RESEARCH ARTICLE

# Screening of *in-vitro* Antioxidant Profile of Different Extracts of the Leaves of *Plumeria alba* Linn

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## ABSTRACT

Production of reactive oxygen species (ROS) causes various diseases and cellular anomalies in human beings. Antioxidants inhibit generation of reactive species by scavenging them. *Plumeria alba* Linn commonly known as White Champa (family: Apocynaceae). It is used to treat ulcers, herpes, scabies and seeds possess haemostatic properties. Till date no antioxidant activity had been reported on the leaves of *Plumeria alba*; therefore, my project work has been conceptualized on the free radical scavenging activity of the leaves of *Plumeria alba.* The present study assessed the phytochemical analysis mainly successive extractive values, thin layer chromatographic profile and phytochemical screening of petroleum ether, chloroform and methanol extracts of *Plumeria alba* leaves. The *in vitro* antioxidant potential by DPPH radical scavenging assay was also estimated for each of the extracts with the reference drug Ascorbic acid. Total chromatographic profile was obtained for each extracts. Presence of phytoconstituents such as flavonoids, polyphenols, terpenoids and glycoside in different extracts were found. The potent free radical scavenging activity was observed on DPPH radical scavenging assay. The different concentrations of all the extracts showed effective free radical scavenging activity on dose dependant manner.

**Keywords:** Antioxidant activity, reactive oxygen species, *Plumeria alba*, chromatographic profile

## INTRODUCTION

Reactive oxygen species [ROS], sometimes called as active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions ($O_2$-.) and hydroxyl radicals (OH.) as well as non-free radical species such as hydrogen peroxide ($H_2O_2$) [1]. These ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations[2]. Living organisms have antioxidant defence systems that protects against oxidative damage by removal or repair of damaged molecules[3]. The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS[4].

Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors[5]. The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of human diseases. The antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions catalyzed by free radical[6]. However, there have been concerns about synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) because of their possible activity as promoters of carcinogenesis.

The natural antioxidant mechanisms maybe insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important[7]. Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature. These facts have inspired widespread screening of plants for possible medicinal and antioxidant properties; the isolation and characterization of diverse phytochemicals and the utilization to antioxidants of natural origin to prevent the diseases[8].

*Plumeria alba* Linn (Apocynaceae) commonly called White Champa, a small laticiferous tree or shrub,

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native of tropical America. It is 4.5m high, occasionally grown in the gardens. The plant is mainly grown for its ornamental and fragrant flowers. Leaves are lanceolate to oblanceolate, flowers white, fragrant in corymbose fascicles[9]. The fruit is edible, latex is applied to ulcers, herpes and scabies and seeds possess haemostatic properties. Moreover its bark is bruised and applied as plaster over hard tumours[10, 11]. Whereas the latter taxon finds used as purgative, cardiotonic, diuretic and hypotensive. Methanolic extract showed antimicrobial activity against *Bacillus anthracis*, *Pseudomonas aeruginosa*[12, 13]. The plant is reported to contain amyrinacetate, mixture of amyrins, ß-sitosterol, scopotetin, the iriddoids isoplumericin, plumieride, plumieride coumerate and plumieride coumerate glucoside [14].

## MATERIALS AND METHODS

**Plant material:** The mature leaves of *Plumeria alba* L. (Family: Apocynaceae) were collected during the month of February 2015 from the fields near by the Ganges at Sodepur, Kolkata, West Bengal, India. The plant material was taxonomically identified by Dr. V. P. Prasad of the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNH/Tech.II/2015/14/287) was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

**Preparation of plant extracts:** The dried powdered material was first extracted (percolation) with petroleum ether (60-80°C), the percentage extractive value was 1.74% w/w. The marc thus obtained was further extracted (percolation) successively with chloroform and methanol for 72 h in a percolator. The solvent was distilled off under reduced pressure resulting in semisolid mass that was vacuum dried to yield the dry extracts and the percentage extractive values were accordingly 3.11 % w/w and 6.35% w/w respectively. The preliminary phytochemical analysis was performed on these three extracts to identify the phytoconstituents present in the extracts[15].

**Reagents and chemicals:** L ascorbic acid (vitamin C) and 1,1-diphenyl-2-picryl-hydrazil (DPPH) were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade obtained commercially.

**DPPH radical scavenging activity:** The antioxidant activity of the extracts was measured in terms of hydrogen-donating or radical scavenging ability, using the DPPH(1,1-diphenyl-2-picryl-hydrazil) method with slight modification [16-18]. Briefly, an 0.1 mM solution of DPPH in methanol was prepared, and 1 ml of this solution was added to 3 ml of the all of the extracts solution respectively in pet ether, chloroform and methanol at different concentrations (2,4,6,8,10,15 µg/ml). The reaction mixture were shaken vigorously and incubated in the dark for 30 min. at room temperature. Then the absorbance of the solution was measured at 517 nm using a UV-Visible spectrophotometer (Genesys 10 UV: Thermo Electron Corporation). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. Ascorbic acid at same concentrations was used as reference. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1) / A_0) \times 100]$$

Where, $A_0$ is the absorbance of the control reaction, and $A_1$ is the absorbance of presence of all of the extract samples and standard. The results have been stated in Table 2.

## Results and Discussion

Preliminary phytochemical screening on the test extracts revealed the presence of terpenoids, steroids in pet ether extracts; terpenoids, steroids, carbohydrate in the chloroform extracts; steroids, terpenoids, carbohydrates, flavonoids, saponins, glycosides, tannins and alkaloids in the methanol extracts of leaves of *Plumeria alba* (Table 1).

The stable DPPH radical model is a widely used, relatively quick and precise method for the evaluation of free radical scavenging activity. The effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progressed, results in the scavenging of the radicals by hydrogen donation. It is visually noticeable that as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidant activity of different antioxidants [19]. It has been reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic disease conditions such as arteriosclerosis [20].

Based on the data obtained from the present study, all the extracts were effective free radical inhibitor or scavenger, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body. Figure 1 illustrates a significant decrease in the concentration of DPPH radicals due to the scavenging ability of the extracts and standard. Free radical scavenging activity also increased with increasing concentration in the range of 2-15 µg/ml.

## CONCLUSION

In conclusion, the results from this study further support the view that all extracts of *Plumeria alba* L. leaves were promising source of good natural antioxidant against various antioxidant systems. From the results, it can be concluded that the antioxidant activity of all the extracts were concentration dependent. The possible mechanism of antioxidant activity of all the extracts include hydrogen-donating ability and scavenging of the free radicals, which may be due to the presence of phytoconstituents such as flavonoids, polyphenols, terpenoids and glycoside present in the pet ether, chloroform and methanol extracts of the leaves of *Plumeria alba* showed the antioxidant activity of the plants. The methanol extract was found to demonstrate the most active free radical scavenging potential even more than that of ascorbic acid at the test concentration, followed by the chloroform and petroleum ether extracts. The present preliminary study confirms remarkable *in vitro* free radical scavenging activity of *Plumeria alba* leaves against DPPH. Further studies are required to identify specific active principles of this plant for the significant antioxidant effect.

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**Table 1:** Phytochemical test of different extracts of leaves of *Plumeria alba* L.

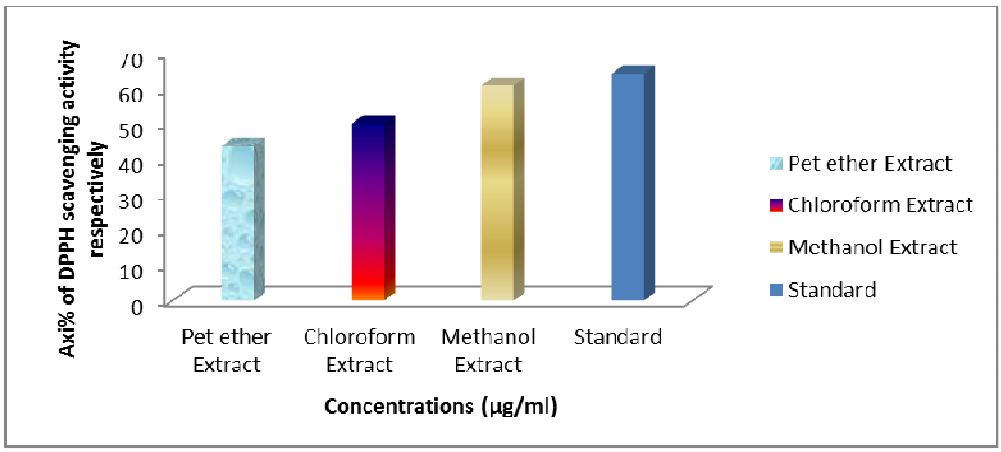
| Serial No. | Phytoconstituents | Pet ether extract | Chloroform extract | Methanol extract |
|---|---|---|---|---|
| 1 | Terpenoid | + ve | + ve | + ve |
| 2 | Flavonoid | - ve | - ve | + ve |
| 3 | Tannin | - ve | - ve | + ve |
| 4 | Alkaloid | - ve | - ve | + ve |
| 5 | Steroid | + ve | + ve | + ve |
| 6 | Saponin | - ve | - ve | + ve |
| 7 | Glycoside | - ve | - ve | + ve |
| 8 | Carbohydrate | - ve | + ve | + ve |
| 9 | Protein | - ve | - ve | - ve |
| 10 | Fixed oil | - ve | - ve | - ve |

**Table 2:** DPPH scavenging power of the different extracts of leaves of *Plumeria alba*

| Extracts | Concentrations (µg/ml) | % of DPPH scavenging activity respectively | Mean ± SEM |
|---|---|---|---|
| Petroleum ether | 2, 4, 6, 8, 10, 15 | 25.18, 28.27, 33.82, 50.51, 57.81, 66.93 | 43.75 ± 4.34 |
| Chloroform | 2, 4, 6, 8, 10, 15 | 29.19, 35.62, 46.18, 54.69, 63.22, 72.18 | 50.18 ± 3.22 |
| Methanol | 2, 4, 6, 8, 10, 15 | 36.71, 45.29, 55.24, 62.29, 78.21, 87.32 | 60.84 ± 5.93 |
| Vitamin C | 2, 4, 6, 8, 10, 15 | 32.18, 43.18, 60.45, 77.54, 82.20, 90.23 | 64.29 ± 5.19 |

SEM - Standard error mean

**Fig.1.** Comparative graphical presentation of DPPH scavenging activity of different extracts of leaves of *Plumeria alba*

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