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Research Article

ANTIMICROBIAL ACTIVITY OF *GLYCYRRHIZA GLABRA* (LICORICE) AGAINST PEPTIC ULCER PRODUCED *HELICOBACTER PYLORI*

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ABSRACT

Ulcer producing organisms have evolved numerous defense mechanisms against antimicrobial agents, hence resistance to old and newly available drugs are increasing at an unprecedented level. The events of antibiotic resistance have lead for screening of several medicinal plants for their potential antimicrobial activity. The aim of this study is to evaluate the antimicrobial efficacy of *Glycyrrhiza glabra* against ulcer producing *Helicobacter pylori* (ULHP). *G. glabra* is a widespread medicinal plant traditionally used in India to treat infectious diseases. Aqueous, acetone and ethanol extracts of leaves of *G. glabra* were tested for antimicrobial activity *in vitro* by the agar well diffusion method. Ethanol extract of leaves exhibited antimicrobial activity against ulcer producing strains ULHP 2 and 4, whereas the aqueous and acetone extracts showed antibacterial activity only against ULHP 3. These antimicrobial properties seem to be related to the presence of tannin, alkaloids and tri-terpenoids in *G. glabra*. It can be concluded that *G. glabra* can be used to discover natural products that may serve as lead for the development of new pharmaceuticals, addressing the major therapeutic needs especially for ulcer producing pathogenic strains.

Keywords: *Helicobacter pylori*, *G. glabra*, Antimicrobial activity.

INTRODUCTION

Helicobacter pylori are a widespread Gram-negative bacterium that infects the stomach of humans. It occurs in spiral and coccoid forms in the human gut.[1] *Helicobacter pylori* helical rod that colonizes the human gastric mucous layer.[2-4]It chronically infects the gastric mucosa causing gastritis in more than 50% of the world's population. The infection can lead to the development of peptic ulcer [5] and gastric mucosa-associated lymphoid tissue lymphoma[6] ; infection with the organism has also been linked with an increased risk of gastric cancer in humans.[7,8] The development of safe anti-*Helicobacter pylori* compounds is desirable due to the problem of antibiotic resistant strains that have emerged.[9]

Numerous studies have been undertaken in order to find antimicrobial agents from plants against organisms ranging from viruses to protozoa.[10] The major concern is the validation in human beings with well-designed clinical trials, and this has also been true for *H pylori* infection. Several *in vitro* studies have looked at the effect of plant extracts on *H pylori*. Anti-microbial effects have been reported for garlic,[11,12] green tea, [13] honey,[14] thyme, [15] some Iranian plants [16] and the essential oils from several species of mint.[17] Some of these studies have been validated in animals and confirmed the potential benefit of using plants as the source of anti-microbial agents against *H pylori*. Although garlic and cinnamon have been tested in human clinical trials with no significant effect,[18] a recent study has shown that consumption of broccoli sprouts is associated with the eradication of *H pylori* in some patients,[19] but more work needs to be done in determining the active ingredients of broccoli as well as performing studies on a larger number of patients.

Licorice *(Glycyrrhiza glabra)* is one of commercially important perennial plant grassy or semi bushy type species from the leguminoceae (Fabaceae) plant family, sub-family papilionaceae. The genus name is driven from the Greek words, *glycy* (or *glulus)* meaning sweet and *rhiza* meaning root.[20] Licorice roots, runners and rhizomes are the commercially desired parts of the plant that contain a number of important chemical compounds. Glycyrrhizin is one of these compounds which shown to be 50 times or more sweet than sugar and demands high prices in the world market.[21,22] These compounds are used in medicine for its non-nutritive sweetness and anti-allergic and anti-inflammatory effects as treatment of bronchial asthma, allergic, eczema. They are also used as food in the confectionery industry such as sweets, alcohol free drinks etc. and in the tobacco industry.[21] Licorice extracts have been used for more than 60 years in Japan to treat chronic hepatitis and also have therapeutic benefit against other viruses, including human immunodeficiency virus (HIV), cytomegalovirus (CMV) and Herpes simplex. Deglycyrrhizinated licorice (DGL) preparations are useful in treating various types of ulcers, while topical licorice preparations have been used to sooth and heal skin eruptions, such as psoriasis and herpetic lesions.

The aim of this study was to substantiate the antimicrobial sensitivity of different extracts of *G. glabra* leaves against clinical isolates of ulcer producing *Helicobacter pylori* strains to lengthen the queue of antimicrobial herbs.

MATERIALS AND METHODS

Collection of Plant Materials

Leaves of *G. glabra* were collected from villages in and around Coimbatore district, South India. Plant leaves were dried under the shadow. The dried leaves were fine powdered and stored in polythene bags at room temperature (30ºC) until use.

Extract preparations

Aqueous extract

To obtain the aqueous extracts, about 10 grams of the dried and finely powdered leaves of G. glabra were homogenized using 100ml of water. They were added to Soxhlet apparatus and the boiling point of water was set up at 100ºC. The water evaporates continuously and was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solution loses the color.[23]

Acetone extract

Ten grams of the dried and finely powdered leaves of G. glabra were homogenized using 100 ml of acetone. They were added to Soxhlet apparatus and the boiling point of acetone was set up at 56.6ºC. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solvent loses its color. The extract was then transferred to a sterile Petri dish and kept for evaporation of acetone at room temperature. Residues of extracts were collected and stored in the refrigerator.

Ethanol extract

Ten grams of the dried and finely powdered leaves of G. glabra were homogenized using 100ml of 70% ethanol. They were added to Soxhlet apparatus and the boiling point of ethanol was set up at

78ºC. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solvent loses its color. The extract was then transferred to a sterile Petri dish and kept for evaporation of ethanol at room temperature. Residues of extracts were collected and stored in the refrigerator.

Antibacterial activity of plant extracts

Antibacterial activity of the aqueous, acetone and ethanol extracts of leaves of *G. glabra* was tested using the agar well diffusion method. Four ulcer producing strains were employed for testing the antimicrobial activity of the aqueous, acetone and ethanol extracts of leaves of *G. glabra*; ULHP 1, ULHP 2, ULHP 3 and ULHP 4.The selection of the test organism was based on the resistant pattern exhibited against the antibiotics used to treat ulcer caused by *H. pylori*. A loop full of culture of each test strain was inoculated into peptone broth and incubated for 2 to 6 hours at 37ºC until it achieved the turbidity of 0.5 McFarland's standard. The test cultures were swabbed on nutrient agar plates, within 15 minutes after adjusting the turbidity of the inoculum's suspension. Wells were made using the sterile well puncture. Different concentrations (200µg to 1000µg) of the sterile aqueous, acetone and ethanol extracts were added to each well. The plates were incubated at 37ºC for 24 hours. The diameter of inhibition zones were measured in millimeter (mm) and the results were recorded.

RESULTS AND DISCUSSION

In vitro antibacterial activities of leaves of *G. glabra* against *Helicobacter pylori* are shown in Table 1. The aqueous and acetone extracts of (200-1000µl) *G. glabra* leaves showed no significant zone of inhibition against the tested strains except ULHP 3 with inhibition zone diameter about 22 and 26 mm, respectively achieved at the highest extracts concentration (1000 µg/ml).

It is clear from the table 1 that the ethanol extract of *G. glabra* leaves have exhibited antimicrobial activity against ULHP 2 and ULHP 4 with maximum zones of inhibition of 22 and 24 mm respectively, but failed to exhibit inhibitory action against ULHP 1 and ULHP 3.

The antimicrobial activities of various plants have been reported by many researchers.[10,24] Umbreen et al. [25] reported the significant activity of methanol and ethyl acetate extracts of leaves and stem of *C. indica* against different bacteria providing a support to the fact that methanol is a better solvent for extraction and isolation of phytochemicals having antimicrobial activity. The present study revealed that the ethanol extract was found to be active against two ulcer producing strains namely ULHP 2 and ULHP 4 and resistant against ULHP 1 and ULHP 3. Dewanjee et al. [26] have reported that methanol extract of *C. grandis* leaves exhibited significant antimicrobial activity.

In this study, the water extract displayed lower antibacterial activity than acetone and ethanol extracts. This is in agreement with earlier studies which reported that use of organic solvents is always better for extraction of antibacterial compounds Varadarajan et al. [27] Furthermore, the effectiveness of the extracts are not due to one main constituent, but to the combined action of other chemical compounds involved in it.[28] Some of them include alkaloids, flavonoids, terpenoids, thymol and other compounds of phenolic nature which are classified as antimicrobial compounds.[29]

CONCLUSION

The results of this study showed that the *G. glabra* leaves have exhibited varied antimicrobial activities against the ulcer producing *H. pylori.* These findings support the claim of the traditional healers that *G. glabra* would be used against ulcer pathogens.

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Table 1: Antimicrobial activity of the different extracts of *G. glabra* leaves by well diffusion method against *Helicobacter pylori*

Plant Extract (1000µg/ml)	Solvent	Ulcer Producing *H. pylori*			
		ULHP 1	ULHP 2	ULHP 3	ULHP 4
G. glabra	Aqueous	-	-	22	-
	Acetone	-	-	26	-
	Ethanol	-	22	-	24

ULHP= Ulcer Producing *Helicobacter pylori*, '**-**'Indicates no significant zone of inhibition

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