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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND IN-VITRO FREE RADICAL SCAVENGING ACTIVITY OF *MELOCHIA CORCHORIFOLIA* PLANT EXTRACTS

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ABSTRACT

The present investigation highlights the free radical scavenging activity of *Melochiacorchorifolia* plant extract by determining DPPH, Nitric oxide, Hydroxyl and Hydrogen peroxide scavenging activity. The shade dried plants were coarsely powdered and subjected to fractional extraction by using petroleum ether, chloroform and ethanol. The extracts were subjected to phytochemical screening and invitro 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 petroleum ether, chloroform and ethanol extract of plant parts 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. The phytochemical screening constitutes flavonoids and tannins and the antioxidant activities may be due to the presence of these phenolic compounds.

Keywords: *Melochiacorchorifolia*, Reactive oxygen species, anti-oxidant, DPPH, Hydroxyl radical.

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 oxideetc. 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]The aim of this research is to determine DPPH, nitric oxide, hydroxyl and hydrogen peroxide scavenging activity of *Melochia corchorifolia* plant extracts.

MATERIALS AND METHOD

Chemicals

Petroleum ether, Chloroform, Ethanol,DPPH, Griess reagent(1%sulphanilamid, 2% H_3PO_4 and 0.1% Naphthylethylene diamine) Sodium nitro prusside, 95% methanol, ferrous

sulphate, salicylic acid, hydrogen peroxide, Gallic acid is gifted sample used as reference standard, all other chemicals and solvents used were of analytical grade.

Preparation of extract

The whole plants of *Melochia corchorifolia* were collected from the surroundings of Surampalem, East Godavari dist, Andhra Pradesh. 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 petroleum ether, followed by 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 phytochemical analysis and evaluation of antioxidant activity.

Phytochemical screening

The freshly prepared extracts of *Melochia corchorifolia* were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts was performed using standard procedures[3,4].

Invitro antioxidant activity

1. DPPHscavenging activity

The free radical scavenging capacity of the extracts was determined by using DPPH method[5]. 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

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

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

2. Nitric oxide radical activity

Nitric oxide radical activity was determined according to the method[6] (Garret et al 1964). 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.

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

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

3. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by the salicylic acid method (Smirnoff and cumbes 1989) with some modifications[7]. 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 at 37^0c 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.

4.Hydrogen peroxide scavenging activity

The ability of the *Melochia corchorifolia* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al (1989)[8]. 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 AND DISCUSSION

Phytochemical screening

Preliminary phytochemical screening of the extract of *Melochia corchorifolia* revealed the

presence of various bioactive components of which flavonoids and tannins were the most prominent and the result of phytochemical test are summarized in (Given in Table No.1).

Invitro antioxidant activity

1. DPPH radical scavenging assay

The scavenging ability of petroleum ether, chloroform and ethanol extracts of *Melochia corchorifolia* DPPH assay is shown in table 2. The percentages of inhibitions were increased with increasing concentrations of the extracts. The IC_{50} value for DPPH scavenging activity of extract was found to be 1.09µg/ml while, The IC_{50} value for Gallic acid was 1.09 µg/ml respectively (Given in Fig No.1, Table No.2).

2. Nitric oxide radical activity

The percentages of inhibitions were increased with increasing concentrations of the extracts. The IC_{50} value for scavenging of nitric oxide for *Melochia corchorifolia* extract was 1.187µg/ml, while the IC_{50} value for Gallic acid was 1.719 µg/ml respectively (Given in Fig No.2, Table No.3).

3. Hydroxyl radical scavenging activity

The scavenging ability of *Melochia corchorifolia* extracts on hydroxyl radical is given in table no.4. The percentage inhibitions were increased with increasing concentrations of the extracts. The IC_{50} value for Hydroxyl radical scavenging activity of extract was found to be 1.99µg/ml while, The IC_{50} value for Gallic acid was 1.485 µg/ml respectively (Given in Fig No.3, Table No.4).

4. Hydrogen peroxide scavenging assay

The scavenging ability of petroleum ether, chloroform and ethanol extracts of *Melochia corchorifolia* Hydrogen peroxide scavenging assay is shown in table no.5. The percentages of inhibitions were increased with increasing concentrations of the extracts. The IC_{50} value for hydrogen peroxide scavenging activity of extract was found to be 1.99µg/ml while, The IC_{50} value for Gallic acid was 1.526 µg/ml respectively (Given in Fig No.4, Table No.5).

Table 1: The Results of Preliminary Phytochemical Screening

S.No	Phytoconstituents	Petroleum ether extract	Chloroform extract	Ethanol extract
1	Carbohydrates	-	+	+
2	Alkaloids	+	+	+
3	Phytosterols	-	+	+
4	Saponins	-	-	+
5	Fixed oils	+	+	+
6	Tannins	+	+	+
7	Flovonoids	+	+	+

+ = Positive, - = Negative

Table 2: DPPH scavenging activity on *Melochia corchorifolia* extracts

Tested material	Concentration (µg/ml)	%inhibition	IC_{50}(µg/ml)
Petroleum Ether Extract	10 25 50 100	63.71±0.905 70.86±0.970 75.41±0.866 84.97±0.584	1.08
Chloroform extract	10 25 50 100	67.54±0.923 74.19±0.472 79.53±1.26 83.44±0.866	1.08
Alcohol extract	10 25 50 100	68.24±1.01 71.68±0.98 75.74±0.99 79.33±0.98	1.09
Gallic acid (Std)	1 2.5 5 10	67.82±1.00 70.3±9.37 77.3±1.56 83.32±0.99	1.09

Values are mean ±SEM n=3

Table 3: Nitric oxide radical activity on *Melochia corchorifolia* extracts

Tested material	Concentration (µg/ml)	%inhibition	IC_{50}(µg/ml)
Petroleum Ether Extract	10 25 50 100	30±1.78 40.3±2.32 43.8±0.29 56.43±2.34	1.187
Chloroform extract	10 25 50 100	39.5±1.37 44.72±2.1 44.98±2.84 53.18±5.22	1.162
Alcohol extract	10 25 50 100	32.79±2.66 60.38±1.23 66.72±2.77 71.9±1.99	1.126
Gallic acid (Std)	1 2.5 5 10	6.32±2.32 18.57±2.77 43.29±2.0 45.62±2.51	1.719

Values are mean ±SEM n=3

Table 4: Hydroxyl radical scavenging activity on *Melochia corchorifolia* extracts

Tested material	Concentration (µg/ml)	%inhibition	IC_{50}(µg/ml)
Petroleum Ether Extract	10 25 50 100	12.595±0.169 36.875±0.496 48.125±0.427 62.125±0.427	1.30
Chloroform extract	10 25 50 100	9.5±0.129 29.325±0.111 35.075±0.801 64.650±1.118	1.69
Alcohol extract	10 25 50 100	12.78±0.147 18.4±0.346 40.1±0.802 65.067±0.636	1.99
Gallic acid (Std)	1 2.5 5 10	13.575±0.415 20.325±0.309 31.925±0.531 40.737±0.511	1.485

Values are mean ±SEM n=3

Table 5: Hydrogen peroxide scavenging activity on *Melochia corchorifolia* extracts

Tested material	Concentration (µg/ml)	%inhibition	IC_{50}(µg/ml)
Petroleum Ether Extract	10 25 50 100	16.987±0.116 27.333±0.441 46.317±3.316 75.113±1.756	1.454
Chloroform extract	10 25 50 100	12.78±0.147 18.4±0.346 40.1±0.802 65.067±0.636	1.99
Alcohol extract	10 25 50 100	12.33±0.176 31.51±0.38 40.167±1.014 56.8±0.115	1.375
Gallic acid (Std)	1 2.5 5 10	12.733±0.176 26.780±0.874 57.750±0.161 79.417±1.536	1.526

Values are mean ±SEM n=3

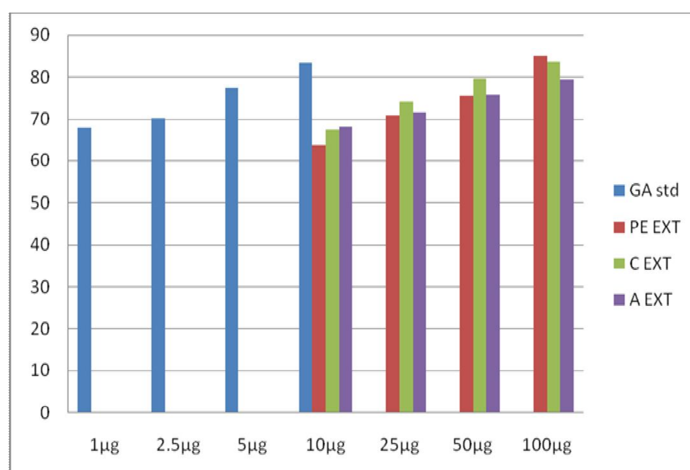

Fig. 1: % inhibition Vs concentration in µg/ml

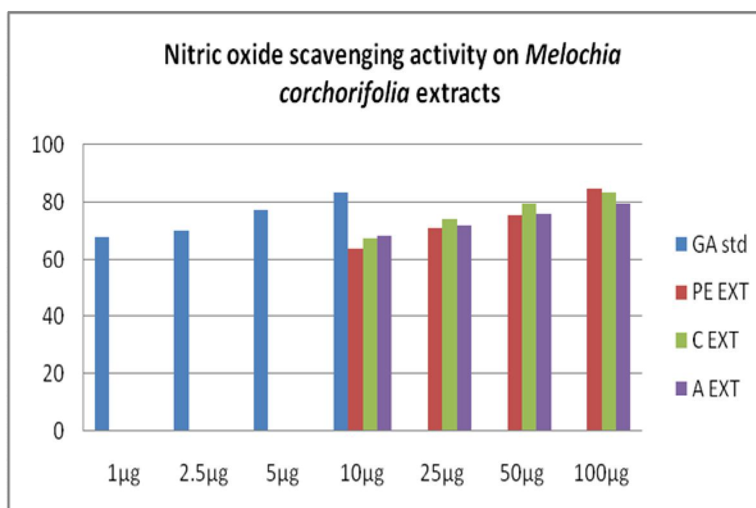

Fig. 2: % inhibition Vs concentration in µg/ml

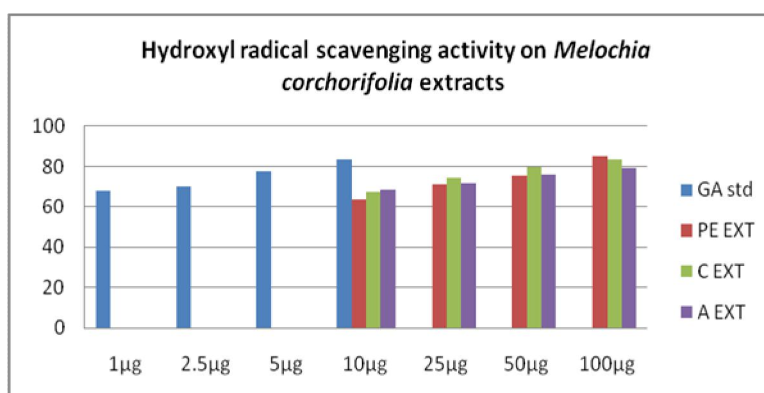

Fig. 3: % inhibition Vs concentration in µg/ml

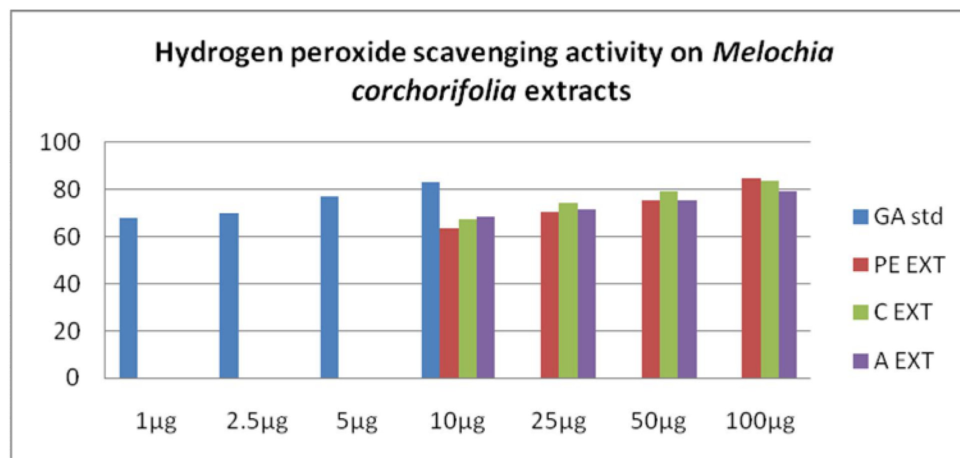

Fig. 4: % inhibition Vs concentration in µg/ml

CONCLUSION

The phytochemical tests results revealed that the plant is a good source of flavonoids and tannins. It is already reported that flavonoids are natural products which have been shown to posses various biological properties related to antioxidant mechanism. The result of free radical scavenging activity showed that the *Melochia corchorifolia* plant has significant antioxidant activity, which can be attributed due to the presence of flavonoids and other phenolic compounds.Further research work to be carried out to isolate bioactive molecules responsible for their activity and to investigate and screen the compounds to evaluate other biological activities.

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REFERENCES

1. Harsha Ramakrishna, Sushma S, Murthy, Divya R, Mamathrani DR and Pandurangamurthy G. “Hydroxyl radical and Dpph scavenging activity of crude protein extract of leucasliniflia: A folk medicinal plant. Asian journal of plant science and research. 2012:2(1):30-35
2. Scarterzini P and Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. J Ethnopharmacology 2000:71-76
3. Trease GE and Evans WC. Pharmacognosy.12th edition, London, Baillieere Tindal; 1983
4. Kokate CK. practical pharmacognosy, 4th edition, vallabhprakashan new delhi(1994) pp 4.29
5. Annie Shirwaiker, Kirti S Prabhu and Punithal S. Invitro antioxidant studies of Sphaeranthusindicus(Linn). Indian journal of experiment biology. 2006: 4:993-996.
6. Green LC, Wagner DA, Glogowski J, Skipper P, Wish Nok JS and Tannenbaum SR. Analysis of nitrate and (15N) nitrate. Anal Biochem. 1982:126:131-138.
7. Smirnoff N, and Cumbes QJ, “hydroxyl radical scavenging activity of compatible solutes” phytochemistry 1989:28:1057-1060.
8. Ruch RJ, Cheng SJ and Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenisis. 10:1003-1008.