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Phytochemical Screening and Antioxidant Activity of Justicia Adhatoda Linn.

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ABSTRACT
Justicia adhatoda Linn. a well-known evergreen shrub belonging to Acanthaceae family is a plant species which is commonly used in preparation of indigenous medicine. It has several pharmacological properties, such as antioxidant activity and antimicrobial activity etc. The present study focuses on Phytochemical screening and analysis of antioxidant activity from three different extracts of Justicia adhatoda prepared by the Magnetic stirrer extraction method and to detect the presence of alkaloids, phenols, flavonoids, steroids and other phytochemicals. In DPPH analysis (1,1-diphenyl-2-picrylhydrazyl), antioxidant activity is characterized as a stable free radical with the delocalization centred at 517a nm.

Keywords - Justicia adhatoda, Acanthaceae, phytochemical, antioxidant.

1. INTRODUCTION

Justicia adhatoda linn is a shrub widespread throughout the tropical regions of Southeast Asia [1,2]. It is commonly known as Vasaka. In India, it has various names based on different languages such as Basak (Bengali); Aradusi, adusa (Gujrati); Arusa, baansa, adulsa (Hindi); Bansa, basuti, bhekkar (Punjabi); Shwetavasa, vasa, vasaka (Sanskrit); and adhatoda (Tamil) [3,4]. It is a perennial, evergreen and highly branched shrub (1.0 m to 2.5 mm height) with bitter taste. It has opposite ascending branches with white, pink or purple flowers [5]. Inflorescences which are in spikes or panicles cymes and the species rarely has solitary, terminal or axillary flowers with a posterior lip that is generally two lobed,an anterior lip that is three lobed, two stamen, a capsule with four seeds,and a basal sterile portion. J. adhatoda is a well-known plant drug in Ayurvedic and Unani medicines [6]. It has been used for the treatment of various diseases and disorders, particularly for the respiratory tract ailments like bronchitis, asthma, tuberculosis, cold and cough [7]. Its main action is expectorant and antispasmodic (bronchodilator) [8]. In Manipur, Justicia adatoda Linn. locally known as nongmangkha angouba is used as an herbal remedy for treating cold, cough,whooping cough and chronic bronchitis and asthma, as sedative expectorant, antispasmodic and anthelmintic. The leaves (figure:1) are used for treating respiratory disorders and the juice from its leaves are used as remedy in treating diarrhoea and dysentery. The alkaloids, vasicine and vasicinone present in the leaves possess respiratory stimulant activity [9]. Whereas vasicine at low concentrations, induced bronchodilation and relaxation of the tracheal muscles. Preparation made from its flower are used to treat tuberculosis. The flower, fruits, and roots are also extensively used for treating cold cough, chronic bronchitis and asthma [10,11].

Utilizing plants meds were arranged which are effectively accessible, protected and less expensive than that present-day engineered drugs [12]. The restorative utilization of plants is because of the phytoconstituents present in them. A portion of these synthetics were bioactive and contains biochemical and produce distinct physiological activities in creatures and people. They are ordinarily called

phytochemicals or optional metabolites which contain flavonoids, alkaloids, tannins, saponin, phenols, glycoside, steroids and terpenoids and so forth [13].

The presence of significant phytochemicals makes the plant valuable against various illnesses and has a solid of giving valuable medications for human use. Phytochemicals are viewed as auxiliary metabolites delivered at next to no sum as the plant has little requirement for them. They are delivered normally in entire pieces of the plant body; like bark, leaves, stem, root, bloom, and so forth [14]. The amount and nature of phytochemicals present in plant parts might contrast from one section to another [15].

The phytochemical compounds gift in the flowers may acts as a complement for the people by natural antioxidants [16]. Many researchers exhibit that plant life consists of the rich assets of antioxidants found in them. Antioxidants are the sources that allow the human body to shield itself from loose radicals at some stage in the oxidation reactions within the body's metabolism [17]. Antioxidants help in controlling and reducing oxidative damage via dilatory or inhibiting oxidation which is a result of Reactive Oxygen Species (ROS) which concurrently increases the self-lifestyles and satisfaction of the meals [18].

Therefore, estimating the antioxidant properties of pure compounds or extracts will result in compounds that can trap free radicals and in turn will help to overcome oxidative damage. Many methods are used that rely on the measurement of radical scavenging activity of a compound against free radicals like the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) and 2,20-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) cation radical (ABTS·+), the superoxide anion radical or the hydroxyl radical [19-22]. The present investigation was conducted to evaluate the presence of some of medicinally important phytochemicals analysis, followed by the antioxidant activity in two different solvent extracts present in Justicia adhatoda Leaf.

FIGURE:1 Justicia adhatoda

2. MATERIALS AND METHODS

CHEMICALS

All chemicals and reagents were used of analytical grade. DPPH, Ethanol, Methanol, Ascorbic Acid, Sulfuric Acid,Millon's reagent, sodium nitrite, Mayer’s reagent, Molisch reagent, ferric chloride, ninhydrin, and chloroform were purchased from Scientific Chemicals, Chennai, Tamil Nadu, India.

COLLECTION OF PLANT

The leaf of Justicia adhatoda was collected in and around the near vegetative area in Chengalpattu, Tamil Nadu, India.

PLANT EXTRACTION PROCEDURE

Freshly collected leaves samples of Justicia adhatoda were washed with fresh water 2–3 times and dried under the shade at room temperature and then blended to powder using an electric blender. Powdered leaves were passed through a 2 mm sieve and stored in a sterile airtight container for further use. Extraction of leaves sample was done in two different extractions Distilled water 100% and methanol 100%. For this purpose, 10 g of dry powdered leaves were placed in the conical flask of 250 ml capacity and 100 ml of different solvents viz. Water and Methanol were added separately. Flasks were tightly sealed with parafilm and allowed to be shaken vigorously on a magnetic stirrer for 48 h. Extracts were filtered using Whatman No.1 filter paper. The filtrates were then stored in an airtight sample bottle in a refrigerator at -4ºC until required.

PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACTS

The presence of phytochemicals is determined based on standard qualitative test procedures [23] of The Aqueous, Ethanol and Methanol Extract of Justicia adhatoda leaf was and these procedures are as follows:

1. **Test for Acids-Million's Test:** To 1.0 ml extract, five drops of Millon's reagent were added, heated on a water bath for 5 min. and allowed to cool followed by the addition of 1% sodium nitrite solution. Then observed for the formation of red colour, which indicates the presence of acids.
2. **Test for Alkaloids-Mayer's Test**: To 2.0 ml extract, 2.0 ml concentrated hydrochloric acid followed by a few drops of Mayer's reagent were added and observed for the formation of green colour or white precipitate, which indicates the presence of alkaloids.
3. **Test for Carbohydrates -Molisch's Test:** To 2.0 ml extract, 1.0 ml Molisch's and a few drops of concentrated sulphuric acid were added and observed for the formation of a purple or reddish ring, which indicates the presence of carbohydrates.
4. **Test for Cardiac Glycosides-Ferric Chloride Test:** To 0.5 ml extract, 2.0 ml glacial acetic acid and a few drops of 5% ferric chloride was added. This was under layered with 1.0 ml concentrated sodium hydroxide. Formation of the brown ring at the interface was observed, which indicates the presence of cardiac glycosides.
5. **Test for Flavonoids-Sulphuric Acid Test:** 1.0 ml extract was treated with a few drops of concentrated sulphuric acid and observed for the formation of orange colour.
6. **Test for Glycosides-Sulphuric Acid Test:** To 2.0 ml extract, 1.0 ml glacial acetic acid, 5% ferric chloride and a few drops of concentrated sulphuric acid were added and observed for the formation of greenish-blue colour, which indicates the presence of glycosides.
7. **Test for Phenols-Ferric Chloride Test:** To 1.0 ml extract, 2.0 ml distilled water, followed by a few drops of 10% ferric chloride were added. Formation of blue or green colour was observed, which indicates the presence of phenols.
8. **Test for Proteins-Ninhydrin Test:** To 2.0 ml extract, a few drops of 0.2% ninhydrin were added and heated for 5 min. and observed for the formation of blue colour. This indicates the presence of proteins.
9. **Test for Quinones-Sulphuric Acid Test:** To 1.0 ml extract, 1.0 ml concentrated sodium hydroxide was added and observed for the formation of red colour, which indicates the presence of quinones.

10. **Test for Saponins-Foam Test:** To 1.0 ml extract, 5.0 ml distilled water was added and shaken well in a graduated cylinder for 15 min. lengthwise. The formation of a 1.0 cm layer of foam was observed, which indicates the presence of saponins.
11. **Test for Starch-Iodine Test:** To 2.0 ml extract, few drops of iodine solution was added and observed for the formation ofblue-purple colour, which indicates the formation of starch.

12. **Test for Steroids-Salkowski Test:** To 5.0 ml extract, 2.0 ml of chloroform and a few drops of concentrated sulphuric acid were added and observed for the formation of red colour, which indicates the presence of steroids.
13. **Test for Tannins-Ferric Chloride Test:** To 1.0 ml extract, 2.0 ml 5% ferric chloride was added and observed for the formation of dark blue or greenish-black colour, which indicates the presence of tannins.
14. **Test for Terpenoids-Sulphuric Acid Test:** To 0.5 ml extract, 2.0 ml chloroform was added and to this, concentrated sodium hydroxide was added carefully. The formation of red-brown colour at the interface was observed, which indicates the presence of terpenoids.

DPPH FREE RADICAL SCAVENGING ANTIOXIDANT ACTIVITY:

DPPH (1,1-diphenyl-2-picrylhydrazyl) is described as a stable free revolutionary by the goodness of the delocalisation of the extra electron over the particle overall, so the atoms don't dimerise, as would be the situation with most other free extremists. The delocalisation additionally leads to the profound violet tone, described by an assimilation band in ethanol arrangement focused at around 520 nm. At the point when an answer of DPPH is blended in with that of a substance that can give a hydrogen particle, then, at that point, this brings about the decreased structure [23] with the deficiency of this violet tone (even though there would be supposed to be a lingering light yellow tone from the picryl bunch present). Addressing the DPPH revolutionary by Z• and the contributor particle by AH, the essential response is Z• + AH = ZH + A• where ZH is the diminished structure and A• is the free extremist created in this initial step. This last extreme will then, at that point, go through additional responses which control the general stoichiometry, or at least, the number of particles of DPPH decreased (decolourised) by one atom of the reductant. [24]. The Antioxidant activity of aqueous and Methanol leaf extract of Justicia adhatoda based on the scavenging property of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free stable radical was obtained by following the procedure.

The antioxidant activity of the samples and the standard were assessed based on the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) – free radical activity method. The Different concentrations of 100-500µg of 1ml Aqueous, Ethanol and Methanol Extract of Justicia adhatoda leaf is diluted. The working solutions of the test samples were prepared in methanol 0.002% of DPPH, 3.7 ml of absolute methanol in all test tubes and 3.8 ml of absolute methanol was added to blank. Add 100µl of Ascorbic Acid to the tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark conditions for 30 minutes. The absorbance of all samples was read at 517nm in a spectrophotometer. For tremendous control, Ascorbic acid becomes used. The DPPH loose radical scavenging assets became the optical density was recorded and % inhibition was calculated using the formula given below.

3. CALCULATION

$$\% \text{ of Antioxidant activity} = \frac{(\text{Absorbance at blank}) - (\text{Absorbance at test})}{(\text{Absorbance at blank})} \times 100$$

STATISTICAL ANALYSIS

Data obtained from the experimental Analysis was expressed in the pattern as Mean and Standard Deviation.

4. RESULT & DISCUSSION

TABLE: 1 - Phytochemical Analysis of Justicia adhatoda

S.no	Test	Method	Aqueous	Ethanol	Methanol
1	Amino Acids	Million's Test	-	-	-
2	Alkaloids	Mayer's Test	+	+	+
3	Carbohydrates	Molisch's Test	-	-	-
4	Cardiac Glycosides	Ferric Chloride Test	+	-	-
5	Flavonoids	Sulphuric Acid Test	+	-	-
6	Glycosides	Sulphuric Acid Test	+	+	+
7	Phenols	Ferric Chlorid e Test	+	+	+
8	Proteins	Ninhydrin Test	+	+	+
9	Quinones	Sulphuric Acid Test	+	-	-
10	Saponins	Foam Test	-	-	-
11	Starch	Iodine Test	-	-	-
12	Steroids	Salkowski Test	+	+	+
13	Tannins	Ferric Chloride Test	+	-	-
14	Terpenoids	Sulphuric Acid Test	-	-	-

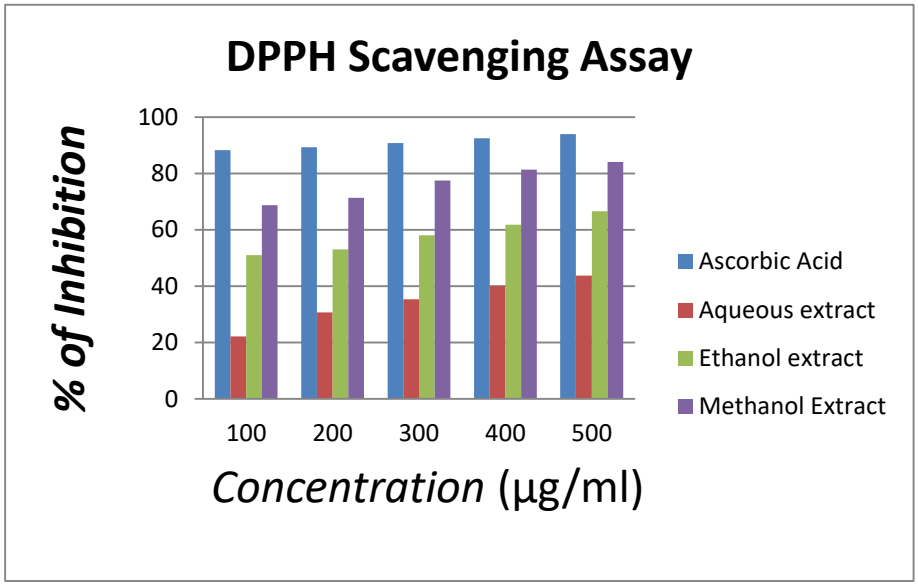
+ = Presence; - = Absence

TABLE: 2- Antioxidant activity of Justicia adhatoda.

DPPH % of inhibition of samples at different concentrations				
Concentrations of the sample (µg/ml)	Standard AscorbicAcid	Aqueous extract	Ethanol extract	Methanol extract
100	88.21±0.29	22.22±0.50	51.01±0.50	68.68±0.50
200	89.22±0.29	30.63±1.05	53.03±0.50	71.38±0.77
300	90.74±0.58	35.35±0.50	58.08±0.50	77.44±1.27
400	92.42±0.50	40.06±0.29	61.78±0.50	81.31±0.50
500	93.93±0.50	43.77±0.29	66.66±0.77	84.17±0.29

Mean ± Standard deviation (SD) for analysis in three replicates

CHART: 1- Total antioxidant activity of Aqueous, Ethanol and methanol extract of Justicia adhatoda and Standard Ascorbic acid.



The healing fee of medicinal plant life lies inside the various chemical compounds processed in them. The bioactive compounds of plant extract are attributed to phytochemical compounds of flora. If the flora wealthy in tannin compound they have got highly effective in controlling the bacteria, because of this character they permit to react with the proteins to form solid water-soluble compounds consequently, it kills the bacterial by way without delay damaging its mobile membrane [25]. The DPPH radical scavenging assay has been broadly used to analyze the perspective of the compounds which include free radical scavengers of the hydrogen donors and used to have a look at the antioxidant interest of plant extract. Phenolic compounds present in plant life act as antioxidants or unfastened radical scavengers because of their OH organizations, which are committed directly to the antioxidant action [26].

Qualitative Analysis of phytochemical screening of the three different extracts of Justicia adhatoda leaves covered the presence of various chemical substance gatherings like. Alkaloids, Cardiac Glycosides, Flavonoids, Glycosides, Phenol, Proteins, saponins, Steroids and Tannins in Aqueous Extracts. Alkaloids, Glycosides, Phenols, Proteins and Steroids in Ethanol Extracts Furthermore, Alkaloids, Glycosides, Phenols, Proteins and Steroids in Methanol Extracts [Table 1].

The Antioxidant activity of Justicia adhatoda using the DPPH radical scavenging assay is based on the ability of antioxidants, to decolourize DPPH. The DPPH revolutionary contains an odd electron, which is liable for the absorbance at517 nm. Cell reinforcements give an electron to DPPH and decolourize it, which can be quantitatively estimated from the changes in absorbance. Each of the evaluated concentrates of Justicia adhatoda had the option to diminish the stable, purple coloured extremist DPPH to the yellow-shaded DPPH.

The leaf extract of Justicia adhatoda and standard ascorbic acid revealed the highest percentage of inhibitory activity in 500 µg/ml (93.93±0.50%) in ascorbic acid, (84.17±0.29%) in Methanol, (66.66±0.77%) in Ethanol and (43.77±0.29%) in Aqueous. The leaf extract of Justicia adhatoda and standard ascorbic acid revealed the least percentage of inhibitory activity in 100 µg/ml (22.22±0.50%) in Aqueous, (51.01±0.50%) in Ethanol, (68.68±0.50%) in Methanol, and (88.21±0.29%) in ascorbic acid. [Table 2 & Chart 1] from the above study, Justicia adhatoda exhibits good Results.

5. CONCLUSIONS

In this current above study, it very well may be pronounced that the phytochemical screening and anti-oxidant DPPH activity of Aqueous, Ethanol and Methanol extract of Justicia adhatoda leaf extract have good properties of Phytochemical and Antioxidant. The methanol Extract has very good properties than the Aqueous and Ethanol Extract. Justicia adhatoda contains the functional groups that can be implemented in pharmaceutical industries to develop drugs to cure many diseases.

6. ACKNOWLEDGEMENTS

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