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VANTAGE POINT

From dirt to data: plant extraction modalities and techniques

BL Lurie, CS van Ballegooyen, AD de Beer

Department of Pharmacology, School for Medicine, University of Pretoria, South Africa
Corresponding author, email: *nieldbeer@gmail.com*

Introduction

Obtaining quantitative information on plant development over different time periods and different socio-cultural areas is crucial for understanding the rationale of plant use in research. Bioprospecting is the identification of valuable biochemical compounds, with the aim of developing new or improving existing medicine. Through analysis of chemical composition of different parts of plants, one can determine biological activity of significant compounds present with the aim of drug discovery with therapeutic and commercial value. However, appropriate guidelines for the analysis of traditional medicine should be maintained and should be used in a way that is culturally sensitive, accessible and one should consider the potential impact of traditional medicine on biodiversity and conservation.

Bioprospecting

The practice of seeking for and researching biological organisms with potential uses in medicine, agriculture, and other industries is referred to as bioprospecting.[1] Examining genetic, biochemical, and physiological properties in order to find active substances, such as enzymes, proteins, and secondary metabolites, may be exploited in the creation of novel medications, treatments, and technologies.[2] Bioprospecting has the ability to provide significant insights into the natural environment while also creating economic opportunities in local communities. However, it is crucial to ensure that bioprospecting is done ethically and sustainably, with adequate safeguards in place to maintain the biodiversity and cultural heritage of the regions being examined. Four significant evaluates such as plant selection, plant preparation, plant screening and plant development need to be considered in terms of plant-based prospecting.

Plant selection can affect the viability and scalability of a research study since certain plants are simpler to nurture and propagate than others, and various species have different features and properties.[3] The next stage is plant preparation, which ensures that the plant samples are of high quality and free from contaminants that might interfere with the precision and reproducibility of the results. Depending on the precise study aims and the qualities of the plant material being investigated, plant preparation may entail a range of processes such as cleaning, drying, grinding, and extraction. Plant screening is a process of identifying potential bioactive compounds or other useful properties in plants.[4] This process involves testing plant extracts or fractions for specific activities or properties, such as antimicrobial, antioxidant, or anti-inflammatory activities and many more. Plant screening can be conducted using a variety of in vitro or in vivo assays, including cell-based assays, animal models, or clinical trials. Drug development can begin after the possible bioactive chemicals in plants have been discovered. This is a multi-step process in which promising bioactive compounds are identified and tested further to establish their safety, effectiveness, and pharmacological features such as toxicity screening, pharmacokinetics, and therapeutic potential.[4,5] Throughout this process, researchers must examine the optimal methods for extracting and purifying active chemicals from plant material, as well as optimising formulations and doses for human administration.[5]

One of bioprospecting's significant benefits is that it allows for the discovery of new and diverse sources of bioactive chemicals that may be employed in the creation of novel drugs. These molecules may have distinct chemical structures and modes of action that differ from those of currently available medications, potentially leading to the creation of more effective and safer therapeutics.[6] Moreover, natural products have better membrane transport properties than synthetic compounds, making them more effective in crossing both the outer and plasma membranes to reach their target.[6,7] Within the Convention on Biological Diversity (CBD) now ratified by most countries, bioprospecting can bring nations with diverse ecosystems and advanced technologies together, providing educational and economic benefits.[7]

Bioactive chemical discovery and separation from natural sources can be a time-consuming and resource-intensive procedure due to their complex nature. Furthermore, many bioactive chemicals derived from natural sources may have low potency, making isolation and identification problematic. In addition, the commercialisation of bioactive substances derived from natural sources may result in biopiracy, inequitable benefit sharing with local populations, informed consent, and cultural sensitivity.[8]

Ethnopharmacology

Ethnopharmacology is a multidisciplinary field that involves the study of traditional medicinal practices and the use of plants and other natural products in different cultures and societies

around the world.[9] Ethnopharmacology helps to bridge the gap between traditional medicine and modern medicine in several ways: Ethnopharmacological research involves the identification and isolation of active compounds from natural products used in traditional medicine and this can help identify potential drug candidates that can be developed further for use in modern medicine. It aims to evaluate the safety and efficacy of natural products, using both in vitro and in vivo methods.[10]

Plant collection and rationale for use

Each part of the plant has its own unique set of bioactive compounds and properties. By studying different parts of the plant, researchers can identify the most effective parts of the plant for specific conditions and isolate the active compounds responsible for their medicinal properties.

Leaves are abundant and easy to harvest, making them readily available; however, the bioactive compounds in leaves can vary greatly depending on the plant species, growing conditions, and harvesting methods used. This can make it difficult to standardise the preparation and dosage of leaf extracts.[11] However, leaves from different plant species can contain a wide range of bioactive compounds, making them a diverse source of potential medicinal compounds. Roots contain a high concentration of bioactive compounds such as alkaloids, glycosides, and flavonoids.[12] However, root harvesting can be damaging to the plant and over-harvesting can lead to depletion of plant populations which can create negative impacts on ecosystems. Bark is a rich source of tannins and other phenolic compounds, which have antioxidant and anti-inflammatory properties. These compounds have been shown to have potential health benefits, such as reducing the risk of chronic diseases like cancer and cardiovascular disease.[13] It is also abundant and easy to harvest. However, one must be cautious of overharvesting as it can also have negative ecological impacts. Bark contains low yields of active compounds, which can make it difficult to isolate and purify the bioactive compounds present. Flowers contain a variety of bioactive compounds, including flavonoids and terpenoids, which have antioxidant, anti-inflammatory, and anti-cancer properties.[14] However, harvesting of flowers depends on seasonal availability, which can limit their availability for research purposes. Fruits are easily available and can be harvested at different stages of ripeness and contain a rich source of vitamins, minerals and antioxidants.[15] They also have a wide variety of bioactive compounds, including flavonoids, carotenoids, and phenolic compounds. However, fruits may also contain low yields of bioactive compounds, and this also depends on the stage of ripeness, harvest season and environmental conditions. Seeds are often readily available and can be easily harvested, and they are easy to store and transport. Fatty acids, phytosterols and antioxidants are found in seeds.[16] However, seeds can be difficult to extract the active compounds from, making isolation of bioactive compounds difficult. Overall, leaves and roots are most commonly used in research.

Phytochemicals

Phytochemicals are categorised into primary and secondary metabolites based on their role in plant metabolism, with thousands of phytochemicals having been identified.[17] Primary metabolites are essential for plant growth and include carbohydrates, amino acids, proteins, lipids, purines, and pyrimidines of nucleic acids. Plant secondary metabolites have significant biological activities and have been used for centuries in traditional medicine.[18] The medicinal properties of plants are attributed to these molecules. Nowadays, plant secondary metabolites are utilised in valuable industries such as pharmaceuticals, cosmetics, and fine chemicals.[18] Therapeutically, alkaloids are particularly well known as anaesthetics, cardioprotective, and anti-inflammatory agents. A well-known example of alkaloids used in clinical settings include morphine, strychnine, quinine, ephedrine, and nicotine.[19] Terpenes can strengthen immune function. Phenolic compounds can inhibit enzymes associated with the development of human ailments, including hypertension, metabolic problems, and neurodegenerative diseases.[17,19] Overall, there are over 4 000 phytochemicals that have been identified, with only 150 analysed in research.[20]

Traditional vs modern medicine

By studying the traditional medicinal practices of different cultures, researchers can identify natural substances that have therapeutic potential. Researchers can also modify these compounds or create new ones based on the knowledge gained from traditional medicine to improve their effectiveness or reduce side effects.[21] Moreover, ethnopharmacological research can also provide insight into the mechanism of action of these isolated compounds. This knowledge can inform the development of new drugs with similar mechanisms of action, potentially leading to more effective and targeted treatments.[10,21]

The use of fresh or dry plant samples

Special consideration needs to be taken when selecting the use of dry or fresh samples. This is a crucial step in plant and extract preparation downstream. Sample collection and preparation needs to be done in a manner whereby it prevents the degradation of bioactive constituents and contamination from bacteria and fungi.[22] The use of fresh and dried plant samples have both advantages and disadvantages. The majority of researchers prefer the use of dried plant samples as it stops chemical processes and reactions within the plant, allowing for a better yield in terms of consistency. Dried plant samples also have a much lower chance of being contaminated and the ease of weighing plays a crucial role in ensuring accuracy in the plant extraction methods. Fresh plant samples tend to produce a large amount of water weight, making it more difficult to accurately weigh the sample. To allow for reproducibility, dried samples are preferred as storage is possible, allowing for use of the same harvest and sample for later experiments and research.

Extraction methods

Correct sample preparation increases the extraction efficiency of phytochemicals and biologically active compounds. Once the correct sample preparation technique has been chosen, important factors regarding the extraction methods need to be taken into consideration as this can impact extraction yield and concentration. Preparation methods to be cognisant of are weighing, mixing, diluting, heating and cooling as these will all impact extraction yield.

Various extraction methods exist and are used based on downstream analysis of the extract. The three most common extraction methods used both in-house and in industry are solvent extraction, maceration, and infusion.

Maceration involves the weighing and grinding of plant material into a fine powder which are left to steep in a closed container for a long period of time. The duration of steeping varies from hours to days and is dependent on the types of downstream process to be done. Maceration is a type of solid/liquid extraction method. The solvent in which the sample is left to steep consists of 70% alcohol. The rationale behind the use of 70% alcohol is to avoid bacterial contamination which the sample is at high risk of contracting due to the long period of time it is left to stand.[23] Researchers use this extraction technique as it is a rather simple, inexpensive technique requiring low energy for extraction. Although, the product produced may take long and the yield obtained from this method is not high and a large amount of solvent is required for extraction.[24]

A common extraction technique used by traditional healers for plant extracts is infusion. Infusion of the plant mimics the preparation of a tea, allowing for easy, non-toxic extraction of the plant. Infusion involves the boiling of water and stirring of the weighted plant material in the boiling water for up to 30 minutes, which is them cooled and filtered. Although this technique is convenient and used by many, it makes use of an aqueous solvent, which may not extract non-polar solvents, which may exert therapeutic effects.[25] The use of heat in samples may lead to denaturation of important proteins that may be required to exert the desired effects and a large number of samples can be lost due to inaccurate filtration techniques.

Solvent extraction is a technique in which a sample's active constituents are separate based on the solubility in various organic solvents like that of methanol, ethanol and hexane. The solubility of certain biological compounds in specific solvents is selective to the extraction of specific phytochemicals, allowing for the high accuracy and specificity of this technique. Various phytochemicals are extracted using different organic solvents. Examples of phytochemicals extracted from ethanol are tannins, terpenoids and flavonoids. Despite the specificity, simplicity, high yield and ease of process, the use of solvent extraction comes with its very own drawbacks. The number of organic solvents used in this type of extraction is rather large, contributing to the high costs of extraction as well as the time taken to extract phytochemicals from samples.[26]

Rationale for fractionation

Fractionation is the process of the separation of crude plant extracts, where the segregation of compounds can take place to isolate a specific group or compound.[27] This could be done to either remove toxic constituents, or to further elucidate the phytochemical constituents within the plant to investigate the compounds that show promise in bioactivity.

There are several methods of fractionation, both working off the physicochemical properties of the compounds found within the crude extract. Physical methods are used in more commercial settings, where large amounts of crude extracts are processed for use as active pharmaceutical ingredients (APIs).[28] Several solvents are used in the processing of plant extracts, including ethanol, methanol and chloroform, as well as membrane filtration methods. These techniques are used today to produce popular APIs such as caffeine, theobromine and bioflavonoids.[29]

Raw materials are extracted using solvent systems and are then purified using either column chromatography or high-speed counter chromatography, in both cases a silica powder is used within a specific solvent system to yield a high amount of specific compounds, and remove impurities.[30] More complex methods such as sublimation (removal of volatile oils such as camphor) or crystallisation are also employed.

These fractions of plant extracts can then be used in either in vitro or animal studies to investigate phytochemical profiles and bioactive compounds, and will be used in identification via several elucidation techniques including mass-spectrometry, nuclear magnetic resonance and infrared spectra.[28] These will make phytochemical fingerprints to establish if the plants are correctly identified, extracted and elucidated in future cases.

Phytochemical regulations and safety

The South African Health Products Regulatory Authority (SAHPRA) has recently introduced more thorough complementary medicines guidelines and registrations. All medicines classified as "complementary medicines", including aromatherapy, Ayurveda, Homeopathy, Traditional Chinese Medicine, Unani Tibb and Western Herbal Medicine, must now be registered under the new licensing laws as "Category D" medicine. This is where each product is submitted and evaluated for claims and safety as monotherapies and combinational products.

Guidelines were published where standardisation and claims for several phytochemical and phytochemical sources (https://www.sahpra.org.za/complementary-medicines-guidelines/) are explained to ensure safety and efficacy, along with protection of the patient and healthcare provider. However, many of these sources for claims and safety data are outdated and have not been put through proper scientific rigour and peer review, with many safety claims coming from either cell-based in vitro studies, or animal studies.[31]

Conclusion

Phytochemicals are a major part of both traditional and modern medicine, and still have a place in the total health care of patients in many countries. There are limitations to the use of crude extracts, as there may be some toxic compounds or unknown interactions with current medications on the market. More studies into the use and safety of plant extracts will provide the safety profile to ensure proper health management in future patients.

Conflict of interest

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ORCID

BL Lurie https://orcid.org/0000-0001-6249-0787
CS van Ballegooyen https://orcid.org/0000-0002-2331-9888
AD de Beer https://orcid.org/0000-0002-5755-5969

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