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Phytochemical Analysis, Antioxidant and Anti-inflammatory effects of aqueous and ethanolic extract of *pongamia pinnata flowers*

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Abstract

In India, plants of therapeutic potential are widely used by all sections of people both as folk medicines in different indigenous systems of medicine like Siddha, Ayurveda, and Unani and also as processed product of pharmaceutical industry. In view of these, current study was aimed to investigate the phytochemicals and antioxidant and anti-inflammatory potential of *pongamia pinnata* flower extracts. Preliminary phytochemical analysis was carried out by standard methods. Antioxidant assay was determined by DPPH assay. Anti-inflammatory property was assessed by protein denaturation assay. Ethanolic extract of *pongamia pinnata flowers* were possess flavonoids, phenolics, tannins, alkaloids, glycosides, saponins and steroids whereas aqueous flower extract have flavonoids, alkaloids, steroids, tannins, polyphenols and carbohydrates,. In DPPH assay plant extracts effectively scavenges the free radical at dose dependant manner; higher free radical scavenging potential was observed at 100µg/ml. At a concentration of 100µg/ml, ethanolic and aqueous flower extracts effectively inhibit the protein denaturation of 80% and 72%, respectively. The present investigation suggests both aqueous and ethanol extracts of *pongamia pinnata* flower posses' biologically active phytochemicals, which may responsible for its strong antioxidant and anti-inflammatory activities.

Keywords: *Pongamia pinnata*, Phytochemicals, Antioxidant and anti-inflammatory activities.

1. Introduction

The therapeutic potential of plant products can be traced back to over five thousand years ago as there is evidence of its use in the treatment of diseases and for revitalizing body systems in Indian, Egyptian, Chinese, Greek and Roman civilizations [1]. India has about 4.5 million plant species and among them estimated only 250,000-500,000 plant species, have been investigated phytochemically for biological or pharmacological activity [2]. The bioactive constituents or plants extracts may be uses for treatment of various diseases and these would be used as a new formulation for the novel drugs discovery in pharmaceutical industries [3]. Medicinal and aromatic plants can play an important role in the subsistence livelihood enhancement rural people, especially women in an environmentally sustainable manner while maintaining the biodiversity of these natural products [4]. Today

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according to the World Health organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary healthcare needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases. Due to fewer communication means, poverty, ignorance and unavailability of modern health facilities, most people especially rural people are still forced to practice traditional medicines for their common day ailments [5]. Medicinal Plant is of the great of the health of individual and communities. The medicinal value of plants lies in some chemical active substances that produce define physiological action on the human body [6]. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents [7]. Secondary metabolites or phytochemicals from plants have eminent pharmacological activities such as anti-oxidative, antiallergic, antibiotic, hypoglycaemic and anti-carcinogenic. These secondary metabolites protect the cells from the damage caused by unstable molecules known as free radicals [8]. There are growing interests in using natural antimicrobial compounds, especially extracted from plants, for the preservation of foods. Hence, there is the need to search for plants of medicinal value [9]. However, the knowledge as well as awareness on the herbal remedies is held by elder males and females of between the age group of 41-70 years. Now, decline in the use of the medicinal plants by the new generation may gradually lead to the fading away [10]. Natural substances present in plants called phytochemicals are essential for fostering favourable human health outcomes. These bioactive compounds support general health via a variety of processes. Phytochemicals have strong antioxidant qualities that aid in scavenging dangerous free radicals from the body. This antioxidant activity can promote cellular health by lowering oxidative stress, a major contributor to ageing and the onset of chronic diseases. Phytochemicals exhibit a dosage-dependent effect, which can either have beneficial or detrimental health outcome. Several in vivo and vitro studies have been conducted that strongly supports the health-promoting properties of phytochemicals in human diets. A diet that is rich in phytochemicals derived from fruits, vegetables, nuts, legumes, and whole grains has been linked to a lower incidence of lipid peroxidation, cardiovascular disorders, cancer, and other illnesses. Compounds such as β-glucans and echinacea stimulate immune responses, enhancing the body's ability to defend against infections and diseases. The study of phytochemicals has expanded the understanding of the biochemical diversity of plants and their potential applications in various fields of human life, including food and nutrition, pharmaceuticals, cosmetics, and agriculture. Hence the present study was aimed to study the phytoconstituent nature, Antioxidant and Anti-inflammatory activity of *pongamia pinnata* flowers.

2. MATERIALS AND METHODS

2.1 Chemicals

Griess reagent and DPPH were purchased from Himedia, Mumbai. All other chemicals and reagents procured for

conducting present study were of analytical grade obtained from SRL, Mumbai.

2.2 Plant material and preparation of leaf extract

The leaves of *pongamiah pinnata* flowers were collected from the plants near the Tirupattur, Tirupattur district, Tamil Nadu. The flowers were washed thoroughly under running tapwater and rinsed in distilled water. They were dried in a shade dry for up to one week, powdered in an electrical grinder which was stored in an airtight container at 5°C until further use.

2.3 Preliminary Phytochemical screening

Preliminary phytochemical analysis was carried out for the extract as per standard methods described by Harbone (11) and Kokate (12).

2.3.1. Detection of Alkaloids

2.3.1.1. Mayer's test

The extract was treated with Mayer s reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

2.3.1.2. Wagner s test

The extract was treated with Wagner s reagent. Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

2.3.2. Detection of Flavonoids

2.3.2.1. Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

2.3.2.2 .Sulphuric acid test

Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

2.3.3. Detection of Steroids

Two ml of acetic anhydride was added to five ml of the extract and then added each with two ml of H_2SO_4. The color was changed from violet to blue or green indicates the presence of steroids

2.3.4. Detection of Terpenoids

2.3.4.1. Salkowski s Test

Taken Five ml of the extract mixed with two ml of chloroform and then added carefully the 3 ml of concentrated H_2SO_4 to form a layer. An appearance of reddish brown colour in the inner face indicates the presence of terpenoids.

2.3.5. Detection of Anthraquinones

2.3.5.1. Borntrager s Test

About five ml of the extract was boiled with10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of Chloroform was added to the filtrate.Few drops of 10% ammonia was added to the mixture and heated. Formation of pink colour indicates the presence of anthraquinones.

2.3.6. Detection of Phenols

2.3.6.1. Ferric chloride test

Taken 10ml of the extract was treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

2.3.6.2. Lead acetate test

Taken 10 ml of the extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

2.3.7. Detection of Saponins

About 0.5ml of the extracts was shaken with five ml of distilled water. Formation of frothing (appearance of creamy of small bubbles) shows the presence of saponins.

2.3.8. Detection of Tannins

A small quantity of extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour was formed. It indicates the presence of tannins.

2.3.9. Detection of Carbohydrates

Taken 0.5ml extracts were dissolved individually in five ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

2.3.10. Detection of Protein and Amino acids

2.3.10.1. Biuret test

To 0.5 ml of extract equal volume of 40% NaoH solution and two drops of one percent copper sulphate solution was added. The appearance of violet colour indicates the presence of protein.

2.3.10.2. Ninhydrin test

About 0.5 ml of extract was taken and two drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates that the presence of proteins, peptides or amino acids.

2.4. Free radical scavenging assays

2.4.1. DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay

The free radical scavenging capacity of the aqueous bark extract of *A. amara* was determined using DPPH [13 Brand Williams et al., 1995]. DPPH (200μM) solution was prepared in 95% methanol. From the stock, flower extract solutions prepared in 0.1 mol L^{-1} TrisHCl buffer (pH- 7.9) 6.25, 12.5, 25, 50 and 100 μg/ml were taken in five test tubes. 0.5 ml of freshly prepared DPPH solution was incubated with flower extracts and kept under light protection for 20 min at room temperature. The decrease of absorbance at 517 nm was measured using the spectrophotometer. Deionised water used as a blank experiment and standard ascorbic acid was used as positive control. The following formula was used to compute the percentage of antioxidants : % of antioxidant activity= [(Ac−As) ÷Ac] × 100 Where: Ac—Control reaction absorbance; As—Testing specimen absorbance.

2.5. Anti-Inflammatory Activity

Bovine Serum Albumin Denaturation Assay

To Dissolved in 8g of sodium chloride (NaCl), 0.2g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na_2HPO_4), and 0.24g of potassium dihydrogen phosphate (KH_2PO_4) in 800 ml distilled water. The pH was adjusted to 6.3 using in hydrochloric acid (HCl) and made the volume to 1000ml with distilled water.

Method

Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (BSA) (0.5% W/V aqueous solution) and 0.05 ml of test solution of various concentrations. Test control solution (0.5ml) consists of 0.45ml of bovine serum albumin

(0.5% W/V aqueous solution) and 0.05ml of distilled water. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5%w/v aqueous solution) and 0.05ml of Diclofenac sodium of various concentrations.

Procedure

About 0.05 ml various concentrations (20, 40, 60, 80 and 100 µg/ml) of *Pongamia pinnata* flower extracts and standard drug Diclofenac sodium were taken respectively and 0.45 ml (0.5% W/V BSA) mixed. The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling and add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 255 nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. The percentage inhibition of protein denaturation can be calculated as: [14 and 15]. Percentage Inhibition = 100 [(optical density of test solution-optical Density of control)/optical density of test)/x 100]

3. RESULTS AND DISCUSSION

The Presence of biologically active plants secondary metabolites of *Pongamia pinnata* flower extract shown in Table 1. Preliminary phytochemical analysis of aqueous flower extract of *Pongamia pinnata* plant shows the presence of various important phytochemicals such as flavonoids, alkaloids, steroids, tannins, polyphenols and carbohydrates. Ethanolic extract of flower contains possess flavonoids, phenolics, tannins, alkaloids, glycosides, saponins and steroids.

Table 1: Phytochemical screening of aqueous and ethanolic flower extracts *Pongamia pinnata*

Phytochemicals	*Pongamia pinnata*	
	Aqueous Extract	Ethanolic extract
Alkaloids	+	+
Flavonoids	+	+
Steroids	+	+
Terpenoids	-	+
Phenols	+	+
Saponins	+	+
Tannins	+	+
Glycosides	+	+
Proteins	-	-
Oils and Resins	-	-

+ indicates presence; - indicates absence

Phytochemicals are derived from ecology as a non-nutrient bioactive compounds exerts several pharmacological and beneficial properties to the human health. The qualitative phytochemical screening of aqueous and ethanolic flower extract of *Pongamia pinnata* evidenced that the flower extract have various important secondary metabolites which readily accounts for the traditional medicinal uses of this *Pongamia pinnata* plant. The various parts of the *Pongamia pinnata* plants are widely used for the ailment of several diseases and disorders. Alkaloids play a vital role in human health by possessing potent pharmacological as well as beneficial effects. Various synthetic and semisynthetic drugs are mainly derived from alkaloids with slight structural modifications. Alkaloids play an extensive role in human

health such as anticancer, antibiotics and various degenerative diseases. Because of their huge pharmacological properties alkaloids contribute great role in the formulation of pharmaceutical drugs especially for cancer as well as inflammatory disorders [16].

Saponins are distributed in wild terrestrial plants warmly involved in our day to day life. It is a structurally and biologically diverse class of triterpenes and steroidal glycosides [17]. Tannins present in the bark extract forms the source for its therapeutic significance. The presence of tannins in the fruits extract forms the basis for its medicinal value. Tannins are considered as important plant secondary metabolite because it possess pharmacological activities like antimutagenic and anticarcinogenic. Tannins in the diet interact with enzymes and proteins and enhance the functions of proteins [18]. Hydroxyl and other functional groups present in the tannins responsible for its significant pharmacological property [19].

Figure 1 represents the *in-vitro* DPPH radical scavenging activity of the *Pongamia pinnata* aqueous and ethanolic flower extracts. The *Pongamia pinnata* flower extracts shows dose dependent scavenging activity against the free radical DPPH. Aqueous flower extract concentration ranges from 6.25, 12.5, 25, 50 and 100 µg/ml shows 8.90 ± 0.81, 15.72 ± 0.97, 24.51 ± 1.90, 38.79 ± 2.01, and 63.01 ± 2.45 percentage inhibition of DPPH radicals. Ethanol extract shows much higher antioxidant activity when compared to aqueous flower extract, it shows 13.81 ± 1.04, 18.62 ± 2.01, 28.03 ± 1.75, 41.94 ± 1.70 and 73.47 ± 1.28. Reduction of DPPH radicals (DPPH*) in methanolic solution under dark condition is the basis of antiradical activity assay.

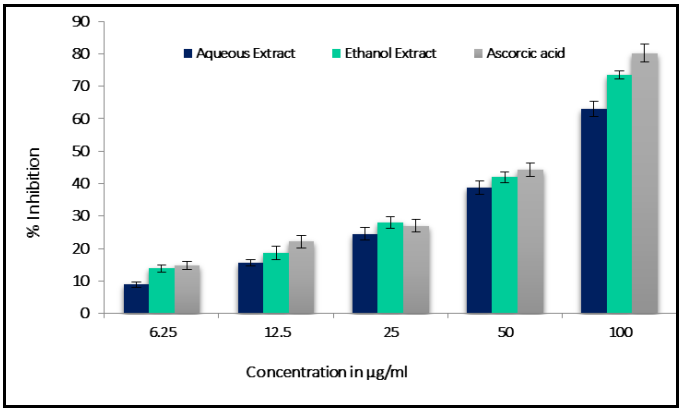

Figure 1: Antioxidant activity of *Pongamia pinnata* flower extracts

DPPH have odd electron configuration, shows absorption maximum very strong at 517 nm [20]. In the presence of hydrogen donor (antioxidants having free radical scavenging property) the odd electrons in the free radical becomes paired off. After getting paired electron from antioxidants the absorption strength of free radical is decreased and the resulting decolorization is stoichiometric with respect to free radical captured electrons number. The free radical scavenging activities of extracts depend on the capacity of antioxidant compounds to lose hydrogen and the structural conformation of these components. Ascorbic acid serves as standard antioxidant compound. Figure 2 demonstrates the anti-inflammatory potential of the flower extracts. The ethanolic and aqueous extracts of *Pongamia pinnata* flower show a clear dose-dependent anti-inflammatory effect. This activity was compared to that of diclofenac sodium, a standard anti-inflammatory drug. The aqueous flower extract inhibit the protein denaturation by 11.05% to 67.36% across

concentrations ranging from 20 to 100 µg/ml, ethanol extract shows 16.62 % to 76.32% at same concentrations.

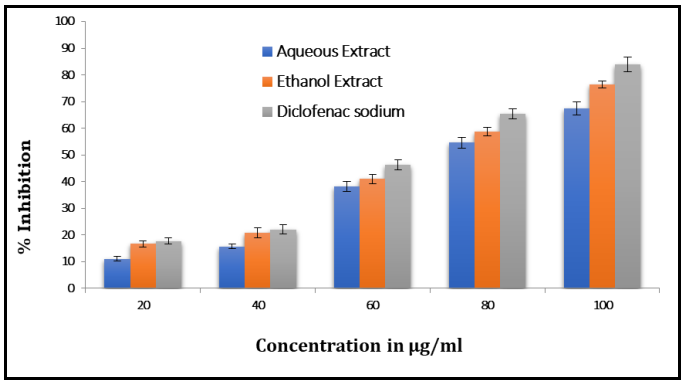

Figure 2: Anti-inflammatory activity of *Pongamia pinnata* flower extracts

Protein denaturation within tissues can lead to the formation of auto-antigens, which in turn contribute to tissue damage, particularly in certain arthritic conditions. Therefore, tissue protein denaturation is considered a significant marker of tissue inflammation and diseases like arthritis [21].

Compounds that prevent protein denaturation are considered promising leads for developing new anti-inflammatory drugs. Consequently, this study utilized an in vitro protein denaturation assay to assess the anti-inflammatory activity of the seed extract across varying concentrations. The results revealed that the extract's ability to inhibit protein denaturation increased with higher concentrations, demonstrating a dose-dependent relationship. This enhanced protein stabilization effect was observed as an increase in absorbance, mirroring the behavior of the standard medication.

4. CONCLUSION

The flowers of possess a rich array of secondary compounds, including carbohydrates, tannins, saponins, alkaloids, flavonoids, terpenoids, and steroids. Analysis of the flower extracts revealed significant antioxidant capabilities, demonstrating a level of free radical scavenging activity, measured by the DPPH assay that approaches that of the standard ascorbic acid. Furthermore, the plant extracts displayed anti-inflammatory properties by inhibiting protein denaturation in a manner that increased with dosage, though it was less potent than the reference drug diclofenac. The research conducted indicates that *Pongamia pinnata* flowers hold promise for addressing health concerns related to both oxidative stress and inflammation.

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Conflict of Interest: Nil

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