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Antioxidant capacity of hydroalcoholic extracts of *Grewia serrulata* DC and *Grewia nervosa* (Lour.) Panigrahi

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ABSTRACT

The current investigation is intended to evaluate the content of phytochemical constituents and antioxidant potential of hydroalcoholic extracts of stem and root of *Grewia serrulata* DC (HAESGS & HAERGS) and leaf and bark of *Grewia Nervosa* (Lour.) panigrahi (HAELGN & HAEBGN). Initially, all the extracts at different concentrations were estimated for their total phenolic content and total flavonoid content. The study was further extended for their antioxidant potential evaluation using various *in vitro* methods such as 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydroxyl radical and superoxide radical scavenging assays. The total phenolic content (mg gallic acid equivalent per gram of extract) was high in HAELGN (170.82±0.19) and HAERGS (123.00±0.48) than HAESGS (111.2±0.26) and HAEBGN (119.60±0.23). The total flavonoid content (mg quercetin equivalent per gram) is greater in HAERGS (71.24±0.50) and HAESGS (65.68±0.27) than HAELGN (55.82±0.35) and HAEBGN (62.38±0.45). The IC_{50} values (µg/ml) of different plant extracts inferred that DPPH radical scavenging activity is greater in HAELGN (42.91±0.88) and HAEBGN (53.87±0.35) than HAESGS (126.73±1.20) and HAERGS (88.87±1.25). However, hydroxyl and superoxide radical scavenging activity is more in HAERGS (135.41±1.19 & 88.00±1.42) and HAELGN (172.28±1.91 & 108.163±1.09) than HAESGS (237.3±1.65 & 110.074±0.87) and HAEBGN (204.7±1.04 & 125.54±1.07). The results of present comprehensive analysis demonstrated that both the plants *Grewia serrulata* DC and *Grewia Nervosa* (Lour.) panigrahi possess high phenolic, flavonoid contents and potential antioxidant activity, and could be used as a valid source of natural antioxidants and might be utilized for pharmacological screening of various therapeutic activities.

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INTRODUCTION

Currently the causes of several diseases such as neurodegenerative diseases, liver cirrhosis, arteriosclerosis, cancer and diabetes have been associated with the presence of free radicals. These radicals may cause oxidative damage by oxidizing bio molecules and it results in cellular death and consequent tissue damage. Therefore, free radical scavenging compounds have a great potential to inhibit or reduce the oxidative damage on the human body (Brighente *et al*., 2007; Moein *et al*., 2007 & Sabu *et al*., 2002).

Many plant derived molecules have shown a promising effect in therapeutics (Lokhande *et al*., 2007). Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in chemo prevention of diseases and aging. Among the plants investigated to date, one showing enormous potential is the genus Grewia. It is a genus of shrubs and trees distributed in the warmer parts of the world. About forty species of this genus occur in India. Diverse bioactivity studies on different species of genus Grewia have been supported ([a]Chandiran *et al*., 2013). *Grewia serrulata* DC and *Grewia nervosa* (Lour.) panigrahi are among them belonging to family tiliaceae and malvaceae respectively ([a]Chandiran *et al*., 2013 & Meena *et al*., 2013). These plants grow as a shrub or a tree (Meena *et al*., 2013).

The aerial parts of *Grewia serrulata* contain phytoconstituents like flavonoids, saponins, glycosides, terpenes, sterols and phenols ([a]Chandiran *et al*., 2013). Leaves and bark of *Grewia nervosa* is enriched with chemical constituents like phenols, saponins, flavonoids and alkaloids. Additionally, leaves of *Grewia nervosa* contain glycosides, terpenoids and phytosterols and the same are absent in bark (Mentham Ramesh *et al*., 2017). *Grewia serrulata* is a cuisine of the popular edible fruit phalsa ([a]Chandiran *et al*., 2013). *Grewia nervosa* is commonly used in Chinese herbal tea (Meena *et al*., 2013). Traditionally both these plants are used for various disease ailments (Meena *et al*., 2013 & [b]Chandiran *et al*., 2013). The current investigation was undertaken to evaluate the content of phytochemical constituents and in vitro antioxidant properties of hydroalcoholic extracts of stem and root of *Grewia serrulata* (HAESGS & HAERGS) and leaf and bark of *Grewia nervosa* (HAELGN & HAEBGN)

MATERIALS AND METHODS

Plant materials

Selected plant parts of *Grewia serullata* and *Grewia nervosa* were collected from Seshachala forest, Tirumala, Chittoor Dt, A.P, India and authenticatedby a registered botanist.

Chemicals

All chemicals [2, 2-diphenyl-picryl-hydrazyl (DPPH), Ethylene Diamine Tetra Acetic acid (EDTA), gallic acid, quercetin, trichloroacetic acid(TCA), thiobarbituric acid(TBA), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide(NADH), ferric chloride($FeCl_3$), potassium ferricyanide, and reagents were of analytical grades or purest quality purchase from 'Sigma Aldrich Chemical Co.'.

Preparation of extracts

After shade drying, stem and root of *Grewia serullata* were blended in to fine powder and used for preparation of hydroalcoholic extracts. 500g of coarsely ground powder of stem and root were taken separately and placed into two separate glass chambers. 1550ml of water and 850ml of ethanol (95%) was added in a 70:30 ratio to get hydroalcoholic extracts of stem and root of *Grewia serullata*. Both the glass chambers were closed to avoid evaporation of menstrum and this alignment was allowed to stand for one week with occasional stirring. The remained menstrum was then strained and the solid residue called marc was pressed to recover as much occluded solution as possible. The strained and expressed liquid thus obtained was mixed and clarified by filtration. The filtration was performed in a beaker using Whatman's filter paper no 1. Two liter of menstrum was obtained for both the extracts which were stored in a refrigerator at 4°C in two beakers. Menstrum was evaporated in china dishes which were then placed on a water bath. A sticky mass was obtained as hydro alcoholic extract. The same procedure was followed for the preparation of hydroalcoholic extracts of leaf and bark of *Grewia nervosa* and all the obtained extracts [HAESGS, HAERGS, HAELGN and HAEBGN] were preserved in a dark colored pre sterilized airtight container until its further usage (Mentham Ramesh *et al*., 2017).

Determination of content of phytoconstituents

Total Phenolic content

Total phenolic content of plant extracts were determined by employing the method involving Folin-Ciocalteau reagent as oxidizing agent and gallic acid as standard (Prior *et al*., 2005). 1ml of plant extract or standard was taken in a test tube and mixed with 5ml Folin-Ciocalteau reagent (prior dilution with water at 1:10v/v) and 5ml of sodium carbonate (7.5%) solution. The test tubes were vortexed for 15 min and allowed to stand for 30 min at 40°C to complete the reaction. Then the absorption of solution was measured at 765nm using a spectrophotometer against blank. Now, the concentration of total phenolic content in samples was determined as milligram of gallic acid equivalent per gram of extract. All the determinations were performed in triplicate manner.

Total flavonoid content

Total flavonoid content [TFC] of all extracts was determined by aluminum chloride colorimetric method (Ordonez *et al*., 2006). Quercetin was used as standard and the flavonoid content was expressed as mg of quercetin equivalent/gram of dried extract. A volume of 1ml of 2% $AlCl_3$ ethanol solution was added to 1ml of extract solution and

left in the dark at room temperature for 1h. The absorbance was measured at 420nm using UV-VIS spectrophotometer. TFC was calculated by extrapolating the absorbance of reaction mixture on calibration curve of quercetin. All the determinations were performed in triplicate and is to be followed for all the extracts.

Free radical scavenging activity

DPPH radical scavenging activity

The antioxidant activity was estimated in terms of hydrogen donating or scavenging ability of radical with the use of stable radical DPPH (2,2- Diphenyl-1-picrylhydrazyl) by following the method of Blois (Blois *et al*., 1958) with few modifications. The alleviation of radical is followed by a decrease in the absorbance at 517nm. A quantity of 2ml of a hydroalcoholic stock solution of the extracts was placed into test tubes and 2ml of 1mM DPPH solution was added. The tubes were enclosed with parafilm and placed further in dark for 1hour. The absorbance was measured at 517 nm and the solution without extract and with DPPH and methanol was used as control. The experiment was performed in triplicate. Ascorbic acid was used as positive control. Inhibition of DPPH free radical in percentage was calculated y the formula

$$\text{DPPH radical scavenging activity (\%)} = (A_{control} - A_{test}) / A_{control} \times 100$$

$A_{control}$ is the absorbance of the control and A_{test} is the absorbance of samples.

The radical scavenging activity of each extract was stated as IC_{50} (concentration required to inhibit DPPH radical formation by 50%) which is calculated graphically using the equation for line.

Hydroxyl radical Scavenging activity

The hydroxyl radical scavenging potency was measured following the modified method of Halliwell (Halliwell *et al*., 1987). Using distilled deionized water as a solvent stock solutions of EDTA (1mM), $Fecl_3$(10mM), ascorbic acid (1mM), H_20_2 (10mM) and deoxyribose (10mM) were prepared. The assay was carried out by admixture of 0.1ml of EDTA, 0.01ml of $FeCl_3$, 0.1ml of H_2O_2, 0.36ml of deoxyribose, 1ml of different respective plant extracts, 0.33ml of phosphate buffer (50mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. For a period of one hour the mixture was incubated at 37°C and then 1ml from this was mixed with 1ml of 10%TCA and 1ml of 0.5% TBA to attain pink color which is measured at 532nm.

$$\text{Hydroxyl radical scavenging activity (\%)} = (A_{control} --- A_{test}) / A_{control} \times 100$$

$A_{control}$ is the absorbance of the control and A_{test} is the absorbance of samples.

The radical scavenging activity of each extract was stated as IC_{50} (concentration required to inhibit hydroxyl radical formation by 50%) which is calculated graphically using the equation for line.

Superoxide radical scavenging activity

Superoxide scavenging was estimated by the nitroblue tetrazolium reduction method (Nishikimi *et al*., 1972). The reaction mixture comprises of 1ml of nitroblue tetrazolium (NBT) solution (1M NBT in 100mM phosphate buffer, pH 7.4), 1ml NADH solution (1M NADH in 100nM phosphate buffer, pH 7.4) and 0.1 ml of different fractions and ascorbic acid(50 mM phosphate buffer, pH 7.4)was mixed. The reaction was initiated by adding 100µl of (PMS) solution (60µM PMS in 100mM phosphate buffer, pH 7.4) in the mixture. The tubes were uniformly illuminated with an incandescent visible light for 15 minutes and the measured its optical density at 530nm before and after the illumination.

$$\text{Superoxide radical scavenging activity (\%)} = (1 - Ae/Ao) \times 100$$

Ao is the absorbance without sample, and Ae is absorbance with sample.

The radical scavenging activity of each extract was stated as IC_{50} (concentration required to inhibit hydroxyl radical formation by 50%) which is calculated graphically using the equation for line.

Statistical analysis

The calibration curves, content of phytoconstituents and IC_{50} values of extracts for free radical scavenging assays were prepared and analyzed using Microsoft excel sheet. The concentration response curves of free radical inhibition potency of extracts were designed by Graph Pad Prism 5.01 version. The data was expressed as mean± Standard error mean (SEM).

RESULTS

Content of phytochemical constituents

In our current investigation, based on the absorbance values of various plant extracts and correlating them with that of standard absorbance, it was revealed with tabulated results (table 1) that hydroalcoholic leaf extract of *Grewia nervosa* [HAELGN] posses high phenolic content (170.82±0.19 mg GAE/gm of extract) and hydroalcoholic root extract of *Grewia serrulata* is enriched with high flavonoid content (71.24±0.50 mg QE/gm of extract). TPC was calculated using the standard curve of gallic acid (standard curve equation: Y=0.0026x + 0.076, R^2 = 0.9951) and TFC was calculated using standard curve of quercetin (standard curve equation: Y= 0.0097x + 0.0593, R^2 = 0.9907) as depicted in figure 1 & 2.

Table 1: Contents of total phenols and total flavonoids

Extract	Total Phenolic Content (mg of GAE/g of extract)	Total Flavonoid Content (mg of QE/g of extract)
HAESGS	111.2 ±0.26	65.68±0.27
HAERGS	123.00±0.48	71.24±0.50
HAELGN	170.82±0.19	55.82±0.35
HAEBGN	119.60±0.23	62.38±0.45

Results signifies mean ± SEM values of triplicates

Table 2: Free radical scavenging activity

Extract	Radical scavenging activity - IC_{50} value (µg/ml)		
	DPPH	Hydroxyl	Super oxide
HAESGS	126.73±1.20	237.3±1.65	110.074±0.87
HAERGS	88.87±1.25	135.41±1.19	88.00±1.42
HAELGN	42.91±0.88	172.28±1.91	108.163±1.09
HAEBGN	53.87±0.35	204.7±1.04	125.54±1.07
Ascorbic acid	9.44±0.08	61.68±1.42	73.596±0.94

Values are expressed as mean ± SEM

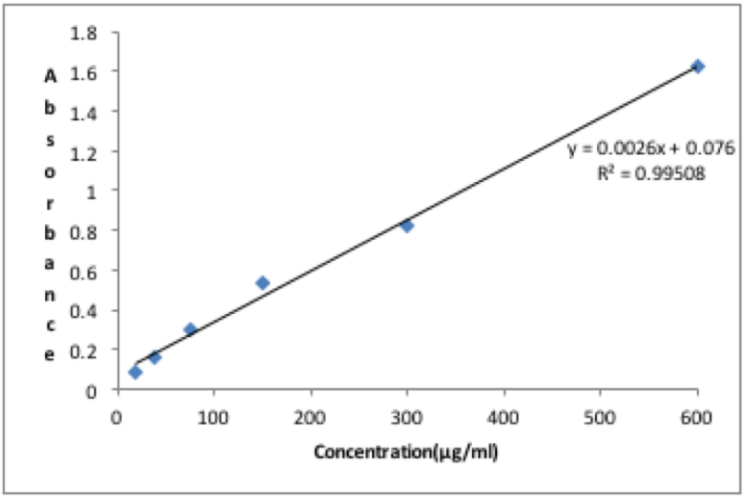

Figure 1: Gallic acid standard curve

Free radical scavenging activity

DPPH radical scavenging assay

Results that elicited from DPPH free radical scavenging activity revealed that among all extracts hydroalcoholic leaf extract of *Grewia nervosa* displayed a significant efficacy in scavenging DPPH of 93.67±1.48 % at a concentration of 500µg/ml. Figure 3 illustrated the dose response curve of DPPH radical scavenging activities of HAESGS, HAERGS, HAELGN, HAEBGN and standard ascorbic acid in terms of their % inhibition. From the depicted table no 2 the strong anti DPPH activity of HAELGN is reinforced with its IC_{50} value (µg/ml) 42.91±0.88 compared to other extracts. The IC_{50} value of standard ascorbic acid was found to be 9.44±0.08.

Hydroxyl radical scavenging assay

In hydroxyl radical scavenging assay hydroalcoholic root extract of *Grewia serrulata* exhibits strong scavenging activity with minimum IC_{50} value (135.41±1.19) and maximum inhibition of hydroxyl radical (88.38±0.64 %) at a concentration of 500µg/ml. The percentage inhibition of hydroxyl radical by the extracts and their IC_{50} values are depicted in figure 4 and table 2. The ascorbic acid achieves minimum IC_{50} value (61.68±1.42) around one third of its value in extracts.

Superoxide radical scavenging assay

Superoxide radicals were generated in a PMS-NADH system and assayed by the reduction of NBT. Depicted data in table no 2 elicits superoxide scavenging activity of four hydroalcoholic extracts of *Grewia serrulata* and *Grewia nervosa* exemplifying with their IC_{50} value. Among these, HAERGS (88.00±1.42) exhibit strong superoxide radical scavenging activity nearer to that of standard drug ascorbic acid scavenging ability (73.596±0.94). While other three extracts show moderate scavenging activity against superoxide radical. Figure 5 represents % inhibition of superoxide radicals

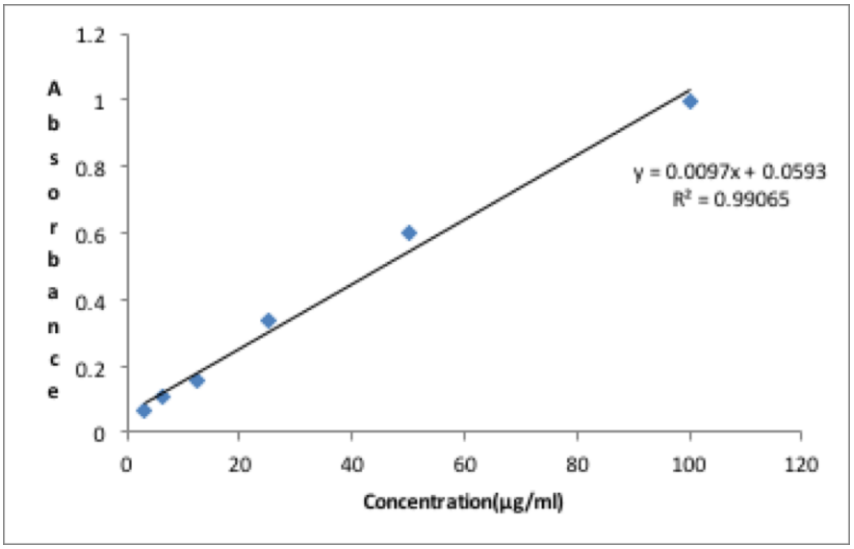

Figure 2: Quercetin standard curve

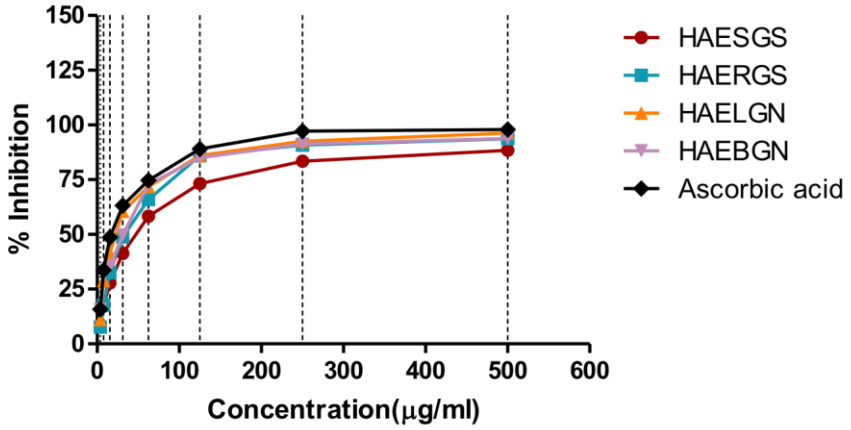

Figure 3: DPPH free radical scavenging activity

with respect to concentration of extracts and HAERGS shows maximum inhibition of 92.34± 1.02 % at concentration of 500µg/ml.

DISCUSSION

Plant phenolics were broadly distributed in various tissues of plants and play a key role as highly effective free radical scavengers with its antioxidant activity. The antioxidant activity of flavonoids is due to their capability to minimize free radical formation and to scavenge them. Consequently, based on the evidenced phytochemical constituents of these two plants, it is vital to evaluate their antioxidant potential. It was revealed that hydroalcoholic extracts of both the plants *Grewia serrulata* and *Grewia nervosa* possess sufficient quantities of phenols and flavonoids. Among these, *Grewia nervosa* leaf extract holds high phenolic content and root extract of *Grewia serrulata* has elevated levels of flavonoids that might be accounted for their strong antioxidant activity compared to other extracts HAELGS and HAEBGN.

Since ancient era, plants are considered to be as an important source of remedy molecules and these plants have been evaluated due to their therapeutics principles (Rosy BA *et al*., 2010). Hence it is of keen importance to evaluate free radical scavenging activity of these two plants *Grewia serrulata* and *Grewia nervosa*. DPPH is a stable free radical which has been widely utilized to appraise the antioxidant activity of various natural products (HU C *et al*., 2000). The more antioxidant present in the extract the more DPPH reduction will occur. Current investigation infers that both the plants have considerable DPPH radical scavenging activity and maximum was elicited with hydroalcoholic leaf extract of *Grewia nervosa*. The resulted antioxidants of extracts may be due to the neutralization of free radicals (DPPH), either by transfer of hydrogen atom or by transfer of an electron (Knezevic SV *et al*., 2011).

Hydrogen peroxide itself is not very reactive, but few cases it causes cell toxicity due to generation of hydroxyl radical. Hence it is vital to eliminate hydrogen peroxide by the antioxidant defense system

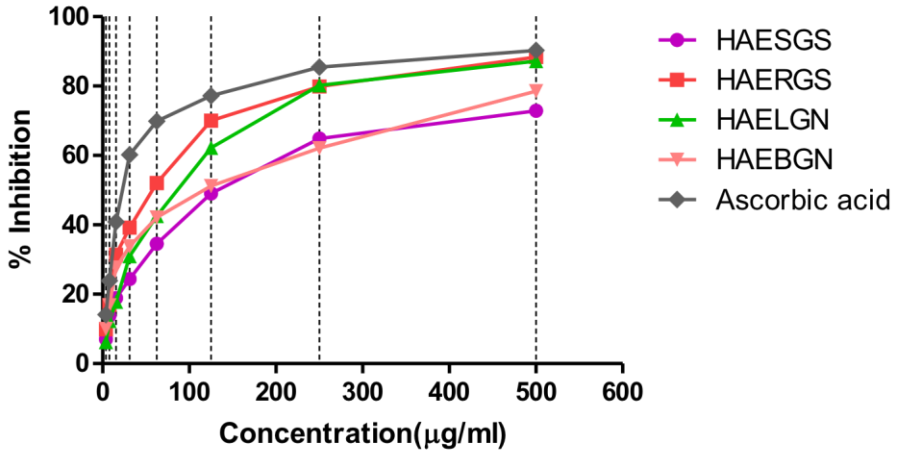

Figure 4: Hydroxyl radical scavenging activity

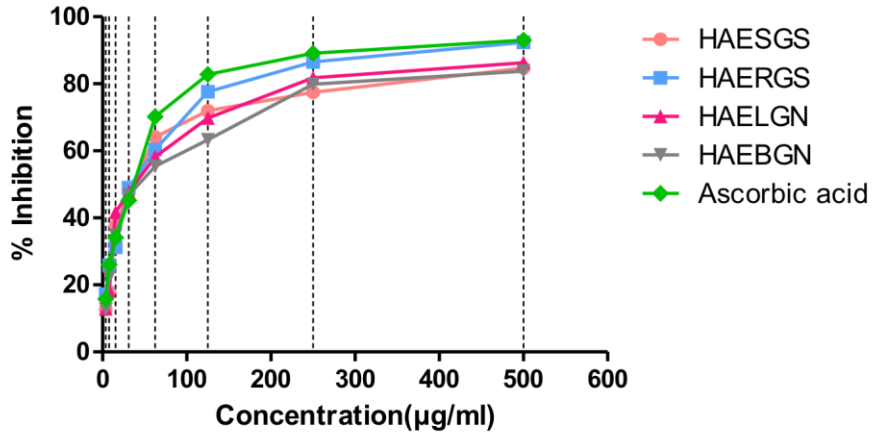

Figure 5: Superoxide radical scavenging activity

of a cell (Battu GR *et al*., 2011). Hydroxyl radical scavenging assay displays the capacity of the extracts to inhibit hydroxyl radical mediated deoxyribose deprivation in a Fe^{3+} -EDTA-ascorbic acid and H_2O_2 reaction mixture called Fenton reaction. It is clear from the results that HAERGS possesses good scavenging activity with minimum IC_{50} value and maximum % inhibition of radicals at a dose 500 µg/ml. Both the plants have shown good hydroxyl radical scavenging activity rendering their utilization in the treatment of various ailments associated with oxidative stress (Liu X *et al*., 2008).

Superoxide radical ($O_2^{\cdot-}$) is another harmful ROS to cellular components in biological system contributing to tissue injure and a range of ailments (B. Halliwell *et al*., 1987). It indirectly initiates lipid peroxidation by generating singlet oxygen. Research on phytomedicine elicited that the antioxidant properties of flavonoids are effective mainly by the scavenging of superoxide anion (Robak J *et al*., 1988). Based on the obtained IC_{50} value and concentration response curve it was revealed that hydroalcoholic root extract of *Grewia serrulata* possesses good superoxide radical scavenging activity as it is rich in flavonoids. *Grewia serrulata* leaf extract and bark extract of *Grewia nervosa* also exhibit moderate superoxide scavenging activity as they also enriched with flavonoids and phenols.

Based on the content of phytochemical constituents and various free radical scavenging activity of *Grewia serrulata* and *Grewia nervosa*, these plants are to be investigated for different pharmacological activities for the treatment of various ailments.

CONCLUSION

In this current investigation, total phenol content, total flavonoid content and free radical scavenging activity of hydroalcoholic extracts *Grewia serrulata* (stem and root) and *Grewia nervosa* (leaf and bark) were estimated. Presence of phenols might be responsible for their DPPH radical scavenging activity and enriched flavonoid content is a key factor for their superoxide and hydroxyl radical scavenging activity. This infers that both the plants *Grewia serrulata* and *Grewia nervosa* contains potential antioxidant bioactive compounds, which under extensive research will elicit chemically interesting

and bioactive molecules that can be screened for diverse pharmacological activities.

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