EVALUATION OF ANTIOXIDANT ACTION OF MIXED EXTRACTS OF PLUMERIA PUDICA AND ARAUCARIA COLUMNARIS

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ABSTRACT

The leaves of Plumeria pudica grow whirling around the stem and have a sessile base. The branchlets of Araucaria columnaris grow in whorls and are having small green, spirally arranged leaves. The extraction yield in methanol was found to be 39.6 % for Plumeria pudica and 43.7 % for Aruacaria columnaris the findings of preliminary phytochemical analysis suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, and flavonoids in the leaf of the Plumeria pudica while alkaloids were not found to be present in Araucaria columnaris. The total phenolic content of the methanolic extracts of P. pudica and A. columnaris were 38.31±1.7 and 54.27±2.1GAE mg/g, respectively. The extracts were individually and in combination (1:1, 1:2 & 2:1) subjected to in vitro determination of antioxidant potential in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH, reducing power using potassium ferrocyanide, hydroxy radical scavenging using Iron-EDTA and ammonium molybdate method. The IC50 value of the DPPH scavenging potential for P. pudica and A. columnaris was found to be 209.3 and 194.4 µg/mL respectively. All the ratios of the extracts exhibited increase in DPPH scavenging potential, the 1:2 ratio was found to be the most synergistically potent with and IC50 value of 56.39 µg/mL. The change in absorbance was observed with increasing the concentration of the extracts ranging from 0.031-0.174 for P. pudica and 0.028-0.135 for A.columnaris in concentration range from50-250µg/mL. On combining the extracts, the extract combination of the ratio 2:1 had reduction potential at dose 150µg/mL better that ascorbic acid. The IC50 value of the percent HRSA for the P. pudica and A. columnaris were found to be 188.17 and 231.95 µg/mL respectively. The IC50 value was found to be 154.86, 137.36 and 125µg/mL for 1:1, 1:2 and 2: 1 ratio of extracts respectively. The IC50 value of the percent reduction of phosphomolybdenum for the P. pudica and A. columnaris were found to be 234.37 and 194.44 µg/mL respectively. The IC50 value of the percent PRP for the combined extracts was found to be 227.27, 170.73, and 206.42µg/mL respectively for 1:1, 1:2 and 2:1 ratios of the extracts. It was found that the 1:2 ratio the extracts were best able to inhibit the phosphomolybdenum complex.

Keywords: - *Plumeria Pudica; Araucaria Columnaris; Antioxidant action, Mice.*

1. INTRODUCTION

Antioxidants may be of great help in improving the quality of life as they can either prevent or postpone the onset of several degenerative diseases. Also, they have a potential for considerable savings in the cost of health care delivery. Plants are known to possess a variety of chemical constituents which render them several biological properties, antioxidant being one of them. The amount of protection against free radicals can be increased by generous ingestion of dietary antioxidants or supplements. Significant evidence reveals that the foods that are rich in

antioxidants and probably in particular the antioxidant nutrients can be helpful in a major way in prevention of diseases. There is, however, a rising consensus among the scientists that an amalgamation of antioxidants, instead of a single antioxidant, may be more successful over long period. In biological system, reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide, hydroxyl, and nitric oxide radicals, can damage the DNA and lead to the oxidation of lipid and proteins in cells [1]. Normally, antioxidant system occurring in human body can scavenge these radicals, which would keep the balance between oxidation and anti-oxidation. Nonetheless, the exposure of cigarette smoking, alcohol, radiation, or environmental toxins induces the production of excessive ROS and RNS, which disrupt the balance between oxidation and anti-oxidation and result in some chronic and degenerative diseases [2-3]. The increment of intake of exogenous antioxidants would ameliorate the damage caused by oxidative stress through inhibiting the initiation or propagation of oxidative chain reaction, acting as free radical scavengers, quenchers of singlet oxygen and reducing agents. The exogenous antioxidants are mainly derived from food and medicinal plants, such as fruits, vegetables, cereals, mushrooms, beverages, flowers, spices and traditional medicinal herbs.

Plumeria is a genus of flowering plants belonging to the Apocynaceae. The species of this genus are deciduous shrubs or small trees. Plumeria pudica is commonly called as Golden Arrow or Gilded spoon. The flowers of Plumeria pudica are white in color with a yellow center and the leaves of the plant are of unusual spoon-like shape. The tree grows up to 1.5 – 6 m and has smooth stem which is rich in white latex. The leaf can be up to 10 cm long and 5 cm wide at the widest point. The leaves occur opposite, rarely whorled or alternating [4].

Figure 1. *Plumeria pudica* plant

Araucaria is a genus of coniferous trees in the Araucariaceae family. Nineteen species in the genus are known to exist, while most of them are used for timber and ornamental purposes Araucaria angustifolia, Araucaria araucana, Araucaria bidwilli, Araucaria cunninghamii and Araucaria heterophylla have been explored for their medicinal properties. Araucaria columnaris is a narrow columnar coniferous tree with short, usually horizontal branches that grow in verticils around the slender, upright trunk that leans towards the tip. These branchlets are dressed with small, green, spirally arranged and overlapping leaves which appear as needles when juvenile and grow triangular and scalar when adult. The bark of the tree exfoliates in thin strips and is rough, resinous and grey in color. The tree can grow up to 50-60 meter in height and 9-15 meter in width [5,6].

Figure 1.2: Araucaria columnaris tree

Taxonomical Classification

- Kingdom : Plantae
- Division : Pinophyta
- Class : Pinopsida
- Order : Pinales
- Family : Auracariaceae
- Genus : Araucaria
- Species : columnaris

2. RESEARCH OBJECTIVE

From the literature it was evident that the Plumeria species especially has being studied widely for its

pharmacological potential. It was also found that the antioxidant potential, the related anti-inflammatory and antidiabetic property of the plant has also been scientifically explored by researchers. Literature also revealed that Araucaria columnaris possesses some sort of pharmacological actions including antioxidant potential. Some studies have also revealed that combining similar solvent extracts from different plants can lead to synergistic effects. It was therefore envisioned that combining the leaf extracts of Plumeria pudica and Araucaria columnaris obtained from various solvent and studying the antioxidant potential would lead to significantly higher antioxidant potential of the plant and be of great applications in phytopharmaceutical applications.

The objective of the study was therefore twofold:

1. To determine the total phenolic contents and the antioxidant potential of the extracts using in vitro models

2. Combining the extracts (of similar solvents) and determining the antioxidant potential of the combined extracts and comparing it to the individual extracts

3. MATERIALS AND METHODS

3.1. Selection of the Plant

Ornamental plants known to have antioxidant potential and found in local region were selected for the study. Both Plumeria pudica and Araucaria columnaris are found to be widely used by households for decorative purposes and hence they were selected for our investigation.

3.2. Extraction of leaves [7]

The plant leaves, after authentication, were washed with distilled water and dried under shade. The dried leaves were powdered using a blender at low speed. The powdered leaves were stored in closed container till use. The powdered leaves of both the plants were used for the extraction process. 100 g of powder was evenly packed in soxhlet apparatus and extracted with 300 ml of methanol by hot continuous extraction process for about 20 h. The extracts were filtered while hot using Whatman filter paper for removal of impurities. The extracts were then concentrated by distillation in order to reduce the volume to one-tenth. The concentrated extracts were transferred to 100 ml beakers and the solvents were evaporated on water bath. The oleo-resinous/semisolid extracts collected and the excessive moisture was removed by placing the extracts in desiccators. The dried extracts were stored in desiccators for further procedures of analysis.

3.3. Preliminary phytochemical screening

The extracts from both the plants were subjected to qualitative phytochemical analysis for testing the presence or absence of common plant secondary metabolites. The evaluation was done for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. Precipitate formation or color intensity was used as analytical response to these tests.

3.3.1 Alkaloids [8]

Test for alkaloids was done using Drangendoff's reagent. Each of the extracts were re-dissolved in 5 ml of 1% HCl and 5 drops of Drangendoff 's reagent was added to each extract solution. A colour change (orange to orange red precipitate) was observed to infer the presence or absence of alkaloids.

3.3.2 Cardiac Glycosides [9]

The Keller-Killani test was performed for detecting the glycosides in the extracts. The plant extracts were dispersed in methanol (5 ml) and were treated with 2 ml of glacial acetic acid, containing one drop of ferric chloride solution. To this was added 1 ml of concentrated sulphuric acid. Brown ring formed at the interface may indicate the presence of deoxysugar cardenoloides. A violet ring may appear just below the brown ring, while in the acetic acid layer, a greenish ring may also form gradually throughout the layer, if the cardiac glycosides are present.

3.3.3 Tannins [10]

The extracts were dissolved in 5 ml distilled water and boiled gently and cooled. To 1 ml of each extract, 3 drops of ferric chloride solution was added. The formation of green coloured precipitate indicates the presence of tannins.

3.3.4 Flavonoids [9]
To a portion of the aqueous filtrate of each plant extract, diluted ammonia solution (5 ml) was added, followed by addition of concentrated sulphuric acid. The formation of a yellow precipitate indicated the presence of flavonoids.

3.3.5 Saponins [11]
The method of persistent frothing was used to detect the presence of saponins in the extracts. 1 g of each extract was boiled with 5 ml distilled water and filtered. To the filtrate was added 3 ml distilled water, shaken vigorously and heated. The samples were observed for the persistence appearance of foam lasting for at least 15 min confirmed the presence of saponins.

3.3.6 Steroids [12]
To 0.5 g of each extract, 2 ml acetic anhydride was added followed by the addition of 2 ml sulphuric acid. Change in color from violet to blue or green indicates the presence of steroids.

3.3.7 Terpenes/terpenoids [13]
The Salkowski test was used to detect the presence of terpenes/terpenoids in the different extracts. Five milliliters of extract were mixed in 2 ml chloroform and 3 ml concentrated sulphuric acid was then carefully added along the walls of the test tube to form a layer. The formation of greyish colour indicates the presence of terpenes/terpenoids.

4. RESULTS AND DISCUSSIONS

The present work focused on preparing methanolic extracts of Plumeria pudica and Araucaria columnaris leaves and establishing the antioxidant potential of the combined extracts in various in vitro models. The results obtained from the investigation are presented in the following sections.

4.1 Extraction Yields
The extraction yield in methanol was found to be 39.6 % for Plumeria pudica and 43.7 % for Aruacaria columnaris in Figure 4.1

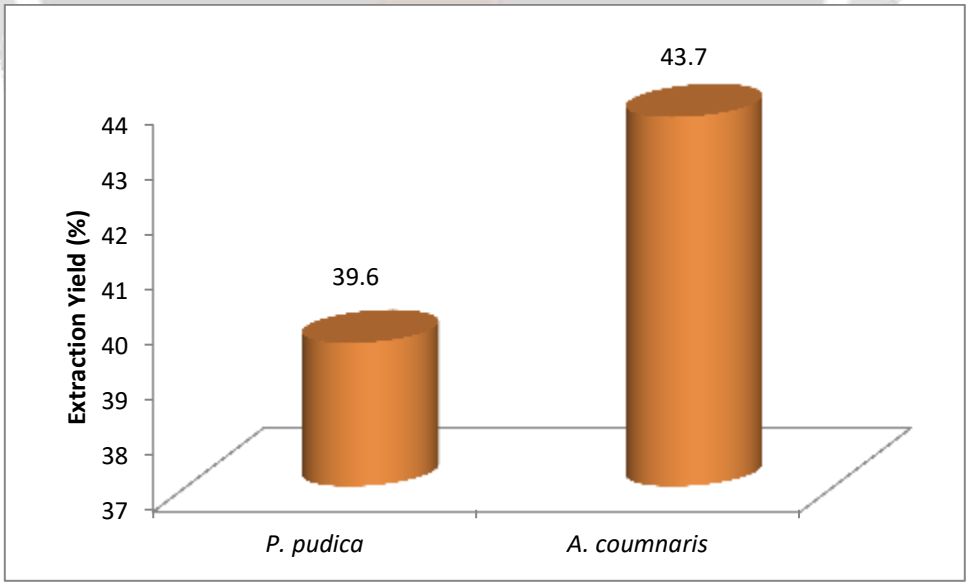


Chart 4.1 Extraction yields in methanol

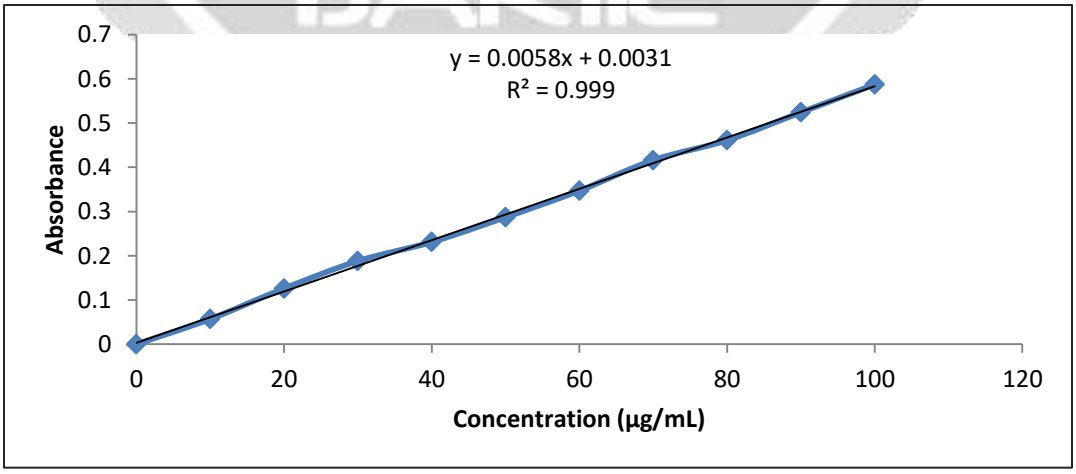
4.2 Total Phenolic content
The total phenolic content in the methanolic extract of Plumeria pudica and Araucaria columnaris was quantified using Folin-Ciocalteu method. Standard curve of gallic acid was calculated and plotted in distilled

water for determining absorption data (Table 4.2). The linear equation of gallic acid was found to be y = 0.0047x - 0.0017 (Figure 4.2). The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in table 4.2.1. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of found in the methanolic extract of Plumeria pudica and Araucaria columnaris were 38.31±1.7 and 54.27±2.1 GAE mg/g, respectively.

Table 4.2 Absorbance data of gallic acid (at 750 nm)

Concentration ppm	Absorbance at 750 nm
0	0
10	0.057
20	0.126
30	0.188
40	0.231
50	0.287
60	0.347
70	0.416
80	0.461
90	0.524
100	0.587

Figure 4. Calibration curve of gallic acid



Plant	Total phenolic content (GAE mg/g)
Plumeria pudica	38.31±1.7
Araucaria columnaris	54.27±2.1

Table 4.2.1 Total phenolic content (Methanolic leaf extract)

Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations

4.3 Determination of Antioxidant Potential

4.3.1 DPPH radicals scavenging activity

The extracts were individually and in combination (1:1, 1:2 & 2:1) subjected to in vitro determination of antioxidant potential in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH, reducing power using potassium ferrocyanide, hydroxy radical scavenging using Iron-EDTA and ammonium molybdate method.

The results obtained from the combined extracts revealed a significant increase in the DPPH scavenging potential. As visible from the results, the individual extracts were not at par with ascorbic acid in scavenging DPPH radical even at the 500 µg/mL dose whereas in the combined extract combination of 1:2, at concentration of 100 µg/mL of the extract the %DPPH scavenging was 84.6±1.435 almost equal to ascorbic acid (90.4±1.166) at the same dose and significantly increased at higher concentrations of the combined extract. Though all the ratios of the extracts exhibited increase in DPPH scavenging potential, the 1:2 ratio was found to be the most synergistically potent with and IC_{50} value of 56.39 µg/mL (Figure 4.3).

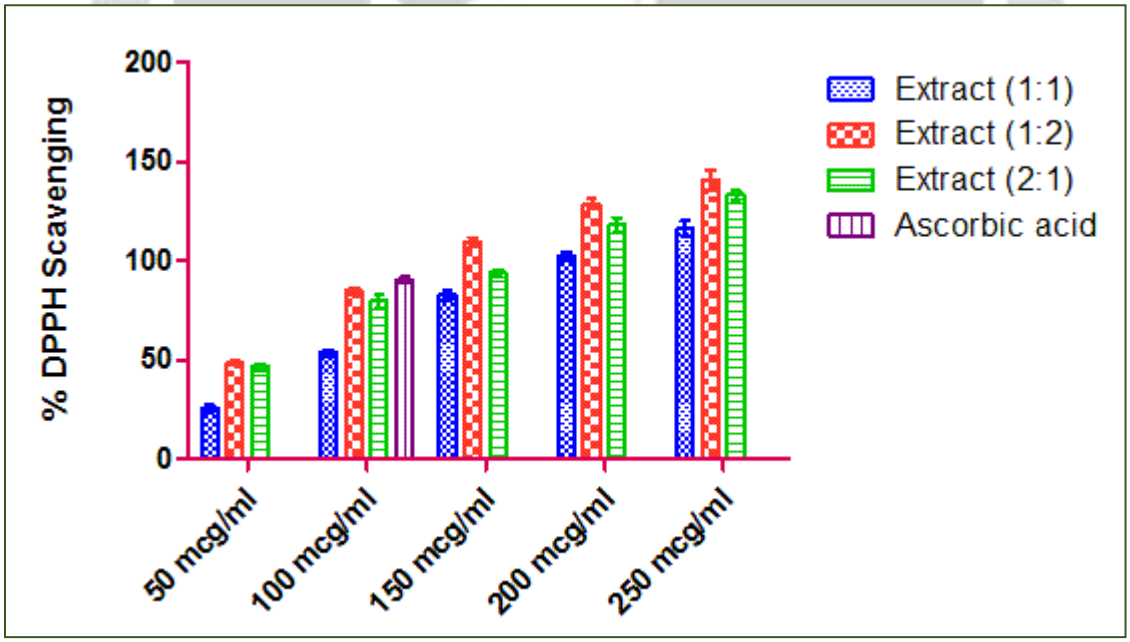


Figure 4.3 % DPPH scavenging potential of combined extracts

5. CONCLUSION

The objective of the present study was to assess the antioxidant potential of combined extracts of Plumeria pudica and Araucaria columnaris using the in vitro models. Methanolic extract of both the plants were found possess some antioxidant action. The results obtained led to the conclusion that mixing extracts of different

species of plants can lead to synergistic bioactivity thereby paving newer therapies for treatment of diseases like inflammation, diabetes and cancer and may also be helpful in formulation of newer nutraceutical supplements.

6. REFERENCES

[1] Fang YZ, Yang S, Wu G. Free radicals antioxidants and nutrition. Nutrition. 2002; 18: 872–879

[2] Peng C, Wang X, Chen J, Jiao R, Wang L, Li YM, Zuo Y, Liu Y, Lei L, Ma KY, et al. Biology of ageing and role of dietary antioxidants. BioMed Research International. 2014; 831-841

[3] Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng,Y. The role of oxidative stress and antioxidants in liver diseases. International Journal of Molecular Sciences. 2015; 16: 26087–26124.

[4] https://en.wikipedia.org/wiki/Plumeria_pudica; assessed on 25/04/2020

[5] https://en.wikipedia.org/wiki/Araucaria_columnaris; assessed on 25/04/2020

[6] https://www.gardenia.net/plant/araucaria-columnaris; assessed on 25/04/2020

[7] Sahira Banu K, Cathrine L. General Techniques Involved in Phytochemical Analysis. International Journal of Advanced Research in Chemical Sciences. 2015; 2(4): 25-32

[8] Harborne JB. Phytochemical methods. 3rd ed. London: Chapman and Hall Ltd. 1973; pp 135–203.

[9] Borokini TI, Omotayo FO. Phytochemical and Ethnobotanical study of some selected medicinal plants from Nigeria. Journal of Medicinal Plants Research. 2012; 6(7): 1106– 1118.

[10] Trease GE, Evans WC. Textbook of Pharmacognosy. 14th ed. London: W.B. Sanders. 1989.

[11] Odebiyi OO, Soforwa EA. Phytochemical screening of Nigerian medicinal plants. Lloydia. 1978; 41(3): 234–246.

[12] Khan AM, Qureshi RA, Ullah F, Gilani SA, Nosheen A, Sahreen S, Laghari MK, Laghari MY, Rehman SU, Hussain I, Murad W. Phytochemical analysis of selected medicinal plants of Margalla Hills and surroundings. Journal of Medicinal Plants Research. 2011; 5(25): 6017–6023.

[13] Olaleye MT, Akinmoladun AC, Crown OO, Ahonsi KE, Adetuyi AO. Homopterocarpin contributes to the restoration of gastric homeostasis by Pterocarpus erinaceus following indomethacin intoxication in rats. Asian Pacific Journal of Tropical Medicine. 2013; 6(3): 200–204.