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FREE RADICAL SCAVENGING ACTIVITY AND PHENOLIC CONTENT ESTIMATION OF GLINUS OPPOSITIFOLIUS AND SESBANIA GRANDIFLORA

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ABSTRACT

The present research work was carried out to investigate the *in vitro* antioxidant activity of methanolic extracts of the whole plant of *Glinus oppositifolius* (MEGO) and leaves of *Sesbania grandiflora* (MESG). The therapeutic effects of tannins and flavonoids can be largely attributed to their antioxidant properties. The quantitative estimation of phenolic content was measured by using UV-spectrophotometric method. The total phenolic content value of MEGO was 12.2±0.12w/w and of MESG was 8.34±0.08 % w/w, respectively, and total flavonoid estimation of MEGO and MESG showed the content values of 4.9±0.02 % w/w and 1.2±0.13 %w/w, respectively, for quercetin and 3.6±0.18 % w/w and 1.56±0.09 % w/w, respectively, for rutin. The results revealed that these plants have antioxidant activity. The antioxidant activity of MEGO was found to be more potent than MESG. So finally it indicates that both the plants contains antioxidant substances which can be used for the treatment of oxidative stress related diseases.

Key words: *Glinus oppositifolius*, *Sesbania grandiflora*, total phenolic content, total flavonid content and free radical.

INTRODUCTION

Glinus oppositifolius (Linn) belonging to family Molluginaceae is a branched herb growing all over India. It is an annual prostrate weed commonly found in paddy fields after harvesting, riversides and open sands of seashores. *Sesbania grandiflora* (Linn) belonging to family Leguminosae: Papilionoideae commonly grows on dikes between rice paddies, along roadsides and in backyard vegetable gardens. The leaves of both the plants are used as leafy vegetable by local peoples. Methanol extract of *Glinus oppositifolius* was found to be hepatoprotective against paracetamol induced liver damage when given orally[1]. *G. oppositifolius* are used by the traditional healers for treating joint pain, inflammation, diarrhoea, intestinal parasites, fever boils and skin disorders [2, 3]. Ethanolic extract of the plant has been reported to depress central nervous activity[4]. The leaves contain spergulagenic, spergulagenin A and a trihydroxy ketone [4]. A bioactive pectic polysaccharide isolated from *G.oppositifolius* is found to possess immunomo dulating property [5]. Bark extracts of *Sesbania grandiflora* is effective on carrageenan induced acute inflammation and adjuvant-induced arthritis in rats [6]. Methanolic extract of leaves of *Sesbania grandiflora* shows strong in vitro anti-tumor promoting activities[7]. The oral administration of an ethanolic extract of *S. grandiflora* leaves has produced significant hepatoprotection against erythromycin estolate induced hepatotoxicity in rats [8]. The increasing interest gained by antioxidants is due to the health benefits provided mainly by natural sourced (exogenous) low molecular weight antioxidants. This consists in preventing the occurrence of oxidative-stress related diseases, caused by the attack of free radicals on key bio components like lipids or nucleic acids [9]. In the present study an attempt has been made to evaluate the antioxidant potential and Phenolic content

estimation of the methanolic extract of *G. oppositifolius* whole plant and *S.grandiflora* leaves.

MATERIALS AND METHODS

Collection and authentication of plant material: *Glinus oppositifolius* and *Sesbania grandiflora* plant materials were collected separately during the month of December from Berhampur and its peripheries with the help of local people. The taxonomical identification of these two plant specimens were done by the concerned expert of Central National Herbarium, Botanical Survey of India, Howrah (Authenticated no. CNH/23/2011/Tech.II/483). Voucher specimens were preserved in the Pharmacognosy department of Royal College of Pharmacy and Health Sciences (R.C.P.H.S), Berhampur for further reference.

Preparation of Plant extract: The whole plant and leaves of *Glinus oppositifolius* and *Sesbania grandiflora* were shade dried and pulverised. Then it was separately extracted by soxhlet apparatus, first with petroleum ether for defating and subsequently with chloroform and methanol. The crude extracts were made solvent free with rotary evaporator.

Chemicals and reagents: 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Steinheim, Germany). Folin Ciatelchu reagnt from Merck, Mumbai, India. All the reagents and chemicals used were of analytical grade.

Phytochemical screening: The preliminary phytochemical screening of methanolic extract of *Glinus oppositifolius* shows presence of carbohydrates, saponins, flavonoids, tannins, alkaloids, reducing sugars, gums and steroids. The methanol extract of *Sesbania grandiflora* shows presence of alkaloids, carbohydrates, glycosides, saponins, phenolic substances, flavonoids, gums and mucilages.

Free radical scavenging assay

H_2O_2 radical scavenging assay: The ability of the extract to scavenge hydrogen peroxide was determined according to the method given by Ruch et al., [10]. Ascorbic acid was used as reference compound.

$$H_2O_2\ activity(\%) = \frac{Abs(Control) - Abs(Sample)}{Abs(Control)} x100$$

Equation No: 1

Where, Abs (control): Absorbance of the control and Abs (sample): Absorbance of the extracts/standard.

Nitric oxide scavenging assay: The activity was measured according to the modified method of Sreejayan and Rao, 1997 [11]. BHT was used as the standard. The percentage of Nitric oxide scavenging activity is calculated from the equation no 1.

1, 1-diphenyl-2-picrylhydrazyl–radical scavenging assay method: The free radical scavenging activities of Methanolic extracts and the standard L-ascorbic acid (vitamin C) were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH.[12,13] The percentage inhibition activity was calculated using equation no 1.

Estimation of total phenolic content by spectrophotometer (Folin-Ciocalteu reagent method): The total phenolic content was estimated by spectrophotometric method using Folin-Ciocalteu reagent method. The total phenolic content (TPC) was determined by using calibration curve (2–12 µg/ml). Three readings were taken for each and every solution to get reproducible and accurate results. The results are shown in Table 7 and Figure 4. The intensity of the solution is directly proportional to the amount of polyphenols present in solution. This test is done by using Tannic acid as standard. The Total Phenolic Content was expressed as mg tannic acid equivalents per 100 g dry weight of sample[14].

Estimation of total flavonoid content by spectrophotometer (Aluminium chloride colorimetric assay method): Total flavonoid contents were measured using aluminium chloride colorimetric assay. Results are shown in Table 8 and Figure 5and 6. Total flavonoid content of the extracts was expressed as percentage of quercetin equivalent per 100 g dry weight of sample [15].

RESULTS AND DISCUSSION

The ability of the extract to scavenge hydrogen peroxide of the methanol extract of *Glinus oppositifolius* and *Sesbania grandiflora* were represented in table 1 in a concentration dependent manner. Their IC50 values are represented in table 2. The IC50 values were found to be 15µg/ml, 68µg/ml and 100µg/ml respectively. The % inhibition of nitric oxide free radical of the plant extract was represented in table 3. The IC50 value of % inhibition of nitric oxide free radical by BHT, MEGO and MESG were 50µg/ml, 141µg/ml and 159µg/ml respectively. It was represented in table 4. Being a stable free radical DPPH**.** is frequently used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm, but upon reduction with an antioxidant, its absorption

decreases due to the formation of its non-radical form, DPPH–H [16]. Thus, the radical scavenging activity in the presence of a hydrogen donating antioxidant can be monitored as a decrease in absorbance of DPPH solution. Figure 3 shows free radical scavenging activity of the ascorbic acid and plant extracts at different concentrations. The % inhibition of DPPH free radical by different concentration of the standard ascorbic acid and methanol extract of the plants were depicted in table 5 and the IC50 values were represented in table 6. The IC50 values of Ascorbic acid, MEGO and MESG were found to be 88µg/ml, 132µg/ml and 145µg/ml respectively.

It is evident that the antioxidant activity in terms of DPPH radical scavenging is influenced by the total flavonoid and phenolic content[17]. Estimation of total phenolic content by spectroscopic method shows presence of 12.2% and 8.34% of tannic acid equivalent of methanol extract of *Glinus oppositifolius* and *Sesbania grandiflora* respectively. Estimation of total flavonoid content by taking quercetin and rutin as standard by spectroscopic method for methanolic extract of Glinus oppositifolius and Sesbania grandiflora. For quercetin standard it was found to be 5% and 3.2% equivalent. For rutin standard it was found to be 6.7% and 4.3% equivalent respectively.

CONCLUSION

In present study, antioxidant activities of the methanol extract of *Glinus oppositifolius* and *Sesbania grandiflora* were investigated. The extracts were found to possess radical scavenging and antioxidant activities, as determined by scavenging effect on the H_2O_2, N_2O and DPPH free radicals. In the present study it is found that the methanol extract of *Glinus oppositifolius* and *Sesbania grandiflora* contains substantial amount of phenolics and flavonoid compounds and it is the extent of phenolics present in this extract being responsible for its marked antioxidant activity as assayed through various in vitro models. Methanol extract of *Glinus oppositifolius* has a better activity as compared to the methanol extract of Sesbania grandiflora this may be due to the higer phenolic and flavonoid content of Glinus oppostifolius. Thus it can be concluded that Glinus oppositifolius and Sesbania grandiflora can be used as an accessible source of natural antioxidants with consequent health benefits.

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Table 1 Percentage inhibition of H_2O_2 free radical by different concentrations of Plant extracts.

Sl no	Conc in µg/ml	% of inhibition of ascorbic acid	% of inhibition of MEGO	% of inhibition of MESG
1	10	32.27±0.46	22.18±0.35	17.92±0.31
2	15	50.49±0.64	33.33±0.64	20.38±0.33
3	25	57.30±0.51	37.75±0.42	28.48±0.42
4	50	70.97±0.51	45.82±0.35	33.6±0.5
5	75	81.57±0.48	56.72±0.44	43.08±0.58
6	100	91.38±0.42	66.98±0.26	51.85±0.43
7	125	95.78±0.37	72.97±0.71	61.63±0.458
8	150	97.82±0.25	76.2±0.38	71.63±0.38
9	200	100.2±0.41	79.13±0.38	76.92±0.27

Values were represented as Mean ± SEM of six parallel data.

Table 2 IC50 values of standard and plant extracts

Sl no	Plant extract/ standard	IC50 Value(in µg/ml)
1	Ascorbic acid	15
2	MEGO	68
3	MESG	100

Table 3 % inhibition of Nitric oxide free radical by different concentration of Plant extracts.

Sl no	Conc.in µgm/ml	% of inhibition BHT	% of inhibition of MEGO	% of inhibition of MESG
1	10	20.4±0.55	8.45±0.40	4.7±0.3
2	15	32.03±0.35	11.37±0.28	7.56±0.26
3	25	40.25±0.54	16.03±0.37	8.76±0.266
4	50	51.72±0.46	21.28±0.4	14.8±0.46
5	75	60.5±0.76	31.03±0.43	22.88±0.47
6	100	69.07±0.55	39.92±0.41	35.12±0.29
7	125	81.15±0.32	47.9±0.3	42.5±0.43
8	150	95.78±0.19	56.02±0.35	51.08±0.331
9	200	98.98±0.3	62.82±0.32	57.17±0.332

Values were represented as Mean ± SEM of six parallel data.

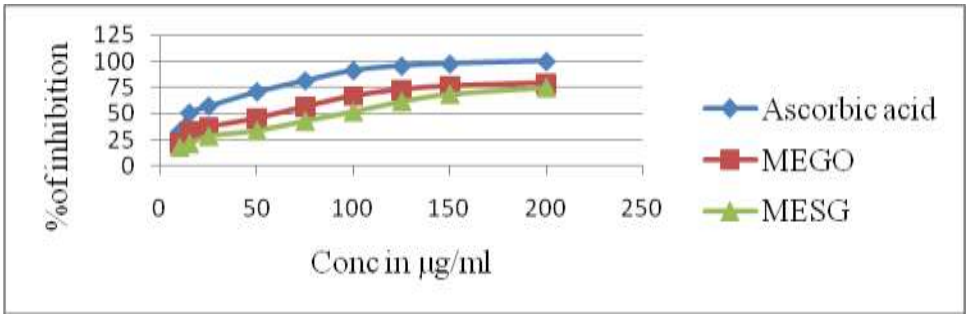

Figure 1 Conc Vs %inhibition curve of H_2O_2 free radical by different concentrations of plant extracts

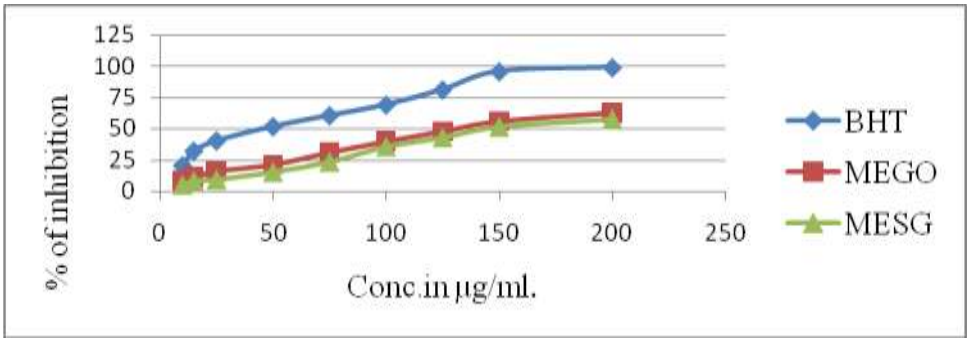

Figure 2 Conc Vs %inhibition curve of Nitric oxide free radical by different concentrations of plant extracts

Table 4 IC50 values of standard and plant extracts

Sl no	Plant extract/ standard	IC50 Value(in µg/ml)
1	BHT	50
2	MEGO	141
3	MESG	159

Table 5 % inhibition of DPPH free radical by different concengtration of Plant extracts.

Sl no	Conc.in µg/ml	% of inhibition of Ascorbic Acid	% of inhibition of MEGO	% of inhibition of MESG
1	50	35±0.35	22.5±0.41	21.62±0.16
2	75	42.77±0.34	24.18±0.45	24.05±0.20
3	100	54.98±0.34	37.87±0.51	35.25±0.31
4	125	64.67±0.31	51.33±0.45	45.65±0.14
5	150	77.37±0.5	61.83±0.61	55.45±0.14
6	200	85.53±0.41	68.97±0.39	64.88±0.17

Values were represented as Mean ± SEM of six parallel data.

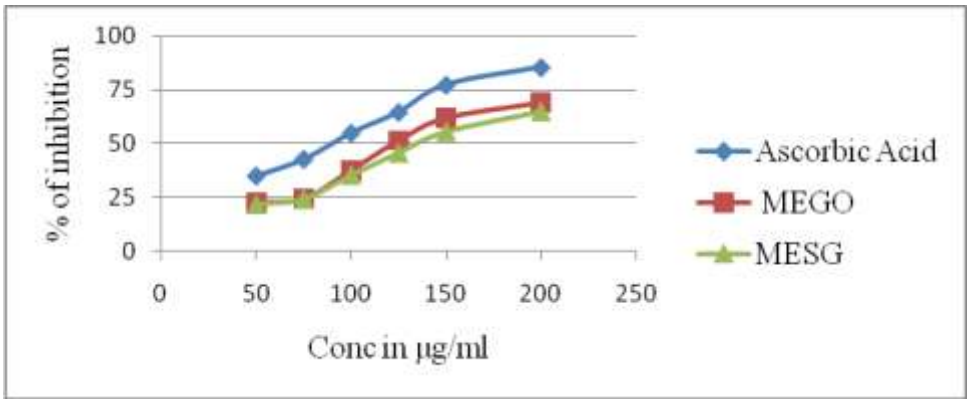

Figure 3 Conc Vs %inhibition curve of DPPH free radical by different concentrations of plant extracts

Table 6 IC50 values of standard and plant extracts

Sl no	Plant extracr/ standard	IC50 Value(in µg/ml)
1	Ascorbic acid	88
2	MEGO	132
3	MESG	145

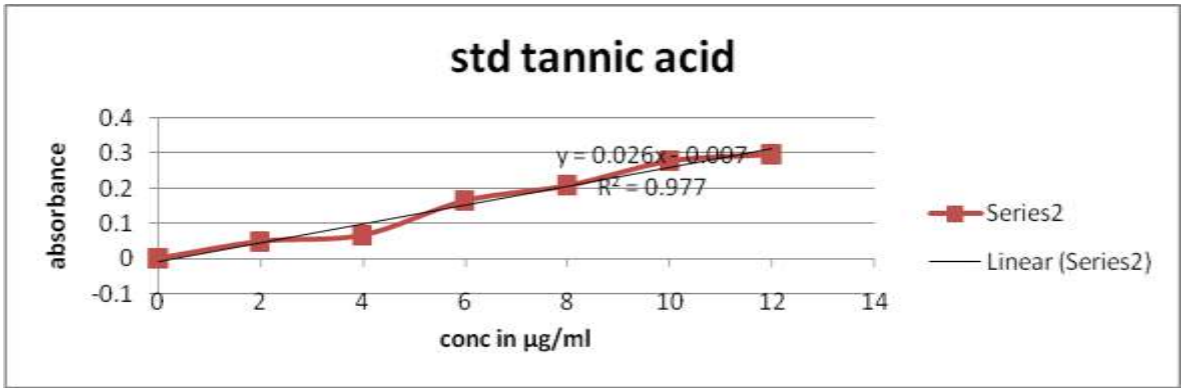

Figure 4 Standard curve of tannic acid

Table 7 Total phenolic content of methanol extract of *Glinus oppositifolius* and S*esbania grandiflora.*

Sl. No	Plant	%w/w of total phenolic content tannic acid equivalent
1	*Glinus oppositifolius*	12.2±0.12
2	*Sesbania grandiflora.*	8.34±0.08

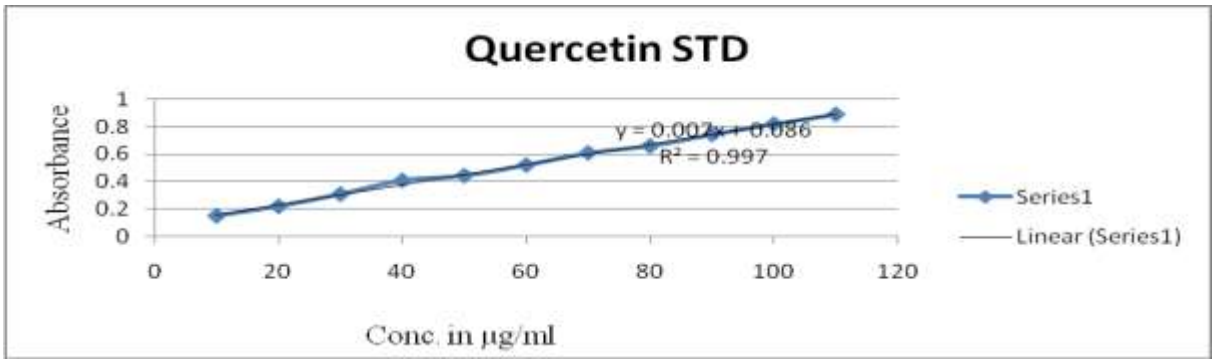

Figure: 5 standard curve of quercetin

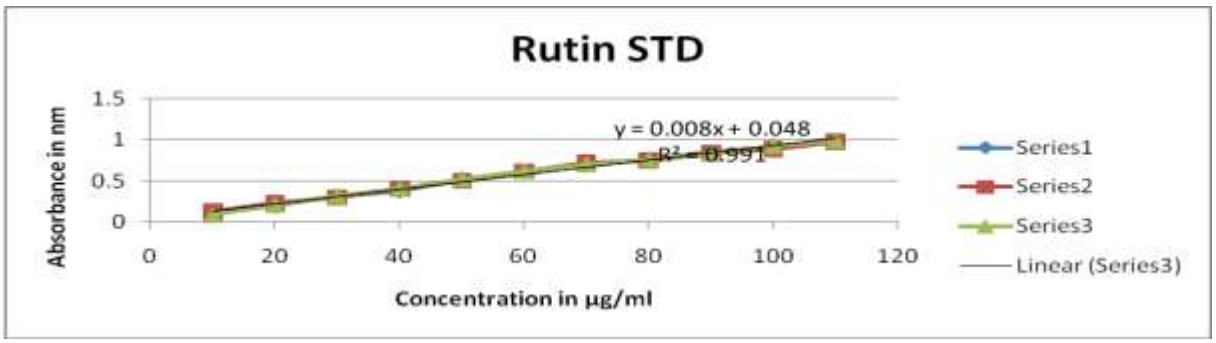

Figure: 6 standard curve of rutin

Table: 8 Total flavonoid content of methanol extract of *Glinus oppositifolius* and *Sesbania grandiflora*.

Sl. No	Plants	%w/w of total flavonoid	
		Quercetin equivalent	Rutin equivalent
1	*Glinus oppositifolius*	4.9±0.02	3.6±0.18
2	*Sesbania grandiflora.*	1.2±0.13	1.56±0.09

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