

# Antimicrobial and Anticancer Potential of Glycyrrhiza Glabra

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**Abstract**— The present study was undertaken to evaluate the antimicrobial and anticancer activity of ethanolic extract of *Glycyrrhiza glabra*. The antimicrobial activity was determined by the disc diffusion method and zone of inhibition against a panel of microorganism for five bacterial strains (i.e. *B.subtilis*, *E.coli*, *P.aerogenosa*, *S.aureus* and *S.flexneri*) and for five fungal strains (i.e. *C.albicans*, *P.crysogenum*, *Rhizopus microporus*, *Trichophyton interdigitale*, *T.viride*). The effect of anticancer activity was determined on HeLa cell line by MTT assay. The results was that the plant extract mildly potent as an antimicrobial agent and anticancer activity having  $IC_{50}$  value of 31.2  $\mu$ g/ml.

**Index Terms**— Disc diffusion, MTT assay, antimicrobial, anticancer

## I. INTRODUCTION

*Glycyrrhiza glabra* is a herbaceous plant belonging to the family Pappilionaceae/Fabaceae, which grows to 1cm in height with pinnate leaves about 7-5 cm [2.8-5.9in] long, with 9-17 leaflets. The flowers are 0.8-1.2 cm long, purple to pale whitish blue, produced in a loose inflorescence. The fruit is an ablong pod, 2-3 m long containing several seeds. The roots are stoloniferous. *Glycyrrhiza glabra* is also known as licorice/Liquorice, sweet wood, mulahatli and yastimadhu (Sanjay Saxena, 2009). The scent of liquorice roots comes from a complex and variable combination of compounds of which anethole is up to 3% of total volatiles.

Much of the sweetness in liquorice comes from glycyrrhizin which has a sweet taste, 30-50 times the sweetness of sugar. Isoflavane glabrene and isoflavane glabridin found in the roots of liquorice. Liquorice which grows best in the well-drained soil deep valley with the full sun is harvested during autumns two to three years after planting. Countries producing liquorice include India, Iran, Italy, Afghanistan, the people's Republic of China, Pakistan, Iraq, Azerbaijan, Uzbekistan, Turkmenistan, Turkey and England (Sanjai Saxena, 2009).

The underground unpeeled or peeled stems or roots are used for the treatment of upper respiratory tract ailments including cough, soariness, sore throat and bronchitis. Ayurveda considers licorice to be a "rasayana" with implicated use in

the treatment of respiratory and digestive disorders. It is also considered as antistress and anabolic agent. Licorice the most prescribed herb in China is used for ailments related to spleen, liver and kidney. Japanese use the herb as an antiviral agent.

It has been shown to decrease circulating levels of testosterone in men [M.M Rafi *et al.*, 2002] and it is reported that licochalcone-A is isolated from *Glycyrrhiza glabra* root and the root has potent of antitumor properties when assayed using cap, breast and leukemia cells [DiPaola *et al.*, 1998]. The roots of *Glycyrrhiza glabra* are rich in bioactive compounds which are responsible for various medical properties like antiviral, anticancer, anti-ulcer, anti-diabetic, anti-inflammatory, antioxidant, antimalarial, antifungal, anti-bacterial, estrogenic, anti-allergenic, anticonvulsant activities [Asad Abbas *et al.*, 2016].

Drug resistance is the reduction in effectiveness of a drug such as antimicrobial, anticancer and an antineoplastic in curing a disease or condition. Antimicrobial resistance challenges clinical care and drive research. When an organism is resistant to more than one drug, it is said to be multidrug resistant. Some of the artificial substances cause some side effects. The purpose of the study is to evaluate the antimicrobial and anticancer potency of the *Glycyrrhiza glabra* root.

## II. MATERIALS AND METHODS

### A. Collection of plant

The *Glycyrrhiza glabra* plant was collected from its natural habitat. The root was separated from the plant and washed with distilled water to remove any adherent particles, shade dried and powdered with the help of a motor.

### B. Extract preparation

25 g of powdered material was weighed and extracted with 300 ml of ethanol by continuous hot percolation with the help of Soxhlet apparatus for 10 hrs. On completion the extract was filtered and concentrated using a rotary evaporator under reduced pressure and controlled temperature of 50<sup>0</sup> C – 60<sup>0</sup> C. The concentrated extract was stored in a refrigerator.

### C. Antimicrobial activity of ethanolic extract

#### C.1. Selection of microorganism

The *G.glabra* root extract was tested against a panel of microorganisms, including five bacteria, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aerogenosa*, *Shigella flexneri* and *Staphylococcus aureus* and five fungi, *Candida albicans*, *Penicillium crysogenum*, *Rhizopus microporus*, *Trichoderma viride* and *Trichophyton interdigitale*. Bacterial strains were cultured overnight at 37<sup>0</sup> C in nutrient broth and fungal strains were cultured overnight at 28<sup>0</sup> C using sabourad dextrose broth.

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C.2. Agar disc diffusion method

Antimicrobial activity of extracts was determined by disc diffusion method on Muller Hinton agar medium for bacterial strains and sabouraud dextrose agar. The agar was prepared, sterilized and poured in the petriplate. After the agar solidified, the inocula were spread on the solid plates with sterile swab moistened with the microorganism suspension. Ampicillin and Amphotericin-B is taken as positive control for bacteria and fungi. Positive control and sample (Concentration: 1000 µg, 750 µg and 500 µg) of 20 µl each were added in sterile discs and placed in agar plates. The plates were incubated at respective temperature for 24 hrs. Then, the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

D. Anticancer activity

Cell lines were obtained from the National Centre for Cell Sciences (NCCS), Pune. The cells were obtained in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (Fetal Bovine Serum), Penicillin (100 U/ml) and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml of CO<sub>2</sub> at 37° C.

*In vitro* assay for anticancer activity (MTT assay) (Mosamann, 1983)

Cells (1×10<sup>5</sup> /well) were plated in 24-well plates and incubated at 37° C with 5% CO<sub>2</sub>. After the cell reached the confluence, the various concentrations of the samples were added and incubated for 24 hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5 mg/ml) of 0.5% 3 - (4, 5 - dimethyl - 2 - thiazolyl) - 2, 5 - diphenyl - tetrazolium bromide (MTT) was added and incubated for 4 hrs. After incubation, 1 ml of DMSO was added in the wells. The absorbance at 570 nm was measured with a UV-Spectrophotometer using DMSO (Dimethyl sulfoxide) as the blank. Measurements were performed and the concentration required for 50% inhibition (IC<sub>50</sub>) was determined graphically. The % cell viability was calculated using the following formula

$$\% \text{ cell viability} = \frac{(A570 \text{ of treated cells})}{(A570 \text{ of controlled cells})} \times 100$$

Then the graph was plotted using the % of cell viability on the Y-axis and concentration of the sample on the X-axis. Cell control and sample control is included in each assay to compare the cell viability assessments.

III. RESULTS AND DISCUSSION

Much attention has been directly towards plant extracts and biological active compounds have been isolated. The use of medicinal plants may play a vital role in converging the basic health needs and these plants may offer a new source of antimicrobial and anticancer agents with significant activity against pathogenic microorganisms (Upadhyay *et al.*, 2010)

A. Antibacterial activity of ethanolic extract

The ethanolic extract of *G.glabra* was tested against some pathogenic microorganism. The results of antibacterial activity from agar disc diffusion method (Table 1) indicated that 1000 µg/ml of extract has a maximum zone of inhibition in *E.coli* and *S.flexneri* (17 and 18 mm) as shown in Figure B and Figure D, in 750 µg/ml has maximum zone of inhibition in *S.flexneri* (15 mm) as shown in Figure D and in 500 µg/ml

has maximum zone of inhibition in *S.flexneri* and *S.aureus* is 13 and 11 mm respectively as shown in Figure D and Figure E. The minimum zone of inhibition was found to be 7 mm in *B.subtilis* at 1000 µg/ml as shown in figure A. The extract activity was compared with standard drug Ampicillin, showed that *G.glabra* root extract have potent against *E.coli* was found as 17 mm comparatively to standard drug (23 mm) in 1000 µg/ml.

Table 1: Antibacterial potential of *G.glabra* root extract against various microorganisms  
Sterile disc diameter = 6mm

| Microorganisms                | Zone of inhibition (mm) |           |           | Standard antibiotic (1mg/ml) |
|-------------------------------|-------------------------|-----------|-----------|------------------------------|
|                               | 1000 µg/ml              | 750 µg/ml | 500 µg/ml |                              |
| <i>Bacillus subtilis</i>      | 07                      | 08        | 09        | 22                           |
| <i>Escherichia coli</i>       | 17                      | 09        | 08        | 23                           |
| <i>Pseudomonas aerogenosa</i> | 10                      | 09        | 08        | 29                           |
| <i>Shigella flexneri</i>      | 18                      | 15        | 13        | 28                           |
| <i>Staphylococcus aureus</i>  | 10                      | 09        | 11        | 34                           |



Fig A: *Baccillus subtilis*



Fig B: *Escherichia coli*

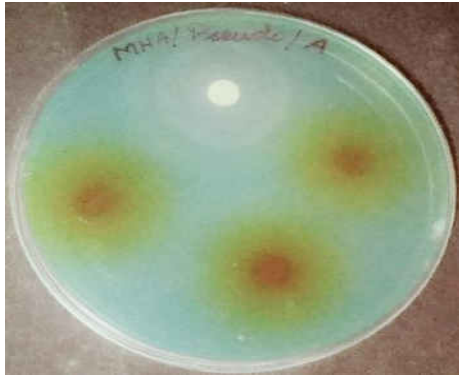


Fig C: *Pseudomonasaerogenus*



Fig D: *Shigella flexneri*



Fig E: *Staphylococcus aureus*

**B. Antifungal activity of ethanolic extract**

The antifungal activity of *G.glabra* root extract was investigated by using five different fungi. The activity of ethanolic extract was undertaken using agar disc diffusion method and the results were shown in Table 2. The results indicated that in 1000  $\mu\text{g/ml}$  ethanolic extract showed good activity against *Rhizopus*, *P.crysogenum* and *Tricophyton*, showing the zone of inhibition 11, 14 and 12 mm respectively as shown in Figure (G, H and I). Differential concentration of extract 750  $\mu\text{g/ml}$  showed the potential against *P.crysogenum*, the zone of inhibition was found to be 15 mm as shown in Figure (G) and for 500  $\mu\text{g/ml}$  concentration showed the effective activity against *C.albicans* and *P.crysogenum*, the zone of inhibition was found as 13 mm as shown in Figure (F and G). All the concentrations of ethanolic extract for antifungal activity were compared with the standard drug Amphotercin B to the concentration 1 mg/ml. The minimum zone of inhibition was found as 08 mm against *Rhizopus* in 750  $\mu\text{g/ml}$  as shown in Figure (H). The root extract have potency against the *P.crysogenum* where the zone of inhibition was found as 15 mm in 750  $\mu\text{g/ml}$  compared to standard drug Amphotercin B were found to be same (15 mm). *T.interdigitale* has maximum zone of inhibition (12 mm in 1000  $\mu\text{g/ml}$  and 11 mm in 750  $\mu\text{g/ml}$ ) and

compared with standard drug, the zone of inhibition in 500  $\mu\text{g/ml}$  (10 mm). The *Rhizopus* has the maximum zone of inhibition (11 mm in 1000  $\mu\text{g/ml}$ , 8 mm in 750  $\mu\text{g/ml}$  and 12 mm in 500  $\mu\text{g/ml}$ ) which is compared to standard drug (7 mm). Hence the root extract of *G.glabra* shows the potency against the fungal species.

Table 2: Antifungal potent ethanolic root extract of *G.glabra* against the fungal species

| Fungi                            | Inhibition zone (mm)  |                      |                      | Standard drug (1 mg/ml) |
|----------------------------------|-----------------------|----------------------|----------------------|-------------------------|
|                                  | 1000 $\mu\text{g/ml}$ | 750 $\mu\text{g/ml}$ | 500 $\mu\text{g/ml}$ |                         |
| <i>Candida albicans</i>          | 11                    | 10                   | 12                   | 21                      |
| <i>Penicillium crysogenum</i>    | 14                    | 15                   | 13                   | 15                      |
| <i>Rhizopus microspores</i>      | 11                    | 08                   | 12                   | 07                      |
| <i>Trichoderma viride</i>        | 12                    | 11                   | 13                   | 25                      |
| <i>Tricophyton interdigitale</i> | 12                    | 11                   | 10                   | 10                      |



Fig F: *Candida albicans*

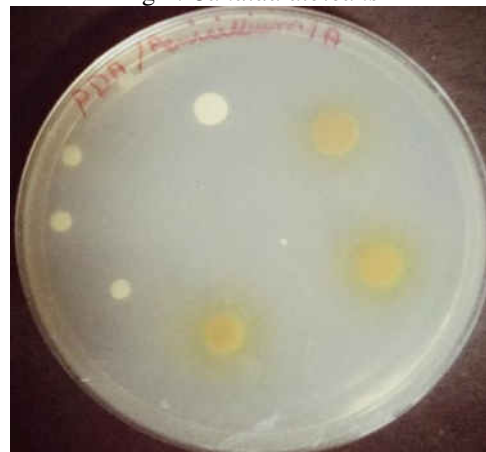


Fig G: *Penicilliumcrysogenum*



Fig H: *Rhizopus microspores*



Fig I: *Tricophyton. Interdigitale*



Fig J: *Trichoderma viride*

C. Anticancer analysis of ethanolic extract of *G.glabra* root

The anticancer effects of an ethanol extract of *G.glabra* on the expression of HSP90, growth and apoptosis in the HT-29 colon cancer cell line was evaluated (Sepide Miraj, 2016). The ethanolic extract has been used in herbal formulation for combating cancers cells like PC-SPE<sub>1</sub>; a polyherbal composition was used for prostate cancer. These extract induced BC<sub>1 2</sub> phosphorylation and G<sub>2</sub>/M cycle arrest in tumor cell lines. The highest dead rate was measured by MTT assay. Then LC<sub>50</sub> value was estimated as 31.2µg/ml as shown in the graph. The results were shown in Table 3 that the ethanolic extract of *G.glabra* root extract is potent to kill cancer cells. The microscopic view of the cancer cells is shown in Figure K and L.

Table 3: Anticancer effect of *G.glabra* on HeLa cell line.

| S.NO | Concentration (µg/ml) | Dilution | Absorbance (O.D) | Cell viability (%) |
|------|-----------------------|----------|------------------|--------------------|
| 1.   | 1000                  | Neat     | 0.234            | 16.13              |
| 2.   | 500                   | 1:1      | 0.339            | 23.37              |
| 3.   | 250                   | 1:2      | 0.438            | 30.20              |
| 4.   | 125                   | 1:4      | 0.528            | 36.41              |
| 5.   | 62.5                  | 1:8      | 0.611            | 42.13              |
| 6.   | 31.2                  | 1:16     | 0.739            | 50.96              |
| 7.   | 15.6                  | 1:32     | 0.847            | 58.41              |
| 8.   | 7.8                   | 1:64     | 0.946            | 6.24               |
| 9.   | Cell control          | -        | 1.450            | 100                |

Graph 1: Anticancer activity of *G.glabra* root extract

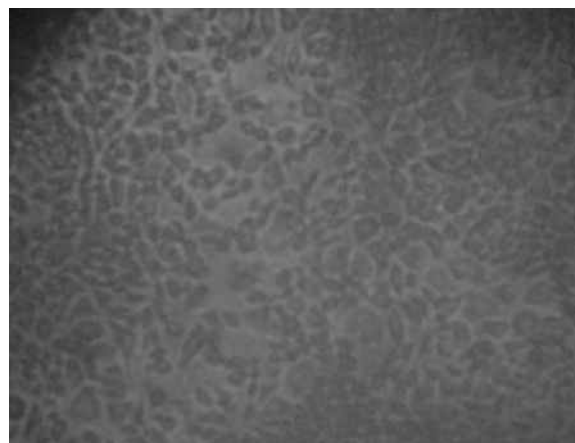
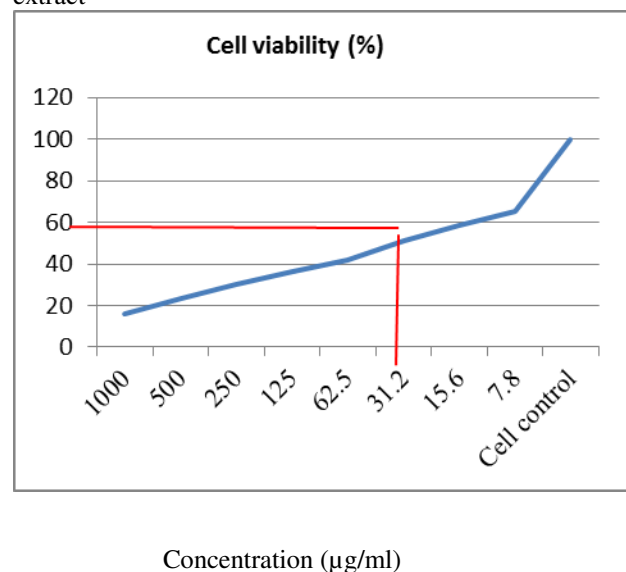


Fig K: Normal cancer cell line

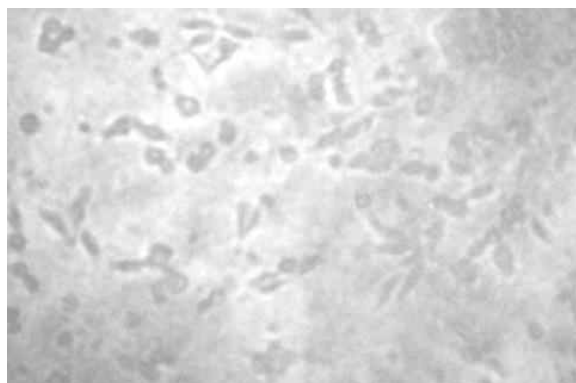


Fig L: Concentration-31.2µg/ml

#### CONCLUSION

The present study concluded that ethanolic extract of *G. glabra* root showed significant potential against antibacterial, antifungal and anticancer activity. It is due to the presence of photochemical substance like saponins, flavonoids, glycosides, alkaloids etc, that this plant is used for alternative drugs in antimicrobial and anticancer drugs. It is able to reduce the side effects of chemotherapeutic agents and control some infections which are caused by microorganisms

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