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RESEARCH ARTICLE

EVALUATION OF ANTIOXIDANT AND ANTIINFLAMMATORY PROPERTIES OF THE TRADITIONAL MEDICINAL PLANT, *CRYPTOLEPIS GRANDIFLORA* WIGHT (APOCYNACEAE)

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ABSTRACT

To evaluate the antioxidant and antiinflammatory effects of methanolic leaf extract of the traditional medicinal plant, *Cryptolepis grandiflora*. The plant leaves were subjected to solvent extraction by using methanol for obtaining crude extract. The crude extract (9.12g/ml) was tested for assaying antioxidant properties by total antioxidant ($ABTS^{\bullet+}$), $DPPH^{\bullet}$, reducing power and metal chelating activities. In addition, inhibition of protein denaturation, proteinase activity and the levels of enzymic antioxidants *viz.,* SOD, CAT and GPx were employed to determine antiinflammatory activity. Methanolic leaf extract was determined to have stronger antioxidant activity by ABTS (2321.4±397.9 µmol TE/g extract), DPPH (35.21% - 50 µg/mL and 74.37% - 250 µg/mL and IC_{50} - 92µg/mL), reducing power (0.231 - 300µg/mL 0.843 - 700µg/mL) and metal chelating (20.18% - 500µg/mL and 52.03% - 900µg/mL) assays in comparison to respective standards. In antiinflammatory study, protein denatturation (91.21% - 200 µg/mL and 93.12% - 400µg/mL), proteinase inhibition (90.02% – 200 µg/mL and 87.12% - 400 µg/mL) and the enzymic antioxidants *viz*., SOD, CAT and GPx were found to be significantly reduced ($p<0.05$) the inflammation in animal model. The leaves of *Cryptolepis grandiflora* is a potential antioxidant and antiinflammatory agent.

INTRODUCTION

Reactive oxygen species (ROS) are continuously produced during cell metabolism. Under normal conditions, they are scavenged and converted to nonreactive species by different intracellular enzymatic and non-enzymatic antioxidant system (Hyman *et al.,* 2005). Certain amount of oxidative damage takes place even under normal conditions; however, the rate of this damage increase during aging and other pathological events as the efficiency of antioxidative and repair mechanism decreases, leading to the condition of oxidative stress (Gil *et al.,* 2006). Antioxidant, thus plays a vital role to protect the human body against damage by reactive oxygen species (Lollinger, 1981; Tutour, 1990). Searching of natural antioxidant and antiinflammatory agents from higher plants is most needed for better health care. Many number of unexplored plant species are available in forest areas of India which are being prescribed by the traditional healers (Pullaiah, 2006). Therefore, the present study is aimed to evaluate the antioxidant and antiinflammatory activities of *Cryptolepis grandiflora* leaves. Inflammation causes pain and involves many events like the increase of vascular permeability, granulosites, mononuclear cell migration and granulomatons tissue proliferation (Deepika *et al.,* 2014).

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Despite the clinical importance of steroidal and non-steroidal drugs for antiinflammatory property, a long term administration by them causes severe ulcers and renal disorders (Warden, 2010). Therefore, developing natural antioxidant and antiinflammatory agents from plants continuing interests among researchers. The plant species, *C. grandiflora*, (Apocynaceae) is distributed in the margins of dry deciduous and scrub forests of southern peninsular India. The leaves of this species may be a good source of drugs for antioxidant and antiinflammatory properties as they are being prescribed for the same purpose by the local healers of Erode and Tirupur districts of Tamil Nadu (Pullaiah, 2006). Hitherto, no scientific work has been carried out on these medicinal properties of this species. To address this lacuna, the present study was aimed to screen both *in vivo* and *in vitro* antioxidant and antiinflammatory potentials of *C. grandiflora* leaves.

MATERIALS AND METHODS

Plant material

The leaves of *C. grandiflora* were collected in August 2013 from the deciduous forests of Sathyamangalam division, Eastern Ghats, Tamil Nadu, India. The identification of the plant was authenticated by the taxonomist, Dr. S. Karuppusamy, The Madura College, Madurai and a voucher specimen (0223) was kept at Sri Ganesan Herbarium, The

Madura College, Madurai. The leaves were cleaned and shade dried and then powdered to a coarse consistency for further use.

Extraction

The leaves were made into fine powder of 40 mesh size using the pulverizer separately. Following that, 100g of powder was filled in the filter paper and successively extracted using 500 mL methanol solvent using Soxhlet extractor for 8 – 10 hours continuous reflux (Gafner *et al.,* 1985). Then the extract was filtered through Whatman No.1 filter paper to remove all undissolved matter.

Antioxidant activity

$ABTS^{\bullet+}$ assay

Total antioxidant activity was estimated as per the standard method (Prieto *et al.,* 1999). Two mg/mL of sample was taken and mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Then the reaction mixture was incubated at 95°C for 90 min. Absorbancy of the reaction mixture was read at 635 nm. Ascorbic acid was used as standard. The antioxidant activity was expressed as ascorbic acid equivalents (mg/g of sample).

DPPH free radical scavenging assay

The standard method is used for assaying free radical scavenging activity by ($DPPH^{\bullet}$) (Blios, 1958). Methanolic leaf extract of *C. grandiflora* at various concentrations (50, 100, 150, 200 and 250 µg/mL) was added separately to each 5 mL of a 0.1 mM $DPPH^{\bullet}$ (HiMedia Laboratories Pvt. Ltd., Mumbai) and allowed to stand for 20 min at 27ºC. After incubation, the absorbance of each solution was measured at 517 nm using spectrophotometer. Butylated Hydroxyl Toluene (BHT) was used as standard. The corresponding blank reading was also taken and $DPPH^{\bullet}$ activity was calculated by using the following formula:

IC_{50} was determined by using the software SPSS v.16.

Reducing power activity

Reducing power assay was determined by following standard method (Yildirim *et al.,* 2000). Different concentrations of methanolic leaf extracts (300, 400, 500, 600 and 700µg/ml) were mixed with 1ml of 200mM sodium phosphate buffer and 1ml of 1% potassium ferric cyanide and then incubated at 50°C for 20 minutes. After adding 1ml of 10% trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant was taken out and mixed with 2ml of distilled water and 0.5ml of 1% ferric chloride. After incubation for 10 minutes, the absorbance was measured at 700nm. Higher absorbance of the reaction mixture indicates reducing potential of the sample extracts. Ascorbic acid was used as reference standard.

Metal chelating activity

Metal chelating activity for the methanolic leaf extract of *C. grandiflora* was determined according to the standard method (Dinis, 1994). Various concentrations of the extracts such as 500, 600, 700, 800 and 900 µg/mL were added with 1 mL of 2mM $FeCl_2$ seperately. The reaction was initiated by the addition of 5mM ferrozine (1mL). Absorbance was measured at 562nm after 10min. EDTA was used as standard.

Antiinflammatory activity

***In vitro* method**

Inhibition of protein denaturation

Protein denaturation inhibition was determined by adapting the method (Elias and Rao, 1988). The reaction mixture (0.5 mL) was prepared by combining 0.45mL of bovine serum albumin (5 % aqueous solution) and 0.05 mL of sample extract (200 and 400 µg/mL). The pH of reaction mixture was adjusted to 6.3 using 1N HCl. The test samples were incubated at 37ºC for 20 min. and then heated at 57ºC for 3 min. After cooling the test samples, 2.5 mL of phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm. For control tests, 0.05 mL distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows:

The control represents 100 % protein denaturation.

Proteinase inhibitory activity

Proteinase inhibitory activity was determined by standard method (Brown and Mackey, 1968). The reaction mixtures (2 mL) were prepared by combining 0.06 mg trypsin, 1 mL of 25 mM tris – HCl buffer (pH 7.4) and 1 mL of methanol extract (200 and 400 µg/mL). The mixtures were incubated at 37º C for 5 min. and then 1 mL of 0.8% (w/v) casein was added. The mixtures were incubated for another 20 min additionaly. Two mL of 70% (v/v) perchloric acid was added to terminate the reaction and the cloudy suspension was centrifuged. The absorbance of the supernatant was measured spectrometrically at 280 nm against buffer as blank. The percentage of inhibition was calculated as follows:

***In vivo* methods**

Animals

Male Wistar rats (120 -150 g) were purchased from small animal breeding station at Mannuthy, Thrissur, Kerala, India and were housed in well-ventilated cages and fed with food and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee of Kongunadu Arts and Science College (Reg.No: 659/02/a CPCESEA), Coimbatore, India.

Acute oral toxicity study

Acute toxicity study was carried out as per the guidelines of Organization for Economic Co-operation and Development (OECD, 2001) 423. Rats were divided into one control group and 7 treated groups (6 animals per group). First group served as control and was treated with normal water. Group 2, 3, 4, 5, 6, 7 and 8 were orally treated respectively with a dose of 50, 150, 300, 500, 1000, 2000 and 3000mg/kg body weight (b.w.)

of methanolic leaf extract of *C. grandiflora.* Observations were made for mortality and clinical signs.

Carrageenan induced paw edema

The antiinflammatory activity of methanolic leaf extract of *C. grandiflora* was investigated in carrageenan induced inflammatory model as per standard method (Vasudevan *et al.,* 2016). For the experiment, the male wistar rats were divided into five groups (n = 6). The animals were fasted overnight prior to the start of the experiment. The first normal group of rats and second group of induced rats received distilled water, while the third induced group was administered with the test extract at the dose of 200 mg/Kg per day p.o. The fourth induced groups were administered with the methanol extracts of *C. grandiflora* at the dose of 400 mg/Kg per day p.o, while the fifth group was treated with the standard drug, indomethacin (10 mg/Kg p.o.). After one hour of the treatment, acute inflammation was produced in the Groups II -V by the subplantar administration of 0.1 mL of 1 % carrageenan (CGN) in the right hind paw of the rats. The thickness (mm) of the paw was measured immediately at 30 min interval for 4 hrs after the carrageenan injection by using vernier caliper.

At the end of experimental regimen, all the animals were subjected to mild diethyl ether anesthesia. Blood was then collected by cardiac puncture and allowed to clot for 20 – 30 min and centrifuged in a refrigerated centrifuge (4º C) at 3000 rpm for 10 min. Fresh serum samples were used to estimate various parameters *viz.,* superoxide dismutase, catalase and glutathione peroxidase.

Determination of enzymic antioxidant status

Estimation of superoxide dismutase activity (SOD)

The SOD activity was studied by following standard method (Marklund and Marklund, 1974). The reaction mixture consisted of 100 µl of the serum and 0.05 mL of pyrogallol solution was made upto the volume, 2.5 mL with tris-HCl buffer, and absorbance was read at 420 nm against reagent blank. A unit of enzyme was defined as the amount which inhibits the reaction by 50%. Specific activity was expressed as 50% inhibition of nitrite /min/mg/mL.

Estimation of catalase activity (CAT)

The CAT activity was estimated by standard method

Table 1. ABTS and DPPH radical scavenging activity of methanolic leaf extract of *Cryptolepis grandiflora*

ABTS radical scavenging activity			
Sample	Total antioxidant activity (µmol TE/g extract)		
Leaf (2mg/mL)	2321.4± 397.9		
DPPH radical scavenging activity			
Sample concentration (µg/ml)	Percentage activity	IC_{50} of sample (µg/ml)	IC_{50} of BHT
50	35.21± 0.40	92	34.74 ±00.26
100	53.02± 0.61		
150	62.87± 1.61		
200	70.06± 1.04		
250	74.37± 0.81		

Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Table 2. Reducing power activity of methanolic leaf extract of *Cryptolepis grandiflora*

S. No.	Sample concentration (µg/mL)	Leaf extract (Absorbance at 700nm)	Sample concentration (µg/mL)	Ascorbic acid (Absorbance at 700 nm)
1.	300	0.231^{a} ±0.05	20	0.417^{a}±0.03
2.	400	0.331^{b} ±0.02	40	0.648^{b}±0.02
3.	500	0.462^{c} ±0.51	60	0.856^{c}±0.01
4.	600	0.724^{d} ±0.21	80	1.098^{d}±0.05
5.	700	0.843^{e}±0.12	100	1.393^{e}±0.02

EDTA reference standard,
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Table 3. Metal chelating activity of methanolic leaf extract of *Cryptolepis grandiflora*

S. No.	Sample concentration (µg/ml)	% of inhibition	
		Leaf extract	EDTA
1.	500	20.18^{a} ± 0.89	50.08^{a} ± 0.19
2.	600	32.02^{b} ± 0.78	62.55^{b} ± 0.33
3.	700	44.36^{c} ± 0.56	72.06^{c} ± 0.20
4.	800	47.57^{c}± 0.56	85.09^{d} ± 0.21
5.	900	52.03^{d}± 0.56	92.69^{e} ± 0.15

EDTA reference standard,
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Table 4. Inhibitory effect of the methanolic leaf extract of Cryptolepis grandiflora on protein denaturation and proteinase enzyme

Sample concentration (µg/ml)	Inhibition of protein denaturation (%)	Proteinase inhibition (%)
200	91.21± 2.02	90.02± 1.33
400	93.12± 1.45	87.12±1.69

Values were performed in triplicates and represented as mean ± SD

Table 5. Paw thickness in the control and experimental rats at 30 min time intervals for 4 hours

Group *	Time (min)								
	0	30	60	90	120	150	180	210	240
I	4.11±0	4.11±0	4.11±0	4.11±0	4.11±0	4.11±0	4.11±0	4.11±0	4.11±0
II	4.26±0.31	5.34±0.21	6.57±0.19	7.30±0.27	7.09±0.29	6.99±0.23	6.95±0.11	6.95±0.28	6.94±0.37
III	4.89±0.29	5.34±0.23	6.27±0.49	6.90±0.33	6.48±0.2	6.12±0.19	5.91±0.21	5.69±0.22	5.54±0.39
IV	4.50±0.21	5.29±0.34	5.96±0.44	5.92±0.29	5.55±0.21	5.21±0.25	5.00±0.17	4.89±0.28	4.67±0.25
V	4.59±0.45	4.98±0.39	5.23±0.48	5.47±0.46	5.02±0.41	4.91±0.27	4.75±0.42	4.68±0.38	4.62±0.46

*Group I - Control, Group II – Induced, Group III – Low dose plat extract of Induced, Group IV – High dose plant extract of Induced, Group V- Standard
Values were performed in triplicates and represented as mean ± SD.

Table 6. Effect of methanolic leaf extracts of *Cryptolepis grandiflora* on paw thickness in carrageenan induced rats

Group	Enzymic Parameter			
	Initial paw thickness (mm)	Paw thickness after 4h (mm)	Increase in paw thickness (mm)	Inhibition (%)
I	4.11±00	4.11±0	0	-
II	4.26±0.31	6.94±0.37	2.68 ± 0.82	62.91 ±0.82
III	4.89±0.29	5.54±0.39	0.65 ±0.08	13.29 ±0.82
IV	4.50±0.21	4.67±0.25	0.17 ±0.02	3.77 ±0.82
V	4.59±0.45	4.62±0.46	0.03 ±0.02	0.65 ±0.16

Values were performed in triplicates and represented as mean ± SD.

Table 7. Enzymic antioxidant status in the control and experimental rats

Group	Enzymic Parameter		
	SOD (50% inhibition of nitrite /min/mg/mL)	CAT (µmoles of H_2O_2 decomposed/min/mL)	GPx (µmoles of glutathione oxidised/min/mL)
I	4.18± 0.39	60.67± 0.22	24.01± 1.86
II	1.08 ± 0.06	32.54± 0.48	11.61± 1.44
III	2.25 ± 0.31	41.97± 0.18	13.50± 1.26
IV	4.39 ± 0.07	61.22± 0.22	24.91± 1.68
V	3.02 ± 0.07	53.74± 1.55	21.95± 1.72
VI	4.62 ± 0.07	61.20± 0.19	24.54± 1.99

Values are expressed as mean±SD. (n=6).

(Sinha, 1972). The reaction mixture of 1.5 mL was made by mixing 1 mL of 0.01 M phosphate buffer (pH – 7.0), 0.1 mL of enzyme preparation and 0.4 mL of 2 M hydrogen peroxide. The reaction was stopped by the addition of 2.0 mL of dichromate acetic acid reagent. The tubes were kept in a boiling water bath for 10 min and cooled. The absorbance was spectrophotometrically measured at 620 nm. A system devoid of enzyme served as control. Activity of catalase was expressed as µmoles of H_2O_2 decomposed/min/mL.

Estimation Glutathione Peroxidase (GPx)

The GPx was estimated as per the method previously described (Ellman, 1959). To 0.4 mL of buffer, 0.2 mL of EDTA, 0.1 mL of sodium azide, 0.2 mL of reduced glutathione and 0.1 mL of H_2O_2 were added separately to two test tubes labelled as test (T) and control (C). To the test tube, T added 0.2 mL of sample and to the test tube,C added 0.2 mL of water. The contents were mixed well and incubated at 37º C for 10 min. The reaction was arrested with the addition of 0.5 mL of 10 % TCA. To determine the glutathione content, 1 mL of supernatant was removed by centrifugation. To that supernatant, 3 mL of buffer and 0.5 mL of Ellman's reagent were added. The colour developed was read at 412 nm. The activity was expressed in terms of µmoles of glutathione oxidised/min/mL.

RESULTS

In vitro antioxidant activity

Total antioxidant assay (ABTS)

The $ABTS^{\bullet+}$ assay is based on the inhibition of the absorbance of radical cation, $ABTS^{\bullet+}$ which has a characteristic wavelength at 635 nm by antioxidants. This study reports that the leaf part of *C. grandiflora* has the antioxidant activity of 2321.4±397.9 µmol TE/g extract (Table 1).

DPPH radical scavenging activity

The percentage of scavenging effect on the DPPH radical was increased with the increase in the concentration of extract from 50 -250 µg/mL (Table 1). The percentage of inhibition of DPPH radical was varying from 35.21% to 74.37% by 50 µg/mL and 250 µg/mL of the extract respectively. The IC_{50} value of the methanolic leaf extract was determined to be 92µg/mL.

Reducing power assay

The absorbance values of reducing power assay was determined to be increased with the increase in the concentration of leaf extract of *C.grandiflora* from 300 to 700µg/mL (Table 2). The reducing ability was varying from

0.231 absorbance for 300µg/mL of extract to 0.843 absorbance for the 700µg/mL extract. However, the activity was lesser than the standard (ascorbic acid) (absorbance 1.39 at 700µg/ml).

Metal chelating activity

The percentage of metal chelating activity was determined to be increased with the increase in the concentration of extract from 500 to 900µg/mL (Table 3). The percentage of inhibition of the metal chelation was varying from 20.18% (caused by 500µg/mL of extract) to 52.03% (caused by 900µg/mL extract) which are not comparable to that of the standard, EDTA.

Antiinflammatory activity

***In vitro* study**

Inhibition of protein denaturation and proteinase inhibition

Two different dose levels of methanolic leaf extract of *C. grandiflora viz.,* 200 and 400µg/mL provided significant protection against denaturation of protein (Table 4). The percentage inhibition of protein denaturation varied from 91.21%, caused by 200µg/mL of extract to 93.12%, caused by 400µg/mL extract. It also exhibited significant proteinase inhibitory activity (200 and 400µg/mL extracts inhibited the proteinase level as 90.02%, and 87.12% respectively).

***In vivo* study**

Acute oral toxicity study

Acute toxicity study showed that the crude extracts possessed high safety profile as no death occurred at any of the doses tested, indicating fairly high margins of safety.

Carrageenan induced paw edema

The changes in the rat paw edema thickness as influenced by the methanolic extracts of *C. grandiflora* are presented in Tables 5 and 6. The paw thickness of the normal rats (Group I) was found to be 4.11 mm. The paw volume of Group II was increased from 4.26 to 6.57 mm at 60 th min, 7.09 mm at 120 th min, 6.95 mm at 180 th min and then to 6.94 mm at 240 th min in carrageenan induced rats. The increase in paw thickness over a period of 4 h indicates the release of first and second stage mediators of inflammation. Upon pretreatment of the animals with the extracts (Group III) of *C. grandiflora* at 200 mg/kg b.wt. one hour prior to induction, the paw thickness was found to be 4.89 mm at initial stage and it was 6.27 mm at 60th min, 6.48 mm at 120th min, 5.91 mm at 180th min and 5.54 mm at 240th min after induction. The group IV rats, one hour prior to induction pretreated with the extracts of *C.grandiflora* at 400 mg/kg b. wt. exhibited the paw thickness, 4.50 mm and 5.96 mm at 60 th min, 5.55 mm at 120th min, 5.00 mm at 180 th min and 4.67 mm at 240 th min. In the standard drug pretreated group (V), one hour prior to induction, the paw thickness was found to be 4.59 mm, and 5.23 mm at 60 th min and 4.62 mm at 240 th min. The increase in paw thickness upon carrageenan induction at 0.1 mL was found to be significantly reduced upon pretreatment of the animals with 400 µg/mL of the extracts, the thickness was considerably minimized upto 0.17 mm in comparison to group II rats that were carrageenan induced control group (Tables 5 and 6). It was comparable to that of the pretreated animal with the standard drug, indomethacin. This considerable decrease in paw thickness upon treatment of extracts indicates the presence of effective antiinflammatory agents in the extracts.

Determination of enzymic antioxidant status

Effect of *C. grandiflora* extracts on serum antioxidants

In the present study, the enzymic antioxidants (SOD, CAT and GP_x) were found to be significantly ($p<0.05$) reduced in the inflammation induced animals, while the same was elevated on treatment with methanolic leaf extracts of *C. grandiflora* (200 and 400 mg/kg b. wt.) and the standard, indomethacin (Table 7). The activity of the enzyme, SOD was found to be decreased from 4.18 (50% inhibition of nitrite/min/mg/Ml) in group I of normal rats to 1.08 (50% inhibition of nitrite /min/mg/mL) in group II of carrageenan induced rats. By the pretreatment of the animals with the extracts of *C.grandiflora* at 200 and 400 mg/kg b. wt., the activity rose upto 2.25 (Group III) and 3.02 (Group V) (50% inhibition of nitrite/min/mg/mL) (Table 7). The activity of the CAT enzyme was also found to be decreased from 60.67 to 32.54 µmoles of H_2O_2 decomposed/min/mL in the carrageenan induced group of animals. For the pretreated animals with the extracts of *C. grandiflora* at 200 and 400 mg/kg b. wt., the activity rose upto 41.97 and 53.74 µmoles of H_2O_2 decomposed/min/mL (Table 7). In the induced group II, the GPx enzyme activity was 11.61 µmoles of glutathione oxidised/min/mL. Pretreatment with the leaf extracts of *C. grandiflora* at 200 mg/kg b. wt. resulted in the increase of activity to 13.50 µmoles of glutathione oxidised/min/mL and in 400 mg/kg b. wt. it was increasing to 21.95µmoles of glutathione oxidised/min/mL. Increase in activity by the standard drug, indomethacin treatment was also observed (Table 7).

DISCUSSION

***In vitro* antioxidant activity**

The main functions of antioxidants are to neutralize the free radicals, which routinely produced in the biological system. Reactive oxygen species (ROS) readily combine and oxidize biomolecules such as carbohydrates, proteins and lipids and thus making them inactive with subsequent damage to cells, tissues and organs (Krötz *et al.,* 2002). *In vitro* antioxidant activity of the methanolic leaf extracts of *C. grandiflora* evaluated by $ABTS^{\bullet+}$, $DPPH^{\bullet}$, reducing power and metal chelating assays indicates that this species possess effective antioxidant activity (Tables 1 - 3). The major phytochemicals of antioxidant properties *viz.,* flavonoids, alkaloids, terpenoids, phenolics etc, reported in the genus, *Cryptolepis* perhaps be the reason for this property (Paulo *et al.,* 1997). Further, the activity level evaluated has proven the effectiveness of the extract in comparison to that of the reference standard antioxidants, BHT and ascorbic acid. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1 – 1 diphenyl – 2 – picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug (Son and Lewis,

2002). The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. In the present study, the scavenging effect of different concentrations of the extracts on the DPPH radical is depicted in Table 1. The extracts had significant scavenging effect on the DPPH radical while the concentration of extracts was increasing from 50 – 250 µg/mL.

The reducing power of the compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al.,* 1995). The present study shows the abilities of the extracts for causing the reduction of Fe^{3+}/Ferric cyanide complex to ferrous form in a concentration dependent manner (Table 2). The extracts had significant reducing effects (P<0.05) on the reducing power, which was increased with the increase in concentrations from 300 – 700 µg/mL. The presence of transition metal ions in a biological system could catalyse the Haber-Weiss and Fenton type reactions, resulting in the generation of hydroxyl radicals (OH). However, these transition metal ions could form chelates with the antioxidants which results in the suppression of OH generation and inhibition of peroxidation processes of biological molecules (Chew *et al.,* 2009). The methanolic leaf extracts of *C. grandiflora* possess statistically high metal ion scavenging activity in dose dependent way (P<0.05) (Table 3) due to the chelating agents, which form sigma bonds with the metal and effective as secondary antioxidants as they reduce the redox potential, thereby the oxidized form of the metal ion.

Antiinflammatory activity

***In vitro* study**

Denaturation of the protein is one of the causes of inflammation. Several antiinflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation (Grant *et al.,* 1970). Production of auto-antigens in certain inflammatory diseases may be due to *in vivo* denaturation of proteins. From the results of the present study (Table 4), it can be known that the methanolic leaf extract of *C.grandiflora* is capable of controlling the denaturation of proteins in inflammatory diseases. Proteinases have been implicated in inflammatory reactions. It was previously reported that leucocyte proteinases play an important role in the development of tissue damage during inflammatory reactions and proteinase inhibitors provided significant level of protection. The methanolic leaf extracts of *C.grandiflora* exhibited significantly higher proteinase inhibitory activity (Table 4). Phenolic compounds are the ideal antioxidants and act by different mechanisms (Olga blokhina *et al.,* 2003). The phenolic compounds has strong reaction in the genus, *Cryptolepis* and certain other members of Apocynaceae (Paulo and Houghton, 2003; Vinegar *et al.,* 1969) and the same may play pivotal role as antioxidant agents in the study species, *C. grandiflora.*

***In vivo* study**

Carrageenan induced paw edema

The development of edema in the paw of the rat after the injection of carrageenan is due to the release of certain inflammatory mediators, istamine, serotonin and prostaglandin like substances. Significantly greater antiinflammatory activity of the leaf extracts of *C. grandiflora* (200 and 400 mg/kg b.wt.) was determined in the present study which may be attributed to the inhibition of such inflammatory mediators by the presence of indole alkaloid as in the genus, *Crypotolepis* (Paulo *et al.,* 2000) (Tables 5 and 6). From the results, it could be confirmed that the extracts of *C. grandiflora* at the doses, 200 and 400 mg/kg b.wt. posses potent antiinflammatory activity.

Determination of enzymic antioxidant status

Effect of *C. grandiflora* extracts on serum antioxidant status

The antioxidant enzymes *viz.,* SOD, CAT and GPx protect aerobic cells against oxygen toxicity and lipid peroxidation. Generally, SOD, CAT and GPx are found to be decreased in carrageenan induced animals which may be explained due to the production of free radicals (Imadaya *et al.,* 1988). The decrease in SOD activity leads to declined production of hydrogen peroxide. As hydrogen peroxide is the substrate for the enzyme, CAT and GPx, they were also found to be decreased. On drug treatment, the activities of SOD, CAT, GPx are brought to near normal levels which may be explained due to the free radical scavenging activity of phenolic compounds (flavonoids and phenolics) reported in the genus, *Cryptolepis* (Wagner *et al.,* 1984).

Conclusion

It is known from the present study that the leaves of *C. grandiflora* have appreciable level of antioxidant and antiinflammatory activities. Therefore, this species can be used as a source of natural drug for treating inflammatory disorders and thus justifying the prescription of this species by the traditional healers of Erode and Tirupur districts, Tamil Nadu for antiinflammatory activity.

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