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In Vitro and *In Vivo* Antioxidant activity of Methanolic Extract of *Solena amplexicaulis (Whole Plant)*

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ABSTRACT

The present study was aimed at investigating the antioxidant activities of the Methanolic extract of Solena amplexicaulis (MESA) Whole plant (Cucurbitaceae). The antioxidant activities of extract have been evaluated by using a range of in vitro assays and in vivo hepatoprotective model. In case of in vitro studies the percentage inhibition values were found to be in DPPH, nitric oxide, hydrogen peroxide radical scavenging assays, respectively. In case of in vivo studies the levels of liver enzymatic, non-enzymatic systems [serum glutamate oxalo-acetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), total bilirubin, total protein, alkaline phosphatase (ALP), catalase (CAT), glutathione (GSH), and lipid peroxidation (LPO)] were restored towards the normal value in treated carbon tetrachloride intoxicated rats. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic (gallic acid content is 63.63 µg/mg, respectively), and flavonoid compounds (41.18 µg/mg) present in MESA. The results obtained in the present study indicate that the Solena amplexicaulis Whole plant is a potential source of natural antioxidant activity.

KEYWORDS: *Solena amplexicaulis ;DPPH, Flavonoid, Total phenolic content, Nitric oxide ,SGOT SGPT and Total phenolic content.*

The effects of free radicals on human beings are closely related to toxicity, disease and aging[1]. Most living species have an efficient defense system to protect themselves against the oxidative stress induced by Reactive Oxygen Species (ROS)[2]. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases including cancer, atherosclerosis and the aging process[3-5]. The antioxidants can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers.

The plant *Solena amplexicaulis* (Lam.) Gandhi (syn: Melotheria heterophylla) belonging to Cucurbitaceae family are widely distributed Indian, Srilanka, China, Taiwan. The tubers, leaves and seeds of the plant are extensively used in traditional system for various ailments like hepatosplenomegaly, spermatorrhoea, thermogenic, appetizer, cardiotonic, diuretics, haemorrhoids and invigorating [6, 7].

Furthermore, literature survey of *Solena amplexicaulis* (MESA) Whole plant revealed that no researcher has yet reported antioxidant activities of this plant. Therefore, it is worth conducting an investigation on the in vitro antioxidant activities of methanolic extract of *Solena amplexicaulis* Whole plant.

MATERIAL & METHODS

Plant material and extraction procedures:

Whole plant of *Solena amplexicaulis* was collected from Thirumala hills of Chithoor district, Andrapradesh. The plant was authenticated by Prof. Dr.K.Madhava chetty ,Department of Botany ,SVU University, Chithoor, Andhra Pradesh (India).The air dried whole plant of S.amplexicaulis was made into coarse powder and extracted with

methanol by Soxhlation and the crude extract was evaporated by using Rotavapour (BUCHI, Germany) under reduced pressure.

Experimental Procedure

***In vitro* antioxidant activity**

Reducing power:

The reducing power of MESA was determined according to the method of Oyaizu [8]. 10 mg of MESA extract in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN_6]$ (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the graph was plotted with the average of three observations.

DPPH radical scavenging activity:

The free radical scavenging activity of MESA was measured by 1,1-diphenyl-2-picryl- hydrazil (DPPH) using the method of Blois [9]. 0.1 mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3 ml of various concentration of MESA and the reference compound (10-125µg/ml). After 30 min, absorbance was measured at 517 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the graph was plotted with the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

$$\text{Percentage inhibition} = \frac{\text{Abs}_{\text{cntrl}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{cntrl}}} \times 100 \quad (1)$$

Nitric oxide scavenging activity:

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction [10,11]. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and MESA and the reference compound in different concentrations (10-125µg/ml) were incubated at 25°C for 150 min. Each 30 min, 0.5 ml of the incubated sample was removed and 0.5 ml of the Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546 nm. Ascorbic acid was used as a positive control compound.All the tests were performed in triplicate and the results averaged. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples using Eq. (1).

Hydrogen peroxide scavenging activity:

Scavenging activity of Hydrogen peroxide (H_2O_2) by the plant extract was determined by the method of Ruch [12.] Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using Eq. (1).

Estimation of total phenolic content:

Total soluble phenolics present within in the MESA were determined using the Folin- Ciocalteu reagent, according to the method of a Slinkard and Singleton13. 0.1 ml of suspension of 1mg of MESA in water was totally transferred into 100 ml Erlenmeyer flask. Then the final volume was adjusted to 46 ml by the addition of distilled water. Afterwards, 1 ml of Folin - Ciocalteu reagent (FCR) was added to this mixture and after 3 min, 3 ml of Na2CO3 (2%) was added. Subsequently, the mixture was shaken on a shaker for 2 h at room temperature and then its absorbance measured at 760 nm. All the tests were performed in triplicate and the results averaged. The concentration of total phenolic compounds in MESA was determined as microgram of Gallic acid equivalent by using an equation that was obtained from the standard Gallic acid graph. The equation is given below;

Absorbance = 0.001 x Gallic acid (µg) + 0.00161 (2)

Total flavonoids determination:

Aluminum chloride colorimetric method was used for flavonoids determination .According to the method of Chang[14]. Each plant extracts (0.5 ml of 1:10 g ml-1) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by gallic acid solution (0 - 100µg/ml) in methanol. The concentration of flavonoid was expressed in terms of µg/ml.

Absorbance = 0.001 x Gallic acid (µg) + 0.00179 (3)

Statistical analysis

Experimental results were mean±SD of three parallel measurements. Linear regression analysis was used to calculate IC_{50} values. Whenever needed.

***In vivo* antioxidant study**

Animals

Female wistar albino rats (100-150 g) procured from M/S Mahaveera Enterprises, Hyderabad (India) and were used for the studies. The animals were housed in large polypropylene cages in a temperature controlled room (37ºC±2ºC) and provided with standardized pellet feed and clean drinking water *ad libitum*. The study protocol was duly approved by the Institutional Animal Ethical Committee.

Experimental design

After seven days of acclimatization, the rats were divided into four groups (n=6). Treatment was done for 8 days as follows:

Group I: Control received the vehicle of 2% gum acacia (1ml/kg/day;p.o)

Group II: Toxic control received (CCl_4: olive oil (1:1);(0.7ml/kg.i.p) at every 72 h

Group III: received standard drug Silymarin (50 mg/kg/day; p.o) for 7 days and simultaniously administered CCl_4 at every 72 h

Group IV**:** received MESA (250 mg/kg/day; p.o) for 7 days and simultaniously administered CCl_4 at every 72 hr.

Group V: received MESA (500 mg/kg/day; p.o) for 7 days and simultaniously administered CCl_4 at every 72 h. After 24 h of the last dose, all the animals were anaesthetized with ether and sacrificed. The blood was collected from retro-orbital plexus. The blood samples were allowed to clot and the serum was separated by centrifugation at 2500 rpm for 15 min at 37° C and the serum was used for biochemical estimation. All the animals were then sacrificed and liver tissues were collected for the evaluation of *in vivo* antioxidant studies.

Biochemical estimation

Serum was analysed for various biochemical parameters like serum glutamic oxaloacetic (SGOT), serum glutamic pyruvic transaminase (SGPT) activities [15], and alkaline phosphatase [16]. The total protein concentration and total bilirubin were also measured by the method of Lowry *et al.*[17] and Mallay and Evelyn [18], respectively. All the analyses were performed by using commercially available kits from Span Diagnostics Ltd.

Estimation of lipid peroxidation (LPO), enzymic (CAT,) non-enzymic (GSH) antioxidant system:

Tissue supernatant preparation for LPO,CAT ,GSH assay:

The livers were quickly removed, weighed and homogenized in phosphate buffer (0.1 M, pH 7.4) .The homogenate was centrifuged at (1000 rpm , 15 min) to remove debris. The supernatant was used to assay the LPO, CAT, and GSH activities.

Determination of lipid peroxidation:

Lipid peroxidation was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA)[19]. To 1 mL of supernatant, 0.5 mL of 30% trichloroacetic acid (TCA) was added followed by ,0.5 mL of 0.8% TBA. The tubes were kept in a shaking water bath for 30 min at 80 °C. After 30 min of incubation the tubes were taken out and kept in ice-cold water for 10 min. These were then centrifuged at 800 g for 15 min. The amount of MDA was assessed by measuring the absorbance of supernatant at 540 nm at room temperature against an appropriate blank. The percentage inhibition of Lipid peroxidation was calculated using the equation:

$$\%\,\text{inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 is the absorbance of the control without extract and A_1 is the absorbance of the sample extract.

Determination of Catalase activity

To estimate the CAT activity by the method [20] the reaction mixture consisted of 1.95 mL phosphate buffer (0.1 M, pH 7.4), 1.0 mL hydrogen peroxide (H_2O_2) (0.019 M), and 0.05 mL of supernatant in a final volume of 3 mL. Changes in absorbance were recorded at 240 nm. . The percentage inhibition of free radicals by CAT was calculated using the equation

$$\%\,\text{inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 is the absorbance of the control without extract and A_1 is the absorbance of the sample extract.

Determination of GSH activity:

Reduced glutathione was determined by the modified method of Ellman (1959) [21].An aliquot of 1.0 ml of liver tissue supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2M,pH 8.0). The absorbance was meserd at 412 nm. The percentage inhibition of free radicals by GSH was calculated using the equation:

$$\%\,\text{inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 is the absorbance of the control without extract and A_1 is the absorbance of the sample extract.

Results and Discussion

***In vitro* antioxidant activity:**

Reductive ability:

Fig. 1 shows the reductive capabilities of MESA extract when compared to the standard Ascorbic acid. Like the antioxidant activity, the reducing power increased with increasing amount of the extract. For the measurement of the reductive ability, the Fe3+-Fe2+ transformation was investigated in presence of the extract. Presence of reductants causes the reduction of the Fe3+/ferricyanide complex to the Fe2+ form. This Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. (**Table-1**)

Table-1: shows the measurement of reductive ability:

Conc (mcg/ml)	Ascorbic acid absorbance	MESA absorbance
10	0.27±0.008	0.20±0.003
25	0.41±0.011	0.34±0.004
50	0.50±0.004	0.40±0.005
75	0.50±0.003	0.51±0.007
100	0.66±0.002	0.54±0.015
125	0.75±0.005	0.60±0.013

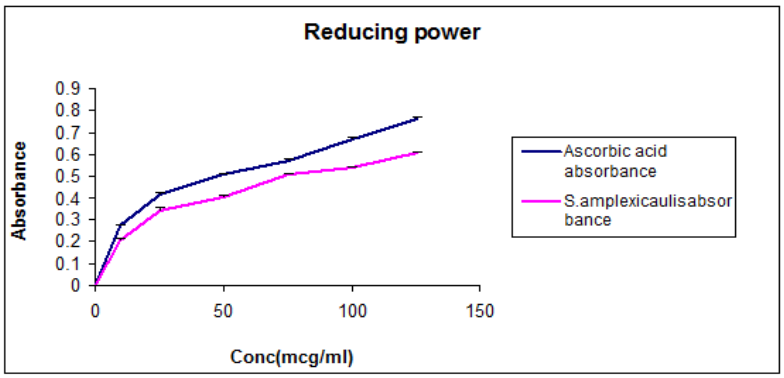


Fig.1 Reductive ability of Ascorbic acid and MESA

DPPH radical scavenging activity

The *Solena amplexicaulis* Whole plant extract demonstrated H-donor activity. The DPPH radical scavenging activity was detected and compared with Ascorbic acid. Fig 2 shows the % inhibition values of Ascorbic acid and MESA was 91.36 %, 85.16 % respectively. DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. DPPH is a stable, nitrogen-centered free radical which produces violet colour in methanol solution. It was reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the extract in a concentration-dependent manner. The IC_{50} value of Ascorbic acid was 36µg/ml,MESA was 57.47µg/ml.

Table-2:DPPH Radical Scavenging Activity

Conc(mcg/ml)	Ascorbic acid absorbance	MESA absorbance	Ascorbic acid % inhibition	MESA % inhibition
10	0.97±0.01	1.27±0.04	44.57±2.34	27.40±4.32
25	0.72±0.01	1.12±0.00	58.66±1.30	35.88±2.13
50	0.56±0.02	0.80±0.01	68.01±1.51	49.94±0.90
75	0.38±0.03	0.57±0.02	78.06±1.61	67.08±2.05
100	0.22±0.02	0.45±0.02	87.50±1.22	74.03±1.87
125	0.15±0.02	0.26±0.02	91.36±0.42	85.16±1.18

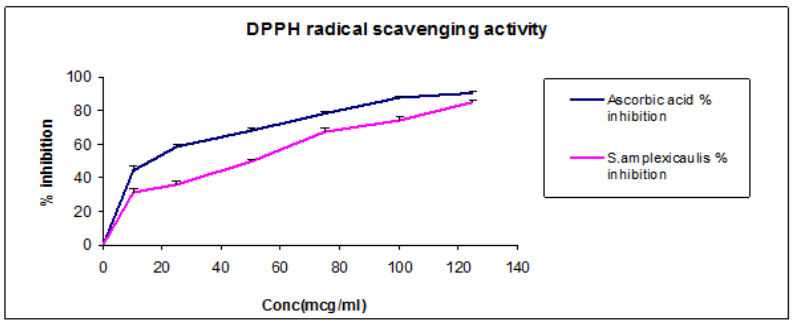


Fig 2. Percentage inhibition of DPPH radicals by Ascorbic acid and MESA

Nitric oxide radical scavenging activity:

MESA effectively reduced the generation of nitric oxide from sodium nitroprusside.Fig.3 shows the % inhibition values of Ascorbic acid and MESA was 85.41%, 80.11% respectively . Scavenging of nitric oxide radical is based on the generation of nitric oxide . sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. The absorbance of the chromophore is measured at 546 nm in the presence of the fractions. MESA decreases the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. The IC_{50} values of Ascorbic acid was 39.02µg/ml, MESA was found to be 50.72µg/ml.

Table-3:

Conc(mcg/ml)	Ascorbic acid absorba	MESA absorbance	Ascorbic acid inhibition	MESA % inhibition
10	1.36±0.07	1.52±0.04	43.11±4.41	36.03±5.91
25	0.91±0.16	1.32±0.03	58.54±5.75	44.85±3.43
50	0.73±0.01	0.95±0.08	69.31±1.68	59.96±5.87
75	0.62±0.01	0.73±0.03	73.73±1.71	69.23±3.34
100	0.51±0.01	0.57±0.02	78.36±1.03	76.00±2.45
125	0.34±0.02	0.46±0.01	85.41±1.41	80.11±0.91

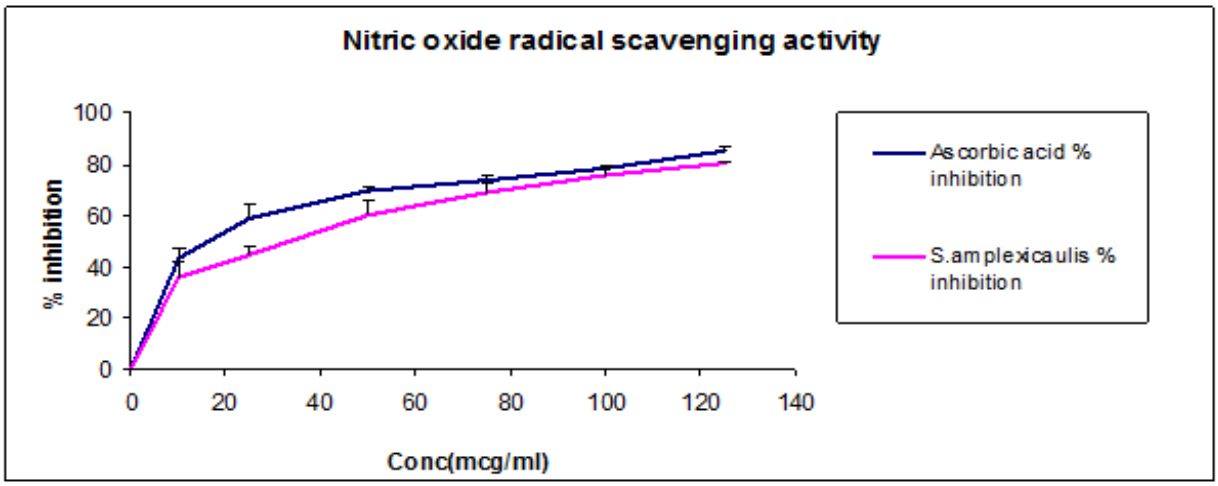


Fig 3. Percentage inhibition of Nitric oxide radicals by Ascorbic acid and MESA

Hydrogen peroxide radical scavenging activity:

Scavenging activity of H2O2 by *S. amplexicaulis* Whole plant extract may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water . The H2O2 scavenging activity was detected and compared with Ascorbic acid. Fig 4 shows the % inhibition values of Ascorbic acid and MESA was 90.20 %, 82.53 % respectively. The absorbance of the chromophore is measured at 230 nm in the presence of the fractions. The extract capable of scavenging H_2O_2 in a concentration dependent manner. The ic_{50} values of Ascorbic acid was found to be 38.74 µg/ml, 51.80 µg/ml.

Table-4:Hydrogen peroxide radical scavenging activity

Conc(mcg/ml)	Ascorbic acid absorba	MESA absorbance	Ascorbic acid inhibition	MESA % inhibition
10	0.88±0.03	1.04±0.01	36.85±1.40	25.12±4.63
25	0.53±0.01	0.67±0.02	61.69±2.79	51.65±2.65
50	0.41±0.01	0.52±0.01	70.31±1.74	62.35±2.73
75	0.35±0.02	0.46±0.02	74.38±2.41	67.11±2.29
100	0.25±0.02	0.36±0.02	81.75±1.81	73.91±2.49
125	0.13±0.01	0.24±0.01	90.20±0.97	82.53±1.13

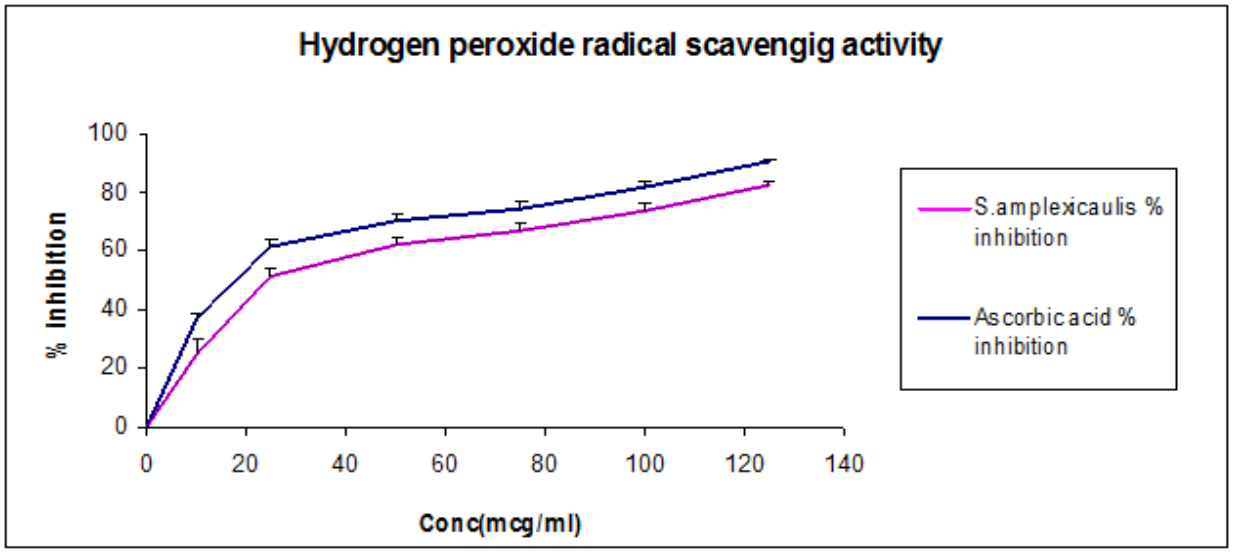
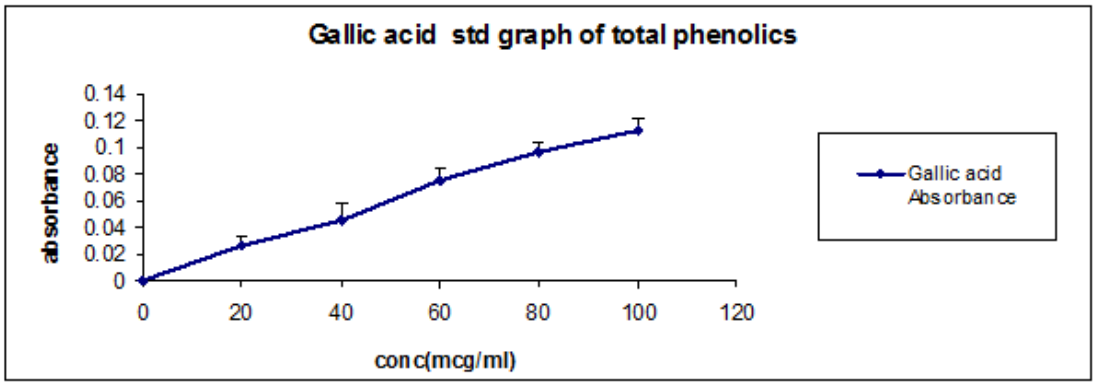


Fig 4. Percentage inhibition of Hydrogen peroxide radicals by Ascorbic acid and MESA

Total phenolic content:

Phenols are very important plant constituents because of their scavenging ability, which is due to their hydroxyl groups [22]. In MESA (1 mg) 63.63µg/mg Gallic acid equivalent of phenols was detected. The phenolic compounds may contribute directly to the antioxidative action [23]. The result indicates strong association between antioxidative activities and phenolic compounds (r^2= 0.9947), suggesting that phenolic compounds are probably responsible for the antioxidative activities of *Solena amplexicaulis*. Phenolic compounds are effective hydrogen donors, making them good antioxidants [24]. Similarly Shahidi and Naczk reported that naturally occurring phenolics exhibit antioxidative activity [25]. Thus, therapeutic properties of the *S. amplexicaulis* may be possibly attributed to the phenolic compounds present. (**Figure-5**)

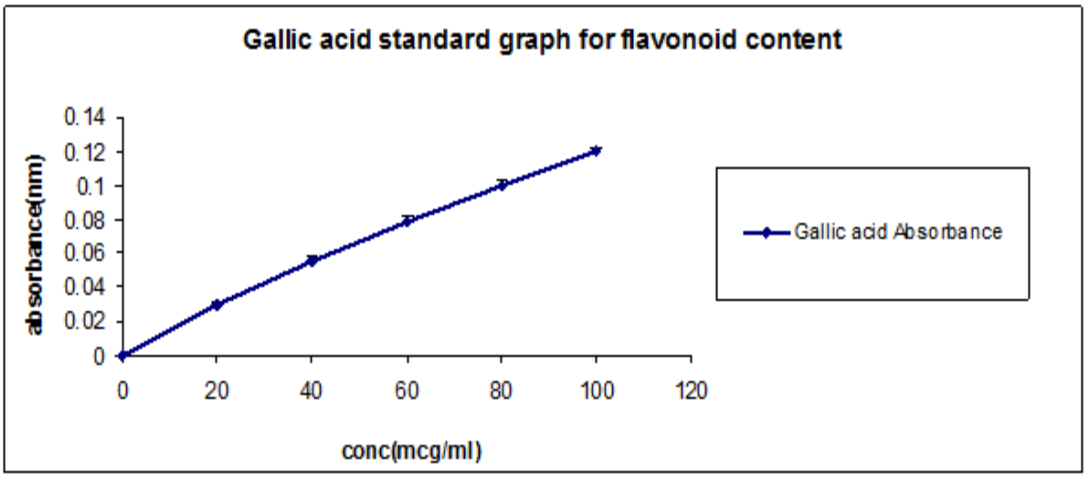
Figure-5 Amount of total phenolic compounds



Estimation of total flavonoid content

Flavonoids are large class of benzo-pyrone derivatives, ubiquitous in plants exhibit antioxidant activity. The flavonoid content of different fruits extract is shown in **Figure 6**. Total flavonoid content of MESA is 41.18 µg/mg of Gallic acid equivalents of extract. The antiradical property of flavonoids is directed mostly toward hydroxyl, superoxide as well as peroxyl and alkoxyl radicals. Furthermore, as these compounds present a strong affinity for iron ions (which are known to catalyze many processes leading to the appearance of free radicals), their antiperoxidative activity could also be ascribed to a concomitant capability of chelating iron. The result indicates strong association between antioxidative activities and flavonoid compounds (r^2= 0.9756), suggesting that Flavonoid compounds are probably responsible for the antioxidative activities of *Solena amplexicaulis*.

Figure-6: Estimation of total flavonoid content



***In vivo* antioxidant study:**

Estimation of biochemical parameters:

Biochemical parameters (SGOT, SGPT, ALP, total protein, total bilirubin) are shown in **Table 5**. The level of SGPT, SGOT, ALP, total protein, total bilirubin is restored towards the normal value in MESA treated carbon tetrachloride intoxicated rats. Peroxidative degradation of cellular membrane due to CCl_4 induction causes functional morphological changes in it resulting in cellular leakage loss of functional integrity of the membrane. It was found from **Table 5** that in CCl_4 toxic control group by the substantial increase in the level of serum marker enzymes (SGOT, SGPT, ALP and total bilirubin). The reduction of the level of total proteins in CCl_4 challenged animals (**Table 5**) is attributed to the damage produced localized in the endoplasmic reticulum which results in the loss of P_{450} leading to its functional failure with a decrease in protein synthesis. The rise in protein

levels in the treated groups suggests the stabilization of endoplasmic reticulum leading to protein synthesis.

Table-5: Effect of methanolic extract of *S. amplexicaulis* (MESA) on serum enzyme levels, total bilirubin, and total protein of CCl_4 intoxicated rats

Groups	SGOT(IU/L)	SGPT(IU/L)	TBL(mg%)	TPL(g%)	ALP(IU/L)
NORMAL	45.24±3.64	52.12±1.78	1.26±0.29	8.85±1.45	48.30±5.94
TOXIC (CCl_4)	133.60±4.68	145.72±5.39	2.73±0.57	4.44±1.86	91.11±9.80
STANDARD (Silymarin)	58.13±2.49***	62.00±1.58**	1.59±0.20**	8.17±1.71***	54.04±2.33***
MESA250 mg)	115.87±3.046*	123.63±5.71*	2.03±0.43**	7.36±2.04*	81.58±5.48*
MESA (500 mg)	72.47±3.97***	87.103±1.85**	1.99±0.57**	7.97±2.22***	63.22±4.44**

Data expressed as Mean±S.D, *n*=6 in each group. *P value<0.05,** P value<0.01,***P value<0.001 compared with toxic group.SGOT=Serum glutamate oxalo transaminase, SGOT=Serum glutamate pyruvate transaminase, TBL=Ttotal bilirubin levels, TPL= Total protein levels, ALP= Alkaline phosphatase. where the significance was performed by One way ANOVA followed by post hoc Dunnett's test.

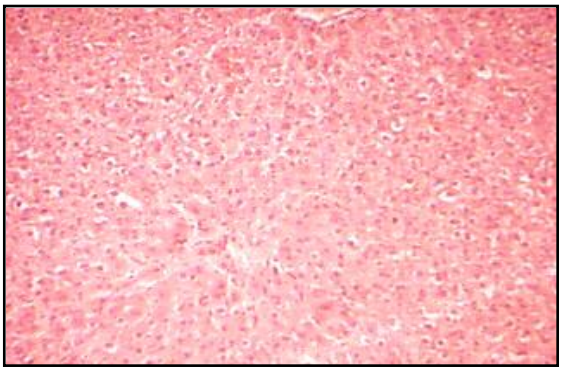
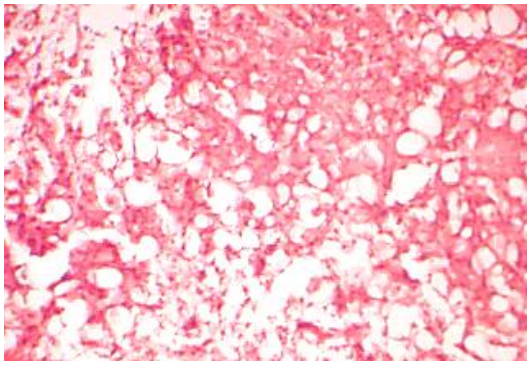
Estimation of lipid peroxidation (LPO), enzymic (CAT) non-enzymic (GSH) antioxidant system:

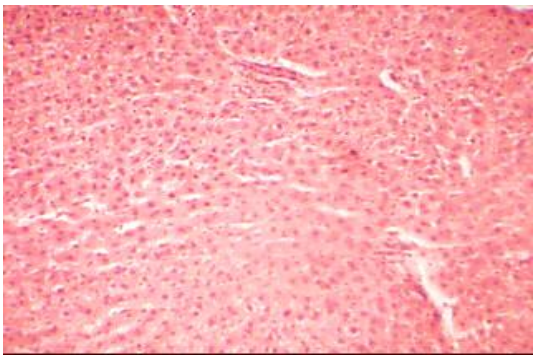
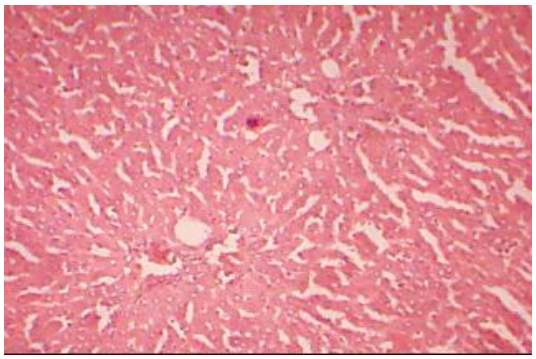
The percentage inhibition of enzymic (CAT), non-enzymic (GSH) antioxidant system and lipid peroxidation (LPO) level of liver homogenate were summarized in **Table 6**. In the present study it is observed that the MESA percentage inhibitions of LPO, CAT and GSH were increased when compared to carbon tetrachloride intoxicated rats. The antioxidant activity or the inhibition of the generation of free radical is important in the protection against CCl_4-induced liver lesion. The percentage inhibition of production of MDA, which is one of the end products of lipid peroxidation in liver tissue, was found to be low in CCl_4 control group (shown in **Table 6**) implying enhanced lipid peroxidation leading to tissue damage failure of antioxidant defense mechanisms against free radicals. Treatment with MESA and Silymarin significantly reversed these changes. Our body has an effective defense system against free radical induced damage. It consists of a set of endogenous antioxidant enzymes, one of the key component of which is catalase (CAT). In the present study it is observed that CCl_4 deplets CAT levels. Regarding non-enzymic antioxidants, reduced glutathione (GSH) is a critical determinant of tissue susceptibility to oxidative damage. It is an intracellular reductant which is extensively found in cells. It protects cells against electrophilic attacks by xenobiotics such as free radicals peroxides. In the present study it is observed that CCl_4 deplets GSH concentration in the rat livers. MESA and Silymarin treatment reverses this effect, which may be due to *de novo* GSH synthesis or GSH regeneration.

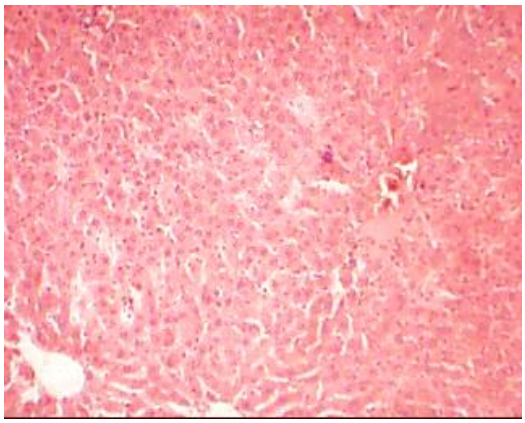
Table 6: Effect of methanolic extract of *S. amplexicaulis* (MESA) on LPO,CAT,GSH of CCl_4 intoxicated rats

GROUP	*LPO*	*CAT*	*GSH*
NORMAL	100	100	100
TOXIC (CCL_4)	49.45	36.75	45.16
STANDARD (Silymarin)	92.77	76.50	90.67
MESA (250 mg)	62.89	55.12	61.25
MESA (500 mg)	78.84	69.86	74.73

Histopathalogical study:

Liver cells of normal rats	*Liver cells of rats intoxicated with CCL_4*
	

Liver cells of rats treated with Standard(Sylimarin) and intoxicated with CCL_4	*Liver cells of rats treated with MESA(250m and intoxicated with CCL_4*
	

Liver cells of rats treated with MESA(500 mg) and intoxicated with CCL_4


CONCLUSION

Free radicals are known to play a definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, alzheimer, hepatic damage etc. Antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. MESA decreases the amount of nitrite generated from the decomposition of sodium nitroprusside, the reducing power of MESA extract, like its antioxidant activity, increases with increasing concentration which implies that extract is capable of donating H atoms in a dose dependent manner. Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities. These also decrease cardiovascular complications. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. MESA showed significantly higher percentage inhibition (stronger hydrogen-donating ability) positively correlated with total phenolic content.

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as antiinflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral, and anticancer activities.In most of the developing countries, the incidence of viral hepatitis is more. So, the investigation for an efficient hepatoprotective drug from the natural resource is an urgent necessity. The changes associated with CCl4-induced liver damage are similar to that of acute viral hepatitis. CCl4 is therefore a useful tool for the induction of hepatic damage in experimental animals. The ability of hepatoprotectivity of MESA to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin is the index of its protective effects.Hence, the present investigation suggests that MESA shows good antioxidant activity, reducing power, free radical scavenging activity and hepatic protection.

REFERENCES

1. Maxwell SJ. Prospects for the use of antioxidant therapies. Drugs (1995) 49: 345
2. Sato M, Ramarathnam N, Suzuki Y, Ohkubo T, Takeuchi M and Ochi H. Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. J. Agri. Food Chem. (1996) 44: 37-41
3. Stajner D, Milic N, Mimica-Dukic N, Lazic B and Igic R. Antioxidant abilities of cultivated and wild species of garlic. Phytother. Res. (1998) 12: 513-514
4. Sanchez-Moreno C, Larrauri JA and Saura-Calixto F. Free radical scavenging capacity an inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res. Intern. (1999) 32: 407-512 Malencic DJ, Gasic O, Popovie M and Boza P.
5. Screening for antioxidant properties of Salvia reflexa Hornem. Phytother. Res. (2000) 14: 546-548
6. Kirtikar K.R, Basu B.D.; Indian Medicinal plants Vol.-II: 3rd Edition 1988, 1162-1163.
7. Indian medicinal plants, Compendium of 500 species, Orient Longman Ltd, 1996, 5:173.
8. Oyaizu M. Studies on product of browning reaction prepared from glucoseamine. Jap. J. Nut. (1986) 44: 307-315
9. Blois MS. Antioxidant determinations by the use of astable free radical. Nature (1958) 29: 1199-1200
10. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JK and Tannenbaum SR. Analysis of nitrate, nitrite and 15N in biological fluids. Anal. Biochem. (1982) 126: 131-136
11. Marcocci L, Maguire JJ, Droy-Lefaix MT and Packer L. The nitric oxide scavenging property of Ginkgo biloba extract EGb 761. Biochem. Biophys. Res.Commun. (1994) 201: 748-755
12. Ruch RJ, Cheng SJ, Klaunig JE (1989). Prevention of cytotoxicity and inhibition of Intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogen., 10: 1003-1008
13. Slinkard K and Singleton VL. Total phenol analyses; automation and omparision with manual methods. Am. J. Enol. Vitic (1977) 28: 49-55
14. Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J.Food Drug Analaysis, 10: 178-182
15. S. Reitman and S. Frankel, Am. J. Clin. Path. **33**, 97 (1957).
16. W. G. Duncombe, Biochem. J. **88**, 7 (1963).
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, J. Biol. Chem. **193**, 265 (1951).
18. H. T. Mallay and K. A. Evelyn, J. Biol. Chem. **119**, 481 (1937).
19. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95:351–8.
20. Clairbone A. Catalase activity. In: Greenwald RA, editor. CRC Handbook of methods for Oxygen Radical Research. Boca Raton, FL: CRC Press; 1985. pp. 283–4.

21. Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch.Bliochem . Biophys., 82: 70-77
22. Diplock AT. Will the 'good fairies' please proves to us that Vitamin E lessens human degenerative of disease? Free Rad. Res. (1997) 27: 511-32
23. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y and Yasuhara. Effects of interaction of tannins with coexisting substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chem. Pharm. bull. (1989) 37: 2016-21
24. (24)Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, and Pridham JB. The relative antioxidant activity of plant derived polyphenolic flavanoids. Free Rad. Res. (1995) 22: 375-383
25. Shahidi F and Naczk M. Method of Analysis and Quantification of Phenolic Compounds. Technomic Publishing Company, Lanchester (1995) 287-293

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