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Application of high performance liquid Chromatography in plant extracts

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A B S T R A C T
Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years in various parts of the world. Globally, 85% of the world peoples are depending on traditional plants. In Ethiopia 90% of the population are relying on the plant based traditional medicines. Natural yields from medicinal plants, either as pure compounds or as standardized extracts, offer unrestricted occasions for new drug discovery because of the ultimate accessibility of chemical diversity. It is difficult to identify the bioactive compounds from plant extracts, but HPLC is the most powerful versatile and effective plant extracts identification and characterization methods. The typical HPLC consists of six key components. There are variants of HPLC, based on the phase system (stationary) in the process. Those are; normal phase HPLC, reversed-phase HPLC, size exclusion HPLC and ion-exchange HPLC. Reversed-phase HPLC is the most common type of HPLC for plant extracts and around 95% of the low molecular weight crude extracts are carried out by RP-HPLC. So the aim of the present review is focused on the importance of HPLC in plant extracts. The review focused on traditional plant, HPLC, plant extracts with scientifically proven efficacy was carried out using electronic databases such as Science Direct, Google Scholar, semantic scholar Cochrane library and PubMed. This review revealed that HPLC is the most relevance method for isolation and characterization of plant extracts.

Keywords*: HPLC, plant extracts, traditional medicine .*

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INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years in various parts of the world [1,2]. In many regions of the world, people still rely on traditional plant-based medicines for their primary healthcare, around 85% of the world populations are dependent of plant based traditional medicine [3,4].This is particularly factual for many rural communities in Africa, parts of Asia, and Central and South America, where plants and knowledge of their traditional use are available and inexpensive. In other countries, many of these traditional plant-based medicines are being combined through regulations into mainstream health systems. For example, in December 2016 the Chinese government announced their aim to integrate Traditional Chinese Medicine (TCM) into their healthcare system by 2020 Filho et al., [5], recognising improved scientific understanding of the plants and their value in treating chronic conditions[5]. In Europe, there is also a trend towards using traditional plant-based ('herbal') medicines alongside pharmaceutical drugs; in Germany, for example, it is estimated that 90% of the population use herbal medicines [3].Research in medicinal plants has gained a renewed focus recently. The principal reason is that plant-based system of medicine being natural come with fewer side effects [6]. However, not everything that is natural is safe so traditional medicine products must be used with care and as indicated, just like any other medication [7].

Secondly, plant products represent an inexpensive virtually inexhaustible reservoir of novel biologically active molecules with enormous structural and chemical diversity, which make them favourable for drug discovery[8]. Lastly, biologically derived secondary metabolites and synthetic compounds derived from them perform better as drugs than do randomly synthesized compounds [9]. Traditional medicine plays an important role in the Ethiopian society. The numerous categories of traditional medicinal practices dealing with different aspects of health include spiritual healing, prevention, as well as curative and surgical practices [10]. It is estimated that about 90% of the Ethiopian population is still dependent on traditional medicine, which essentially involves the use of plants [11]. Traditional based medicinal plants are a source of drug discovery, with 80% of all synthetic drugs are deriving from them. Many beneficial biological activities such as anticancer, antimicrobial, antioxidant, anti-inflammatory, analgesic and wound healing skin, antimalarial activity were reported[12]. In many cases the people claim the good benefit of certain natural plants or herbal products[13]. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim. Despite their wide use in the traditional health care, only a little study has been done both phytochemically and pharmacologically. When one ponders that a single plant may encompass up to

thousands of ingredients, the opportunities of making new discoveries become evident. The crucial factor for the ultimate success of an investigation into bioactive plant constituents is thus the selection of plant material.

In view of the large number of plant species potentially available for study, it is essential to have efficient systems available for the rapid chemical and biological screening of the plant extracts selected for investigation must be available [14]. Because of the plant extracts having various secondary active compounds of different degrees of polarity and is still a common problem and key challenges to encounter in botanicals and herbal preparations for their extraction, isolation and characterization. By combining basic biological assays with High-Performance Liquid Chromatography (HPLC) analyses, this can be accomplished. HPLC is an extremely flexible technique; it is the best, most effective, and quickest chromatographic technique for crude plant species quality control. It is an important qualitative and quantitative technique that is commonly used for pharmaceutical and biological sample estimation [15]. Therefore, the aim of this study is to review the use of HPLC in plant extracts.

Identification and characterization techniques of plant extracts

Natural yields from medicinal plants, either as pure compounds or as standardized extracts, offer unrestricted occasions for new drug discovery because of the ultimate accessibility of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds [16]. Because the plant extracts contain various active compounds or phytochemicals with different polarities it still remains difficult for the process of isolation and characterization of them [17]. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC, should be used to obtain pure compounds [17]. Amongst of those HPLC is very versatile fastest plant extraction technique. So the present review is focused on the techniques of High performance liquid Chromatography (HPLC) in plant extracts.

High performance liquid chromatography

High-performance liquid chromatography (HPLC) developed during the 1960s as a direct side-shoot of classic column liquid chromatography through advances in the technology of columns and instrumental components (pumps, injection valves, and detectors). Originally, HPLC was the acronym for high-pressure liquid chromatography, reflecting the high operating pressures generated by early columns. By the late 1970s, however, high-performance liquid chromatography had become the preferred term, emphasizing the effective separations achieved. In fact, newer columns and packing materials offer high performance at moderate pressure (although still high pressure relative to gravity-flow liquid chromatography [18]. HPLC is the most powerful, versatile, robust, and widely used method for the isolation as well as purification of natural products, HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture [19-21]. At the moment, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants [22]. Natural products are frequently isolated following the evaluation of a relatively crude extract in a biological assay in order to fully characterize its properties. The resolving power of HPLC is ideally suited to the rapid processing of such multi component samples on both an analytical and preparative scale [18]. Different scientists were reported that HPLC is used for characterization and identification of secondary metabolites in plant extracts, mainly phenol compounds, steroids, flavonoids, alkaloids [23-30].

Components of HPLC

Based on the principle of chromatographic separation, HPLCs are used to determine the composition of complex mixtures (such as plant extracts) to measure the extent of a reaction, or to verify the purity of a product[31-32]. The typical HPLC consists of six components. Solvent is pumped at high pressure through a column packed with reprivatized silica gel. Upstream of the column is an injector to allow for introduction of a sample. Downstream of the column is a detector (typically one that measures UV-Vis absorbance). The sample is introduced into the HPLC system by way of an injector. When the injector is turned to the “load” position (counter clockwise), a syringe is used to fill up (or load) a sample loop of a precise volume. When the injector is then turned to the “inject” position, the loop is placed in line with the column [32]. Column: Provides separation through high pressure created by the small particles. Detector: It quantifies and identifies the sample components and provides information to the computer. Computer: Takes the signals from the detector and displays the retention times and quantity of the components [33].

Types of HPLC

There are variants of HPLC, based on the phase system (stationary) in the process. Those are; Normal phase HPLC, Reversed-phase HPLC, Size exclusion HPLC and Ion-Exchange HPLC [34].

Normal phase chromatography: Also known Normal Phase HPLC (NP-HPLC), this method separates analyses based on polarity. NP-HPLC uses a polar stationary phase and a non-polar mobile phase. The polar analysed interacted with

and is retained by the polar stationary phase. Adsorption strengths increase with increased analyses polarity and the interaction between the polar analyses and the polar stationary phase increases the elution time[23].Polar compounds in the mixture being passed through the column will stick longer to the polar silica while the non-polar compounds will pass through.

Reversed-phase chromatography: Reversed-phase chromatography is the most commonly used HPLC separation mode. It is far superior to the other modes in the variety of target compounds it can handle. The dominant phenomenon retaining the sample in the column in reversed-phase chromatography is the hydrophobic interaction between the solid phase and sample. Two types of reversed-phase chromatography column packing are used: one type is a silica gel matrix with chemically bonded alkyl chains and the other is resin-based packing. Except in special circumstances, the silica gel matrix type is used due to its high number of theoretical plates. A resin-based packing must be used if the pH of the mobile phase used for separation is set outside the range that can be used with silica gel or if unreacted silanol groups remaining on the silica gel surface have a detrimental effect on separation and this problem cannot be resolved by changing the composition of the mobile phase. However, these are comparatively rare cases. Typical alkyl groups that are chemically bonded to the silica gel include the octadecyl group, the octyl group, and the trimethyl group [35].Non-polar compounds (hydrophobic) in the mixture being passed through will stick longer to the column while the polar compounds will pass through first.

According to Ravi et al. [36] review report, ninety percent (90%) of low molecular weight of plant extracts are separated by reversed HPLC[36]. Likewise, Prathap et al. [37] reported that 60% of all HPLC separations are carried out by the reversed phase HPLC[37]. The reasons for this include the simplicity, versatility, and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass for example, to identify secondary plant metabolites [38]. In addition, the colloquial term used for the mobile phases in reversed phase chromatography is "buffer". However, there is little buffering capacity in the mobile phase solutions since they usually contain strong acids at low pH with large concentrations of organic solvents. Adequate buffering capacity should be maintained when working closer to physiological conditions [37].

Ion-exchange chromatography(IEC): is part of ion chromatography which is an important analytical technique for the separation and determination of ionic compounds, together with Ion-partition (interaction)and ion-exclusion chromatography [39]. Ion chromatography separation is based on ionic (or electrostatic) interactions between ionic and polar analytics, ions present in the eluent and ionic functional groups fixed to the chromatographic support. Two distinct mechanisms as follows; ion exchange due to competitive ionic binding (attraction) and ion exclusion due to repulsion between similarly charged analyses ions and the ions fixed on the chromatographic support, play a role in the separation in ion chromatography. Ion exchange has been the predominant form of ion chromatography to date [40]. Positively charged column/beads, negatively charged compounds in the mixture being passed through will interact with the beads while the positively charged compounds will pass through. This chromatography is one of the most important adsorption techniques used in the separation of peptides, proteins, nucleic acids and related biopolymers which are charged molecules in different molecular sizes and molecular nature [41-44]. The separation is based on the formation of ionic bonds between the charged groups of bio molecules and an ion-exchange gel/support carrying the opposite charge [45]. Bio molecules display different degrees of interaction with charged chromatography media due to their varying charge properties [46].

Size exclusion chromatography: Size exclusion chromatography (SEC), also called as gel permeation chromatography or gel filtration chromatography mainly separates particles on the basis of size. It is also useful for determining the tertiary structure and quaternary structure of proteins and amino acids. This technique is widely used for the molecular weight determination of polysaccharides [47].

HPLC in identification and characterization of plant extracts

Using HPLC, chemical identification can be completed by using the fact that, depending on polarities given a specific column and mobile phase, some compounds have different migration rates. The separation rate is calculated mainly by the option of the stationary phase and the mobile phase. The method of separating or removing the target compound from other (possibly structurally related compounds or contaminants is the purification of the crude extract of interest using HPLC. Under certain chromatographic conditions, each compound should have a characteristic peak [15]. So, to identify any compound by HPLC, a detector must first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed. UV detectors are popular among all the detectors because they offer high sensitivity [48]]; and also because majority of naturally

occurring compounds encountered have some UV absorbance [49], phenolics are frequently identified using UV-VIS and Photodiode Array (PDA) detectors at wavelengths 190-380 nm [50].

The high sensitivity of UV detection is bonus if a compound of interest is only present in small amounts within the sample. Besides UV, other detection methods are also being employed to detect phytochemicals among which is the Diode Array Detector (DAD) coupled with Mass Spectrometer (MS). Liquid Chromatography coupled with Mass Spectrometry (LC/MS) is also a powerful technique for the analysis of complex botanical extracts. It provides abundant information for structural elucidation of the compounds when tandem Mass Spectrometry (MS) is applied. Therefore, the combination of HPLC and MS facilitates rapid and accurate identification of chemical compounds in medicinal herbs, especially when a pure standard is unavailable [51]. The processing of a crude source material to provide a sample suitable for HPLC analysis as well as the choice of solvent for sample reconstitution can have a significant bearing on the overall success of natural product isolation [5]. The source material, e.g., dried powdered plant, will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into solution. In the case of dried plant material, an organic solvent (e.g., methanol, chloroform) may be used as the initial extracting and following a period of maceration, solid material is then removed by decanting off the extract by filtration [52,48]. The filtrate is then concentrated and injected into HPLC for separation. The usage of guard columns is necessary in the analysis of crude extract. Many natural product materials contain significant level of strongly binding components, such as chlorophyll and other endogenous materials that may in the long term compromise the performance of analytical columns. Therefore, the guard columns will significantly protect the lifespan of the analytical columns [53].

Advantages of HPLC over other types of chromatography

The use of HPLC in the isolation and purification of complex compounds is increasing tremendously due to its flexibility and efficiency. It has several advantages over traditional methods of isolation and purification:

1. Variety of separating techniques.
2. Variety of column packing for different techniques.
3. Separation optimized by alteration of the mobile phase.
4. Mobile phase easily manipulated in gradient systems.
5. RP technique separates very similar and very different compounds simultaneously.
6. HPLC can be used as a preparative method.
7. HPCL can be used as a purification technique. More than one detector can be connected in series (e.g. UV and evaporative light scattering detector).
8. Most sample analysis is carried out at room temperature.
9. Short analysis runs. More than 70% of HPLC separations are performed on UV detectors and 15% rely on fluorescence without any derivatization[18-19].

METHOD

Information sources and search strategy

A search strategy combining both MeSH and free-text terms for HPLC, Medicinal plants and Extracts was developed to retrieve articles of interest in the following databases: Medline, PMC, Cochrane, PubMed, Semantic scholars, Gogle scholar; Medline In-Process and the Cochrane Library.

CONCLUSION

Plant contains several secondary active compounds and it is important to identify and characterize its components. Globally, 85% of the world peoples are relying on plant based traditional medicinal plants and 90% of Ethiopian peoples are also depending on plant based traditional medicinal plants. There are different methods to identify active compounds occurring in plant extracts, meanwhile they comprise of multi-component mixtures, and their isolation purpose still makes difficulties. However, HPLC is a versatile, robust, and widely used method for the isolation as well as purification of plant secondary compounds. It has also extensive applications in different fields in term of isolation, quantitative and qualitative estimation of active molecules. There are several types of HPLC based on the phase system those are: Normal phase HPLC, Reversed-phase HPLC, Size exclusion HPLC and Ion-Exchange HPLC. RP-HPLC is the most common types of HPLC for identification and separation of natural products. Moreover, this review indicated that an overview of advanced identification techniques to isolate and purify of compounds from plant based sources principally is by HPLC technique.

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