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Phytochemical analysis and antioxidant studies of leaf, stem, root and fruit of *Malva parviflora* L.

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Malva parviflora is a common edible weed in Pakistan. The different parts of this weed i.e., leaf; stem, root and fruit are part of the folk medicine. These different plant parts are used in different illnesses. Antioxidant activity of the whole plant has already been established. The rationale of the existing research is to evaluate the antioxidant potential of different parts of indigenous medicinal plant *M. parviflora*. Methanolic extract of different parts (leaf, fruit, root and stem) were prepared. Phytochemical analysis represented that leaf and fruit have more amount of phenolics as well as flavonoids content. Five methods were used to evaluate the antioxidant capacity of methanolic extract of different parts of the plant. These methods included: FTC (Ferric thiocyanate analysis), FRAP (Ferric reducing antioxidant power) analysis, DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical inhibition activity and total antioxidant activity as well as lipid peroxidation. The results revealed that leaf extract exhibited highest antioxidant activity as compared to other plant parts.

Keywords: *Malva parviflora*, phytochemical analysis, FTC, FRAP, DPPH

INTRODUCTION

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation reactions can be produced by the formation of free radicals (Panche et al. 2016). These free radicals are derived from reactive oxygen (ROS) and reactive nitrogen (RNS) species, they are generated in our bodies in result of various physiological and pathological states (Kumar et al. 2018). These free radical species damage to protein and result in various enzymatic activities, damage to DNA can result in various mutagenesis and carcinogenesis (Chithiraikumar et al. 2017). In a normal healthy human body, the generation of pro-oxidants in the form of ROS and RNS are effectively kept in check by the various

levels of antioxidant defense. However, when it gets exposed to adverse physicochemical, environmental or pathological agents such as atmospheric pollutants, cigarette smoking, ultraviolet rays, radiation, toxic chemicals, over nutrition and advanced glycation end products (AGEs) in diabetes, this delicately maintained balance is shifted in favor of pro-oxidants resulting in 'oxidative stress'.

Antioxidants, capable of neutralizing free radicals or their actions, act at different stages. Antioxidants may be natural or synthetic. The most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Propylwerdsgallate (PG) and butylated hydroquinone (Aryal et al. 2019).

Malva parviflora belong to family Malvaceae. It is found worldwide, especially in warm climates. There are roughly 100 genera and 2000 species (Morhardt and Morhardt, 2004). Plants of the Mallow family are usually herbaceous or shrubby and slightly hairy (Kirkpatrick et al. 2008). *Malva* tea was used for soothing to a dry, irritating cough and bronchial irritation. Mallow prevent formation of kidney stone, also used in resolving boils, abscesses and removing splinter tissue (Kane, 2006). A decoction of roots or leaves has also been used as a hair rinse to remove dandruff and to soften the hair (Moerman, 1998). Leaves or roots tea was also used for angina and stomachaches (Vizgirdas and Vizgirdas, 2006). Bhattacharjee indicated that *M. parviflora* was used in the treatment of swollen glands of throat, in fever, in pneumonia, in headache and also used for the induction of menstrual flow (Bhattacharjee, 2001). Cooked Leaves of *M. parviflora* was used to treat constipation (Hayat et al. 2008). Hexane extract of whole herb also showed anti-inflammatory activity (Shale, 2005), also have wound healing property (Lanes et al. 2007). So the present research work to evaluate the antioxidant potential of *Malva parviflora* therefore it is important to report *in vitro* antioxidant potential of methanolic fractions of Leaves, Stem, Fruit, Roots of this species.

MATERIALS AND METHODS

Collection and identification of plant material

The plant was collected from Sheikhpura, Punjab, Pakistan in May 2019. The plant was identification by Mr. Muhammad Ajaib of the Department of Botany, GC University, Lahore and a voucher specimen has been deposited in the herbarium (voucher specimen numbers are GC.Herb.Bot.1600) of the same university.

Chemicals and standards

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, 2,4,6-tripyridyl-s-triazine (TPTZ), trolox, gallic acid, Folin-Ciocalteu phenol reagent and butylated hydroxytoluene (BHT) were obtained from the Sigma Chemical Company Ltd. (USA) and the organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol), sulphuric acid, sodium phosphate, ammonium molybdate, ferric chloride from Merck (Pvt.) Ltd. (Germany).

Extraction of plant material

About 10 kg fresh plant was collected and different plant parts were separated. All separated

parts were dried under shade for 2 weeks, after that they were grinded to a fine powder. The methanolic extract was prepared by soaking dry powdered of each plant part in methanol (2×4L) at room temperature for few days so that alkaloids, terpenoids and other organic constituents get extracted from each plant part. Extracts were prepared by using rotary evaporator in final form.

Phytochemical screening

Qualitative phytochemical screening were performed by using standard procedures to identify the phytochemical constituents, *i.e.*, alkaloids, terpenoids, saponins, tannins, sugars, phenolics, flavonoids and cardiac glycosides, using standard procedures (Akbar et al. 20121).

Sample preparation for Antioxidant activity

The sample solutions of different extracts were prepared by dissolving 0.02g of the extract in 20ml of the methanol (1000µg/ml).

Determination of antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging activities of leaf, stem, root and fruit of plant were examined by comparison with that of a known antioxidant *i.e.* butylated hydroxytoluene (BHT), using a reported method. IC50 values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation. Synthetic antioxidant reagent, BHT was used as reference positive (Chaves et al. 2020). Inhibition free radical DPPH was calculated in following way:

$$I (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100$$

Each sample was assayed in triplicate and the mean values were calculated.

Total antioxidant activity by the phosphomolybdenum method

It was evaluated by the phosphomolybdenum complex formation method. Briefly, 500 µg/mL of each sample was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in sample vials. The blank solution contained 4mL of reagent solution. The vials were capped and incubated in water bath at 95 °C for 90minutes. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm against the blank. The antioxidant activity was expressed relative to that

of BHT. All determinations were assayed in triplicate and mean values were calculated (Untea et al. 2018).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to Chaves with some modifications. The stock solutions included 300 mM acetate buffer, 10 mM TPTZ soln in 40 mM HCl, and 20 mM ferric chloride hexahydrate solution. The solutions of plant part and that of trolox were prepared in methanol (500µg/mL). 10µL of each fractions and BHT soln were taken in separate test tubes and 2990µL of FRAP soln was added to each to make a total volume upto 3 mL. The fractions were allowed to react with the FRAP solution in the dark for 30 minutes. The absorbance of the coloured product (ferrous tripyridyltriazine complex) was checked at 593 nm. The FRAP values are determined as micromoles of trolox equivalents (TE) per mL of the sample using the standard curve constructed for different concentrations of trolox. The results were expressed in TE µM/mL (Chaves et al. 2020).

Ferric thiocyanate (FTC) assay

The antioxidant activities of leaf, stem, root and fruit of *M. parviflora* on the inhibition of linoleic acid peroxidation were assayed by the thiocyanate method. 0.1 ml of each of sample solution (0.5mg/ mL) was mixed with 2.5 mL of linoleic acid emulsion (0.02 M, pH 7.0) and 2.0 mL of phosphate buffer (0.02 M, pH 7.0). The linoleic emulsion was prepared by mixing 0.28 g of linoleic acid, 0.28 g of Tween-20 as emulsifier and 50.0 mL of phosphate buffer. The reaction mixture was incubated for 5 days at 40 °C. The mixture without extract was used as the control. The 0.1 mL aliquot of the mixture was taken and mixed with 5.0 mL of 75 % ethanol, 0.1 mL of 30 % ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5 % hydrochloric acid and allowed to stand at room temperature. Precisely 3 min after the addition of ferrous chloride to the reaction mixture, the absorbance was recorded at 500 nm (Mogadem et al. 2021). The antioxidant activity was expressed as percentage inhibition of peroxidation (IP %):

Inhibition of lipid peroxidation (%)

$$= [1 - \frac{A_{sample}}{A_{control}}] \times 100$$

The antioxidant activity of BHT as reference standard was assayed for comparison.

Total content of phenolics

The total phenolics of the various fractions of plant were determined with the Folin Ciocalteu reagent by a reported method. The 0.1 mL (0.5 mg /mL) of sample was combined with 2.8mL of 10 % sodium carbonate and 0.1 mL of 2N Folin–Ciocalteu reagent. After 40 minutes, the absorbance at 725 nm was measured using a UV–visible spectrophotometer. The total phenolics were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample using a standard calibration curve constructed for different concentrations of gallic acid. The curve was linear between 50µg/ mL and 400 µg/mL of gallic acid. Results were expressed in GAE µg/mL (Sembiring et al. 2018).

RESULTS

The phytochemical analysis conducted on *Malva parviflora* extracts revealed the presence of tannins, flavonoids, sugars, alkaloids, terpenoids, phenolics and saponins in methanolic extracts of root, stem, leaf and fruit. The results were tabulated in Table1.

DPPH radical scavenging assay

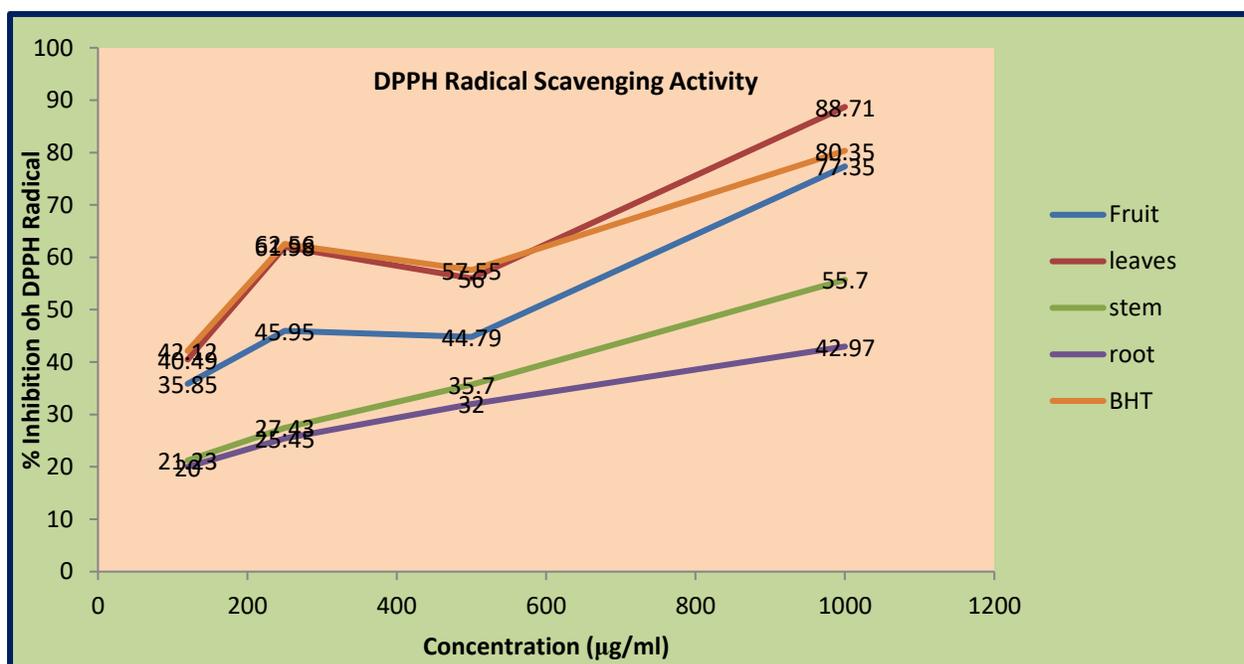
Among the four (4) extracts of different parts (leaf, root, stem, fruit) of *Malva Parviflora* and standard tested for the in vitro antioxidant activity using the DPPH method. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution.

Total antioxidant capacity

The antioxidant capacity of the fractions was measured spectrophotometrically through phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 765 nm. A high absorbance value of the sample indicates its strong antioxidant activity. The present study demonstrated that methanolic extract of *Malva* fruit exhibited the highest antioxidant capacity for phosphomolybdate reduction which is 0.486±0.0138 (Fig 2)

Table1: Phytochemical Analysis of Leaf, stem root and fruit of *Malva parviflora*.

Phytochemical	Test	Methanolic extract (leaf)	Methanolic extract (fruit)	Methanolic extract (stem)	Methanolic extract (root)
Terpenoids	Salkowaski	+	+++	++	+
	Liebermann's	-	+++	+	+
Sterols	Salkowaski t	+	+++	++	+++
	Liebermann's	+	+++	++	+
	Sulphur test	+	+	+	+
Glycosides	Bromine water	+	++	+	+++
	Keller killani	++	+++	++	+++
	Legal 's test	-	-	-	-
Flavonoids	Ferric chloride	-	-	-	-
	Alkaline reagent	+++	+	+	-
	Lead acetate	+	++	++	+
Alkaloids	Mayer's	+++	++	+	-
	Wagner's	+++	++	+	+
	Hager 's Dragendroff 's	+++	+++	+++	+++
		+	++	+	+
Proteins	Millon 's	-	-	-	-
	Ninhydrin	+	+++	+++	+++
	Biuret	-	-	-	-
Carbohydrates	Barfoed 's	+	++	-	-
	Molisch 's	++	+++	+++	+++
	Benedicts	-	+++	+++	-
Saponins	Foam	+	-	-	+
	Bromine water	-	+++	++	++
	Legal 's	+	+++	++	++
Lipids	Soap formation	-	-	-	-
Tannins	Ferric chloride	-	-	-	-

**Figure 1: Comparison of Percentage Inhibition of DPPH Radical of different Concentrations of Leaf, Fruit, Stem, Root extracts of *Malva parviflora* and BHT.**

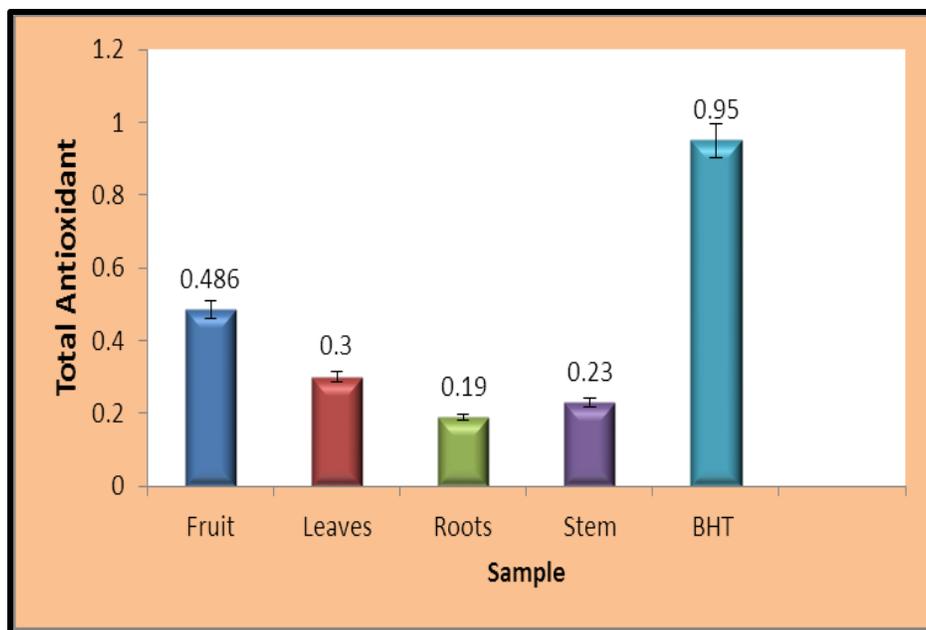


Figure 2: Comparison of Total Antioxidant contents of Fruit, Leaves, Root, Stem extracts of *Malva parviflora* and BHT.

Total phenolic contents

Phenolic compounds are considered secondary metabolites and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants and are diversified. Phenolic compounds of plants are also very important because their hydroxyl groups confer scavenging ability. Phenolic compounds of plants fall into several categories; chief among these are the flavonoids which have potent antioxidant activities. Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. In present study, leaves of *Malva* Plant are found to have highest value of Total Phenolic contents as shown in Fig 3.

Ferric reducing antioxidant power assay (FRAP)

The reducing capacity of the extracts Fe^{3+} /ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity. The existence of reductones are the key of the reducing power, which exhibit their

antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom. The presence of reductants (i.e., antioxidants) causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

Inhibition of lipid peroxidation by ferric thiocyanate method

In this method the antioxidant activity of all four extracts was determined by peroxidation of linoleic acid. [Fig 4]. During linoleic acid peroxidation, peroxides were formed and these compounds oxidized Fe^{2+} to Fe^{3+} . The Fe^{3+} ion formed a complex with SCN^- , which had a maximum absorbance at 500 nm. Thus, a high absorbance value was an indication of high peroxide formation during the emulsion incubation (Figure 4).

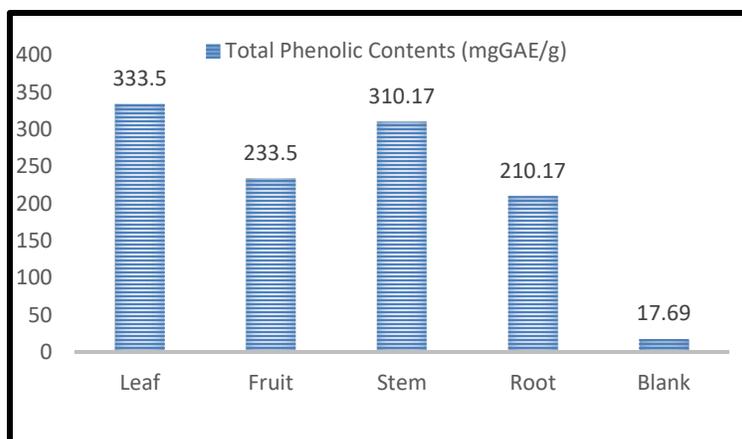


Figure 3: Comparison of Total Phenolic Contents (mgGAE/g) of Leaf, Fruit, Stem and Root extracts of *Malva parviflora*

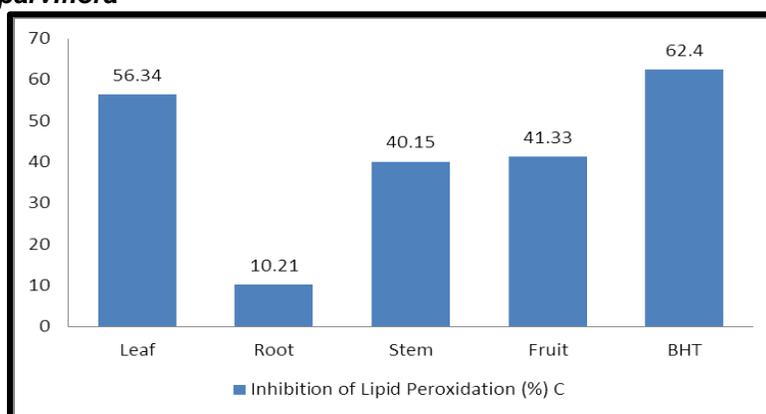


Figure 4: Comparison of Percentage inhibition of lipid peroxidation of Leaf, Root, Stem, Fruit extracts of *Malva parviflora* and BHT.

Statistical Analysis

All results are presented as mean \pm standard mean error of three assays. * $p < 0.05$ when compared with negative control i.e. blank/solvent ($p < 0.05$ is taken as significant). ** $p < 0.05$ when compared with reference standard (BHT).

DISCUSSION

The phytochemical analysis indicated the presence of various phyto constituents i.e. alkaloids, glycosides, tannins, and flavonoids in the crude methanolic extract. Several of such compounds are known to possess potent antioxidant activity. Some of these constituents have already been isolated from this plant (Helmy et al. 2021). Hence, the observed antioxidant activity may be due to the presence of any of these constituents. The plant leaf exhibited strong anti-inflammatory, wound-healing, antiviral and several other activities. These properties may be due to its antioxidant activity.

Leaf, root, stem and fruit methanolic extract of *Malva parviflora* were used for the in vitro antioxidant activity using the DPPH method (Yan-Hwa et al. 2000). A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test. Initial DPPH radical concentration by 50% in 30 minutes (IC₅₀). Lowest IC₅₀ values indicated that the highest antioxidant capacity (Sharma et al. 2007). The methanolic extracts of Leave, fruit, stem and root showed antioxidant activity, with IC₅₀ values of 13.59 ± 0.4811 , 257.13 ± 1.12 , 451.25 ± 0.8348 , 740.35 ± 1.22 $\mu\text{g/ml}$, respectively. It showed that leave extract has strong antioxidant activity while root extract has weak antioxidant activity. The IC₅₀ value for BHT was 12.32 ± 0.1 $\mu\text{g/ml}$. The results indicate that the antioxidant activity of the leave extract of *M. parviflora* is nearly the same as that of BHT. However, the other extracts from fruit, stem and root of *M. parviflora* were found to be less active than BHT since their IC₅₀ values were found to be higher (Table 2 and 3).

Table 2: Free radical scavenging activity of leaf, stem, root and fruit of *Malva parviflora* using 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH)

Sr. No.	Sample	Concentration in assay ($\mu\text{g/ml}$)	% Scavenging of DPPH (\pm S.E.M ^a)
1	Leaf extract	1000	88.71 \pm 1.20
		500	61.98 \pm 0.89
		250	56.0 \pm 1.05
		120	40.49 \pm 0.71
2	Fruit extract	1000	70.35 \pm 0.58
		500	44.79 \pm 1.14
		250	45.95 \pm 1.31
		120	35.86 \pm 0.87
3	Stem extract	1000	55.70 \pm 1.02
		500	35.70 \pm 1.11
		250	31.23 \pm 0.99
		120	27.43 \pm 0.75
4	Root extract	1000	42.97 \pm 0.79
		500	32.0 \pm 1.21
		250	25.45 \pm 0.45
		120	20.0 \pm 0.3
6	BHT ^{b)}	1000	93.35 \pm 0.14
		500	89.16 \pm 0.08
		250	72.57 \pm 0.05
		120	60.32 \pm 0.13

Table 3: IC50, Total antioxidant activity, Frap values, Lipid peroxidation inhibition and Total phenolics values of leaf, stem, root and fruit of *Malva parviflora*

Sr. No.	Sample	DPPH-radical scavenging activity (IC50; $\mu\text{g/mL}$)	Total antioxidant activity (abs. at 695 nm)	FRAP value (TE mM/mL)	Total phenolics (GAE mg/g)	Inhibition of lipid peroxidation (%)
1	Leaf extract	13.67 \pm 0.48	0.3 \pm 0.008	116.9 \pm 0.368	333.50 \pm 0.67	56.34 \pm 0.68
2	Fruit extract	257.13 \pm 1.12	0.486 \pm 0.013	82.17 \pm 0.206	233.50 \pm 0.38	46.33 \pm 1.53
3	Stem extract	451.25 \pm 0.83	0.23 \pm 0.003	79.59 \pm 0.479	310.17 \pm 0.433	40.15 \pm 1.46
4	Root extract	740.35 \pm 1.22	0.19 \pm 0.015	68.65 \pm 0.267	210.17 \pm 0.4080	10.21 \pm 0.95
6	BHT	12.32 \pm 0.354	0.95 \pm 0.06	-	-	62.48 \pm 1.07
7	Blank ^{b)}	-	-	18.64	17.69	-

The antioxidant capacity of all four parts was measured spectrophotometrically through phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V). A high absorbance value of the sample indicates its strong antioxidant activity. The present study demonstrated that methanolic extract of *Malva* fruit exhibited the highest antioxidant capacity for phosphomolybdate reduction which is 0.486 ± 0.0138 while root extract exhibited lowest antioxidant capacity which is 0.19 ± 0.0047 . Recent studies have shown that many flavonoid and

related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants (Sharififar et al. 2009).

Phenolic compounds of plants are also very important because their hydroxyl groups confer scavenging ability. Phenolic compounds of plants fall into several categories; chief among these are the flavonoids which have potent antioxidant activities (Lee et al. 2015). Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. In present study, leaves of *Malva* are found to have highest value of total Phenolic contents as shown in Fig.2.

The reducing capacity of the extracts Fe³⁺/ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity (Meir et al. 1945). Among the extracts of four different parts (leaf, fruit, stem, root) of Malva Plant, Leaf extract has the highest FRAP value as shown in Fig 3. While Root has the lowest FRAP value. Fruit and stem extracts have nearly similar FRAP values.

In lipid peroxidation method the antioxidant activity of extracts was determined by peroxidation of linoleic acid (Zou et al. 2004). (Fig 4). During linoleic acid peroxidation, peroxides were formed and these compounds oxidized Fe²⁺ to Fe³⁺ (Meir et al. 1945). The Fe³⁺ ion formed a complex with SCN⁻, which had a maximum absorbance at 500 nm (Ferreira et al. 2007). Thus, a high absorbance value was an indication of high peroxide formation during the emulsion incubation. As shown in Figure 10, among four extracts of different parts, leaf has the highest percentage inhibition (56.34%) and is slightly less than BHT (62.4%). These results indicate that leaf extracts of Malva can significantly inhibit the peroxidation of linoleic acid and reduce the formation of hydroperoxide, thus implying that leaf of Malva has powerful antioxidant activity.

CONCLUSION

From present research work it is concluded that all parts of *Malva parviflora* have good antioxidant activities but leaves have stronger antioxidant activity among all other parts. These evaluations provide scientific basis for the use of different parts of this plant to cure/prevent different diseases.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SA wrote the manuscript and performed the experimental work, SI rechecked the manuscript and designed the experimental work. KS and MYR helping in preparation of all extracts, UR, SR and SR helped in performance of antioxidant activity while SR and AM helped in calculation of IC₅₀ value of each assay. MNK supported financially.

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