IN VITRO ANTIOXIDANT ACTIVITY OF ROOTS OF *THESPESIA LAMPAS* DALZ AND GIBS

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ABSTRACT

The free radical scavenging potential of roots of *Thespesia lampas* Dalz and Gibs was studied by different antioxidant models. Free radicals are implicated for more than 80 diseases including Diabetes mellitus, arthritis, cancer, ageing etc. In the treatment of theses diseases, antioxidant therapy has gained an utmost importance. Current research is directed towards finding naturally occurring antioxidant of plant origin. In Indian system of medicine, *Thespesia lampas* is an important medicinal plant and its root juice has been used in various ailments and as health tonic. To understand the mechanisms of pharmacological actions, the *in vitro* antioxidant activity of aqueous extract of *Thespesia lampas* was investigated for the activity of scavenging superoxide anion radicals, nitric oxide radical and lipid peroxidation assay. In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals, metal chelating, reducing power or inhibition of lipid peroxidation. These results clearly indicate that *Thespesia lampas* is effective against free radical mediated diseases.

Keywords: Antioxidant, nitric oxide, Lipid peroxidation, superoxidation, *Thespesia lampas.*

INTRODUCTION

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals (Gutteridgde, 1995). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Tiwari, 2001). The most common reactive oxygen species (ROS) include superoxide, anion, hydrogen peroxide (H202), peroxyl (ROO^-) radicals, and reactive hydroxyl (OH^-) radicals. The nitrogen derived free radicals are nitric oxide (NO_2) and peroxynitrite anion ($ONOO^-$). ROS have been implicated in over a hundred of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (Joyce, 1987). In treatment of these diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage by free radical and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (Buyukokuroglu, 2001). Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability (Auudy, 2003). Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc (Miller, 1996). They were also suggested to be a potential iron chelator (Boyer, 1998; Harsteen, 1983). Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties. Roots of *Thespesia lampas* Dalz and Gibs (family Malvaceae) are used as Gonorrhea and syphilis (The Wealth of India, 1976), anti-microbial (Vasaraj, 1997) and hepatoprotective (Sangameswaran, 2008).

Plant material

The plant was collected from the foot hills of Yercaud, Salem, in the month of September 2005. The plant was identified and authenticated by Dr P. Jayaraman, Professor in Botany, Plant Anatomy Research, Center, Chennai, Tamil, Nadu India. A voucher specimen (Herbarium No: RTL-58) has been kept in our institute museum for future reference.

MATERIALS AND METHODS

Chemicals

Nitro blue tetrazolium (NBT), ethylene diamine tetra acetic acid (EDTA), sodium nitroprusside (SNP), trichloro acetic acid (TCA), thio- barbituric acid (TBA) and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

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Anti-oxidant activity

Nitric oxide scavenging activity assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions. This can be determined by the use of the Griess Illosvoy reaction 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 250C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 546 nm was measured with a spectrophotometer (fig. 1). The nitric oxide radicals scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A0-A1) / A0 \times 100)$$

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

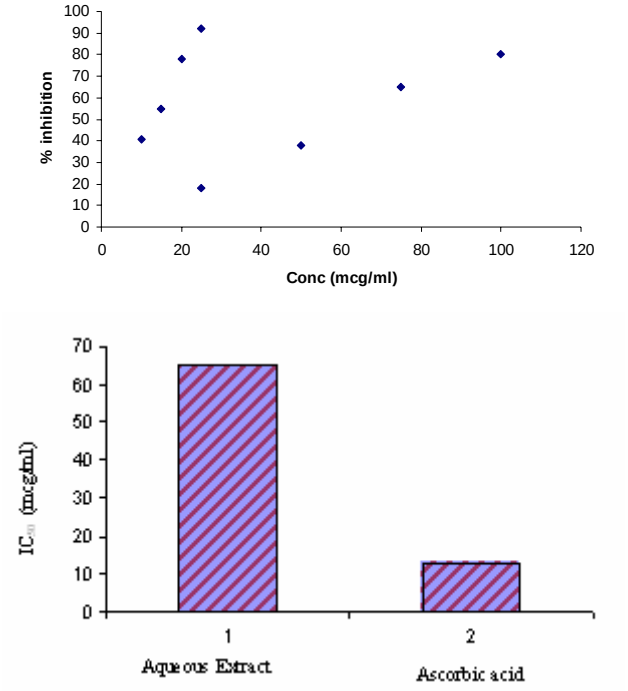

Fig. 1: Nitric oxide radical scavenging of *T. lampas*

Determination of the scavenging of superoxide radicals

The scavenging activity of the *T. lampas* Dalz and Gibs towards superoxide anion radicals was measured by the method of Liu, Ooi, and Chang (Liu, 1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMSNADH) system through the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 ml of Tris- HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 µM) solution, 0.75 ml of NADH (936 µM) solution and 0.3 ml of different concentrations of the extract. The reaction was initiated by adding 0.75 ml of PMS (120 µM) to the mixture (fig. 2). After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = (A0-A1) / A0 \times 100)$$

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

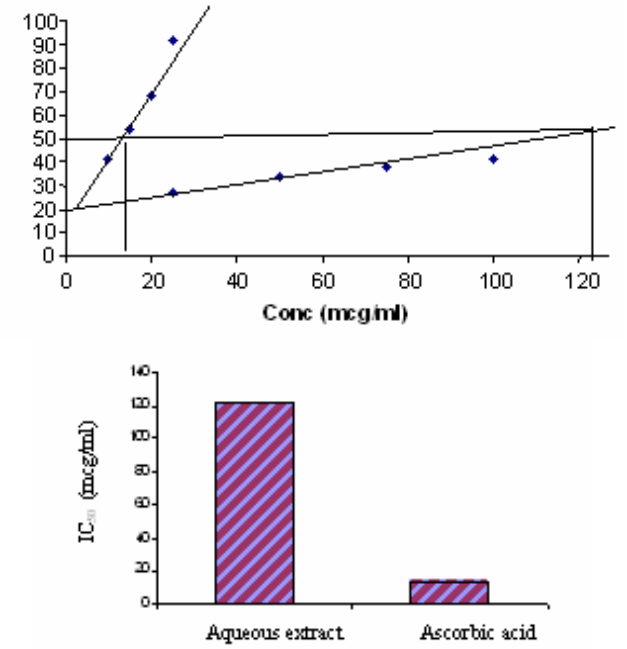

Fig. 2: Super oxide radical scavenging *T. lampas.*

Anti-lipid peroxidation activity

The extract of lipid peroxidation in rat liver homogenate was measured *in vitro* in terms of formation of thiobarbituric acid reactive substances. Different concentration of the extract (25-100 µg/ml) was made up with water. The aqueous extract was expressed in terms of dry weight (mg/ml) in water. These samples were individually added to the liver homogenate (0.5 ml). This mixture was incubated with 0.15 *M* KCL (100µg/ml). Lipid peroxidation was initiated by adding 100 µl of 15 m *M* $FeSO_4$ solution. The reaction mixture was incubated at 37^{o}C for 30 min. an equal volume of TBA: TCA (1 : 1, 1 ml) was added to the above solution followed by the addition of 1 ml BHT. This final mixture was heated on a water bath for 20 min at 80^{o}C and cooled, centrifuged and absorbance read at 532 nm using a spectrophotometer

Table 1: *In vitro* antioxidant activity of aqueous extract of roots of *T. lampas*

Group	Conc (µg/ml)	% Inhibition		
		Nitric oxide	Super oxide	Lipid peroxidation
Aqueous extract of *T. lampas*	25	17.85 ± 0.23**	27.24±.1.70**	21.16±0.38**
	50	38.11 ± 0.42**	33.42±1.37**	43.78±0.47**
	75	64.95 ± 0.50**	38.13±1.04**	73.67±0.96**
	100	80.07 ± 0.64**	41.04±0.44**	84.73±0.25**
Ascorbic acid	10	40.74±0.41	41.48±0.84	47.07±0.16
	15	54.91±0.35	53.52±1.42	62.60±0.52
	20	78.24±0.21	68.30±0.65	88.19±0.33
	25	91.84±0.35	91.84±0.35	94.13±0.463

Result expressed as mean ± SEM from six observation $^{**}P<0.01$
Data were analyzed by one way ANOVA followed by Dunnette's Multiple Comparison test

(Shimadzu 160 IPC) (fig. 3). The percentage inhibition of lipid peroxidation was calculated by comparing the results of test with those of controls not treated with the extract as per the formula:

$$\text{Inhibition (\%)} = \frac{\text{(Control – Test)}}{\text{Control}} \times 100$$

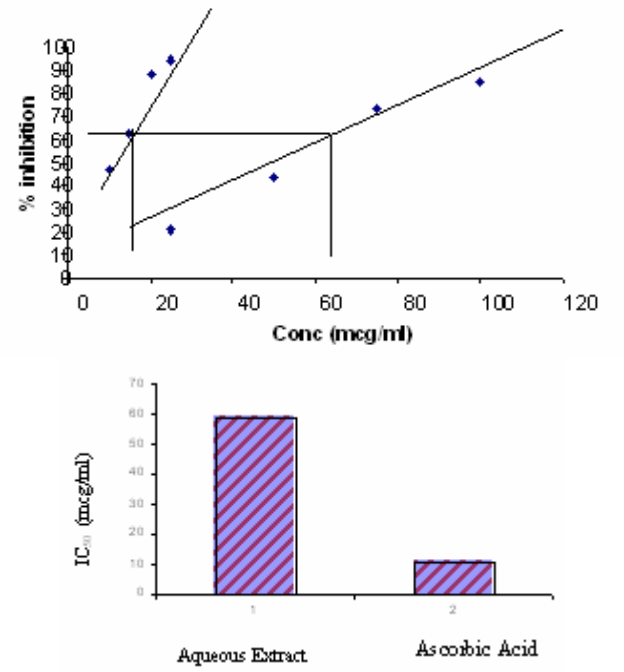

Fig. 3: Anti-lipid peroxidation activity *T. lampas.*

Determination of ascorbic acid (vitamin C)

5 g of the sample was weighed into an extraction tube and 100 ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture was shaken for 30 min, and transferred into a centrifuge tube and centrifuged at 800g for about 20 min. It was transferred into a 100 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipetted into a volumetric flask and 1% starch indicator was added and titrated with 20% CuSO4 solution to get a dark end point (Baraka, 1973). The amount of vitamin C was calculated as mg/100 ml of plant sample.

RESULTS

Several concentrations, ranging from 25-100 µg/ml of the aqueous extract of *T. lampas* were tested for their antioxidant activity in different *in vitro* models. The percentage of inhibition was observed that free radicals were scavenged by the test compounds in a concentration dependent manner upto the given concentration in all the models (table 1).

The percentage of inhibition in nitric oxide in different concentration like 25, 50, 75, 100 µg/ml were observed in 17.85 ± 0.23, 38.11 ± 0.42, 64.95 ±0.54 and 80.07 ± 0.64 respectively where as the percentage inhibition of ascorbic acid in concentration like 10, 15, 20, 25 µg/ml were found to be 40.74±0.41, 54.91± 0.35, 78.24±0.21 and 91.84 ± 0.35 respectively.

Superoxide free radicals were scavenged in concentration like 25, 50, 75, 00 µg/ml were observed in 27.24 ±1.70, 33.42±1.37, 38.13±1.04 and 41.04±0.44 respectively where as the percentage inhibition of ascorbic acid in concentration like 10, 15, 20, 25 µg/ml were found to be 41.48±0.84, 53.52±1.42, 68.30±1.42, 68.30±0.65 and 91.84±0.35 respectively.

Anti-lipidperoxidation free radicals were scavenged in concentration like 25, 50, 75 µg/ml were observed in 21.16±0.38, 49.78±0.47, 73.67±0.96 and 84.73±0.25 respectively. However, the extract showed encouraging response in Ic_{50} values were given table 2.

Table 2: Data showing Ic_{50} values

IC_{50} values (µg/ml)	Aqueous extract of roots of *T. lampas*	Ascorbic acid
Nitric oxide	65	13
Super oxide	121	14
Lipid peroxidation	59	11

DISCUSSION

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases and inflammatory conditions cancer and ageing (Marx, 1987). Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid per oxidation and by many other mechanisms and thus prevent disease (Braugghler, 1986).

The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. Initiation of lipid per oxidation by ferrous sulphate takes place either through ferryl-perferryl complex (Gutteridge, 1985) or through $^{-}$OH radicals by Fenton reaction (Halliwell, 1978) thereby initiating a cascade of oxidative reactions. The results obtained in the present studies may be attributed to several reasons *viz*, the inhibition of ferryl- perferyyl complex formation; scavenging of OH or superoxide radicals or by changing the ratio of Fe^{3+}/ Fe^{2+}; reducing the rate of conversions of ferrous to ferric or by chelating of the iron itself (Braugghler, 1986). The moderate activity of the extract may probably be due to the rapid and extensive degradation of the antioxidant principles in an *ex vivo* state thereby corroborating the finding that was observed in a study carried out in Australia with a group of human volunteers (Recard, 2000). It is also known that the $^{-}$OH radical which initiates lipid peroxidation has a difficult to investigate by conventional methods (Pryor, 1986).

Nitric oxide is a free radicals product in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases (Ialenti, 1993). In the present study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25^0 C was reduced by the aqueous extract of *T. lampas*. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite.

Superoxide dismutase catalyses the dismutation of the highly reactive superoxide anion to oxygen and hydrogen peroxide (Kamalakkannan, 2003). Superoxide anion is the first reduction product of oxygen (Ray, 2002). This is measured in terms of inhibition of generation of O_2.

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