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Screening of phytochemical, antimicrobial and antioxidant activity of *Glycyrrhiza glabra* root extract

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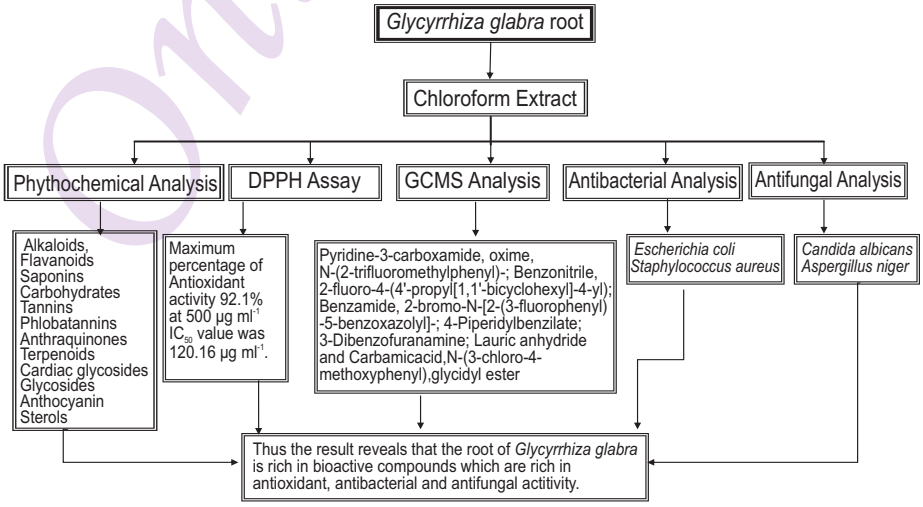
Abstract

Aim : *Glycyrrhiza glabra* Linn, an Indian folklore plant, has many therapeutic values. The present study was designed screening of phytochemical components, antioxidant and antimicrobial activity and also GC-MS analysis of the root extract of *Glycyrrhiza glabra.*

Methodology : Chloroform extract was prepared with powdered root sample of *Glycyrrhiza glabra* Linn. The crude extracts were characterized by phytochemical screening, DPPH assay and GC-MS analysis followed by antimicrobial assay.

Results : Preliminary phytochemical analysis revealed the presence of various bioactive components such as alkaloids, flavonoids, saponins, carbohydrates, tannins, phlobatannins, anthraquinones, terpenoids, cardiac glycosides, glycosides, anthocyanin and sterols. Determined antioxidant activity showed maximum activity of 92.1% at 500 μgml^{-1} and the IC_{50} value was 120.16 μgml^{-1}. GC-MS study confirmed the presence of seven metabolites such as Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-; Benzonitrile,2-fluoro-4-(4'-propyl[1,1'-bicyclohexyl]-4-yl); Benzamide, 2-bromo-N-[2-(3-fluorophenyl)-5-benzoxazolyl]-; 4-Piperidylbenzilate; 3-Dibenzofuranamine; Lauric anhydride and Carbamicacid,N-(3-chloro-4-methoxyphenyl),glycidyl ester. The crude extract exhibited satisfactory antimicrobial (*Escherichia coli* and *Staphylococcus aureus)* and antifungal (*Candida albicans* and *Aspergillus niger)* activity.

Interpretation : The results of the antimicrobial study revealed efficient activity of crude extract which might be due to the presence of soluble bioactive components and were confirmed by phytochemical analysis, DPPH assay and GC-MS analysis. Thus, it was observed that the roots of *Glycyrrhiza glabra* could be used for the extraction of medicinally important bioactive compounds.



Introduction

Glycyrrhiza glabra Linn an Indian folklore traditional medicinal plant have many therapeutic values. It is a perennial herbaceous plant that belongs to Leguminosae family. It measures about 1.5m in height with wrinkled woody, brown and sweet taste roots. The leaves are unequally branched in 4-7 pairs found in subtropical and warm temperate regions. Flowers are violet colored with pods of 3-5 brown seeds (Jatav *et al.,* 2011). *G. glabra* is commonly known as "liquorice" or "sweet wood". The roots and rhizomes of licorice have complex physiological properties with wide therapeutic uses. Herbs with medicinal properties are useful and effective source of treatment for various diseases (Asl and Hossein, 2008; Geetha and Roy, 2012). The main components of plant are flavonoids, saponins, essential oils and tannins. The aqueous extracts of liquorice contain 5-10% of a sweet, white, crystalline diglucuronide known as glycyrrhizin. The roots also contain 5-10% sugars (sucrose, dextrose), starch, an acid resin, malic acid and some proteinous, fatty and inorganic matters. The roots of these plants have anti-inflammatory, expectorant, carminative, hypolipidemic, antiviral, hypotensive, hepatoprotective, spasmolytic, anti-diuretic, anti-mutagenic, antipyretic, antiulcer, anxiolytic, antioxidant and aphrodisiac activities. They are useful in treating hyperdipsia, cough and bronchitis, ulceration of urinary tract, pharyngitis, epilepsy and anaemia. It is used as an expectorant and wound-healing agent in ruminants. It detoxifies and protects the liver. Medicinally, it is used internally for Addison's disease, asthma, bronchitis, peptic ulcer, arthritis and allergic complaints (Nirmala and Selvaraj, 2011; Chopra *et al.,* 2013).

Glycyrrhizin, a triterpenoid compound, accounts for the sweet taste of *G. glabra* root (Yamamura *et al.,* 1997**;** Kalaiarasi and Pugalendi, 2009). The yellow colour of *G. glabra* is due to the flavonoid contents like liquiritin, isoliquiritin (a chalcone) and other compounds. On acid or enzymatic hydrolysis of glycyrrhizin, it yields triterpenoid glycyrrhetinic acid and two mols of glucuronic acid. *G. glabra* is one of the important medicinal plants reported in the literature for its biological activities such as anti-inflammatory and expectorant, controls coughing and hormonal effects (Saxena, 2005). The extracts are often used as a flavouring agent in modern medicine (Damle, 2014). Licorice inhibits the growth and cytopathology of many unrelated DNA and RNA viruses. Glycyrrhizic acid inhibits cyclooxygenase activity and prostaglandin formation (specifically prostaglandin E2), as well as indirectly inhibiting platelet aggregation (Damle, 2014). Specifically, glycyrrhizin has antiviral activity against *Herpes simplex* and is capable of irreversibly inactivating the virus. Glycyrrhizin has also been shown to inhibit viral replication and infectivity of HIV (Ammar *et al.,* 2012).

Treatment with various herbal plants and its combinations has even practiced now-a-days without knowing the active ingredients or the bioactive compounds present in it. Various solvent has been used for the preparation of crude extract of Glycyrrhiza *glabra* and in the current study chloroform was used due to the intermediate polarity for the better extraction. The present study focused to screen the phytochemical components present in the chloroform root extract of *Glycyrrhiza glabra* followed by the antioxidant and antimicrobial assay to analyze its effectiveness and also GC-MS study, to decipher the presence of various bioactive components.

Materials and Methods

Plant collection and extraction : Fresh roots of *Glycyrrhiza glabra* Linn commonly known as athimathuram in Tamil and Mulethi in Hindi were collected from Kancheepuram, Chennai, Tamil Nadu. They were washed and shade dried for 5 to 6 days. The dried samples were finely ground into powder. The fine powdered roots of *Glycyrrhiza glabra* were weighed to determine the dry weight. The extraction was carried out by Soxhlet apparatus using chloroform. About 10g of dried *G. glabra* was dissolved in 100 ml of chloroform and stirred using stirrer. The sample and solvent was maintained at 1:10 ratio. After extraction, the contents were filtered using Whatmann filter paper No.1. The wet weight of the sample was recorded. The filtrate was kept on a hot water bath at 75ºC to concentrate the product by evaporating the residual solvents in the filtrate.

Characterization of chloroform root extract : The extracts were characterized by phytochemical screening, DPPH assay and GC-MS analysis followed by antimicrobial assay.

Phytochemical screening : Two grams of extract was dissolved in 20 ml of chloroform and filtered to remove debris. The extract were qualitatively tested for various phytochemical components like alkaloids, saponins, flavanoids, tannins, Phlobotannins, terpenoids, sterols, glycosides, Cardiac Glycosides, quinines, anthroquinones, carbohydrates, proteins, Oxalates and anthocyanins (Harborne, 1998; Devmurai, 2010; Savithramma *et al.,* 2012; Somkuwar and Kamble, 2013).

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay: Antioxidant assay was carried out using ascorbic acid as standard. IC_{50} value (50% inhibition) was calculated from the graph of inhibition percentage plotted against extract concentration (Patel and Patel, 2011).

GC-MS Analysis : Isolation of pure, pharmacologically active constituents from plants remain a long and tedious process. GC-MS analysis of chloroform root extract of *G. glabra* were performed at SGS Private Limited, Chennai, using 5975 inert XL MSD(Agilent Technologies, CA, USA) equipped with J &W DB-5ms capillary column (size=30m×0.25 mm; film thickness=0.25 µm). The initial temperature was set at 40°C which increased to 150°C at the rate of 10°C min^{-1}. At the rate of 5°C min^{-1}, the temperature was again increased to 230°C. The process continued till temperature reached 280°C at the rate of 20°C min^{-1}

which was held for 8 min. Injector port temperature remained at 280°C and detector temperature was 250°C. Helium was used as carrier gas with a flow rate of 1ml min^{-1}. The samples were injected in split mode as 110:1 and the ionization voltage was 70eV.

Antibacterial activity by disc diffusion method : Antibacterial activities of solvent extracts of *G. glabra* were determined by disc diffusion method on Muller Hinton agar (MHA) medium. The sample concentrations were 1000 µg ml^{-1}, 500 µg ml^{-1}, 250 µg ml^{-1}, 125 µg ml^{-1} and 62.5 µg ml^{-1}. Streptomycin on MHA plates served as positive control while DMSO served as negative control. The test microorganisms were *Escherichia coli* and *Staphylococcus aureus.*

Antifungal activity by disc diffusion method : Antifungal activities of solvent extracts of *G. glabra* were determined by disc diffusion method on potato dextrose agar medium. The sample was loaded in the concentration of 1000 µg ml^{-1}, 500 µg ml^{-1}, 250 µg ml^{-1}, 125 µg ml^{-1} and 62.5 µg ml^{-1} with negative control as DMSO and positive control as amphotericin B on respective disc and placed on PDA plates. *Candida albicans* and *Aspergillus niger* were selected as test organisms for the study. The plates were incubated for 24 hrs at 37ºC. Microbial growth was determined by measuring the diameter of zone of inhibition.

Results and Discussion

Ten percent (W/V) of finely powdered root of *G. glabra* were subjected to chloroform extraction using soxhlet apparatus in order to achieve a final concentration of 3 mg ml^{-1}. Table 1 represents the presence of phytoconstituents such as alkaloids, flavonoids, saponins, carbohydrates, tannins, phlobotannins, anthraquinones, terpenoids, cardiac glycosides, glycosides, anthocyanin and sterols which was confirmed through preliminary routine qualitative analysis. Chloroform extraction yielded more components (Table 1) than earlier reported presence of steroids in ethanolic extract and presence of tannins, flavonoids, sugars in petroleum ether extracts respectively (Chopra *et al.,* 2013). Polarity, structural stability and mass transfer parameters such as diffusibility, coefficient, molecular stability and concentration gradient might attribute to the presence of more components in chloroform extract than others (Harborne, 1998). Based on the preliminary studies, it is hypothesized that chloroform may prove to be a better solvent with respect to *G. glabra* root extract.

Free radical scavenging activity is a potent indicator for the bioactive compounds that acting as an effective phytotherapeutics. Table 2 shows the antioxidant activity of *G. glabra* root extract with 100 µg and 500 µg exhibiting minimal 70.33±5.67 and maximal 87.70±4.09 scavenging activity. Flavonoids and other phenolic compounds act as reducing agent by an effectual electron donors/hydrogen atom acceptors. Free radical centred purple coloured DPPH (2,2-diphenyl-1-picrylhydrazyl) converted into yellow coloured non radical form of 1,1-diphenyl-2-picryl hydrazine when it was treated with bioactive compounds. Presence of prominent antioxidant components in the extract reported positive correlation between the extract concentration and antioxidant activity (Vaya *et al.,* 1997; Mambro and Fonseca, 2005; Chin *et al.,* 2007). Inhibition concentration (IC_{50}) of plant extract was 120.16 µg ml^{-1}.

GC-MS analysis revealed the presence of seven metabolites such as Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-; Benzonitrile, 2-fluoro-4-(4'-propyl[1,1'-bicyclohexyl]-4-yl); Benzamide, 2-bromo-N-[2-(3-fluorophenyl)-5-benzoxazolyl]-; 4-Piperidylbenzilate; 3-Dibenzofuranamine; Lauric anhydride and Carbamicacid, N-(3-chloro-4-

Table 1 : Screening of phytochemicals in chloroform root extract of *G. glabra*

Phytocompounds	Test/chemical reagent	Chloroform
Alkaloids	Mayer's test	+
	Dragendorff's test	+
Flavonoids	NaOH test	+
	Shinoda's test	-
Saponins	Foam test	+
	Lead acetate test	+
Carbohydrates	Benedict's test	-
	Molisch's test	+
Protein	Biuret test	-
	Ninhydrin test	-
	Xanthoprotein test	-
Tannins	Ferric chloride test	+
	Lead acetate test	+
Phlobatannins	HCl Test	+
Quinones	NaOH Test	+
	HCl Test	+
Anthraquinones	HCl Test	-
Terpenoids	Salkowski's test	+
Glycosides	Fehling's test	+
Cardiac Glycosides	Keller Kelliani's test	+
Sterols	Leibermann Burchard test	+
Coumarins	NaOH and $CHCl_3$ test	-
	UV fluorescent test	-
Anthocyanin	NaOH test	+
Oxalate	Ethanolic glacial acid test	-

Table 2 : Antioxidant activity by DPPH assay for chloroform root extract of *G. glabra*

Concentration (µg)	% of antioxidant activity
100	70.33±5.67
200	72.83±3.54
300	78.53±6.07
400	86.3±4.51
500	87.70±4.09
IC_{50} Value	120.16 µg ml^{-1}

Values are mean of triplicates ± SD

Table 3 : Phytocomponents identified in the chloroform root extract of *G. glabra* by GCMS

Constituents name	Retention time factor	Area %
Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	24.635	29.80
Benzonitrile,2-fluoro-4-(4'-propyl[1,1'-bicyclohexyl]-4-yl)-	24.829	5.95
Benzamide, 2-bromo-N-[2-(3-fluorophenyl)-5-benzoxazolyl]-	27.483	33.48
4-Piperidylbenzilate	27.689	19.09
3-Dibenzofuranamine	27.753	5.15
Lauric anhydride	27.788	1.08
Carbamicacid,N-(3-chloro-4-methoxyphenyl)-,glycidyl ester	27.894	5.44

Table 4 : Antibacterial activity of chloroform root extract of *G. glabra*

Microorganisms	Zone of Inhibition (mm)						
	1000 µg	500 µg	250 µg	125 µg	62.5 µg	DMSO	Streptomycin (10 µg)
Antibacterial activity							
Escherichia coli	8.00± 1.50	7.00±1.35	7.66±1.15	-	-	-	8.00±1.00
Staphylococcus aureus	17.83±1.75	22.50±1.32	23.66±1.15	16.83±1.60	19.33±1.52	-	15.66±3.05

Values are mean of triplicates ± SD

Table 5 : Antifungal activity of chloroform root extract of *G. glabra*

Microorganisms	Zone of Inhibition (mm)						
	1000 µg	500 µg	250 µg	125 µg	62.5 µg	DMSO	Amphotericin B (20 µg)
Antifungal activity							
Candida albicans	11.00±1.00	15.33±1.51	13.00±1.00	10.33±1.08	11.33±1.51	-	4.66±1.52
Aspergillus niger	10.00±1.00	7.33±1.52	5.66±1.15	-	-	-	9.33±1.51

Values are mean of triplicates ± SD

methoxyphenyl), glycidyl ester. Major components present in the chloroform extract were Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-; Benzamide, 2-bromo-N-[2-(3-fluorophenyl)-5-benzoxazolyl]- and 4-Piperidyl benzilate (Table 3).

Among these seven compounds Carbamicacid,N-(3-chloro-4-methoxyphenyl)-,glycidyl ester was abundant present in the chloroform root extract of *G. glabra*. Whereas methanolic root extract of G. Glabra showed the presence of 3-Pyridinol; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy- 6-methyl-; Benzofuran; Isosorbide; 2-Methoxy-4-vinylphenol; Methylparaben; D-Allose; Octanal,2-(phenylmethylene)-; Hexadecanoic acid, methyl ester; n-Hexadecanoic acid and Dibutyl phthalate (Rattan *et al*., 2014).

The extracts were tested for their antibacterial and antifungal activity using disc diffusion method and the zone of inhibition is shown in Table 4 and 5 respectively. The maximum zone of inhibition in the chloroform extract was observed as 23±1.32 mm at concentration of 250 µgml^{-1} with *S. aureus*. The best result was shown in *C. albicans* with chloroform extract of 500 µgml^{-1}. The maximum zone of inhibition was found to be 15.33 mm for *C. albicans* when compared to *A. niger*. Observed results exhibited the effective antibacterial and antifungal activity of the root extract of *G. glabra* which might be due the presence of phytocomponents. Available literatures also advocate that the presence of phytoconstituents in the plant extract exhibited antimicrobial, antimycobacterial and antifungal activity and also it could act as phytotherapeutical component. (Gupta *et al.,* 2008; Sadul *et al.,* 2012; Sharma and Agarwal, 2013; Ates and Erdourul, 2003; Sedighinia *et al.*, 2012; Helan and Suhashini, 2015).

Confirmed availability of seven bioactive compounds by GC-MS in the chloroform root extract could be responsible for the antimicrobial activity. Inhibition in the bacterial test might be due to the presence of pyridine-3-carboxamide oxime n-(2-trifluoromethyl phenyl)- in the extract has 2 hydrogen donor bonds and 6 hydrogen acceptor bonds which was categorized as a strong antibacterial activity with respect to *S. aureus*. Higher inhibition was found at 250 µg ml^{-1} rather than 1000 µg ml^{-1}, where protein would have been modulated at higher concentration because of MgrA protein present in *S. aureus* (NCBI, 2016a). Inhibition in bacterial activity might also be due to lipoteichoic acid which is present in cell wall

polymer of Gram positive bacteria where Pyridine-3-carboxamide oxime n-(2-trifluoromethyl phenyl)- blocks phosphatidylglycerol binding to LtaS, a protein present in virulent strains of *S. aureus* (NCBI, 2016b). The results of antimicrobial and antifungal study revealed the efficient activity of crude extract which might be due to the presence of soluble bioactive components and were confirmed by phytochemical analysis, DPPH assay and GC-MS analysis. Thus, the roots of *Glycyrrhiza glabra* can be used for the extraction of phytotherapeutics. Current study is the first of its kind but it has to be warranted and validated by further experimental methodologies.

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