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A REVIEW ARTICLE: IDENTIFICATION AND ISOLATION PLANTS CONSTITUENTS BY HPLC

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ABSTRACT:

Medicinal plants are still a chief portion of the medicine in advance countries, may be used to treatment the divers human disease through utilized various plants material. Therefore, the researcher focus on analysis the plants material by using high performance liquid chromatography HPLC technique. HPLC technique is sensitive and rapid technique, utilized to identification the qualification and quantification various constituents in traditional medicine plants. Plants constituents as alkaloids, phenols, saponins, tannins, anthocyanin, flavonoids, anthraquinones, cardiac glycosides, and cyanogenic glycosides. The present review chiefly focuses on the plants constituents' identification by HPLC for preparations diverse pharmaceutical products.

KEYWORDS: Plants constituents, HPLC, Medicinal plants.

INTRODUCTION:

PHYTOCHEMICALS CONSTITUENTS OF PLANTS:

Plants are a main source of modern natural drugs although the availability of diverse methods for the finding of therapeutic constituents for drugs product, natural products still continue as one of the greatest sources of new therapeutic products. The uniform plants extracts, provide limitless chances for modern drug discover due to availability of divers of chemical constituents (1). Phytochemicals are chemical constituents which occurrence naturally in the plant kingdom. Some of them responsible for the medicine properties from natural sources in present. The term is usually utilized to represent those chemicals which have biological significance, as carotenoids, coumarins, flavonoids, coumarins, but not essential nutrients for plants. There are many or 4,000 diverse phytochemicals have activity against numerous sicknesses as cancer, metabolic or degenerative diseases (2).World Health Organization (WHO) indicated more than 80% of the world's people depend on herbal medicine for their primary health-care requests, herbal medicine have a broad range of constituents which may be utilized to treat sever infectious sicknesses (3). Large number of researches is studied the properties of plant extracts and a microorganism in different part of the word (4). Plants active constituents comparatively recently have been isolated. The plants active constituents are the chemicals which have a therapeutic effect for human. The plants active constituents, are chemicals that have a marked, define physiological and therapeutic activity for the human. The constituents and their activity which used by human also as their pharmacology. Phytochemicals constituents may be divided into chief groups: alkaloids, phenols, saponins, tannins, anthocyanins, flavonoids, anthraquinones, cardiac glycosides, and cyanogenic glycosides (5).

ALKALOIDS:

Alkaloids are one of the major groups of secondary metabolites of plant, existing in numerous economically families of plant.

Alkaloids are heterocyclic ring having a nitrogen atom, and low molecular weight as shown in figure (1). Alkaloids are used to fight oral intoxication, antitumoral vinblastine and vincristine. They are may play as defense constituents in plants, effective against predators and pathogens due to their toxicity. Generally, toxic effects based on specific dosage, individual features, exposure time, site of action, sensitivity, and developing stage. Understanding the alkaloid mechanisms action and biosynthesis is important to enhance production the alkaloids for discovering new bioactive constituents (6).

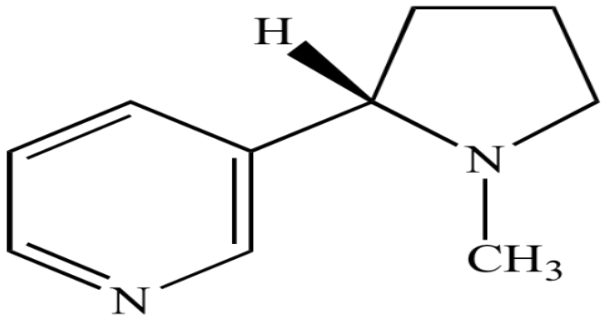

Figure (1) Alkaloids structure

PHENOLS:

The term phenolic or polyphenol is may be defined as constituents which include an aromatic ring bearing one or more hydroxyl groups. Generally, phenols contain double or more hydroxyl groups as shown in figure (2) they have biological active constituent which occurrence in food plants typically used up by a number of people (7).

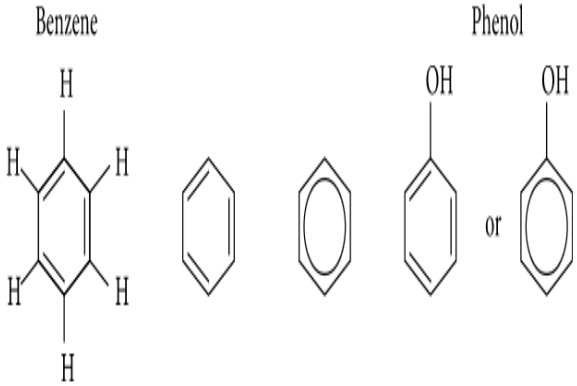

Figure (2) Phenolic structure

Phenolic compounds of plants act as antioxidants, anti-inflammatory, anti-aging, and anti-proliferative activities. Thus, it is useful to eat plant foods which have a great amounts of antioxidant content that will defense from some chronic sicknesses, such as cancers, diabetes, and cardiovascular diseases, by the control of oxidative stress (8).

SAPONINS:

Saponins are steroid and triterpenoid glycosides which show various biological properties. A broad existence in plants, in addition have pharmaceutical application, this led to extraction and identification the saponin from several species (9).

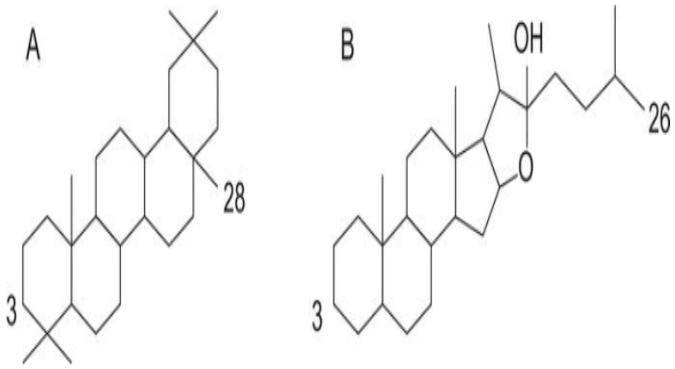

Figure (3) (A) Triterpenoid, (B) Steroidal saponins

TANNINS:

Tannins or polyphenols, sometimes named plant polyphenols. The properties distinguished tannins from polyphenols of other types of plants are mostly the features of the former: basic compounds, binding to proteins, large molecular compounds, pigments, and metallic ions, and have antioxidant proprieties. These proprieties of tannins lead to quantitative and qualitative analytic variances between tannins and other polyphenols (10).

ANTHOCYANINS:

Anthocyanins are purple, blue, red pigments occurrence in plants, particularly flowers, tubers, and fruits. Anthocyanin seems

like red pigment in acidic condition, while seem blue pigment in alkaline conditions. They are considered as one of the flavonoids, it has a positive charge at the oxygen atom of the C-ring of basic flavonoid structure as shown in figure (4) (11). broadly studied have been indicated anthocyanins in plant, they have therapeutic values as antidiabetic, anti-inflammatory, anticancer, anti-obesity, antimicrobial effects, in addition to prevention the cardiovascular diseases (CVDs) (12). Hence, anthocyanins isolated from plants for pharmaceutical constituents.

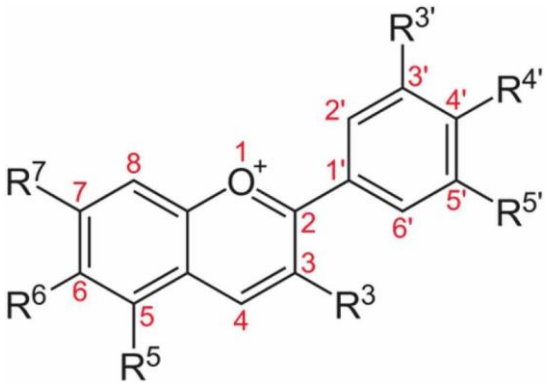

Figure (4) Basic anthocyanin structure

FLAVONOIDS:

Flavonoids are phenolic compounds, isolated from a broad range of vascular plants, with over 8000 individual constituents recognized. They act in plants as photoreceptors, antioxidants, feeding repellants, antimicrobials, and visual attractors. Many studies have proposed the flavonoids exhibition biological activities, having antiviral, antiallergenic, anti-inflammatory, and vasodilating actions. Still, most interest and developing the antioxidant compounds of flavonoids, due to their capacity to decrease free radical formation and to scavenge free radicals (13).

ANTHRAQUINONE:

Anthraquinone occurrence naturally in many plants, insects, and fungi, it occurs to the coloring pigment of these organisms. property, the anthraquinone is utilized commercially to manufacture the dyes. Anthraquinone, also named dioxoanthracene or anthracenedione is an aromatic (a hydrocarbon categorized by general changing double and single bonds between carbons) organic compound as shown in figure (5) (14).

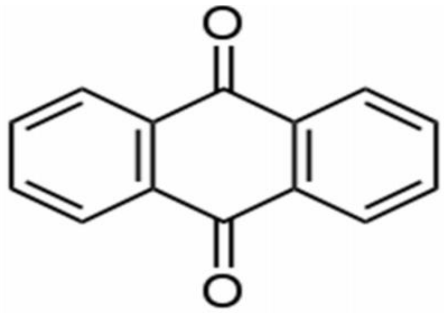

Figure (5) Structure of 9,10–Anthraquinone

CARDIAC GLYCOSIDES:

Cardiac glycosides are a various family of naturally derived from plants which bind to and inhibit Na+ /K+ ATPase. Cardiac glycosides clinically used for many years for healing the atrial arrhythmia and heart failure, and new therapeutic for several diseases. markedly, the increased weakness the cancer cells, therefore, these compounds used as cancer therapies (15).

CYANOGENIC GLYCOSIDES:

Cyanogenic glycosides occurrence in a broad range of plant. The toxicity of this compounds get up from enzymatic degradation to lead hydrogen cyanide, that severe poisoning (16).

Phytochemical constituents are utilized in the diseases treatment which effect on population. Therefore, the human which consumed them should be knowledge them chemical structure (17). Herbal plants are broadly utilized currently for the product numerous pharmaceutical formulae, or like food flavors; therefore, the researcher concentrated on analysis methods by HPLC for structure knowledge (18).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):

HPLC is an important technique, usually utilized for assessment quantitative and qualitative of pharmaceutical constituents and biological samples. It is the multipurpose, dependable, fastest, and safest chromatographic practice for the quality control of drugs products (19). HPLC is really important technique, the reversed-phase way ability to control of compounds of a diverse molecular mass and polarity. Reversed-phase chromatography has present in analytical, separation and purification the phytochemical compounds. Suitable plant has been selected, appropriate isolation process may be belonging to isolate the active compounds (20). Numerous writers describe the utilized of HPLC for quantification and characterization of secondary constituents in plant extracts, chiefly alkaloids, phenol, flavonoids, and steroids compounds (21,22). Plant extracts generally exist like a mixture of several types of bioactive constituents, phytochemicals with diverse polarities, therefore, their separation still continues as large test for the method of identification and separation of them. It is a common technique in separation of these bioactive constituents. Number of diverse separation techniques like Thin-Layer Chromatography (TLC), column chromatography, Sephardim chromatography, flash chromatography and High-Performance Liquid Chromatography (HPLC) (23). HPLC chiefly uses column which holds packing substance (stationary phase), a pump which moves to (mobile phase) by the column, the detector which displayed the retention times of the substance. Retention time differs based on the connections between the stationary phase, the substance actuality analyzed, and the solvent utilized (24). Presently, this technique is having popularity between several investigative techniques like the chief choice for fingerprint study for the quality controller of medicinal plants (25). Plants natural products are recurrently isolated subsequent the assessment of a comparatively crude extract in a biological test in order to fully properties. HPLC resolving power is perfectly suitable to the fast processing of like multi-constituent samples on both preparative and analytical scale. Numerous researchers define the utilized of HPLC for description and quantification of plant secondary metabolites (26,27). HPLC profile of the leaves of some plants presented of the maximum peaks for the secondary compounds as protein source. Same kind of earlier tests determines the process of a crude substantial to afford appropriate sample for HPLC investigation in addition to the choice of solvent for sample reconstruction may have a significant bearing on the complete success of plants product isolation (26). The source substance like dried powdered of plant, will primarily want to be treated in such a method to ensure which the substance professionally liberated into solution, an organic solvent such as chloroform, and methanol can be utilized as the primary extracting, then solid substance is removed through filtration (28,29). The filtrate then is concentrated and injected in HPLC for separation. The isolation and purification the plant products as secondary constituents and proteins by HPLC which is very beneficial for researchers and industrialist, those who focus on quality (30). Liquid chromatography application is the qualification or quantification assessment of a specific structure of samples which obtained from natural origin. The qualitative analysis is estimated based on the constancy the retention time of reference standards and in the analyzed sample. The quantitative assessment is complete by based on the standard curve created after reference standards are injected at diverse concentration levels (31). HPLC technique still the perfect analytic separation technique utilized generally

for qualitative and quantitative analysis of natural products from herbal preparations or plant samples (32).

DETECTOR:

The output is documented like a series of peaks, each one representing a constituent in the combination passing by the detector and absorbance UV light. The area below the peak is represented the amount of constituent, which passed by detector, this area may be automatically calculated by the computer linked to the display (19).

APPLICATION:

The information about using HPLC contains identification, qualification, quantification, and resolution of a constituents. Biochemical separations is based on the some compounds contain diverse migration rates given a specific column and mobile phase, the degree of separation is generally investigated through the choice of stationary phase and mobile phase (19).

PURIFICATION:

Purification is clear a process of separation or extraction the target constituents from a combination. All constituents displayed a distinguishing peak under some chromatographic conditions (19).

IDENTIFICATION:

Generally, analysis of constituents are carried by utilizing HPLC. The parameters of this analysis must be a clean peak of the recognized sample which is observed from the chromatograph. The identification the peak should contains a practical retention time and would be fine separation from inessential peaks and the detection levels which the analysis will be achieved (19).

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