Research Article

Comparative Evaluation of Free Radical Scavenging Efficacy of Aqueous and Ethanolic Root Extracts of *Malva neglecta*

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ABSTRACT

These findings suggest that *Malva neglecta* possesses notable potential as a natural source of antioxidants, which may be beneficial for developing functional foods or supplements aimed at combating oxidative stress. Further studies could explore the specific compounds responsible for this activity and their mechanisms of action. The present study concluded that the presence of phytochemicals in both the aqueous & ethanolic root extracts of *Malva Neglecta* and both extracts exhibited antioxidant activity when subjected to the free radical scavenging tests. The *Malva neglecta* results (IC_{50}) for the DPPH free radical scavenging assay were found to be 80.50 µg/ml, and for the H_2O_2 radical scavenging test, they were found to be 58.97µg/ml. The *Malva neglecta* results (IC_{50}) for the DPPH free radical scavenging assay were found to be 119.38 µg/kg, and for the H_2O_2 radical scavenging test, they were found to be 117.00 µg/ml.

Keywords: *Malva neglecta*, DPPH and Hydrogen Peroxide, Ascorbic acid, Anti-oxidants.

INTRODUCTION

Since nature has been a source of therapeutic (medicinal) chemicals for centuries, an impressive number of new or novel drugs have been derived from natural sources[1]. Recently it is seen that there has been phenomenal rise in the preparation and usage of plants derived health products in both developed and developing countries, which results in ensuring an exponential growth of herbal products globally. In an effort to identify all medicinal plants used globally the WHO has identified more than 22,000 species[2]. According to a WHO survey, 80% of the population in developing nations uses traditional herbal medicine as their main source of healthcare[3-4]. *Malva Neglacta* is one of the several plants whose medicinal uses are being investigated which is commonly known as wind tree (English) and malva (Hindi). In this paper, author intends to provide information of the chemical constituents present in various parts of *Malva Neglacta* as well as the various pharmacological actions, which results in various uses of *Malva Neglacta* for the treatment of various diseases[5].

Plant profile

Plant Name: *Malva Neglecta*

Figure 1: *Malva Neglecta* Plant leaves

Figure 2: *Malva Neglecta* Plant root

Scientific Classification:

- Kingdom: Plantae;
- Subkingdom: Tracheobionta;
- Super division: Spermatophyta;
- Division: Magnoliophyta;
- Class: Magnoliopsida;
- Subclass: Dilleniidae;
- Order: Malvales;
- Family: Malvaceae;
- Genus: Malva L.;
- Species: Malva neglecta

Health Benefits

The root and petals have traditionally been used as a treatment for bruising, inflammation, and insect bites, as well as to treat urinary and respiratory tract infections internally. Sore throat, constipation, throat infection, wounds, women's sterility, hemorrhoids, miscarriage swellings, stomachache, indigestion, rheumatic pain,

abdominal discomfort, renal illnesses, cough, common cold, bronchitis, and peptic ulcer were all treated with the root and flowers in the past[6].

MATERIALS AND METHODS

Preparation of extracts:

The root extract of *Malva Neglecta* was collected and reduced to small size after drying in shade for one month or till dry and crushed to form coarse powder. The powdered drug (250 gm) was subjected to continuous hot extraction with the help of a Soxhlet apparatus using water & ethanol. The plant material was dried in a hot air oven at 50°C for an hour. After the effective extraction, the solvents were distilled off, and both extracts were then concentrated on a water bath to become dried. The obtained extract was weighed and stored in an airtight container**.**

In-Vitro Antioxidant Evaluation

DPPH free Radical Scavenging Assay[7-8]

The DPPH assay of leaf extracts was determined by according Pin Der Duh et. At., 1995.

Preparation of Standard Ascorbic acid solutions:

Various solutions of the ascorbic acid were prepared in 90% methanol to obtain different concentrations (1-100 µg/mL). A 200 µM solution of DPPH (in methanol) was prepared, and 1.5 ml of this solution was added to 1.5 ml of a methanolic ascorbic acid solution of different concentrations and incubated for 30 min (at room temperature) in the dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as a blank) at 517 nm.

Preparation of Test solutions:

Various solutions of ethanolic or aqueous root extract of *Malva neglecta* were prepared in 90% methanol to obtain different concentrations (10-100 µg/ml). A 200 µM solution of DPPH in methanol was prepared, and 1.5 ml of this solution was added to 1.5 ml of a methanolic root extract of *Malva Neglecta* solution of different concentrations and incubated for 30 min (at room temperature) in the dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as a blank) at 517 nm.

Preparation of Control solution:

For control, 1.5 ml of methanol was mixed with 200 µM DPPH solution and incubated for 30 min at room temperature in the dark. The absorbance of the control was taken after 30 min against methanol (as a blank) at 517 nm.

The antioxidant activity of aqueous or ethanolic root extract of *Malva Neglecta* and ascorbic acid was calculated by using the following formula in terms of % inhibition:

$$\% \text{ Inhibition} = \frac{\text{Ac 230} - \text{At 230nm}}{\text{Ac 230nm}} \times 100$$

Where:- Ac = Absorbance of control, At =Absorbance of ascorbic acid / root extract.

Hydrogen Peroxide radical Scavenging Assay[9-10]

The ability of the ethanolic root extract of *Malva Neglecta***,** to scavenge hydrogen peroxide was determined according to the method of Ruch et.al. (1989).

Preparation of Standard Ascorbic Acid solutions:

Different concentrations of the ascorbic acid were prepared in distilled water to give the solutions of varying concentrations (1-100 µg/ml). 1 ml of each solution of ascorbic acid was mixed with 2.4 ml of 0.1 M phosphate buffer and 600 µl of 40 mM H_2O_2 solutions. After 10 minutes, absorbance of different samples was taken at 230 nm using a phosphate buffer as a blank.

Preparation of test solutions:

Various concentrations of the aqueous or ethanolic root extract of *Malva neglecta* were prepared in distilled water to give solutions of varying concentrations (1-100 µg/ml). 1 ml of each solution of aqueous or ethanolic root extract of *Malva neglecta* was mixed with 2.4 ml of 0.1 M phosphate buffer and 600 µl of 40 mM H_2O_2 solutions. After 10 minutes, absorbance of different samples was taken at 230 nm using a phosphate buffer as a blank.

Preparation of Control Solution:

For control, 2.5 ml of 0.1 M phosphate buffer solution was mixed with 600 µl of 40 mM H_2O_2 solution. After 10 minutes, absorbance of the control was taken at 230 nm.

H_2O_2 radical scavenging activity of aqueous or ethanolic root extract of *Malva Neglecta* and ascorbic acid were calculated by using the following formula

$$\text{Inhibition} = \frac{\text{Ac 230} - \text{At 230nm \%}}{\text{Ac 230nm}} \times 100$$

Where:- Ac = Absorbance of control (0.1M phosphate buffer solution and H_2O_2)

At = Absorbance of ascorbic acid / root extract.

Statistical Analysis

The data of results obtained were subjected to statistical analysis and expressed as regression curve and % Inhibition curve value with help of EXCEL. The data were statically analyzed by Graph pad prism Software version (7.1).

RESULT AND DISCUSSION

In-Vitro Antioxidant Assay

Antioxidant activity was assessed on the basis of different assay methods i.e. DPPH radical scavenging, hydrogen peroxide scavenging activities (Table 1, 3, 5,7) and IC_{50} (Table 2, 4, 6,8).

DPPH Free Radical Scavenging Assay

The DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay is widely used to assess antioxidant activities in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to burn into a stable diamagnetic molecule.

The DPPH assay for ethanolic root extract was performed by using an ascorbic acid solution as a standard. The absorbance data were recorded against the selected concentrations (1-10 µg/ml for ascorbic acid and 10-100 µg/ml for fresh ethanolic root extract) at 517 nm.

The percentage inhibition curves for the DPPH free radical scavenging assay of ascorbic acid and ethanolic root extract were plotted, from which IC_{50} values of percentage inhibition of DPPH by ascorbic acid and ethanolic root extract were calculated using a regression equation.

It was observed that ethanolic root extract has significant activity in the DPPH assay in the concentration range of 10-100 µg/mL. The IC_{50} for ascorbic acid was found to be 70.82 µg/mL, while for ethanolic root extract, it is 80.50 µg/mL.

Table 1: The percentage Inhibition data of DPPH free radical scavenging assay by ascorbic acid and ethanolic extract of *Malva neglacta*

S.No	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Ascorbic acid) At	% Inhibition	Absorbance (Absorbance of ethanolic extract), At	% Inhibition
1.	20	0.700	0.590	15.71	0.610	12.85
2.	40		0.505	27.85	0.525	25.00
3.	60		0.410	41.42	0.430	38.57
4.	80		0.315	55.00	0.365	47.85
5.	100		0.195	72.14	0.260	62.85

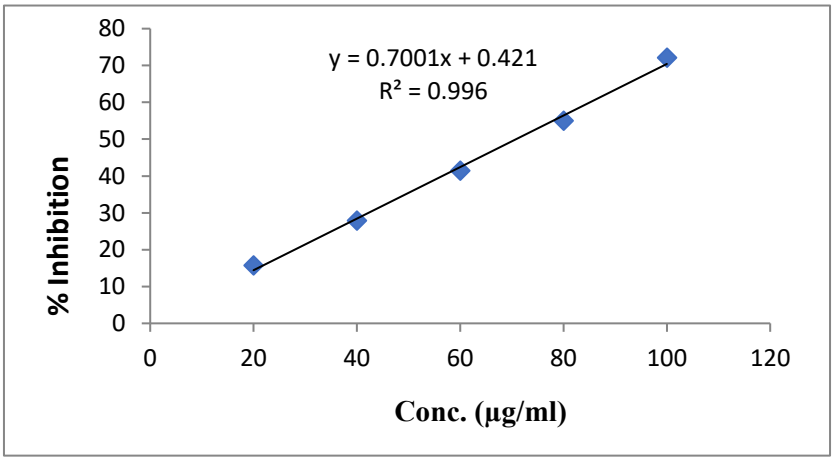

Figure 3: Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method

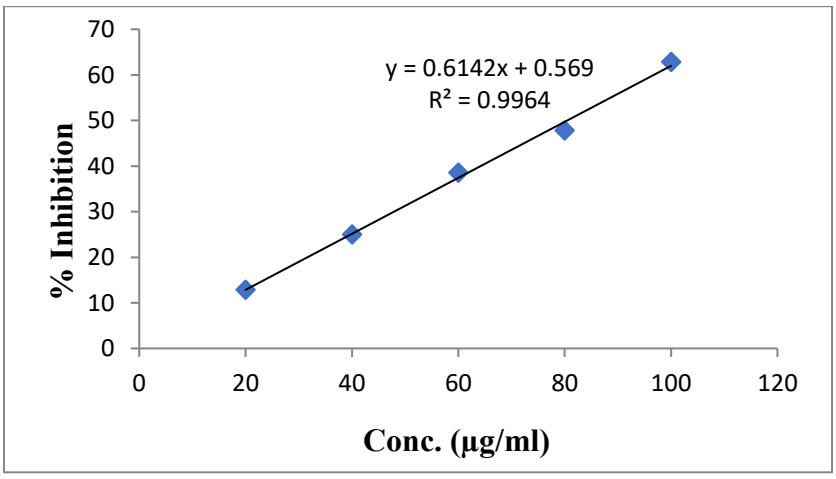

Figure 4: Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method

Table 2: IC_{50} value and Statistical analysis of ascorbic acid and ethanolic extract of *Malva neglacta*

Sample	IC_{50}	Equation	R^2 value	F value	Dfn and Dfd value	P Value
Ascorbic acid	70.82 µg/ml	y =0.700x + 0.421	R^2 = 0.996	718.2	1,3	<0.0001
ethanolic extract	80.50 µg/ml	y =0.614x + 0.569	R^2 = 0.996	614.6	1,3	<0.0001

Table 3: Percentage Inhibition data H_2O_2 of free radical scavenging assay by ascorbic acid and ethanolic extract of *Malva neglacta*

S.No	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Ascorbic acid) At	% Inhibition	Absorbance (Absorbance of ethanolic extract), At	% Inhibition
1.	20	0.660	0.610	07.57	0.615	06.81
2.	40		0.525	20.45	0.540	18.18
3.	60		0.425	35.60	0.445	32.57
4.	80		0.312	52.72	0.355	46.21
5.	100		0.210	68.18	0.245	62.87

Hydrogen peroxide radical scavenging activity

The hydrogen peroxide radical assay is a method used to assess antioxidant activities in a relatively short time. H_2O_2 radical scavenging of ethanolic root extract was estimated by using the ascorbic acid solution as a standard. The absorbance data was recorded against the selected concentration (10-100 µg/ml for ascorbic acid and ethanolic root extract).

The standard curves for H_2O_2 radical scavenging of ascorbic acid and ethanolic root extract were plotted, from which IC_{50} values of percentage inhibition of hydrogen peroxide radical scavenging of ascorbic acid and *Malva neglecta* were calculated using regression equation.

The IC_{50} value was calculated by using straight-line equations. In the H_2O_2 scavenging assay, it was observed that the root extract served as a good scavenger of hydrogen peroxide in the concentration range of 10-100 µg/ml. The IC50 for ascorbic acid was found to be 53.26µg/ml, while that for the ethanolic extract of *Malva neglecta was found to* be 58.97µg/ml.

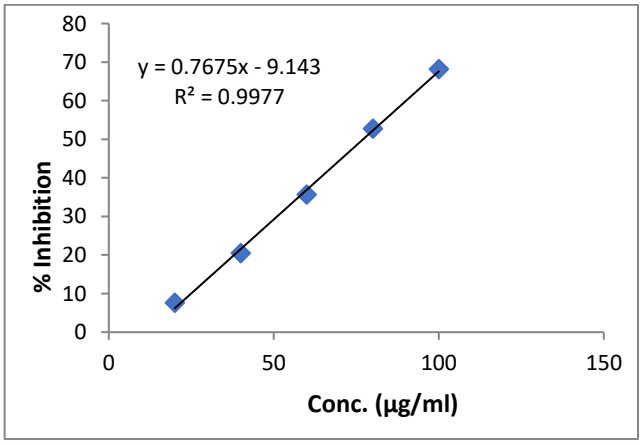

Figure 5: Representing % inhibition curve and regression curve of ascorbic acid by H_2O_2 assay method

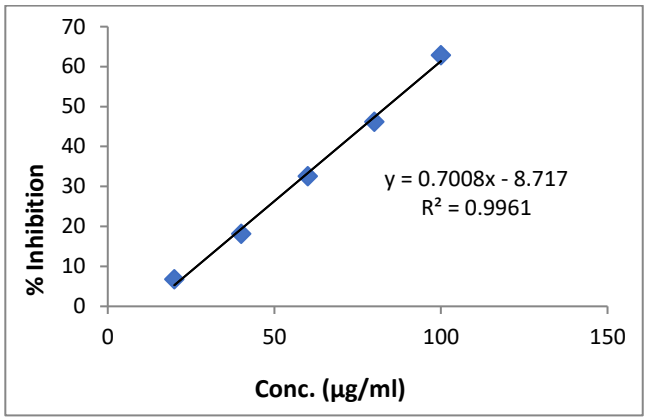

Figure 6: Percentage Inhibition curve and regression curve of root extract *malva neglecta* by H_2O_2 assay method

DPPH Free Radical Scavenging Assay (aqueous extract of *malva neglecta* root)

It was observed that ethanolic root extract significant activity in DPPH assay in the concentration range of 10-100µg/ml. IC_{50} for ascorbic acid was found to be 154.33µg/ml while for ethanolic root extract is 119.38µg/ml.

Hydrogen peroxide radical scavenging activity (aqueous extract of *malva neglecta* root)

The hydrogen peroxide radical assay is a method used to assess antioxidant activities in a relatively short time. H_2O_2 radical scavenging of aqueous root extract was estimated by using the ascorbic acid solution as a standard. The absorbance data were recorded against the selected concentration (10-100 µg/ml for ascorbic acid and aqueous root extract). The standard curves for H_2O_2 radical scavenging of ascorbic acid and ethanolic root extract were plotted, from which IC_{50} values of percentage inhibition of hydrogen peroxide radical scavenging of ascorbic acid and aqueous extract of *Malva neglecta* root were calculated using regression equations.

Table 4: IC_{50} value and Statistical analysis of ascorbic acid and ethanolic extract of *Malva neglacta*

Sample	IC_{50}	Equation	R^2 value	F value	Dfn and Dfd value	P value
Ascorbic acid	53.26 µg/ml	y =0.767x + 9.143	R^2 = 0.997	453.7	1,3	<0.0002
Root extract	58.97 µg/ml	y =0.700x +8.717	R^2 = 0.996	420.1	1,3	<0.0001

Table 5: The percentage Inhibition data of DPPH free radical scavenging assay by ascorbic acid and ethanolic extract of *malva neglacta* root

S.No	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Ascorbic acid) At	% Inhibition	Absorbance (Absorbance of aqueous extract), At	% Inhibition
1.	20	0.700	0.580	17.14	0.611	12.45
2.	40		0.509	27.28	0.542	22.85
3.	60		0.410	43.93	0.489	32.85
4.	80		0.360	48.57	0.411	41.42
5.	100		0.295	57.85	0.340	51.42

Table 6: IC_{50} value and Statistical analysis of ascorbic acid and aqueous extract of *Malva neglacta*

Sample	IC_{50}	Equation	R^2 value	F value	Dfn and Dfd value	P Value
Ascorbic acid	154.33 µg/ml	y =0.300x + 3.699	R^2 = 0.998	728.2	1,3	<0.0001
Aqueous extract	119.38 µg/ml	y=0.397x + 2.603	R^2 = 0.989	814.6	1,3	<0.0001

Table 7: Percentage Inhibition data H_2O_2 of free radical scavenging assay by ascorbic acid and aqueous extract of *malva neglacta*

S.No	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Ascorbic acid) At	% Inhibition	Absorbance (Absorbance of aqueous extract), At	% Inhibition
1.	20	0.660	0.510	22.72	0.535	18.93
2.	40		0.460	30.30	0.490	25.75
3.	60		0.415	37.12	0.410	26.86
4.	80		0.352	46.66	0.370	43.93
5.	100		0.280	57.57	0.295	55.30

Table 8: IC_{50} value and Statistical analysis of ascorbic acid and aqueous extract of *malva neglacta root*

Sample	IC_{50}	Equation	R^2 value	F value	Dfn and Dfd value	P value
Ascorbic acid	103.86 µg/ml	y =0.468x + 1.389	R^2 = 0.994	473.7	1,3	<0.0002
aqueous extract	117.00µg/ml	y =0.386x +4.923	R^2 = 0.996	170.1	1,3	<0.0001

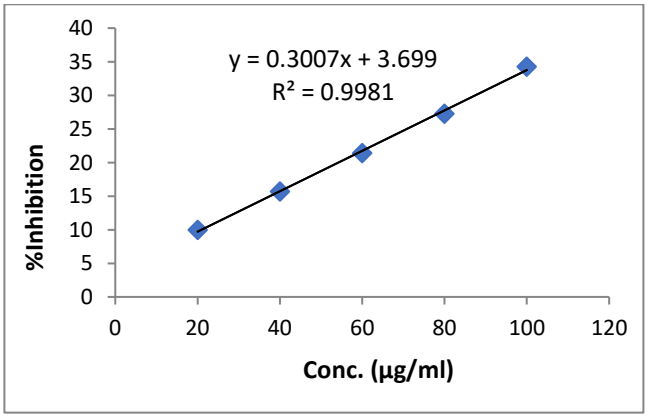

Figure 7: Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method

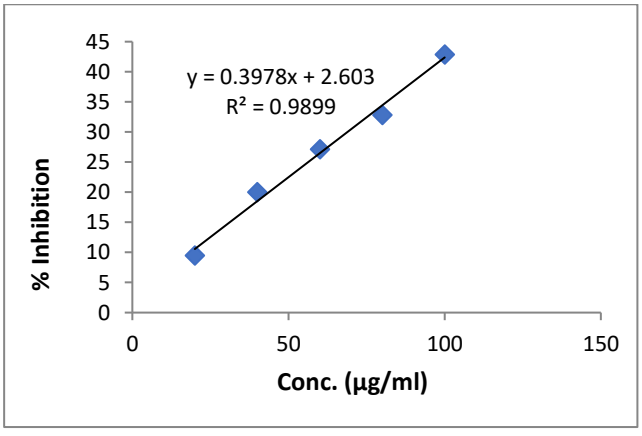

Figure 8: Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method

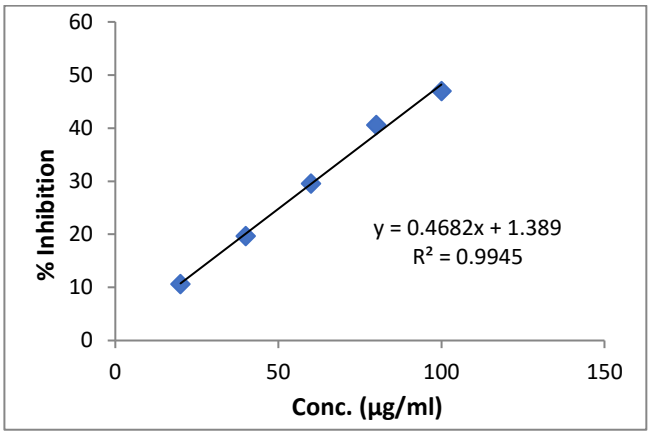

Figure 9: Representing % inhibition curve and regression curve of ascorbic acid by H_2O_2 assay method

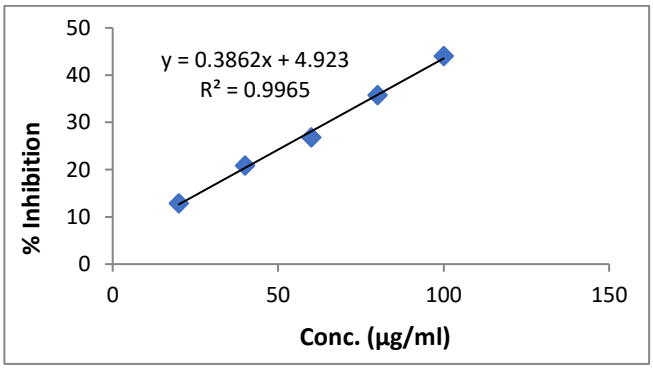

Figure 10: Percentage Inhibition curve and regression curve of aqueous extract of *Malva neglacta* root by H_2O_2 assay method

The IC_{50} value was calculated by using straight-line equations. In the H_2O_2 scavenging assay, it was observed that the root extract served as a good scavenger of hydrogen peroxide in the concentration range of 10-100 µg/ml. The IC_{50} for ascorbic acid was found to be 103.86 µg/ml, while that for the aqueous extract of *Malva neglecta* root was found to be 117.00 µg/ml.

CONCLUSION

Result was concluded that the both aqueous and ethanolic root extract antioxidant activities i.e. DPPH free radical and hydrogen peroxide scavenging activities of both extracts.

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AUTHOR CONTRIBUTION STATEMENT

Mr. Deepak Sharma conceptualized and gathered the data with regard to this work. Dr. Mohit Shrivastav analyzed these data, and necessary inputs were given towards the designing of the manuscript. Both authors discussed the methodology and results and contributed to the final manuscript.

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