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Antimycobacterial Activity of Glycyrrhiza Glabra Linn. Root Extract against Mycobacterium Smegmatis

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Abstract: The present study was aimed to investigate the fluorescent and antimycobacterial properties of cold and hot extracts of *Glycyrrhiza glabra* against *Mycobacterium smegmatis*. Upon fluorescence analysis under visible light, *G. glabra* powder showed brown, green and brown colour under short and long UV light respectively and different colours were observed after the application of different reagents exposed under short and long UV. Maximum extractives were recorded for cold extraction process with a highest percentage of 2.75 each in chloroform and ethyl acetate extracts and the lowest percentage was obtained in acetone extracts (0.5) of *G. glabra*. Out of six extracts tested, chloroform extract recorded significant activity with an inhibitory zone of 22 mm in hot extract and 24 mm in cold extract followed by dicholoromethane. No antimycobacterial activity was recorded in hexane, ethyl acetate and methanol extracts. Out of thirteen column fractions tested, only 2 fractions recorded antimycobacterial activity and the highest activity was recorded by 25% hexane + 75% dichloromethane fraction (14mm) and the lowest was recorded in 75% chloroform + 25% methanol fraction with an inhibition zone of 12mm. The other fractions recorded no activity.
Keywords: Antimycobacterial, drug resistant, *Glycyrrhiza glabra*, *Mycobacterium smegmatis,* thin layer chromatography.

Introduction

Tuberculosis (TB) is an airborne bacterial infection caused by *Mycobacterium tuberculosis* (MTB) and this is principally a disease of poverty, with 95 percent of cases and 98 percent of deaths occurring in developing countries.[1] World over, TB is the most common cause for death due to a single infectious agent in adults and in 1993 World Health Organization (WHO) took an unprecedented step to declare TB as a global emergency based on its severity[2] as it causes severe socio economic consequences to the infected people.

Tuberculosis is the major opportunistic infection of HIV/AIDS in developing countries.[3] The World Health Organization estimates that a person with both HIV and TB infection is thirty times more likely to become ill with TB than a person with *Mycobacterium tuberculosis* infection alone.[4] In 2012, 8.6 million people fell ill with TB and 1.3 million died from it, including 320000 among people who were HIV- positive. Drug resistance to tuberculosis is a major public health problem that threatens progress made in TB care and control worldwide. Drug resistance arises due to improper use of antibiotics in chemotherapy of drug-susceptible TB patients. In 2012, there were an estimated 450000 new cases of multidrug-resistant TB.[5] Multidrug-resistant tuberculosis (MDR-TB) infections are a global health security risk and carries grave consequences for those affected. Globally in 2012, WHO estimates that 450000 people fell ill with MDR-TB and there were 170000 MDR-TB deaths.[6]

Glycyrrhiza glabra (family, Leguminosae) is also known as licorice and sweet wood, is native to the Mediterranean and certain areas of Asia. It is one of the oldest and widely used herbs from the ancient medical history of Ayurveda, both as a medicine and also as a flavoring agent to disguise the unpleasant flavor of other medications.[7] The licorice shrub is a member of the pea family and grows in subtropical climates in rich soil to

a height of four or five feet. The leaflets are oval, leaves multifoliate, imparipinnate, flowers in axillary spikes, papilionaceous, lavender to violet in colour, pods compressed, containing reinform seeds, white to purplish flower clusters, an extensive root system with a main taproot and numerous runners. The main taproot, which is harvested for medicinal use, is soft, fibrous and has a bright yellow interior.[8]

Glycyrrhiza plays an important part in Hindu medicine and is one of the principle drugs of the 'Susruta'. Licorice is used to relieve 'Vata' and 'Kapha' inflammations, eye diseases, throat infections, peptic ulcers, arthritic conditions, and liver diseases in Indian Ayurveda system.[9] The chemical constituents present in the roots were previously reported include, glycyrrhizin (a saponin glycoside which is about 50 times sweeter than cane sugar), glycyrrhizinic acid,[10] glabridin, glabrene, glabrol, licoflavonol, glycyrol, licoricone, formononetin, phaseollinisoflavan, hispaglabridin A & B, 3-hydroxy glabrol, 3-methoxy glabridin,[11,12] glabranin isomer, narigenin, lupiwightenone.[13,7] The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties.[14] Hence, the present study was designed to investigate the fluorescent, antimycobacterial properties of cold and hot extracts of *G. glabra*. Column chromatography was used to find the active fraction showing positive antimycobacterial activity against *M. smegmatis*.

Experimental

Chemicals used

All the solvents (hexane, chloroform, dichloromethae, ethyl acetate, acetone and methanol) used for the extraction process were of analytical grade and procured from SD Fine Chemicals Pvt. Ltd., Mumbai, India. Silica gel (100-200 mesh) used for column chromatography was procured from Merck Limited, Germany. Microbiological media (nutrient agar and Mueller Hinton agar) were procured from Hi-Media Laboratories Private Limited, Mumbai, India.

Collection and authentication of plant material

Fresh and healthy root samples of *G. glabra* were procured from local market in Thiruvananthapuram, Kerala state, India. The plant material was identified by Dr. Shaji P.K., Scientist, Environmental Resources Research Centre (ERRC), P.B. No. 1230, P.O. Peroorkada, Thiruvananthapuram, Kerala state, India. The plant materials were initially cleaned, dried under shade and then pulverized to coarse powder in an electric grinder. The powder was then stored in airtight bottles for further studies.

Fluorescence analysis

The fluorescent characteristics of the powdered root samples were studied under short UV (254 nm), long UV (366 nm) and visible light as per the method described by Kokoshi et al.[15] A small quantity of root powder was taken and two to three drops of different organic solvents like 5% H_2SO_4, 5% HCl, 5% $FeCl_3$, 5% NaOH, 5% KOH, 1N NaOH in water, ethanol, nitric acid, acetic acid, chloroform, acetone and distilled water were added separately and mixed well. The slides were then placed inside the UV chamber and observed under visible light, short and long ultra violet radiations.

Preparation of extracts

The 30 grams of *G. glabra* powder was subjected to hot and cold extraction using each of the 250 ml solvents (hexane, chloroform, dichloromethane, ethyl acetate, acetone and methanol) in an increasing order of polarity. The final filtrate of each extract was concentrated using a rotary vacuum evaporator (IKA, RV 10 digital, Germany). The extracts were collected, evaporated to dryness and stored in vials for further studies. The percent extractive of hot and cold extracts were calculated by using the formula

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Bacterial strain

The mycobacterial strain, *Mycobacterium smegmatis* (MTCC 994) was used for this study and it was procured from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India.

Antimycobacterial activity

The antimycobacterial sensitivity assay was carried out by disc diffusion method[16] and different solvent extracts of roots were tested against *M. smegmatis*. The bacterial culture was evenly spread over Mueller Hinton agar plates using a sterile cotton swab. The sterile discs (6 mm in diameter) were impregnated with 100 µl of different solvent extracts and placed in the inoculated agar. The plates were then incubated at 37°C for 2 to 4 days. After incubation, the inhibition zones developed were measured with the scale to the nearest in mm.

Fractionation of chloroform crude extract

The chloroform layer was dried over Na_2SO_4 and evaporated to dryness under reduced pressure yielding 600 mg of extract, which was dissolved in chloroform and chromatographed on a silica gel 60 column (100-200 mesh) with 100 ml hexane, linear gradient of hexane and dichloromethane (v/v, 75:25 to 25:75), 100 ml of dichloromethane, 100 ml of linear gradient of dichloromethane and chloroform (v/v, 75:25 to 25:75), 200 ml of chloroform, 100 ml of linear gradient of dichloromethane and methanol (v/v, 75:25 to 25:75) and finally with 100 ml of 100% methanol. About 16 fractions measuring 100 ml were collected and concentrated using rotary evaporator. The collected fractions were stored at -20ºC for further analysis.

Antimycobacterial activity of the column fractions

Antimycobacterial activity of each column fractions was tested by disc diffusion method as mentioned above.

Results and Discussion

Globally, tuberculosis still remains a major public health problem. India is a high TB burden country contributing to 26 per cent of global TB burden.[17] Many researchers are screening plants to identify bioactive compounds which can act against Mycobacterium and *G. glabra* is one among them. Lot of phytochemicals has been isolated from licorice, including a water-soluble, biologically active complex that accounts for 40-50 percent of total dry material weight. This complex is composed of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and various other substances.[18]

Fluorescence analysis

The fluorescence analyses under visible light, *G. glabra* powder showed brown, green and brown colours under short and long UV light respectively and the colors observed by application of different reagents in various radiations are given in Table 1. Similar fluorescence analysis was carried out by many authors[19, 20] to ascertain the purity. The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostic evaluation of crude drugs.[15] Fluorescence studies help in the identification and authentication of the plant material and this information can act as reference information for exact identification of a particular plant and will also be useful in making a monograph of the plant. In addition to this, it will act as a tool to detect adulterants and substituent and will help in maintaining the quality, reproducibility and efficacy of natural drugs.[21]

Table 1: Fluorescence analysis of root powder of *G. glabra*

Particulars of treatment	Under short UV light	Under long UV light	Under visible light
Powder as such	Green	Brown	Brown
5% H_2SO_4	Green	Dark Green	Brown
5% HCl	Green	Dark Red	Dark Red
5% $FeCl_3$	Black	Black	Black

5% NaOH	Black	Black	Black
5% KOH	Black	Black	Black
1N NaOH in water	Black	Black	Black
Ethanol	Green	Black	Brown
Nitric acid	Green	Black	Red
Acetic acid	Green	Black	Red
Chloroform	Green	Black	Brown
Acetone	Green	Dark Red	Dark Brown
Water	Green	Black	Dark Red

In the case of percent extractives of *G. glabra,* maximum extractives was recorded for cold extraction process with a highest percentage of 2.75 each in chloroform and ethyl acetate extracts and the lowest percentage was exhibited by acetone extracts (0.5). The corresponding values for other extracts are given in Table 2.

Table 2: Percent extractive of *G. glabra* root extracts

Plant	Extraction process	Hexane	Chloroform	Dichloro methane	Ethyl acetate	Acetone	Ethanol
G. glabra	Hot	1.06 %	2.7%	0.73%	0.4%	0.3%	0.9%
	Cold	1.125%	2.75%	0.75%	2.75%	0.5%	1.75%

Antimycobacterial activity of the extracts

Antimycobacterial activity of the various cold and hot extracts was shown in Table 3. Out of six extracts tested chloroform extract recorded the significant activity with an inhibitory zone of 22 mm in hot extract and 24 mm in cold extract followed by dichloromethane. No activity was recorded in hexane, ethyl acetate and methanol extracts.

Table 3: Antimycobacterial activity of different extracts of *G. glabra*

Test organism	Name of solvent	Extraction process and zone of inhibition in (mm)	
		Hot	Cold
M. smegmatis	Hexane	Nil	Nil
	Chloroform	22±.01	24±1
	Dichloromethane	20±2	22±.01
	Ethyl acetate	Nil	Nil
	Acetone	12±0.81	16±0
	Methanol	Nil	Nil

Ethanolic and aqueous extracts of *G. glabra* exhibited a strong antimicrobial activity against *Candida albicans* and gram positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis and Enterococcus faecalis.*[22] The antimicrobial activity of *G. glabra* is well known[23] and glabridin has been reported to possess antibacterial activities against some bacterial strains.[24] Glabridin has been reported to exhibit multiple pharmacological activities such as antimicrobial activity against *Helicobacter pylori,*[25] methicillin resistant *Staphylococcus aureus.*[24] The isoflavones, glabridin and hispaglabridins A and B have significant antioxidant activity[26] and both glabridin and glabrene possess estrogen-like activity.[27]

Antimycobacterial activity of column fractions

Out of thirteen column fractions tested, only 2 fractions recorded antimycobacterial activity (Table 4). Highest activity was recorded by 25% hexane + 75% dichloromethane fraction (14mm) and the lowest was recorded in 75% chloroform + 25% methanol fraction with an inhibition zone of 12mm. Thus these fractions could be considered as a source of interesting antimicrobial compounds.

Table 4: Zone of inhibition of different column fractions of chloroform extract

Test organism	Name of solvents	Diameter of zone of inhibition (mm)
Mycobacterium smegmatis	100% Hexane	Nil
	75% Hexane + 25% Dichloromethane	Nil
	50% Hexane + 50% Dichloromethane	Nil
	25% Hexane + 75% Dichloromethane	14±1
	100% Dichloromethane	Nil
	75% Dichloromethane + 25% Chloroform	Nil
	50% Dichloromethane + 50% Chloroform	Nil
	25% Dichloromethane + 75% Chloroform	Nil
	100% Chloroform	Nil
	75% Chloroform + 25% Methanol	12±1
	50% Chloroform + 50% Methanol	Nil
	25% Chloroform + 75% Methanol	Nil
	100% Methanol	Nil

The antitubercular phenolic compounds from *G. glabra* and *G. inflate* were previously identified as licoisoflavone and licochalcone A.[28] In the present study the antimycobacterial activity of *G. glabra* may be attributed to the presence of glabridin, licoisoflavone and licochalcone A. Further studies can confirm the active principle behind this activity and this can be beneficial to the search for active antimycobacterial compounds.

Conclusion

G. glabra is well known for its antioxidant, antimicrobial, hepatoprotective activities and from the present study we conclude that the chloroform extract recorded the maximum antimycobacterial activity against *M. smegmatis*. Further fractionation of chloroform crude extract revealed that the fraction 25% hexane + 75% dichloromethane exhibited good antimycobacterial activity and this fraction could be considered as a source of interesting antimycobacterial compounds.

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