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Original Article

Comparative study on *in-vitro* antioxidant activity of flowers and leaves of *Plumeria Alba L.*

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ABSTRACT

The present investigation was aimed to evaluate the phytochemicals and invitro antioxidant activity of *Plumeria alba L.* Phytochemicals were analysed in aqueous and ethanolic extracts of flowers and leaves by qualitative method and the result confirmed the presence of alkaloids, sterols, terpenoids, flavonoids, amino acids, volatile oils, tannins and phenolic compounds etc., The invitro antioxidant property of aqueous and ethanolic extract of both parts of *Plumeria alba L.* were evaluated using total antioxidant capacity, reducing power assay, hydrogen peroxide scavenging activity and nitric oxide scavenging activity. Among all the extracts, ethanolic flower extract showed highest antioxidant activity than other extracts. All the results were compared with standard ascorbic acid. In conclusion, the ethanolic extract of *Plumeria alba* L. flower possess high antioxidant activity which may be due to presence of high content of various phytochemicals.

Keywords: Phytochemical, Antioxidant, *Plumeria alba* L.

INTRODUCTION

An antioxidant is a substance capable of preventing or slowing the oxidation of other molecules. Generally, an antioxidant can protect against metal toxicity by trapping free radicals thus terminating the chain reaction by chelating metal ion and preventing the reaction with reactive oxygen species or by chelating metal and maintaining it in a redox state leading to its incompetency to reduce molecular oxygen. Substances which protect biomolecules from free radical mediated damage both in vivo and in vitro fall under this category. Many antioxidant compounds, naturally occurring in plant sources, have been identified as free radical scavengers or active oxygen scavengers. A number of plants have been investigated for their biological activities and antioxidant properties[1]. Recently, an interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants[2]. In addition, natural antioxidants have the capacity to improve food quality and stability and also act as neutraceuticals to terminate free radical chain reaction in biological systems, and thus may provide additional health benefits to consumers. There is an increasing interest in the measurement and use of plant antioxidants for scientific research as well as for industrial (dietary, pharmaceutical and cosmetics) purposes. This is mainly due to its strong biological activity, excluding those of many synthetic antioxidants, which have possible activity as promoters of carcinogenesis. Therefore, the need exists for safe, economic powerful and natural antioxidants to replace these synthetic ones. Obviously, there has been an increasing demand to evaluate directly the antioxidant properties of plant extracts[3]. Keeping this view, our present study was designed to evaluate the phytochemicals and invitro antioxidant avtivity of *Plumeria alba* flowers and leaves. *Plumeria alba* (Family: Apocynaceae) is a evergreen a small laticiferous tree or shrub commonly called White Champa, spread to all tropical areas of the world[4]. The plant is medicinal which contains amyrinacetate, mixture of amyrins, β-sitosterol, scopotetin, the iriddoids isoplumericin, plumieride, plumieride coumerate and plumieride coumerate glycoside[5]. The flowers are part of a traditional medical preparation taken as a vermifuge or as a laxative. Decoction of leaves are applied for cracks and eruption of the soles of the feed. Infusion or extract from leaves is used to control asthma. It's bark is used as plaster over hard tumors, purgative and febrifuge. The milky sap of the stem and leaf is applied to skin diseases such as herpes, scabies and ulcers. Decoction of bark is used as counter irritant on the

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gum for tooth ache. An extract of bark is used internally and externally for syphilitic ulcers[6]. The latex is used as purgative, cardiotonic, diuretic and hypotensive[7]. The latex is mixed with coconut oil warmed and applied to affected area to treat arthritis, rheumatism, pruritic skin lesion.

MATERIALS AND METHODS

Collection of plant material: The flowers and leaves of the plant *Plumeria alba* were collected from in and around Athamangalam, Nagai district Tamilnadu. The collected samples were carefully kept in polythene bags. These plant samples were authenticated by Dr. S. Johnbritto, The Director, The Rabinet Herbarium Centre for Molecular Systematic, St. Joseph's College, Tiruchirappalli and a voucher specimen (Voucher No R.M 001/2013) was deposited in the Department of Biochemistry, S.T.E.T Women's College, Mannargudi, Thiruvarur, Tamil Nadu. The flowers and leaves were dried in shade and stored in air tight containers until further studies.

Chemicals: All the chemicals used in this assay were purchased from Rankem Laboratory, Chennai.

Extraction of plant material: Aqueous and ethanol extracts were prepared according to the methodology of Indian pharmacopoeia. The shady dried plant materials were subjected to pulverization to get coarse powder. The coarse powder material was subjected to soxhlet extraction separately and successively with ethanol. For aqueous extract, The plant material was mixed with and it was six parts of water was added, boiled. The extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°C). The aqueous and ethanolic extracts put in airtight container and stored in refrigerator.

Phytochemicals analysis: Aqueous and ethanolic extracts of *Plumeria alba* Linn., flowers and leaves were subjected to qualitative test for the identification of various plant constituents[8].

Invitro antioxidant activity: In the present study, we evaluated the invitro antioxidant activity of *Plumeria alba* by various methods like total antioxidant activity[9], Reducing power assay [10], H_2O_2 scavenging activity[11] and nitric oxide scavenging activity[12].

RESULT

In this study, we analysed the phytochemicals and Invitro antioxidant activity of flowers and leaves extract of *plumeria alba*. Aqueous and ethanolic extract of flowers and leaves were screened for their antioxidant activity. Results were compared with standard ascorbic acid.

Phytochemical analysis: Table 1 shows the qualitative analysis of phytocontituents of flowers and leaves of *plumeria alba.* The result showed the presence of alkaloids, flavonoids, phytosterols, terpenoids, triterpenoids, amino acid, volatile oils, tannins, phenolic, and absence of steroids, proteins, glycosides, saponins, phlobatannins in all the plant extracts.

Invitro antioxidant activity: The invitro antioxidant activity of aqueous and ethanolic extract of *plumeria alba* L. were evaluated by different invitro models, including total antioxidant capacity, reducing power assay, hydrogen peroxide scavenging activity, nitric oxide scavenging activity. The obtained results were represented in table 2.

Total antioxidant capacity: Total antioxidant capacity of aqueous and ethanolic flowers and leaves extract of plumeria *alba*. were analysed. Aqueous and ethanolic extract of flowers showed highest antioxidant capacity of 28.4%, 84.8% respectively than aqueous and ethanolic leaves extract of 25.6%, 74.4%. Among all the extracts, ethanolic flower extract showed highest activity which was nearer to that of standard antioxidant (92%).

Reducing power assay: Table 2 showed the reducing power assay of aqueous and ethanolic extract of *plumeria alba*. The reducing power of aqueous leaves and flowers extract were 34.6% and 36.5% respectively. Ethanolic leaves and flowers extract were 56.3% and 74.8% respectively and ascorbic acid showed 72.3%. Among all the extracts, ethanolic flowers extract exhibited highest value than that of standard ascorbic acid.

Hydrogen peroxide scavenging activity: Hydrogen peroxide scavenging activity of *plumeria alba* flowers and leaves were analysed. The hydrogen peroxide scavenging activity of aqueous leaves and flowers showed 28.4% and 26.5% respectively. In ethanolic leaves and flower extract, the hydrogen peroxide scavenging activity were 56.4% and 72% respectively and ascorbic acid showed 65.3%. Among all the extract, the ethanolic extract of flowers exhibited highest value than the standard.

Nitric oxide scavenging activity: Table 2 also showed the nitric oxide scavenging activity of aqueous and ethanolic extract of *plumeria alba*. Aqueous leaves and flowers extract showed 23.4% and 21.5% of scavenging activity respectively. In

ethanolic leaves and flowers extract, nitric oxide scavenging activity were 68.4% and 76.8% respectively and ascorbic acid showed 70.33%. Among all the extract, ethanollic flower extract exhibited highest value which was higher than that of the standard.

DISCUSSION

Plants are the source of energy for the animal kingdom. In addition, Plants can synthesize a large variety of chemical substances that are of physiological importance [13]. Medicinal, herbal and aromatic plants constitute a large segment of the flora, which provide raw materials for use by pharmaceutical, cosmetic, fragrance and flavour industries. They have been used in the country for a long time for their medicinal properties[14]. In the present study, phytochemical of ethanolic and aqueous extract of *Plumeria alba L.* were analysed qualitatively and the results revealed the presence of alkaloids, flavonoids, phenol, volatile oils, phytosterols, terpenoids, triterpenoid, amino acid, tannins and the absence of phlobatannins, protein, steroids, glycosides, saponins, compound. It was proposed that the antioxidant activity of plant extract could possibly be related to flavonoids [15]. Flavonoids, which are well known antioxidant and free radical scavenger, such as kaempferol 3-rhamnoside and kaempferol 3- rhamnogalactoside have been reported to be present in *plumeria alba* L.[16]. Reactive oxygen species generated in the human body cause oxidative damage and responsible for many degenerative diseases such as coronary heart disease, atherosclerosis, diabetes and cancer in living organisms[17]. The activity of reactive oxygen species are counteracted by antioxidant however there is a widespread agreement that various synthetic antioxidant are present in the market live, putylhydroxyanisole and putyl hydroxyl toluene but they have toxigological affects and carcinogenic potential and have prompted the need from natural alternative in the last few decades[18]. *Plumeria alba* L have been used in traditional medicine for various ailments which are closely associated with free radical formation. Hence in the present investigation, we have evaluated the free radical scavenging activity of *plumeria alba*.

Total antioxidant capacity: The total antioxidant capacity of the extracts was calculated based on the formation of phosphomolybdenum complex which was measured spectrometrically at 694 nm. Hydroxyl radical are most reactive species, initiating the peroxidation of the cell membrance[19]. The lipid radical thus generate would initiate chain reaction in the presence of oxygen, giving rise to lipid peroxidation, which break down to aldehydes, such as malondialdehyde, which are known to be mutagenic and carcinogenic[20]. In the presence study, the ethanolic extract and it is function showed potent inhibition of lipid peroxide.

Reducing power assay: The reducing capacity of compounds may serve as a significant indicator of its potential antioxidant activity[21]. However the antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging[22,23]. For the measurement of the reductive ability, we investigated Fe^{3+} to Fe^{2+} transformation in the presence of the aqueous and ethanolic extract of *Plumeria alba* using the method of Oyaizu, 1986[10]. Our study revealed that the ethanolic flower extract exhibited potent reducing ability than the standard ascorbic acid.

Nitric oxide scavenging activity: NO is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecules in diverse biological systems including neuronal messenger, vasodilation, antimicrobial and antitumour activities. Scavengers of NO compete with oxygen leading to the reduced production of NO. In the present investigation, aqueous and ethanolic extract of *Plumeria alba* compete with NO and thus inhibit peroxynitrite formation[24]. Our finding suggests that the phenolic compounds present in the extract might be responsible for NO scavenging effect. Phenol are known as powerfull chain breaking antioxidant because of their hydroxyl group and can be used as easily accessible source of natural antioxidants[25]. The presence of rich amount of structural chemistry for free radical scavenging and they have been to be more effective antioxidant properties than vitamin E and ascorbic acid[26].

Hydrogen peroxide scavenging activity: Hydrogen peroxide is genrated invitro by several peroxidase enzymes. In this method, when an antioxidant is incubated with hydrogen peroxide, the decayed or loss of hydrogen peroxide is measured spectrophotometrically[27]. Hydrogen peroxide is a weak oxidizing agent which inactivates enzymes by oxidation of the essential thiol (SH-) group. It rapidsly transverses cell membranes and once inside the interior, interact with $Fe^{2+}$and CU^{2+} to form hydroxyl radical which is harmful to the cell[28]. The ethanolic flower

extract of *plumeria alba* showed good scavenging effect than standard ascorbic acid. Based on the result indicated, the ethanolic extract of *Plumeria alba* L., were found to more effective than that of aqueous extract. Among the ethanol exctracts, flower extract showed highest antioxidant activity than leaves. When compared with standard ascorbic acid, ethanolic flower extract exhibited highest antioxidant and radical scavenging activity except in model of total antioxidant activity. In conclusion, the ethanolic extract of *plumeria alba* Linn. flowers exhibited the greatest antioxidant activity which may be due to the presence of active constituents. Thus, the study ascertain the value of *Plumeria alba* L. used in Ayurveda, which could be of considerable interest to be development of new drugs.

Table 1: Qualitative phytochemical screening of *Plumeria alba* flowers and leaves

Phytochemicals	***Plumeria alba***			
	Aqueous extracts		**Ethanol extracts**	
	Leaves	Flowers	Leaves	Flowers
Alkaloids	+	+	+	+
Carbohydrates	_	_	_	_
Phytosterols	+	+	+	+
Steroids	_	_	_	_
Terpenoids	+	+	+	+
Triterpenoids	+	+	+	+
Flavonoids	+	+	+	+
Proteins	_	_	_	_
Aminoacids	+	+	+	+
Glycosides	_	_	_	_
Volatile oils	+	+	+	+
Tannins	+	+	+	+
Phenolics	+	+	+	+
Saponins	-	-	-	-
Phlobatannins	-	-	-	-

(+)-presence: (-)-absence

Table 2: Invitro antioxidant activities of *Plumeria alba* flowers and leaves

Antioxidant Models	***Plumeria alba***				**Standard ascorbic acid**
	Flowers		**Leaves**		
	Aqueous extract	**Ethanol extract**	**Aqueous extract**	**Ethanol extract**	
Total antioxidant capacity (%)	28.4	84.8	25.6	74.4	92
Reducing power assay (%)	36.5	74.8	34.6	56.3	72.2
Hydrogen peroxide scavenging activity(%)	26.5	72.2	28.5	56.4	65.3
Nitric oxide scavenging activity (%)	21.5	76.8	23.4	68.4	70.3

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