ISOLATION, IDENTIFICATION AND ANALYSIS OF PHYTOCONSTITUENT (GLYCRRHIZINIC ACID) BY CHROMATOTRON

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Abstract

This review is an effort to emphasis the phytochemical and chemical constituents of G. glabra and their isolation and analysis by chromatotron. G . glabra is an old age medicinal plant that belongs to Leguminosae/Fabaceae/Papilionaceae family and commonly known as mulaithi in north India.Chemical constituents of G. glabra like isoliquiritine, isoflavones, glycyrrhetic acid, saponin and their derivatives have been examined for their pharmacological activities. Most critical chemical compounds which isolated from the root/stem extract of the G. glabra are Glycyrrhizin and Glycyrrhetic acid. Chromatotron is a convenient, reliable and economic method for preparative scale separation of natural products. Chromatotron also known as centrifugal thin layer chromatography (CTLC). Centrifugal thin layer chromatography which makes use of centrifugal force for separation of multi-component system offers extensive platform for the isolation of phytoconstituents from medicinal plants.CTLC instrument are operated by the same principal involving movement of the mobile phase by centrifugal forces through a thin layer of sorbent coated on either a circular glass or plastic plate with the aid of a binder. Centrifugal forces are generated by the planar circular motion of the coated plate mounted on the inner chamber of the rotor. Additionally, this article also highlights on various applications of isolatedchemical constituents of G glabra

Index Terms : Chromatotron , Glycrrhizinic Acid , Glycrrhiza glabra , Antitussive , Antiulcer , Immunomdulatory .

INTRODUCTION

Phytoconstitents are the substances found inside the plants that work alone in correct to enhance the effect of another, and their pharmacological action provides the scientific basis for their usage in modern medicine. Leaves, flower,rootd, stems, barks, fruits and seeds as well as fully harvested, processed (dried), and stored plant material are frequently used as medication or in the manufacturer of medicines. [1]

Glycyrrhiza glabra has long been well-known in pharmacy. It was considered first-class drugs in the old Chinese pharmacy and the rejuvenating quality was attributed to it when ingested for long periods. Licorice was widely used in ancient Egypt, Greece and Rome. Theophrastus had alluded to this. The use from that time on,until now, proves the effectiveness. Trade content comes from wild plants and "semi-wild" plants grown in

the former Soviet Union, Turkey, Iran, China, India, Pakistan, Afghanistan, Syria, Italy and Spain . Crude plant based drugs in Pakistan cost around Rs. 120 million per year.[2] That is a good indicator of medicinal plants 'potential economic value. Aromatic and medicinal plants have regional and international markets of significantscale . According to World Health Organization 80% of the world population depends upon indigenous medicinal plant remedies. The medicinal value of these plants lies in some chemical constituents that produce a definite physiological action on the human body. The most important of these bioactive substances of plants are saponin, flavonoids triterpenoid, tannins, alkaloids, and phenolic compounds. *G. glabra* is native to Europe andAsia.[3]

Isolation of constituents from plants with highest purity is a long and tedious process and requires expertise and profound knowledge of phytochemistry and separation chemistry .There are a number of techniques available for isolation of marker compounds or bio actives from medicinal plants such as preparative thin layer chromatography (PTLC), preparative HPLC, droplet counter current chromatography (DCCC), centrifugally accelerated thin layer chromatography (CTLC), etc.[4] CTLC is a preparative chromatographic technique where centrifugal force is used for separation of multi component system .It is a preparative, centrifugally accelerated, radial thin layer chromatographic technique. It allows rapid separations using centrifugal action of the spinning rotor driving the mobile phase through the adsorbent layer. Thus, CTLC offers a widespread platform for the fractionation, separation and purificationof plant molecules.

Fig: Glyrrhiza glabra plant

Fig:: Glyrrhiza glabra root

2. Scientific Classification

Kingdom: Plantae
Family: Leguminosae Division: AngiospermaeGenus: *Glycyrrhiza* Class: DicotyledoneaeSpecies: *glabra* Linn
Order: Rosales

G. glabra is herbaceous perennial, growing to 1 m in height, with pinnate leaves about 7– 15 cm long, with 9–17 leaflets. Usually flowers are of 0.8–1.2 cm long, purple to pale whitish blue color, and it is produced in a loose inflorescence. The fruit is an oblong pod, length of 2–3 cm, consists of several numbers of seeds.[5] The *Glycyrrhiza* shrub is a member of the pea family and grows in subtropical climates in rich soil. Below ground, the *G. glabra* plant has an extensive root system with a main taproot and numerous runners. The main taproot which is soft and fibrous has a bright yellow interior color and harvested for medicinal use.[6] Glycyrrhizin is the major bioactive compound in the *Glycyrrhiza* plant root which possesses a wide range of pharmacological properties and it is commonly used worldwide as a natural sweetener. Because of this it gives an economic value to the plant and biosynthesis of glycyrrhizin has received considerable attention.

ISOLATION OF GLYCYRRHINIC ACID FROM GLYCYRRHIZAGLABRA.

METHOD 01:-

➢ Required quantity of coarse powder of Glycyrrhiza roots is extracted with boiling water,filtered and concentrate the extract to obtain a crude liquorice extract.

➢ Then this extract is again extracted in water and acidified with HCl to maintain pH 3-3.4 toprecipitate Glycyrrhetinic acid and filter.

➢ The residue is washed with water to yield Glycyrrhetinic acid.[7]

METHOD 02:-

➢ Transfer 20g powedered liquorice +50ml acetone +2 ml dil. HNO3, mix, macerate for 2hrswith stirring.

➢ Filter, transfer the marc to stoppered conical flask & add 20 ml acetone & filterCombine both filtrate, conc. under vacuum, Add dil. NH3 sol.

➢ For ppt. of NH4+ glycyrrhizinate Separate ppt by filtration and wash it with 5ml acetone(2x) followed by drying & weigh the product.

METHOD 03:-

➢ Weigh accurate qty of liqurice powder + CHCl3 in soxhlet apparatus for 3hrs.Filter the content of flask & discard the filtrate.

➢ The residue left on filter paper is then extracted with 0.5M H2SO4 for few hrs.Filter the content of flask. Transfer the filtrate in and extract with CHCl3 Separate & combine the CHCl3 layer.

➢ The combined CHCl3 layer is evaporated to dryness to get glycyrhetinic acid

IDENTIFICATION TEST:-

- **Libermann test:** 3ml of concentrate and 3ml of acidic anhydride is warmed and cooled. To this a drops of conc. H2SO4 is added. Blue color is seen which show the presence of triterpenoid.
- **Libermann-Burchard test:** In 3ml of extract, 2ml of chloroform, 1ml of acetic anhydride and one drop of conc. H2SO4 is added. Blue-green to red orange color is observed which indicate the presence of triterpenoid or steroids .[8]

ANALYSIS BY CHROMATOTRON (CTLC):

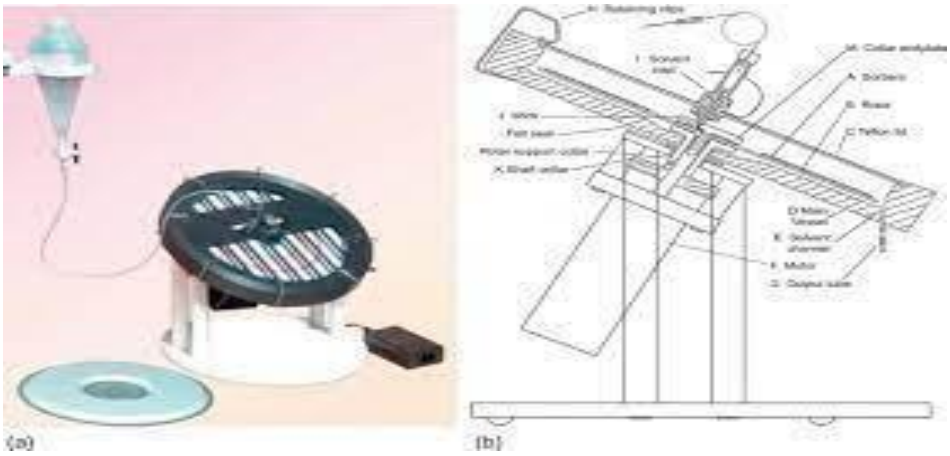

Fig : CTLC [Chromatotron]

***Sample preparation* :** 1mg of Glycyrrhetinic acid is dissolved in 1ml of methanol: Chloroform (1:1)

Standard sample : Glycyrrhetinic acid

Stationary phase: The various sorbents used are Silica gel (TLC standard grade with gypsum), acidified silica gel, aluminium oxide GF- 254,silica gel containing silver nitrate and gypsum. Along with sorbent, calcium sulphate hemihydrates is frequently used as binder.

Mobile phase : The selection of mobile phase depends upon the sample to be separated. Mostly the solvent system giving a low Rf (i.e. 0.2- 0.4) are preferred. The solvent used for mobile phase system areToluene: Ethyl acetate: Glacial acetic acid (12.5:7.5:0.5).

Detection : For detection of bands, UV lamp is placed over the Teflon lid of the instrument. Detection is generally done at short wavelength (254 nm). Compounds that do not absorb UV rays can be analyzed by conventional TLC after collection.

Colour of the band :- Purplish colour band.

Pharmacological activity

Antitussive and expectorant

G. glabra powder and its extracts were found to be helpful in curing ailments like cough, sore throat and bronchial catarrh. Due to the presence of glycyrrhizin and demulcent, antitussive and expectorant loosing activities can be seen. Therefore, it helps to expel congestion in the respiratory tract. [9]

ANTIMICROBIAL ATIVITY

Glycyrrhiza Linn and its species are recognized to have selective antimicrobial activity due to isoprenoid phenols present in it. Most recent studies have shown that there are significant anti-bacterial properties against gram positive and gram negative pathogens in hydromethanolic extracts of *G. glabra* .[10] Specially chloroform, ethanol, methanol and diethyl ether extracts of *G. glabra* have been tested against number of species like *Salmonella typhimurium, Pseudomonas aeruginosa*, *Escherichia coli, Staphylococcus aureus* and *Bacillus subtillus* by using agar well diffusion assay.

Antiulcer Activity

As a result of the ability of *G. glabra* extract to inhibit delta13-prostaglandin reductase and 15-hydroxyprostaglandin dehydrogenase, *G. glabra* extract exhibit anti-ulcer effect. Antiulcer genic effect can be exhibit by Carbenoxolone which is present in *G. glabra* extract by inhibiting secretion of gastrin and also it can raise the prostaglandin concentration in the digestive system to induce mucus secretion in the stomach.[11]

Immunomodulatory Activity

G. glabra roots consist of Glycyrrhizic acid has the ability to inactivate virus particles and inhibit the virus growth as a potent source of immunomodulatory. A peptide known as N- acetylmuramoyl is glycyrrhizin analogue which have the potential in *in vitro* immune- stimulation and it can mediate the virus by restricting the virus replication. This was tested and confirmed in animal studies. Even in plants which contain Glycyrrhizic acid, can protect its self from virus due to the action of Glycyrrhizic acid as it inhibits the virus growth and inactivate virus particles as a source of immunomodulatory.[12]

ANTIOXIDANT ACTIVITY

High content of phenolic component in ethanolic extract of Liquorice (*Glycyrrhiza glabra L*) is responsible for its powerful antioxidant activity. Liquorice flavonoids have exceptionally strong antioxidant activity. [13].Antioxidant activity of liquorice flavonoids was found to be over 100 times stronger than that of antioxidant activity of vitamin E. Liquorice extract can be efficiently used to formulate cosmetic products for the protection of skin and hair against oxidative damage.

ANTI-HYPERGLYCIMMIC ACTIVITY

The effect of liquorice extract on serum lipid profile and liver enzymes was studied in albino mice. Root extract of *Glycyrrhiza glabra* was found to have anti-lipidemic and anti- hyperglycemic activity at low doses.[14]

HAIR GROWTH STIMULATORY AVTIVITY

The hydro-alcoholic extract of liquorice showed good hair growth promoting activity. Comparison between liquorice extract and the standard drug used (Minoxidil 2%) showed that, 2% concentration of liquorice extract showed better hair growth stimulatory activity than 2% Minoxidil. Thus, after efficacy and safety analysis, it has been be concluded that, liquorice has a significant hair growth activity and it can be safely used in herbal formulations in treatment of various types of Alopecia.[15]

Conclusion:

liquorice is a plant with ethnopharmacological importance. The present review was mainly focused on isolation , identification , analysis and various pharmacological activities of glycrrhizinic acid. Also focused on currently developed Centrifugal thin layer chromatography which also known as chromatotron or cyclograph.

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