

EVALUATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF *GLYCYRRHIZA GLABRA* ROOT EXTRACTS

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ABSTRACT

Objectives: The present study was designed to investigate the phytochemical analysis, antioxidant potential, and antibacterial activities of the traditionally used medicinal plant *Glycyrrhiza glabra*.

Methods: The plant secondary metabolites were extracted through cold percolation using methanol (MeOH) as a solvent. The MeOH extract was further fractionated in different solvents in increasing order of polarity. The antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl assay. The antibacterial activity was studied by agar well diffusion method.

Results: The antioxidant potential IC_{50} was found 43.13, 104.83, and 200.11 $\mu\text{g/ml}$ for ethyl acetate (EtOAc), MeOH, and chloroform (CHCl_3) extracts, respectively. The EtOAc fraction showed the potent antioxidant with IC_{50} 43.13 $\mu\text{g/ml}$ compared to the standard ascorbic acid 58.76 $\mu\text{g/ml}$. The antimicrobial activity exhibited by MeOH extract against *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 6538P) zone of inhibition was 18 mm and 17 mm, for chloroform extracts 15 mm and 13 mm, and for EtOAc fraction 11 mm against *Bacillus subtilis*. The highest dilution that yielded no single bacteria colony on the nutrient agar plates for *Bacillus subtilis* and *S. aureus* of MeOH extract was found 0.39 mg/ml and 6.25 mg/ml, for chloroform extract 3.125 mg/ml and 6.25 mg/ml and EtOAc fraction against *Bacillus subtilis* was 12.50 mg/ml as minimum bactericidal concentration.

Conclusion: The plant extracts showed potent antioxidant and antibacterial activity. The results support for using the *G. glabra* in bacterial infection which provides partial scientific validation for using the plant against bacterial infections.

Keywords: *Glycyrrhiza glabra*, zone of inhibition, Minimum bactericidal concentration, Antioxidant, Antibacterial, Medicinal plant.

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INTRODUCTION

Nepal is regarded as the country of a showroom of biodiversity in terms of species richness at the global and continental level, respectively. Within its narrow stripe, it includes diverse physiographic structures, a wide range of climatic conditions, varied topography, and abundant ecological habitats. Nepal has prosperous biodiversity of flora and fauna. Around 7000 plant species are found in Nepal, among them, 1800 species are currently in use for folk medicine [1,2]. Despite our rich heritage and knowledge of the uses of medicinal plants to cure different diseases as drugs, little attention has been paid to harness the inexpensive remedies to modern requirements. Natural product is the high-value chemical entities derived from plant or microbial sources [3]. Natural product concerns to the primary and secondary metabolites. Since the origin of life, human beings are depending on nature for their basic needs such as shelter, food, clothing, means of transportation, and medicines [4]. Through trial and error, traditional healers found that some plants had healing power, and this knowledge was passed down through the generations. This knowledge is systematized and used in Ayurveda, Tibetan medicine, homeopathy, and Unani system [5]. Medicinal plants have minimal toxicity, are cost-effective, and pharmacologically active hence they provide an easy remedy for many human ailments as compared to the synthetic drugs which are a subject of adulteration and side effects [6].

Thus, biologically active phytochemicals can be isolated from the crude plant extract and after its isolation, dose-based activity can be determined in *in-vitro* and in *in-vivo* conditions to develop a novel drug. Partially purified samples of medicinal plants are used for the management of different diseases as antioxidant, anticancer, antibiotic, antidiabetic, etc. Oxidative stress is the state of imbalance between the generation of reactive oxygen species and detoxification of the reactive intermediates

or repairing of the resulting damage. Oxidative stress is produced during the normal metabolic process in the body as well as induced by a variety of environmental factors and chemicals [7]. These antioxidants play a vital role in delaying, intercepting, or preventing oxidative reactions, catalyzed by free radical through scavenging or chelating process. This antioxidant activity might be due to the presence of polyphenolic compounds such as flavonoids, phenolic acids, and phenolic diterpenes [8]. Antibiotics are a type of chemical compound used in the treatment and prevention of bacterial infections in animals and human beings by killing or inhibiting the growth of microorganisms. Due to extensive and haphazard use of commercial antimicrobial drugs in the treatment of infectious diseases, multiple drug resistance has developed, making it a global growing problem [9]. Natural products found in high altitudes may give a new source of antimicrobial agents with possible novel drug candidates with a new mechanism of action. Thus, screening of plant extracts from higher plants can be the solution to this global problem [10]. The present study focused on the collection of traditionally used medicinal plant *Glycyrrhiza glabra*, extraction of plant secondary metabolites, and performed the antioxidant and antibacterial activity.

METHODS

Chemicals

Most of the chemicals used were of analytical grade. Methanol (MeOH) (Collagen Scientific), hexane (Merck), ethyl acetate (EtOAc) (Merck), and chloroform (Merck) were purchased from the market. Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium nitrite, aluminum chloride, potassium hydroxide, and sodium hydroxide were available in the laboratory. Reagents and solvents used during phytochemical analysis were prepared in the laboratory with the chemicals provided in the laboratory.

Equipment

Electric grinder, mortar, and pestle, digital weighing balance (GT 210), hot air oven (Griffin-Grundy), rotatory evaporator (Buchi RE 111) with a water bath (Buchi 461), spectrophotometer (WPA, supplied by Philip Harris Shenstone, England), iodine chamber, cuvettes, burettes, pipettes, micropipettes (Erba BIHOT), thermometer, condenser, beakers, conical flasks, test tubes, reagent bottles, etc., were used during this work.

Collection and identification of samples

The fresh root of *G. glabra* was collected from local market New Road, Kathmandu. The taxonomic identification of the plant was done at the Central Department of Botany, Tribhuvan University Kirtipur.

Extract preparation

The collected fresh root of *G. glabra* was washed with tap water to remove the contaminants. Then, the roots were shade dried. The shade dried roots were grounded into powder form in an electric grinder and stored in a clean plastic bag until further use.

A powdered root (250 g) of *G. glabra* was kept separately in clean and dry conical flasks. Five hundred milliliter MeOH was added to each flask and kept for 3–4 days with frequent shaking. The mixtures were decanted and filtered with the help of a cotton plug and thus obtained filtrates were concentrated with the help of a rotatory evaporator. The concentrated filtrates were kept in a beaker wrapping with aluminum foil containing small pores to facilitate the evaporation of the solvent. After complete evaporation of the solvent, (semi) solid MeOH extracts were obtained as the test sample. The crude plant extract was fractionated using different solvents such as MeOH, EtOAc, chloroform, and hexane by solvent-solvent extraction based on their polarity.

Phytochemical analysis

The phytochemicals present in different plant extracts were analyzed by following the protocol adopted by Ciulei I. (1982) [11].

Antimicrobial activity

Inhibition of bacterial growth was tested using an agar well diffusion plate and zone of inhibition (ZOI) was measured [12]. The bacterial strains were *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, and *Staphylococcus aureus*. The organisms were identified as ATCC. In bioassay, the Mueller–Hinton Agar plates were labeled with the name of the bacteria and the number of plates. The inoculums of bacteria were transferred into a petri dish containing solid nutrient media of agar using a sterile swab. One swab was used for one species of bacteria only. Then, 15 µl of the working solution of the plant extracts were loaded into the respective wells with the help of micropipette. Ofloxacin was used as a positive control in the separate well. The plates were then left for half an hour covered with a lid so that extracts diffuse into the media. Then, Petri plates were incubated overnight at 37°C. After 24 h, Petri plates were then observed for ZOI produced by the antibacterial activity of plant extracts. Minimum bactericidal concentration (MBC) value was determined by subculturing the test dilution (which showed no visible turbidity) on to freshly prepared nutrient agar media. The plates were incubated further for 24 h at 37°C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC. The inhibition zones were measured by the use of a scale. The negative control was taken as dimethyl sulfoxide (DMSO) and MeOH.

Antioxidant activity

Ascorbic acid solution (2 ml) from each concentration was pipetted out and mixed with 2 ml 0.2 mM DPPH solution and kept in the dark for 30 min. Two milliliters MeOH was mixed with 2 ml 0.2 mM DPPH solution and kept in the dark. Then, the absorbance was measured at 517 nm using a spectrophotometer against MeOH and DPPH as a blank. Similarly, absorbance for extract and DPPH solution was measured following the same procedure taking ascorbic acid as standard. Finally, a curve was drawn, taking sample concentration in X-axis and percent

radical scavenging activity in Y-axis for both ascorbic acid and sample solution.

The radical scavenging activity percentage was calculated using the following relation,

$$\% \text{radical scavenging activity} = \frac{(A_0 - A)}{A_0} \times 100\%$$

Where, A_0 = Absorbance of the blank, A = Absorbance of the sample. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of scavenging percentage against extract concentration graphically using the regression equation.

RESULTS AND DISCUSSION

Yield percentage

The percentage yield of methanol EtOAc, chloroform, and hexane extracts of *G. glabra* is shown in Fig. 1.

The MeOH fraction showed the highest yield percentage (8.14), whereas chloroform (2.55) and EtOAc (2.13) showed nearly the same yield percentage, whereas hexane (1.32) showed the lowest yield percentage. The result showed that the MeOH fraction is the rich source of plant secondary metabolites as compared to the chloroform, EtOAc, and hexane fractions.

Phytochemical analysis

The results of the phytochemical analysis are shown in Table 1. All the extracts MeOH, EtOAc, chloroform, and hexane of *G. glabra* were found a rich source of secondary metabolites.

The chemical constituents in different fractions are considered as secondary metabolites which are known to be biologically active ingredients. They are directly responsible for different activities such as antimicrobial and antioxidant [13]. The most

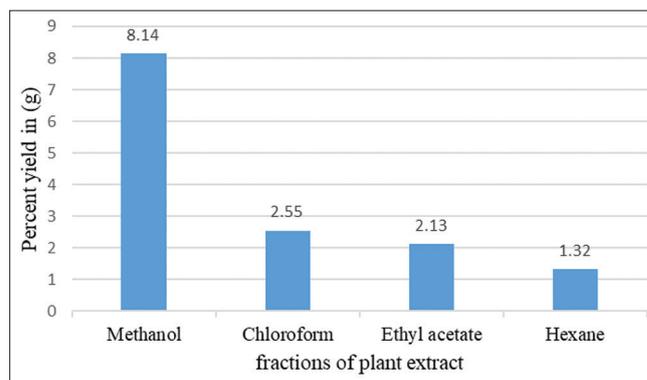


Fig. 1: Yield percentage of extracts in different solvents

Table 1: Phytochemical analysis of plant extracts

Phytochemicals	Hexane	Ethyl acetate	Chloroform	Methanol
Volatile oils	+	-	-	-
Alkaloids	-	-	-	-
Terpenoids	-	+	+	+
Coumarins	+	-	-	-
Flavonoids	-	+	+	+
Quinones	-	-	-	-
Polyphenols	-	+	+	+
Glycosides	+	-	-	-
Reducing sugars	-	+	+	+
Saponins	-	+	+	+
Tannins	-	-	-	+

(+) present (-) absent

important secondary metabolites such as flavonoids are present in polar extracts. Many scholars have reported that flavonoids and phenolic compounds in plants proved to exhibit multiple biological activities such as antioxidant, anti-inflammatory, antimicrobial, antiangiogenic, anticancer, and anti-allergic. In general, phenolic compounds and their derivatives are also considered as free radical scavengers [14].

Antioxidant activity

The results of antioxidant activity are shown in Fig. 2, in which the graphs are plotted concentration of plant extract in different solvent against the radical scavenging in which ascorbic acid is used as standard.

The antioxidant activity of MeOH, EtOAc, and chloroform extracts of *G. glabra* was measured by the ability to scavenge DPPH free radicals comparing with ascorbic acid. The scavenging effects of both plant extracts and the standard DPPH radical were expressed as half-maximal inhibitory concentration (IC_{50}) values; the results are shown in Table 2. Lower IC_{50} value reflects higher DPPH radical scavenging activity. The result showed that the EtOAc extract of *G. glabra* showed significant DPPH activity with the IC_{50} value of 43.13 $\mu\text{g/ml}$, while IC_{50} of ascorbic acid as standard was 58.76 $\mu\text{g/ml}$. The MeOH and chloroform extracts showed the poor antioxidant potential with IC_{50} 104.83 and 200.11 $\mu\text{g/ml}$, respectively. The poor activity recorded for the plant extracts may be attributed to the fact that the extracts being in crude form, contain a very small amount of bioactive compounds. The result was found comparable to the antioxidant activities of medicinal plant extracts reported by the previous researchers. Several researchers have reported a linear correlation between antioxidant activity and phenolic content of plant extracts [15].

IC_{50} value of different extracts was calculated and compared with an IC_{50} value of ascorbic acid to evaluate their potential antioxidant activity. Antioxidant activity is inversely proportional to the IC_{50} values, that is, extract or fraction or compound having small IC_{50} values are more potent. The IC_{50} values for MeOH, EtOAc, and chloroform extract of *G. glabra* were found 104.83, 43.13, and 200.11 $\mu\text{g/ml}$, respectively. The IC_{50} value shown by the EtOAc fraction (43.13 $\mu\text{g/ml}$) is found higher than that of the standard ascorbic acid (58.76 $\mu\text{g/ml}$) whereas methanol and chloroform fractions showed mild antioxidant activity. The antioxidant activity showed by *G. glabra* was found comparable to the results reported by the previous researcher in the extract of *G. lucida* fruits [16].

Antimicrobial activity

The results of antimicrobial activity of EtOAc, chloroform, and MeOH extract of the root of *G. glabra* are shown in Tables 3 and 4.

MBC was performed for only those organisms which showed a zone of inhibition and was sensitive to the plant extracts in the previous antimicrobial assay by agar well diffusion method.

The antibacterial activity for a MeOH extract of *G. glabra* root resulted ZOI 18 mm and 17 mm against *Bacillus subtilis* and *S. aureus*, respectively, while the rest of the test microorganisms were found resistant. The plant extracts that were found effective as an antibacterial agent was later tested to determine the MBC values for each strain. The MeOH extract exhibited the MBC against two bacterial strains such as *Bacillus subtilis* (ATCC 6051) and *S. aureus* (ATCC 6538P), as 0.39 mg/ml and 6.25 mg/ml, respectively. Similarly, the antimicrobial activity for chloroform extract of *G. glabra* root showed 15 mm and 13 mm against *Bacillus subtilis* and *S. aureus*, respectively, while the rest of the test microorganisms were found resistant. The MBC exhibited by the chloroform extract against two bacteria *Bacillus subtilis* (ATCC 6051) and *S. aureus* (ATCC 6538P) were found 3.125 mg/ml and 6.25 mg/ml, respectively. Furthermore, the antimicrobial activity for EtOAc extract of *G. glabra* root showed 11 mm against *Bacillus subtilis*, while the rest of the test microorganism were found resistant. The MBC showed by EtOAc extract against *Bacillus subtilis* was found 12.50 mg/ml. In this study, it was found that the zone of inhibition shown by the plant extracts is lower than that of the positive control, indicating poor antibacterial agent. The bacterial inhibition depends upon the solvent used for extraction, plant extracts, organism tested, etc. A previous study reported that although the fruit extracts of some seeds had antibacterial activity against *E. coli*, they had not any effect against some other bacteria [17]. Another study reported that fruit and leaf extracts of some plants had an antibacterial effect against *Bacillus megaterium*, *Pseudomonas aeruginosa*, and *S. aureus*, but their leaf extracts do not exhibit any effect against *E. coli* [18,19].

Table 2: The IC_{50} values for antioxidant activity of different extracts of *G. glabra* and standard ascorbic acid

<i>G. glabra</i> extracts/ascorbic acid	IC_{50} ($\mu\text{g/ml}$)
Ascorbic acid	58.76
Methanol extract	104.83
Ethyl acetate extract	43.13
Chloroform extract	200.11

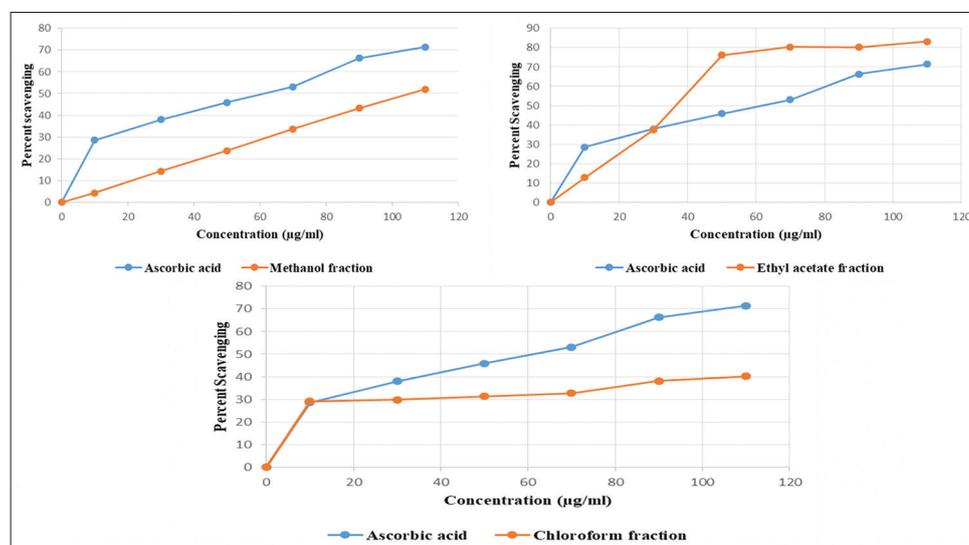


Fig. 2: Percent scavenging against the concentration of methanol, ethyl acetate, and chloroform extracts of *Glycyrrhiza glabra*

Table 3: Antibacterial activity showed by methanol, EtOAc, and chloroform extracts of *G. glabra*

Microorganism	Reference culture	Zone of inhibition ZOI (mm)			EtOAc fraction CHCl ₃ fraction	
		Positive control	Negative control	MeOH fraction		
<i>Bacillus subtilis</i>	ATCC 6051	25	-	18	11	15
<i>Enterococcus faecalis</i>	ATCC 29212	19	-	-	-	-
<i>Escherichia coli</i>	ATCC 8739	27	-	-	-	-
<i>Klebsiella pneumonia</i>	ATCC 700603	18	-	-	-	-
<i>Proteus vulgaris</i>	ATCC 6380	22	-	-	-	-
<i>Pseudomonas aeruginosa</i>	ATCC 9027	13	-	-	-	-
<i>Salmonella typhi</i>	ATCC 29630	13	-	-	-	-
<i>Shigella dysenteriae</i>	ATCC 13313	12	-	-	-	-
<i>Staphylococcus aureus</i>	ATCC 6538P	23	-	17	18	13

Table 4: MBC showed by the different fractions of *G. glabra*

Microorganisms	Reference culture	Methanol fraction (MBC mg/ml)	Ethyl acetate fraction (MBC mg/ml)	Chloroform fraction (MBC mg/ml)
<i>Bacillus subtilis</i>	ATCC 6051	0.39	12.5	3.12
<i>Enterococcus faecalis</i>	ATCC 29212	-	-	-
<i>Escherichia coli</i>	ATCC 8739	-	-	-
<i>Klebsiella pneumonia</i>	ATCC 700603	-	-	-
<i>Proteus vulgaris</i>	ATCC 6380	-	-	-
<i>Pseudomonas aeruginosa</i>	ATCC 9027	-	-	-
<i>Salmonella typhi</i>	ATCC 29630	-	-	-
<i>Shigella dysenteriae</i>	ATCC 13313	-	-	-
<i>Staphylococcus aureus</i>	ATCC 6538P	6.25	-	6.25

CONCLUSION

Phytochemical analysis shows plant extracts are found a good source of secondary metabolites and could be used to isolate the pharmacologically active chemical constituents as drug candidates. The EtOAc fraction exhibits the potential antioxidant, whereas the MeOH and chloroform extract showed mild antioxidant potential. This study offers a scientific rationale for the traditional use of this medicinal plant, particularly for infectious diseases. This study also provides partial scientific support for the traditional use of *G. glabra* in the treatment of infectious diseases. The results of the present investigation revealed that the plant extract of *G. glabra* contains pharmacologically active substances with antibacterial properties. It also points out the possibility of isolating active antibacterial compounds from the active plant extract. In the present study, the MeOH extract of *G. glabra* shows potent inhibition against *S. aureus* which is a predominant organism of burn and wound infection. The study also supports that MeOH extract is the source to isolate the active chemical compound that could use in the treatment of infectious diseases caused by resistant bacteria.

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AUTHORS' CONTRIBUTIONS

Sharma *et al.* analyzed the data and wrote the manuscript, whereas Sushil Bhusal carried out the laboratory work. Both the authors read and approved the final manuscript.

CONFLICTS OF INTEREST

Both authors declare that there are no conflicts of interest in publishing the paper.

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