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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *JUSTICIA TRANQUEBARIENSIS* AND *BAUHINIA RACEMOSA*

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ABSTRACT: Introduction: The phytochemicals in medicinal plants are defined as bioactive non-nutrient plant compounds in fruits, vegetables, and grains that have been linked to reducing the risk of major chronic diseases. The medicinal plant is promising to have such have effective medicinal property due to the presence of phytochemical compounds like alkaloids, tannins, flavonoids, and phenolic compounds. The present study aimed to find the phytochemicals present in hexane and ethyl acetate extracts of *Justicia tranquebariensis* and *Bauhinia racemosa* and also find out the antioxidant property of these plant extracts. **Methods:** Chemical methods identified phytochemicals present in the hexane and ethyl acetate extract of *Justicia tranquebariensis* and *Bauhinia racemosa*. The antioxidant property was done by DPPH free radical scavenging method. **Results:** In *Justicia tranquebariensis*, hexane extract showed positive for flavonoids, alkaloids, and coumarins. Ethyl acetate extract showed positive for flavonoids, alkaloids, cardiac glycosides, terpenoids, coumarins, and steroids. The hexane extract of *Bauhinia racemosa* showed positive for flavonoids, alkaloids, quinines, cardiac glycosides, terpenoids, and coumarins. Ethyl acetate extract showed positive for flavonoids, alkaloids, cardiac glycosides, terpenoids, coumarins, and steroids. Ethyl acetate showed better extraction of phyto-chemicals than hexane. Hexane and ethyl acetate extracts of *Justicia tranquebariensis* exhibited 66% and 90% respectively of free radical scavenging activity at a concentration of 150 µg. Extracts of *Bauhinia racemosa* showed less antioxidant activity. **Conclusion:** This study suggests that the *Justicia tranquebariensis* extracts have good antioxidant property, which might help prevent of slowing the progress of diseases involved as a result of oxidative stress.

INTRODUCTION: Medicinal plants, as a source of remedies, are widely used as alternative therapeutic tools for the prevention or treatment of many diseases.

The recent studies have investigated that the antioxidant effect of plant products is mainly attributed to phenolic compounds such as flavonoids, phenolic acids, tannins, *etc.*^{1,2}

Accumulation of free radicals can cause pathological conditions such as ischemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, the aging process and perhaps dementia. Natural antioxidants have become the target of a great number of research studies in finding the sources of potentially safe,

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effective and cheap antioxidants³. Herbal drugs containing free radical scavengers are known for their therapeutic activity⁴. In the present study, the phytochemical screening and antioxidant activities of two medicinally important herbs *Justicia tranquebariensis* and *Bauhinia racemosa* were taken to identify its phytoconstituents and radical scavenging capacity.

Justicia tranquebariensis (Acanthaceae) is a small shrub, which is widely distributed in the southern parts of India. Some species of the genus *Justicia* have been used in the traditional system of medicine for the treatment of fever,⁵ pain⁶, inflammation⁷, diabetes⁸, diarrhea⁹ and liver diseases¹⁰. The juice of small and somewhat fleshy leaves of this species of *Justicia* is considered by the natives of India as cooling and aperients, and is prescribed for the children in smallpox, in the doses of a table-spoonful or two, twice daily; bruised leaves are also applied to blows and other external injuries¹¹. As far as our literature survey could ascertain, no information was available on the protective and curative effect of *Justicia tranquebariensis* leaf extract. Therefore this study aimed to investigate the protective and curative effect of *Justicia tranquebariensis* from India.

Bauhinia racemosa Lam. (Caesalpinaceae) is a small bushy tree with drooping branches. The leaves are green and broader than long. The flowers are white or pale yellow, terminal or leaf-opposed racemes^{12, 13, 14}. A new tetracyclicupeol, betulin, β -sitosterol, and tetracyclic 2, 2-dimethylchroman have been isolated from the roots^{15, 16}. The seed contains flavonoids, crude protein, and lipid^{17, 18}. A methanolic extract of the stem and bark are used as an anti-inflammatory, analgesic and antipyretic¹⁹. A methanolic extract of the flower buds is used in the treatment of peptic ulcer²⁰. The whole plant is used as veterinary medicine in central India.

MATERIALS AND METHODS:

Chemicals and Reagents: DPPH (2, 2-diphenyl-1-picrylhydrazyl) and all other chemicals and reagents used were of the highest analytical grade commercially available²¹.

Collection and Identification of Plant Materials: Fresh leaves of *Justicia tranquebariensis* and

Bauhinia racemosa were collected from Chinnapaliyapattu, Tiruvannamalai, Tamil Nadu, India and Presidency College, Chennai, Tamil Nadu, India. The Taxonomist identified the plants at the Institute (NIPRD).

Phytochemical Tests: Preliminary phytochemical tests were carried out for aqueous extract of *A. deliciosa* to identify different phytoconstituents²¹.

Test for Carbohydrates: To 2 ml of plant extract, 1 ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates²².

Test for Tannins: To 1 ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins²³.

Test for Saponins: To 2 ml of plant extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Formation of a 1 cm layer of foam indicates the presence of saponins²⁴.

Test for Flavonoids: To 2 ml of plant extract, 1 ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids²⁵.

Test for Alkaloids: To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids²⁶.

Test for Quinines: To 1 ml of extract, 1 ml of concentrated sulphuric acid was added. Formation of red color indicates the presence of quinones²⁷.

Test for Glycosides: To 2 ml of plant extract, 3 ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates the presence of glycosides²⁸.

Test for Cardiac Glycosides: To 0.5 ml of extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of the brown ring at the interface indicates the presence of cardiac glycosides²⁵.

Test for Terpenoids: To 0.5 ml of extract, 2 ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red-brown color at the interface indicates the presence of terpenoids²⁵.

Test for Phenols: To 1 ml of the extract, 2 ml of distilled water followed by a few drops of 10% ferric chloride was added. Formation of blue or green color indicates the presence of phenols²⁷.

Test for Coumarins: To 1 ml of extract, 1 ml of 10% NaOH was added. Formation of yellow color indicates the presence of coumarins²⁷.

Phlobatannins: To 1 ml of plant extract a few drops of 2% HCl was added the appearance of red color precipitate indicates the presence of phlobatannins²⁵.

Steroids and Phytosteroids: To 1 ml of plant extract equal volume of chloroform is added and subjected with few drops of the concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish-brown ring indicates the presence of phytosteroids²⁹.

Anthraquinones: To 1 ml of plant extract, a few drops of 10% ammonia solution were added, appearance pink color precipitate indicates the presence of anthraquinones²⁵.

Antioxidant Activity: The ability of the extracts to annihilate the DPPH radical (1, 1-diphenyl-2-

picrylhydrazyl) was investigated by the method described by (Blois 1958). A stock solution of leaf extracts was prepared for the concentration of 1mg/ml. 100µg of each extract was added, at an equal volume, to a methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. BHT was used as standard controls. The annihilation activity of free radicals was calculated in % inhibition according to the following formula:

$$\% \text{ of Inhibition} = (A \text{ of control} - A \text{ of Test}) / A \text{ of control} \times 100$$

RESULTS: In *Justicia tranquebariensis*, hexane extract showed positive for flavonoids, alkaloids, and coumarins. Ethyl acetate extract showed positive for flavonoids, alkaloids, cardiac glycosides, terpenoids, coumarins, and steroids. The hexane extract of *Bauhinia racemosa* showed positive for flavonoids, alkaloids, quinines, cardiac glycosides, terpenoids, and coumarins. Ethyl acetate extract showed positive for flavonoids, alkaloids, cardiac glycosides, terpenoids, coumarins, and steroids. Ethyl acetate showed better extraction of phytochemicals than Hexane. Hexane and ethyl acetate extract of *Justicia tranquebariensis* exhibited 66% and 90% respectively of free radical scavenging activity at a concentration of 150 µg. Extract of *Bauhinia racemosa* showed less antioxidant activity **Fig. 1, 2, 3, & 4** and **Table 1 & 2**.

TABLE 1: PHYTOCHEMICAL SCREENING OF JUSTICIA TRANQUEBARIENSIS EXTRACTS

Phytochemical test	<i>Justicia tranquebariensis</i>	
	Hexane	Ethyl acetate
Carbohydrates	-	-
Tannins test	-	-
Saponin test	-	-
Flavonoid test	+	+
Alkaloid test	+	+
Quinones	-	-
Glycosides test	-	-
Cardiac glycosides test	-	+
Terpenoids test	-	+
Triterpenoids	-	-
Phenols	-	-
Coumarins	+	+
Proteins	-	-
Steroids and Phytosteroids	-	+
Phlobatannins	-	-
Anthraquinones	-	-

TABLE 2: PHYTOCHEMICAL SCREENING OF BAUHINIA RACEMOSA EXTRACTS

Phytochemical test	<i>Bauhinia racemosa</i>	
	Hexane	Ethyl acetate
Carbohydrates	-	-
Tannins test	-	-
Saponin test	-	-
Flavonoid test	+	+
Alkaloid test	+	+
Quinones	+	-
Glycosides test	-	-
Cardiac glycosides test	+	+
Terpenoids test	+	+
Triterpenoids	-	-
Phenols	-	-
Coumarins	+	+
Proteins	-	-
Steroids and Phytosteroids	-	+
Phlobatannins	-	-
Anthraquinones	-	-

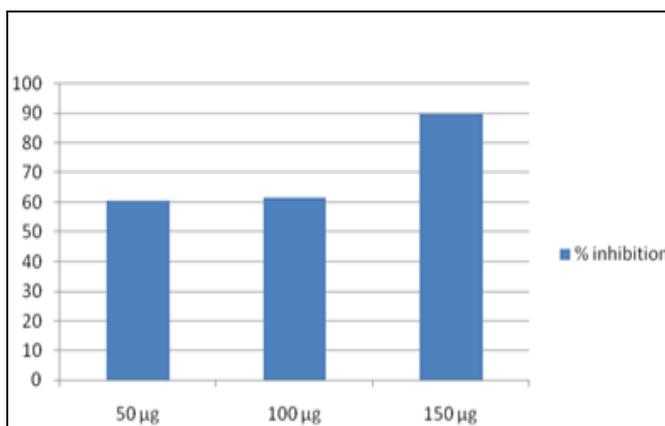


FIG. 1: ANTIOXIDANT ACTIVITY ETHYL ACETATE EXTRACT OF *J. TRANQUEBARIENSIS*

Concentration µg/ml, X-axis – concentration of the sample, Y-axis – the percentage of inhibition

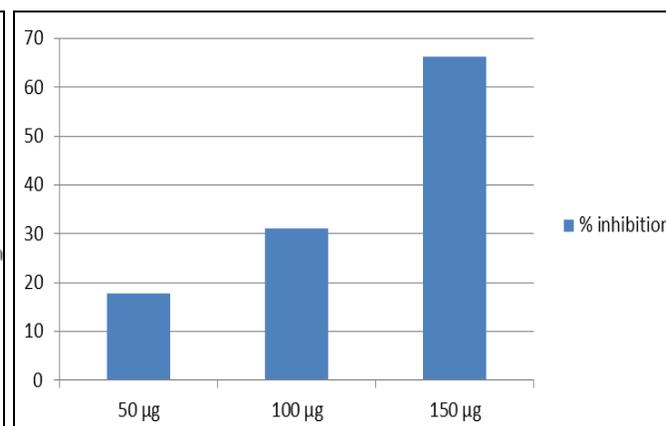


FIG. 2: ANTIOXIDANT ACTIVITY HEXANE EXTRACT OF *J. TRANQUEBARIENSIS*

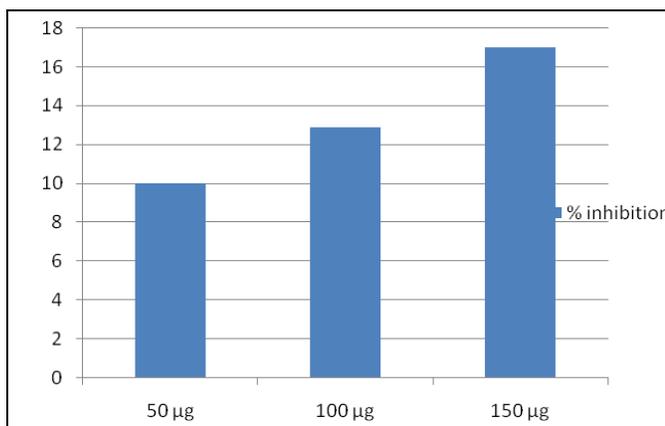


FIG. 3: ANTIOXIDANT ACTIVITY ETHYL ACETATE EXTRACT OF *BAUHINIA RACEMOSA*

Concentration µg/ml, X-axis – concentration of the sample, Y-axis – the percentage of inhibition

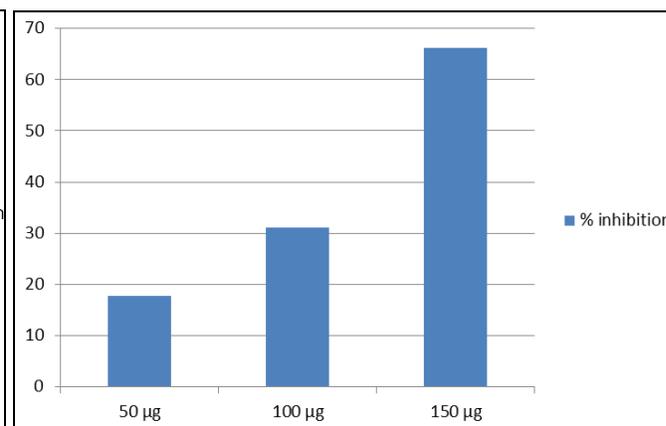


FIG. 4: ANTIOXIDANT ACTIVITY HEXANE EXTRACT OF *BAUHINIA RACEMOSA*

DISCUSSION: The importance of medicinal values of plants has been accepted in the past few decades. Plants produce a very diverse group of

secondary metabolites with antioxidant potential. Antioxidant blocks the action of the free radicals which have been implicated in the pathogenesis of

many diseases and the aging process. The free radicals are governing the important biological processes which are necessary for the body. The free radicals are necessary but at the same time harmful for the body. Hence it has some mechanisms are minimized the free radical-induced damage. The enzymes involved in repairs, the damage caused by free radicals are superoxide dismutase, catalase, glutathione, peroxidase, and glutathione reductase^{30, 31, 32}. In our study, hexane and methanol extract of *Bauhinia racemosa* showed positive for the presence of flavonoid, alkaloid, quinones, cardiac glycosides, terpenoids, coumarins steroids. Manohas *et al.*,³³ reported that methanol and aqueous extracts showed positive for carbohydrates, glycosides, alkaloids, phytosterol, saponins, flavonoids, gums and mucilage, tannins, fixed oil, and fats.

Akilandeswari *et al.*,³⁴ reported that the leaf of *Justicia tranquebariensis* showed, positive for phytosteroids, flavonoids, glycosides, and absence of triterpenoids, alkaloids, saponins, tannins. From our study, hexane and ethyl acetate extracts of *Justicia tranquebariensis* exhibited positive for the presence of flavonoids, alkaloids, cardiac glycosides, terpenoids, coumarins, and steroids. Balamurugan *et al.*,³⁵ reported that ethanol extract of the aerial portions of *Justicia tranquebariensis* showed free radical scavenging activity. In this study, hexane and ethyl acetate extracts of *Justicia tranquebariensis* exhibited 66% to 90% free radical scavenging activity at a concentration of 150 µg.

CONCLUSION: Phytochemical screening of hexane and ethyl acetate extracts of *Justicia tranquebariensis* and *Bauhinia racemosa* had revealed the presence of flavonoids, alkaloids, cardiac glycosides, terpenoids, coumarins, steroids. From this study, it has been concluded that *Justicia tranquebariensis* extracts have shown significant antioxidant activity than *Bauhinia racemosa*. This plant might help prevent or slowing the process of diseases involved as a result of oxidative stress.

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CONFLICT OF INTEREST: Nil

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