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Evaluation of Reactive Oxygen and Nitrogen Species Scavenging Activities of *Pergularia daemia* Leaves Extract

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Abstract: Drugs derived from plants have a long and storied history of usage in alternative medical practises across the globe. In this research, we examined the leaves of *Pergularia daemia* to see how effective they were at neutralising free radicals. Scavenging of several free radicals (DPPH, superoxide anion, hydroxyl radical, nitric oxide, and hydrogen peroxide) was used to evaluate the plant extract's free radical scavenging capability. The effects were concentration dependant and were equivalent to those of the gold standard antioxidant ascorbic acid. It was shown that the phytochemical content of *Pergularia daemia* leaves, which included antioxidants such as polyphenols and flavonoids, significantly correlated with the extract's free radical scavenging activity and electron-donating capacity. Observed free radical scavenging action lends credence to *Pergularia daemia*'s purported medicinal usage, while further *in vitro* and *in vivo* investigations are needed to determine the risk against benefit ratio at therapeutic dosages.

Keywords: Antioxidants, *Pergularia daemia* leaves, Free radical scavenging activity, Medicinal plants

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Introduction

Activation of the enzyme cyclooxygenase results in the production of reactive oxygen species (ROS) during the inflammatory response (Vapaatalo, 1986). Endothelial cells, Kupffer cells, neutrophils, and macrophages produce Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) as a defence mechanism against foreign or infectious diseases; these cells are among the most prominent sources of these inflammatory mediators (Halliwell *et al.*, 1988). Inadvertently, several diseases, including cancer, rheumatoid arthritis, transplanted organ

rejection, atherosclerosis, amyotrophic lateral sclerosis, sepsis, and ageing, may be exacerbated by the persistent overproduction of reactive oxygen species and reactive nitrogen species and/or the decrease in antioxidant defences (Mouithys *et al.*, 2000). The reactive oxygen and nitrogen species (ROS), peroxyl radical (ROO°), hydroxyl radical (HO°), superoxide radical (O_2°-), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl) in addition to the RNS nitric oxide (°NO) and peroxynitrite anion (ONOO°) play important roles in the aforementioned pathophysiologies, and thus are potential targets for the chemotherapy of inflammation (Kataoka *et al.*, 1997).

Inhibiting the oxidative pathways that cause degenerative illnesses, antioxidant substances including phenolic acids, polyphenols, and flavonoids scavenge free radicals like peroxide, hydroperoxide, and lipid peroxyl (Wu *et al.*, 2011). Herbal plants have historical reputations as potent antioxidants. Antioxidants found in nature have the ability to shield cells from free radical damage. The oxidation of lipids in meals and medicines is slowed or stopped entirely by the presence of natural antioxidants. Over the last three decades, a number of pharmaceuticals and formulations based on antioxidants have been developed for the treatment and prevention of serious illnesses including cancer and Alzheimer's (Aqil *et al.*, 2006). Numerous plant compounds, such as polyphenols, terpenes, and different plant extracts have been demonstrated to have an antioxidant effect in recent research (Zahin *et al.*, 2009; Olugbami *et al.*, 2015). Plants' secondary metabolites, such as phenolic and flavonoid compounds are thought to be powerful free radical scavengers. They permeate the whole plant, from leaves to fruits to seeds to bark (Mathew and Abraham, 2006; Velavan, 2015). This research was carried out to assess the relative free radical scavenging abilities of the leaves of the *Pergularia daemia* plant.

Materials and Methods

Collection of plant:

In March 2022, locals in Thanjavur, Tamil Nadu, India collected the leaves of the plant *Pergularia daemia*. The leaves of *Pergularia daemia* were washed many times with distilled water to eliminate any remaining contaminants. After being air dried at room temperature, the leaves were ground into a coarse powder in a mixer grinder.

Preparation of extract:

There was an extraction using a powdered form of *Pergularia daemia* leaves that weighed 10 g. We used the maceration technique for cold extraction into an ethanol solvent, and then we "intermittently shaken" the mixture for 24 h to get the extract. The assay continued using the filtrate obtained after filtering the extract with Whatman filter No. 1 paper.

In vitro radical scavenging activity:

The ability to scavenge DPPH radicals was measured according to Shimada *et al.* (1992) protocol. Scavenging activity against superoxide anion radicals was evaluated using Liu et al (1997). Fenton reaction was used to test hydroxyl radical scavenging activity using Yu *et al*. (2004). The ability to scavenge nitric oxide radicals was measured in accordance with a technique described by Garratt (1964). This extract's ability to scavenge hydrogen peroxide was calculated using the method of Zhang (2000).

Statistical analysis:

Three distinct experiments each had three sets of test results. The linear regression approach in Ms- Windows based graphpad Instat (version 3) software was used to visually predict the quantity of sample required to inhibit free radicals concentration by 50%, IC_{50}. Mean SD plots were used to depict the results.

Results and Discussion

Free radicals, also known as reactive oxygen species, are a byproduct of the biological combustion involved in many processes. Oxidative stress, caused by an overload of free radicals, has been linked to a wide range of health

issues, including asthma, cancer, cardiovascular disease, liver disease, muscle degeneration, and other inflammatory processes (Sen *et al.*, 2010). Protein, nucleic acid, DNA, and RNA are all susceptible to damage from oxidative stress, which is described as an imbalance between oxidants and antioxidants (Dröge, 2002). Therefore, it is considered that antioxidants and the equilibrium between reactive species or free radicals is a crucial idea for maintaining a healthy biological system. Antioxidants help prevent cell damage caused by free radicals by scavenging them, neutralising them with reducing agents, quenching singlet oxygen molecules, and promoting the activity of antioxidative enzymes. Many studies have revealed that consuming plant products is inversely associated to death from age-related illnesses; this may be because plants contain a wide variety of antioxidant chemicals, the most reactive of which are phenolics.

Plant foods that contain antioxidants aid in the activation of the body's cellular defence system and biological system to combat oxidative damage.

Antioxidants may be found in abundance in medicinal plants (Rice-Evans, 2004). In addition to lowering the likelihood of developing cancer, cardiovascular disease, and stroke, natural antioxidants also boost the body's natural defences against these illnesses (Prior and Cao, 2000). Antioxidants are believed to affect DPPH because of their capacity to donate hydrogen (Baumann, 1979). Scavenging free radicals is crucial in preventing cancer and other illnesses caused by free radicals. Screening the antioxidant activity of plant extracts by their ability to scavenge DPPH free radicals is a common practise. Direct interactions of hydroxyl radicals with DNA lead to DNA breakage and contribute significantly to cancer development, which is why free radicals have a mutagenic capability (Scully, 1993).

DPPH radical scavenging activity:

Figure 1 represents DPPH radical scavenging activity of *Pergularia daemia* leaves extract and compared with ascorbic acid. The *P. daemia* and ascorbic acid showed maximum DPPH inhibitory activity which were 83.61% and 92.01% at 100 µg/ml concentration range while minimum inhibitory activity were 16.80% and 20.16% at 20 µg/ml concentration range, respectively. The half inhibition concentration (IC_{50}) of *Pergularia daemia* leaves extract and ascorbic acid were 62.38 µg/ml and 53.01 µg/ml, respectively (Table 1).

Superoxide Scavenging Activity:

Superoxide Scavenging activity of *Pergularia daemia* leaves extract was compared with ascorbic acid. At the minimum concentration of *P. daemia* and ascorbic acid (20 µg/ml) the activities were 19.45% and 21.62%, respectively while at the maximum concentration (100 µg/ml), the corresponding activities were 87.56% and 90.27%, respectively. The half inhibition concentration (IC_{50}) of *Pergularia daemia* leaves extract and ascorbic acid were 57.02 µg/ml and 52.01 µg/ml, respectively (Table 2; Fig. 2).

Hydroxyl radical scavenging activity:

Figure 3 illustrates the hydroxyl radical scavenging activity of *Pergularia daemia* leaves extract and compared with ascorbic acid. The leaves of *Pergularia daemia* and ascorbic acid showed hydroxyl radical scavenging activity from 17.64 to 84.42% and 19.72 to 91.34% at concentration range from 20 to 100 µg/ml, respectively. The half inhibition concentration (IC_{50}) of *Pergularia daemia* leaves extract and ascorbic acid were 59.88 µg/ml and 53.06 µg/ml, respectively (Table 3).

Nitric oxide scavenging activity:

Figure 4 depicts the nitric oxide scavenging activity of *Pergularia daemia* leaves extract and ascorbic acid. At the minimum concentration of *P. daemia* and ascorbic acid (20µg/ml), the activities were 18.62% and 22.26%, respectively while at the maximum concentration (100 µg/ml), the corresponding activities were 85.02% and 91.90%, respectively. The half inhibition

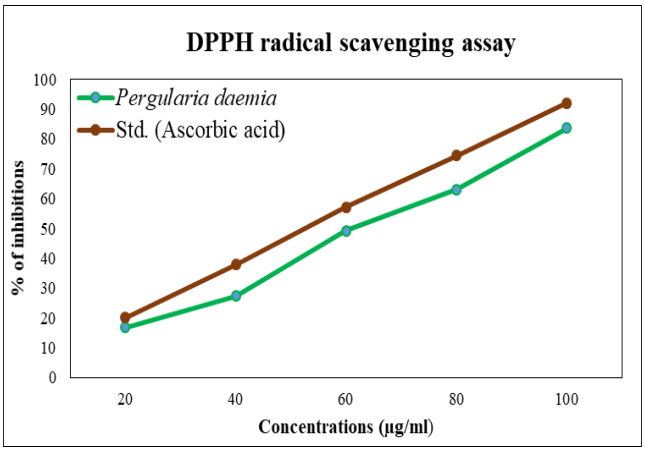

Fig. 1: DPPH radical scavenging activity of *Pergularia daemia* leaves extract and ascorbic acid.

Table 1: DPPH radical scavenging activity of *Pergularia daemia* leaves extract and ascorbic acid

Concentrations (µg/ml)	**% of inhibitions**	
	Pergularia daemia	**Std. Ascorbic acid**
20	16.80±0.05	20.16±0.17
40	27.31±0.15	37.81±0.24
60	49.15±0.37	57.14±0.45
80	63.02±0.64	74.36±0.69
100	83.61±0.73	92.01±0.85
IC_{50} (µg/ml)	62.38	53.01

Values expressed as Mean ± SD for triplicate

Table 2: Superoxide Scavenging activity of *Pergularia daemia* leaves extract and ascorbic acid

Concentrations (µg/ml)	**% of inhibitions**	
	Pergularia daemia	**Std. Ascorbic acid**
20	19.45±0.16	21.62±0.27
40	33.51±0.22	40.00±0.32
60	54.05±0.38	57.83±0.67
80	68.10±0.59	74.59±0.86
100	87.56±0.77	90.27±1.03
IC_{50} (µg/ml)	57.02	52.01

Values expressed as Mean ± SD for triplicate

concentration (IC_{50}) of *Pergularia daemia* leaves extract and ascorbic acid were 60.71 µg/ml and 52.58 µg/ml, respectively (Table 4).

Hydrogen peroxide scavenging activity:

Figure 5 illustrates the hydrogen peroxide scavenging activity of *Pergularia daemia* leaves extract and ascorbic acid. The *P. daemia* and ascorbic acid showed maximum inhibitory activity which was 83.33% and 90.58% at 100 µg/ml concentration range while minimum inhibitory activities were 16.86% and 20.78% at 20 µg/ml concentration range, respectively. The half inhibition concentration (IC_{50}) of *Pergularia*

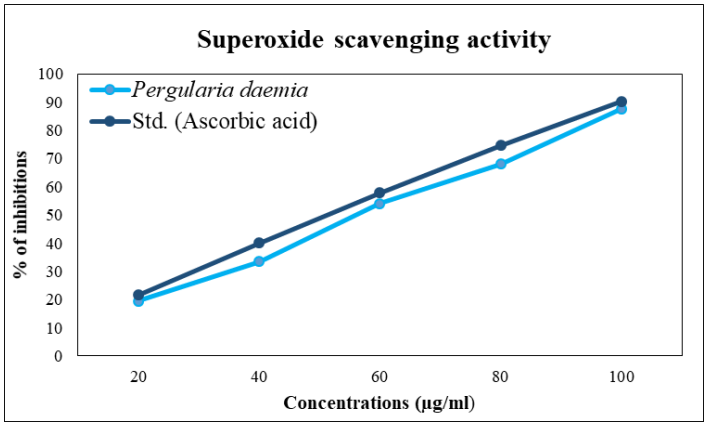

Fig. 2: Superoxide Scavenging activity of *Pergularia daemia* leaves extract and ascorbic acid.

Table 3: Hydroxyl radical scavenging activity of *Pergularia daemia* leaves extract and ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Pergularia daemia	**Std. Ascorbic acid**
20	17.64±0.10	19.72±0.23
40	28.37±0.29	37.71±0.37
60	50.51±0.42	57.43±0.59
80	69.55±0.67	75.08±0.71
100	84.42±0.86	91.34±0.89
IC_{50} (µg/ml)	59.88	53.06

Values expressed as Mean ± SD for triplicate

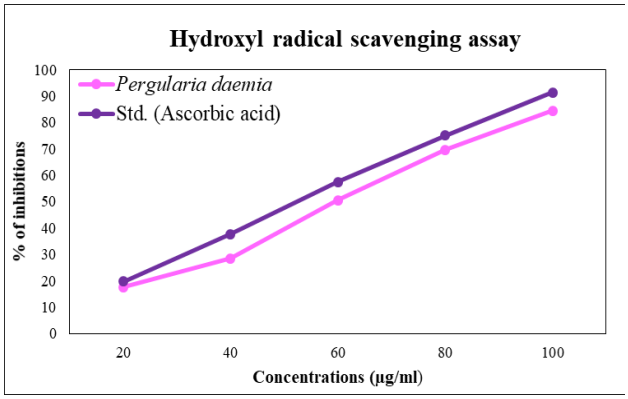

Fig. 3: Hydroxyl radical scavenging activity of *Pergularia daemia* leaves extract and ascorbic acid.

daemia leaves extract and ascorbic acid were 61.78 µg/ml and 54.15 µg/ml, respectively (Table 5).

Natural product studies commonly draw on ethnobotanical data, and many currently used medications were originally derived from plants traditionally utilised by indigenous peoples. In recent years, the pharmacological effects of certain medicinal plants have been a primary focus of ethnopharmacological study (Heinrich, 2013; Atanasov *et al.*, 2015). Plants used in traditional medicine have been shown to be

Table 4: Nitric oxide scavenging activity of *Pergularia daemia* extract and ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Pergularia daemia	**Std. Ascorbic acid**
20	18.62±0.13	22.26±0.28
40	27.53±0.28	38.05±0.37
60	49.39±0.44	55.87±0.52
80	66.39±0.69	74.49±0.79
100	85.02±0.86	91.90±1.04
IC_{50} (µg/ml)	60.71	52.58

Values expressed as Mean ± SD for triplicate

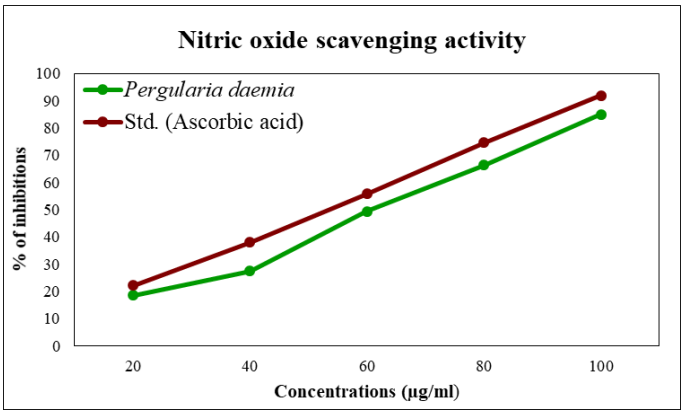

Fig. 4: Nitric oxide scavenging activity of *Pergularia daemia* extract and ascorbic acid.

Table 5: Hydrogen peroxide scavenging activity of *Pergularia daemia* leaves extract and ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Pergularia daemia	**Std. Ascorbic acid**
20	16.86±0.12	20.78±0.19
40	28.23±0.26	36.27±0.32
60	46.86±0.38	54.70±0.57
80	67.05±0.59	73.52±0.79
100	83.33±0.77	90.58±1.03
IC_{50} (µg/ml)	61.78	54.15

Values expressed as Mean ± SD for triplicate

useful models for pharmacological investigations in a number of studies. Consequently, extracts from plants and other natural sources have been studied as a potential alternative treatment for a wide range of illnesses (Majouli *et al.*, 2017; Singh *et al.*, 2017). Any agent that prevents or slows down the oxidation of a certain molecule is considered to be an antioxidant (Yamagishi and Matsui, 2011). Antioxidants are distinguished by their primary property, which is to mop up harmful free radicals.

Pergularia daemia leaf extract was evaluated for its radical scavenging activity *in vitro* utilizing a number of different in vitro models. Many illnesses may trace their origins and progression back to oxidative stress (Adwas *et al.*, 2019). Because of the reaction products' part in the radical scavenging activity, some writers argue

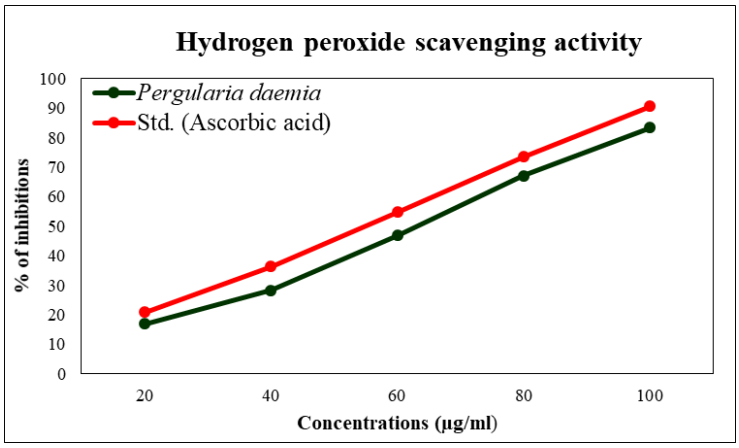

Fig. 5: Hydrogen peroxide scavenging activity of *Pergularia daemia* leaves extract and ascorbic acid.

that some methodologies can not properly assess the structure-activity link or correlate their findings with the antioxidant activity found by other techniques (Oszmianski *et al.*, 2007). Antioxidant activity were shown to be proportional to sample concentration, chemical composition, and degree of polymerization (Gil *et al.*, 2000). This is a common approach of determining whether or not a plant extract has antioxidant properties. An antioxidant or free radical scavenger, DPPH works by giving hydrogen ions to oxidised substances (Soare *et al.*, 1997).

The hydroxyl radical, which is the neutral form of the hydroxide ion (OH), is very reactive. This means its existence is fleeting. In addition to DNA, hydroxyl radicals may damage a number of other cellular membranes. No endogenous enzymatic scavenging routes exist inside the body to neutralise this radical, and instead it is neutralised by a combination of glutathione, melatonin, and antioxidants obtained from nutrition (Garratt, 2012). An essential bioactive molecule, nitric oxide (NO) performs a number of vital physiological roles (Marcocci *et al.*, 1994). These findings corroborate earlier research indicating the importance of plant phenolic chemicals for their free radical scavenging properties (Enechi *et al.*, 2013; Bera *et al.*, 2015).

Conclusion

There is preliminary evidence that an extract of the leaves of the *Pergularia daemia* plant have antioxidant properties. Using DPPH, superoxide anion scavenging, hydroxyl radical scavenging, nitric oxide scavenging, and hydrogen peroxide scavenging at varying doses, the potential antioxidant activity of *Pergularia daemia* ethanol extract of leaves was demonstrated. There was a strong association between the free radical scavenging activity and the electron donating capacity of *Pergularia daemia* leaves extract, which was caused by the phytochemicals present in the extract, such as polyphenol and flavonoid contents. When compared with ascorbic acid, the activity exhibits a higher proportion of free radical inhibition. The pharmaceutical and food sectors might benefit from such research.

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