



Phytochemical Screening and Antioxidant Activity of *Meyna laxiflora* Species Found in Imphal West District of Manipur

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ABSTRACT

Aqueous and methanol extracts of *Meyna laxiflora* leaf, fruit pulp and seed were screened for the presence of phytochemical constituents. Total phenolic content, total flavonoid content and antioxidant activity in the extracts were also determined. Phytochemicals were extracted using soxhlet extraction method. Phytochemical screening showed the presence of most of the phytoconstituents in aqueous and methanol extracts of various parts of *M. laxiflora*. Methanol was found to be a better solvent for extraction of phytochemicals from *M. laxiflora*. Total phenolic content is expressed in terms of gallic acid equivalent (GAE) and was found to be highest in methanolic fruit pulp extract (129.54 ± 0.25 mg /g of extract). Total flavonoid content is expressed in terms of quercetin equivalent (QE) and methanolic leaf extract recorded the highest ($80.45 \mu\text{g} \pm 0.16$ /100g of dried extract) followed by fruit pulp and seed extracts. Results indicate that aqueous and methanol extracts of various parts of *M. laxiflora* were found to possess DPPH scavenging activity, ferric reducing power and total antioxidant activity in dose dependent manner. In the entire antioxidant assay performed methanol extracts were found to have higher activity than aqueous extracts and among the various parts of *M. laxiflora* used for the study, fruit pulp was showing higher activity than leaf and seed which may be due to higher total phenolic content.

Keywords: *Meyna laxiflora*, leaf, fruit pulp, seed, phytochemical, antioxidant.

INTRODUCTION

Phytochemicals means plant chemicals which are naturally occurring biochemicals in plant that give plants their colour, flavour, smell and texture. They are bioactive non-nutritive plant compounds in fruits, vegetables, grains and other plant foods that have health related effects.^{1,2} Free radicals are compound with one or more unpaired electrons in its outer most orbital. They are extremely reactive and are known to be the underlying cause of several degenerative diseases. Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant in the body. Antioxidants are agents which scavenge free radicals. Many synthetic antioxidants are available but caused toxicity to human. Many studies have found that plants are potential source of immense chemicals for the treatment of various ailments and known to be rich source of natural antioxidant. In recent years research in natural antioxidant, especially of plant origin has increased tremendously.

Meyna laxiflora Robyns. which belongs to family Rubiaceae commonly known as muduna (Hindi, Bengali) and heibi (Manipuri) is a small tree or a spinescent. It found mainly in North-east, West Bengal, Western UP and Deccan Peninsula.³ Different parts of the plant were used in the treatment of boils, dysentery, diphtheria etc. Fresh leaves with dry fish, little common salt and chillis are eaten as blood purifier. Dry fruits are consumed as such in case of boils and dysentery.⁴ Fresh leaves smeared with coconut oil and then slightly heated are wrapped on

goiter or swellings.⁵ Five pinches of seed powder is mixed in water thoroughly and is given twice a day for 10-15 days to treat kidney stone.⁶ In Manipur, from ancient time fresh leaves of *M. laxiflora* are boiled with rice water and used for washing hair to give soft and lustrous hair, oil extract from fruit pulp are applied on skin to prevent skin from dryness and local people consume its leave and fruit frequently in various ways. Noticing the medicinal uses of various parts of *M. laxiflora* and frequent consumption by local people, the present study was carried out to analyze the phytochemical constituents and antioxidant activity of various parts and to compare them.

MATERIALS AND METHODS

Plant sample

M. laxiflora leaves and fruit were collected from Imphal West district of Manipur, Northeast India. The plant species was identified by L. Somarjit Singh, Associate Professor, Department of Botany, Imphal College, Imphal. Leaves and fruits were washed with tap water and then rinsed with distilled water and shade dry. Completely dried leaves were ground into powder. Fruit pulp was separated out from seed then dried separately and ground into fine powder.

Soxhlet extraction

i) Leaf: 40g powdered leaf of *M. laxiflora* was extracted with 400ml each of distilled water and methanol respectively by soxhlet until the solvent become



colourless in main chamber of the soxhlet extractor. The extracts were evaporated to dryness and crude extracts were obtained.

ii) Fruit pulp: *M. laxiflora* powdered fruit pulp of 40g was extracted separately using 400ml each of distilled water and methanol respectively by soxhlation until the solvent become colourless in main chamber of the soxhlet extractor. The extracts were evaporated to dryness and crude extracts were obtained.

iii) Seed: 40g powdered seed of *M. laxiflora* was extracted using 400ml each of distilled water and methanol respectively by soxhlation until the solvent become colourless in main chamber of the soxhlet extractor. Evaporated the extracts to dryness and crude extracts were obtained.

Phytochemical screening of aqueous and methanol extracts of leaf, fruit pulp and seed of *M. laxiflora* was carried out using standard protocol.⁷⁻¹¹

1. Test for alkaloids

Hager's test: Extract was dissolved in dilute HCl and filtered. Filtrate was treated with Hager's reagent (saturated picric acid solution). Yellow colour precipitate indicates the presence of alkaloid.

2. Tests for carbohydrates

Benedict's test: Extract was filtered. Filtrate was treated with Benedict's reagent and heated gently. Formation of orange red precipitate indicated the presence of reducing sugars.

Fehling's test: Filtrate was mixed with equal volume of Fehling's A and Fehling's B solutions and heated. Formation of brick red precipitate of cuprous oxide indicated the presence of reducing sugars.

3. Test for amino acids

Ninhydrin test: Amino acids and proteins when boiled with few drops of 5% solution of ninhydrin, violet colour appear.

4. Test for proteins

Xanthoproteic test: The extract was treated with a few drops of conc. nitric acid. Formation of yellow colour indicated the presence of proteins.

Biuret test: To the test solution 4% NaOH solution and few drops of 1% CuSO₄ solution were added, appearance of violet colour indicates the presence of protein.

5. Test for flavonoids

Alkaline reagent test: To the test solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

6. Test for phenolic compounds

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Formation of white precipitate indicated the presence of phenolic compounds.

7. Test for tannins

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicated the presence of tannin.

Ferric chloride test: To the test solution, a few drops of ferric chloride solution were added. An intense green, purple, blue or black colour indicated the presence of tannin.

8. Test for steroids and terpenoids

Salkowski's test: Extract was treated with chloroform and filtered. The filtrate was treated with few drops of conc. sulphuric acid, shaken well and allowed to stand. Appearance of red colour in the lower layer indicated the presence of steroids. Formation of reddish brown colour of interface after addition of conc. sulphuric acid to the side carefully (without shaking) indicated the presence of terpenoids.

9. Test for saponins

Froth test: Extract was added to 2-3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicated the presence of saponin.

10. Test for cardiac glycosides

Keller Killiani test: To the test solution, 2ml of glacial acetic acid containing a few drops of FeCl₃ solution was added. 1ml of conc. H₂SO₄ was added along the side of the test tube carefully. A brown ring at the interface indicated the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer indicating the presence of cardiac glycosides.

11. Test for oil and fat

A small quantity of the extract was pressed between the two filter papers. Oil stain on the filter papers indicated the presence of oil.

12. Test for phlobatannin

Extract was boiled with 2 ml of 1% hydrochloric acid. No red precipitate was formed indicating the absence of phlobatannin.

13. Test for diterpenes

Copper acetate test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicated the presence of diterpenes.



Determination of Total Phenolic Content

The amount of phenol content in aqueous and methanol extracts of three different parts of *M. laxiflora* i.e. leaf, fruit pulp and seed were determined with Folin-Ciocalteu reagent.^{12,13} 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na₂CO₃ (2% w/v) were added to 0.5 ml of the sample (3 replicates) of each parts of the plant extract solution (1mg/ml). The resulting mixture was incubated at 45°C for 15 min. The absorbance of each sample was measured at 760 nm using UV Visible Spectrophotometer (UV-2700). Gallic acid (50-300 µg/ml) was used as a standard compound. The gallic acid standard calibration curve was established by plotting concentration (µg/ml) versus absorbance (nm) ($y = 0.003x + 0.073$; $R^2 = 0.998$), where y is absorbance at 760 nm and x is concentration (**Figure-1**). Total phenolic content in the plant extract was expressed as gallic acid equivalent (mg of gallic acid equivalent/g of sample) and was calculated by the formula¹⁴:

$$T = (C \times V) / M$$

Where, T = total content of phenolic compounds, mg/g plant extract, in GAE; C = concentration of gallic acid established from the calibration curve, µg/ml; V = volume of extract, ml; M = weight of water extract of the plant, g.

Estimation of total flavonoid content Aluminium Chloride Colorimetric Method

The basic principle of Aluminium chloride colorimetric method is that Aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids. Quercetin is reported to be suitable for building the calibration curve. Therefore standard Quercetin solutions of various concentrations were used to build up the calibration curve. In this method, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λ_{max} of quercetin) with a spectrophotometer (UV-2700). Aluminium chloride, 1% and potassium acetate, 1M solutions were prepared.

Stock Solution of Extracts: 100 mg of each extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

Preparation of Test Solutions: 0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank of all extracts were prepared and their absorbance was measured at 415 nm. All prepared solutions were filtered through Whatmann filter paper before measuring.^{15,16}

Determination of free radical scavenging assay

The free radical scavenging capacity of the aqueous and methanol extracts of various parts of *M. laxiflora* was determined using DPPH assay.¹⁷ DPPH solution (0.004% w/v) was prepared in methanol. Stock solution (1mg/ml) of each extracts and standard ascorbic acid (0.5mg/ml) were prepared using methanol. Various concentrations (30-150µg/ml) of the extracts and ascorbic acid were taken in test tubes and 1ml of freshly prepared DPPH solution were added, the test tubes were protected from light by covering with aluminum foil. The final volume in each test tube was made to 2ml with methanol and incubated in dark for 30 minutes at room temperature. After incubation, the absorbance was read at 517nm using a spectrophotometer (UV-2700). Control sample was prepared containing the same volume of methanol and DPPH without any extract and reference ascorbic acid. Methanol was served as blank.

% scavenging activity of the DPPH free radical was calculated by using the following equation:

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

Estimation of reducing power

Various concentrations of the aqueous and methanol extracts of various parts of *M. laxiflora* (1mg/ml) in corresponding solvents were mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml).

This mixture was kept at 50°C in water bath for 20 minutes. After cooling, 2.5ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10min whenever necessary.

The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and freshly prepared 1% ferric chloride solution (0.5ml). The absorbance was measured at 700 nm.

Control was prepared in similar manner excluding samples. Ascorbic acid (0.5mg/ml) at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.¹⁸

Determination of total antioxidant activity

The phosphomolybdenum method was used to evaluate the total antioxidant activity of the extracts.¹⁹

Antioxidants can reduce Mo (VI) to Mo (V) and the green phosphate / Mo (V) compounds at acidic pH, which have an absorption peak at 695 nm, were generated subsequently. 0.3 ml of the sample (1mg/ml) as well as ascorbic acid (0.5mg/ml) was mixed with 3.0ml of the reagent solution separately.

Reaction mixture was incubated at 95°C for 90min under water bath. Absorbance of all the mixtures was measured at 695 nm after cooling.

Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in microgram per milliliter of extract.

Total antioxidant activity was calculated by using the formula.

Total antioxidant = O.D. of test x concentration of standard in μg x made up volume of sample

RESULTS AND DISCUSSION

Phytochemical screening of aqueous and methanol extracts of *M. laxiflora* leaf, fruit pulp and seed reveals the presence of phytoconstituents as listed in the following table.

Table 1: Phytochemicals present in various parts of *M. laxiflora*

Phytochemicals	Test performed	<i>Meyna laxiflora</i>					
		Leaf		Fruit pulp		Seed	
		Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Alkaloids	Hager's test	-	+	-	-	+	+
Carbohydrates	a) Benedict's test	+	+	+	+	+	+
	b) Fehling's test	+	+	+	+	+	+
Amino acid	Ninhydrin test	+	+	-	-	-	-
Proteins	a) Xanthoproteic test	+	+	+	+	+	+
	b) Biuret test	+	+	-	-	+	-
Flavonoids	Alkaline reagent test	+	+	-	+	+	+
Phenolic compounds	a) Lead acetate test	+	+	+	+	+	+
Tannins	a) Lead acetate test	+	+	+	+	+	+
	b) Ferric chloride test	+	+	-	-	-	-
Steroids & terpenoids	Salkowski's test	+	+	-	+	+	+
Saponins	Froth test	+	+	+	+	+	+
Cardiac glycosides	Keller Killiani test	+	+	+	+	+	+
Oil & fats		-	+	-	+	-	+
Phlobatanin		-	-	-	-	-	-
Diterpene	Copper acetate	-	-	-	+	-	-

Key: + = presence and - = absence

Table 2: Total phenolic and total flavonoid content in various parts of *M. laxiflora*

Sample	Total phenolic content in mg /g of extract (in GAE)		Total flavonoid content in $\mu\text{g}/100\text{g}$ of dried extract (in QE)	
	Aqueous	Methanol	Aqueous	Methanol
Leaf	100.50 \pm 0.19	111.86 \pm 0.22	21.26 \pm 0.07	80.45 \pm 0.16
Fruit pulp	94.18 \pm 0.14	129.54 \pm 0.25	19.51 \pm 0.06	51.70 \pm 0.15
Seed	91.66 \pm 0.09	50.3 \pm 0.17	10.01 \pm 0.03	35.21 \pm 0.12

Assays were performed in triplicates. Values are expressed as means \pm SD.

Almost all the phytochemicals were present in aqueous and methanol extracts of *M. laxiflora* leaf, fruit pulp and seed. Alkaloid is present in leaf methanol extract and aqueous and methanol seed extracts while it is absent in fruit pulp extract. Flavonoid, steroid and terpenoid were found to be absent only in fruit pulp aqueous extract. Oil and fats is present in methanol extract of the three parts used in the study. Methanol is found to be a better solvent for the extraction of phytochemicals from various parts of *M. laxiflora* when compared to aqueous. Rathod

and Valvi worked on antinutritional factors of some wild edible fruits and found the presence of saponin and tannin in *M. laxiflora*.²⁰

Standard curve of gallic acid and quercetin is shown in the figures 1 and 2. Total phenolic content and total flavonoid content in aqueous and methanol extracts of various parts of *M. laxiflora* were obtained from the respective standard curve. Table 2 indicates the total phenolic and total flavonoid content in various parts of *M. laxiflora*.

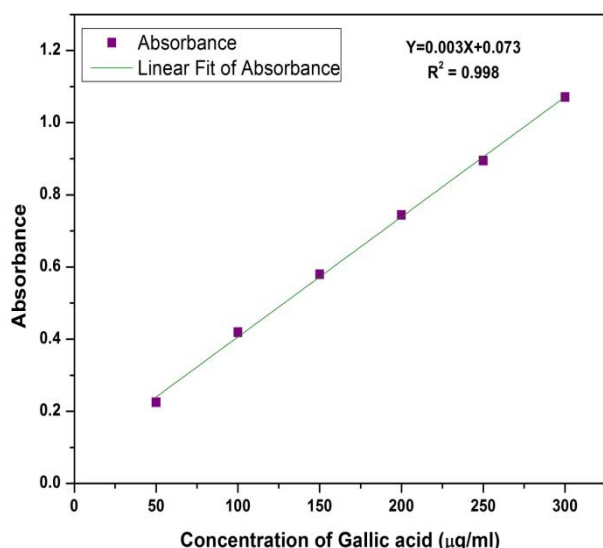


Figure 1: Standard curve of Gallic acid

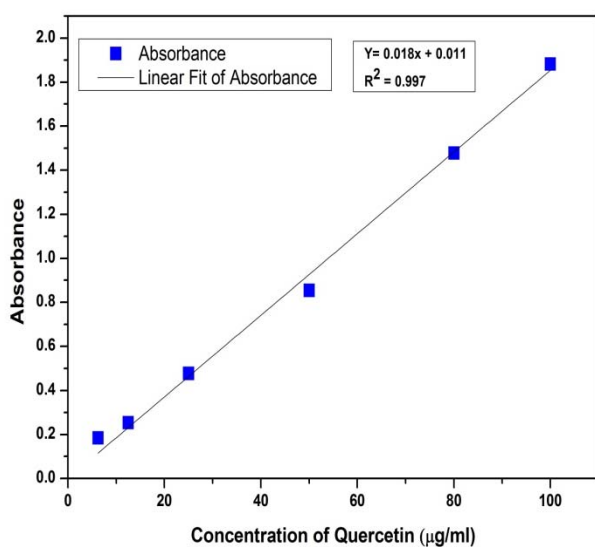


Figure 2: Standard curve of Quercetin

The total phenolic content in aqueous and methanolic leaf extracts in terms of gallic acid equivalent is 100.50 and 111.86 mg/g of extract powder respectively. Total phenolic content in aqueous and methanolic fruit pulp extracts in terms of gallic acid equivalent were 94.18 and 129.54 mg /g of extract powder whereas in aqueous and methanolic seed extracts in terms of gallic acid equivalent were 91.66 and 50.3 mg/g of extract powder respectively. Result indicates that methanol extract of leaf and fruit pulp of *M. laxiflora* showed higher total phenolic content than aqueous extract while for seed aqueous extract was found to have higher total phenolic content than methanol extract.

The phenolic compounds act as free radical terminators and the mechanism of action are through scavenging or chelating process.²¹ Phenolic compounds are having wide bioactivity including antioxidant properties/activity. The antioxidant activity of phenolic compound is due to hydroxyl functional group, however other factors eg.,

presence of electron withdrawing or releasing group in the aromatic ring having hydroxyl moiety will increase or decrease the activity. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties.²²

Total flavonoid content was found to be higher in methanol extract than aqueous extract for all the parts of *M. laxiflora* used in the study. Total flavonoid content was expressed in µg in terms of quercetin equivalent per 100g of the dried extract. Methanolic leaf extract showed the highest total flavonoid content of 80.45 µg/100g of dried extract followed by fruit pulp (51.70 µg/100g of dried extract) and least was noted in seed extract (35.21 µg/100g of dried extract).

Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2.²³ Flavonoids serve as health promoting compound as a results of its presence as anion radicals.²⁴ The compounds such as flavonoids, which hold hydroxyl groups, are responsible for the radical scavenging activity in the plants.^{25,26} It has been acknowledged that flavonoids show significant antioxidant action on human health and fitness. It is known that flavonoids act through scavenging or chelating process.²⁷

DPPH is a relatively stable free radical and the assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. DPPH method allows estimation of hydrogen radical donating ability of the extract.²⁸ DPPH scavenging activities of various parts of *M. laxiflora* were determined taking ascorbic acid as standard and are illustrated in figure 3.

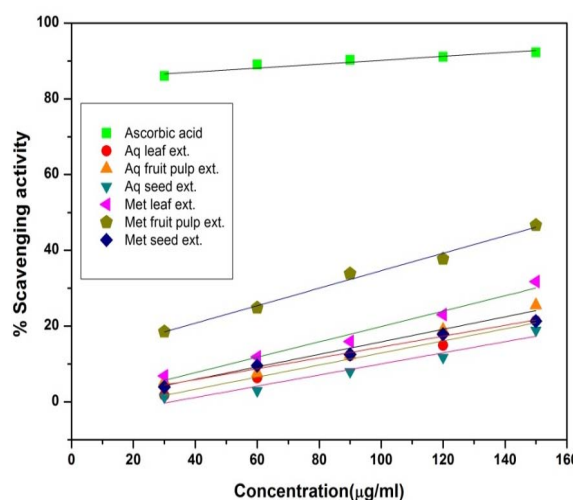


Figure 3: DPPH scavenging activity of standard and various extracts of *M. laxiflora*

DPPH scavenging activity showed a dose dependent manner with increase in concentration scavenging activity increases for the standard ascorbic acid and extracts of various parts of *M. laxiflora*. Methanol extract have higher scavenging activity than aqueous extract. DPPH

scavenging activity of aqueous and methanol extract of leaf, fruit pulp and seed was in the following order:

Fruit pulp > leaf > seed

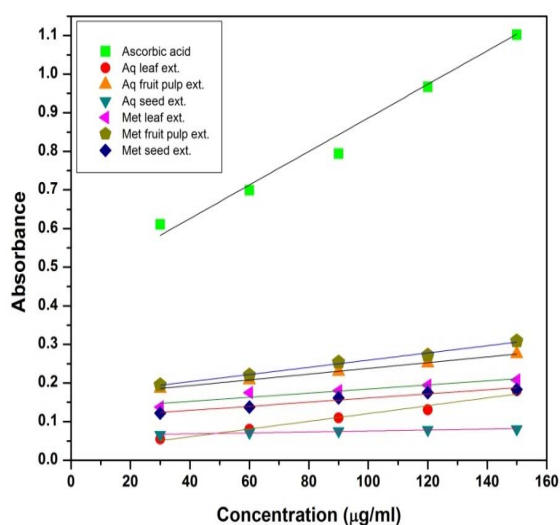


Figure 4: Reducing power of standard and various extracts of *M. laxiflora*

DPPH scavenging activity at 150 µg/ml concentration for methanolic leaf, fruit pulp and seed extracts were 31.7%, 46.6% and 21.3% respectively. Higher DPPH scavenging activity of fruit pulp extract may be due to higher total phenolic content in the extract.

DPPH assay allows determining the intrinsic ability of the antioxidant compound to donate hydrogen atoms or electrons to this reactive species in a homogenous system. The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for

the visible deep colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolourized which can be quantitatively measured from the changes in absorbance. Higher reduction of DPPH is related to the high scavenging activity performed by particular sample.²⁹

Reducing power assay is another way to analyze the antioxidant activity of the extract. In reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe³⁺ to Fe²⁺ by donating an electron.³⁰ The amount of Fe²⁺ complex can then be monitored by measuring the formation of Perl's blue at 700 nm. Reducing power of all the three parts of *M. laxiflora* were estimated using ascorbic acid as standard and is shown in figure 4.

From the above graph, reducing power increases with increased in concentration for the standard ascorbic acid and various extracts of *M. laxiflora*. Increasing absorbance indicates an increase in reductive ability. Methanolic extracts showed higher reducing power than aqueous extracts. Among the various parts of *M. laxiflora* used for the study, fruit pulp was showing highest reducing power followed by leaf and least was noted in seed. As reducing power assay measures the electron donating capacity of an antioxidant, it is associated with the presence of reductones. Reductones exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom and also reported to react with certain precursor of peroxide thereby preventing peroxide formation.^{31,32,33} As phytochemicals were extracted best in methanol than aqueous, this may be responsible for higher antioxidant activity of methanolic extracts when compared to aqueous extracts.

Table 3: Total antioxidant activity of various parts of *M. laxiflora*

Concentration (µg/ml)	Total antioxidant activity in µg/ml of extract (in AAE)					
	Leaf		Fruit pulp		Seed	
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
30	17.1 ± 0.01	19.2 ± 0.04	16.5 ± 0.03	17.1 ± 0.03	12.9 ± 0.05	20.1 ± 0.05
60	22.5 ± 0.04	31.35 ± 0.05	20.4 ± 0.02	39.3 ± 0.03	15.6 ± 0.04	21.9 ± 0.01
90	27.6 ± 0.06	33.9 ± 0.09	25.5 ± 0.03	57.03 ± 0.1	21.7 ± 0.01	29.4 ± 0.06
120	30.75 ± 0.01	38.1 ± 0.1	28.5 ± 0.07	73.8 ± 0.1	24.3 ± 0.06	38.1 ± 0.04
150	34.95 ± 0.02	60.75 ± 0.05	37 ± 0.1	77.4 ± 0.05	35.4 ± 0.1	44.1 ± 0.09

Assays were performed in triplicates. Values are expressed as means ± SD.

The total antioxidant activity assay also indicates a dose dependent manner with methanol extract having higher activity than aqueous extract. Total antioxidant activity is expressed in terms of ascorbic acid equivalent (AAE) in µg per ml of extract. The total antioxidant activity of aqueous and methanolic extracts of leaf at highest concentration used for the experiment was 34.95 and 60.75 µg/ml of extract while that of fruit pulp was 37 and 77.4 µg/ml of extract and for seed it was found to be 35.4 and 44.1 µg/ml of extract respectively.

Total antioxidant activity of both aqueous and methanol extracts of various parts of *M. laxiflora* showed the following trend:

Fruit pulp > leaf > Seed

CONCLUSION

The present study indicates that various parts of *M. laxiflora* possess antioxidant activity. Methanol was found to be a better solvent for extracting phytochemicals from *M. laxiflora*. Total phenolic content was highest in

methanolic fruit pulp extract while methanolic leaf extract showed the highest flavonoid content. Among the various parts used for the study fruit pulp was found to have highest antioxidant activity which may be attributed to higher total phenolic content in the extract. Further work can be carried out to identify the bioactive components responsible for higher antioxidant activity in fruit pulp.

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