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Preliminary finger printing of Chloroform, Ethanol and Aqueous root extracts of *Glycyrrhiza glabra* by comparing various mobile phase

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ABSTRACT

Herbal medicine has a long history in the treatment of several kinds of diseases. Scientific evaluation of plants and preparations of plant-origin medications have received more attention. In the current study, various sources (chloroform, ethanol and double distilled water) of root extracts of *Glycyrrhiza glabra* were subjected to Thin Layer Chromatography (TLC) using various mobile phases. Different mobile phase combinations were used but bands were observed in Chloroform: Methanol (8:2), Chloroform: Acetone (8:2), Toluene: Ethyl acetate (9:1) and Toluene: Ethyl acetate: Acetic acid (5:4.2:0.8). Among these combinations, Toluene: Ethyl acetate (9:1) showed the best result, for all the extracts. TLC plates were observed under UV 254nm and 365nm. To identify the compound in a mixture the retention factor (R_f) value of a compound is compared with the R_f of a known standard. Based on the R_f value calculated for each mobile phase combinations, presence of Glycyrrhizin could be confirmed.

KEY WORDS: Thin Layer Chromatography (TLC), profiling, Leguminosae, Licorice.

1. INTRODUCTION

Medicinal herbs are effective source of treatment for various ailments (Geetha and Anitha, 2012). The plant *Glycyrrhiza glabra* Linn, commonly known as "licorice" and "sweet wood" belonging to Leguminosae family is a traditionally used herb to treat many diseases. In modern medicine, licorice extracts are prepared as flavouring agent to mask bitter taste, and as an expectorant in cold and cough preparations (Damle, 2014). Licorice was effectively used in acidity, leucorrhoea, bleeding, jaundice, hiccup, hoarseness, bronchitis, vitiated conditions of vatadosha, gastralgia, diarrhea, fever with delirium and anuria (Sheth, 2005).The plant *G. glabra* is a vital ingredient in medicinal oils used for the treating rheumatism, hemorrhagic diseases, epilepsy and paralysis (Kaur et al., 2013).The principal constituent of our plant is glycyrrhizin which gives sweet taste and other constituents present are triterpene saponins, flavonoids, iso-flavonoids, polysaccharides, pectins, simple sugars, amino acids and colouring matter. The yellow colour is due to the anthoxanthin glycoside, isoliquiritin (chalcone) which undergoes partial conversion to liquiritin and also possess medicinal property.

Glycyrrhizin inhibits growth and cytopathology of many unrelated DNA and RNA viruses. Glycyrrihizic acid was down-regulated the expression of Latency Associated Nuclear Antigen (LANA) in B lymphocytes. This caused natural cell death (apoptosis) of the KSHV virus. Glycyrrhizic acid prevents cyclooxygenase activity and prostaglandin formation (specifically prostaglandin E2), and also indirectly inhibiting platelet aggregation. Chronic hepatitis and liver cirrhosis were treated by glycyrrhizin and its effectiveness was proved (Khare, 2004). Glycyrrhizin and glycyrrhizic acid have been shown to act against the growth of numerous RNA and DNA viruses, including hepatitis A and C, HIV, herpes simplex and CMV (Varsha Sharma et al., 2013). Glycyrrhizin, specifically, is capable of irreversibly inactivating the virus. Glycyrrhizin has also been shown to inhibit viral replication and infectivity of HIV (Evans, 1958; Pompei et al., 1979; Ohuchi and Tsurufuji, 1982; Okimasu et al., 1983; Ammar et al., 2012). In the present study, the attempt has been made towards the effective and economical profiling from chloroform, ethanol and aqueous root extract of *Glycyrrhiza glabra* subjected to TLC using various mobile phase.

2. MATERIALS AND METHODS

Ethanolic, Chloroform and Aqueous root extracts of *G.glabra* were dissolved in 500µl of their respective solvents and the same were subjected for the TLC to obtain the profile.

Thin layer chromatography: TLC method was carried out for these extracts on 10 x 20 cm aluminum sheets coated with 0.2 mm thickness Silica gel 60 (Merck). Each extracts (10µL) was applied on TLC plate at equal distance with the help of micropipette. The extracts loaded plates were kept in chromatography chamber. The samples were allowed to run in the mobile phases tabulated in Table-1. Iodine vapor test was carried out as per the method developed by Mathuravalli and Eswaralakshmi (2012) and Ranganathan (2004). The colour change indicates the presence of phenols and the respective retention factor (R_f) values were measured. R_f value is the ratio of the distance travelled by the compound and distance travelled by the solvent.

TLC principle: TLC principle works on a solubility rule "Like Dissolves Like" and is followed on separation of mixture of polar, non-polar, mid-polar compounds from the extracts on a static phase (Silica gel) and movable phase or combination of movable phase such as Ethyl acetate, Chloroform, Hexane and Methanol that runs on static phase. About 2µL to 5µL of crude extract (mixture of compounds) is spotted at 1cm position from the bottom of TLC plate using a capillary spotter. The plate allowed to develop in developing chamber with a suitable movable phase or combination of movable phase, which is of appropriate level well below the spotted sample applied (Harborne, 1998).

The mobile phase is drawn up through the stationary phase by capillary action. Few compounds in mixture would dissolve in mobile phase and go over the plate, and conversely, some compound in mixture will remain on the stationary phase. The movement of compounds from the mixture relies on the physical properties, molecular structure and functional groups. If the physical property of compound from mixture is similar to the mobile phase, the compound will remain longer in mobile phase and will travel a longer distance on TLC plate (Scott, 2006). The compounds that are not soluble in mobile phase will have an affinity for stationary phase and will travel to a smaller extent than the soluble compounds**.** An R_f value is "retardation factor" or "ratio to front" or "retention factor" which can be calculated by using the formula.

$$R_f = \frac{Distance\ traveled\ by\ compound}{Distance\ traveled\ by\ solvent}$$

These R_f values can be calculated by observing spots on TLC plates under UV- transilluminator at 254nm and 365nm.

3. RESULTS AND DISCUSSION

TLC profiling of root extracts of *G. glabra* with different solvent system confirms the presence of diverse group of phytochemicals. In the present study, the most suitable mobile phase system was Toluene: Ethyl acetate (9:1) with the largest discriminating power for all extracts. Seven bands were obtained in this mobile phase system. Standard R_f value of glycyrrhizin was found to be 0.38 ± 0.01 (Ajay Kumar Meena et al., 2010). The R_f values for the bands obtained were calculated in Tables 2, 3, 4 and 5. The TLC results have showed the best compound separation in Toluene: Ethyl acetate (9:1) with maximum value was 0.617±0.038 and minimum value was 0.115±0.023 for Chloroform extract. For Ethanol extract, the maxima and minima were 0.580±0.078 and 0.107±0.017. Least compounds were separated in Chloroform: Acetone (8:2), as it showed two bands with R_f values 0.470±0.039 and 0.552±0.035 for Chloroform and 0.428±0.030 and 0.454±0.039 for Ethanol extracts. The mobile phase Toluene: Ethyl acetate: Acetic acid (5:4.2:0.8) yielded four bands each for Chloroform and Ethanol extracts and Chloroform: Methanol (8:2) produced five bands for Chloroform extract and six bands for Ethanol extract. No bands were observed in aqueous extract in any of the mobile phase combinations. This method was applied as we found it to be simple, precise, sensitive and accurate and could also be used for the quantification of Glycyrrhizin (Yamamura, 1992). It can also be used in routine quality control of herbal materials as well as formulations containing any of these compounds (Damle, 2014).

Table.1.List of all the solvent combinations for mobile phase

Solvent Combinations	Ratio
Chloroform: Methanol	8:2
Chloroform: Acetone	8:2
Toluene:Ethyl Acetate	9:1
Toluene:Ethyl Acetate: Acetic Acid	5:4.2:0.8
Chloroform: Acetone	9:1
Toluene:Ethyl Acetate	9.3:0.7
Chloroform: Ethyl Acetate	8:2
Hexane: Acetone	9:1
Hexane: Ethyl Acetate	9:1
Chloroform: Hexane	8:2
Ethyl acetate: Hexane	7:3
Dichloromethane: Methanol	7:3

Table.2.TLC Profiling shows the R_f values of *G.glabra* root extract for Toluene: Ethyl Acetate (9:1)

Mobile Phase used	Extract of *G.glabra*	R_f value
Toluene : Ethyl Acetate (9:1)	Chloroform	R_f1 = 0.115±0.023
		R_f2= 0.193±0.047
		R_f3= 0.323±0.069
		R_f4= 0.338±0.049
		R_f5= 0.487±0.031
		R_f6= 0.558±0.059
		R_f7= 0.617±0.038
	Ethanol	R_f1 = 0.107±0.017
		R_f2= 0.44±0.203
		R_f3= 0.389±0.091
		R_f4= 0.432±0.038
		R_f5= 0.511±0.014
		R_f6= 0.542±0.038
		R_f7= 0.580±0.078
	Aqueous	--

Table.3.TLC Profiling shows the R_f values of *G.glabra* root extract for Toluene: Ethyl Acetate: Acetic Acid (5:4.2:0.8)

Mobile Phase used	Extract of G.glabra	R_f value
Toluene : Ethyl Acetate: Acetic Acid (5:4.2:0.8)	Chloroform	R_f1 = 0.738±0.041
		R_f2= 0.805±0.049
		R_f3= 0.902±0.017
		R_f4= 0.911±0.086
	Ethanol	R_f1 = 0.708±0.060
		R_f2= 0.817±0.030
		R_f3= 0.905±0.026
		R_f4= 0.933±0.057
	Aqueous	--

Table.4.TLC Profiling shows the R_f values of *G.glabra* root extract for Chloroform: Methanol (8:2)

Mobile Phase used	Extract of G.glabra	R_f value
Chloroform: Methanol (8:2)	Chloroform	R_f1 = 0.452±0.083
		R_f2= 0.525±0.043
		R_f3= 0.819±0.024
		R_f4= 0.89±0.016
		R_f5= 0.938±0.025
	Ethanol	R_f1 = 0.504±0.006
		R_f2= 0.525±0.044
		R_f3= 0.901±0.008
		R_f4= 0.968±0.024
		R_f5= 0.156±0.050
		R_f6= 0.189±0.066
	Aqueous	--

Table.5.TLC Profiling shows the R_f values of *G.glabra* root extract for Chloroform: Acetone (8:2)

Mobile Phase used	**Extract of G.glabra**	**R_f value**
Chloroform: Acetone (8:2)	Chloroform	R_f1 = 0.470±0.039
		R_f2= 0.552±0.035
	Ethanol	R_f1 = 0.428±0.030
		R_f2= 0.454±0.039
	Aqueous	--

4. CONCLUSIONS

The present investigation was made to profile *G. glabra* root extract prepared with chloroform, ethanol and aqueous extract with various mobile combinations. Among which Toluene: Ethyl acetate (9:1) shows better results against various solvent mobile phase combinations. This method is rapid, safe, accurate and economical. The results obtained in the present work suggested that the roots of *G. glabra* are good sources of phytochemicals. These studies could help researchers further to evaluate the various properties about the plant. Also, the profiling procedure used plays an important role in characterization of diverse phytochemicals economically to locate the novel leads (Cai, 2014).

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