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Comparative assessment of antimicrobial efficacy of *Tinospora cordifolia* and *Glycyrrhiza glabra* against synergistic pathogens

Kanchan Kumari, Manoj Sharma, Pushpa Jagarwal and Mala Agarwal

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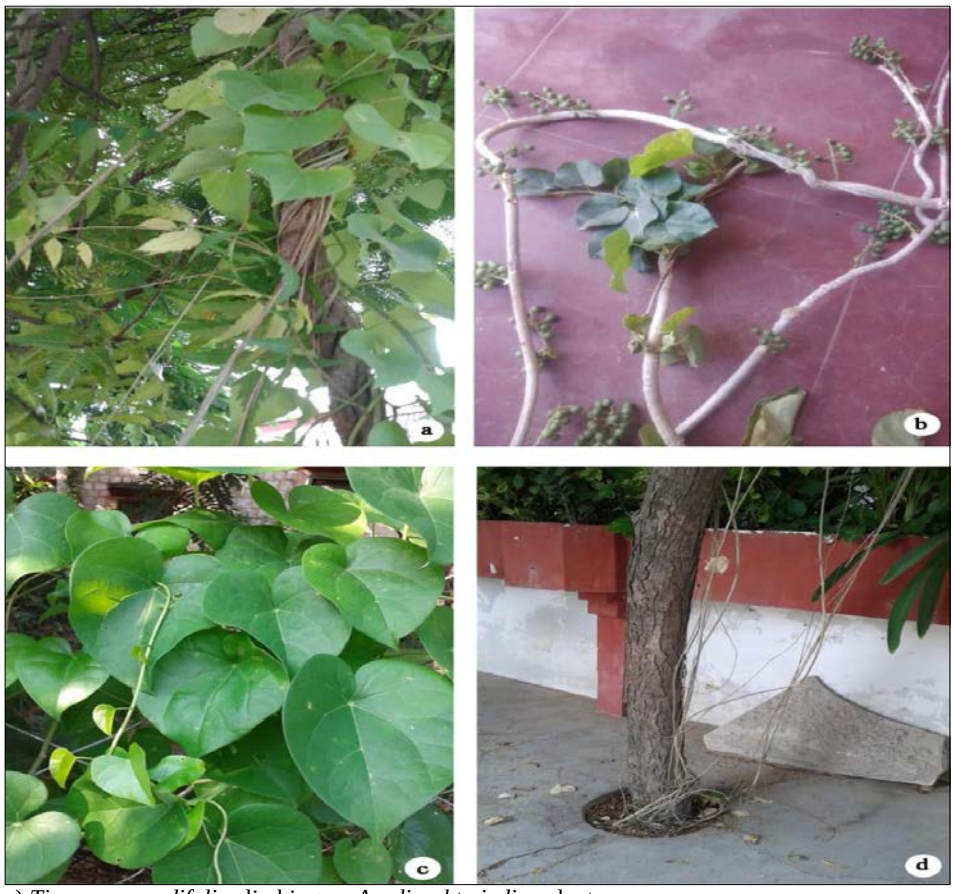
Abstract
Tinospora cordifolia and *Glycyrrhiza glabra* are well-known in traditional medical systems for its therapeutic effects, as it is efficacious in the management of infectious disorders while also reducing many of the adverse effects of synthetic antimicrobials. In an antibacterial bioassay, the biological activity of ethanol and benzene extracts of different plant components (leaves and stem) and callus of *T. cordifolia* and *G. glabra* was assessed. They could be used as lead compounds in the pharmacy sector or as the foundation for novel antibiotic research. Using the agar well diffusion method, the antibacterial activity of crude ethanol and benzene extracts of various plants parts (leaf and stem) and callus induced from nodal was assessed *in vitro*. The antimicrobial activity was determined using some *S. aureus*, *B. subtilis*, *E. coli*, *S. grisveus*, and fungal isolates *viz. T. reesei*, *F. oxysporium*, *C. albicans* and *P. funiculosum.* The ethanolic and benzene extract of callus showed maximum zone of inhibition in *G. glabra* and in *T. cordifolia*, leaf extract showed maximum toxicity against bacterial pathogens. Ethanolic and benzene extract of callus and stem was showed maximum zone of inhibition in *T. cordifolia* and *G. glabra* respectively against fungal pathogens. The leaves, stem and callus of *T. cordifolia* show that it had greater potential to inhibit the growth of all fungal pathogens but less toxic effect on *C. albicans* and *F. oxysporum*. The present study was carried out to estimate the antimicrobial activity of plants parts (leaf and stem) and callus extract to justify the pharmaceutical aspect of the plant to provide herbal plant products as phytochemistry in Ayurveda as well as ethno botanical aspect of the plant.

Keywords: *Tinospora cordifolia*, *Glycyrrhiza glabra*, pathogen, agar well diffusion, ethanol, benzene

Introduction
Natural plant products are a significant source of synthetic and traditional medicines. The majority of the world population, especially in developing countries, is dependent on herbal formulations for their primary health care needs. *Tinospora cordifolia* and *Glycyrrhiza glabra* are a popular medicinal plant and possess many pharmacological properties. *Tinospora* be a member of family Menispermaceae, which is part of the larger group Angiosperms. They are present in Asia, Africa, and Australia's tropical and subtropical regions. *T. cordifolia* and *T. crispa* are the most widespread species. The genus *Tinospora* contains many plants species, and native peoples utilised some plants as conventional remedies. In Asia, Africa, and Australia's tropical and subtropical regions (Bisset and Nwaiwu, 1983) [3]. The Menispermaceae have been used in traditional pharmacopoeia, and medicines derived from these plants are widely used in modern medicinal world (Xie *et al*., 2015) [29]. *Glycyrrhiza glabra* is a flowering plant in the Fabaceae bean family from which a sweet, fragrant flavouring may be harvested. *G. glabra* (family Fabaceae), also known as Licorice, is an herbaceous perennial that has been used for thousands of years as a flavouring ingredient in meals and medical treatments (Sharma *et al*., 2013) [24]. Since ancient times, licorice root has been commonly used to cure coughs all throughout the world. Due to having steroid-like structures, glycyrrhizin and glycyrrhetinic acid are regarded the primary active components and are strong inhibitors of cortisol metabolism (Sharma *et al*., 2018) [25].
Tinospora species were discovered a genus of essential medicinal herbs used as home remedies for treatment of nausea, pain in head, throat infection, flu, diarrhoea, ulcer in mouth and stomach, asthma, to produce more digestive juice, and rheumatoid arthritis in a comprehensive literature review (Spandana *et al*., 2013) [26]. Ethnomedicinal properties like antistress, antiulcer, anticancer and antitumor activity (Veloso *et al*., 2014) [28], anti-diabetic and hyperglycaemic activtiy, antitussive (anticough), immunomodulatory are indicated in literature (Pushpangadan *et al*., 2013) [16].

The crude extracts of members of family Menispermaceae possessed antibacterial, antifungal, anti-inflammatory, plasmodial, hepatoprotective, antioxidant, anti-inflammatory, anti-arthritis, anti-cancer, anti-tumoral activity etc. hence used as traditional therapeutic purpose to abate a number of chronic diseases from ancient periods (Choudhury *et al*., 2014) [6]. Licorice has also been found to have anti-diabetic, anti-inflammatory, antioxidant and antibacterial effects. As a result, from ancient times, it has been used in traditional remedies as an antiallergic, irritation, emollient, and fungicide (Badkhane *et al*., 2014) [4]. It is also used in different illnesses, including coughs, asthma, arthritis, peptic ulcers, Addison's disease, bronchitis, and allergy symptoms. Glycyrrhizin and glycyrrhizic acid, both derived from licorice, exhibit impressive biological active properties (Sharma *et al*., 2018) [25]. They may act as lead compounds for the pharmaceutical industry or as the base for the development of new antimicrobials.

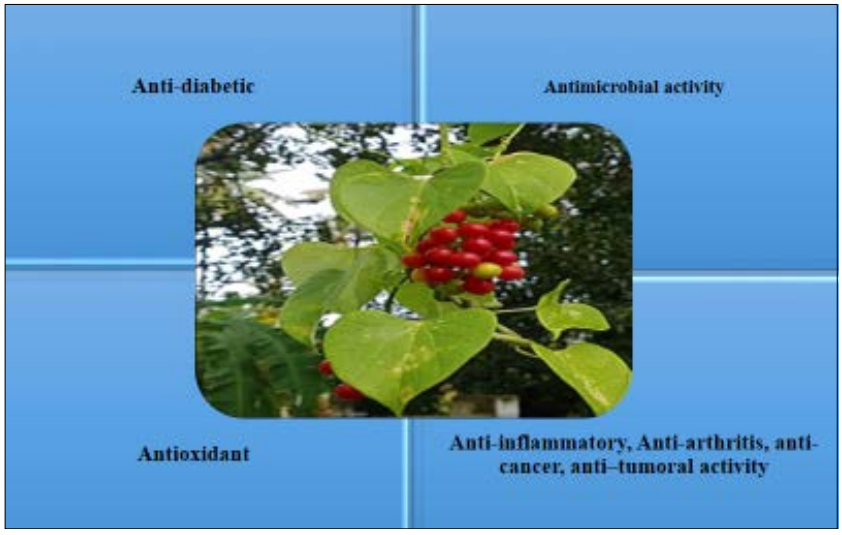
These traditional medicinal herbs' anti-COVID-19 action may be mediated by direct suppression of viral replication or entrance (Shree *et al*., 2020) [23]. *T. cordifolia* and *G. glabra* both plants are huge source of phenols and antioxidants. Further, biological active compounds which are present in both plants did splendid miracle in speedy recovery from SARS CoV-2 induced acute inflammation (Armanini *et al*., 2020; Sagar and Kumar, 2020) [2, 22]. Furthermore, phytoconstituents has been use to hinder the protease activity in treatment of Covid-19 patients (Sinha *et al*., 2020; Jena *et al*., 2021) [21, 10].



a) *Tinospora cordifolia* climbing on *Azadirachta indica* plant,
b) *Tinospora cordifolia* fluting stage
c) Leaves of *Tinospora cordifolia*
d) Aerial roots of *Tinospora cordifolia*

This plant has many economic importance hence investigated for its *in vitro* growth and propagation. *In vitro* germination and callus were induced in *T. cordifolia* (Raghu *et al*., 2006) [20]. *In vitro* antimicrobial activity was also checked (Agarwal *et al*., 2019) [1]. Callus cultures may also be used to start suspension cultures of *T. cordifolia* for berberine research (Pillai and Siril, 2019) [18]. *In vitro* germination and callus were induced in *T. cordifolia* (Raghu *et al*., 2006) [20].

The effectiveness of a particular antimicrobial agent results in the production of growth- inhibition zones that appear as clear areas surrounding the disc from which the agent diffused. The diameter of the zones can be measured and the results of such an experiment are represented as zone of inhibition in mm. In the present investigation ethanol and benzene extract of various plant parts (leaf and stem) and callus of *Tinospora cordifolia* and *Glycyrrhiza glabra* been studied for their antimicrobial efficiency.



a) *Glycyrrhiza glabra* plant
b) Twig of *Glycyrrhiza glabra*
c) Leaves of *Glycyrrhiza glabra*

Materials and Methods

Tinospora cordifolia and *Glycyrrhiza glabra* leaves, stem and callus were shade dried and extracted with benzene and ethanol, respectively.

I) Callus culture

In the instance of *T. cordifolia*, nodal segments were cut aseptically and placed on Murashige and Skoog's medium (Murashige and Skoog, 1962) [12] having 30 g/l sucrose and enriched with 0.5 mg/l 2, 4-D + 0.2 mg/l NAA (-Naphthalene Acetic Acid). For *G. glabra*, standard medium was 0.5 mg/ml 2, 4-D + 2.0 mg/ml BAP, which was cultivated at 22 2°C with 16 hours of light and 8 hours of darkness. Calli were subcultured on MS Basal media after 3 weeks and collected after 4 weeks.

II) Preparation of extract

The crude extract was made up of dry powder of plant leaf, stem and callus (30g) in respective solvents for 24 h on a rotating shaker. The extract was centrifuged for 15 minutes at 5000 g, and dried to reduced pressure. The sample was held in sealed bottles at 4 °C.

III) Culture and maintenance of clinical isolates

Pure cultures of bacterial isolates of *Staphylococcus aureus* (MTCC-3381), *Bacillus subtilis* (MTCC-10619), *Escherichia coli* (MTCC-443), *Streptomyces grisveus* (MTCC-4734), and fungal isolates *viz. Trichoderma reesei* (ATCC-13631), *Fusarium oxysporium* (ATCC-62506), *Candida albicans* (ATCC-11568) and *Penicillium funiculosum* (ATCC-11797) obtained from S.M.S. Medical College, Jaipur. Before each antimicrobial assay, a suspension of analyse organism in solution which is saline was prepared by agar slant.

IV) Determination of anti-bacterial assay

The crude methanol extract's antibacterial activity was tested *in vitro* bacterial strains using the agar well diffusion process (Perez *et al*., 1990) [17]. The Mueller Hinton agar no. 2 (Hi Media, India) is bacteriological medium. The extracts were dissolved in 100% Dimethylsulphoxide (DMSO) at five mg/mL concentrations. The Mueller Hinton agar was melted to cool down at 48-50 °C before adding a standardised inoculum (1.5108 CFU/mL, 0.5 McFarland) aseptically to the molten agar which is melt and spread after pour into autoclaved petri dishes to form a stable plate. Wells were made in the planted agar plates. The petri plates were incubated at 37 °C overnight. The extract's antimicrobial spectrum was calculated in terms of zone inhibition were compared to those of streptomycin, a commercial control antibiotic.

V) Determination of anti-fungal assay

The antifungal behaviour of the investigational plant was studied using the agar well diffusion process (Bonjar *et al*., 2005) [5]. The subculture of yeasts and saprophytic fungi were done into Sabouraud's dextrose agar and leave experimental condition 37 °C for 24 hours and 25 °C for 2 - 5 days, respectively. Fungal spore suspensions were made in sterile PBS and calibrated to a concn of 106 cells/ml. A disinfected swab was dipped into the fungal suspension and rolled on the agar medium's surface. At 37 °C, the plates were incubated. Bioactivities were measured after a 24-hour incubation by calculating the diameter of ZOI (in mm). All tests were performed in three-time, mean and standard error were computed.

Results

Antibacterial Bioassay of *Tinospora cordifolia* and *Glycyrrhiza glabra*

Tinospora cordifolia

The biological activity of ethanolic and benzene extracts of different plant parts (leaves and stem) and callus of *T. cordifolia* were evaluated for antibacterial bioassay. The leaves, stem and callus of *T. cordifolia* show that it had greater potential to inhibit the growth of all bacteria in benzene extract comparison to ethanolic extract. Contrary to this observation, the callus showed highest inhibitory activity than stem followed by leaves (Table 1; Fig 1). Ethanolic leaf extracts were showed nontoxic effect on bacteria. Ethanolic callus extracts were showed maximum zone of inhibition than callus extract of benzene (Plate 1). Ethanolic extract of callus was observed most toxic on *S. griseus* with zone of inhibition 19±1.03mm followed by *B. subtilis* with 18±1.29 mm and minimum toxicity in *S. aureus* 12±0.56 mm (Plate 2). The AI ranged 0.5 to 0.77 in E. coli and 0.5 to 1.00 in S. griseus significant at $p< 0.01$. The stem of this plant also followed the same trend and demonstrated AI as 0.28 and 0.5 in ethanolic extract and 0.29 and 0.00 in benzene extract in two bacteria respectively.

Glycyrrhiza glabra

The Table 2 shows the impact of ethanol and benzene extract of leaf, stem and callus on bacteria. The ethanolic extract of leaf had no impact on all four bacteria namely *E. coli*, *B. subtilis*, *S. aureus* and *S. griseus*. The best results were gathered from callus extract in ethanol and benzene against pathogen *S. griseus* where ZOI was obtained 20±0.23 mm and 12±0.56 mm respectively with significant at $p<0.01$. The AI ranged 0.45 to 1.14 mm at all concn in B. subtilis indicated the toxic nature *G. glabra*. Ethanol extract of callus showed toxicity on E. coli with ZOI 21±0.33 mm. Ethanolic extract of callus showed its highest antibiotic potential against *S. aureus* (ZOI 12±0.56 mm) in significant at $p<0.01$ and benzene extract of callus was showed minimum toxicity (Plate 3; Fig). Callus showed more toxicity in *B. subtilis* than stem and leaf (Plate 4).

Antifungal Bioassay of *Tinospora cordifolia* and *Glycyrrhiza glabra*

Tinospora cordifolia

The antifungal activity of ethanolic extracts of different plant parts (leaves and stem) and callus of *T. cordifolia* were showed maximum zone of inhibition than benzene extracts (Table 3; Plate 5). The leaves stem and callus of *T. cordifolia* show that it had greater potential to inhibit the growth of all fungal pathogens but less toxic effect on *C. albicans* and *F. oxysporum*. In contrast to this result, the callus exhibited the greatest inhibitory action in both the ethanolic and benzene extracts. Ethanolic callus extracts had a greater zone of inhibition than benzene callus extracts (Plate 6). Ethanolic extract of callus was observed most toxic on *P. funiculosum* (18±0.29 mm) than *T. reesei* (16±0.23 mm) and *F. oxysporum* (16±0.96 mm) followed by *C. albicans* (15±0.33 mm) at $p<0.01$. Benzene extracts of callus was showed maximum potential to inhibit the *P. funiculosum* with zone of inhibition 15±0.76 mm. *T. reesei* pathogen was more susceptible in both extract with ZOI range from 11±0.29 mm to 19±0.56 mm.

Glycyrrhiza glabra

The antifungal activity of ethanolic extracts of leaves, stem and callus was showed greater toxicity than benzene extracts. Leaves, stem and callus shown a better ability to suppress the growth of all fungal pathogens (Plate 7 & 8). Callus showed less toxicity in both the extracts than stem and leaf (Table 4; Fig.). Callus was showed no sign of toxicity on *C. albicans* in benzene extract and on *F. oxysporum* in both ethanol and benzene extract. Ethanolic extracts of leaf were showed toxicity range from 6±0.33 mm to 16±0.59 mm. The maximum zone of inhibition was observed against *F. oxysporum* (17±0.19 mm) in ethanolic extract and *P. funiculosum* (12±0.19 mm) in benzene extract of stem. Toxicity of callus was observed highest in *C. albicans* with inhibition zone 15±0.53 mm and lowest in *T. reesei* with 11±0.97 mm in ethanolic extract. The ethanol and benzene extract of stem produced highest antifungal effect against all four fungal pathogens significant at $p<0.05$.

Table 1: Antibacterial activity of *Tinospora cordifolia* in different crude extracts

Bacterial pathogen	Ethanolic extract			Benzene extract			Standard
	Leaf	Stem	Callus	Leaf	Stem	Callus	
Zone of Inhibition (mm)							
E. coli	-	-	17±0.93**	8±0.23	6±0.23**	11±0.19**	22±1.09
AI	0.00	0.00	0.77	0.36	0.27	0.5	
B. subtilis	-	16±1.09*	18±1.29**	6±0.29*	14±0.39**	14±0.29*	17.53±0.33
AI	0.00	0.91	1.02	0.34	0.71	0.71	
S. aureus	-	6±0.23**	12±0.56**	9±0.33**	4±0.29*	5±0.19*	21.05±1.23
AI	0.00	0.28	0.57	0.42	0.19	0.23	
S. griseus	-	9±0.33*	19±1.03*	15±0.56*	-	18±1.09**	18.00±0.23
AI	0.00	0.5	1.05	0.83	0.00	1.00	

Standards: Ciprofloxacin; Activity index = ZOI of test sample/ZOI of standard. Mean ± SE, * $p< 0.05$, ** $p<0.01$, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software

Table 2: Antibacterial activity of *Glycyrrhiza glabra* in different crude extracts

Bacterial pathogen	Ethanolic extract			Benzene extract			Standard
	Leaf	Stem	Callus	Leaf	Stem	Callus	
Zone of Inhibition (mm)							
E. coli	20±0.33**	18±0.93**	21±0.33**	17±0.58**	19±0.29**	10±0.33**	22±1.09
AI	0.90	0.81	0.95	0.77	0.86	0.45	
B. subtilis	18±0.75**	20±0.75**	16±0.39**	18±0.39**	18±0.33**	8±0.23*	17.53±0.33
AI	1.12	1.14	0.91	1.02	1.02	0.45	
S. aureus	21±1.03**	17±0.29**	19±0.26**	21±1.13**	19±0.19**	9±0.29*	21.05±1.23
AI	1.00	0.80	0.90	1.00	0.90	0.42	
S. griseus	21±1.22**	16±0.23**	20±0.23**	20±0.93**	19±0.93**	12±0.56**	18.00±0.23
AI	1.16	0.88	1.11	1.11	1.05	0.66	

Standards: Ciprofloxacin; Activity index = ZOI of test sample/ZOI of standard. Mean ± SE, * $p< 0.05$, ** $p<0.01$, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software.

Table 3: Antifungal activity of *Tinospora cordifolia* in different crude extracts

Fungal pathogen	Ethanolic extract			Benzene extract			Standard
	Leaf	Stem	Callus	Leaf	Stem	Callus	
Zone of Inhibition (mm)							
C. albicans	4±0.23**	-	15±0.33*	10±0.39*	-	13±0.56**	20.00±1.73
AI	0.2	0.00	0.75	0.5	0.00	0.65	
P. funiculosum	16±0.96**	10±0.33*	18±0.29**	13±0.23**	18±0.33**	15±0.76**	19.00±0.93
AI	0.84	0.52	0.94	0.68	0.94	0.78	
T. reesei	19±0.56**	18±0.77**	16±0.23**	12±0.96**	14±0.93**	11±0.29**	22.00±0.23
AI	0.86	0.81	0.72	0.54	0.63	0.5	
F. oxysporum	-	6±0.33	16±0.96**	-	6±0.76	15±0.33**	16.00±0.16
AI	0.00	0.37	1.00	0.00	00.37	0.93	

Standards: Ketoconazole; Activity index = ZOI of test sample/ ZOI of standard. Mean ± SE, *$P< 0.05$, **$P<0.01$, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software.

Table 4: Antifungal activity of *Glycyrrhiza glabra* in different crude extracts

Fungal pathogen	Ethanolic extract			Benzene extract			Standard
	Leaf	Stem	Callus	Leaf	Stem	Callus	
Zone of Inhibition (mm)							
C. albicans	6±0.33	10±0.39**	15±0.53**	7±0.29*	6±0.28*	-	20.00±1.73
AI	0.3	0.5	0.75	0.35	0.3	0.00	
P. funiculosum	8±0.29*	10±0.33**	13±0.26**	6±0.26*	12±0.19**	6±0.23*	19.00±0.93
AI	0.42	0.52	0.68	0.31	0.63	0.31	
T. reesei	12±0.96**	8±0.29*	11±0.97**	6±0.23*	6±0.19*	9±0.29*	22.00±0.23
AI	0.54	0.36	0.5	0.27	0.27	0.40	
F. oxysporum	16±0.59**	17±0.19**	-	12±0.29**	11±0.23**	-	16.00±0.16
AI	1.0	1.06	0.00	0.75	0.68	0.00	

Standards: Ketoconazole; Activity index = ZOI of test sample/ ZOI of standard. Mean ± SE, *$P< 0.05$, **$P<0.01$, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software.

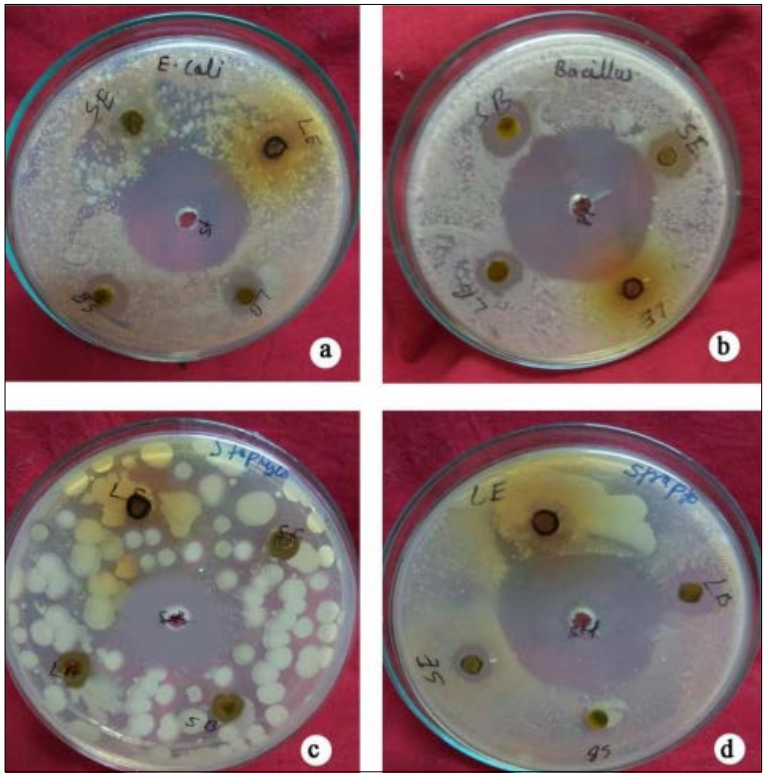
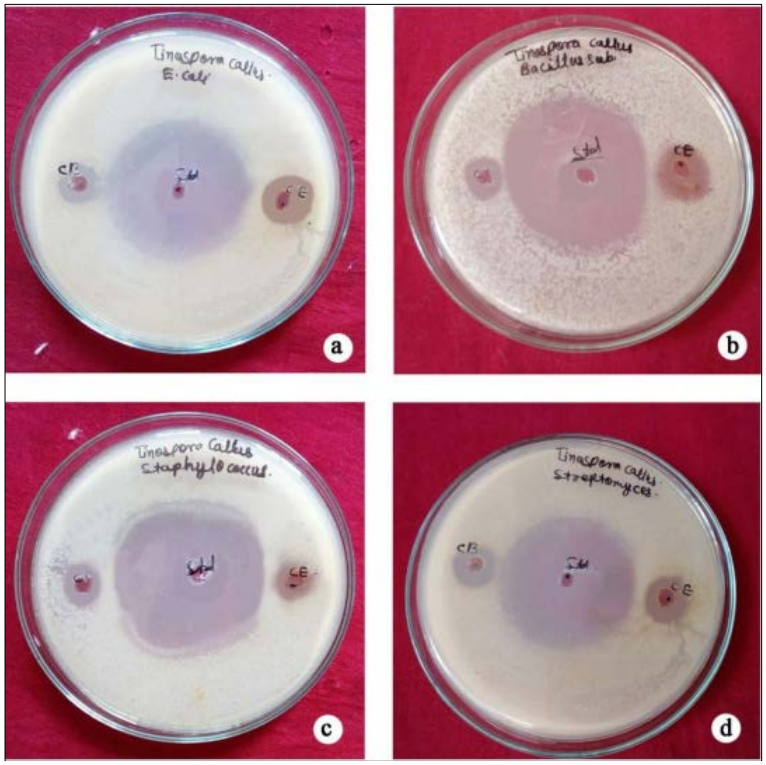


Plate 1: Antibacterial activity of *Tinospora cordifolia* (leaf and stem extract) A) *E. coli* B) *B. subtilis* C) *S. aureus* D) *S. griseus*



Abbrivations	**Abbrivations**
LE - Leaf ethanolic extract	CE - Callus ethanolic extract
SE-Stem ethanolic extract	CB- Callus benzene extract
LB-Leaf benzene extract	Std - Standard
SB Stem Benzene extract	
Std - Standard	

Plate 2: Antibacterial activity of *Tinospora cordifolia* (callus extract) *E. coli* B) *B. subtilis* C) *S. aureus* D) *S. griseus*

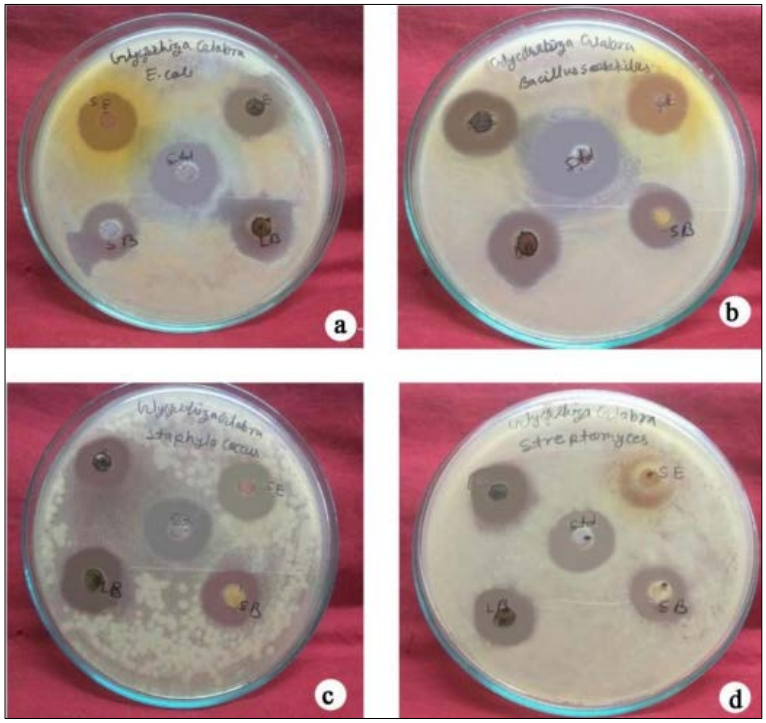
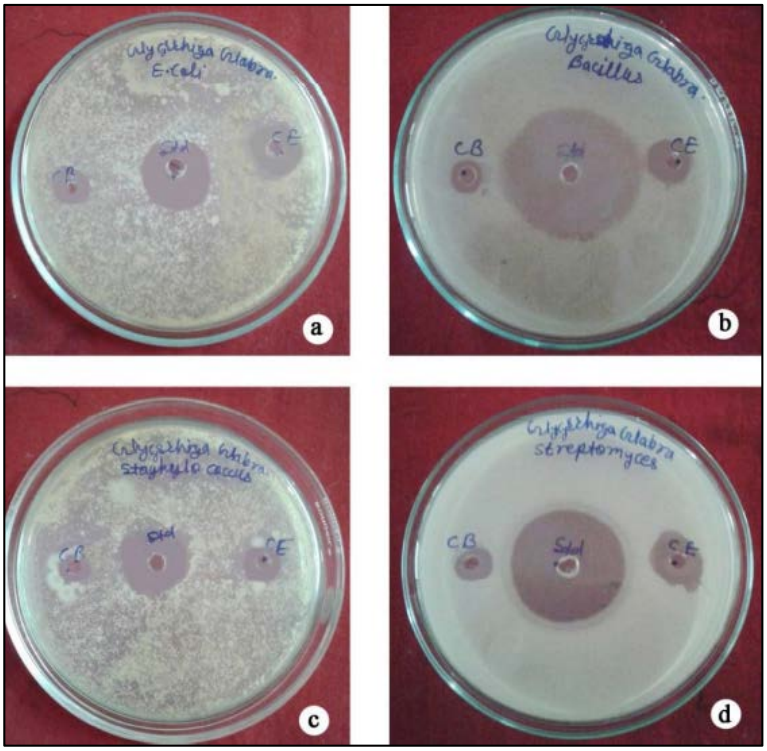


Plate 3: Antibacterial activity of *Glycyrrhiza glabra* (leaf and stem extract) A) *E. coli* B) *B. subtilis* C) *S. aureus* D) *S. griseus*



Abbrivations
LE - Leaf ethanolic extract
SE-Stem ethanolic extract
LB-Leaf benzene extract
SB Stem Benzene extract
Std - Standard

Abbrivations
CE - Callus ethanolic extract
CB- Callus benzene extract
Std - Standard

Plate 4: Antibacterial activity of *Glycorrhiza glabra* (callus extract) *E. coli* B) *B. subtilis* C) *S. aureus* D) *S. griseus*

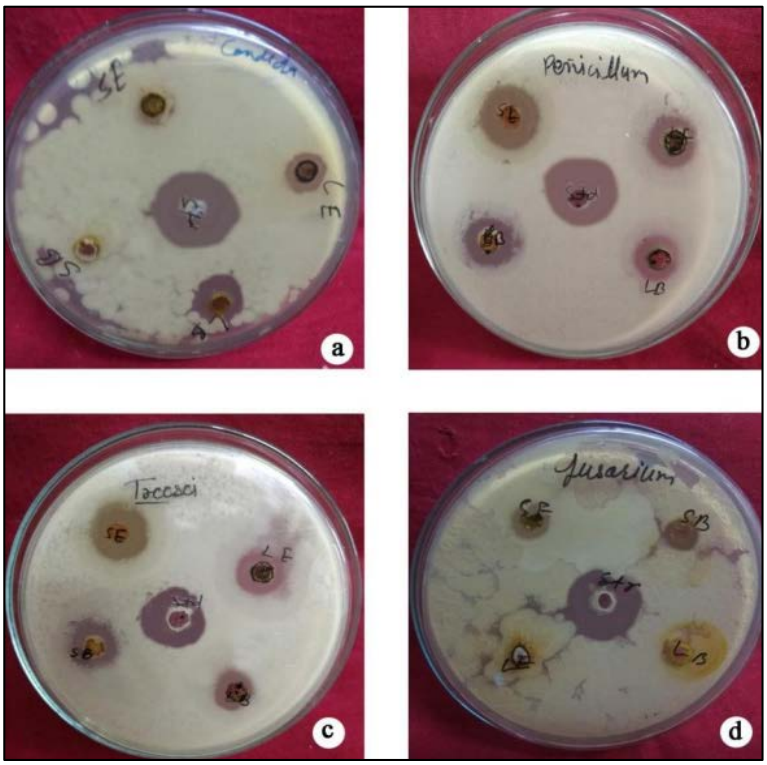
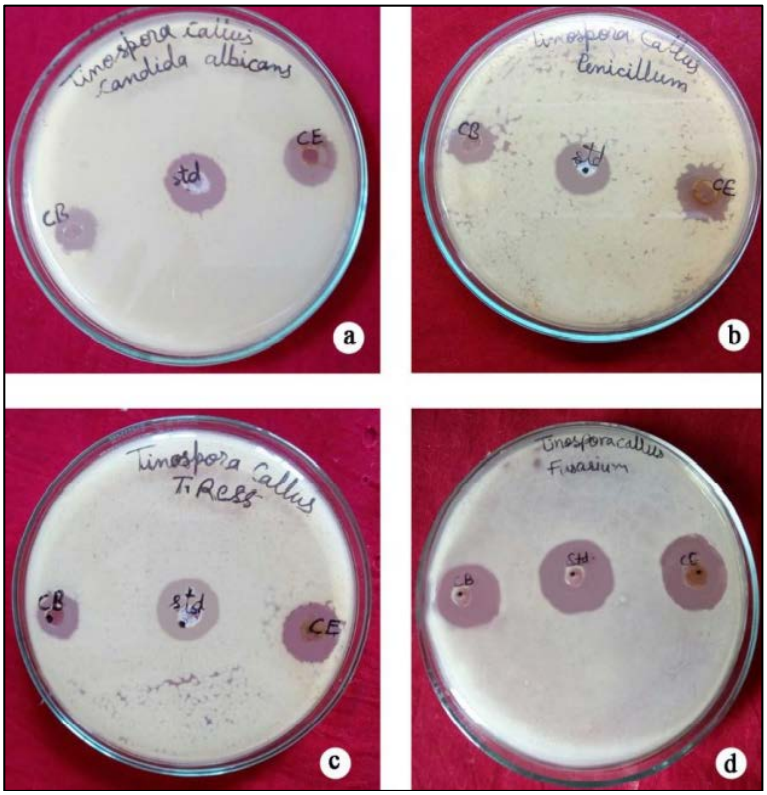


Plate 5: Antifungal activity of *Tinospora cordifolia* (leaf and stem extract) A) *C. albican* B) *P. funiculosum* C) *T. reesei* D) *F. oxysporum*



Abbrivations	**Abbrivations**
LE - Leaf ethanolic extract	CE - Callus ethanolic extract
SE-Stem ethanolic extract	CB- Callus benzene extract
LB-Leaf benzene extract	Std - Standard
SB Stem Benzene extract	
Std - Standard	

Plate 6: Antifungal activity of *Tinospora cordifolia* (callus extract) A) *C. albican* B) *P. funiculosum* C) *T. reesei* D) *F. oxysporum*

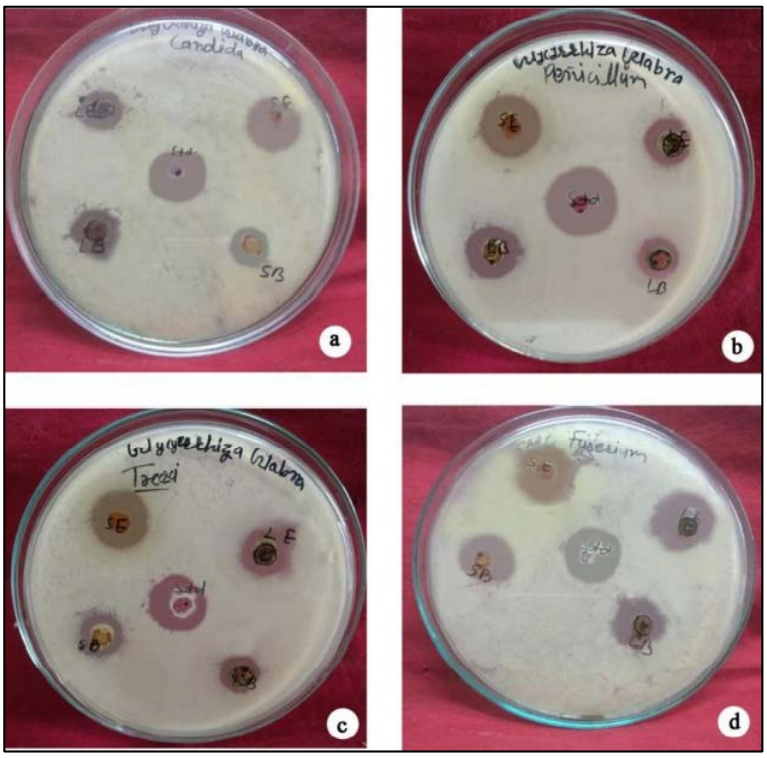
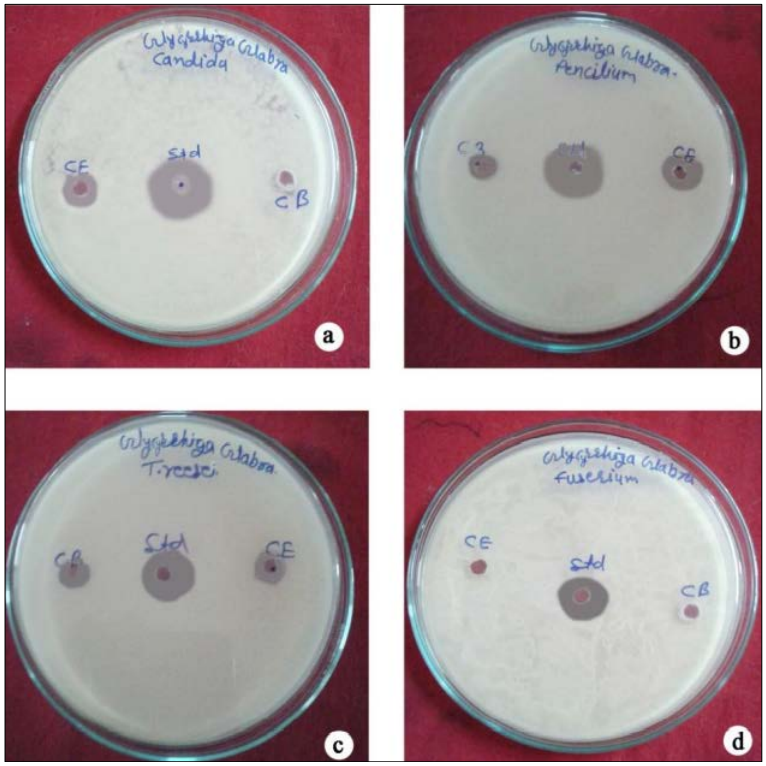


Plate 7: Antifungal activity of *Glycyrrhiza glabra*(leaf and stem extract) A) *C. albican* B) *P. funiculosum* C) *T. reesei* D) *F. oxysporum*



Abbrivations
LE - Leaf ethanolic extract
SE-Stem ethanolic extract
LB-Leaf benzene extract
SB Stem Benzene extract
Std - Standard

Abbrivations
CE - Callus ethanolic extract
CB- Callus benzene extract
Std - Standard

Plate 8: Antifungal activity of *Glycyrrhiza glabra* (callus extract) *C. albican* B) *P. funiculosum* C) *T. reesei* D) *F. oxysporum*

Discussion

This is in agreement with previous research, antibacterial and antifungal bioassays reveal that ethanol extract of both plants (*T. cordifolia* and *G. glabra*) possessed strong potential especially against bacteria *S. aureus*, *B. subtilis*, *S. griseus*, and *E. coli* and fungi *T. reesei*, *P. funiculosum* and *A. niger* in the present study. Similarly, both exhibited strong antibacterial potential towards *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*, *S. pyogenes* and *S. pneumoniae*. The present findings on antibacterial properties of both plants are in corroboration with earlier reports. *T. cordifolia* had the greatest antibacterial activity in a volume of 40 µl at a

concentration of 2%, with a zone of inhibition of 19 mm (Agarwal *et al*., 2019) [1]. *T. cordifolia* was shown to have antibacterial action against *S. aureus, B. subtilis* and *E. coli*. The ethanolic and benzene extracts of the aforementioned plant species were shown to be more powerful, which may effectively minimize pathogenic bacteria contamination (Upadhyay *et al*., 2011; Mishra *et al*., 2014) [27, 14]. The antibacterial efficacy of water, ethanol, and chloroform extracts of *T. cordifolia* stems against *Escherichia coli*, *Proteus vulgaris*, *Enterobacter faecalis*, *Salmonella typhi*, *Staphylococcus aureus*, and *Serratia marcesenses* was investigated using the disc diffusion method (Jeyachandran *et al*., 2003) [11]. The ethanolic and benzene extract of callus showed maximum zone of inhibition 19±1.03 mm and 18±1.09 mm, respectively against *S. griseus*. Ethanolic leaf extracts were showed nontoxic effect on bacteria and benzene leaf extract was showed toxic effect on all bacterial pathogens i.e. *E. coli*, *B. subtilis*, *S. aureus* and *S. griseus*. Ethanolic extract of stem was more toxic on all the bacterial pathogens than benzene extract. The antifungal activity of ethanolic extracts of different plant parts (leaves and stem) and callus of *T. cordifolia* were showed maximum zone of inhibition than benzene extracts. The leaves, stem and callus of *T. cordifolia* show that it had greater potential to inhibit the growth of all fungal pathogens but less toxic effect on *C. albicans* and *F. oxysporum*. *G. glabra* was recommended as a suitable option to assist us in controlling dental cavities and endodontic infections (Sedighinia *et al*., 2012) [19]. When compared to conventional bactericide and fungicide medicines, the aqueous and ethanolic extracts of *G. glabra* roots considerably reduced the development of microorganisms. The fungicidal efficacy of the diethyl ether fraction against *Candida albicans* was significant. Positive controls included gentamycin, streptomycin, and fluconazole (Patil *et al*., 2009) [15]. Glabridin, an active component of *G. glabra* roots, was discovered to be active against filamentous fungus. Glabridin also showed resistance-modifying action against drug-resistant mutants of *Candida albicans* at a minimum inhibitory concentration of 31.25-250 g/mL (Fatima *et al*., 2009) [7]. *G. glabra* root alcohol extract has been shown to have antifungal action against *Candida albicans* (Motsei *et al*., 2003) [13] as well as other fungus *Arthrinium sacchari* and *Chaetonmium funicola* (Hojo and Sato, 2002) [9]. The ethanol root extract of *G. glabra* demonstrated a considerably broader spectrum of action in the current investigation, being effective against different *C. albicans* strains as well as filamentous fungus including filamentous dermatophytes and nondermatophytes. *G. glabra*'s antibacterial action is widely recognised (Gupta *et al*., 2008) [8]. Antibacterial activity of *G. glabra* was not reported much but antifungal activity was noticed on different fungal pathogens.

In present study, the impact of ethanol and benzene extract of leaf, stem and callus on bacteria is studied. The ethanolic extract of leaf had no impact on all four bacteria namely *E. coli*, *B. subtilis*, *S. aureus* and *S. griseus*. The best results were gathered from callus extract in ethanol and benzene against pathogen *S. griseus* where ZOI was obtained 19±1.03 mm and 18±1.09 mm respectively in *T. cordifolia*. The maximum zone of inhibition was shown against bacterial pathogens from 8±0.23 mm to 21±1.22 mm in *G. glabra*. Antifungal activity of callus was showed maximum in both ethanol and benzene extract in *T. cordifolia* than *G. glabra*. As a result, the current study point‟s future researchers in the right path to conduct research on the both plant in order to obtain some medicinally significant medicines. Because of its safety and efficacy, *T. cordifolia* and *G. glabra* is a viable source of medicines due to its biological active constituents, pharmacological and therapeutic properties. With the development of glabridin and tinosporin as a powerful and important molecule to cure bacterial and fungal diseases, our findings support ethnomedical usage of *G. glabra* and *T. cordifolia* to heal coughs and chest associated illnesses. These plants extracts may be utilised as a good source for beneficial medicines, and their quantitative values can be employed as a key instrument for establishing a drug's quality control profile. It is possible to conclude that thick stems can be used to make high-quality goods.

Conclusion

Because of their high activity, *T. cordifolia* and *G. glabra* can develop antibiotics that are more intriguing therapeutically than recognised antibiotics. Both pharmaceutical businesses and research institutes involved in the development of antimicrobial medications may find the current study of economic and commercial value. There have been no side effects or toxicity reports on this plant for many years, therefore there is plenty of room for more investigation with this approach.

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References

1. Agarwal S, Ramamurthy PH, Fernandes B, Rath A, Sidhu P. Assessment of antimicrobial activity of different concentrations of *Tinospora cordifolia* against Streptococcus mutans: An *in vitro* study. Dental Research Journal (Isfahan). 2019;16(1):24-28.
2. Armanini D, Fiore C, Bielenberg J, Sabbadin C, Bordin L. Coronavirus-19: possible therapeutic implications of spironolactone and dry extract of *Glycyrrhiza glabra* L. (Licorice). Frontiers in Pharmacology. 2020;11:110-115.
3. Bisset NG, Nwaiwu J. Quaternary alkaloids of Tinospora species. Planta Medica. 1983;48(08):275-279.
4. Badkhane Y, Yadav AS, Bajaj A, Sharma AK, Raghuwanshi DK. *Glycyrrhiza glabra* L. a miracle medicinal herb. Indo American Journal of Pharmaceutical Research. 2014;4:355-359.
5. Bonjar GHS, Farrokhi PR, Aghigi SL, Bonjar S, Aghelizadeh A. Antifungal characterization of actinomycetes isolated from Kerman, Iran and their future prospects in biological strategies in greenhouse and field conditions. Plant Pathology. 2005;4:78-84.
6. Choudhury SS, Handique PJ. TDZ enhances multiple shoot production from nodal explants of *Tinospora cordifolia* - a commercially important medicinal plant species of NE India. Research Journal of Biotechnology. 2014;8:31-36.
7. Fatima A, Gupta VK, Luqman S, Negi AS, Kumar JK, Shanker K, *et al*. Antifungal activity of *Glycyrrhiza glabra* extracts and its active constituent glabridin. Phytotherapy Research: An International Journal Devoted

to Pharmacology and Toxicology Evaluation of Natural Product Derivatives. 2009;23(8):1190-1193.
8. Gupta VK, Fatima A, Faridi U, Negi AS, Shanker K, Kumar JK, *et al*. Antimicrobial potential of *Glycyrrhiza glabra* roots. Journal of Ethnopharmacology. 2008;116(2):377-380.
9. Hojo H, Sato J. Antifungal activity of licorice (*Glycyrrhiza glabra* Linn) and potential applications in beverage foods. Journal of Food Ingredients. 2002;203:45-49.
10. Jena S, Munusami P, Balamurali MM, Chanda K. Computationally approached inhibition potential of *Tinospora cordifolia* towards COVID-19 targets. Virus Disease. 2021;32(1):65-77.
11. Jeyachandran R, Xavier TF, Anand SP. Antibacterial activity of stem extracts of *Tinospora cordifolia* (Willd) Hook. f and Thomson. Ancient Science of Life. 2003;23(1):40.
12. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco callus. Physiologia Plantarum. 1962;15:473-497.
13. Motsei ML, Lindsey KV, Van Staden J, Jäger AK. Screening of traditionally used South African plants for antifungal activity against Candida albicans. Journal of Ethnopharmacology. 2003;86(2-3):235-241.
14. Mishra A, Kumar S, Pandey AK. Scientific validation of the medicinal efficacy of *Tinospora cordifolia*. The Scientific World Journal. 2013;11:212-218.
15. Patil SM, Patil MB, Sapkale GN. Antimicrobial activity of *Glycyrrhiza glabra* Linn. roots. International Journal of Chemistry Sciences. 2009;7(1):585-591.
16. Pushpangadan P, Dan VM, Ijinu TP, George V. Phytochemical characterization of medicinal plant in herbal drugs. Chemistry, Biochemistry and Ayurveda of Indian Medicinal Plants; c2013. p. 1-12.
17. Perez C, Pauli M, Bazerque P. An antibiotic assay by agar-well diffusion method. Acta Biologiae et. Medecine Expweimentaalis. 1990;15:113-115.
18. Pillai SK, Siril EA. Enhanced production of berberine through callus culture of *Tinospora cordifolia* (Willd.) Miers ex Hook F. and Thoms. India Section B Biology Science; c2019. p. 1-9.
19. Sedighinia F, Afshar AS. Antibacterial activity of *Glycyrrhiza glabra* against oral pathogens: an *in vitro* study. Avicenna Journal Phytomedicine. 2012;2(3):118-124.
20. Raghu AV, Geetha SP, Martin G, Balachandran I, Ravindran PN. *In vitro* clonal propagation through mature nodes of *Tinospora cordifolia* (Willd.) Hook. F. and Thoms.: an important ayurvedic medicinal plant. *In Vitro* Cellular and Developmental Biology-Plant. 2006;42(6):584-588.
21. Sinha SK, Prasad SK, Islam MA, Gurav SS, Patil RB, AlFaris NA, *et al*. Identification of bioactive compounds from *Glycyrrhiza glabra* as possible inhibitor of SARS-CoV-2 spike glycoprotein and non-structural protein-15: a pharmacoinformatics study. Journal of Biomolecular Structure and Dynamics; c2020. p. 1-15.
22. Sagar V, Kumar AH. Efficacy of natural compounds from *Tinospora cordifolia* against SARS-CoV-2 protease, surface glycoprotein and RNA polymerase. Virology. 2020;8(2):1-10.
23. Shree P, Mishra P, Selvaraj C, Singh SK, Chaube R, Garg N, *et al*. Targeting COVID-19 (SARS-CoV-2) main protease through active phytochemicals of ayurvedic medicinal plants-Withania somnifera (Ashwagandha), *Tinospora cordifolia* (Giloy) and Ocimum sanctum (Tulsi)-a molecular docking study. Journal of Biomolecular Structure and Dynamics. PMC7484581. 2020.
24. Varsha S, Agrawal RC. *Glycyrrhiza glabra* plant for the future. Mintage Journal of Pharmacology and Medicine Science. 2013;2(3):15-20.
25. Sharma V, Katiyar A, Agrawal RC. *Glycyrrhiza glabra*: chemistry and pharmacological activity. Sweeteners; c2018. p. 87-93.
26. Spandana U, Ali SL, Nirmala T, Santhi M, Babu SS. A review on *Tinospora cordifolia*. International Journal of Current Pharmacology Review and Research. 2013;4(2):61-68.
27. Upadhyay RK, Tripathi R, Ahmad S. Antimicrobial activity of two Indian medicinal plants *Tinospora cordifolia* (Family: Menispermaceae) and Cassia fistula (Family: Caesalpinaceae) against human pathogenic bacteria. Journal of Pharmaceutical Research. 2011;4(1):167-170.
28. Veloso A, Kirkconnell KS, Magnuson B, Biewen B, Paulsen MT, Wilson TE, *et al*. Rate of elongation by RNA polymerase II is associated with specific gene features and epigenetic modifications. Genome Research. 2014;24(6):896-905.
29. Xie D, He J, Huang J, Xie H, Wang Y, Kang Y, *et al*. Molecular phylogeny of Chinese Stephania (Menispermaceae) and reassessment of the subgeneric and sectional classifications. Australian Systematic Botany. 2015;28(4):246-255.