



Research Article

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Phytochemical Screening and Evaluation of Antioxidant Activity of hydroalcoholic extract of *Justicia procumbans* leaf

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ABSTRACT

Objective: To perform Phytochemical Screening and Evaluation of Antioxidant Activity of hydroalcoholic extract *Justicia procumbans* leaf. **Methods:** Proximate analysis in terms of ash value, extractive value was performed as per the standard method. Presence of secondary metabolites in the extract of *Justicia procumbans* was performed through various chemical tests. Folin-Ciocalteu assay method was used to determine total phenolic content and the in-vitro antioxidant activity was investigated in a dose-dependent manner with the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide free radical scavenging method. **Results:** Present studies revealed that the hydro-alcoholic extract of leaf contains secondary metabolites such as alkaloids, steroids, flavonoids, carbohydrates, proteins, and tannins. Total phenolic content was found to be 79.32±0.02 mg/gm by using the Linear Equation. IC₅₀ value by DPPH and H₂O₂ methods was found to be 68.83µg/ml and 56.02 µg/ml respectively. **Conclusion:** It has been observed that the plant has high phenolic compounds and antioxidant activity. Therefore his plant can be the potent source of natural antioxidants as compared to synthetic compounds.

Keywords: *Justicia procumbans*, DPPH, Hydrogen peroxide scavenging method, Total phenolic content.

INTRODUCTION

Indian sub-continent due to its varied geographical and agro-climatic regions is a rich source of plant and animal wealth. At present Indian medical care conveyance comprises of traditional and modern systems of medicines. Traditional systems of medicine like Ayurveda, Siddha, Unani and folk medicine have been prospering admirably. Siddha and Ayurveda are of Indian origin and 75% of rural Indian population depends on these traditional systems [1].

The Konkan region is a storehouse of medicinal plants which is the coastal belt of western state of Maharashtra. The world famous Western Ghats is lying in the Konkan region. The Western Ghats of India comprise a biodiversity hotspot and have more than 2000 endemic vascular plant species. This area is a treasury of around 700 medicinal plants. Some of them are utilized for traditional and folk medicinal practices. The Western Ghats presents an entire scope of angles, both altitudinal just as latitudinal in climatic components, for example, all out yearly precipitation, greatest temperatures. The heat and humidity commended by hefty precipitation from southwest rainstorm and positive edaphic factors make an ideal condition for the lush development of plant, which can be seen uniquely in barely any pieces of the world [2].

The restricted information on the differed utilization of the therapeutic plants, their accessibility debilitates the approaches to use these assets effectively. In this way, it is needed to get the data different sources into one rooftop.

Western Ghats was and will stay a territory where the therapeutics of nature can be uncovered. Henceforth, endeavors must be taken to bring to spotlight the utilization of nature's primary care physicians to support their reality for the people in the future [1]. With the support of home grown meds and their items expanding, there is a dire need to moderate the endemic variety in the therapeutic plants before it is cleared out from nature. In this way assortment and development of such species, recognizable proof of their various species and Pharmacological assessment is the current days call for researcher.

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Hence present study deals with the pharmacognostic, phytochemical and pharmacological evaluation of *Justicia procumbans*, one of the plant found in Konkan region [2].

The plant Ghatipittapada (*Justicia procumbens*) commonly known as Water Willow belongs to the family Acanthaceae [3]. It is a small perennial shrub, distributed throughout the world and endemic to India, found commonly in humid areas, at altitudes up to 1500 m. The plant is slender; stems diffuse, with many divaricate branches, rootings at the lower nodes, the flowers pale purple, glabrous or pubescent [4]. It is bitter in taste and can be utilized in the form of decoction for treating variety of diseases including venereal diseases, boils, tumors, skin diseases, apthous ulcer, cancer, diabetes, fever, headache, inflammation, arthritis and diverse gastrointestinal disorders [5, 6].

MATERIALS AND METHODS

Chemicals and instrumentation

All chemicals utilized were of analytical grade or higher. Gallic acid and DPPH were purchased from Sigma–Aldrich (Mumbai, India). UV–Visible absorption was measured on a UV–Visible Spectrophotometer (UV–1800, Shimadzu, Japan). Extract was ultrasonicated using Power Sonic 420.

Materials

The leaves of plant *Justicia procumbans* were collected locally from Ratnagiri region in the month of January 2020. It was identified by Prof. Dr. S A Apaté, Department of Botany, R.P. Gogate College of Arts and Science, Ratnagiri, affiliated to University of Mumbai, Mumbai.

Methods

1) Proximate analysis of JP leaves [7]

The powdered leaves of JP were subjected to evaluate various ash contents such as total ash, acid insoluble ash, water soluble ash, water and alcohol soluble extractive values.

a) Total ash content

1 g powdered drug was taken in a tared silica dish previously dried and weighed. It was ignited in a furnace until free from carbon. The ash obtained was weighed.

Acid insoluble ash

To the crucible containing the total ash, 25 ml of dilute hydrochloric acid was included, covered with a watch glass and bubbled tenderly for 5 minutes. The insoluble matter was gathered on an ash less filter paper, washed with hot water until the filtrate is neutral. It was dried and ignited to consistent weight. The residue was allowed to cool in a suitable dessicator for 30 minutes, and afterward weighed immediately.

Water-soluble ash

To the crucible containing the total ash, 25 ml of water was included and bubbled tenderly for 5 minutes.. The insoluble matter was

gathered on an ash less filter paper, washed with hot water, and ignited in a crucible for 5 minutes. The residue weight was subtracted from the weight of total ash.

b) Extractive value (Water & Alcohol)

About 2 g of precisely weighed homogenized drug was placed in a glass stoppered conical flask. It was macerated with 100 ml of solvent for 6 hours, shaking frequently and afterward was permitted to stand for 18 hours. Concentrate was separated quickly taking consideration not to lose any dissolvable. 25 ml of the filtrate was transferred to a tared flat –bottom dish and evaporated to dryness on a water bath. The residue was dried at 105°C till its weight got steady, cooled in a dessicator for 30 minutes and weighed immediately.

2) Extraction of plant material

The matured leaves of *Justicia procumbans* were washed in order to remove dust, debris and sand under running tap water. The plant material were dried in shade under ordinary environmental condition and subjected to size reduction. The powdered leaves were subjected to maceration at room temperature with hydro alcohol (8:2 v/v) and the filtrates were concentrated using Rotary vaccum evaporator which was then freeze-dried for further studies. Residue was stored in glass bottle.

3) Phytochemical screening [8, 9, 10]

Qualitative phytochemical screening of leaf extract was performed for the existence of the phytochemicals like Flavonoids, Alkaloids, Steroids, Saponins, Tannins, Carbohydrate and Protein by using the standard methods.

4) Estimation of total phenolic content (TPC) [10]

Folin-Ciocalteu method is used to determine total phenolic content of the sample. The Folin-Ciocalteu method is an electron move based assay, and gives decreasing limit which is communicated as phenolic content. 5 ml of Folin-Ciocalteu reagent (10 % v/v) is added to 1 ml (10mg/5ml) of the sample and is vortexed for 5 min, followed by addition of 5 ml of sodium carbonate(7.5%). This reaction mixture was incubated for 2 hr at room temperature. From these flasks pipette out 2ml of extract solution and make up the volume of test tubes to 5ml with distilled water. Then allowed to stand for 15 minutes and absorbance was measured at 765 nm. The same procedure was followed for the standard solution of gallic acid (stock solution 5mg/100ml). From stock of gallic acid 6 dilutions were made, each containing gallic acid 50µg, 100µg, 150µg, 250µg, 350µg, 500 µg in 50ml.

5) Antioxidant Assay

Two different chemical methods namely DPPH and H₂O₂ assays were utilized for assessing the antioxidant activity of hydroalcoholic extract. Stock solutions of crude extracts and standard (Ascorbic acid) were prepared in methanol at 10 mg/10ml concentration from dry weight. These stock solutions were diluted in methanol to provide five different concentrations; 20, 40, 60, 80 and 100 µg/ml for each.

a) Hydrogen peroxide (H₂O₂) radical scavenging activity assay [11, 12]

Solution of 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide solutions were prepared according to the Indian Pharmacopoeia 1996 standards. 50 ml potassium dihydrogen phosphate solution was put in a 200 ml volumetric flask and 39.1 ml of 0.2 M sodium hydroxide solution was added and finally volume was made up to 200 ml with distilled water to prepare phosphate buffer (pH-7.4). 50 ml of phosphate buffer solution was added to equal amount of hydrogen peroxide to generate the free radicals and solution was kept aside at room temperature for 5 min to finish the reaction. Extracts (1 ml) in distilled water were added to 0.6 ml hydrogen peroxide solution and the absorbance was estimated at 230 nm in a spectrophotometer against a blank solution containing phosphate buffer solution without hydrogen peroxide. The percentage of scavenging of H₂O₂ by extract was determined by using the following equation:

$$\text{Percent scavenge (H}_2\text{O}_2) = \frac{A_0 - A_1}{A_0} \times 100$$

Where A₀ is the absorbance of the control and A₁ is the absorbance in the presence of the extract and standard

b) DPPH free radical scavenging assay [13, 14, 15]

Solution of DPPH (0.1 mM) in methanol was prepared. The solution was kept in darkness for 30 min to finish the reaction. 9 ml of DPPH solution was added to 1 ml of plant extract/ascorbic acid and permitted to stand at room temperature for 30 min. An equal amount of methanol (1ml) was used as a blank (control) with 9 ml of DPPH solution. All the examples were set up in three-fold. The decrease in absorbance of each sample was measured against methanol as blank on UV-Visible spectrophotometer at 517 nm. The free radical scavenging activity was calculated as using following formula,

$$\% \text{ inhibition} = \frac{A_c - A_t}{A_c} \times 100$$

Where, A_c is the absorbance of control and A_t is the absorbance of test sample.

The results were reported as IC₅₀ value, a lower IC₅₀ value represents a stronger DPPH scavenging capacity.

RESULTS

Proximate analysis (Ash and extractive values)

Total ash, water soluble ash, acid insoluble ash, water soluble and alcohol soluble extractive values were determined and results are tabulated in Table 1.

Table 1: Determination of ash and extractive value of JP leaf

| | | |
|-----------------------|--------------------|-------------|
| Ash values (%) | Total ash | 3.92±0.12% |
| | Acid insoluble ash | 1.61±0.03% |
| | Water soluble ash | 2.11±0.09% |
| Extractive values (%) | Water soluble | 14.36±0.04% |
| | Alcohol soluble | 3.58±0.11% |

Extraction

Percentage yield of hydroalcoholic extract of JP leaf was found to be (16.66%)

Phytochemical Screening

Preliminary phytochemical screening of hydroalcoholic extract was done to uncover the presence of different primary and secondary metabolites. The hydroalcoholic extract showed the presence of steroids, flavonoids, carbohydrates, alkaloids, proteins and tannins. (Table 2)

Table 2: Phytochemical screening of JP leaf extract

| Plant constituents | Test/Reagent | Hydroalcoholic extract |
|--------------------|--------------------------|------------------------|
| Steroids | Salkowski reaction | + |
| | Liebermann-Burchard test | + |
| Alkaloids | Dragendorff's reagent | - |
| | Mayer's reagent | + |
| | Hager's reagent | + |
| | Wagner's reagent | + |
| Tannins | Ferric chloride test | + |
| | Lead acetate test | + |
| | Potassium dichromate | + |
| Flavonoids | Shinoda test | + |
| Carbohydrates | Molish's test | + |
| | Barfoed's test | - |
| Proteins | Biuret test | - |
| | Xanthoproteic test | + |
| Saponins | Foam test | - |

+: found to be present
-: Found to be absent

Total Phenolic Content

Total phenolics content was estimated by Folic-Ciocalteu method. The data obtained from experiment was tabulated and calibration curve was drawn by Microsoft Office Excel 2007. The total phenolic content was calculated from linear regression equation and the outcomes were expressed as mg of gallic acid equivalent per g dry weight. Total phenolic content was found to be 79.32±0.02 mg/g by using the Linear Equation y = 0.0011x, R² Value= 0.9933 (Fig. 1)

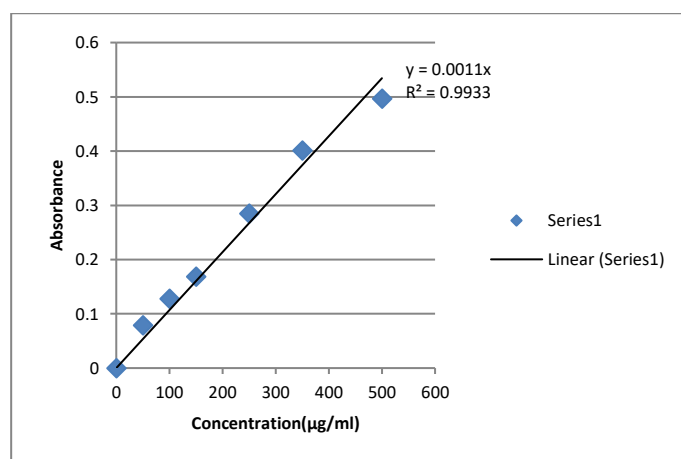


Figure 1: Standard Gallic acid calibration curve for total phenolic content

Antioxidant Assay

H₂O₂ radical scavenging activity assay

Hydrogen peroxide radical scavenging activity of extract was found to be dose dependent. The scavenging activity of extract was 30.9±1.1%, 46.59±1.9%, 51.49±1.21%, 61.06±1.2% and 68.95±0.98% at the concentrations of 20, 40, 60, 80 and 100µg/ml respectively. Similar dose dependent scavenging was observed with ascorbic acid (standard). The IC₅₀ value of the extract was 56.02 µg/ml whereas for ascorbic acid it was 23.19 µg/ml using linear regression equation (Table 3, Fig. 2)

Table 3: The scavenging ability of the extract and standard on H₂O₂

| Sr No. | Extract | Concentration (µg/ml) | % Inhibition |
|--------|---------------------|-----------------------|--------------|
| 1 | JPE | 20 | 30.9±1.1 |
| | | 40 | 46.59±1.9 |
| | | 60 | 51.49±1.21 |
| | | 80 | 61.06±1.2 |
| | | 100 | 68.95±0.98 |
| 2 | Std (Ascorbic acid) | 20 | 46.51±0.5 |
| | | 40 | 60.9±1.3 |
| | | 60 | 74.39±1.8 |
| | | 80 | 86.51±1.9 |
| | | 100 | 95.61±1.1 |

Mean ±SD; (n =3)

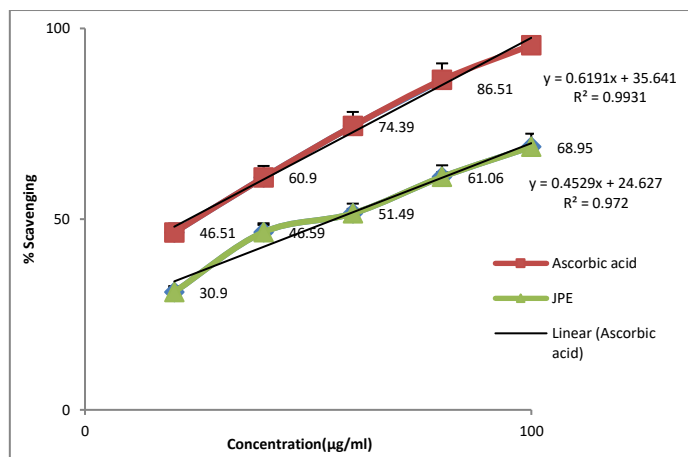


Figure 2: The scavenging ability of the extract and standard on H₂O₂

DPPH free radical scavenging assay

The capacity of samples to scavenge DPPH radical was estimated on the basis of their concentrations providing 50% inhibition (IC₅₀). The result indicates significant difference of mean percentage scavenging between different concentrations of tested extract. The scavenging activity of extract was well pronounced at higher concentrations of 80 µg/ml and 100 µg/ml with a mean percentage of 60.49±0.87 % and 65.21±0.32% respectively, which was lower as compared with standard antioxidant, ascorbic acid but exhibits similar pattern of concentration dependent free radical scavenging effect. The IC₅₀ values of extract (68.83 µg/ml) and ascorbic acid (35.37 µg/ml) were obtained using the linear regression equation. (Table 4, Fig. 3)

Statistical Analysis

All information was introduced as the mean of three separate investigations and error bars are displayed with standard error. The IC₅₀ value was determined from linear regression analysis utilizing Microsoft excel with its Data Analysis add-in.

Table 4: The scavenging ability of the extract and standard on DPPH

| Sr No. | Extract | Concentration (µg/ml) | % Inhibition |
|--------|---------------------|-----------------------|--------------|
| 1 | JPE | 20 | 22.33±0.92 |
| | | 40 | 35.25±0.57 |
| | | 60 | 42.21±0.31 |
| | | 80 | 60.49±0.87 |
| | | 100 | 65.21±0.32 |
| 2 | Std (Ascorbic acid) | 20 | 39.51±0.65 |
| | | 40 | 54.97±0.98 |
| | | 60 | 63.18±1.14 |
| | | 80 | 75.32±1.1 |
| | | 100 | 82.65±0.94 |

Mean ±SD; (n =3)

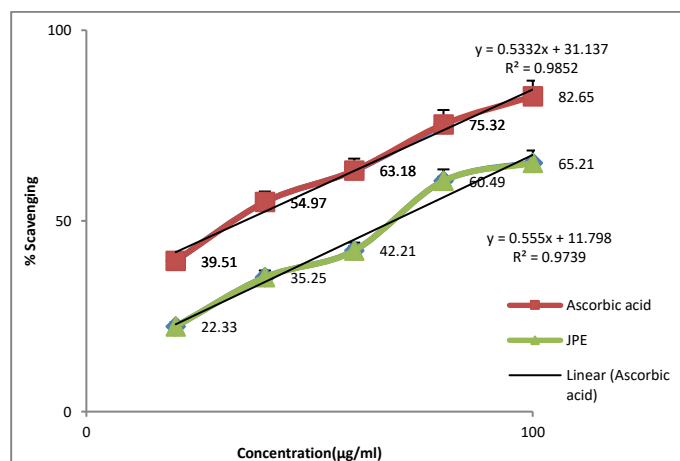


Figure 3: The scavenging ability of the extract and standard on DPPH

DISCUSSION

The physical quantitative examination like total ash, water soluble ash, acid insoluble ash were determined which are useful identifying parameters to validate and verify the drug and could be valuable in the preparation of herbal monograph for its evaluation. These investigations additionally help to detect adulteration or any unintentional mixture in original drug as well as purity of the sample.

Extractive value provides an idea about the nature of the phytochemicals present and their solubility. The result reported higher percentage of water-soluble extractive than alcohol soluble extractive. This indicated that maximum phytoconstituents of *Justicia procumbans* leaves were more soluble in polar solvents.

The qualitative determination of plant constituents through various chemical tests is necessary to identify the presence of various groups of plant secondary metabolites and also ascertain the therapeutic efficacy. Present studies revealed the presence of secondary metabolites such as alkaloids, steroids, flavonoids, carbohydrates,

proteins, and tannins in hydro alcoholic extract of *Justicia procumbans* leaf.

In the present research work total phenolic content was estimated. Plant materials wealthy in phenolics are progressively being utilized in the pharma industry since they hinder oxidative debasement because of their hydroxyl extremist. Phenolic compounds demonstrated to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases. Antioxidants battle against free radicals and shield us from different sicknesses. The extract exhibited the highest total phenolics content. This bioactive constituent could be the root factor for a potential antioxidant activity. Thus it was thought to evaluate its antioxidant capacity.

DPPH and Hydrogen peroxide techniques have been utilized to decide the antioxidant activity. The electron donation ability of natural products can be measured by 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured solution bleaching. The method is based on scavenging of DPPH through the addition of antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. An enormous diminishing in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test. In the current investigation hydroalcoholic extract demonstrated fundamentally higher inhibition percentage and positively correlated with total phenolic content. Aftereffects of this investigation recommend that the plant extract contain phytochemical constituents that are fit for giving hydrogen to a free extremist to rummage the likely harm.

Hydrogen peroxide occurs normally at low focus levels noticeable all around, air, water, human body, plants, microorganisms and food. H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH\cdot$) that can start lipid peroxidation and cause DNA damage. Hydroalcoholic extract of *Justicia procumbans* leaf effectively scavenged hydrogen peroxide which might be credited to the presence of phenolic groups that could donate electrons to hydrogen peroxide, subsequently neutralizing it into water.

CONCLUSION

The present study on physicochemical parameter provides important information which may be helpful in authentication and adulteration for quality control of raw material.

The replacement of synthetic antioxidants with natural may be invaluable. In the present study analysis of free radical scavenging activity demonstrated that this plant can be the potent source of natural antioxidants. Therefore, further examinations be done to assess its bioactivity utilizing unadulterated compounds in developing potential pharmaceutical drugs.

However, In future there is further scope for isolation, screening of isolated constituents for antioxidant activity and identification of active constituents by different spectrophotometric techniques such as UV-Visible, IR, NMR, Mass spectroscopy.

The present study adds to the existing knowledge of *Justicia*

procumbans and while be very useful for development of a formulation for treating various diseases.

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