

In Vitro Antioxidant Activity of Plant *Pongamia Pinnata* (Linn.)

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ABSTRACT

DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colour stoichiometrically with the number of electrons consumed, which is measured spectrophotometrically at 517 nm. As shown in *Pongamia pinnata* Linn. extract strongly scavenged DPPH radical with the IC₅₀ being (0.4157 mg/ml). The scavenging was found to be dose dependent. The total antioxidant capacity of the extract was calculated based on the formation of the phosphomolybdenum complex which was measured spectrophotometrically at 695 nm. The total antioxidant capacity of the extract was found to be 0.3299 nmol/g ascorbic acid. The total antioxidant capacity of the Ethanolic (50%) and water extract measured spectrophotometrically at 695 nm based on the formation of the phosphomolybdenum complex was found to be 0.3299 nmol/g ascorbic acid in 50% Hydroalcoholic & 4.5950 nmol/g of ascorbic acid in Water.

Key word:- Antioxidant, *Pongamia pinnata* Linn, Ethanolic extract

1. INTRODUCTION

Variety of reasons has been cited for the need for studying medicinal plants. Most of the traditional knowledge about medicinal plants was in the form of oral knowledge that had been lost with persistent invasions and cultural adaptations. There was no uniform or standard procedure for maintaining the inventory of these plants and the knowledge about their medicinal properties. There is a prevalence of using plants and plant based products in various contemporary and traditional systems of medicines, without any written documentation or regulation. Therefore, it is essential that such uses of natural products be documented and studied for systematic regulation and wide-spread application. These include comminuted or powdered herbal substances, tincture, extracts, essential oils, expressed juices and processed exudates. Originally an Indo-Malaysian species, it is now found in many countries. *Pongamia pinnata* (Linn.) Pierre (Synonyms: *Pongamia glabra* Vent., *Derris indica* (Lam.) Bennet, *Cytisus pinnatus* Lam.) is a member of the Fabaceae family (Papilionaceae; Leguminosae). *Pongamia pinnata* is a medium-sized, glabrous, semi-evergreen tree, growing up to 18 meters or more in height, with a short bole, spreading crown, and grayish-green or brown bark.

Pongamia pinnata (*Pongamia*)

pinnata (synonyms: *Millettiapinnata*, *Cytisus pinnatus*, *Derris indica*, *Pongamia glabra*; common names: Karanj, Indian Beech Tree, Honge Tree, Pongam Tree) is a perennial oleaginous legume (Leguminosae) with nitrogen-fixing capability and medicinal properties. This plant is native to the Indian subcontinent and grows on marginal land with no direct competition with food crops. It can thrive in areas with annual rainfall ranging from 500 to 2500 mm with the maximum temperature ranging from 27 to 38 °C and the minimum from 1 to 16 °C (Sangwan et al., 2010). Mature trees can withstand waterlogging, slight frost, and high salinity. It is used to control soil erosion and for binding dunes because of its dense network of lateral roots.

3. MATERIALS AND METHODS

Antioxidant studies Ohkawa et al., (1979)

The ethanolic (50%) extract of seeds of *Pongamia pinnata* Linn. was used for the evaluation of antioxidant activity.

IN VITRO STUDIES

DPPH scavenging activity R. Govindraj et al., (2006)

DPPH scavenging activity or the Hydrogen donating capacity was quantified in presence of stable DPPH radical on the basis of Blois method (Blois, 1958). Briefly, to a methanolic solution of DPPH (100 µM, 2.95 ml), 0.05 ml of test compounds dissolved in

methanol was added at different concentration (2 -10 mg/ml). Reaction mixture was shaken and absorbance was measured at 517nm at regular intervals of 30 seconds for 5 minutes, and the reading was taken till 20 min. Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract. Shown in table no.5

$$\text{Scavenging effect (\%)} = (1 - B/A) \times 100$$

Where A = Absorbance of DPPH control with solvent (517nm)

B = Absorbance of decolorized DPPH in presence of test sample (517nm)

Total antioxidant capacity

Total antioxidant capacity was measured according to spectrophotometric method of Preito, ml of the extract (10mg/ml) dissolved in water was combined in an eppendorf tube with 1ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695nm against a blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid. Shown in table no.5

4. RESULT & DISCUSSION

IN VITRO ANTIOXIDANT

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defenses. In most diseases, increased oxidant formation is a consequence of the disease activity. Potential antioxidant therapy therefore should include either natural free radical scavenging antioxidant principles or agents, which are capable of augmenting the activity of the antioxidant enzymes. ROS are capable of damaging biological macromolecules such as DNA, carbohydrates or proteins. ROS is a collective term, which includes not only the oxygen radicals (O_2 , and OH^\cdot) but also some non-radical derivatives of oxygen these include H_2O_2 , $HOCl$ and ozone (O_3). If human disease is believed to be due to the imbalance between oxidative stress and antioxidative defense, it is possible to limit oxidative tissue damage and hence prevent disease progression by antioxidant defense supplements. DPPH is a stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colour stoichiometrically with the number of electrons consumed, which is measured spectrophotometrically at 517 nm. As shown in **Table No.5**, *Pongamia pinnata* Linn. extract strongly scavenged DPPH radical with the IC_{50} being (0.4157mg/ml). The scavenging was found to be dose dependent. The total antioxidant capacity of the extract was calculated based on the formation of the phosphomolybdenum complex which was measured spectrophotometrically at 695 nm. The total antioxidant capacity of the extract was found to be 0.3299 nmol/g ascorbic acid. Thus establishing the extract as an antioxidant. Photochemical apparatus and method allowed precise as well as time and cost effective determination of the integral antioxidative capacity of the *Pongamia* extract. Free radicals are generated in the instrument by means of photo sensitizer. The free radical thus generated were detected by their reaction with a chemiluminescent substance. Luminol acts both as photosensitizer as well as the detecting reagent. In the presence of radical scavengers in the extract the intensity of the PCL was attenuated as a function of concentration. In this way the antioxidative capacity of the extract could be quantified. The antioxidative capacity was found to be 0.99557 nmol ascorbic acid/gequivalents. The Change in colour of DPPH is directly proportional to the amount of antioxidant present in the reaction mixture (antioxidant reacts with stable free radical i.e DPPH) and the 50% Hydroalcoholic extract was found to have active free radical scavenging activity increase from 14.7108% (100µg/ml) to 88.1802 % (800µg/ml).

TableNo:5AntioxidantactivityofdifferentextractofdriedseedsofPongamiapinnataLinn.

<	DPPHScavengeing%				
	Butanol	Chloroform	Water	50%Hydroalcohol	Ascorbic Acid
100	3.0612	3.9115	11.9528	14.7108	20.4081
200	15.4761	17.9421	13.2996	17.7721	25.8503
400	26.8707	20.8333	25.4208	57.4829	69.8129
800	32.0578	48.3843	56.4625	88.1802	93.9625
I.C50(mg/ml) (at20min.)	0.9945	0.9823	0.7080	0.4157	0.396884

TotalAntioxidant

The total antioxidant capacity of the Ethanolic (50%) and water extract measured spectrophotometrically at 695 nm based on the formation of the phosphomolybdenum complex was found to be 0.3299 nmol/ g ascorbic acid in 50% Hydroalcoholic & 4.5950 nmol/g of ascorbic acid in Water.

5. CONCLUSION

Thus establishing the extract as an antioxidant. Photochem apparatus and method allowed precise as well as time and cost effective determination of the integral antioxidative capacity of the pongamia extract. Free radicals are generated in the instrument by means of photosensitizer. The free radicals thus generated were detected by their reaction with a chemiluminescent substance. Luminol acts both as photosensitizer as well as the detecting reagent. In the presence of radical scavengers in the extract the intensity of the PCL was attenuated as a function of concentration. In this way the antioxidative capacity of the extract could be quantified. The antioxidative capacity was found to be 0.99557 nmol ascorbic acid/gequivalents. The Change in colour of DPPH is directly proportional to the amount of antioxidant present in the reaction mixture (antioxidant reacts with stable free radical i.e. DPPH) and the 50% Hydroalcoholic extract was found to have active free radical scavenging activity increase from 14.7108% (100 µg/ml) to 88.1802 % (800 µg/ml).

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