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ORIGINAL RESEARCH ARTICLE

Comparative *Invitro* Antioxidant Activty of *Pongamia pinnata* Linn. Leaves Extracts and Isolated Compound

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ABSTRACT

The word herb, as used in herbal medicine, is also known as botanical medicine or as phytotherapy or phytomedicine which means a plant or plant part is used to make medicine to assist in the healing process during illness and disease. *Pongamia pinnata* Linn. Pierre commonly known as 'Karanj' has been used in different system of traditional medicines for the treatment of various diseases. It contains various phytoconstituents belonging to alkaloids, glycosides, flavonoids, fixed oils and carbohydrates. The roots of *Pongamia pinnata* are good for cleaning foul ulcers, cleaning teeth, strengthening gums and gonorrhoea. The root paste is used for local application in scrofulous enlargement. The fresh bark of *Pongamia pinnata* is sweet and mucilaginous to taste, soon become bitter and acrid. It is anthelmintic and is useful in beriberi, ophthalmology, dermatopathy, vaginopathy and ulcers. Leaves of *Pongamia pinnata* are digestive, laxative, anthelmintic and are good for diarrhoea, leprosy, dyspepsia and cough. A hot infusion of leaves is good for cleaning ulcers and wounds. Flowers are useful to quench dipsia in diabetes and for alleviating *vata* and *kapha*. The seeds are anthelmintic, bitter, acrid, haematinic and carminative. They are useful in inflammation, chronic fevers, anaemia and haemorrhoids. The oil is anthelmintic, styptic and recommended for ophthalmia, leprosy, ulcers, herpes and lumbago. Its oil is a source of biodiesel. Our aim with this study was to evaluate the antioxidant properties of *Pongamia pinnata.* The antioxidant activity of isolated compound and various extracts of *Pongamia pinnata* was evaluated *in vitro* by DPPH free radical scavenging activity. Our results showed that *Pongamia pinnata* displayed potent antioxidant properties, supporting the ethnomeidcal use given to this plant for treatment of diseases.

Keywords: Antioxidant; DPPH; Free radical scavenging; *Pongamia pinnata*

INTRODUCTION

The role of free radicals and reactive oxygen species (ROS) in the pathogenesis of human diseases such as cancer, aging, inflammatory response syndrome, respiratory diseases, liver diseases and atherosclerosis has been widely recognized.[1] Electron receptors such as molecular oxygen, readily react with free radical to become free radicals themselves

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also referred as ROS with chemical species such as super oxide anions (O_2), hydrogen peroxide (H_2O_2) and hydroxyl free radicals (OH) which is known to induce damage to biomembranes, proteins and DNA. [2] A prominent manifestation of free radical activities in biological systems is lipid peroxidation and it is involve in the development of different diseases. The primary targets of free radical attack on lipids are poly unsaturated fatty acids (PUFA). Lipid peroxidation is usually proceed as a chain reaction: alkyl reaction are form during the initiation step by the attack of lipid by free radical or other reactive species, followed in the propogation phase by the formation of alkyl peroxyl (POO) and alkoxyl (RO) radicals and terminating with the formation of lipid hydroperoxide (ROOH). [3] However this product readily decomposed to other relatively more stable substances such as aldehydes (Malondealdehyde, hydroxynonenal, dienols, etc.) are isoprostanes, which has been used to assess lipid peroxidation *in vivo*. Among the numerous analytical approaches for the estimation of oxygen radicals mediated damage in biological systems determination of malondialdehyde (MDA) as one of the major aldehyde species, has been employed more frequently. The main method utilize is the reaction of MDA with thiobarbituric acid (TBA). The reaction of TBA with MDA and linked chromgens to lipo peroxide in biomaterial, result in the well known method, "thiobarbituric acid reactive substances" (TBARS). Malondialdehyde is three carbon molecules that constitute one of the major secondary decomposition products of peroxides PUFA. MDA is inferred to be cytotooxic and it has been found at elevated levels in various diseases thought to be related to free radical damage[3-5]. Therefore, research has focused on the use of antioxidants, with particularly emphasis on naturally derived antioxidants, which may inhibit ROS production and may display protective effects.

Plants phenolics, in particular phenolic acid [6-8] tannins [9-10] and flavonoids [11] are known to be potent antioxidant and occur in vegetables, fruits, nuts, seeds, roots, barks and leaves. In addition to their antioxidant properties, these compound display a vast variety of pharmacological activities such as anti-inflammatory, anti-carcinogenics, antibacterial or antiviral activities which may explain, at least in part, its use as alternative or supportive treatments in various degenerative diseases. [12-15]

Pongamia pinnata Linn. Pierre (synonyms- Indian beech) commonly known as Karanj belonging to family Fabaceae. It is also called *Pongamia glabra*. Pongam is a fast-growing evergreen tree, which reaches 40 feet in height and spread, forming a broad, spreading canopy casting moderate shade. The three-inch-long, pinnately compound, glossy green leaves are briefly deciduous, dropping for just a short period of time in early spring but being quickly replaced by new growth. In spring, Pongam is at its finest when the showy, hanging clusters of white, pink, or lavender, pea-like, fragrant blossoms appear, the clusters up to 10 inches long. These beautiful blossoms and the glossy, nearly evergreen leaves imparipinate, shiny, young and deep green, leaflets 5-9, the terminal leaflet larger than the others, help make Pongam a favorite for use as a specimen, shade, or windbreak. It has also been planted as a street tree, but dropping pods often litter the ground. However, the seeds which are contained within the oval, 1.5 inch-long, brown seedpods are poisonous, a fact which should be considered in placing the tree in the landscape, if many children are present.[1,2] It is an indo-Malaysian species, a medium-sized evergreen tree, and common on alluvial and coastal situations from India to Fiji, from sea level to 1200m. Now found in Australia, Florida, Hawaii, India, Malaysia, Oceania, Philippines and Seychelles. [16-18]

Since this plant contain flavonoids as a active constituents so our aim is to study in vitro antioxidant activity of isolated compound and various extracts from leaves of plant.

MATERIALS AND METHODS

Plant material and extraction

The leaves of the plants were collected from the local areas of Ujjain (MP) and authenticated by Dr. S.K. Billore (Professor and Head of the Botany Department, Vikram University, Ujjain). The voucher specimen number (MIPS/P/004/2008) was submitted in the pharmacognosy deprtment, MIPS Ujjain. Leaves were dried under the shade, powdered and extracted with alcohol and water separately. The extraction was done for 72 hrs. After extraction the extract was separated from marc.then the extract was concentrated and convert into syrupy mass and finally in to dried mass.

Preliminary Phytochemical Investigation [19-21]

Preliminary phytochemical screening of extracts was carried out to know the difffernet constituents present in it as per the standara procedures. The extracts were tested for alkaloids, sterols, glycosides, phenolic compounds, flavonoids, carbohydrates, saponins and fats.

Isolation of Phytoconstituent [22-25]

Isolation of phytoconstituent was done by column chromatography. The lower end of the column was plugged with glass wool. Hexane was poured in to the glass wool to release the air bubbles, which might the tapped with the flat end of the packaging rod. The column was clamped in the vertical position. It was filled up to three fourth the length of the column after mixing the adsorbent (silica gel) in to a slurry with the solvent and pouring the mixture in the glass tube. The stopper at the lower end was opened and the solvent was run of until the level falls to about 1 cm above the adsorbent. After the adsorbent settled, a filter paper disc was placed. Dissolved about 5 gm of ethyl acetate extract in solvent and concentrated it to 10 ml volume and added at the toped of the column by mean of pipette. Care was taken to ensure that it dose not adhere to the wall of the column. The solvent was run off and sample was settled on the top of the column.

***IN VITRO* ANTIOXIDANT ACTIVITY** [26-28]

DPPH Method

DPPH scavenging activity was measured by the spectrophometric method. A stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol gave an initial absorbance of 0.973. Decrease in the absorbance in presence of sample extract at different concentration (10-125 µg/ml) was noted after 15 min. IC_{50} was calculated from % inhibition.

Protocol for DPPH Free Radical Scavenging Activity

Preparation of stock solution of test sample: 100 mg of extract was dissolved in 100 ml of methanol to get 1000 µg/ml solution.

(i) Dilution of test solution: 100, 200, 300, 400 and 500 µg/ml solution of test were prepared from stock solution.

(ii) Preparation of DPPH solution: 15 mg of DPPH was dissolved in 10 ml of methanol. The resulting solution was covered with aluminum foil to protect from light.

(iii) Estimation of DPPH scavenging activity:

75 µl of DPPH solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading.

75 µl of DPPH and 50 µl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol.

Absorbance at zero time was taken in UV-Visible at 517 nm for each concentration.Final decrease in absorbance of DPPH with sample of different concentration was measured after 15 minute at 517 nm.

Percentage inhibitions of DPPH radical by test compound were determined by the following formula.

$$\% \text{ Reduction} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

Calculation of IC_{50} value using graphical method.

RESULTS

Preliminary Phytochemical Investigation

The qualitative chemical test performed revealed the presence of alkaloids, glycosides, flavonoids, saponins, carbohydrates, phenolic compounds, tannins and fats.

DPPH radical scavenging activity

As shown in tables *Pongamia pinnata* Linn. Extracs and isolated compound strongly scavenge in dose dependent manner. IC_{50} value for isolated compound, aqueous extract and alcoholic extract was found to be 313, 380 and 417µg/ml respectively.

Table- 1 DPPH Free Radical Scavenging Activity of Isolated Compound
Standard absorbance: - 0.945 of blank i.e. DPPH and 0.019 nm of standard i.e. ascorbic acid (98.05% reduction).

S. No.	Conc. (µg/ml)	% Reduction	IC_{50} Value (µg/ml)
1.	100	22.54	417
2.	200	27.91	
3.	300	38.57	
4.	400	47.14	
5.	500	56.37	

For Isolated Compound $R^2 = 0.995$, equation: $Y= 0.180X + 15.79$

Table- 2 DPPH Free Radical Scavenging Activity of Aqueous Extract Standard absorbance: - 0.945 of blank i.e. DPPH and 0.019 nm of standard i.e. ascorbic acid (98.05% reduction).

S. No.	Conc. (µg/ml)	% Reduction	IC_{50} Value (µg/ml)
1.	100	28.11	313
2.	200	36.17	
3.	300	47.55	
4.	400	59.17	
5.	500	70.94	

For Aqueous Extract $R^2 = 0.986$, equation: $Y= 0.0950X + 14.45$

Table- 3 DPPH Free Radical Scavenging Activity of Alcoholic Extract
Standard absorbance: - 0.945 of blank i.e. DPPH and 0.019 nm of standard i.e. ascorbic acid (98.05% reduction).

S. No.	Conc. (µg/ml)	% Reduction	IC_{50} Value (µg/ml)
1.	100	25.94	380
2.	200	31.24	
3.	300	42.92	
4.	400	51.25	
5.	500	63.45	

For Alcoholic Extract $R^2 = 0.992$, equation: $Y= 0.0868X + 12.44$

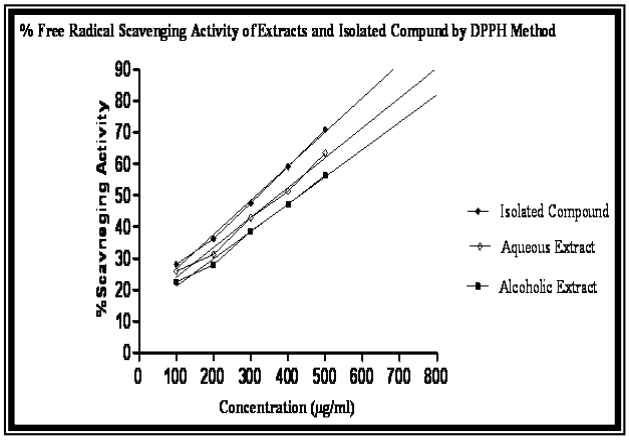

Fig. 1 Free radical scavenging activity by DDPH method

DISCUSSION

DPPH is stable nitrogen centered free radical that can accept an electron is hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agent than losing stiochiometrically with the number of electrons consumed which is measured spectrometrically at 517 nm. Ascorbic acid is a potent free radical scavenger [29-30]. So when compared to such pure component, IC_{50} of isolated compound and extracts shows that *Pongamia pinnata* is potent free radical scavenger. In very recent year, potent free radical scavengers have attracted a tremendous interest as possible therapeutic against free radical mediated diseases. Free radical are constantly generated *in vivo* both by accident of chemistry and for specific metabolic purpose. When an imbalalnce between free radical generation and body defence mechanism occurs, oxidative damage will spread over all the cell target (DNA, protein and lipid). It has been reported that a series of human illness such as cancer, atherosclerosis, cardio and cerbrovascular diseases, diabetes, immune system impairment, neurodegenerative diseases, Parkinson's and Alzheimer's diseases and arthritis as well as premature body aging, can br linked to the damaging action of extremely reactive free radical. Many phenolis, such as flavonoids, flavonols and flavones have potent antioxidant capacity. *Pongamia pinnata* contains flavones, pongamones, furanoflavones, pongamol, pongagalabrone and pongapin, pinnatin and kanjone which have potent antioxidant activity. Potent free radical scavenging capacity of *Pongamia pinnata* can be a attributed to different flavones present in the extracts. So plant may be useful in the management of free radical mediated diseases.

The hydroxyl radical is extremely reactive free radical found in the biological system and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotide in DNA and cause strand breakage which contribute to carcinogenesis, mutagenesis and cytotoxicity. In addition these species can be considered to be one of the quick imitator of lipd peroxidation process, abstracting hydrogen atom from the unsaturated fatty acid.it is found that the flavonoids are potent OH radical scavenging agent

CONCLUSION

The data presented here indicate that the marked antioxidant activity of *Pongamia pinnata* seems to be due to presence of flavonoids like flavones, flavanes, flavonols, which may act as a similar fashion as redutones by donating the electrons and reacting with free radicals to convert them into ,more stable product and terminate free radical chain reaction. The qualitative chemical tests also revealed the presence of flavonoids.

There is a good scope in examining the leaves for *in vivo* model and antioxidant free radical scavenging activity.

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