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Phytochemical constituents and antibacterial activity of *Tinospora cordifolia* and *Glycyrrhiza glabra*

Shivani Gupta, RK Sharma, Anju Nayak, Vidhi Gautam and Shreya Dubey

Abstract
Phytochemical study was undertaken to determine active principles in the ethanolic and aqueous extracts of *T. cordifolia* and *G. glabra*. Results showed presence of alkaloids, reducing sugar, glycosides, steroids, proteins and amino acids in ethanolic and aqueous extracts of *T. cordifolia*. Whereas ethanolic and aqueous extracts of *G. glabra* showed presence of alkaloids, reducing sugar, tanins and saponins. The *in vitro* antibacterial activity of ethanolic and aqueous extract of *T. cordifolia* and *G. glabra* was evaluated against *Escherichia coli*, *Klebsiella pneumonia*, *Samonella* Typhimurium, *Staphyloccocus aureus*, *Streptococcus pyogens* and *Bacillus cereus*. No zone of inhibition was found against any of the bacteria.

Keywords: Phytochemical, antibacterial, extract, *T. cordifolia*, *G. glabra*

Introduction
Herbal medicines represent one of the most important fields of traditional medicine all over the world. To promote the use of herbal medicine and to determine their potential as a source for new drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified way (Joshi *et al*., 2015) [1]. Plants are the main source of herbal medicine and are safe, economic and easily available. Various guidelines have been developed and set for application to prevent foodborne diseases and food spoiling microorganisms; it is completely impossible to be free of them. Reports have indicated that *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* are not only multidrug resistant pathogens but also pan drug-resistant bacteria.

Tinospora cordifolia is an herbaceous shrub, well known for its medicinal properties. The Hindi name of the plant is Giloy, a Hindu mythological term that cites to heavenly elixir used by Celestial beings to stay off the aging and to stay young forever (Jalalpurea *et al*., 2006) [2]. An immunomodulator is a substance which stimulates or suppresses the components of immune system including both innate and adaptive immune responses (Mukherjee *et al*., 2014) [3]. *Tinospora cordifolia*, also called 'Guduchi' has been examined for its immunomodulatory properties. According to Ayurveda used for the cure of jaundice, skin diseases, diabetes, anemia, emaciations and various infections for its anti-spasmodic, anti-inflammatory, anti-arthritic and anti-allergic properties. It has also been reported that it improves the phagocytic and bactericidal activities in patients suffering from polymorphism in surgical jaundice (Bhardwaj *et al*., 2012) [4]. The extract of stem of *Tinospora cordifolia* has wide range of phytoconstituents including alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, steroids and triterpenoids (Mehra *et al*., 2013) [5]. The antibacterial activity of *Tinospora cordifolia* stem extracts was investigated against bacteria causing UTIs viz. uropathogens, *Escherichia coli* and *Staphylococcus aureus* (Salkar *et al*., 2017) [6].

Glycyrrhiza glabra commonly known as licorice and Mulethi in Hindi, is an herbaceous perennial and its root has been widely used around the world to treat cough since ancient times. It contains active compounds, including glycyrrhizin, glycyrrhetinic acid, flavonoids, isoflavonoids and chalcones. Glycyrrhizin and glycyrrhetinic acid are considered as its main active components. *Glycyrrhiza glabra* root extracts showed various antibacterial activities against the bacterial organisms viz., *Bacillus coagulans*, *Escherichia coli* and *Salmonella* Typhimurium were tested (Nirmala and Selvaraj, 2011) [7].

Keeping the above facts in mind following study was conducted, to evaluate the phytochemical constituents, percent extractability and *in vitro* antibacterial activity from

aqueous and alcoholic extract of *Tinospora cordifolia* and *Glycyrrhiza glabra* in poultry.

Material and Methods

The study was carried out in the Department of Veterinary Pharmacology and Toxicology College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur, Madhya Pradesh.

Experimental Animals: Present study was conducted on 96-day old chicks of Cobb broilers procured from Phoenix group of poultry, Jabalpur. Chicks was divided in 08 groups consisting of 12 birds in each group with two replicate each. The birds were kept in experimental poultry house of College of Veterinary Science and Animal Husbandry, Jabalpur and was maintained under standard hygienic condition, ration and water (ad lib).

Indigenous Plant: Stem of *Tinospora cordifolia* and roots of *Glycyrrhiza glabra* was obtained from the Department of Botany, Jawaharlal Nehru Krishi Vishwa Vidhyalay, Jabalpur. Stem of *Tinospora cordifolia* and roots of *Glycyrrhiza glabra* was dried, crushed and used for supplementation of diet in chicks/birds.

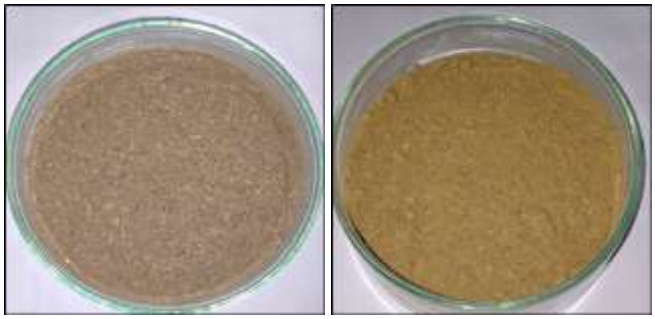

Fig 1: Stem powder of *Tinospora cordifolia* (A), Root powder of *Glycyrrhiza glabra* (B)

Table 1: Reference Bacterial Strains

S. No.	Bacteria	ATCC Catalogue No.
1.	*Escherichia coli*	25922
2.	*Klebsiella pneumoniae*	700603
3.	*Salmonella* Typhimurium	13311
4.	*Bacillus cereus*	11778
5.	*Staphylococcus aureus*	6538
6.	*Streptococcus pyogenes*	12386

Preparation of *Tinospora cordifolia* and *Glycyrrhiza glabra* extract

The stem and roots were shade dried at room temperature. Powdered stem and roots were sieved through a sterile muslin cloth. The powder was used for preparing the aqueous and alcoholic extract.

Preparation of Aqueous extract of *Tinospora cordifolia* and *Glycyrrhiza glabra*

One gram of stem powder of *Tinospora cordifolia* and root powder of *Glycyrrhiza glabra* was boiled in sterile 10 ml distiled water for ten minutes over boiling water bath. The extract was cooled and filtered through Whatmann filter paper no. 1 and volume of the filtrate was adjusted as per requirement by adding sterile distilled water.

Preparation of Alcoholic extract of *Tinospora cordifolia* and *Glycyrrhiza glabra*

Ethanolic extracts of stem powder of *Tinospora cordifolia* and root powder of *Glycyrrhiza glabra* was prepared using Soxhlet apparatus as per the method described by Pandey and Shrivastava (1989) [8]. Stem powder and root powder was weighed and kept in thimble and then in the extraction assembly. Ethanol 96 percent was used for extraction. The extraction was continued till the thimble became colourless. The diluted extracts were heated in a petridish over hot water bath to obtain semisolid alcoholic extracts which was then covered and kept in a refrigerator at 4 °C.

Estimations

Percent Extractability: The percent extractability of aqueous and ethanolic extracts of *Tinospora cordifolia* and *Glycyrrhiza glabra* was calculated by the formula described by Tandle *et al*. (1986) [9].

$$\text{Percent extractability} = \frac{\text{Total amount of extract obtained}}{\text{Total amount of powder taken for extraction}} \text{X100}$$

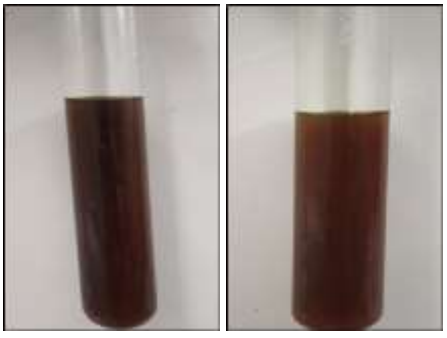

Fig 2: Aqueous extract of *Tinospora cordifolia* (A), Aqueous extract of *Glycyrrhiza glabra* (B)

Fig 3: Photograph showing preparation of ethanolic extract of stem powder of *Tinospora cordifolia* and root powder of *Glycyrrhiza glabra* by Soxhlet apparatus

Phytochemical Study

The chemical analysis of aqueous and alcoholic extract of *Tinospora cordifolia* and *Glycyrrhiza glabra* was done as per the method described by Tandle *et al*. (1986) [9]. The study was conducted as follows:

Test for Alkaloid: A minute quantity of extract was taken in five ml of 1.5 percent Hydrochloride acid (v/v) and was immediately filtered. The filtrate was subjected for the presence of alkaloid by using following methods:

- **Dragendroff's Reagent:** The sample solution in dilute HCl was applied on the dragendroff's reagent sprayed paper using a capillary tube. Development of an orange red colour indicated the presence of alkaloid.
- **Wagner's Reagent:** The filtrate i.e. acid solution of the extract was added to Wagner's reagent. Appearance of brown flocculent precipitate indicated presence of alkaloid.

Test for Reducing Sugar: The extract was dissolved in warm distilled water and tested with Benedict's and Fehling's reagents respectively for the presence of reducing sugar and glycosides.

- **Benedict's Reagent:** Five ml of extract solution was poured in a test tube and equal quantity of Benedict's reagent was added and heated, the appearance of brown, red precipitate indicated the presence of reducing sugar.
- **Fehling's Reagent:** To two ml of the extract solution 0.5ml of Fehling's reagent was mixed (Fehling's solution A and B each mixed equally immediately before use) and thereafter two ml of 10 percent sodium hydroxide solution was added. The mixture was heated on boiling water bath 10 minutes. Appearance of red precipitate indicated the presence of reducing sugar.

Test for Glycosides

- **Fehling's Reagent:** The solution already obtained in Fehling's test was used for the presence of glycosides. Few drops of HCl were added to solution and it was boiled for five minutes for hydrolysing the glycosides. Fehling's reagent (0.5 ml) was added to note any further reduction, which indicates the presence of glycosides.
- **Benedict's Reagent:** The solution obtained in Benedict's test was filtered and dilute HCl was added. The equal quantity of Benedict's reagent was added and boiled. Appearance of brownish precipitate revealed the presence of glycoside.

Test for Tannin: Methanol was added to the residue of the extract. The solution was heated and filtered through Whatman filter paper. With this filtrate following tests were carried out using different reagents.

Lead Acetate Test: Two or three drops of lead acetate solution were added to the above-mentioned extract solution. The formation of precipitate indicated presence of tannin.

Ferric Chloride Test: Few drops of ferric chloride solution were added in the above filtrate. A green coloration in filtrate of ethanolic extract indicated the presence of tannin.

Test for Resin: A small amount of extract residue was dissolved in alcohol and few drops of distilled water were added. The appearance of turbidity was considered as a positive test for resin.

Test for Sterol

Salkowski Reaction: One-gram residue of the extract was taken in two ml of chloroform. Thereafter, two ml of concentrated sulphuric acid was added by the side of test tube. The tube was shaken for a few minutes. Development of the red colour in chloroform layer and greenish yellow fluorescence in the lower layer indicated the presence of sterol.

Test for Fixed Oil: A drop of extract was put on filter paper, appearance of oily base (spot) indicated positive test for presence of oil. To five ml of the extract solution, one ml of the mixture containing equal part of concentrated sulphuric and concentrated nitric acid was added. A colour reaction indicated presence of fixed oil.

Test for Amino Acid

Ninhydrin Test: One ml of 0.1 percent solution of ninhydrin alcohol was added to the extract. Development of violet or purple colour indicated the presence of amino acid.

Test for Protein

- **Xanthoprotein Test:** One ml of extract was taken in two ml of water and to it 0.5 ml of concentrated nitric acid was added. The appearance of white or yellow precipitate indicated the presence of protein.
- **Biuret Test:** One-gram residue was taken in water and one ml of four percent sodium hydroxide solution was added. One drop of one percent solution of copper sulphate was also added. Violet pink colour indicated presence of protein.

Test for Saponin

Foam Test: One ml of extract was taken in a test tube and small amount of sodium bicarbonate and water was added. It was shaken vigorously. Formation of froth indicated presence of saponin.

Test for Anthraquinone

Bontrager's Test: A small amount of the extract was boiled for a few minutes with five ml of 10 percent sulphuric acid and filtered immediately while hot. The filtrate was cooled and shaken with benzene. The benzene layer was separated and shaken with half of its volume of 10 percent ammonia. The ammoniacal layer acquiring pink colour indicated the presence of anthraquinone.

Test for Flavonoid: Five ml extract was dissolved in five ml ethanol (95 percent) and heated with 2-3 drops of concentrated HCl and 0.5 gram of magnesium tunings. Development of pink or magnet red colour indicated the presence of flavonoid.

Antibacterial Activity

***In vitro* study**

Preparation of Bacterial Broth: The known culture of bacteria was inoculated in brain heart infusion broth and incubated at 37°C for 2 to 6 hours. Bacterial concentration was determined by as per the method described by Henric *et al*. (1956) [10].

Preparation of Discs (Discs Impregnation Method): Sterile discs were soaked in aqueous and alcoholic extract of *Tinospora cordifolia* and *Glycyrrhiza glabra* in sterile petridish for 24 hours and dried in laminar air flow, then dried discs was used immediately for discs impregnation in the inoculated plates as per the method described by Kirubaharan *et al*. (1999) [11] with slight modifications.

Antibiotic Sensitivity Test (AST): Fresh 2 to 6 hours bacterial culture of *Escherichia coli*, *Klebsiella pneumonia*, *Samonella* Typhimurium, *Staphyloccocus aureus*, *Streptococcus pyogens* and *Bacillus cereus* was spread on sterile Mueller Hinton agar plate as per the method described by Bauer *et al*. (1969) [12]. Microbial growth inhibition effect was studied by the disc diffusion method. The dried discs inoculated with plant extract samples was kept on each plate at a definite distance. A disc was also used for negative and positive controls. All the plates will be incubated at 37 °C for 24 hours. The antibacterial activity was observed by the formation of zone of inhibition. Zone of inhibition was measured by antibiotic zone inhibition reader.

Result and Discussion

Percent Extractability: The extractability of *Tinospora cordifolia* stem in aqueous and ethanol was 12 and 30 percent. The extractability of *Glycyrrhiza glabra* root in aqueous and ethanol was 10 and 25 percent.

Phytochemical Analysis: Phytochemical study was undertaken to determine the active principles in the aqueous and ethanolic extracts of *Tinospora cordifolia* and *Glycyrrhiza glabra*. The result is shown in Table 03 and Table 04.

Table 3: Phytochemical constituents in aqueous and ethanolic extracts of *Tinospora cordifolia*

Active Principle	Test applied	Aqueous extract	Ethanolic extract
Reducing sugar	Benedict Test	Positive	Positive
Glycosides	Benedict test for sugar and then hydrolysis with N/10 HCL	Positive	Positive
Resins	Alcohol containing extract in distilled water	Negative	Negative
Tannins	Ferric chloride	Negative	Negative
Alkaloid	Dragendroff, picric acid	Positive	Positive
Fixed oils	Filter paper oil	Negative	Negative
Flavonoids	Magnesium Test	Positive	Positive
Sterols	Salkowski Test	Positive	Positive
Proteins	Biuret	Positive	Positive
Saponin	Foam	Positive	Positive
Anthraquinone	Bontrager's Test	Negative	Negative
Amino acids	Ninhydrin Test	Positive	Positive

Table 4: Phytochemical constituents in aqueous and ethanolic extracts of *Glycyrrhiza glabra*

Active Principle	Test applied	Aqueous extract	Ethanolic extract
Reducing sugar	Benedict Test	Positive	Positive
Glycosides	Benedict test for sugar and then hydrolysis with N/10 HCL	Positive	Positive
Resins	Alcohol containing extract in distilled water	Negative	Negative
Tannins	Ferric chloride	Positive	Positive
Alkaloid	Dragendroff, picric acid	Positive	Positive
Fixed oils	Filter paper oil	Negative	Negative
Flavonoids	Magnesium Test	Negative	Negative
Sterols	Salkowski Test	Positive	Positive
Proteins	Biuret	Positive	Positive
Saponin	Foam	Positive	Positive
Anthraquinone	Bontrager's Test	Negative	Negative
Amino acids	Ninhydrin Test	Positive	Positive

The chemical composition of aqueous and ethanolic extract *Tinospora cordifolia* indicate presence of reducing sugar, alkaloids, flavonoids, glycosides, sterol, proteins and amino acid. Whereas *Glycyrrhiza glabra* indicate presence of reducing sugar, tannins, alkaloids, glycosides, sterol, proteins and amino acid.

Pradhan *et al*. (2013) [13] strengthened result of present finding who reported presence of phenols, flavonoids, alkaloids, saponins, cardiac glycosides, steroids, carbohydrate and proteins in *T. cordifolia*. Kaur *et al*. (2016) [14] reported the

presence of carbohydrates, glycosides, flavonoids, phenols, tannins and amino acids in extract of *T. cordifolia*. Similar result was shown by Lalam (2020) [15] and Radha and Rajaathi (2015) [16] who found glycosides, flavonoids, steroids, phenols, saponins, alkaloids and tannins in methanolic extract of *G. glabra*. The result of these findings correlates with result of present research.

In vitro Antibacterial Activity of *Tinospora cordifolia* and *Glycyrrhiza glabra*

For determination of antibacterial activity, the disc was immersed in different concentration of aqueous and ethanolic extract of stem of *Tinospora cordifolia* and root of *Glycyrrhiza glabra*. Ciprofloxacin was used as control drug to compare the effect of *in vitro* antibacterial activity of extracts.

The antibacterial activity of aqueous and ethanolic extract of *Tinospora cordifolia* and *Glycyrrhiza glabra* were evaluated against various Gram positive and Gram-negative micro-organisms namely, *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella* Typhimurium.

Results were recorded for zone of inhibition around the disc. The inhibitory zone around the disc indicated absence of bacterial growth and reported as sensitive, whereas the absence of zone of inhibition reported as negative.

In vitro Antibacterial Activity of *Tinospora cordifolia*

The *in vitro* antibacterial activity of ethanolic extract of *Tinospora cordifolia* against different Gram positive and Gram-negative bacteria are shown in Table 05. No antibacterial activity was found at any concentration of ethanolic extract.

The *in vitro* antibacterial activity of aqueous extract of *Tinospora cordifolia* against different Gram positive and Gram-negative bacteria are shown in Table 06. No antibacterial activity was found at any concentration of aqueous extract.

Table 5: *In vitro* antibacterial activity of *Tinospora cordifolia* ethanolic extract against Gram positive and Gram-negative bacteria

Bacteria	10 percent	50 percent	70 percent	90 percent
Bacillus cereus	R	R	R	R
Staphylococcus aureus	R	R	R	R
Streptococcus pyogenes	R	R	R	R
Escherichia coli	R	R	R	R
Klebsiella pneumonia	R	R	R	R
Salmonella Typhimurium	R	R	R	R

R-Resistant

Table 6: *In vitro* antibacterial activity of *Tinospora cordifolia* aqueous extract against Gram positive and Gram-negative bacteria

Bacteria	10 percent	50 percent	70 percent	90 percent
Bacillus cereus	R	R	R	R
Staphylococcus aureus	R	R	R	R
Streptococcus pyogenes	R	R	R	R
Escherichia coli	R	R	R	R
Klebsiella pneumonia	R	R	R	R
Salmonella Typhimurium	R	R	R	R

R-Resistant

In vitro Antibacterial Activity of Glycyrrhiza Glabra

The *in vitro* antibacterial activity of ethanolic extract of *Glycyrrhiza glabra* against different Gram positive and Gram-negative bacteria are shown in Table 07. No antibacterial activity was found at any concentration of ethanolic extract.

The *in vitro* antibacterial activity of aqueous extract of *Glycyrrhiza glabra* against different Gram positive and Gram-negative bacteria are shown in Table 08. No antibacterial activity was found at any concentration of aqueous extract.

Table 7: *In vitro* antibacterial activity of *Glycyrrhiza glabra* ethanolic extract against Gram positive and Gram-negative bacteria

Bacteria	10 percent	50 percent	70 percent	90 percent
Bacillus cereus	R	R	R	R
Staphylococcus aureus	R	R	R	R
Streptococcus pyogenes	R	R	R	R
Escherichia coli	R	R	R	R
Klebsiella pneumonia	R	R	R	R
Salmonella Typhimurium	R	R	R	R

R-Resistant

Table 8: *In vitro* antibacterial activity of *Glycyrrhiza glabra* aqueous extract against Gram positive and Gram-negative bacteria

Bacteria	10 percent	50 percent	70 percent	90 percent
Bacillus cereus	R	R	R	R
Staphylococcus aureus	R	R	R	R
Streptococcus pyogenes	R	R	R	R
Escherichia coli	R	R	R	R
Klebsiella pneumonia	R	R	R	R
Salmonella Typhimurium	R	R	R	R

R-Resistant

Our findings are supported by Jeyachandran *et al.* (2003) [17] who reported no antibacterial activity of stem extract of *Tinospora cordifolia* against *Salmonella* Typhimurium, *Staphylococcus aureus* and *Serratia marcescens*. In contrast to previous research paper by Mehra *et al.* (2013) [5] 30 and Tambekar *et al*. (2009) [18] 50 who reported zone of inhibition in *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhimurium, *Salmonella paratyphi* and *Shigella flexineri* in methanolic extract of *Tinospora cordifolia*. Irani *et al*. (2010) [19] reported that ethanolic root extract of *Glycyrrhiza glabra* showed sensitivity for *B. subtilis*, *S. aureus* and *E. faecalis* and aqueous root extract showed sensitivity for *B. subtilis*, *S. aureus*, *E. faecalis* and *C. albicans*. These findings do not support result of present research.

Conclusion

Important bioactive principles were found in *T. cordifolia* and *G. glabra* which may lead to the drug discovery and development. Antibacterial activity was not found in extract of *T. cordifolia* and *G. glabra*. Thus, study should be extended to search the pure bioactive molecules, responsible for various activities of *T. cordifolia* and *G. glabra*. Further studies are required to carry out *in vivo* antibacterial activity of *T. cordifolia* and *G. glabra*.

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