discovery from plants. Research has been conducted using these techniques and publications are available on the same. However, it is important to put them together and discuss their fundamental descriptions including comparative advantages and disadvantages. The aim of this work is to provide choices in the field of drug discovery and allied research areas on the optimal techniques that can be employed. Through a basic literature review method on the techniques that are systematically employed in drug discovery from natural sources, students, researchers and project managers can make an informed choice of the techniques to employ in their projects.

Keywords: Extraction; isolation; characterization; elucidation; drug discovery.

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1. Introduction

Drug discovery from natural sources demands a pursuit of multifaceted approach to scientific investigation. It requires a good understanding of the ethno uses, chemistry, pharmacology, biology, and toxicology, among other disciplines, all to understand drug or drug-like activities [1]. Drugs from plants are embedded well inside complex plant tissue framework. Drug discovery from these natural plant sources demands to know the actual active ingredients responsible for a specific or multiple pharmacological activities; its nature and properties. The first step involves the acquisition of the knowledge on its ethno use which directs scientists to appropriate extraction methods to use. Chemistry and other scientific investigation disciplines play a central role in drug discovery, where the active drug substance has to be isolated from the complex framework of plant materials, characterized and tested [2]. Various techniques are currently applied to free the active plant compound, separate it from others and identify it. Depending on the nature of the desired group of compounds, if already known, compounds are extracted at high, low or normal temperatures. Drug discovery criteria include therapeutic and quality evaluation of the drug and pharmaceutical product; their purity, uniformity, content, physical and chemical stability, bioavailability and identity characterization [3] which requires the extracted plant drug materials to be isolated identified and developed further. Evaluation of ethno use of plants against diseases is done through community surveys, previous literature reviews, outcomes of community interventions, media reports and community observations [4, 5]. Extraction is carried out using different techniques in various solvent systems [6]. Isolation is done using chromatographic techniques and then majorly, spectroscopic techniques are used to identify the characteristics of the compounds isolated [7]. Reference resource materials to researchers, students and policy makers are required in the field of drug discovery. This review paper presents various compounds' extractions, isolation and characterization techniques in general that are basically essential in drug discovery.

2. Extraction methods

Plant material extraction is a crucial process in the isolation of natural plant compounds and their purification. Plant matrices naturally are complex, containing a wide range of compounds that have various physical and chemical properties [8]. It is therefore imperative to carefully, isolate from the rest of the plant, matrices and make pure, compounds of interest in plants for their characterization. There are several ways extraction methods can be categorized [9]. In this chapter, they have been categorized based on the temperatures they work under.

2.1 Low or room Temperature methods

2.1.1 Cold extraction method

The method has been described in literature [10, 11]. Briefly, dried plant parts samples (Cut, crushed or milled) are put in various solvents for seven days, with shaking every 24 hours. The samples are then filtered using a Whatman filter paper and dried under vacuum at room temperature on pre-weighed watch glasses and the mass of yield determined by difference. One common example of cold extraction is maceration. In this method, coarsely powdered plant part or whole plant material is stored in contact with a solvent for some time with

regular shaking. This releases soluble matter dissolved in the solvent [12]. The overall advantage of low temperature methods of extraction is that they are simple, cheap, environmentally friendly [6] and can be done even in the field. Their disadvantage is that they may not avail all compounds from the plant matrices.

2.1.2 Enzyme Assisted Extraction (EAE)

This method employs solvents with various enzymes selected depending on the environments they perform the best and the pathway in mind that the scientist needs the compounds to be catalyzed. Some of the enzymes generally used in the extraction are protease, lipase and phospholipase and they effectively reduce the use of solvents [13]. Specifically for essential oils, pectinase and α -amylase are the mostly used enzymes. The method is non-degrading to compounds but the setup is costly. It is also too demanding in terms of nutrients required, oxygen presence and temperature optimization.

2.1.3 Plant tissue homogenization

Fresh, wet or dried parts of plants are ground and soaked in solvents. The mixture is either vigorously shaken for 5 to 10 minutes or let stand for 24 hours with regular shaking and then the extract is filtered. The extract filtrates may be centrifuged for clarified concentration then dried. Sometimes the filtrates may be directly dried under reduced pressure and then re-dissolved using solvents of interest [14]. Most times, this requires a laboratory set up, and cannot be easily done in the field.

2.1.4 Ionic Liquid Extraction

In this method, organic salts in liquid form interact selectively with polar and nonpolar compounds using π -stacking interactions, hydrogen bonding, ion exchange and hydrophobic interactions [15]. It is a very good method that recovers organic and inorganic ligands in high yields. Due to the ionic interactions, the quality and efficiency of the extraction is very high.

2.2 High temperature extraction methods

Extraction in high temperatures should be conducted on compounds that are known to be thermally stable [16, 17]. Although extraction under elevated temperatures is feared by many to be denaturing to essential compounds, it is not all compounds that get destroyed. A research done on extraction of *Asiatica cantella* at 90°C, reported increased phenolic compounds and antioxidant activities [18]. It is therefore important to understand the nature of the compounds of interest in the plant materials.

2.2.1 Decoction

This method involves boiling in water for 15 minutes, cooling, straining and passing appropriate amounts of cold water into the drug to give required volumes. Heat stable and water soluble compounds of interest from herbal plants can be extracted using this method [17]. This method yields more oil-soluble compounds than infusion and maceration due to the high temperatures [18]. This method is cheap and can be used in a field or

homestead set up as reported to be used by some herbalists [19].

2.2.2 Soxhlet extraction

This is best suitable when the compounds of interest are known to have limited solubility in a solvent and when impurities in that solvent are insoluble. Thermolabile compounds are not passed through this method as it can degrade them. The advantage of this method is that it does not require multiple passes of hot solvents, just one pass is enough as the same gets recycled [16]. It requires a laboratory set up and Soxhlet apparatus.

2.2.3 Microwave assisted extraction

This is basically the traditional solvent extraction but the solvent and sample mixture is heated using microwave energy. The microwave energy penetrates through plant materials targeting and evaporating the smallest traces of moisture in plant cells. The cell walls rupture due to pressure created by the heating inside the cells. This rupturing exudates active compounds from within the cells, making this method more yielding of phyto-constituents [10].

2.2.4 Automated solvent digester extraction method

Recent use of universal extraction systems (BUCHI systems for example) employs thimbles which contain the test samples suspended in small glass jars containing various extraction solvents and temperature is set just below the solvent's boiling point. The extracted extract is filtered and concentrated using vacuum concentrator, ready for phytochemical determinations [10, 11].

2.2.5 Pressurized Liquid Extraction (PLE)

This employs an apparatus set up that uses heat of upto 200°C and pressure of between 35 and 200 Bars [13]. Samples are put in a sample holder, usually with water as a solvent. When the temperature is raised, the heat reduces viscosity of the solvent, making it easily penetrate the plant matrices. High pressure keeps the solvent in the liquid phase. The makeup of the apparatus protects oxygen and photo sensitive compounds from degradation. The method is ecofriendly but expensive. It is selectively good for thermally stable compounds.

2.3 Optional temperature extraction

The following methods can be undertaken optionally, depending on the knowledge of the nature of the constituents of interest in the samples.

2.3.1 Serial exhaustive extraction

This is extraction in a series of solvents from the least polar (usually n-hexane) to the most polar (Usually methanol) to ensure extraction of compounds at a wider range of polarities. It is one of the most common methods of extraction which can go either under elevated temperatures (eg. Soxhlet) or normal temperatures, particularly for thermolabile compounds [17].

2.3.2 Infusion and digestion

This method is used only when there are readily available components of the drug in crude form, like tea in tea bags. Infusions are freshly prepared by maceration of the ready drug components in hot or cold water. A closely related method is digestion, where during the preparation of the extract, a steady gentle heat is applied to the maceration process to enhance the release of active components [17].

2.3.3 Supercritical fluid extraction method

This method involves use of liquefied gases, usually CO₂ which is pumped through a cylindrical channel contained the test sample material at around 32°C or higher and 74 bars [13].

The mixture is then taken to a separating chamber where the gases are recovered for re-use and the extract separated completely from the solvent. This is what makes this method better as there remains no trace of solvent on the extracts [20]. At lower temperatures, it gives high yields of thermal labile compounds like terpenes and terpenoids (B.P ~ 150°C) [13]. It is non-toxic, easy to recover, mainly essential oils, and nonflammable. It is however an expensive set up.

2.3.4 Sonication

In this sonochemistry based method, ultrasound waves (20 to 2000 KHz) are used to penetrate the sample materials. It can be used under normal temperatures but mostly on a hot plate at varied elevated temperature [21], thereby increasing cell wall permeability. Choice of solvents is critical based on the viscosity, polarity, surface tension and vapor pressure which influence the cavitation phenomena [13]. Methanol, ethanol and hexane are the most common solvents, to which sometimes water is added. This method is effective in releasing components but its disadvantages include high installation and operational costs, modification of some active compounds and the formation of free radicals in the samples which are likely to falsify results [22].

3. Analytical techniques in the Separation and purification of plant constituents

Due to the variability of the nature of phenolic compounds (Polarity, chemical structure, glycosidic linkages and spectral characteristics) there is no single method that may be universally appropriate for the separation of all extracts, and should be carefully selected [23].

The identification and isolation of bioactive compounds from herbal extracts is the starting point for drug development for potentially new mechanisms against human diseases [24]. To purify, samples are subjected to a range of solvents of varied polarities and then separated using chromatographic techniques [25, 26]. There are two broad types of chromatography; liquid and gas chromatography.

Liquid chromatographic techniques are techniques where the mobile phase is a liquid whilst gas chromatography has gas phase as the mobile phase. The mechanisms of interaction vary depending on the states of the stationary phase and type of equilibrium reached. This section discusses basic mechanisms of both types

with examples.

3.1 Liquid chromatographic techniques

In this chromatographic technique, molecules in test samples are separated based on their shapes, sizes and charges [27]. A solvent containing analyte which is the mobile phase is passed through a molecular sieve, called the stationary phase that categorically separates components of the extracts.

Chromatographic techniques can be categorized based on phase states and mechanisms, phase polarities, separation region geometries, Gradient of experimental parameters and duration and column dimensions. This chapter describes the techniques based on phase states and mechanisms.

3.1.1 Adsorption chromatography

This is also called liquid/solid or displacement chromatography, based on solute interactions with active sites on the solid stationary phase. The stationary phase particularly interacts with specific functional groups in the mobile phase by non-polar interactions, non-covalent bonds, hydrophobic interactions and Van-Der-Vaals forces [28]. The compounds in the mobile phase get separated on the basis of similarities with the nature of the stationary phase, eluting loosely bound molecules first.

3.1.2 Partition chromatography

Also known as liquid/liquid chromatography, the method is based on the interaction of the molecules to be separated, with two immiscible liquid phases relative to their solubility, where the stationary liquid phase is adsorbed on a solid.

The components that are soluble in one are held strongly by it, if mobile phase, then they are the first to be eluted, but if they are held more strongly by stationary phase, they will be delayed within the system [29].

3.1.3 Affinity chromatography and ion chromatography

Extracts are introduced into the columns and they interact with the stationary phase ligands. If they have a high affinity to the ligands, they are drawn towards the stationary phase. If they have low or no affinity, they get washed away easily through the system using buffers of different pH or higher ionic strength, resulting in early elution. Usually, the desired components are bound to the ligands [10]. On the other hand, closely related ion chromatography separates ionic components and polar molecules in extracts based on their electrical properties [30]. The stationary phase is made of ion resins.

3.1.4 Size exclusion chromatography

This technique is also known as gel permeation, molecular sieve and gel filtration chromatography and is based on molecular size, relative to the sizes of the permeation spaces on the stationary phases. It enables measurements of molecular weights and their distribution for compounds, particularly polymeric compounds

[31]. Liquid stationary phase forms interstices on a polymeric solid. Phase Equilibrium is achieved through sieving or partitioning [32]. There is no chemical interaction in this technique.

3.1.5 Bonded phases

In this method, the stationary phase is an organic species bonded to a solid surface. If the mobile phase is a liquid, the method is called liquid-bonded phase where equilibrium is based on the partition between the liquid and the bonded surface [33].

If the mobile phase is a gas, the method is called a gas-bonded phase where the equilibrium is based on the partition between gas and the bonded surface [7].

3.2 Examples of Liquid chromatography

3.2.1 Planar chromatographic techniques

These are techniques that allow solute in a solvent to run on a plane of an adsorbent material laid on a plane.

3.2.1.1 Paper chromatography

A special sheet of paper is used to provide an inert platform for separation. Test samples are placed gently near the bottom of the paper, then the paper is placed in a chromatographic chamber containing a solvent which moves upwards by capillary action carrying respective soluble molecules along.

The paper needs to have high porosity for high rate of capillary action and to be thick to accommodate a reasonably higher amount of samples better [34]. The advantage of paper chromatography is that it is relatively cheaper and has a considerable reproducibility of retention factor (RF) values right on the paper [35].

3.2.1.2 Thin layer chromatography

This is an adsorption chromatography [36] where the separation is based on the interaction of samples with a thin adsorbent layer attached to a plate. This works best for low molecular weight molecular compounds. The advantage of this technique over paper chromatography is that it is versatile, more sensitive and quick to tell the researcher possible number of compounds in a sample [8]. Adsorbents are carefully selected depending on the components they are good at separating. The most commonly used adsorbents with components they separate are Silica gel (Alkaloids, amino acids, lipids, sugars and fatty acids among others), Aluminum (Phenols, alkaloids, carotenes, steroids and vitamins), Celite (Inorganic cations and steroids), Starch (amino acids) and Sephadex (proteins and amino acids) [10].

3.2.1.3 Column and Pressurized techniques

These are chromatographic techniques that involve the use of solid phase packed columns as a separation housing. Sometime, they use regulated pressure to pump mobile phase through columns or a planar chamber

laid with an adsorbent stationary phase.

High performance liquid chromatography

This technique is used for separation, quantification and identification of inorganic and organic solutes in industrial, environmental, biological or pharmaceutical samples [37]. It is based on solute interactions in the mobile phase (usually polar combinations of water and other solvents) with tightly packed solid particles of the stationary phase (usually non-polar particles like C18). High pressure of between 250 to 400 bars is required for analyte elution through the column to the detector. Diode Array Detector (DAD) is one of the most common detectors that measure analyte spectra. This technique is better for samples that are thermally labile and cannot be vaporized, providing a good complement to gas chromatographic technique in sample analyses [38].

High performance thin layer chromatography

This technique is an advanced technology to TLC, with better efficiency and resolution, an auto sampler and automated visualization of spots and capability to allow for quantitative analysis [39]. This technique does not use columns but rather, chambers which contain the separation plates.

The preparation of HPTLC has plate adsorbent sizes of between 5 and 7 microns and coating layer of between 150 and 200 microns, which is thicker than TLC. Mobile phase is pumped over the plates using steady pressure [40]. HPTLC is also used in combination with spectroscopic techniques to maximize analytical potential of these techniques [40].

Optimum performance lamina chromatography

This technique combines principles of TLC and HPLC, presenting a preparative and analytical tool suitable for both research and quality control laboratories. A liquid mobile phase is pumped through a column embedded with solid stationary phase of silica or bonded phase media (Amino, diol, cyano, ion exchange, C8 and C18). A pressure of up to 50 bars is enough to push the mobile phase through the planar columns with a constant velocity [10]. Depending on convenience, ease of separation due to nature of compounds involved and availability of materials and tools, purification may involve one or more techniques above. To purify phenolics from *Tripodanthus acutifolius* leaves [41] made use of column chromatography and reverse phase HPLC while only silica gel column chromatography was used to purify phenolics from roots of piper betle [42].

3.3 Gas chromatographic techniques

This technique is used to separate volatile and stable compounds, where species are distributed between the gas (Mobile) and liquid (stationery) phases. Samples are vaporized and injected into a chromatographic column where it gets transported by an inert gas. The stationary phase is embedded onto an inert solid material. The distribution of species in the test sample gives a measure of separation, where some gets well mingled into the stationary phase and delay or does not elute with the gas phase at all and those that distribute well into the gas phase will elute as the gas does [17, 43]. Gas chromatography has three most common categories based on

phase states and mechanism. The gas-bonded phase has already been described in bonded phases section. The second one is gas-liquid, where a liquid adsorbed onto a solid acts as the stationary phase and equilibrium is reached through partition between gas and liquid. The last one is gas-solid technique, where a solid is the stationary phase and equilibrium is reached by adsorption.

3.4 Non-chromatographic techniques

3.4.1 Immunoassay

This method employs monoclonal antibodies against low molecular mass bioactive natural compounds and drugs [44]. They provide very high sensitivity for receptor binding analyses, qualitative and quantitative analyses and enzyme assays. Compared to chromatography, enzyme linked immunosorbent assay (ELISA) are more sensitive in most cases [8]. This method is efficient but very involving to get reagents, ethical clearance for *in vivo* assays and costly for *in vitro* assays on cell lines.

3.5 Structure elucidation using spectroscopic techniques

Structural elucidation basically involves the use of spectroscopic techniques to characterize chemical constituents in samples [25]. Kind and Fiehn (2010) defined structure elucidation as the full *de novo* identification of structures, completely resulting into molecular connections with right stereo-chemical assignments. This is done with a combination of spectroscopic techniques, each one of which provides vital piece(s) of information to be linked together with the data provided by the other. The structures are identified from the interpretation of spectra or by searching and comparing with already discovered, identified and known data from spectral libraries. Public chemical compound repositories such as ChemSpider [45] and PubChem [46] and drug and metabolism databases such as HMDB[47], DrugBank [48], KEGG [49], MZedDB [50] and ChEBI [51] provide a web-based search of formulae and masses of molecular compounds already known with any of their available biological test results [52]. This section presents techniques in the identification of structures and subsequently names of compounds isolated from plant extracts.

3.5.1 Fourier-transform infrared spectroscopy (FTIR)

This technique is useful in the identification of functional groups present in the isolates from plant extracts [53, 54]. A drop of liquid samples is placed between two plates of sodium chloride to form a small film. For solid samples, they are ground to the finest powder possible and mixed homogeneously with potassium bromide (KBr). The mixture is then compressed into a pellet and placed in a sample holder, ready for analysis. The analysis produces peaks at specific wave numbers, characteristic to functional groups detected in the samples [17].

3.5.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

This technique is valuable for the determination of structural frameworks and their orientations relative to central isotopic carbons. The technique uses magnetic fields to determine the structural forms. C-NMR helps to identify the kind of isotope present in a region and how they related to positions of one another. ¹H-NMR largely detects relative positions of protons in the structure. Other types of NMR include Time Domain NMR

which gives information on molecular dynamics in solutions. If the sample is solid, Solid State NMR can be used to directly determine the structure of the solid sample. In industrial applications, NMR techniques are replacing X-ray crystallography techniques due to the former's versatility [10].

3.5.3 Mass spectrometry (MS)

This technique is useful in elemental determination in plant extracts and other products. It is also important in providing partial insights in partial structural make up of using fragmentation of mass spectra [55].

There are four types of mass spectroscopy, categorized according to stages of advancement. According to Wang (2007), Ion trap MS is a versatile instrument and quadruple MS is used to work for general purposes. Triple quadruple MS is very sensitive and usually, used for targeted samples. For very high mass accuracy and resolution, Fourier transform instruments are used for structural determination as it provides information about the mass of the compound as a whole and masses of different fragments of the compound [10].

4. Conclusion

Cost of some operational apparatus and equipment may be a limiting factor in extraction and isolation. A combination of simpler and cheaper methods could overcome this limitation. Due to a variety of compounds in plants for potential drug development, a single method may not be ideal to extract and to isolate them. Sometimes, efficiency may be achieved when two or more methods at the extraction and isolation stages are combined. Structural elucidation is already done in combination of various techniques for meaningful interpretation of spectral data. However, there is need for a single robust piece of equipment that can do all the techniques at once to determine structures of compounds.

The limitation to this work was that it did not include quick test methods available commercially on the market. This limitation does not affect the methods described because even the quick test methods still employ fundamentals described in this review.

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5. Recommendations

This work recommends that before embarking on the use of any extraction solvents and methods, isolation phases and characterization procedures, scientists must first understand all underlying properties that the sample and most importantly, the compounds of interest in the sample have. This helps to avoid unknowingly compromising the intended compounds through physical means and/or associated chemical reactions.

References

- [1] S. K. Burley and F. Park, "Meeting the challenges of drug discovery: a multidisciplinary re-evaluation of current practices," *Genome Biol.*, vol. 6, p. 330, 2005.
- [2] S. Visht and S. Chaturvedi, "Isolation of Natural Products," *Current Pharma Research*, vol. 2, pp. 584-599, 2012.
- [3] M. S. Butler, "Natural products to drugs: Natural product-derived compounds in clinical trials.," *Nat Prod Rep.*, vol. 25, pp. 475–516., 2008.
- [4] K. K. Ajibesin, D. N. Bala, and U. F. Umoh, "Ethno medicinal survey of plants used by the indigenes of Rivers State of Nigeria," *Pharm Biol.*, vol. 50, pp. 1123-43, 2012.
- [5] S. Das and M. D. Choudhury, "Ethno- medicobotanical observations on some tribal communities of Tripura, India," *Pleione* vol. 3, pp. 89-95, 2009.
- [6] T. Dhanani, S. Shah, N. A. Gajbhiye, and S. Kumar, "Effects of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*," *Arabian Journal of Chemistry*, vol. 10, pp. S1193-S1199, 2017.
- [7] S. Bocian, A. Nowaczyk, and B. Buszewski, "Synthesis and characterization of ester-bonded stationary phases for liquid chromatography.," *Talanta.*, vol. 131, pp. 684-92, 2015.
- [8] S. Sasidharan, Y. Chen, D. Saravanan, K. M. Sundram, and L. Y. Latha, "Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts," *Afr J Tradit Complement Altern Med*, vol. 8, pp. 1-10, 2011.
- [9] M. M. Cowan, "Plant Products as Antimicrobial Agents," *Clin Microbiol Rev.*, vol. 12, pp. 564-582, 1999.
- [10] K. P. Ingle, A. G. Deshmukh, D. A. Padole, M. S. Dudhare, M. P. Moharil, and V. C. Khelurkar, "Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts.," *Journal of Pharmacognosy and Phytochemistry.* , vol. 6 pp. 32-36 2017.
- [11] J. B. Harborne, *Phytochemical methods*. London.: Chapman and Hall Ltd., 1973.
- [12] N. S. Ncube, A. J. Afolayan, and A. I. Okoh, " 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., 2008.
- [13] C. DeMonte, S. Carradori, A. Granese, G. B. DiPierro, C. Leonardo, and C. DeNunzio, "Modern extraction techniques and their impact on the pharmacological profile of *Serenoa repens* extracts for the

- treatment of lower urinary tract symptoms," *BMC Urol*, vol. 14, 2014.
- [14] K. Das, R. K. S. Tiwari, and D. K. Shrivastava, "Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends.," *Journal of Medicinal Plants Research*, vol. 4, pp. 104-111, 2010.
- [15] B. Tang, W. Bi, M. Tian, and K. H. Row, "Application of ionic liquid for extraction and separation of bioactive compounds from plants," *J Chromatogr B.*, vol. 2012, pp. 1–21., 2012.
- [16] S. B. Nikhal, P. A. Dambe, D. B. Ghongade, and D. C. Goupale, "Hydroalcoholic extraction of *Mangifera indica* (leaves) by Soxhletion.," *International Journal of Pharmaceutical Sciences.*, vol. 2, pp. 30-32., 2010.
- [17] K. S. Banu and L. Catherine, "General Techniques Involved in Phytochemical Analysis.," *International Journal of Advanced Research in Chemical Science.*, vol. 2, pp. 25-3, 2015.
- [18] N. N. Azwanida, "A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation.," *Med Aromat Plants.*, vol. 4, p. 196, 2015.
- [19] A. G. K. Mtewa, "Antibacterial potency stability, pH and phytochemistry of some Malawian ready-to-serve aqueous herbal formulations used against enteric diseases," *International Journal of Herbal Medicine*, vol. 5, pp. 01-05, 2017.
- [20] P. S. Patil and R. Shettigar, "An advancement of analytical techniques in herbal research," *J Adv Sci Res.*, vol. 1, pp. 08-14. , 2010.
- [21] N. A. Salehan, A. Z. Sulaiman, and A. Ajit "Effect of temperature and sonication on the extraction of gallic acid from *Labisia pumila* (Kacip fatimah)." *Journal of Engineering and Applied Sciences.*, vol. 11, pp. 2193-2198., 2016.
- [22] S. S. Handa, S. P. S. Khanuja, and G. Longo, *Extraction Technologies for Medicinal and Aromatic Plants*. Trieste: International centre for science and high technology., 2008.
- [23] E. Miniati, "Assessment of phenolic compounds in biological samples.," *Ann Ist Super Sanità.* , vol. 43, pp. 362-368. , 2007.
- [24] M. J. Lee, S. Prabhu, X. Meng, C. Li, and C. S. Yang, "An improved method for the determination of green and black tea polyphenols in biomatrices by high performance liquid chromatography with coulometric array detection.," *Analytical Biochemistry.*, vol. 279, pp. 164-169., 2000.
- [25] S. L. Jothy, Z. Zakaria, Y. Chen, Y. L. Lau, L. Y. Latha, L. N. Shin, and S. Sasidharan, "Bioassay-directed isolation of active compounds with antiyeast activity from a *Cassia fistula* seed extract.," *Molecules.*, vol. 16 pp. 7583-7592. , 2011.

- [26] S. Sasidharan, Y. Chen, D. Saravanan, K. M. Sundram, and L. Y. Latha, "Extraction, isolation and characterization of bioactive compounds from plants' extracts.," *African Journal of Traditional, Complementary and Alternative Medicine.*, vol. 8, pp. 1–10. , 2011.
- [27] F. Heftmann, *Chromatography: Fundamentals and Application of Chromatographic and Electroforetic Techniques.* Amsterdam.: Elsevier., 1992.
- [28] S. H. Pine, "Separation by adsorption chromatography: An experiment on a colorless mixture," *Journal of Chemical Education*, vol. 43, p. 672 1966.
- [29] F. Akter, S. Saito, Y. Tasaki-Handa, and M. Shibukawa, "Partition/Ion-Exclusion Chromatographic Ion Stacking for the Analysis of Trace Anions in Water and Salt Samples by Ion Chromatography," *Anal Sci.*, vol. 34, pp. 369-373, 2018.
- [30] J. Weiss and T. Wesiss, *Handbook of Ion Chromatography.* Weinheim.: Wiley-VCH., 2004.
- [31] E. Uliyanchenko, "Size-exclusion chromatography-from high-performance to ultra-performance.," *Anal Bioanal Chem.*, vol. 406, pp. 6087-94, 2014.
- [32] B. W. Pirok, P. Breuer, S. J. Hoppe, M. Chitty, E. Welch, T. Farkas, S. Van-der-Wal, R. Peters, and P. J. Schoenmakers, "Size-exclusion chromatography using core-shell particles," *J Chromatogr A.*, vol. 1486, pp. 96-102, 2017.
- [33] S. Bocian, A. Nowaczyk, and B. Buszewski, "New alkyl-phosphate bonded stationary phases for liquid chromatographic separation of biologically active compounds," *Anal Bioanal Chem.*, vol. 404, pp. 731-740, 2012.
- [34] J. Sherma and G. Zweig, *Paper Chromatography.* New York, USA.: Academic Press., 1971.
- [35] J. B. Harborne, *Phytochemical Methods: A guide to modern techniques of plant analysis.*, 2nd ed. vol. 3. Springer. Germany.: Chapman and Hall publishers, 1998.
- [36] E. Hahn-Deinstrop, *Applied Thin Layer Chromatography: Best practice and avoidance of Mistakes.* Weinheim, Germany.: Wiley-VCH., 2000.
- [37] W. S. Hancock, *High Performance Liquid Chromatography in Biotechnology.* New Jersey, U.S.A: Wiley-Interscience., 1990.
- [38] K. Grosser and N. M. VanDam, "A Straightforward Method for Glucosinolate Extraction and Analysis with High-pressure Liquid Chromatography (HPLC)," *J Vis Exp.*, vol. 121, 2017.
- [39] E. Reich and A. Schibli, "Stationary Phases for Planar Separations - Plates for Modern TLC," *LC GC.*, vol. 23, pp. 58–69., 2005.

- [40] M. Attimarad, K. K. M. Ahmed, B. E. Aldhubaib, and S. Harsha, "High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery," *Pharm Methods*, vol. 2, pp. 71-75, 2011.
- [41] J. R. Soberón, M. A. Sgariglia, D. A. Sampietro, E. N. Quiroga, M. G. Sierra, and M. A. Vattuone, "Purification and identification of antibacterial phenolics from *Tripodanthus acutifolius* leaves.," *Journal of Applied Microbiology.*, vol. 108, pp. 1757-1768. , 2009.
- [42] C. F. Lin, T. L. Hwang, C. C. Chien, H. Y. Tu, and H. L. Lay, "A new hydroxychavicol dimer from the roots of Piper betle.," *Molecules.*, vol. 18, pp. 2563-2570. , 2013.
- [43] W. A. König and D. H. Hochmuth, "Enantioselective Gas Chromatography in Flavour and Fragrance Analysis.," *J.Chromatogr. Sc.*, vol. 42, pp. 423-439., 2004.
- [44] T. L. Eberhardt, X. Li, T. F. Shupe, and C. Y. Hse, "Chinese Tallow Tree (*Sapium Sebiferum*) utilization: Characterization of extractives and cell-wall chemistry. ," *Wood Fiber Sci.*, vol. 39, pp. 319–324., 2007.
- [45] A. J. Williams, "A perspective of publicly accessible/open-access chemistry databases.," *Drug Discov Today.*, vol. 13, pp. 11-12, 2008.
- [46] Y. Wang, J. Xiao, T. O. Suzek, J. Zhang, J. Wang, and S. H. Bryant, "PubChem: a public information system for analyzing bioactivities of small molecules. ," *Nucleic Acids Res* 37, vol. 37, pp. W623–W633, 2009.
- [47] D. S. Wishart, C. Knox, A. C. Guo, R. Eisner, N. Young, B. Gautam, and D. D. Hau, "HMDB: a knowledgebase for the human metabolome.," *Nucleic Acids Res.* , vol. 37, pp. Wishart DS, Knox C, Guo AC, Eisner R, Young N, Gautam B, Hau DD, Psychogios N, Dong E, Bouatra S. HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res.* 2008;37:D603–D610. doi: 10.1093/nar/gkn810. , 2008.
- [48] D. S. Wishart, "DrugBank and its relevance to pharmacogenomics.," *Pharmacogenomics.*, vol. 9, pp. 1155–1162., 2008.
- [49] M. Kanehisa, M. Araki, S. Goto, M. Hattori, M. Hirakawa, M. Itoh, T. Katayama, S. Kawashima, S. Okuda, and T. Tokimatsu, "KEGG for linking genomes to life and the environment.," *Nucleic Acids Res.*, vol. 36, pp. D480-D484, 2008.
- [50] J. Draper, D. P. Enot, D. Parker, M. Beckmann, S. Snowdon, W. Lin, and H. Zubair, "Metabolite signal identification in accurate mass metabolomics data with MZedDB, an interactive m/z annotation tool utilising predicted ionisation behaviour ‘rules’ *BMC Bioinformatics.*," *BMC Bioinformatics.*, vol. 10, p. 227, 2009.

- [51] K. Degtyarenko, P. DeMatos, M. Ennis, J. Hastings, M. Zbinden, A. McNaught, R. Alcantara, M. Darsow, M. Guedj, and M. Ashburner, "ChEBI: a database and ontology for chemical entities of biological interest.," *Nucleic Acids Res.*, vol. 36, pp. D344-D350, 2008.
- [52] M. Brown, W. B. Dunn, P. Dobson, Y. Patel, C. L. Winder, S. Francis-McIntyre, P. Begley, K. Carroll, D. Broadhurst, A. Tseng, N. Swainston, I. Spasic, R. Goodacre, and D. B. Kell, "Mass spectrometry tools and metabolite-specific databases for molecular identification in metabolomics," *Analyst*, vol. 134, pp. 1322-32, Jul 2009.
- [53] T. L. Eberhardt, X. Li, T. F. Shupe, and C. Y. Hse, "Chinese Tallow Tree (*Sapium Sebiferum*) utilization: Characterization of extractives and cell-wall chemistry.," *Wood Fiber Sci.*, vol. 39, pp. 319-324. , 2007.
- [54] K. M. Hazra, R. N. Roy, S. K. Sen, and S. Laska, "Isolation of antibacterial pentahydroxy flavones from the seeds of *Mimusops elengi* Linn.," *Afr. J Biotechnol.*, vol. 6, pp. 1446-1449, 2007.
- [55] T. Kind and O. Fiehn, "Advances in structure elucidation of small molecules using mass spectrometry.," *Bioanal. Rev.*, vol. 2, pp. 23-60, 2010.