**ORIGINAL RESEARCH ARTICLE**

# Effect of various solvent fractions on antioxidant potential of *glycyrrhiza glabraL*. (Mulhatti) Roots

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**ABSTRACT**
The effect of widely used methods and solvents on the antioxidant activity of Mulhatti root has been studied. The dry powder of Mulhatti root is extracted in methanol and by liquid-liquid separation of different solvent fractions, namely dichloromethane (DCM), ethyl acetate (EA), n-butanol (nB), residual aqueous solution (RA) and non-aqueous (NAR) residues were prepared. The antioxidant activity of all the different solvent fractions of Mulhatti root was evaluated using antioxidant assays such as the 2,2 diphenyl-1-picryl hydrazyl free radical scavenger assay and the total antioxidant activity. Total phenol and flavonoid content was also measured. For methanol extraction, the aqueous fraction was considered to be the best method for phenol and flavonoid extraction, but the ethyl acetate fraction showed the lowest $IC_{50}$ (41.08), followed by DCM (41.77), non-aqueous residues (64.50), n-butanol (351.78) and 461.50 µg/mL in residual water. The outcomes confirmed that the extracting solvent considerably altered the antioxidant of Mulhatti roots.

**Keywords**-Antioxidant activity, fractions, phenolic acids, flavonoids

## 1.0 Introduction
In particular, herbal medicines have been extensively studied for their antioxidant activity, and foods rich in natural antioxidants have been linked to a reduced risk of degenerative diseases such as cardiovascular disease and cancer [1]. There are three main classes of these phytochemicals: terpenoids, phenols, and alkaloids [2]. Among them, the most important phenolic compounds for dietary use include phenolic acids (hydroxybenzoic acid, hydroxycinnamic acid), polyphenols (hydrolyzed and condensed tannins), and flavonoids. These phytochemicals protect plants, fruits and vegetables from oxidative damage and are used by humans as antioxidants [3]. Currently, the search for new and safe antioxidants from natural sources is of great interest for use in natural antioxidants, nutraceuticals and nutraceuticals [4]. Glycyrrhiza glabra also has good medicinal plants and belongs to the Leguminosae family. It is commonly known as Mulhatti (Hindi), Sweet Wood (English) and Madhuka (Sanskrit) and is a perennial plant in the family Fabaceae found in the wild, mainly in the southern part of Europe and parts of Asia. It is one of the most widely used herbs as medicine and spice in the ancient history of Ayurvedic medicine [5]. It is a good source of natural antioxidants and also has antioxidant properties. Methods for obtaining antioxidants from plants include Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water



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extraction, and ultrasonic extraction. However, the extraction yield and antioxidant activity depend on the extraction method as well as the solvent used for extraction. Therefore, the presence of different antioxidant compounds with different chemical properties and polarities may or may not be soluble in certain solvents [6]. To concentrate and obtain polyphenol rich fractions before analysis, strategies including sequential extraction or liquid-liquid partitioning and solid phase extraction (SPE) based on polarity and acidity have been commonly used (7).The evaluation of phytochemicals and antioxidant activity in*Glycyrrhiza glabra* L. was performed by Soxhlet extraction by preparing extracts/fractions in different solvents, and various extracts/fractions (DCM, ethyl acetate, butanol, residual aqueous and non-aqueous residues).*Glycyrrhiza glabra* L is one of the best plant for the isolation of natural products and estimation of phytochemicals and evaluation of antioxidant potential. The aim of this study was to investigate the effect of solvent fractions on the extraction of polyphenols from *Glycyrrhiza glabra* L. and to evaluate the antioxidant activity of these extracts/fractions.

## 2.0 Materials and methods

### 2.1 Plant material collection

Root samples of *Glycyrrhiza glabra* L. collected in 2017 from the Experimental Area of Medicinal, Aromatic and Potential Crops (MAPCS) of Chaudhary Charan Singh Haryana Agricultural University, Department of Genetics and Plant Breeding in Hisar (between 28º59' to 29º49 N latitude and 75º11' to 76º18'E longitude).

### 2.2 Preparation of plant extract by normal refluxing method

A 100 g powder sample of Mulhatti root was weighed and placed in a 3000 ml round bottom flask half filled with methanol. Extraction was performed by heating at the boiling point of methanol (40 ~ 45 °C), and the solvent vapor was raised to a condenser to cool the solvent. After extraction for 18 hours, it was filtered through a muslin cloth. After filtration and distillation, when about 100 ml of the extract remained, it was used for further processing to obtain a viscous mass of the extract.

### 2.3 Liquid-liquid partitioning to prepare various fractions

After concentrating the remaining 100 ml extract on a rotary vacuum evaporator under reduced pressure, the viscous mass was obtained on the collector evaporator, and the methanol extract of the viscous mass was washed with petroleum ether (60-80° C.) and washed at 3-4 wash times. After that, the viscous mass was dissolved in 100 ml of 10% methanol (methanol: water, ratio 1:9), and precipitation took place. Separation was carried out by filtration into aqueous layer and non-aqueous residues**.**

Further, the aqueous layer was distributed in various fractions of the solvent, and the non-aqueous layer was restored to methanol. The liquid bulkhead was achieved by shaking the aqueous layer when the solvent is added to the split funnel. Depending on the polarity, dichloromethane (50, 50, 50 mL), ethyl acetate (50, 50, 50 mL) and n-butanol (50, 50, and 50 mL) are continuously separated into dichloromethane. DIMLS is formed of ethyl acetate on the aqueous layer in dichloro mete and ethyl acetate to form an emulsion separation of the solvent, and then saved for a long time, even after saving a long time, Subsequently, a small amount of ethanol (56 ml) is then added to the emulsion, and each fraction of the three distribution steps



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of the solvent was combined, and the amount of these were pointed out. The water residual water (precipitate) was not restored to a moderate amount of methanol, and about 100 ml and volume of volume were weakened. The resulting fractions were used for subsequent experimental studies.

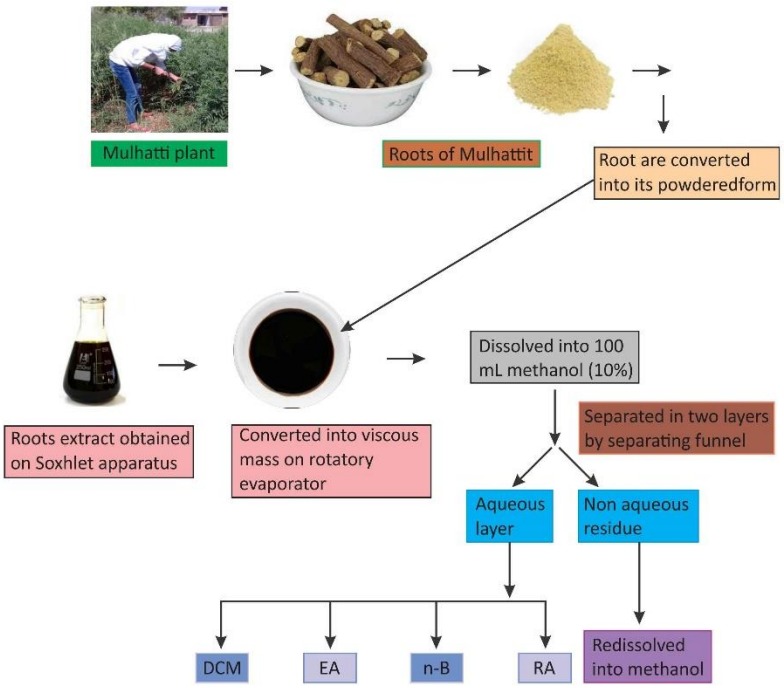


*Figure 1: Liquid-liquid partitioning of methanolic extract of Glycyrrhiza glabraroots*

**2.4 Determination of extract yield**

AOAC, (2000). Official methods of analysis. Gaithersburg, MD, Washington, USA (8).

**2.5 Quantitative phytochemical analysis**

**2.5.1 Determination of total phenolic content**

The total content of phenolic compounds in the different extracts/fractions (DCM, ethyl acetate, n-butanol, aqueous and non-aqueous residues) of Mulhatti root was determined by the Folin Ciocalteu method [9]. For the determination of phenolic compounds; 0.2 ml of each extract diluted with the respective solvent to adjust the absorbance within the calibration limit was introduced into a test tube, then one ml of Folin Ciocalteu reagent was added and two ml of $Na_2CO_3$ (20%) were mixed. , w/v). Add distilled water to the final volume to 10 ml. The mixture was kept for 8 min then centrifuged at 6000 rpm for 10 min. White is prepared in the same way. A suitable solvent was used instead of the sample. The optical density of the supernatant was then measured on a blank prepared in a UVVIS dual-beam spectrometer at 730 nm, UV 1900 model (Shimadzu). Total phenols present in the various extracts/fractions of root are calculated from a standard curve and expressed in mg GAE/g.



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### 2.5.2 Estimation of flavonoids content

The flavonoid content of different extracts/fractions (DCM, ethyl acetate, n-butanol, aqueous and non-aqueous residues) of Mulhattiroot was determined by aluminum chloride colorimetric analysis [10]. Take 1 ml of each extract to measure the flavonoid compounds, add 4 ml of distilled water, 0.3 ml of $NaNO_2$ (5%), after 5 minutes add 0.3 ml of $AlCl_3$ (10%). Immediately add 2 ml of NaOH (1 M) and add distilled water to the test tube to obtain a final volume of 10 ml. White is prepared in the same way. A suitable solvent was used instead of the sample. After sufficient stirring of the solution, absorbance was measured at 510 nm on blanks prepared in a double-beam, UV 1900 model UV-VIS spectrometer (Shimadzu).The amount of flavonoids present in the various extracts/fractions of root was estimated using a standard curve and expressed in mg CE/g.

### 2.5.3 Total antioxidant capacity

The total antioxidant capacity of various extracts/fractions (DCM, ethyl acetate, n-butanol, residual aqueous and non-aqueous residues) of Mulhatti root was determined using a modified phosphomolybdenum method [11]. To assess overall antioxidant capacity; 1 ml of each extract was taken, 3 ml of phosphomolybdenum reagent was added to a glass vial, the lid was closed, and the solution was thoroughly mixed. They were incubated for 90 minutes at 95° C. Then the contents of the vial were cooled and the absorbance measured at 695 nm in a double beam UV-VIS spectrophotometer model UV 1900 (Shimadzu) on the prepared blanks. The blank was prepared in the same way. Appropriate solvents were used at the sampling site. Total antioxidant capacity in various extracts/fractions of root was calculated using a standard curve and expressed in mg AAE/g.

### 2.5.4 DPPH free radical scavenging assay

The antioxidant activity was evaluated in different extracts/fractions (DCM, ethyl acetate, n-butanol, aqueous and non-aqueous residues) of Mulhatti root using DPPH free radical scavenging method [12]. After adding 10 ml of the aqueous extract to the beaker and drying it completely, the dry mass was recorded and the dry mass of the aqueous extract was re-dissolved in an appropriate amount of 50% (v/v, methanol: water). Stock solution of 10,000 µg/ml according to the dry weight of the extract (since it was not completely dissolved in methanol). Concentrations between 10 µg/ml and 5000 µg/ml were obtained from the stock solution by appropriate dilutions of 50% (v/v) water: methanol. Evaluation of the activity of the free radical scavenger DPPH; 1 ml extracts of each concentration were withdrawn and capped and added to 2'diphenyl/pirylhydrazyl glass vials (DPPH; 0.1 mM, 50% (v/v) water: methanol). Shake gently for 5 minutes. For control, a solvent was used instead of the sample. After incubation in the dark for 30 minutes, absorbance was measured for blanks containing pure methanol at 517 nm in a UV-VIS dual-beam spectrophotometer, model UV 1900 (Shimadzu), and each sample was transferred in triplicate. Using Microsoft Excel software, histograms were graphed between DPPH free radical scavenging activity (%) on the y-axis and extraction concentration (µg/ml) on the x-axis, and a quadratic regression equation was obtained. two ($y = ax^2 + bx + C$). The resulting equation is converted to the form ($ax^2 + bx + c = 0$) by setting y = 50%. Using the equation ($ax^2 + bx + c = 0$), the $IC_{50}$ value is calculated using the formula.



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$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$ Where, x = $IC_{50}$ (µg/mL)

The percentage DPPH scavenged activity (% DPPH*$_{SC}$) was calculated using:

$$\%\,\mathrm{DPPH}^*{}_{sc} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

*Where, $A_{control}$= control absorbance, $A_{sample}$= sample absorbance*

**2.6 Statistical analysis**

All experiments were performed in triplicate for statistical studies and expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to evaluate the significant difference between sample means in online statistical analysis (OPSTAT). The $IC_{50}$ value for DPPH free radical scavenging activity was calculated using a quadratic regression equation (Table 1). The correlation between IC50 values of total phenol, total flavonoid, and DPPH free radical scavenging capacity and total antioxidant capacity was determined using the Karl Pearson method in Microsoft Excel and all Other measurements were also performed in Microsoft Excel 2014.

*Table 1: Quadratic regression equations for $IC_{50}$ (µg/mL) values of Glycyrrhiza glabra roots in various solvent fractions (DCM, ethyl acetate, n-butanol, residual aqueous and non-aqueous residue) of methanolic extracts*

| Fractions | Roots |
|---|---|
| **DCM** | $y = -0.0037x^2 + 1.278x + 3.0733$<br>$R^2 = 0.9919$ |
| **Ethyl acetate** | $y = -0.0053x^2 + 1.4894x - 2.2451$<br>$R^2 = 0.9963$ |
| **n-Butanol** | $y = -1E-04x^2 + 0.1729x + 1.5522$<br>$R^2 = 0.9987$ |
| **Residual aqueous** | $y = -5E-07x^2 + 0.0117x + 6.7687$<br>$R^2 = 0.9978$ |
| **Non-aqueous residue** | $y = -0.002x^2 + 0.8507x + 3.4481$<br>$R^2 = 0.9966$ |

**3.0 Results and discussion**

**3.1 Extractive yield**

The dry powder of Mulhatti roots was extracted in methanol and various solvent fractions were prepared by liquid-liquid partitioning and separation was done by filtration into aqueous and non-aqueous residue. The yields of the extract were 0.01, 0.03, 0.05, 0.09 and 0.07 g/100 g in DCM, ethyl acetate, n-butanol, water residue and no water residue, respectively, in Mulhatti root segments. The extract yield of wild parsley (*Torilisleptophylla*) in various solvent fractions has been determined but did not show a regular trend as indicated above [13]. The highest extraction yield was observed in the residual aqueous solution (8.2 g/100 g), followed by, ethyl acetate (6.1 g/100 g), butanol (4.8 g/100 g), and chloroform (4.3 g/100 g). Therefore, the present data are consistent with the literature data.



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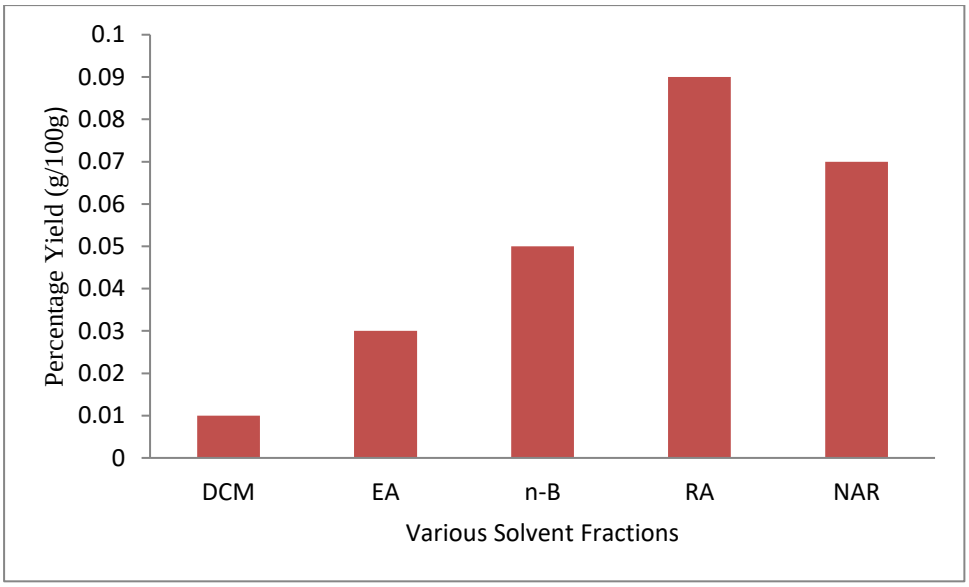


*Fig 1: Extractive yield (percent) of Mulhattirootsin various solvent fractions of methanolic extracts*

**3.2 Total phenolics**

Total phenol content (mg GAE / g) (gallic acid per gram) using the formula (y = 0.0104x + 0.0079, $R^2$ = 0.9989) obtained from the calibration curve of gallic acid used as a standard material. Acid equivalent milligrams) are calculated. Maximum values were shown for the residual aqueous fraction (0.66), followed by n-butanol (0.11), ethyl acetate (0.09), DCM (0.08), and 0.05 mg GAE / g in non-aqueous residue. The high amount of phenolic compounds in the protictic solvent fraction may be due to the presence of polar phenolic compounds such as gallic acid, quercetin and dicatin. The total amount of phenolic compounds in Terminalia arjuna was measured after extraction and showed higher values in water, followed by ethyl acetate and hexane extracts at 3.6, 4.1, and 1.0 mg GAE / g, respectively [14]. Total phenolic compounds in various solvent fractions of Hamana (Cleome gynandra) were measured, n-butanol (133.02 mg GAE / g), followed by ethyl acetate (97.90 mg GAE / g), DCM (37.380 mg GAE / g). The higher phenol in was measured. ) And n-hexane (20.72 mg GAE / g) [15].

The total phenolics was estimated using different solvents (methanol, ethanol, petroleum ether, n-hexane and chloroform) in root extract of *Asparagus racemosus* and reported that the total phenolic content was found to be in order, ethanol (108.78 ± 2.77), methanol (92.97 ± 3.50), chloroform (79.74 ± 4.77), petroleum ether (55.47 ± 1.83), n-hexane (22.47 ± 3.14) in mg/gm GAE respectively (16).

**3.3 Total flavonoids**

Similarly, using equation (y = 0.0018x + 0.0038, $R^2$ = 0.998) acquired from the calibration curve of catechin used as a standard and well-known quantity of flavonoids content (mg CE/g) had been decided. The most flavonoids content was located in non-aqueous residue (2.00) followed by n-butanol (0.51), residual aqueous (0.49), DCM (0.29), 0.19mg GAE/gin ethyl acetate. Similarly, other researchers further resolved different amounts of flavonoids in the



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exclusive fraction of the solvent. The flavonoids were estimated *in vivo* in red clover(*Trifolium pratense* L.) and results showed that the higher amount of flavonoids was present in methanol (26.61 ± 0.92) followed by chloroform (19.56 ± 1.11), n-hexane (16.06 ± 1.58 and ethyl acetate fraction (11.71 ± 1.43)[17]. Therefore, the current data is consistent with the document data. Morescott et al. [18] also measured the content of flavonoids in aqueous solution, butanol, ethyl acetate and hexane and similar variants were obtained as in the present data. That is, there is no regular tendency towards flavonoids. The highest amount of flavonoids was in ethyl acetate (85.75 mg QE/g) followed by butanol (78.87 mg QE/g), aqueous (68.05 mg QE/g) and n-hexane (51.16 mg QE/g).

### 3.4 Total antioxidant capacity

Total antioxidant capacity (mg AAE/g) was calculated using the equation ($y = 0.0066x + 0.0036$, $R^2 = 0.999$) derived from the calibration curve for ascorbic acid used as the standard. Maximum total antioxidant capacity was found for non-aqueous residue (3.42), residual aqueous (2.17), n-butanol (1.40), ethyl acetate (0.57) and DCM0.35 mg AAE/g. The phenolic compounds, flavonoids and also total reducing power in solvent fractions of n-hexane, chloroform, and ethyl acetate of crude methanol extracts and aqueous fractions of pseudo-Ashok (*Polyalthia longifolia*) leaves were evaluated and the provided data did not show trends according to polarity [19]. Both phenolics and flavonoids content and total reducing power were in the fractions were in the following order; ethyl acetate > chloroform > methanol extract > n-hexane > water soluble fraction

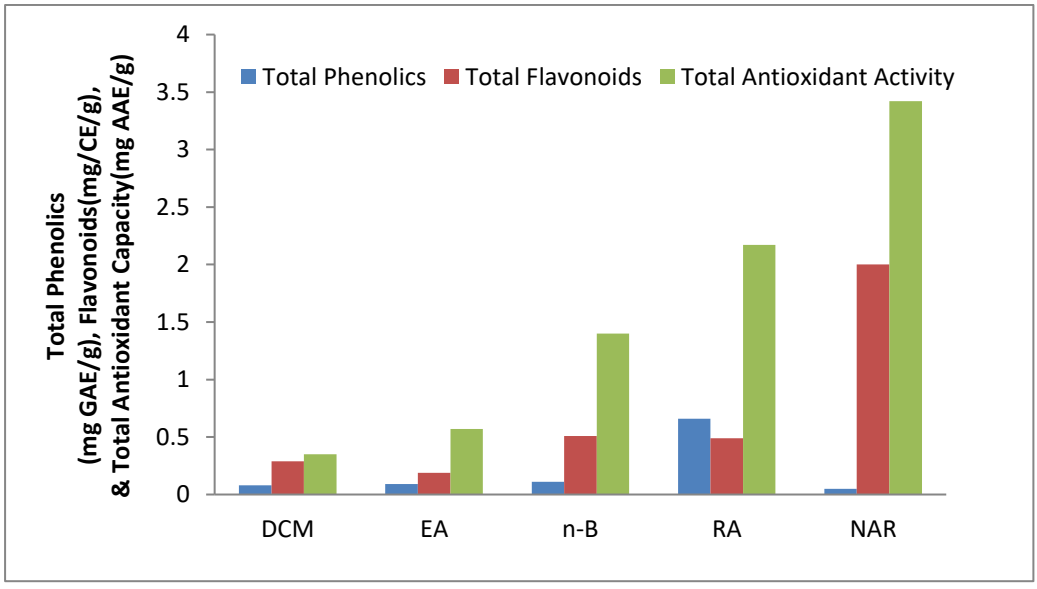


*Figure: 2 Graphical representation of quantitative number of phytochemicals in root extracts/fractions of Glycyrrhiza glabra*

### 3.5 Antioxidant activity

Percent activity and $IC_{50}$values (µg/mL) for DPPH free radical scavenging activity were calculated in different extracts/fractions (DCM, ethyl acetate, n-butanol, aqueous and non-aqueous residues) of methanol extract of *Glycyrrhiza glabra* roots and are listed in Table 3. The



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lowest $IC_{50}$ value was determined in ethyl acetate (41.08), followed by DCM (41.77), non-aqueous residues (64.50), n-butanol (351.78) and in the residual water it was 461.50 µg/mL. The antioxidant activity by DPPH radical scavenging activity in fractions prepared from the methanolic extract of *Glycyrrhiza glabr*a was determined and the results showed that the chloroform fraction was found to be an effective antioxidant. (87.7%) compared to other fractions lacking the polarity of the solvent [20]. In the methanol extract of the bark of Acacia nilotica, the total phenol and flavonoid content and antioxidant activity in the different solvent fractions (butanol, residual solution fractions, dichloromethane, chloroform and hexane) were also evaluated [21] and reported that the ethyl acetate fraction contained the most phenolic compounds (9,369 mg EC/g) and flavonoids (3,229 mg EC/g), and also showed the highest antioxidant activity (IC50 24, 9 µg/ml). Hurka (Rumex hastatus D. Don.) leaves different phytochemicals and DPPHs The radical scavenging activity and total antioxidant capacity in different solvent fractions have been evaluated and shown to be different [22]. The greatest number of phenolic compounds are present in the ethyl acetate fraction, and flavonoids are present in the butanol fraction and DPPH radical scavenging activities in the following order: butanol > methanol > acetate d ethyl > chloroform > hexane and water fractions and total capacity The antioxidant capacity of different fractions of methanol extracts of RH leaves can be classified in the order butanol > methanol > chloroform > ethyl acetate > hexane > water fraction. They concluded that the strong DPPH scavenging activity and antioxidant capacity could be attributed to the presence of phytochemicals, i.e. phenols (hydrogen transfer capacity of phenolic compounds) and similar types of variants was found in our data.

*Table 2: DPPH free radical scavenging activity (%) and $IC_{50}$value (µg/mL) of roots of Glycyrrhiza glabra in various solvent fractions of methanolic extracts*

| Fractions Name | DPPH free radical scavenging activity (%) at different concentrations (µg/mL) | | | | | | | | | $IC_{50}$ Value (µg/mL) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2500 | 1000 | 500 | 250 | 100 | 50 | 25 | 10 | 5 | |
| **DCM** | A | A | A | 93.56 | 92.63 | 56.59 | 39.57 | 14.26 | A | **41.77** |
| **EA** | A | A | A | A | 93.34 | 59.11 | 31.54 | 13.95 | 0.95 | **41.08** |
| **n-B** | A | 92.34 | 64.21 | 37.97 | 18.75 | 10.31 | 6.56 | 3.13 | 2.81 | **351.78** |
| **RA** | 96.96 | 88.19 | 52.42 | 29.52 | 15.92 | 3.22 | 2.50 | 1.61 | A | **461.85** |
| **NAR** | A | A | A | 90.39 | 66.77 | 41.73 | 25.51 | 13.54 | 7.87 | **64.50** |

*'a' represent absent of DPPH free radical scavenging activity*

The correlation coefficients were also determined to see the relationship between total phenolic content, flavonoids and total antioxidant capacity with $IC_{50}$ value of DPPH free radical scavenging activity in various solvent fractions of roots of *Glycyrrhiza glabra* and a regression analysis was done. Pearson's correlation coefficient analysis was performed. Pearson's correlation coefficient is significantly negative if $0.61 \leq r \leq 0.97$ and significantly positive if $0.61 \leq r \leq 0.97$ [23]. The present study showed that total phenolic content in various solvent fractions of roots of Mulhatti had significant and positive correlation with their $IC_{50}$ of DPPH scavenging activity ($r = 0.789$,$P<0.05$) and it can be predicted that phenolic compounds are the main contributor in antioxidant activity. Total flavonoids also had significant and strong positive correlation with their total antioxidant capacity ($r = 0.893$, $P<0.01$) and it can be predicted that



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flavonoids are the main contributor in total antioxidant capacity of Mulhatti roots by phosphomolybdenum method.

**4.0 Conclusion**

This present study concluded that the effect of various solvent fractions/extracts on phytochemicals and antioxidant potential of Mulhatti roots and results data indicated that the extraction method and different types of solvent fractions had a significant effect on the antioxidant properties of the resulting methanol extract. Because multiple properties and reaction mechanisms are likely to be involved, a single assay cannot accurately reflect all antioxidants in a mixed or complex system. Therefore, in order to fully describe the full antioxidant capacity profiles of the different solvent fractions, we used various antioxidant capacity analyzes in this study. The results showed that the total phenol content was maximum in the aqueous fraction, i.e. 0.66 mg GAE/g, the flavonoid content and total antioxidant capacity were highest in the non-aqueous residue, i.e. 2.00 mg GAE/g and 3.42 mg AAE/g, respectively. DPPH had the highest antioxidant activity by trapping radicals in ethyl acetate having minimum $IC_{50}$i.e. 41.08 µg/ml. Our results indicated the potent and superior antioxidant activity of *Glycyrrhiza glabra*L. extract/fraction. Further studies are needed to isolate pure bioactive compounds that can be considered as potential sources of biomolecules for the pharmaceutical and food industries. The results of current studies on antioxidant assays confirm and partially confirm the extensive use of tested plants and previous literature. The excellent antioxidant activity of test plants is the use of antioxidants to prevent the oxidation of foods, and further research is needed to investigate the in vivo efficacy of these root extracts/fractions.

**5.0 Conflict of interest**

The authors declare no conflict of interest.

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