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Comparative Phytochemical study of total compound of *Glycyrrhiza glabra* (Yasthimadhu) and *Tinospora cordifolia* (Giloy) and their antimicrobial activity

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ABSTRACT

The study was carried out to identify the phytochemicals present in total compound of the medicinal plants *Glycyrrhiza glabra* (Yasthimadhu) and *Tinospora cordifolia* (Giloy). The antimicrobial sensitivity of the total compound was checked against Gram Negative Bacteria (*Escherichia coli*) and Gram Positive Bacteria (*Staphylococcus aureus*). Water and solvent extract of both the samples were tested for different phytochemical test. In case of solvent extract of total compound of both samples S1 and S2, sample1 (*Glycyrrhiza glabra*) showed the better antimicrobial activity result then sample2 (*Tinospora cordifolia*) against both microorganism *Staphylococcus aureus* (gram +ve) and *Escherichia coli* (gram -ve). Where was in case of water extract of total compound of both sample S1 and S2, S1 and S2 showed the almost same antimicrobial activity against *Staphylococcus aureus* (gram +ve) but against *Escherichia coli* (gram -ve) sample2 (*Tinospora cordifolia*) showed the better antimicrobial activity then sample1 (*Glycyrrhiza glabra*).

Key words : *Glycyrrhiza glabra* (Yasthimadhu), *Tinospora cordifolia*(Giloy), solvent extract, Water extract, Total compound, Antimicrobial activity, Phytochemical.

Introduction

According to WHO 80% of the total population depends on traditional medicine which is made of plant extract on their active constituents. India is following a tradition of producing medicine from plants since ancient times (Ayurveda, Siddha, Unani, Amdi and local health tradition). India is a country which has large variety of indigenous herbal plants due to the favorable climatic conditions and plays an important role in traditional medicine production (Pandey *et al.*, 2008).

In countries like India, Egypt, China and Greece the practice of treatment by medicinal plant product is carried out since back to over 5,000 years ago. In

India there are about 4.5 million plant species and out of it 2,50,000-5,00,000 plants species are studied and known to have pharmacological or biological activity. The use and development of traditional medicine by the use of medicinal plant has considerable economic benefits. Under the circumstances such as lack of modern medicine facility or poverty most people of rural area still used the traditional plant medicine in their day to day life to treat few diseases (Bamola *et al.*, 2018).

Phytochemicals plays an important role in preventing chronic disease like cancer, diabetes and coronary heart diseases. The phytochemicals functions as antioxidants, detoxifying agents, anti-cancer, dietary fiber, neuro-pharmacological and immu-

nity potentiating agents. The source of phytochemicals is medicinal plants. They are naturally occurring chemical compounds. For example tannins, alkaloids, saponins, steroids, phenol, flavanoids, terpenoids and glycosides (Krebs, 2001).

Flavonoids possess the biological property such as antimicrobial, cytotoxicity, anti-inflammatory and also anti-tumor activity. The main property of flavonoid is that it acts as anti-oxidants and it protects our body from free radicals and reactive oxygen species. Plant extracts which contents tannins are used as astringents, against diarrhea, as diuretics against stomach and duodenal tumors, anti oxidants, antiseptic and also anti-inflammatory. Alkaloids containing plant extract have properties of antimonial activity and also hypersensitive effect. Terpenoids containing plant extract have the properties to act as an anti-inflammatory, anti-cancer, antimonial activity. Saponin containing plant extract have ant carcinogenic property and also act as an antiviral and antifungal (Srinivasan *et al.*, 2017).

Selection of plant

Glycyrrhiza glabra is a medicinal plant (glykys-sweet, rhiza-root) and is commonly known as Yasthimadhu. It belongs to the family *Fabaceae*. It is mainly a shrub and its height ranges up to 120cm (Kumar *et al.*, 2017). It has woody root and stolen. Its leaves are arranged in a multi-foliolate manner and the flower contains spikes in an auxiliary manner (Kushwaha *et al.*, 2017). It has various properties such as anti-inflammatory, antiviral, anti-bacterial and ant hepatotoxic. It is sweet in taste and also very widely used in various Ayurveda medicines (Premanath and Lakshmideri, 2010).

Taxonomical classification: Kingdom: Plantae
Division: Tracheophyta, Class: Magnoliopsida
Order: Ranunculales, Family: Fabaceae
Genus: *Glycyrrhiza*, Species: *glabra*

Tinospora cordifolia is a medicinal plant that is being most widely used as a medicinal plant. It belongs to the family *Menispermaceae*. It is a large, deciduous, extensively spreading, climbing shrub with several elongated twining branches. Leaves are simple, alternate and heart shapes (Gupta *et al.*, 2018).

Taxonomical classification
Kingdom: Plantae, Division: Magnoliophyta
Class: Magnoliopsida, Order: Ranunculales

Family: Menispermaceae, Genus: *Tinospora*
Species: *cordifolia*

This present study was designed to extract total compound with water and solvent (Hexane) from *Glycyrrhiza glabra* and *Tinospora cordifolia* and their phytochemical analysis and to also perform antimicrobial activity of the total compounds on Gram +ve And Gram -ve Bacteria.

Materials and Method

Plant material

Glycyrrhiza glabra (S1)

Stem of *Glycyrrhiza glabra* commonly known as **Yasthimadhu** was collected from Local market of Guwahati, Assam. It was cut in small pieces with the help of a knife and then was grinded to get powder form.

Tinospora cordifolia (S2)

Fresh stem of *Tinospora cordifolia* commonly known as Giloy was collected from local market of Udalguri, Assam. It was washed with distilled water and cut into small pieces and then dried under sunlight till complete dry. It was then grinded to get powder form with the help of a mixture grinder.

Chemical

Bacteriological media used in this study were purchased from Hi-media laboratories and chemicals of phytochemical analysis were obtained from Sigma-Aldrich. Co., St. Louis, USA, Himedia laboratories and Sisco Research Laboratory, Mumbai, India. Solvent were obtained from Sisco Research Laboratory, Mumbai, India. Molisch's reagent, Fehling's reagent were obtained from Himedia laboratories, Mumbai, India. Known antimicrobial disk was obtained from Himedia laboratories, Mumbai, India.

Extraction of total compound from plant sample

Water extraction

10 g of S1 and S2 were weighed and dissolved in 100 ml of distilled water respectively. It was then homogenized and kept in a hot water bath (GeNei Ultrasonic bath XUBA3) for 3 hours at 60°C. Then it was filtered through Whatman filter paper and the filtrate was lyophilized by the help of racy biotech freeze dryer to get the powder form. And weighed

300 mg of powder form of water extract and dissolve it 30 ml of distilled water to get standard solution of total compound (10 mg/ml) and stored it at 4 °C for further phytochemical analysis.

Solvent extraction

Solvent extraction was done a method slightly modified from Franz Von Soxhlet in 1879. The solvent in the flask is evaporated in a rotary evaporator and thus the extract is obtained [11]. Hexane as solvent was used for the extraction. Extraction of total compound from both S1 and S2 were done by the solvent as hexane (ratio 1:10) through Soxhlet apparatus (U R Biocoction SXA-43-6) for 24 to 30 hour. The solvent were then evaporated by the help of rotary evaporator to get powder form of total compound as extract. And then weighed 300 mg of powder form of solvent extract and added 6 ml of DMSO (20 % DMSO) and then dissolved completely into 24 ml of dist. water to (total 30 ml) get standard solvent extract solution of total compound (10 mg/ml) and stored it at 4 °C for further phytochemical analysis.

Pytochemical screening

Preliminary assay were performed to detect the presence of various phytochemicals in the total compound of plant extract. Tests were performed for S1 and S2 with the standard (10 mg/ml) water extract and solvent extract (Hexane).

Test for terpenoids

2 ml of each extracts were treated with Chloroform (1 ml) followed by a few drops of concentrated sulphuric acid. Formation of brown ring at the junction indicated the presence of terpenoids.

Test for carbohydrate

Molisch Test- Treat extract with few drops of molisch reagent. Add concentrated sulphuric acid slowly along the sides of the test tube. If purple to violet color ring appears it confirms the presence of carbohydrates.

Fehling's Test- Fehling A and Fehling B reagents were mixed and few drops of the extract is added and boiled. A brick red colored precipitate of cuprous oxide formation confirms the presence of carbohydrates.

Test for flavonoid

Few drops of 20% sodium hydroxide solution were

added to 2 mL of extracts. Formation of intense yellow color, which becomes colorless on addition of dilute hydrochloric acid, confirms the presence of flavonoids.

Test for saponin

Foam Test- The extract was diluted with 20 ml of distilled water in a test tube and it was shaken by hand for 15 min. A layer of foam formed at the top of the test tube indicated the presence of Saponin.

Test for glycoside

Keller-Kiliani Test- In 2 ml plant extract, 2 ml acetic acid, one drop of 2% FeCl₃ and concentrated H₂SO₄ were added. Reddish brown color ring appears at the junction of the two liquid layers indicates the presence of glycosides.

Test for tannins

Ferric Chloride Test- 1 ml each of plant extract and 3-4 drops of ferric chloride is added. A transient greenish to the black color indicated the presence of Tannins.

Lead Sub Acetate Test- 1 ml of plant extract added with 3-4 drops of 1% of lead acetate solution. A creamy gelatinous precipitation indicates a positive test for Tannins.

Test for steroid

In 2 ml of plant extract, 2 ml of chloroform and 2 ml of concentrated H₂SO₄ was added and shaken well. If Chloroform layer appeared red and acid layer greenish yellow fluorescent. It confirms the presence of steroids.

Test for alkaloids

Mayer's reagent- It is used for the detection of alkaloids. 2-3 ml of plant extract, few drops of Mayers's reagent. It will produce a creamy white precipitation if alkaloids are present.

Picric acid test- 2ml of plant extract is treated with few drops of 1% picric acid. A yellow precipitate indicates the presence of alkaloids.

Anti-microbial activity test

For determining the antimicrobial activity media was prepared by Mueller Hinton Agar and distilled water in required proportions. The media was autoclaved and poured into the dishes (14X90 mm Tarson, Cat. No. 460090) and left for solidification.

After solidification of the media two particular bacteria *Staphylococcus aureus* and *Escherichia coli* from pure culture in peptone water media and microorganisms were spreading by the help of swabs stick all over the Mueller Hinton Agar media. Paper disk were placed on to the solid media plate. Then 10 micro liter of water as well as solvent (hexane) extract of total compound for both sample 1 and 2 with different concentration (1mg /ml, 5 mg/ml and 10mg/ ml) were poured on that paper disk with respect to known antibiotic disk. The dishes are then kept for overnight in incubator (U R Biocoction). The plates were then observed regularly after 24 hrs. and 48 hours respectively and the diameter of zone of inhibition was then measured.

Statistical analysis

Statistical analysis was carried out in triplicates (n=3) and standard error (SE) was calculated.

Results and Discussion

Phytochemical test

The outcome of qualitative phytochemical analysis of total compound of *Glycyrrhiza glabra* (S1) and *Tinospora cordifolia* (S2) are presented in Table 1. "+" sign indicates Presence and "-" sign indicates absence of compound in the total compound of water as well as solvent extract.

In-vitro Antimicrobial activity of total compound

In this study the anti-microbial activity of total compound of water and solvent extract of *Glycyrrhiza glabra* (S1) and *Tinospora cordifolia* (S2) were tested against *Staphylococcus aureus* and *Escherichia coli*. As shown in **Fig 1** zone of inhibition measured against *Staphylococcus aureus* for solvent extract of S1 and S2



Fig. 1. Solvent extract of Yasthimadu (S1) and Giloy (S2) and their comparative antimicrobial activity against *Staphylococcus aureus* (Gram positive bacteria).

were 8.9 and 6.46 mm with 10mg/ml. The concentration of solvent extract (10 mg/ml) showed the best result for both sample against *Staphylococcus aureus* (Graph 1). In Fig 2 the zone of inhibition measured against *Escherichia coli* for solvent extract of S1 and S2 were 8.5 and 7.33 mm with 10mg/ml. As shown in Graph 2 concentration of solvent extract (10 mg/ml) measured the best inhibition result for both the sample. As shown in Fig 3 zone of inhibition measured against *Staphylococcus aureus* for water extract of S1 and S2 were 6.766 mm and 6.766 mm with 10 mg/ml of total compound. As shown in

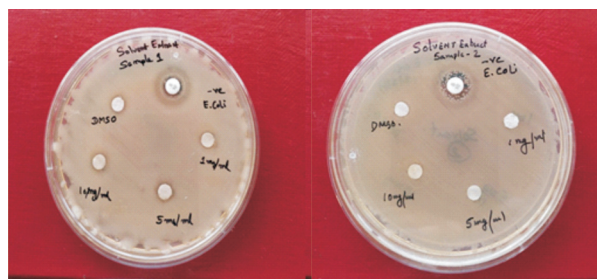


Fig. 2. Solvent extract of Yasthimadu (S1) and Giloy (S2) and their comparative antimicrobial activity against *Escherichia coli* (Gram negative bacteria).

Table 1. Qualitative phytochemical analysis of total compound of *Glycyrrhiza glabra* (S1) and *Tinospora cordifolia* (S2).

SL No.	Phytochemicals	Result			
		Aqueous extract (Water)		Solvent extract (Hexane)	
		S1	S2	S1	S2
1	Terpenoids	+	+	+	+
2	Carbohydrate	+	+	+	+
3	Flavanoids	+	+	+	+
4	Saponins	+	+	-	-
5	Glycosides	+	-	+	-
6	Tanins	-	-	-	-
7	Steroids	-	-	-	-
8	Alkaloids	-	-	-	-

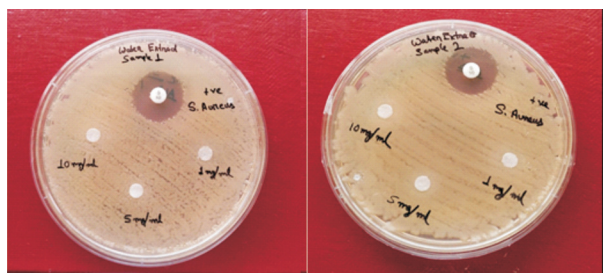


Fig. 3. Water extract of Yasthimadu (S1) and Giloy (S2) and their comparative antimicrobial activity against *Staphylococcus aureus* (Gram positive bacteria).

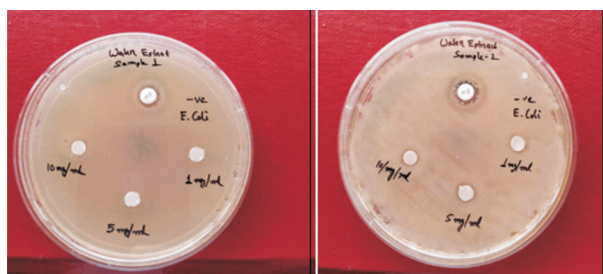
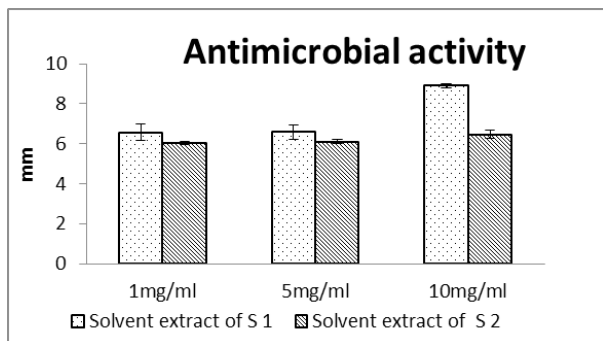


Fig. 4. Water extract of Yasthimadu (S1) and Giloy (S2) and their comparative antimicrobial activity against *Escherichia coli* (Gram negative bacteria).

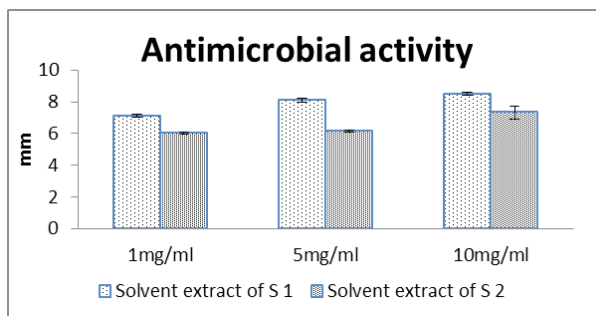


Graph 1. Solvent extract of Yasthimadu (S1) and Giloy (S2) and their comparative antimicrobial activity against *Staphylococcus aureus* (Gram positive bacteria)

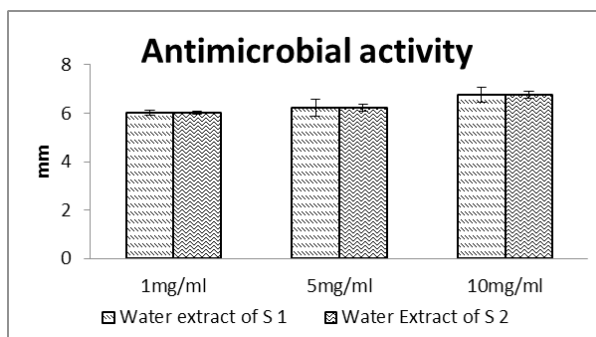
Graph 3 again water extract of total compound of both samples (10 mg/ml) gave the best inhibition. According to Fig 4 zone of inhibition against *Escherichia coli* for water extract of both samples (S1 and S2) measured as 7.1 mm and 8.1 mm. Based on the Graph 4 concentration of water extract for both sample (10mg/ml) showed the best inhibition result.

Conclusion

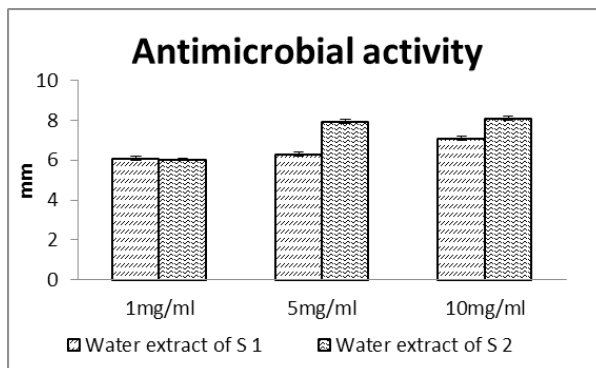
In case of solvent extract of total compound of both samples S1 and S2, sample1 (*Glycyrrhiza glabra*)



Graph 2. Solvent extract of Yasthimadu (S1) and Giloy (S2) and their comparative antimicrobial activity against *Escherichia coli* (Gram negative bacteria).



Graph 3. Water extract of Yasthimadu (S1) and Giloy (S2) and their comparative antimicrobial activity against *Staphylococcus aureus* (Gram positive bacteria).



Graph 4. Water extract of Yasthimadu (S1) and Giloy (S2) and their comparative antimicrobial activity against *Escherichia coli* (Gram negative bacteria).

showed the better antimicrobial result then sample2 (*Tinospora cordifolia*) against both microorganism *Staphylococcus aureas* (gram +ve) and *Escherichia coli* (gram -ve). Where was in case of water extract of total compound of both sample S1 and S2, S1 and S2 showed the almost same antimicrobial activity

against *Staphylococcus aureus* (gram +ve) and against *Escherichia coli* (gram -ve) sample2 (*Tinospora cordifolia*) showed the better antimicrobial activity than sample1 (*Glycyrrhiza glabra*).

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