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Antioxidant, cytotoxic and hypolipidemic activities of *Plumeria alba* L. and *Plumeria rubra* L.

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Abstract: Methanolic flower extracts of *Plumeria alba* and *P. rubra* were tested for in vitro antioxidant potential, cytotoxicity and hypolipidemic activities. DPPH assay of methanolic extract of *Plumeria* revealed that 81% and 72% inhibition by *P. alba* and *P. rubra* respectively and the total phenolic content was found as 173.9 µg ml^{-1} and 167.3 µg ml^{-1}. Significant free radical scavenging activities of 1.74 mg $ml^{-1}$and 1.67 mg ml^{-1} were observed due to the higher phenolic content. Anti-chlolesterol assay of the extracts demonstrated *P. rubra* has highest hypolipidemic activity (60%) followed by *P. alba* (52%). MTT assay using HCT 116 cell lines revealed the antiproliferative and cytotoxic activities of *P. alba* with an IC50 value of 259.9 µg ml^{-1}. *Plumeria* species could be a new source of drugs for the treatment of colon cancer and hyperlipidemic conditions due to higher antioxidant potential and phenolic content which was revealed from the study.

Keywords: *Plumeria*, Antioxidant, Anticancer, Anti-Cholesterol, Colon Cancer

1. Introduction

Medicinal plants are an important source of new chemical entities with potential therapeutic values. More than 75% of the total population in developing countries relies on traditional medicines based on plant products [1]. Cancer has a high morbidity and mortality worldwide and the treatment comprise radiation therapy, surgery, chemotherapy, immunotherapy and hormonal therapy [2]. Most of the therapies being used for cancer treatment often lead to serious side effects and novel anticancer drugs developed from natural resources may increase the efficacy of conventional cancer treatment methods. Natural products from plants are important sources of new drugs [3]. Unlike synthetic medicines, medicinal plants promote the natural functions of the body [4]. Some of these agents compared to purely synthetic drugs have higher activity and lower toxicity [5]. Many plants have been identified as having potential antioxidant activities The antioxidant capacities of plant extracts are due to their radical scavenging activity, binding of transition metal ion catalysts, increasing endogenous status of antioxidant enzymes to prevent oxidative damage [6]. Hyperlipidemia is considered as a risk factor involved in the development of cardiovascular disease [7]. Pharmacologists and chemists have been perplexing by the characteristic profiles of toxic side effects of hypolipidemic drugs. There are reports of herbal medicines exerting good hypolipidemic actions [8-10].

Plumeria commonly known as Frangipani belongs to the family Apocyanaceae. *Plumeria alba* grows as 4-5 m high tree and leaves are lanceolate. The flower of the plant is white with yellow center [11]. *Plumeria rubra* grows as a spreading shrub or small tree to a height of 2-8 m and similar width. The large green leaves can reach 30-50 cm long and are arranged alternately and clustered at the end of the branches. The flowers are terminal, appearing at the ends of branches, often profuse and very prominent, strongly fragrant and have five petals. The colours range from common pink to white with shades of yellow in the centre of the flower [12]. *Plumeria* species have been reported to have antimicrobial [13, 14], anticancer [15-18], antipyretic [19] and antioxidant [20] activities. The present attempt is to determine the antioxidant, anti-cancer and hypolipidemic activities of *Plumeria alba* and *P. rubra* flower extracts.

2. Materials and Methods

2.1. Plant Material Collection and Extraction

Fresh flowers of *Plumeria alba* and *P. rubra* were collected from local areas of Bangalore, air dried, ground into powder and sieved (60 mesh). About 100 g of dried flower powder was extracted in methanol (1:7 w/v) at 25°C for 24 hours. The extracts were centrifuged at 10,000 rpm for 10 minutes and the supernatant was filtered using Whatman No.1 filter paper and concentrated to dryness under reduced pressure in rotary vacuum evaporator. The final extract was stored in air tight containers at 4°C until used.

2.2. DPPH Assay

The capacity of the extract to scavenge the stable 2, 2'-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method reported by Gyamfi et al., [21]. In brief, 50 µl of the methanolic flower extract (100 µg ml^{-1}) was mixed with 1.8 ml of 0.5 mM DPPH in methanol solution. Methanol (50 µl) was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured at 517 nm. The percent inhibition was calculated from the following equation:

$$\text{Inhibition (\%)} = \frac{(\text{Control}_{\text{A517}} - \text{Sample}_{\text{A517}})}{\text{Control}_{\text{A517}}} \text{ x } 100$$

2.3. Determination of Total Phenolic Content

The amount of total phenolics in the flower extract was determined with Folin-Ciocalteu (FC) reagent [22]. To 200 µl of sample (3 replicates), 1 ml of 1:2 dilution of FC reagent and 800 µl of sodium carbonate (7.5% w/v) were added and the resulting mixture was incubated at room temperature for 30 minutes. The absorbance of the sample was measured at 765 nm using a spectrophotometer and the results were expressed as milligram of gallic acid equivalent per gram of dry weight.

2.4. Antioxidant Potential Assay

The Antioxidant potential assay was carried out by phosphomolybdenum reduction assay [23]. To 200µl of flower extract, 1ml of the reagent containing 4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulphuric acid were added and the resulting mixture was incubated at 37°C for 60 minutes. The absorbance of samples were measured at 695 nm using a spectrophotometer against blank using methanol. The antioxidant potential activity was expressed as mg of ascorbic acid equivalent per gram of dry weight.

2.5. In Vitro Anti-Cholesterol Assay

The anti-cholesterol assay was carried out as described by Iswantini et al., [24] and Cholesterol Enzymatic Endpoint Method [25]. Cholesterol was dissolved in chloroform until achieving 25 mg/10 ml. 10 µl of the flower extract (two concentrations) were pipetted into micro titre plate followed by the addition of 2000 µl of Randox reagent and 10 µl of cholesterol as sample. 20 µl of distilled water and 2000 µl of Randox reagent were used as blank. Negative control comprised of 20 µL cholesterol and 2000 µl Randox reagent; standard comprised of 20 µl simvastatin and 2000 µl Randox reagent. The contents were mixed and incubated for 10 minutes at room temperature. The absorbance was read at 500 nm in a microplate reader against reagent blank.

2.6. MTT Assay

The flower extracts were tested for in vitro cytotoxicity using HCT-116 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [26]. HCT 116 (70-80%) confluent cell lines were trypsinized followed by viability checking and centrifugation. In a 96 well plate, 5 x 10^4 cells/ well were seeded and incubated for 24 hrs at 37°C in a humidified 5% CO_2 incubator. Plant extracts of varying concentrations ranging from 0-320 µg/ ml (two fold variations) in RPMI media without fetal bovine serum (FBS) and antibiotics were incubated for 24 hrs. After incubation with flower extracts, the media was removed from the wells and added 100µl/well (50 µg/well) of the MTT (5 mg/10 ml of MTT in 1x PBS, the solution was filtered through a 0.2 µ m filter and stored at 2-8 °C for frequent use or frozen for extended periods) working solution was added and incubated for 3 to 4 hours. After incubation with MTT reagent, the media was removed from the wells and added 100 µl of DMSO to rapidly solubilize the formazan. The absorbance for each well was measured at 590 nm in a microtitre plate reader and the percentage inhibition was calculated using the formula.

$$\text{Inhibition (\%)} = \frac{(\text{Control}_{\text{A590}} - \text{Sample}_{\text{A590}})}{\text{Control}_{\text{A590}}} \text{ x } 100$$

3. Results

Methanolic flower extracts of *Plumeria alba* and *P. rubra* were used for biological assays. DPPH assay of methanolic extract of *Plumeria* revealed that 81% and 72% inhibition by *P. alba* and *P. rubra* respectively thereby confirming the presence of antioxidants (Fig-1). The total phenolic content of *P. alba* and *P. rubra* was determined spectrophotometrically by the Folin-Ciocalteu method using gallic acid as internal standard (Fig-2) and it was found as 173.9 µg ml^{-1} and 167.3 µg ml^{-1} (Fig-3). Total antioxidant potential was carried out using ascorbic acid (Fig-4) and was determined as 1.74 mg $ml^{-1}$and 1.67 mg ml^{-1} (Fig-5). In vitro anti-cholesterol activity of *Plumeria* flowers were examined using simvastatin as the standard drug for a total half an hour at 10 minutes intervals. The purple colour developed during the reaction was decreased with increasing time and 52% activity was observed at the end of the reaction for *P. alba* whereas 60% activity was found with *P. rubra* (Fig-6). The standard drug, simvastatin

has exhibited 94% anticholesterol activity under in vitro conditions. Cytotoxic assay using colon cancer cell lines (HCT 116) revealed the antiproliferative activity of *Plumeria* against HCT 116 cell lines. *Plumeria alba* has exhibited dose dependent cytotoxicity whereas *P. rubra* was completely failed to control the proliferation of colon cancer cells. Loss of viability of the dying cells as evidenced by the morphological changes was scrutinized by microscopy. Depending on the concentration, the extract exhibited different levels of cytotoxicity like cell shrinkage, aggregation and cell death with an IC50 value of 259.9 µg ml^{-1} (Fig-7) was observed with *P. alba*.

4. Discussion

Research studies have found that methanol is the best reagent for extraction because it can easily penetrate the cell wall. In addition, many useful compounds have been found in methanol extracts [27]. In this study, 98% methanol was used in the extraction of *Plumeria* flowers. Natural antioxidants are generally more desirable for consumption than the synthetic one such as butylated hydroxyanisole (BHA) which was reported to be carcinogenic to humans [28]. The antioxidant activity is mainly due to their redox properties, which can play an important role in chelating transitional metals and scavenging free radicals [29]. For evaluation of free radical scavenging properties of *P. alba* and *P. rubra*, DPPH assay was performed using ascorbic acid as standard compound. Higher scavenging activity of free radicals was observed in both *P. alba* and *P. rubra* flower extracts which could be attributed by the presence of high phenolic content.

Cancer is a class of diseases characterized by unregulated cell growth. The World Health Organization has reported that approximately 13% of all deaths in the world are caused by cancer each year. Colorectal cancer is one of the most commonly diagnosed cancers worldwide. Death from colon cancer has risen to be the fourth highest among all cancer-related deaths. In our study, the human colon cancer cell (HTT-116) line was used as the target. Approximately 60% of drugs approved for cancer treatment are of natural origin [30, 31]. Plants are important sources for the development of potential anticancer agents which many of them have been purified and used as drugs for cancer prevention or treatment [32]. The results of MTT assay revealed that the *Plumeria* extract exert significant cytotoxicity and antiproliferative action on HCT 116. Oxidative stress is suggested as a mechanism underlying hyperlipidemia, which is one of the major risk factors for coronary artery diseases [33]. In our study, *Plumeria* species with high phenols showed a good antioxidant activity, which encouraged us to check the hypolipidemic activity in vitro. Both species of *Plumeria* were exhibited hypolipidemic activity with highest percentage observed in *P. rubra* (60%).

5. Conclusion

In conclusion, the results from this study further support the view that *Plumeria* species are promising source of natural antioxidants. *Plumeria alba* flowers were found as potential source of antioxidants and cytotoxic activity whereas, *P. rubra* was significant in exhibiting hypolipidemic activity in vitro. The in vivo antioxidant properties of *Plumeria* flowers should be the objective of future research in new formulation for pharmacological applications.

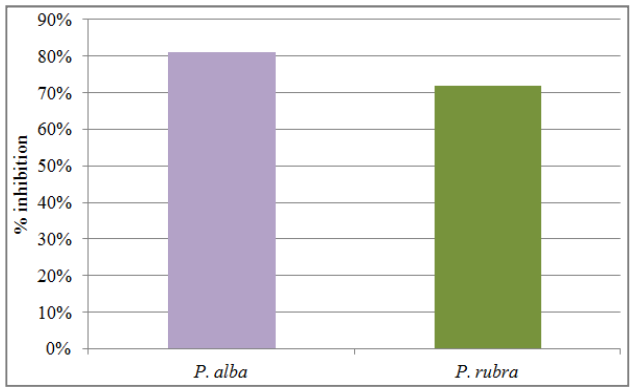

Fig 1. *DPPH assay of Plumeria alba and Plumeria rubra*

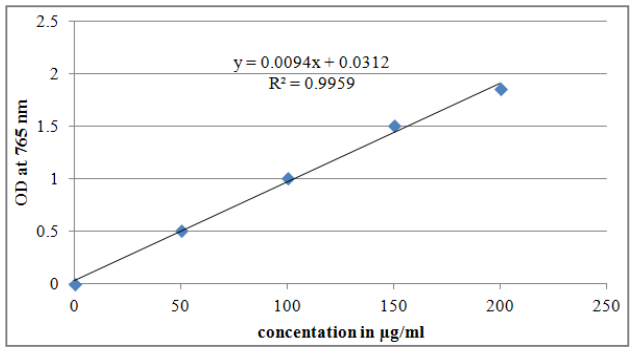

Fig 2. *Standard curve for total polyphenols using gallic acid*

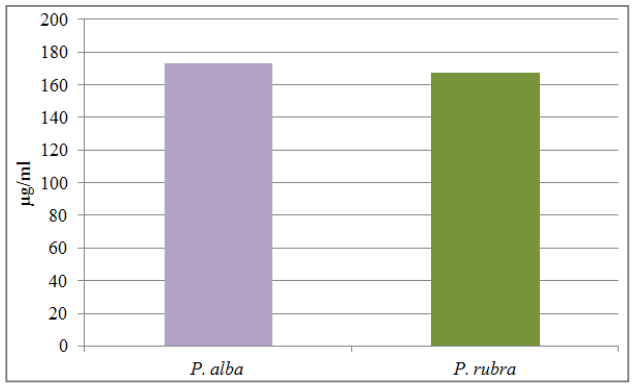

Fig 3. *Total phenolic content of P. alba and P. rubra (µg ml^{-1})*

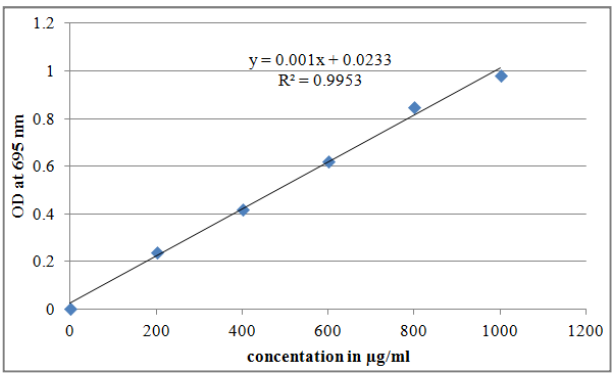

Fig 4. *Standard curve for antioxidant potential using ascorbic acid*

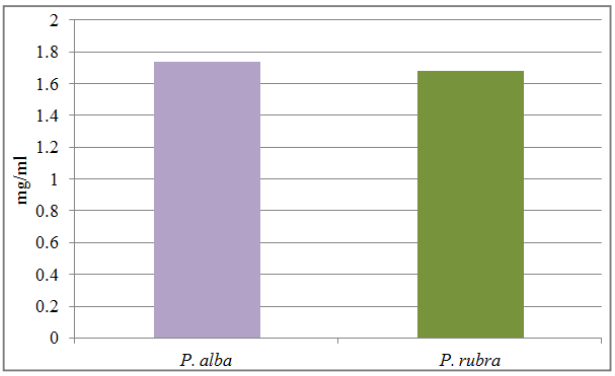

Fig 5. *Total antioxidant activity of P. alba and P. rubra (mg ml^{-1})*

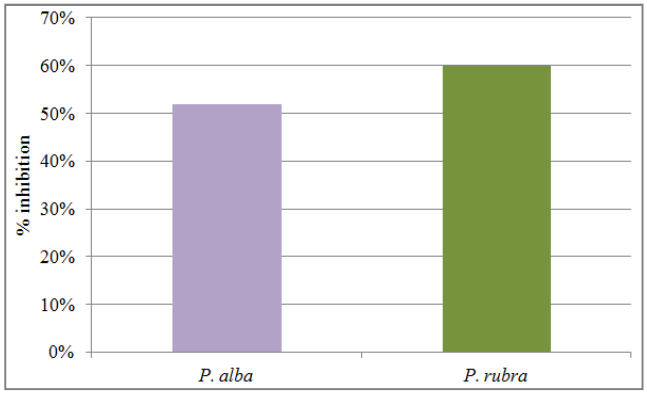

Fig 6. *Hypolipidemic activity of P. alba and P. rubra*

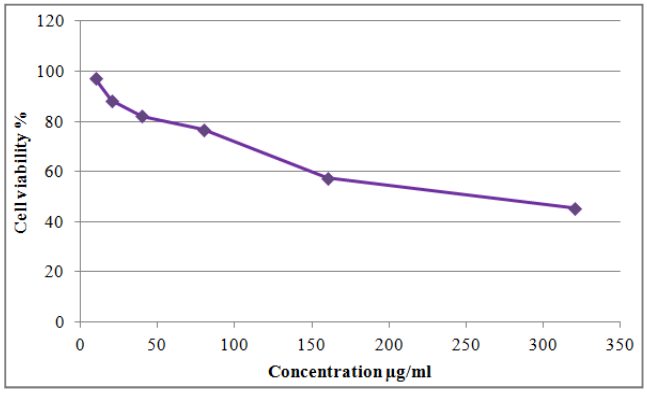

Fig 7. *MTT cytotoxic assay of Plumeria alba flowers against HCT 116 cell lines*

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