HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC) METHOD FOR ESTIMATION OF GLYCYRRHIZIC ACID (GA) IN A HERBAL EXTRACT OF TAVERNIERA CUNEIFOLIA

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ABSTRACT
The roots and stolon of liquorice plant, Glycyrrhiza glabra comprise glycyrrhizic acid (GA), the most applied phytochemical component in the field of herbal medicines. This compound is also present in roots of plant Taverniera cuneifolia, well known Indian liquorice. Extract of this plant is examined against ulcer, inflammations, hepatotoxin, viral and radical scavenging activities across the globe. The elementary part of these studies describes the method of extraction of the compound, and are applied in the industrial large-scale extraction procedure. Thus, in this study we demonstrate the High-performance thin-layer chromatography (HPTLC) method for purification and identification of glycyrrhizic acid (GA). In this method solvent system was an optimized mixture of 1-butanol, glacial acetic acid and water (7:1:12 v/v). The peaks were measured at 254nm absorbance. We also discuss different methods of extraction and the one by which we can achieve the optimum volume of glycyrrhizic acid (GA).

INTRODUCTION
Liquorice plants belonging to genus Glycyrrhiza and Taverniera produced Glycyrrhizic acid (GA), the multiplicity of medicinal properties and applied in food industries in Asian countries. GA in liquorice roots of small leguminous shrubs Glycyrrhiza glabra L. and Taverniera cuneifolia where the latter is well known as Indian liquorice plant or the Jethimadh; the main compound being (20-β-carboxy-11-oxo-30-norolean-12-en-3β-yl-2-O-β-d-glucopyranosyl-a-d-glucopyranosiduronic acid (C42H62O16, MW 822.92) (Li et al. 2014), is a triterpenoid glycoside (saponin) with glycyrrhetinic acid, which is condensed with O-β-d-glucuronosyl-(1→2)-β-d-glucuronic acid (Figure 4 of m processing of this compound from the plant extract, it yields as crude ammonium glycyrrhizin (AG), with further treatment yield as crystalline mono-ammonium glycyrrhizin (MAG), where both differ in solubility and sensitivity to pH (Mukhopadhyay and Panja 2008). Taverniera cuneifolia has been studied as an alternative or a substitute for G. glabra, being a scientific and valid indigenous or wild variety of G. glabra. T. cuneifolia is indigenous endemic plant to African and south Asian countries, is in demand in Pakistan and Afghanistan for their valuable medicinal purposes.

GA is on the FDA's generally recognized safe' (GRAS) list; thus, proving it as the main component as flavourings, sweeteners (50 times sweeter than sugar). At levels of 30–300 mg kg\(^{-1}\), it enhances the flavour of cocoa and chocolate-flavoured products, flavours and sweetens candy, confectionery, and beverages, and masks the bitter taste of pharmaceuticals (Glória 2003). With concerning to its application in herbal medicine, GA is applied to improve health, detoxifies, cures injury, viral hepatitis, allergic dermatitis, has corticoid activity, influencing steroid metabolism to maintain blood pressure and volume to regulate glucose/glycogen balance. (Fukai et al. 2003 https://www.ncbi.nlm.nih.gov/pubmed/14630165). Moreover, GA has been a traditional prescription for treating conditions like asthma, dry cough, and other "pectoral diseases" (Banarjee and Giri 2016). Also, GA shows apoptotic effect on tumor cells, and it has emerged as an attractive drug candidate for cancer therapy (Hibasami et al., 2005). Zore et al. 2008 discussed the chemo-profile and bioactivities of T. cuneifolia.

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Marjan Ghasemi, High-Performance Thin-Layer Chromatography (HPTLC) Method For Estimation of Glycyrrhizic Acid (Ga) In A Herbal Extract of Taverniera Cuneifolia

RESULTS

The study revealed that Glycyrrhiza glabra showed best results in water: ethanol (60:40 v/v) solvent system and result in extraction of GA by HPTLC method. The best extraction was at 254 nm with eight phytoconstituents. The HPTLC images shown in Figure 1 indicate that all sample constituents were separated without any diffusion and result in 8 peaks and was comparable with standards. The Rf values ranged from 0.23 to 0.29. Among all the peaks, the peak with RF value 0.28 was more predominant and comparable with RF value of standards (Figure 2). The standard GA was compared with GA extracted from the plant following correlation analysis by best correlation coefficient r2 of 0.994 was achieved.

Methods of extraction best suited for GA

GA extraction from the cultured roots (1 g of dry wt) was done using 10 ml mixture of water/ethanol (60:40v/v) at 50°C on shaker incubator (100 rpm) for 60 min and filtered, as described by Tian et al. (2008). Companies such as Sigma Aldrich Standard supply GA using which stock solution was prepared (10 mg/ml) in distilled water. For high performance thin layer chromatography (HPTLC) analysis, pre-coated E-Merck (Darmstadt, Germany). CAMAG HPTLC system (Muttenz, Switzerland) supplied TLC plates (Silica Gel F254, 0.2 mm thickness, 20×20 cm), with a Linomat-V sample applicator for the sample application and further analysis. Five bands of (2, 4, 6, 8 and 10 μl each) the standard GA solutions (6 mm length each) were applied using linomat fitted with a 100 μl Hamilton syringe. For quantitative determination of GA from cultured roots, 10 μl of the filtered extract was applied on each plate with 6 mm band length.

An optimized mixture of 1-butanol, glacial acetic acid, and water (7:1:12v/v) in a separating funnel was prepared, mixed well and allowed to stand for 30 min; the upper layer was used as a solvent system. Plates were developed over a path of about 8 cm and removed from the chamber; the solvent front was marked and dried in air. Dry plates were examined under UV light (254 nm) and scanned using CAMAG TLC scanner 3. UV spectra, Rf values, % Area under the curve (AUC) and λmax of each band were documented as described by Zore et al. (2008).

DISCUSSION

This study is a pilot-scale analysis of an extract of Glycyrrhiza acid (GA) from plant Taverniera cuneifolia. This compound can be used as a drug by in vitro technique in future analysis indeed as a replacement for the natural liquorice plant, Glycyrrhiza glabra. This plant extract showed a positive result for several compounds such as phenols, flavonoids, tannins, etc., which can be further confirmed by HPTLC analysis.

Tian et al. 2008 investigated extraction and separation of GA and glabridin from liquorice plant following various extraction solvents, temperature and procedure for standardization of the process. The study says that ethanol: water (30:70 v/v) as the best-suited solvent in 60 min dipping time and at 50 degrees. The authors used reverse phase HPLC method. A previous study wherein Shanker et al. 2007 analyzed in a similar survey the RP-HPLC for extraction of GA from Glycyrrhiza glabra.

with successful testing for cytotoxicity, viral, anti-HIV, anti-tumor and germ tube formation in Candida albicans.

In due course of its application, preparation and extraction methods play a vital role in their qualitative extract in industries and other novel investigations. The table 1 of Mukhopadhyay and Panja 2008 and studies by Awad et al. 2011; Zore et al. 2008; Mangalorkar et al. 2014, summarize some of the essential methods for extraction of glycyrrhizin from Taverniera cuneifolia. The methods signify diversified methods ranging from necessary bead beating, ultra-sonication bath, solvent extraction to the advanced reserve phase HPLC, liquid-phase extraction systems and pressurized hot water extraction. A comparatively simple, sensitive, precise and robust high-performance thin-layer chromatographic (HPTLC) method can be relayed on for the estimation of any compound in herbal extracts and pharmaceutical dosage forms (Jain et al. 2010, https://www.sciencedirect.com/science/article/pii/S1319610310000712). HPTLC is promising above all other methods where the UV chromatogram aids in quantification of GA content together with their qualitative analysis. Looking into the commercial demand for Indian liquorice plant and standardization of their extraction and considering research on the methodology as an essential objective, this study demonstrates the extraction of glycyrrhizic acid from the root cultures of Taverniera cuneifolia (roth) Arn., by HPTLC analysis.
using acetonitrile-water containing 2% AcOH (70:30) as an eluent. While the Chauhan et al. 2018 studied the extraction of GA from licorice roots and evaluated for its phytochemical constituents and anti-inflammatory tests. The results showed the presence of fewer impurities in the extract, with the potential anti-inflammatory activity. Our study also suggest similar and comparatively significant outcomes for glycyrrhlic acid from the plant extract of Taverniera cuneifolia.

With the advancement of techniques, several extraction methods are using different solvents, wherein this study use of water: ethanol (60:40 v/v) showed a maximum number of peaks in wavelength 254 nm and results were compared with the standard solution of glycyrrhlic acid. This investigation revealed the plant Taverniera cuneifolia has medicinal properties and can be incorporated as a future drug and applied in several medicines.

GA from different plants are implied extensively in medicinal industries as anti-inflammatory, anti-allergic and anti-cancerous agents (Mukhopadhyay and Panja 2008). Food and industries have the wide advantage of this extract as it is used in confectionery and pharmaceutical products, such as cough syrups, herbal supplements, chewing gums, drinks, and candy because of its natural sweetness, 50–170 times sweeter than sucrose. Thus, the same extract from the other plant Taverniera cuneifolia found and indigenous to India is of wide application in near future industries for quantitative estimations.

**CONCLUSION**

From all the above studies, it can be concluded that Glycyrrhiza acid is majorly studied across globe but restricted to extraction by one plant, licorice roots, i.e., Glycyrrhiza glabra, however, the present investigation of extraction and evaluation of Taverniera cuneifolia for GA was effectively performed following HPTLC method and correlated with the standard component. The study results can be further achieved to understand the different phytochemical properties of the plant and to establish long term extraction of the compound for various medicinal and industrial purposes.

**Reference**


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