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A Prospective Phyto Chemical and *In-vitro* Antioxidant Screening of Leaves of *Pongamia Pinnata*

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A B S T R A C T

Traditional system of medicine consists of large number of plants with various medicinal and pharmacological importances and hence represents a priceless tank of new bioactive molecules. *Pongamia pinnata Linn. Pierre* commonly known as 'Karanj' belonging to Fabaceae has been used in different system of traditional medicines for the treatment of various diseases. *Pongamia pinnata* leaves were collected and extraction was done by maceration process using ethanol as a solvent. Phytochemical investigation of extract was done and phytochemical tests for confirmation of the presence of alkaloids, tannins, flavonoids, steroids, glycosides, carbohydrates, aminoacids, proteins, fats & oils. *In-vitro* Antioxidant activity of the ethanolic extract of *Pongamia pinnata* was determined by hydrogen peroxide scavenging method. *Pongamia pinnata* had provided useful information for its identification. Leaves have alkaloids, flavonoids, tannins and saponins which have therapeutic value. Hydrogen peroxide is very important because of its ability to penetrate biological membranes. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radicals in the cells. Thus removing hydrogen peroxide is very important for the protection of living systems. Therefore the results indicate that *Pongamia pinnata* had strong hydrogen peroxide scavenging activity in dose dependent manner. Among all the concentrations 60µg/ml had shown the maximum activity.

Keywords: *Pongamia pinnata*, Phytochemical investigation, Hydrogen peroxide, Antioxidant activity.

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1. Introduction

Pongamia pinnata (Linn.) Pierre is a medium sized glabrous tree popularly known as Karanja in Hindi, Indian Beech in English and Pongam in Tamil.[1] Most of the physicians of Indian system of traditional medicine Ayurveda and Siddha use *Pongamia pinnata* to treat various kinds of diseases including diabetes mellitus. It is a medicinal plant native to Western Ghats and chiefly found in tidal forests of India[2]. *Pongamia pinnata* also called as Derris indica, is a monotypic genus and grows abundantly along the coasts and riverbanks in Myanmar. The tree is known for its multipurpose benefits and as a potential source of biodiesel[3]. The seeds are reported to contain on an average about 28– 34% oil with high percentage of polyunsaturated fatty acids. Historically, Pongamia has been used as folk medicinal plant, particularly in Ayurvedha and Siddha systems of Indian medicine. All parts of the plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, itches, abscess, painful rheumatic joints wounds, ulcers, diarrhea etc.[4] Besides, it is well known for its application as animal fodder, green manure, timber and fish poison. It has also been recognized to possess applications in agriculture and environmental management, with insecticidal and nematicidal activity. More recently, the effectiveness of P. pinnata as a source of biomedicines has been reported, specifically as antimicrobial and therapeutic agents. [5]

Pongamia pinnata is a medium sized semi evergreen glabrous tree with a short bole and spreading crown up to 18 m or more in height, bark grayish green or brown , very often mottled with dark brown dots, specks, lines or streak; leaves compound, leaflets 5-7 ovate, acuminate or elliptic; Flowers lilac or pinkish white, fragnant, in axillary racemes; fruits thick, woody, smooth, compressed, with a short curved beak, seeds 1 or 2 per pod, reniform to nearly round, smooth or wrinkled, testa reddish brown leathery. [6,7]

Taxonomy [8]:

Kingdom	-	Plantae
Subkingdom	-	Tracheobionta
Superdivision	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Subclass	-	Rosidae
Order	-	Fabales
Family	-	Fabaceae
Genus	-	Pongamia
Species	-	Pinnata

Vernacular Names [9]:

Sanskrit : Ghrtakarauja Karanjaka, Naktahva, Naktamala
Bengali : Dahara karanja, Karanja, Natakaranja
Assamese : Korach
Kannada : Honge, Hulagilu
Marathi : Karanja
Gujrati : Kanaji, Kanajo
Punjabi : Karanj
Telugu : Ganuga, Kanugu
Hindi : Karuaini, Dithouri
Oriya : Karanja
Tamil : Pungai, Pongana
Urdu : Karanj
Malayalam : Pungu, Ungu, Unu, Avittal

Figure 1: *Pongamia pinnata* tree

1.2 Traditional Uses: [10,11]

1.2.1. Seed oil

Applied to skin disease, in scabies, sores, herpes and the like cases of eczema have been benefited by applying a mixture of the oil and zinc oxide. Internally the oil has sometimes been used as stomachic and cholagogue in case of sluggish liver. Oil is styptic, anthelmintic, and good in leprosy, piles, ulcers, chronic fever and in liver pain. Useful in rheumatism arthritis scabies, whooping cough.

1.2.2. Leaves

Decoction of leaves is applied as bath or fomentation to rheumatic joints. Leaves are also used in diarrhea and in cough. Juice of leaves in treatment of flatulency, dyspepsia and diarrhea. Young leaves are applied to bleeding piles. Juice of leaves is used for cold, cough, diarrhea, dyspepsia, flatulence, gonorrhea, leprosy.

1.2.3. Stem

Juice of stem in remedy for Gonorrhoea. Aqueous extracts of stem bark exhibit significant CNS sedative and antipyretic activity.

1.2.4. Root

Juice in treatment of Gonorrhea, urethritis, good for cleaning foul ulcer, cleaning teeth, strengthening gums and gonorrhea. Juice of roots with coconut milk and lime water used for treatment of gonorrhea. Roots are bitter anti-helmintic and used in vaginal and skin diseases. Juice of the root is used for cleansing foul ulcers and closing fistulous sores.

1.2.5. Seed

Pulp of seed is an application in leprosy. Commonly used in Bronchitis and whooping cough. Used for keloid tumors. Used in hypertension, skin ailments and rheumatic arthritis. Seed powder valued as a febrifuge, tonic and in bronchitis and whooping cough. Useful in inflammations, pectoral diseases, chronic fevers, hemorrhoids and anemia.

1.2.6. Bark: Useful internally in bleeding piles. It is anthelmintic and alexeteric and useful in hemorrhoids, beriberi, opthalmopathy, dermatopathy, vaginopathy and

ulcer.For bleeding piles, for beriberi, reduce swelling of the spleen. Useful in mental disorder, cough and cold.

1.2.7. Flowers

Dried flowers in powder in combination with other ingredients is given as decoction in diabetes to quench thirst. Useful to quench dysepsia in diabetes, for bleeding piles.

1.2.8. Fruits

Fruits used for abdominal tumors. Useful in ailments of female genital tract, leprosy, tumors, piles, ulcers and upward moving of the wind in the abdomen. Seeds, leaves, roots and oil are Antiparasitic.

2. Materials and Methods

1.1. Collection of Plant:

Leaves of *Pongamia pinnata* were collected in the month of December 2014 from its natural habitat from nearby sathupally. The leaves were cleaned and dried under the shade to avoid degradation of volatile oil. The samples which were shade dried are powdered and stored in a container.

1.2. Preparation of extracts by maceration:

In this process 100 gm of leaf powder of *Pongamia pinnata* were weighed accurately then the drug was added with ethanol at an ratio of 1:3 allowed to stand at room temperature for a period of at least 3 days with frequent agitation with magnetic stirrer until the soluble matter has dissolved. The mixture then is strained. After that the extract was filtered and filtrate was poured into china dishes and air dried till it was dried completely to get dried residue. Then the dried residue was weighed and stored.[12]

Methods:

- Phytochemical investigation of leaf extract
- Evaluation of Antioxidant activity

Phytochemical Investigation of Leaf Extract

Table 1: Test for Carbohydrates

Test	Observation	Inference
Molish test:- Take 2-3ml of aqueous extract add few drops of α-Naphthol Solution in alcohol shake and add Concentration H_2So_4 from sides of the test tube	Violet ring is formed at the junction's of 2 liquids	Carbohydrates are present
Fehlings test:- Take 1ml of Fehling A and B Solution was mixed and boiled for one minute add equal volume of test Solution	Yellow colored Precipitate was not observed	Reducing sugars are absent

Figure 2: *Pongamia pinnata* leaves

heat in boiling water bath for 5-10min		
Benedicts test:- Equal volume of Benedicts reagent and test Solution in test tube were mixed heated in boiling water bath for 5min	Greenish yellow or red color not appeared	Reducing sugar's are absent
Bar fords test:- Equal volume of Bar ford's reagent and test Solution were added and heated for 1-2min in boiling water and cooled	Red Precipitate is not observed	Mono saccharine are absent

Table 2: Test For Proteins

Test	Observation	Inference
Biuret test: take 3ml test Solution add 4% NaOH and few drops of 1% $CuSo_4$ Solution	Violet/pink color	Protein's are present
Millon's test: Mix 3ml of test Solution with 5ml of Millon's reagent	White Precipitate warm the Precipitate is turn to brick red	Protein's are present
Xanthoprotein test: Mix 3ml test Solution with 1ml concentration H_2So_4	White Precipitate does not form's	Protein's containing tyrosine (or)tryptophan is absent
Mix 5ml test Solution with	On boiling Solution turns	Proteins containing

2ml 40% NaOH and 2drops of 10% lead acetate Solution.	black(or) brown due to formation	sulphurs are present

Table 3: Test for Aminoacids

Test	Observation	Inference
Ninhydrin's test:- Heat 3ml of test Solution and 3 drops 5% Ninhydrin's Solution in boiling water bath for 10min	Purple (or)bluish color does't appear	Amino acid is absent
Melons test: Heat 3ml of test Solution and 3 drops of melons reagent	Dark red color does't appear	Tyrosine is absent
To 3ml of test Solution add few drops of glycoralic acid and concentration H_2So_4	Reddish violet ring at junction of the two layer does't appear	Tryptophan is absent
To 5ml of test Solution add few drops of 40% NaOH and 10% lead acetate Solution and boil	Black Precipitate of lead sulphate is not formed	Cysteine is absent

Table 4: Test for Fats and Oils

Test	Observation	Inference
Place a thin section of drug on glass slide add a drop of sudan red III reagent after 2 min wash with 50% alcohol mount in glycerin and observe under microscope	Oil, globules does't appear red	Fat's and oil's are absent
To thin rection of drug add a drop of 1% osmic acid after 1min observe under microscope	Oil drops does't appear black	Fats and oils are absent

Table 5: Test for Alkaloids

Test	Observation	Inference
Mayer's test: Mix 2-3ml of filtrate and add few drops of Mayer's reagent.	Precipitate is formed	Alkaloids are present
Hager's test: Mix 2-3ml of filtrate with Hager's reagent.	Precipitate is formed	Alkaloids are present
Wagner's test: Mix 2-3ml of filtrate and add few drops of Wagner's reagent	Yellow Precipitate is formed	Alkaloids are present

Table 6: Test for Steroids

Test	Observation	Inference
Salkowski reaction: To 2ml of extract add chloroform and 2ml of concentration H_2So_4 shake well	Chloroform layer appears red and acid layer shows greenish yellow fluorescence	Steroids are present
Liberman – burchard reaction: Mix 2ml of extract with chloroform add 1-2ml of acetic anhydride and 2drops concentration H_2So_4 from the side of test tube	First red then blue and finally green	Steroids are present
Liebermann's reaction: Mix 3ml extract with 3ml acetic anhydride heat, cool and add few drops of concentrationH_2So_4	Blue color was appears	Steroids are present

Table 7: Test for Tannins and Phenolic Compounds

Test	Observation	Inference
To 2-3ml of aqueous or alcoholic extract add few drops of 5% $Fecl_3$ Solution	Deep blue/black color	Tannins and phenolic compounds are present
To 2-3ml of aqueous or alcoholic extract add few drops of lead acetate Solution	White Precipitate	Tannins and phenolic compounds are present
To 2-3ml of aqueous or alcoholic extract add few drops of acetic acid Solution	Red color Solution	Tannins and phenolic compounds are present
To 2-3ml of aqueous or alcoholic extract add few drops of dil.HNO_3	Reddish to yellow color	Tannins and phenolic compounds are present

Table 8: Test for Flavanoids

Test	Observation	Inference
Shinoda test: To the dried powder or extract	Pink color observed	Flavonoids are present

add 5ml 95%ethanol few drops of concentration Hcl and 0.5 gr of magnesium turning		
To small quantity of residue add lead acetate solution	Yellow colored Precipitate is formed	Flavonoids are present
Addition of amount of sodium hydroxide to the residue	Shows yellow coloration which de coloration after addition of acid	Flavonoids are present
Sodium picrate test: Soak a filter paper strip first in 10% picric acid then in 10% sodium carbonate dry it in a conical flask place moistened powdered drug cock it place a blue filter paper strip in the slit in cork	The filter paper turns to brick red or maroon	Cyanogenetic glycosides are present

Table 9: Test for the Glycosides

Test	Observation	Inference
Baljets Test: In section of drug add sodium picrate	Section shows yellow to orange color	Cardiac glycosides are present
Legal's test: To alcoholic extract add 1ml pyridine and 1ml sodium nitro prusside	Pink to red color appears	Cardiac glycosides are present
Kellar-killani test: To 2ml extract add glacial acetic acid one drop5%$Fecl_3$ and Concentration H_2So_4	Reddish brown color appears	Cardiac glycosides are present
Lieberman's test: To 3ml extract add 3ml acetic anhydrate heat and cool add few drops of concentration H_2So_4	Blue color appears	Cardiac glycosides are present
Brontagers's test: To 3ml extract add dil.H_2So_4.boil and filter to cold filtrate add equivalent volume of benzene or chloroform shake well and separate the organic solvent and add ammonia	Ammonia layer turns to pink or red	Anthraquinone glycosides are present

Evaluation of Antioxidant activity:

Hydrogen peroxide radical scavenging (H_2O_2) Assay [13, 14]

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. It is widely used as a bleaching agent in the textile, paper and pulp industries. Human beings exposed to H_2O_2 indirectly via the environment are estimated as 0.28 mg/kg/day with intake from leaf crops contributing most to this exposure. Hydrogen peroxide enters the human body through inhalation of vapor or mist and through eye or skin contact. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage.

The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of Ruch et al. (1989). [15] A solution of hydrogen peroxide (40 mm) is prepared in phosphate buffer (50mMpH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (20 – 60 µg/ml) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide [17].

The percentage of hydrogen peroxide scavenging is calculated as follows:

% Scavenged (H_2O_2) = (A0 – A1 / A0) X100.

3. Results and Discussion

3.1. Phytochemical investigation of *Pongamia pinnata* Extracts:

The plant extract i.e Ethanolic leaf extract were subjected for phytochemical investigation and the results are tabulate. The phytochemical investigation of leaf extract of *Pongamia pinnata* respectively passes the tests for carbohydrates, proteins, steroids, flavonoids, glycosides, alkaloids and Tannins but the amino acids and fats were absent.

Table 10: Phytochemical Investigation

Test	Reagents/test	Ethanolic leaf extract
Test for Carbohydrates	Molish test	+
	Fehling's test	-
	Benedict's test	-
Test for Proteins	Millon's test	+
	Xantho protein test	-
Test for Amino acids	Ninhydrin's test	-
	Millon's test	-
Test for Steroids	Salkowski test	+
Test for oils & Fats		-
Test for Flavonoids	Shinoda test	+
Test for Glycosides	Keller killani test	+
Test for Alkaloids	Dragondroff's test	+
	Mayer's test	+
Test for Tannins	5% $FeCl_3$ &Lead acetate	+

3.2. *In-vitro* Antioxidant Activity of *Pongamia pinnata* leaf Extract:

Table 11: Antioxidant Activity of *Pongamia pinnata*

S.NO	Control	Concentrations of Pongamia Pinnata				
		ELE 20	ELE 30	ELE 40	ELE 50	ELE 60
1	0.70	0.48	0.45	0.35	0.32	0.28
2	0.68	0.49	0.45	0.34	0.32	0.28
3	0.69	0.49	0.45	0.35	0.31	0.26
4	0.70	0.48	0.43	0.35	0.32	0.24
Mean	0.69	0.49	0.45	0.35	0.32	0.27
CT-TEST		0.21	0.25	0.35	0.38	0.43
(A0-AT/A0)X100		29.96	35.74	49.82	54.15	61.73

Control mean absorbance value is 0.69(A0)

The current study was performed on the model hydrogen peroxide scavenger activity, in this study we are compared the OH^- ions scavenger capacity of ethanolic extract, the extract was made into 20, 30,40,50,60 micro grams/ml concentrations, the activity of extract is observed under the UV Spectroscopy at 230nm, the obtained absorbance values are compared with control and the percentage inhibition was calculated according to the formula.

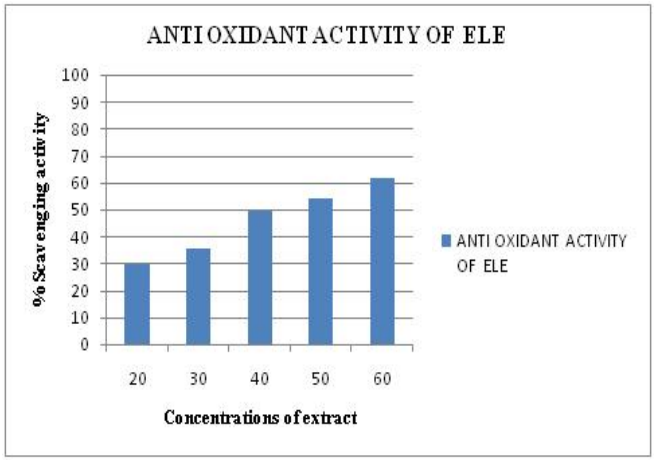

Figure 3: Antioxidant Activity of *Pongamia pinnata*

Discussion: The Phytochemical screening of present findings exhibited that the extract shown the presence of alkaloids, flavonoids, tannins and saponins which have therapeutic value. The extracts showed ascending inhibitory effect with respect to their concentrations. The effect was showed high in the 60 microgram/ml concentration in all the extracts, the percent inhibition of extracts is 61.73 respects to *Pongamia pinnata*. The results are shows comparable effects with the control. Concentration dependent protection of Antioxidant activity was seen with the ethanolic leaf extract. The higher concentration i.e 60 µg/ml shown maximum percentage protection out of those all the concentrations the Leaf extract showed significant response with the standards. Thus the leaves have potent antioxidant activity.

4. Conclusion

In recent years of scientific investigations, attention has been drawn to the health promoting activity of plant foods and its active components. Many herbal remedies have been recommended in various medical treatises for the cure of different diseases. This plant is a multipurpose tree with immense medicinal and economic value. The present study on phytochemical evaluation of leaves *Pongamia pinnata* Linn of family Fabaceae had provided useful information

for its identification. Leaves have alkaloids, flavonoids, tannins and saponins which have therapeutic value. The hydrogen peroxide scavenging activity was determined according to the method of HORAC assay. Hydrogen peroxide is very important because of its ability to penetrate biological membranes. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radicals in the cells. Thus removing hydrogen peroxide is very important for the protection of living systems. Therefore the results indicate that *Pongamia pinnata* had strong hydrogen peroxide scavenging activity in dose dependent manner. Among all the concentrations 60µg/ml had shown the maximum activity.

5. References

1. Krentz A.J. and Bailey C.J., Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs, **2005**, 65: 385-411.
2. Gupta Y.K. and Briyal S., Animal models of cerebral ischemia for evaluation of drugs. Indian J Physiol Pharmacol. **2004**, 48: 379–94.
3. Amadou C.K., Promoting alternative medicine.Africa Health J. **1998**, 2: 20-5.
4. Krishnamurthi A. **1969**. The Wealth of India, vol.VIII. Publication and Information Directorate CSIR, New Delhi, India.
5. Punitha R. and Manoharan S., Antihyperglycemic and antilipidperoxidative effects of *Pongamia*
6. *pinnata* (Linn.) *Pierre* flowers in alloxan induced diabetic rats. J Ethnopharmacol. **2006**, 105: 39–46.
7. Naik M., Meher L.C., Naik S.N. and Dasa L.M.,Production of biodieselfrom high free fatty acid Karanja (*Pongamia pinnata*) oil. Biomass and Bioenergy, **2008**, 32: 354–7.
8. Sarma A.K., Konwer. D. and Bordoloi P.K., A comprehensive analysisof fuel properties of biodiesel from Koroch seed oil. Energy Fuels, **2005**, 19, 656–7.
9. Meera B., Kumar S., Kalidhar S.B., A review of the chemistry and biological activity of *Pongamia pinnata*. J Medicinal Aromatic Plant Sci, **2003**, 25, 441–65.
10. Shoba G.F. and Thomas M., Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. J Ethnopharmacol, **2001**, 76(1), 73–6.
11. Brijesh S., Daswani P.G., Tetali P., Studies on *Pongamia pinnata* (L.) Pierre leaves: understanding the mechanism(s) of action in infectious diarrhea. J Zhejiang Univ Science B **2006**, 7(8), 665–74.
12. Chopade VV, Tankar AN, Pande VV, Tekade AR, Gowekar NM, Bhandari SR, Khandake SN. *Pongamia pinnata*: Phytochemical constituents, traditional uses and pharmacological properties: A review. *Int. J. Green. Pharm*, **2008**, 2: 72-5.
13. Duke, A Handbook of energy crops, unpublished. Available at: www.hort.purdue. edu; **1985**.
14. Scott, PT, Pregelj, Chen, N, Hadler, JS, Djordjevic, MJ and Gresshoff, PM., *Pongamia pinnata*: an untapped resource for the biofuels industry of the future. Bioenergy Research, **2008**, 1: 2-11.
15. Agroforestry Database: World Agroforestry Centre, *Pongamia pinnata*. Available at: www.worldagroforestrycentre.org
16. Shameel S, Usmanghani K, Ali MS. Chemical constituents from seeds of Pongamia pinnata (L.) Pierre Park, J Pharm Sci, **1996**, 9: 11-20.
17. Warrier P K, Nambiar VPK, Ramankutty C. Indian medicinal plants: a compendium of 500 species, volume 4. Orient Longman private Limited, Hyderabad, **1995**, 339-344.
18. The Ayurvedic Pharmacopoeia of India, Vol-IIND, Part-I; 80-88.
19. Narayanrao UB, Indigenous Medicinal Specialities, Biotech Books, NewDelhi, **2001**, 30.