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Analyzing the phytochemical composition of *Justicia neesii* Ramam)

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Abstract

Phytochemical profile is an important aspect as it will give an over view of possible pharmacological properties of the plant. *Justicia neesii* is a plant belongs to Acanthaceae family, on which no significant phytochemical and pharmacological was done. The objective of the present study is to elucidate the phytochemical profile and analysis of antioxidant properties by TLC method. The phytochemical analysis was done for screening the maximum number of phytochemicals using standard methods. The TLC plates were developed with a solvent system containing methanol: chloroform: hexane at a ratio of 7:2:1. Ascorbic acid was used as positive control and a blank TLC plate was used as negative control in the experiment. The diluted DPPH in methanol was sprayed on the developed plates and observed under UV light. The preliminary phytochemical analysis shows the presence of flavonoids, glycosides, lactones, lignins, phenols, phytosterols, quinins, reducing sugars, saponins and terpenoids. The TLC analysis has shown the higher intensity of yellow color for the test spots which indicating the higher antioxidant potential of plant extract compared to standard ascorbic acid after treatment with DPPH solution. The plant is having good antioxidant potential. The plant was also composed of many significant phytochemicals.

Keywords: *Justicia neesii*, Phytochemical, Antioxidant, TLC analysis.

Introduction

Natural products are the primary and most significant source for developing new leads in drug discovery process. Plant parts like leaves, roots, bark are well established and most trusted medicines in India during earlier days of 1900. At that time people kept their trust on plant derived medicine and relayed largely on plants for curing every kind of diseases. In the later part of synthetic era people started working on natural products and developed many leads. 75% of anti infective drugs and 60% of anticancer agents are derived from natural product and also natural products are continued to play their roles even in the competition of new drug discovery strategies.¹ With respect to the constant contribution of natural products in human medicine basic pharmacological researchers are placing their interest mostly on plants and plant derived products.

Justicia neesii is a plant belongs to family Acanthaceae, grows in tropical regions of India as a perennial herb. It is also called as *Justicia micrantha* Wall. Acanthaceae family is composed of nearly 2500 species of plants belongs to 250 genera. This family of plants mainly contains alkaloids, tannins, diterpenoids, cyanogenetic compounds and saponins.² The ethno pharmacological information suggested that the plants belongs to *Justicia* genera can be used for treating variety of diseases including cancer, diabetes, fever, headache, inflammation, arthritis and different gastrointestinal disorders.³ In the previous studies on this plant reported the presence of various types of lignans. Three β -apolignans including 1,4-Dihydroxytaiwanin C, Jusneesiin, Jusneesiinol⁴ and two aryl-naphthalide lignans including juscimicranthin and justirumalin are found to be present.^{5,6} The plant was also found to contain diphyllin glycosides like Neesiinoside A and Neesiinoside B.⁷ The lignan glycosides derived from *Justicia ciliata* showed good anti-inflammatory activities.⁸ The lignan glycosides derived from *Justicia procumbens* showed good cytotoxic activities.⁹ The review of the scientific literature did not reveal any significant information on pharmacological properties of *Justicia neesii*. So it was consider worthwhile to analyze different phytochemicals present in the plant and make a clear profile of the possible pharmacological properties.

Materials and Methods

Collection and Identification of Plant

Plant material was collected from different areas of East Godavari district, Andhra Pradesh during the month of February 2014 on day time. The plant was taxonomically identified by the experts of Botanical Survey of India, Hyderabad (BSI/DRC/2013-14/Tech./915-A).

Extraction of Plant Material

Whole plant parts including leaves, stem, twigs, flowers, seeds, roots were separated and made free from soil matter. They were dried and powdered by using hand pulveriser to a coarse powder. Then the powder was extracted with ethanol by using Soxhlet apparatus at a temperature of 50-55°C for 8h. The extracts were concentrated using vacuum evaporator and the semisolid mass was dried in vacuum desiccators.

Phytochemical Analysis

The phytochemical analysis was carried out on the ethanolic extracts of the plant by following standard methods.¹⁰⁻¹⁷

Test for Alkaloids:

0.2 g of extract was added to 5mL of 2N hydrochloric acid and heated on boiling water bath. The mixture was filtered and cooled. To the two equal halves of the filtrate, Mayer's reagent was added to one portion and Wagner's reagent to other. Both the extracts form turbidity upon addition indicating the presence of alkaloids.

Test for Anthraquinones:

0.5 g of the extract was dissolved in 10% hydrochloric acid and boiled in water bath for few minutes. The solution was then filtered and cooled. To the cool filtrate equal volume of chloroform and few drops of 10% ammonia was added, mixed well and heated on water bath. The formation of rose pink colour will indicate the presence of anthraquinones.

Test for Carbohydrates:

0.2 g of the extract was dissolved in 2mL of distilled water and add few drops of α -naphthol solution in alcohol. Mix well and add concentrated sulphuric acid from sides of the test tube. Appearance of violet colour ring at the junction of two liquids will indicate the presence of carbohydrates.

Test for Carotenoids:

0.5 g of the extract was dissolved on chloroform and filtered. To the filtrate add 85% sulphuric acid. Blue colour at the interface of two layers will indicate the presence of carotenoids.

Test for Coumarins:

0.2 g of extract was dissolved in 2mL of distilled water. The test tube was covered with a filter paper which is previously soaked in sodium hydroxide solution and allowed to boil for few minutes. The paper was then observed under ultra violet light. The appearance of yellow colour fluorescence will indicate the presence of coumarins.

Test for Flavonoids:

0.2 g of the extract was dissolved in 5mL of distilled water and few drops of 10% sulphuric acid followed by 1mL of ammonia was added. The formation of greenish yellow precipitate indicates the presence of flavonoids.

Test for Fixed oils ad Fatty acids:

Spot the extract on a filter paper. Oil staining on the filter paper will indicate the presence of fixed oils and fatty acids.

Test for Glycosides:

0.2 g of extract was dissolved in 5 mL of distilled water and few drops of glacial acetic acid were added. Then few drops of 5% ferric chloride and concentrated sulphuric acid were added to the mixture. The appearance of bluish green colour in the upper layer and formation of reddish brown colouration at the junction of two liquids will indicate the presence of glycosides.

Test for Gums and mucillages:

0.5 g of the extract was dissolved in 10 ml of distilled water and added to 25 mL of absolute alcohol with constant stirring and then filtered. The precipitate was dried in air and observed for its swelling properties and carbohydrates content.

Test for Lactones:

0.2 g of the extract was dissolved in 2 mL of distilled water and add few drops of sodium picrate solution. Appearance of yellow to orange colour will indicate the presence of lactone ring.

Test for Lignins:

0.2 g of the extract was dissolved in 2 mL of distilled water. To this few mL of phloroglucinol alcoholic solution followed by few drops of hydrochloric acid was added. Appearance of red colour will indicate the presence of lignins.

Test for Phlobatanins:

0.5 g of the extract was dissolved in few mL of distilled water and filtered. To the filtrate 2% hydrochloric acid was added and boiled in water bath for few minutes. No red precipitate shows the absence of phlobatanins.

Test for Phenols:

The diluted extract was spotted on a filter paper and a drop of phosphomolybdic acid was added. The blue colouration of the spot upon exposure to the ammonia vapours will indicate the presence of phenols.

Test for Phytosterols:

0.2 g of the extract was dissolved in 2 mL of chloroform and add 1 mL of concentrated sulphuric acid from the sides of the test tube. Appearance of reddish brown colour in chloroform layer will indicate the presence of phytosterols.

Test for Proteins and Amino acids:

0.2 g of extract was dissolved in 2 mL of distilled water and 4% sodium hydroxide and few drops of 1% copper sulphate. Appearance of violet or pink colour will indicate the presence of proteins.

Test for Quinones:

0.2 g of the extract was dissolved in 2 mL of distilled water and few mL of alcoholic potassium hydroxide solution was added. The formation of red to blue colour indicates the presence of quinones.

Test for reducing sugars:

0.5 g of the extract was dissolved in 5mL of distilled water and boil on a water bath for 5 min and filtered. The filtrate was made alkaline to the litmus paper with 20% sodium hydroxide solution. The resultant solution was boiled on water bath with equal volume of Benedict's solution. The formation of brick red precipitate indicates the presence of reducing sugars.

Test for Resins:

0.2 g of the extract was dissolved in 2 mL of distilled water and add 3 mL of copper sulphate solution. The solution was mixed well and allowed to separate. The formation of green colour precipitate will indicate the presence of resins.

Test for Saponins:

0.2 g of the extract was dissolved in distilled water and then boiled in water bath. The mixture was cooled and shaken vigorously to form foam and observed for 20 min. The

formation of foam indicates the presence of saponins and the height of froth indicates the amount of saponins present.

Test for Steroids:

0.5 g of the extract was dissolved in two mL of acetic anhydride and 2 mL of concentrated sulphuric acid was added drop wise through the walls. The colour changes from violet to blue or green indicate the presence of steroids.

Test for Tannins:

0.2 g of extract was dissolved in 10mL of distilled water and warm on water bath. The mixture was filtered and to the filtrate 1mL of 5% Ferric chloride was added. The appearance of dark green solution indicates the presence of tannins.

Test for Terpenoids:

0.2 g of the extract was shaken with 2 mL of chloroform and then 3 mL of concentrated sulphuric acid was added through the walls of the test tube. Reddish brown coloration at the interface will indicate the presence of terpenes.

Test for Volatile oils:

0.2 g of the extract was dissolved in 2 mL of distilled water and add 0.1 mL of sodium hydroxide solution and shake well. Then add small amount of diluted hydrochloric acid and shake the solution. The formation of white precipitate will indicate the presence of volatile oils.

Thin layer chromatography test for antioxidants

For the determination of antioxidant composition of extract the thin layer separation followed by treatment with 2, 2 –diphenyl – 1-picrylhydrazyl (DPPH) was done. In this method about 2µL of the extract was loaded on the TLC plates (Merck 10 x 10 cm²) and developed with a solution of methanol: chloroform: hexane (7:2:1) for the separation different natural products present in the extract. Ascorbic acid was used as positive control and a blank TLC plate was used as negative control in the experiment. The developed plates are air dried and observed under UV cabinet for various spots and the Rf values are noted. Then 0.05% DPPH in methanol was sprayed on the developed plates and incubated for 15 min at room temperature. The antioxidant constituents will appear as a yellowish white band on the violet colour background. The intensity of the bands compared to the positive control was noted.^{18, 19}

Results and Discussion

The yield of ethanolic extract was found to be 10.58%. The yield of the plant extract may be affected by different chemical and environmental factors including the season of plant collection.²⁰

The preliminary phytochemical analysis was done for finding various chemical constituents present in the ethanolic extract of plant which shows the presence of flavonoids, glycosides, lactones, lignins, phenols, phytosterols, quinins, reducing sugars, saponins and terpenoids. High positive values are observed for the presence of glycosides, lactones, phenols and saponins. Flavonoids are the compounds which possess antiprotozoal, antibacterial and antiviral actions. These compounds having the capability of inhibiting important viral enzymes like reverse transcriptase and protease.²¹ Flavonoids will also show their effects on biochemical enzymes like aldose reductase, xanthine oxidase, phosphodiesterase, Ca⁺²-ATPase, and lipoxygenase. Several mechanisms for anti-inflammatory action of flavonoids have been reported.²² Glycosides are the well known and well established class of phytochemicals which are having cardiotoxic, antibacterial,

antiviral, anticancer, antioxidant, anti-inflammatory, neuroprotective, hepatoprotective and immunomodulatory actions. They can also inhibit the oxidase enzymes like tyrosinase and useful in dermatological treatments.²³ Sesquiterpene lactones are having biological activities like antimicrobial, cardioprotective, anticancer and anti-inflammatory activities.²⁴ Lignins and lignans are also having various biological activities including anti-inflammatory activity.²⁵ The phenolic compounds in the plants are the major source of primary antioxidants.²⁶ They also have antimicrobial action. In humans they will change the redox status of the cells by interacting with the receptors or enzymes involved in signal transduction.²⁷ Phenols also contain the wound healing potential by providing materials to the lignification of cell.²⁸ In addition to these phenolics can also act as anticancer, anti-inflammatory, anti-allergic, estrogenic and immunomodulatory agents.²⁹ Phytosterols are known mainly for their effect on lipid metabolism.³⁰ Some studies also stated the anti-inflammatory activities of phytosterols in in-vitro models.^{31, 32} Saponins are a kind of glycosides which produce the foaming aqueous solution. These compounds contain hemolytic, antifungal and anti-inflammatory properties.^{33, 34} Triterpenoids are reported to show antimicrobial properties.³⁵ Based on the above literature we can state that the test plant may contain mainly antimicrobial, anti-inflammatory and anticancer properties.

The TLC analysis was carried out to find the possible antioxidant constituents present in the plant extracts and the developed plates are observed in UV cabinet, before and after treating with DPPH solution (Figure 1). The positive antioxidant activity was noted by the fading of purple color of DPPH to yellow color spots. The plant extract has shown four spots before treating with DPPH and out of these three distinct yellow TLC bands are observed after treating with DPPH, under long UV light. Out of the three, two are showing high intensity of yellow color. Higher intensity of yellow color was observed for the test spots which indicating the higher antioxidant potential of plant extract compared to standard ascorbic acid.

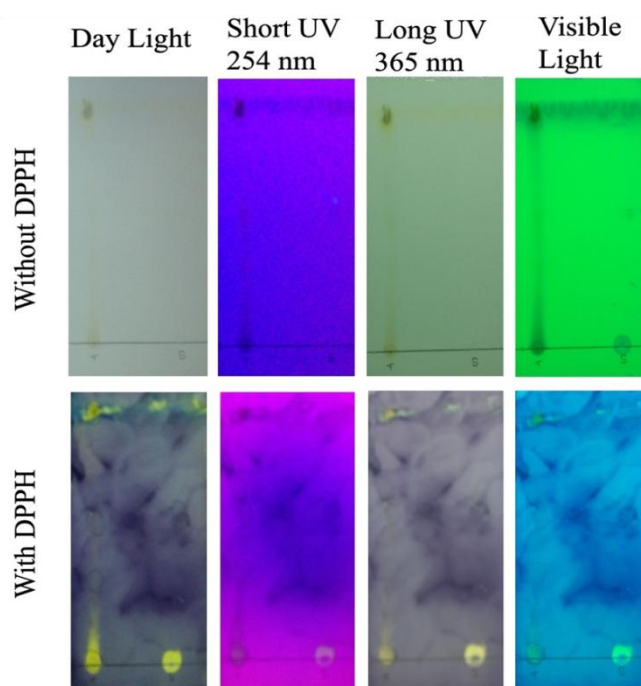


Figure 1: Analysis of antioxidant activity by TLC method

Conclusion

Complete pharmacological profile of a synthetic drug will be helpful in assessing the possible positive and negative effect on human body. Likewise a complete phytochemical study on the plant was helpful in assessing the possible pharmacological effects. By this study we are concluding that the plant is having good antioxidant potential. The plant was also composed of many significant phytochemicals and it will be worthwhile to conduct different pharmacological assays with respect to the phytochemical present in this plant extract.

References

1. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. *Journal of Natural Products* 2003; 66 (7): 1022–1037.
2. WC Evans, WB Saunders, Editors. *Trease and Evans Pharmacognosy*. 15th ed. London: Cambridge University Press; 2002. p. 35.
3. Geone M Correa, Antonio F de C Alcantara. Chemical constituents and biological activities of species of *Justicia* - a review. *Brazilian Journal of Pharmacognosy* 2012; 22(1): 220-238.
4. Dodda Rajasekhar, Gottumukkala V Subbaraju. *Justicia lignans*- V. Three new beta-apolignans from *Justicia neesii* Ramamoorthy. *Tetrahedron* 1998; 54:13227-13236.
5. Dodda Rajasekhar, Mulabagal Vanisree, Gottumukkala V Subbaraju. *Justicia lignans*: Part 4- Two new aryl-naphthalide lignans from *Justicia neesii* Ramamoorthy. *Indian Journal of Chemistry* 1999; 38B: 713-717.
6. Kovuru Gopalaiah, Jakka Kavitha, Raju V Kanumuri, Dodda Rajasekhar, Gottumukkala V Subbaraju. *Justicia lignans*: Part 9- Two new lignans from *Justicia neesii* Ramamoorthy. (White flower variety) *Indian Journal of Chemistry* 2001; 40B: 596-600.
7. Gottumukkala V Subbaraju, Dodda Rajasekhar, Jakka Kavitha, Jorge I Jimenez. *Justicia lignans*: Part 7-Two new diphyllin glycosides from *Justicia neesii* ramamoorthy. *Indian Journal of Chemistry* 2001; 40B: 313-319.
8. Day SH, Chiu NY, Tsao LT, Wang JP, Lin CN. New lignan glycosides with potent anti-inflammatory effect, isolated from *Justicia ciliate*. *Journal of Natural Products* 2000; 63: 1560-1562.
9. Day SH, Lin YC, Tsai ML, Tsao LT, Ko HH, Chung MI et al. Potent cytotoxic lignans from *Justicia procumbens* and their effects on nitric oxide and tumor necrosis factor-production in mouse macrophages. *Journal of Natural Products* 2002; 65: 379-381.
10. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour HR. Phytochemical screening of some species of Iranian plants. *Iranian Journal of Pharmaceutical Research* 2003; 4: 77-82.
11. Harborne JB. *Phytochemical methods. A guide to modern techniques of plant analysis*. 3rd ed. London: Chapman and Hall; 1998. p 40-96.
12. Khandelwal KR. *Practical Pharmacognosy*. 19th ed. Pune: Nirali Prakashan; 1995. p. 149-155.
13. Kokate CK, Purohith AP, Gokhale SB. *Pharmacognosy*. 44th ed. Pune: Nirali Prakashan; 2009. p. 6.14-6.17.
14. Rizk AM. Constituents of plants growing in Qatar. *Fitoterapia* 1982; 52: 35-42.
15. Siddiqui AA, Ali M. *Practical Pharmaceutical chemistry*. 1st ed. New Delhi: CBS Publisher and Distributors; 1997. P. 126-31.
16. WC Evans, WB Saunders, Editors. *Trease and Evans Pharmacognosy*. 15th ed. London: Cambridge University Press; 2002. p. 336-93.
17. Abayomi Sofowora. *Medicinal plants and Traditional Medicine in Africa*. 2nd ed. New Jersey: John Wiley and son Ltd; 1993. p. 150-153.
18. Kannan R, Arumugam R, Meenakshi S. Thin layer chromatography analysis of antioxidant constituents from seagrasses of Gulf of Mannar Biosphere Reserve, South India. *International Journal of Chem Tech Research* 2010; 2: 1526-1530.
19. Shreya Mandal, Arpita Patra, Animesh Samanta, Suchismita Roy, Arpita Mandal, Tapasi Das Mahapatra et al. Analysis of phytochemical profile of *Terminalia arjuna* bark extract with antioxidative and antimicrobial properties. *Asian Pacific Journal of Tropical Biomedicine* 2013; 3(12): 960-966.
20. Sharma R, Amin H, R G, Prajapati PK. Seasonal variations in physicochemical profiles of Guduchi Satva (starchy substance from *Tinospora cordifolia* [Willd.] Miens). *Journal of Ayurveda and Integrative Medicine* 2013; 4:193-7.
21. Havsteen BH. The biochemistry and medical significance of the flavonoids. *Pharmacology and Therapeutics* 2002; 96(2-3):67-202.
22. Permender Rathee, Hema Chaudhary, Sushila Rathee, Dharmender Rathee, Vikash Kumar, Kanchan Kohli. Mechanism of Action of Flavonoids as Anti-inflammatory Agents: A Review. *Inflammation and Allergy-Drug Targets* 2009; 8(3):229-35.
23. Fu G, Pang H, Wong YH. Naturally occurring phenylethanoid glycosides: potential leads for new therapeutics. *Current Medicinal Chemistry* 2008; 15 (25):2592-613.
24. Devdutt Chaturvedi. Sesquiterpene lactones: Structural diversity and their biological activities. Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry 2011; 37: 313-334.
25. Cyril Heitner, Don Dimmel, John Schmidt. *Lignin and Lignans: Advances in Chemistry*. 1st ed. Florida: CRC press; 2010. p. 585.
26. Hatano T, R Edamatsu, M Hiramatsu, A Mori, Y Fujita et al. Effect of interaction of tannins with co-existing substances VI. Effect of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chemical and Pharmaceutical Bulletin* 1989; 37:2016-2021.
27. Mudgal V, N Madaan, A Mudgal, S Mishra.. Dietary polyphenols and human health. *Asian Journal of Biochemistry* 2010; 5: 154-162.
28. Miller NJ, MB Ruiz-Larrea. Flavonoid and other plant phenols in diet: Their significance as antioxidants. *Journal of Nutritional and Environmental Medicine* 2002; 12:39-51.
29. Tawaha K, FQ Alali, M Gharaibeh, M Mohammad, T El-Elimat. Antioxidant activity and total phenolic content of selected Jordanian plant species. *Food Chemistry* 2007; 104: 1372-1378.
30. Elzbieta Bartnikowska. Biological activities of phytosterols with particular attention to their effects on lipid metabolism. *Polish journal of food and nutrition science* 2009; 59(2): 105-112.
31. Awad AB, Toczek J, Fink CS. Phytosterols decrease prostaglandin release in cultured P388D1/MAB macrophages. *Prostaglandins Leukot Essent Fatty Acids* 2004; 70(6):511-520.

32. Navarro A, De las Heras B, Villar A. Antiinflammatory and immunomodulating Properties of a sterol fraction from *Sideritis foetens* Clem. Biological and Pharmaceutical Bulletin 2001; 24(5):470-473.

33. Figen Mert-Turk. Saponins versus plant fungal pathogens. Journal of Cell and Molecular Biology 2006; 5: 13-17.

34. Takagi K, Hee PE, Histoshi K. Anti-inflammatory activities of hedergenin and crude saponin from *Sacchindus mukarassi*. Chemical Bulletin 1980; 28(4): 1183.

35. Ghosh P, Mandal A, Chakraborty P, Rasul MG, Chakraborty M, Saha A. Triterpenoids from *Psidium guajava* with biocidal activity. Indian Journal of Pharmaceutical Sciences 2010; 72:504-507.