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Research Article

Comparative *In-vitro* antioxidant activity on *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* leaf extracts and their Phenolic contents

M. N. Palaksha[*], K. Ravishankar[1] & V. Girija Sastry[2]
Research scholar, Department of pharmacy, JNTUK; Kakinada- 533431
[1]Aditya College of Pharmacy, Surampalem- 533437.
[2]A.U. College of Pharmaceutical Sciences, Visakhapatnam-530017.

***Corresponding Author:**
Mr. M. N. Palaksha
Email: palaksha.mn@gmail.com

Abstract: The present investigation highlights the free radical scavenging activity of *Melochia corchorifolia*, *Sida acuta* and *Saccharum officinarum* leaf extracts and their Phenolic contents by determining DPPH, Nitric oxide, Hydroxyl and Hydrogen peroxide scavenging activity. The shade dried leaves were coarsely powdered and subjected to fractional extraction by using chloroform and ethanol. The extracts were subjected to in-vitro antioxidant activity by various antioxidant assays. The antioxidant activities were compared with that of standard Gallic acid from the results obtained, it was suggested that the Phenolic contents of these leaves exhibit strong free radical scavenging activity in all the tested methods and shows maximum scavenging of DPPH, Nitric oxide, hydroxyl and hydrogen peroxide at 100µg/ml concentration compared to chloroform and ethanol extract of leaves. The phytochemical screening of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* leaf extracts constitutes flavonoids and tannins and the antioxidant activities may be due to the presence of these phenolic compounds.
Keywords: *Melochia corchorifolia*, Reactive oxygen species, anti-oxidant, DPPH, Hydroxyl radical, nitric oxide and hydrogen peroxide

INTRODUCTION:

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. Various metabolic processes, UV radiations, smoke etc, trigger the production of free radicals. Reactive oxygen species (ROS) includes superoxide anions (02,$^{-}$), hydroxyl radical (OH), singlet oxygen hydrogen peroxide (H_2O_2), ferric ion, nitric oxide etc. excessive production of free radicals leads to oxidative stress, the disease associated with the ROS mainly depend on the balance of the pro-oxidant and the antioxidant concentration in the body. Pro-oxidant condition dominates either due to the increased generation of free radicals or due to the excessive oxidative stress of the depletion of the dietary antioxidant. Plants and other organisms have evolved a wide range of mechanisms to contend with this problem, with a variety of antioxidant molecules and enzyme. Recent evidences suggested that involvement of oxidative stress in the pathogenesis of various diseases and had attracted much attention of scientists and general public on the role of antioxidants in the maintenance of human health and prevention and treatment of diseases [1]. Free radical reactions have been implicated in the pathology of many human diseases like atherosclerosis, ischemic heart disease, diabetes and neurodegenerative disease etc., and disease conditions like ageing process, inflammation, immunosuppresion, etc. A number of plants and plant isolates have been reported to protect free radical induced damage in various experimental models [2].

Melochia corchorifolia L., Malvaceae, is a wild crop and grows in most parts of India as a weed. Some species of the genus Melochia have been used in folk medicine, such as dysentery, abdominal swellings and water-snake bites, bronchitis and cough [3].

Sida acuta (malvaceae), is an erect perennial shrub found throughout the hotter parts of Nepal and India. It is used for various medicinal purpose such as liver disorders, asthma, fever, headache (migrane), cough, cold, ulcer, anthelmintic, snake bite, urinary diseases, female disorders, antifertility agent and sedative, diuretic & abortifacient in ayurvedic preparations[4].

Saccharum officinarum is popularly known as noble cane due to its high sucrose content and low fiber content is one of the most important industrial crops of the world. The sugar cane juice contains flavonoids. The roots and stems of sugar cane are used in medicine to treat skin and urinary tract infections as well as for bronchitis, heart conditions, loss of milk production,

cough anemia, constipation as well as general debility. It is also used to treat jaundice and lowering blood pressure [5].

The aim of this research is to determine DPPH, nitric oxide, hydroxyl and hydrogen peroxide scavenging activity of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* leaf extracts and their Phenolic contents.

MATERIALS AND METHOD

Chemicals used

Petroleum ether, Chloroform, Ethanol, DPPH radical, Griess reagent(1%sulphanilamid, 2% H_3PO_4 and 0.1% Naphthyl ethylene diamine) Sodium nitro prusside, 95% methanol, ferrous sulphate, salicylic acid, hydrogen peroxide, Gallic acid as gifted sample used as reference standard , all other chemicals and solvents used were of analytical grade.

Instruments used

UV/Visible double beam spectrophotometer, Heating mantle, Centrifuge machine, Digital balance, pH meter, Electronic balance, Micropipettes, Pipettes etc.,

Preparation of extract

The leaves of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* were collected from the surroundings of Surampalem, East Godavari dist, Andhrapradesh. The plants were identified and authenticated by the taxonomist Dr.T.V.Raghavarao, Maharani College, Peddapuram. The plants were dried under shade, coarsely powdered and 100gm of powder was extracted exhaustively in a soxhlet apparatus with chloroform and ethyl alcohol separately for 72 hour at 50^0c. The solvent was completely evaporated and obtained gummy exudates. These crude extracts were stored at low temperature at refrigerator and used for evaluation of antioxidant activity.

Extraction of Phenolic content from the extract

Weigh 1gm of crude extracts of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* was defatted with n-hexane then extracted with 90% ethyl alcohol for 1hr. Then filter the extract and evaporate the solvent then dissolve it in 30% acetone and add solid sodium chloride and add phosphoric acid to obtain a pH of 5.5 filters the solvent and dry the precipitate. This is used for various antioxidant assays.

In-vitro antioxidant activity

DPPH scavenging activity

The free radical scavenging capacity of the extracts was determined by using DPPH method [6]. A DPPH solution 0.1mM was prepared in 95% methanol and 1ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (10,25,50,100µg/ml) of petroleum ether chloroform and ethyl alcohol, 30min later the absorbance was measured at 517nm. A blank was prepared without adding extract. Gallic acid at 1, 2.5, 5, 10µg/ml concentration was used as standard. The experiment was repeated triplicate and the scavenging activity was calculated by

$$\%Scavenging\ activity = \frac{\mathrm{Ao} - \mathrm{A1}}{\mathrm{Ao}} \times 100$$

Where Ao = Absorbance of control
A1= Absorbance of sample extract

Nitric oxide radical activity:

Nitric oxide radical activity was determined according to the method [7]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrate ions which can be determined by the use of greiss reagent. Sodium nitro prusside 5mM in phosphate buffer saline was mixed with different concentrations of the extracts (10, 25, 50, 100µg/ml) dissolved in the suitable solvent systems and incubated at 25^0c for 150 min. The samples above was reacted with griess reagent (1%sulphanilamid, 2% H_3PO_4 and 0.1% naphthyl ethylene diamine) was read at 546nm. Gallic acid at (1, 2.5, 5, 10µg/ml) concentration was used as standard. The experiment was repeated triplicate and the % decrease in absorbance was calculated.

$$\%Scavenging\ activity = \frac{\mathrm{Ao} - \mathrm{A1}}{\mathrm{Ao}} \times 100$$

Where Ao = Absorbance of control
A1= Absorbance of sample extract

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by the salicylic acid method with some modifications [8]. Briefly the plant extracts were dissolved in distilled water at (10, 25, 50,100) µg/ml. A 1ml extract was mixed with 1ml of 9mmol/l salicylic acid, 1ml of 9mmol /l ferrous sulphate and 1ml of 9mmol /l hydrogen peroxide. The reaction mixture was incubated for 60 min at37^0 c in a water bath after incubation the absorbance of the mixtures was measured at 510nm using a UV/Vis spectrophotometer. The %hydroxyl radical scavenging activity of test sample was determined accordingly in comparison with negative control. Negative control was without antioxidant or extract. Gallic acid was taken as the positive control.

Hyhdrogen peroxide scavenging activity

The ability of the *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.* [9]. A solution of hydrogen peroxide 40mM was prepared in phosphate buffer pH 7.4. Extracts of petroleum ether, chloroform and ethnol of 10, 25, 50, 100µg/ml in distilled water were added to

hydrogen peroxide solution 0.6ml (40mM). Absorbance of hydrogen peroxide at 230nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide

RESULTS

A. *In-vitro* antioxidant activity of leaf extracts

- **DPPH radical scavenging assay:** The scavenging ability of chloroform and ethanol extracts of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* on DPPH assay was shown in table 1. The percentages of inhibitions were increased with increasing concentrations of the extracts. (Given in Table No.1).
- **Nitric oxide radical activity:** The percentages of inhibitions were increased with increasing concentrations of the extracts. (Given in Table No.2).
- **Hydroxyl radical scavenging activity:** The scavenging ability of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* extracts on hydroxyl radical is given in table no.3. The percentage inhibitions were increased with increasing concentrations of the extracts. (Given in Table No.3).
- **Hyhdrogen peroxide scavenging assay:** The scavenging ability of chloroform and ethanol extracts of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* on Hydrogen peroxide scavenging assay is shown in table no.4. The percentages of inhibitions were increased with increasing concentrations of the extracts. (Given in Table No.4).

Table-1: DPPH scavenging activity of Leaf extracts:

Tested material	Concentration (µg/ml)	%inhibition of *M. corchorifolia*	%inhibition *Sida acuta*	%inhibition *S.officinarum*
Chloroform extract	10	67.54±0.923	68.18±1.01	61.52±1.01
	25	74.19±0.472	78.14±0.98	65.58±0.98
	50	79.53±1.26	83.15±0.99	72.84±0.99
	100	83.44±0.866	86.82±0.98	79.64±0.98
Alcohol extract	10	68.24±1.01	56.14±0.923	55.94±0.923
	25	71.68±0.98	61.13±0.472	61.30±0.472
	50	75.74±0.99	64.52±1.26	69.71±1.26
	100	79.33±0.98	68.96±0.866	76.53±0.866
Gallic acid (Std)	1	67.82±1.00	67.82±1.00	67.82±1.00
	2.5	70.3±9.37	70.3±9.37	70.3±9.37
	5	77.3±1.56	77.3±1.56	77.3±1.56
	10	83.32±0.99	83.32±0.99	83.32±0.99

Values are mean ±SEM n=3

Table-2: Nitric oxide radical activity of leaf extracts

Tested material	Concentration (µg/ml)	%inhibition of *M. corchorifolia*	%inhibition *Sida acuta*	%inhibition *S.officinarum*
Chloroform extract	10	39.5±1.37	18.62±1.78	30±1.78
	25	44.72±2.1	26.13±2.32	40.3±2.32
	50	44.98±2.84	40.06±0.29	43.8±0.29
	100	53.18±5.22	42.18±2.34	51.03±2.34
Alcohol extract	10	32.79±2.66	16.12±1.37	28.12±1.37
	25	60.38±1.23	18.92±2.1	31.58±2.1
	50	66.72±2.77	28.10±2.84	42.01±2.84
	100	71.9±1.99	36.19±5.22	44.88±5.22
Gallic acid (Std)	1	6.32±2.32	6.32±2.32	6.32±2.32
	2.5	18.57±2.77	18.57±2.77	18.57±2.77
	5	43.29±2.0	43.29±2.0	43.29±2.0
	10	45.62±2.51	45.62±2.51	45.62±2.51

Values are mean ±SEM n=3

Table-3: Hydroxyl radical scavenging activity of Leaf extracts

Tested material	Concentration (µg/ml)	%inhibition of *M. corchorifolia*	%inhibition *Sida acuta*	%inhibition *S.officinarum*
Chloroform extract	10	9.5±0.129	11.15±0.169	12.59±0.169
	25	29.325±0.111	17.12±0.496	36.87±0.496
	50	35.075±0.801	28.63±0.427	46.35±0.427
	100	64.650±1.118	35.12±0.427	52.55±0.427
Alcohol extract	10	12.78±0.147	10.98±0.129	9.5±0.129
	25	18.4±0.346	16.15±0.111	23.25±0.111
	50	40.1±0.802	21.92±0.801	35.17±0.801
	100	65.067±0.636	33.12±1.118	43.28±1.118
Gallic acid (Std)	1	13.575±0.415	13.57±0.415	13.57±0.415
	2.5	20.325±0.309	20.32±0.309	20.32±0.309
	5	31.925±0.531	31.92±0.531	31.92±0.531
	10	40.737±0.511	40.73±0.511	40.73±0.511

Values are mean ±SEM n=3

Table-4: Hydrogen peroxide scavenging activity of Leaf extracts

Tested material	Concentration (µg/ml)	%inhibition of *M. corchorifolia*	%inhibition *Sida acuta*	%inhibition *S.officinarum*
Chloroform extract	10	12.78±0.147	5.05±0.116	2.08±0.116
	25	18.4±0.346	7.12±0.441	9.48±0.441
	50	40.1±0.802	12.14±3.316	16.44±3.316
	100	65.067±0.636	21.90±1.756	34.08±1.756
Alcohol extract	10	12.33±0.176	3.12±0.147	1.08±0.147
	25	31.51±0.38	5.32±0.346	8.24±0.346
	50	40.167±1.014	9.48±0.802	12.44±0.802
	100	56.8±0.115	17.64±0.636	30.79±0.636
Gallic acid (Std)	1	12.733±0.176	12.733±0.176	12.733±0.176
	2.5	26.780±0.874	26.780±0.874	26.780±0.874
	5	57.750±0.161	57.750±0.161	57.750±0.161
	10	79.417±1.536	79.417±1.536	79.417±1.536

Values are mean ±SEM n=3

***In-vitro* antioxidant activity of Phenolic content of leaf extracts**

DPPH radical scavenging assay

The scavenging ability of Phenolic content of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* was subjected to DPPH ra*dical scavenging* assay and the results were shown in Table 5. The percentage inhibition was increased with increasing concentrations of the extract. Phenolic content of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* shows maximum inhibition 62.1±0.17, 88.02±0.16 and 84.8±0.72 respectively at 100µg/ml, and Gallic acid (STD) shows maximum inhibition 83.32±0.99 at 10µg/ml (Given in Table No.5)

Nitric oxide radical activity

The Phenolic content of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* subjected to nitric oxide scavenging assay and the results showed that the inhibitions were increased with increasing concentrations of the extracts. Phenolic content of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* shows maximum inhibition 54.12±0.81, 46.91±0.92 and 64.10±0.24 respectively at 100µg/ml and standard Gallic acid shows maximum inhibition 45.62±2.51 at 10µg/ml (Table No. 6).

Hydroxyl radical scavenging activity

The Phenolic content of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* was subjected to Hydroxyl radical scavenging activity. The results showed the percentage inhibitions were increased with increasing concentrations. Phenolic content of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* shows maximum inhibition 44.16±0.82, 39.16±0.51and 56.13±0.5 respectively at 100µg/ml and Gallic acid shows maximum inhibition 40.73±0.511 at 10µg/ml. (Given in Table No. 7)

Hydrogen peroxide scavenging assay

The Phenolic content of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* was subjected to Hydrogen peroxide scavenging assay. The results showed the percentages of inhibitions were increased with increasing concentrations of the extract. The Phenolic content of *M. corchorifolia* shows maximum inhibition 71.7±0.42at 100µg/ml and Gallic acid (Std) shows maximum inhibition 79.417±1.536 at10µg/ml (Table No. 8.).

Table-5: DPPH scavenging activity on *Phenolic content of* Leaf extracts

SI. No.	Sample	Concentration (µg/ml)	%inhibition of *M. corchorifolia*	%inhibition of *Sida acuta*	%inhibition of *S.officinarum*
1.	Phenolic content	10 25 50 100	50±0.81 57±1.15 60.5±0.25 62.1±0.17	77.7±1.22 83.9±0.82 85.7±0.17 88.02±0.16	71.3±0.82 75.3±1.32 78.7±0.56 84.8±0.72
2.	Gallic acid (Std)	1 2.5 5 10	67.82±1.00 70.3±9.37 77.3±1.56 83.32±0.99	67.82±1.00 70.3±9.37 77.3±1.56 83.32±0.99	67.82±1.00 70.3±9.37 77.3±1.56 83.32±0.99

Values are mean ±SEM n=3

Table-6: Nitric oxide radical activity on Phenolic content of Leaf extracts

S. No.	Tested material	Concentration (µg/ml)	%inhibition of *M. corchorifolia*	%inhibition of *Sida acuta*	%inhibition of *S.officinarum*
1.	Phenolic content	10 25 50 100	9.05±1.26 20.17±0.82 43.15±0.82 54.12±0.81	18.13±0.12 26.11±0.5 42.16±0.86 46.91±0.92	26.2±0.62 34.15±0.52 48.92±0.42 64.10±0.24
2.	Gallic acid (Std)	1 2.5 5 10	6.32±2.32 18.57±2.77 43.29±2.0 45.62±2.51	6.32±2.32 18.57±2.77 43.29±2.0 45.62±2.51	6.32±2.32 18.57±2.77 43.29±2.0 45.62±2.51

Values are mean ±SEM n=3

Table-7: Hydroxyl radical scavenging activity on Phenolic content of Leaf extracts

SI. No.	Tested material	Concentration (µg/ml)	%inhibition of *M. corchorifolia*	%inhibition of *Sida acuta*	%inhibition of *S.officinarum*
1.	Phenolic content	10 25 50 100	14.5±1.2 18.6±0.82 29.14±1.3 44.16±0.82	15.12±1.32 19.10±1.42 25.0±0.36 39.16±0.51	28.32±0.13 35.12±0.87 48.11±1.32 56.13±0.5
2.	Gallic acid (Std)	1 2.5 5 10	13.57±0.415 20.32±0.309 31.92±0.531 40.73±0.511	13.57±0.41 20.32±0.30 31.92±0.51 40.73±0.51	13.57±0.415 20.32±0.309 31.92±0.531 40.73±0.511

Values are mean ±SEM n=3

Table-8: Hydrogen peroxide scavenging activity on Phenolic content of Leaf extracts

SI. No.	Tested material	Concentration (µg/ml)	%inhibition of *M. corchorifolia*	%inhibition of *Sida acuta*	%inhibition of *S.officinarum*
1	Phenolic content	10 25 50 100	23.5±1.6 49.7±0.56 62.4±1.5 71.7±0.42	16.6±0.87 32.2±0.16 47.7±1.33 56.8±0.74	21.0±1.2 38.0±.82 51.1±0.72 64.6±0.65
2	Gallic acid (Std)	1 2.5 5 10	12.7±0.17 26.7±0.87 57.7±0.16 79.4±1.51	12.73±0.17 26.78±0.87 57.75±0.16 79.41±1.53	12.73±0.17 26.78±0.87 57.75±0.16 79.41±1.53

Values are mean ±SEM n=3

RESULTS AND DISCUSSION

This study highlights the free scavenging activity of *Melochia corchorifolia*, *Sida acuta* and *Saccharum officinarum* leaf extracts as well as its Phenolic content by using DPPH scavenging activity, nitric oxide free scavenging activity, hydroxyl scavenging activity and hydrogen peroxide scavenging activity. The Gallic acid was used as standard

The DPPH assay method is used to evaluate scavenging activity of plant extracts, food materials or chemicals, due to its simplicity and rapid results [10]. The extracts contain antioxidants which react with free radicals and neutralize the free radicals [11, 12] and control free radical damage in the body. The DPPH scavenging activity of leaf extracts shows similar results with that of standard Gallic acid.

Nitric oxide is another free radical generated in the body, at physiological pH and was found to be inhibited by these extracts and Gallic acid. The generated nitric oxide [13] was determined by use of Greiss reagent. These extracts are scavengers of Nitric oxide which fight against oxygen radical there by reduced production of nitric oxide. Leaf extracts of *Melochia corchorifolia, Saccharum officinarum* and *Sida acuta* had comparably more nitric oxide scavenging activity than Gallic acid.

In biological systems OH radical are the most powerful radicals evolved from hydrogen peroxide and superoxide anions in metal ions presence. Hydroxyl radical can damage any cells in the body and responsible for many pathological conditions. This can even cause conjugate to nucleotides in DNA, lipids and proteins and can cause mutagenesis, cancer and cytotoxicity [14, 15]. The leaf extracts of *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* had strong hydroxyl scavenging activity.

The ability to penetrate biological membranes H_2O_2 is one of the important radical. Because H_2O_2 itself is not reactive but rarely it can cause harmful effects due to formation of hydroxyl radical in the body, to protect the living system the removal of H_2O_2 is very[16]. The leaf extracts of *Melochia corchorifolia, Saccharum officinarum* and *Sida acuta* had had strong hydrogen peroxide scavenging activity similar to Gallic acid.

Phytochemical screening of leaf extracts of *Melochia corchorifolia* [17], *Sida acuta* [4] and *Saccharum officinarum* [5] had reported the presence of glycosides, phytosterols, saponins, tannins, flavonoids, etc.,

From the results, it was suggested that the *Melochia corchorifolia, Sida acuta* and *Saccharum officinarum* leaf extracts shows good activity in all the tested methods at 100µg/ml concentration. However, Phenolic contents of *Melochia corchorifolia,* shows better antioxidant activity when compared to leaf extracts. *Melochia corchorifolia, Saccharum officinarum* and *Sida acuta* Phenolic content shows good scavenging of all the tested methods at 100µg/ml concentration, when compared to that of whole extracts.

CONCLUSION

It was already reported that flavonoids are natural products which have been shown to posses various biological properties related to antioxidant mechanism. The results of free radical scavenging activity showed that the *Melochia corchorifolia, Saccharum officinarum* and *Sida acuta* leaves has significant antioxidant activity, which can be attributed due to the presence of flavonoids and other phenolic compounds.

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