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Pharmacognostic, Phytochemical and Pharmacological Evaluation of *Justicia glauca*

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Abstract: Several different kinds of activities were carried out by the *Justicia* species, as indicated by the ethnomedical data presented in the current study. In order to authenticate and identify the plant for the sake of further study, the leaves have been subjected to pharmacognostic parameter assessment. TLC and HPTLC analyses that were performed on the ethanolic extract of *Justicia glauca*, enabled us to create a fingerprint of the plant profile for future reference. The ethanolic extract of *Justicia glauca* was tested using the DPPH method, and the results showed that it has significant antioxidant activity as well as the potential to protect against radicals. It is possible that the ethanolic extract of *Justicia glauca* is a major medicinal plant that is used in the production of several semi-synthetic drugs to treat life-threatening illnesses such as cancer, congestive heart failure, and bacterial and fungal infections.

Keywords: *Justicia glauca*, Antioxidant activity, Herbalism, DPPH, Hydrogen peroxide

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Introduction

There are a few other names for herbal medicine, including herbalism and botanical medicine (Ramachandran *et al.*, 2022). Herbal medicine refers to the practice of using plants for their medicinal or therapeutic characteristics. The term "herb" refers to a plant or a piece of a plant that is highly valued for its aromatic and medicinal capabilities (Emmanuel *et al.*, 2017). Plants are responsible for the production and discovery of a vast variety of chemical substances that have an effect on the body. Through the utilization of various plant components, such as leaves, flowers, stems, fruits, and roots, herbalists are able to prevent, treat, and cure illness (Chhabra and Kulkarni, 2020). In the realm of scientific inquiry, a significant number of herbal medicines are considered to be experimental. However, the fact of the matter is that herbal medicine has a strong reputation and a long history. A great number of well-known pharmaceuticals that were developed in the 20th century originated from traditional medicinal methods that made use of certain plants to treat a variety of medical ailments (Ibrahim *et al.*, 2017). The medicinal properties of a great number of botanicals have been recognized by modern science, and the constituent elements of these plants have been extracted. There are now a great number of plant components that are being synthesized in large laboratories for use in pharmaceutical formulations (Agila *et al.*, 2022).

Approximately four billion people, which accounts for eighty per cent of the world's population, are presently receiving fundamental medical care through the use of herbal medicine, as stated by the World Health Organization (WHO) (Toplan *et al.*, 2020). Herbal medicine is an essential component of many different medical practices, including Ayurvedic, homoeopathic, naturopathic, traditional oriental, and Native American Indian medicine. All indigenous peoples have included it into their traditional medicinal methods, making it an essential component (Marathakam *et al.*, 2012).

According to the World Health Organization (WHO), around 74% of the 119 pharmaceutical medications that are derived from plants are used in modern medicine in ways that are strongly tied to their historical usage as plant cures by indigenous civilizations. Research is now being conducted by large pharmaceutical businesses to investigate the possible medical applications of plant materials that have been obtained from rain forests and other sites (Rani and Sharma, 2013).

Instead of utilizing the entire plant in order to acquire the active chemicals, a pharmacologist will separate, isolate, extract, and synthesise particular components of a plant; this is done in order to obtain the active substances (Rajoka *et al.*, 2020). This might be an issue due to the fact that plants include other compounds that are vital to the medicinal properties of particular herbs. These compounds include minerals, vitamins, volatile oils, glycosides, alkaloids, and bioflavonoids, in addition to the active components that are found in plants (Tewari *et al.*, 2022).

Medical experts and government bodies want to see scientific studies before they are willing to acknowledge the possibility that plants might be utilized as a kind of therapy. However, despite the fact that a substantial amount of research is being carried out in other countries, pharmaceutical companies and laboratories in the United States have not yet taken the choice to devote a significant number of finances or resources to the study of botanicals (Chan *et al.*, 2018). Furthermore, as a consequence of this, herbal medicines are not accorded the same level of acceptance or prestige in the United States as they are in other countries (Sahib *et al.*, 2019).

The beneficial effects of cardioprotective herbs have been well investigated, both in terms of their chemical makeup and the clinical evaluations that have been conducted. Herbs that are considered to be cardioprotective are beneficial to the heart because they contain a high concentration of bioflavonoids. Particularly beneficial to the cardiovascular system, it enhances the body's ability to make use of calcium and oxygen (Kamal *et al.*, 2022).

Bradycardia was induced by the majority of the cardio tonic herbs in rats with normal blood pressure as well as animals with low blood pressure. Additionally, they demonstrated anti-arrhythmic effects in all of the experimental kinds of arrhythmia and effectively reduced arterial blood pressure by a substantial amount. The cardio tonic herbs also demonstrate strong negative chromotropic, positive inotropic, and coronary dilating activities. These actions are important for the cardiovascular system (Meena *et al.*, 2011).

Numerous herbs have the potential to cure a variety of heart-related conditions, including congenital heart failure, hyperlipidemia, hypertension, intermittent claudication, and venous insufficiency, among others. According to study conducted in vitro, garlic has the potential to reduce overall blood pressure via inhibiting platelet NO synthase (Tiwati *et al.*, 2023). The process by which *Gingko biloba* operates includes the elimination of free radicals, the inhibition of platelets, the reduction of inflammation, the vasodilation of blood vessels, and the reduction of blood viscosity.

Howthorn is a prickly shrub that contains flavonoids and oligomeric procyanthins. These flavonoids and procyanthins have potential antihyperlipidemic, inotropic, vasodilatory, and antioxidant activities (Studzińska-Sroka *et al.*, 2022). Additionally, they decrease the capillary permeability. One possible explanation for positive inotropic action is that it is caused by the inhibition of myocardial Na+/K+ ATPase, which is an essential membrane enzyme that maintains the resting potential of the heart. During the early stages of congestive heart failure, it also reduces blood pressure, which in turn raises the patient's tolerance for physical activity (Ramachandran *et al.*, 2022).

Because the bioflavonoids included in herbs are used to expand the blood vessels in the coronary and peripheral arteries, people who suffer from angina use certain herbs. Some people believe that the contents of procyanidins are responsible for the vasorelaxant effects that they have (Das *et al.*, 2022). Additionally, there have been indications that the glycoside component of hawthorn is responsible for an increase in the vagal tone of the heart. *Ephedra sinica*, more often referred to as Ma Huang, is a naturally occurring source of ephedrine that has powerful sympathomimetic effects. The glutathione status of red blood cells and the mitochondria of the heart are both improved by Dang-Gui Buxue tang, which in turn strengthens the body's resistance to the effects of oxidative stress. More than 2500 B.C., people have been using *Terminalia arjuna* because of its reputation for being beneficial to the heart. Treatment for hypertension, angina, hyper-lipidemia, hypercholesterolemia, and heart attacks is provided by this medication (Ban *et al.*, 2020).

Plants offer a wide range of possible uses, including those that protect the cardiovascular (Tiwari *et al.*, 2022) system from the effects of toxic substances and those that fight cancer. As a result, studies are being conducted to determine whether or not the herb *Justicia glauca*, which is relatively unknown, can be of use in treating the illnesses that have been stated above (Tiwari *et al.*, 2021).

Critical information on the physical, microscopical, and morphological characteristics of crude pharmaceuticals may be obtained by the conduct of a comprehensive pharmacognostical study. Eventually, they will be able to be employed in order to identify the plant in a botanical sense (Shukla *et al.*, 2020). The results that have been generated as a result of multiple pharmacognostic examinations that have been carried out on numerous key pharmaceuticals have been included into a range of herbal pharmacopoeias. Such investigations have been carried out on numerous medications. As of yet, the identification of the plant source for certain crude medicines by scientific means has not yet been completed. Therefore, pharmacognostic research offers scientific information regarding the quality and purity of medications that are in their unrefined form (Tiwari *et al.*, 2023).

Materials and Methods

Plant Collection and Extract preparation:

Justicia glauca's leaves were collected and after thorough washing, leaves were dried in the shade. Then pulverized for use in further use (Tiwari *et al.*, 2023). For maceration, 25 L of petroleum ether (60–80°C) were used to break down about 1 kg of dried and ground *Justicia glauca* leaf powder. The liquid was separated after filtering, and the marc was dried. Then three times 70% ethanol was added to it. The extraction process was performed. Rota gas was used to turn the mixed liquid into a solid after it had been filtered (Tiwari *et al.*, 2023).

In vitro evaluation of antioxidant activity:

As the name suggests, an antioxidant is any chemical that can stop other molecules from oxidizing. When a substance gives up electrons or hydrogen to an oxidizing agent, this is known as oxidation (Tiwari *et al.*, 2022). The process of burning can make free radicals. After that, these radicals can start a chain reaction. If there is a chain reaction, a cell could get hurt or even die. Antioxidants stop these chain reactions and stop more oxidation by getting rid of the free radicals that are in the middle (Sonawane *et al.*, 2023). Thiols, ascorbic acid, and polyphenols are all antioxidants. Because they work by being oxidized, they are usually reducing agents. Antioxidants protect against many diseases, such as cancer, heart disease, diabetes, and getting older, by getting rid of the free radicals that are created by reactive stress. Degenerative diseases happen when the cell's antioxidant defence system is overloaded by ROS and free radicals start to damage the cell's proteins, lipids, and carbs. Because of this, there is a strong case for using antioxidants to avoid and treat diseases where oxidative stress plays a major role in their development. Antioxidants protect cells from ROS damage in two ways: they can either get rid of reactive chemicals or change them into molecules that are less reactive, which stops ROS from being made. The activity tells us about how antioxidants work at first, while the antioxidant capacity tells us about how long they last. Because of this, antioxidants, both natural and man-made, are becoming more and more important for staying healthy (Thakur and Ashawat, 2023).

Diphenyl picryl hydrazyl (*DPPH*) *method*:

As a "scavenger" or trap for other radicals, DPPH is a well-known radical. The amount by which a chemical reaction slows down when DPPH is added is used to figure out how radical the reaction is. Due to a wide absorption band centred at about 520 nm, the DPPH radical is deep violet when it is in solution. When it is neutralised, it changes to light yellow or white. The EPR signal of the DPPH or the change in optical absorption at 520 nm can be used to figure out how many starting radicals there are (Ceremuga *et al.*, 2020).

Procedure:

Different amounts of 4 ml of DPPH were added to 1 ml of test samples. The test drug was not used in the control group. When making the blank, ethanol was used instead of DPPH. The response was left to finish in the dark for about 30 min. At 517 nm, the absorption of the test mixes was then found. The amount of DPPH radical scavenging was used to figure out and describe the blockage.

A picture of concentration vs. percentage blockage was made. The sample concentration needed for a 50% drop in absorbance was found using linear regression analysis (IC50). The findings are shown in Table 2 (Surana *et al.*, 2024).

Nitric oxide scavenging activity assay:

Principle:

To figure out the nitric oxide scavenging ability Green *et al.* (1982) method was used. When sodium nitropruside is mixed with water at a normal pH, it creates nitric oxide on its own. This oxide then mixes with oxygen to make nitrite ions. The Griess-Illosvoy reaction can be used to figure out what kind of ions these are. The nitrite ions change sulphanilamide into a diazonium salt. This salt then mixes with NN naphthyl ethylene diamine dihydrochloride to make a pink chromophore that absorbs light best at 546 nm. When nitric oxide (NO) and superoxide anion come together, they can make peroxinitritre ($ONOO^-$), which can be dangerous. NO is a strong pleiotropic mediator in healthy conditions and a free radical that can spread in unhealthy ones. When it is protonated, peroxinitrous acid (ONOOH) is a strong oxidizer (Somtimuang *et al.*, 2018).

Procedure:

1 ml of sodium nitroprusside was mixed with 2.5 ml of phosphate-buffered seawater (pH 7.4). The solution above was mixed with varying volumes of extracts (1 ml), and it was then allowed to settle at 25°C for ½ h. To 1.5 ml of the simmering mixture, add 1 ml of sulfanilamide in phosphoric acid and

Table 1: Per cent yield of successive solvent extraction of leaves of *Justicia glauca*

S. No.	Extracts	Extraction Method	Physical Nature of extract	Colour of extract	Percentage yield (w/w)
1.	Non polar (Hexane)	Continuous hot percolation method	Semisolid	Pale green	10.2
2.	Semi polar (Ethyl acetate)		Sticky	Greenish black	11.2
3.	Polar (Ethanol)		Semisolid	Brownish black	11.6

Table 2: *J. glauca* ethanolic extract and standard ascorbic acid percentage inhibition of DPPH at 517 nm

S. No.	Conc. (µg/ml)	Percentage inhibition byascorbic acid	Percentage inhibition by *J. glauca*
1	40	90.13± 0.16	31.11± 0.29
2	80	92.98± 0.31	61.69± 0.31
3	120	99.89± 0.32	87.32± 0.25
4	160	96.11± 0.54	91.17± 0.41
5	200	97.12± 0.61	93.63± 0.52
	IC_{50}	20.19 µg/ml	79.64 µg/ml

Values are mean±SD (n=3)

0.5 ml of naphthyl ethylene diamine dihydrochloride. The absorbance was measured at 546 nm. In order to maintain clarity, ascorbic acid was used. To determine the IC_{50}, linear regression analysis was used (Khan *et al.*, 2021).

Scavenging of hydrogen peroxide activity:

Principle:

A mild oxidising agent called hydrogen peroxide can quickly stop some enzymes from working. It does this by oxidising important thiol (-SH) groups. A lot of the bad things that hydrogen peroxide does may start with the creation of hydroxyl radicals, which could mix with Fe^{+2} and maybe even Cu^{+2}. From a biochemical point of view, this means that cells should control how much hydrogen peroxide they let build up (Surana and Mahajan, 2022).

Procedure:

It was made to have 40 mM hydrogen peroxide in phosphate buffer (pH 7.4). By measuring absorption at 230 nm with a spectrophotometer, the amount of hydrogen peroxide was found. Extracts (0.01 to 1.0 mg/ml) were mixed with pure water and added to the hydrogen peroxide solution. (0.6 ml, 4 mM). A blank solution with phosphate buffer but no hydrogen peroxide was used to compare the absorbance of hydrogen peroxide at 230 nm after 10 min. The same method was used with regular vitamin C (Tiwari *et al.*, 2022).

Results and Discussion

Phytochemical Investigation:

Percentage yield of successive solvent extraction of Justicia glauca:

Extraction was carried out with solvents of increased polarity for the leaves of *Justicia glauca* and percentage yield was determined and tabulated (Table 1). The polar-extract (ethanol) had a higher percentage yield than the other extracts.

Evaluation of antioxidant activity:

Diphenyl picryl hydrazyl (DPPH) Assay:

Table 2 displays the findings of the radical scavenging activity against DPPH. According to the table, at a concentration of 200 µg/ml, ascorbic acid exhibited a percentage inhibition of

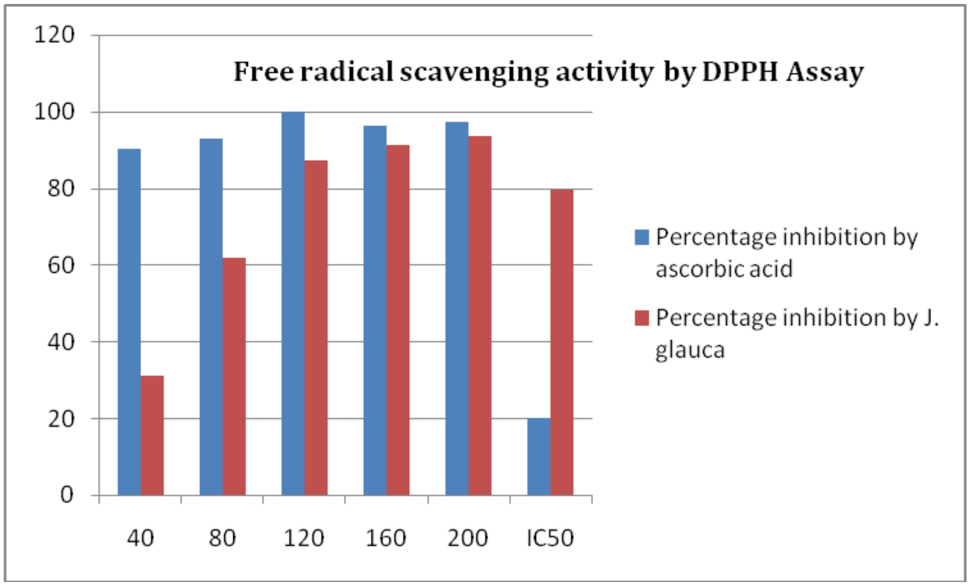

Fig. 1: Ascorbic acid and a 70% ethanolic *J. glauca* extract scavenge nitric oxide radicals.

Table 3: *Justicia glauca* ethanolic extract's percentage suppression of nitric oxide at 546 nm

S. No.	**Conc. (μg/ml)**	**Percentage inhibition by ascorbic acid**	**Percentage inhibition by extract**
1	40	58.59±0.52	54.45 ± 0.45
2	80	58.41±0.19	55.55 ± 0.33
4	120	71.35±0.41	58.25 ± 0.22
5	160	76.11±0.39	60.13 ± 0.27
6	200	83.12±0.31	78.41 ± 0.10
	IC_{50}	88.59 μg/ml	156.62 μg/ml

Values are mean±SD (n=3)

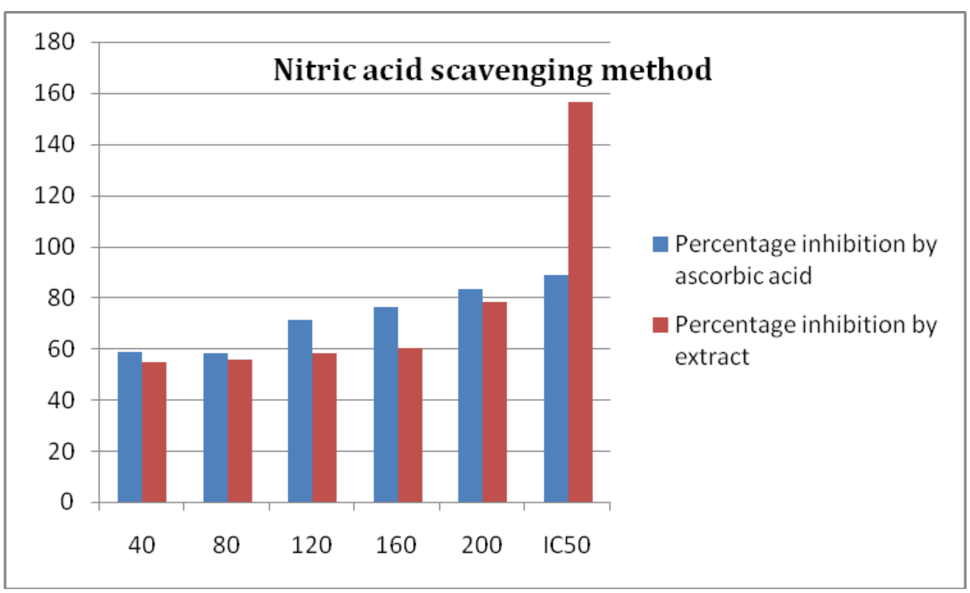

Fig. 2: Ascorbic acid and a 70% ethanolic *J. glauca* extract scavenge nitric oxide radicals.

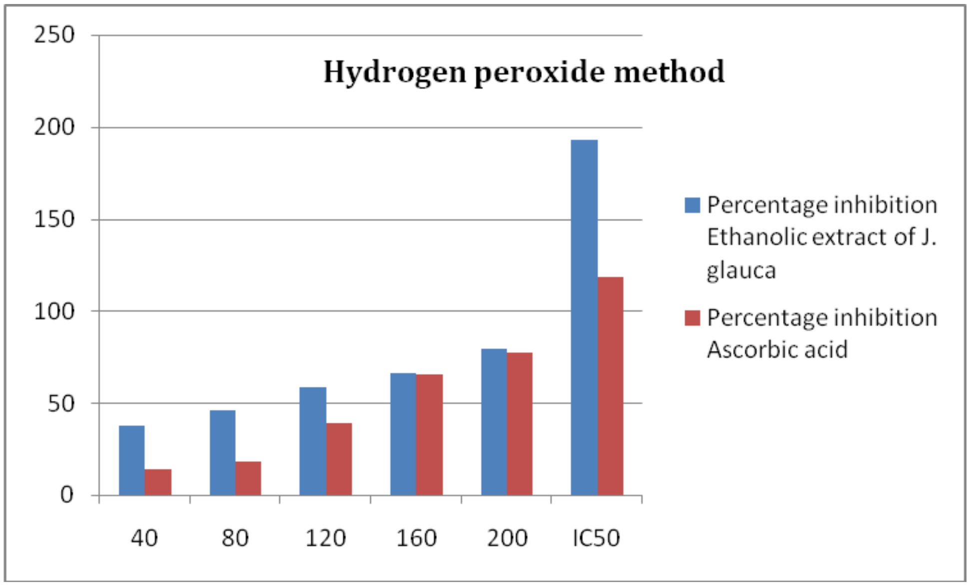

Fig. 3: Scavenging activity by hydrogen peroxide method.

97.12±0.61, but the ethanolic extract of *J. glauca* showed a percentage inhibition of 93.63± 0.52. For ethanolic extract and ascorbic acid, the IC_{50} values determined by linear regression analysis which were 20.19 and 79.64 µg/ml, respectively (Fig. 1). The extract has a strong ability to scavenge radicals.

Nitric oxide scavenging activity:

The results of the free radical scavenging action against the nitric oxide radical are shown in Table 3. The percentage of inhibition at 500 µg/ml of ascorbic acid was 83.12±0.31, while the percentage of inhibition at 78.41± 0.10 for the ethanolic extract of *J. glauca* is shown in Table 3. Through linear regression analysis, the IC_{50} values for ethanolic extract and ascorbic acid were found to be 156.62 and 88.59 µg/ml, respectively (Fig. 2).

Determination of scavenging activity against hydrogen peroxide:

The findings of hydrogen peroxide scavenging are shown in Table 4. The table presents the results that ascorbic acid inhibited 77.21±0.81 at a concentration of 200 µg/ml, whereas the ethanolic extract of *J. glauca* inhibited 79.22±0.25 at the same concentration. By employing the technique of linear regression analysis, the values of the IC_{50} for ethanolic extract and ascorbic acid were determined to be 193.11 and 118.45 µg/ml, respectively (Fig. 3).

Conclusion

The relevance of a great number of undiscovered plants that have considerable medicinal efficacy is still not fully acknowledged, despite the fact that they are significant. The field of science is something that they have not been exposed to. In this study, pharmacognostic, phytochemical, and pharmacological research are conducted on the medicinal plant *Justicia glauca*, which belongs to the Acanthaceae family and is readily accessible. In order to authenticate and identify the plant for the sake of further study, the leaves have been subjected to pharmacognostical parameter assessment. The ethanolic extract of *Justicia glauca* was tested using the DPPH method, and the results showed that it has significant antioxidant activity as well as the potential to protect against radicals. It is possible that the ethanolic extract of *Justicia glauca* is a major medicinal plant that is used in the production of several semi-synthetic drugs to treat life-threatening illnesses such as cancer, congestive heart failure, and bacterial and fungal infections.

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