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Studies of antioxidant activities in leaf extract of *Pisonia alba* span. (Nyctaginaceae)

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Abstract
The present study was aimed at the estimation of the antioxidant potentialities of leaf homogenate of *Pisonia alba.* The results of the reducing power assay showed a steady increase in the reducing power, with increase in the concentration of the leaf homogenate. Similarly the free radical scavenging activity also showed a dose dependent relation, ie the scavenging of DPPH free radical and nitric oxide free radical increased with increase in the concentration of the homogenate. The antioxidant potential expressed by the leaf homogenate of *Pisonia alba* during the current study may be due to the presence of diverse types of phytochemical constituents such as flavanoids, phenolics, oxalic acid and tannins. Hence it may be concluded that *Pisonia alba* can play an important role in the reduction as well as prevention of free radical induced pathogenesis.

Keywords: antioxidant activity, leaf extract, *Pisoniaalba* span. nyctaginaceae

Introduction
Sixty percent of the factors responsible for individual health and quality of life are correlated to lifestyle. Millions of people follow an unhealthy lifestyle which may be in the form of malnutrition, unhealthy diet, smoking, alcohol consumption, drug abuse, stress and so on. One of the major metabolic outputs associated with such unhealthy lifestyle is the generation of free radicals above an optimum level [1]. Free radicals are agents with an unpaired electron and are important intermediates in many natural processes [2]. They are highly unstable and react quickly with other compounds and try to capture the required electron to gain stability. Hence, these radicals have high reactivity and are capable of oxidizing various bio-molecules like DNA, proteins and lipids [3].

Free radicals are derived mainly from oxygen (Reactive Oxygen Species / ROS) and nitrogen (Reactive Nitrogen Species / RNS). Oxygen which is most essential for the very existence of living organisms can thus become mostly dangerous by receiving such free electrons[4]. However, the presence of excess amount of free radicals within the body adversely affects the human due to the development and progression of many metabolic disorders [5]. The deleterious reactions triggered by reactive free radicals can be inhibited by antioxidants. Antioxidants are the compounds that can scavenge or prevent the free radical formation and in turn reduces the risk. Hence, their presence is critical in maintaining health and well-being [6]. Antioxidants are either produced within the body or are received through diet. In a healthy body a balance between free radicals and antioxidants are maintained, where antioxidants protect the living organisms form free radical damage [7].

The safe, effective and natural source of antioxidants is plant products like fruits, vegetables and spices. These natural antioxidants are much preferred to protect human body against oxidative damage caused by free radicals [8]. The identification of plants with antioxidant potentiality needs laborious biochemical analysis. So far, many assay protocols has been developed, which can be employed to identify the antioxidant potentials very precisely. *Pisoniaalba* is known widely for its food and nutritional value. The medicinal properties of *Pisoniaalba* is well exploited in various traditional systems of medicine [9]. It has been used traditionally as medicinal ingredient against a number of ailments. Phytochemical studies have revealed the presence of a wide variety of bioactive compounds in the leaf, root and bark of *Pisoniaalba* [10]. In this context, the present work is an attempt to investigate the antioxidant potentiality of leaf homogenate *Pisoniaalba* using various standard assay systems.

Materials and Methods
About the plant
Pisoniaalba Span., the grand devil's claw, is a species of flowering tree in the bougainvillea family, Nyctaginaceae. It is an evergreen shrub commonly known as 'leechai kottai keerai, Maduracheera or maracheera. It is endemic to regions like South India and Australia. Leaves, stems and roots of this species are extensively used by the tribals for the preparation of several dishes and also in folk medicines.

Collection of plant material
The plant part used for the present study was leaves. They were collected from *Pisoniaalba* plant grown in the Botanical garden of St. Joseph's College, Kozhikode.. The plant was authenticated with the help of latest literature and voucher specimens were deposited in the Herbarium of St. Joseph's College, Kozhikode.

Preparation of the leaf homogenate
The leaf homogenate was prepared fresh for each experiment. Mature healthy leaves of *Pisoniaalba* were used for the preparation of homogenate. The leaves were cleaned with running water and water was removed completely using tissue paper. They were cut into small pieces and were ground well using mortar and pestle. It was then filtered to remove the debris. The filtrate was then centrifuged at 10,000 rpm at 4^{o}C for 10 minutes. The clear

supernatant thus obtained (100% homogenate) was used for the experiments.

Doses studied

Five different concentrations of the homogenate, such as 0.05%, 0.1%, 0.2%, 0.4% and 0.8% were used in the present study. Experiments using the different doses, were conducted in triplicate to minimize the chance for errors.

Solvent used

Double distilled water was used as the solvent for diluting the homogenate during the different assays.

Assays conducted

1. Reducing power assay
2. DPPH radical scavenging assay
3. Nitric oxide radical scavenging assay

Reducing power assay

Reducing power of the leaf homogenate was determined according to the method of Yen and Chen [11]. All the experiments were done in triplicate as follows. 1.25 ml of each of the dilution was mixed with 1.25ml of 0.2M phosphate buffer at p^H 6.6. It was mixed with 1.25ml of 1% potassium ferricyanide. This mixture was incubated at 50°C for 20 minutes using a BOD incubator.

Afterwards 1.25ml of 10% trichloro acetic acid was added and the mixture was centrifuged at 1,500 rpm for 10 minutes. 1.25ml of the supernatant was transferred to a fresh tube and was mixed with 1.25ml of distilled water and 0.25ml of 1% ferric chloride. This mixture was incubated at room temperature for 10 minutes. The absorbance of resulting solution was measured at 700 nm using spectrophotometer. The reaction mixture without plant extract was used as blank.

The reducing power was expressed in relation to the reducing power of ascorbic acid, which was used as the positive control. Ascorbate Equivalent Antioxidant Capacity (AEAC) was calculated by following equation.

$AEAC = C_A \times A_S / A_A$

Where, C_A – final concentration of ascorbic acid in µg/ml

A_S - absorbance of the sample

A_A – absorbance of ascorbic acid.

Increase in absorbance of reaction mixture indicated the increase in reduction power. The results were analyzed statistically and were expressed in terms of arithmetic mean and standard error.

DPPH radical scavenging assay

Determination of the scavenging of DPPH (2, 2-Diphenyl-1-picryl hydrazyl), a commercially available and stable free radical, was carried out with different concentrations of the homogenate. This method is based on the reduction of DPPH solution in presence of a hydrogen donating antioxidant, resulting in the formation of the non-radical form of DPPH called diphenyl picrylhydrazine. In its radical form, DPPH has an absorption maximum at 515nm and disappears on reduction by antioxidant.

Reaction mixture was prepared by mixing 1.8ml of 0.1mM freshly prepared DPPH solution (in methanol) and 0.2ml of the different concentrations of the plant extracts. The mixture was incubated at room temperature under darkness for five minutes and the absorbance was measured at 515nm [12]. Reaction mixture, where methanol was added instead of plant extract was used as the control.

Percentage of DPPH radical scavenging activity was expressed as percentage of inhibition calculated using the formula,

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The results were analyzed statistically and were expressed in terms of arithmetic mean and standard error.

Assay of nitric oxide radical scavenging

The assay was conducted according to the procedure developed by Green *et al.* [13]. Nitric oxide is generated from sodium nitroprusside in an aqueous solution at physiological pH (7.2 – 7.4). The amount of nitrate ions produced by the interaction of nitric oxide with oxygen was measured by Griess reaction.

All the experiments were done in triplicate. 3 ml of reaction mixture containing 300µl sodium nitroprusside (10mM) in phosphate buffered saline and 2700 µl of various concentrations of plant extract were incubated at 25^0C for 150 minutes. At the end of incubation, 1.5ml of Griess reagent (Equal volume of 1% sulphanilamide, 2% phosphoric acid and 0.1% naphathalene diamine dihydrochloride) was added to 1.5ml of the above reaction mixture. The absorbance of the reaction mixture was measured at 546nm. A control group was maintained without any plant extract, but with an equal amount of buffer.

The percentage of inhibition of nitric oxide generation was measured by comparing the absorbance value of control group and that of the reaction mixtures containing different concentrations of plant extracts using the formula,

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The results were analyzed statistically and were expressed in terms of arithmetic mean and standard error.

Results and Discussion

The results obtained during the present investigation aimed at determining the reducing power, DPPH radical scavenging activity and nitric oxide radical scavenging activity of different concentrations of leaf homogenate of *Pisonia alba* are mentioned below. The plants homogenate responded positively to all the three assay system and exhibited remarkable antioxidant activity.

Reducing power assay

The reducing ability was measured by the ferric to ferrous transformation that may occur in the assay system in presence of different concentrations of leaf homogenate of *Pisonia alba*. The increase absorbance of the reaction mixture indicates an increase reducing power.

The reducing power of the different concentrations of leaf homogenates was compared with the reducing power of the ascorbic acid as standard using the same assay system. The data obtained as Ascorbate Equivalent Antioxidant Capacity (AEAC) were tabulated in Table 1 and are represented in Fig 2. The AEAC values showed a dose dependent increase

with increase in concentration of the homogenate. The present result obviously proves the high reducing power of *Pisonia alba.*

Table 1: AEAC values of leaf homogenate of *Pisonia alba*

Sl. No.	Concentration of the leaf homogenate (%)	AEAC value (Mean ± Standard error)
1.	0.05	13.42 ± 0.98
2.	0.1	18.34 ± 0.56
3.	0.2	24.65 ± 0.09
4.	0.4	36.29 ± 0.72
5.	0.8	42.91 ± 0.12

Antioxidants are compounds that inhibit or retard the many oxidation reactions caused by free radicals, thereby preventing or delaying damage to the cells and tissues. They limit the free radicals from oxidizing the vital bio-molecules [14].

DPPH radical scavenging assay

The results of the DPPH radical scavenging assay using the different concentrations of leaf homogenates of *Pisonia alba* showed a dose dependant antiradical activity (Table2, Fig 2). At the lowest concentration of the homogenate, the percentage of inhibition of DPPH radical was 36.38 ± 0.23 and at the highest concentration of the homogenate, the percentage of inhibition of DPPH radical was 78.55 ± 0.10. These results clearly indicate the capacity of the bioactive ingredients present in the leaf homogenate *Pisoniaalba* to scavenge the DPPH radical. This in turn elucidates the antioxidant potential of *Pisoniaalba*.

The free radicals / oxidants have very short life, high reactivity and damaging activity towards macromolecules. During their attempt to get stabilized, they may attack other bio-molecules leading to the activation of these bio-molecules, which in turn results in cell damage triggered by the formation of new free radicals from the chain of reactions [15]. Chemical compounds capable of generating free radicals are referred to as pro-oxidants. On the other hand, compounds and reactions that disposes these species, scavenging them, suppressing their formation or opposing their action are called antioxidants. In a normal cell there is an appropriate pro-oxidant antioxidant balance [16].

Table 2: DPPH radical scavenging activity of leaf homogenates of *Pisoniaalba*

Sl. No.	Concentration of the leaf homogenate (%)	Percentage of inhibition of DPPH radical (Mean ± Standard error)
1.	0.05	36.38 ± 0.23
2.	0.1	46.11 ± 0.43
3.	0.2	58.50 ± 0.80
4.	0.4	66.32 ± 0.62
5.	0.8	78.55 ± 0.10

Nitric oxide radical scavenging assay

During this assay nitric oxide is generated from sodium nitroprusside in aqueous solution at near neutral pH. The concentration of the nitric oxide was measured by Griess reaction. The results of the present study indicated the dose dependent inhibition of the nitric oxide radical generation from sodium nitroprusside by different concentrations of leaf homogenates of *Pisoniaalba.* The percentage of inhibition of nitric oxide generation was 43.87 ± 0.10 at the lowest concentration of the leaf homogenate, whereas the percentage of inhibition at highest concentration of leaf homogenate was 82.26 ± 0.80. The data obtained were tabulated in Table 3 and are shown in Fig 2.

Table 3: Nitric oxide radical scavenging activity of leaf homogenate of Pisoniaalba.

Sl. No.	Concentration of the leaf homogenate (%)	Percentage of inhibition of nitric oxide generation (Mean ± Standard error)
1.	0.05	43.87 ± 0.10
2.	0.1	52.74 ± 0.00
3.	0.2	72.54 ± 0.71
4.	0.4	79.51 ± 0.90
5.	0.8	82.26 ± 0.80

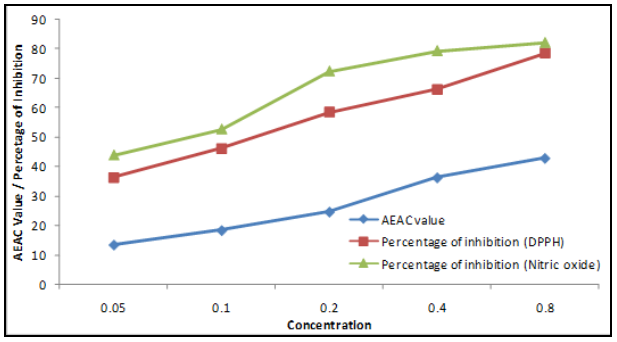

Fig 1: Graph showing Reducing power, DPPH radical scavenging activity and Nitric oxide radical scavenging activity of different concentrations of leaf homogenates of *Pisonia alba.*

The results obtained from three different antioxidant assays showed somewhat similar pattern of observations. The results clearly indicated antioxidant activity for the leaf homogenate of *Pisoniaalba*.

The results of the current experiments showed that the leaf homogenate of *Pisoniaalba* have high reducing power as well as the ability to scavenge free radicals. Since these two are used as absolute measure of antioxidant capacity, it is established evidently that the *Pisoniaalba* has high antioxidant activity. They have the ability to prevent the deleterious effects of free radicals formed in the body during biochemical reactions. This may be because of certain biochemical ingredients present in it. It has been established that various compounds such as flavanoids, tannins, coumarins, xanthones, phenolics etc present in plants are responsible for their antioxidant nature [17]. The antioxidant activity of these compounds is mainly due to their redox properties which can play an important role in absorbing and neutralizing free radicals [18].

Palanivel *et al.* [19]., reported the presence of alkaloids, steroids, saponins, tri-terpenes, flavonoids and polyphenolic compounds in the ethanol extract of leaves of *Pisonia aculeata*. Phytochemical and GC-MS analysis of methanolic extract of *Pisonia grandis* carried out by Pradheesh *et al.*, [20] also proved the presence of chemical constituents such as alkaloids, flavonoids, phenols, tannins and oxalate. Among the phytochemicals reported in *Pisonia* - flavanoids, oxalic acid and tannins are examples for standard reducing agents. The antioxidant potential of the leaf homogenate of *Pisoniaalba* observed during the present study may be due to the presence of these biochemical substances. The present study suggested that the leaf homogenate of *Pisoniaalba* have high antioxidant potential.

Hence it may be concluded that this plant and its products should be viewed as promising therapeutic agents against free radical pathogenesis.

Conclusion

The present work was aimed at the estimation of the antioxidant potentialities of 0.05%, 0.1%, 0.2%, 0.4% and 0.8% solutions of leaf homogenate of *Pisoniaalba*. The experimental procedures such as reducing power assay (Yen and Chen, 1995), DPPH radical scavenging activity assay (Blois, 1958) and nitric oxide radical scavenging assay (Green *et al.*, 1982) were adopted during this study. The results of the reducing power assay showed a steady increase in the reducing power, with increase in the concentration of the leaf homogenate. Similarly the free radical scavenging activity also showed a dose dependent relation, ie the scavenging of DPPH free radical and nitric oxide free radical increased with increase in the concentration of the homogenate.

The high reducing power as well as the free radical scavenging activity can be taken as an indication of the antioxidant potentiality of this plant. The antioxidant potential expressed by the leaf homogenate of *Pisonia alba* during the current study may be due the presence of diverse types of phytochemical constituents such as flavanoids, phenolics, oxalic acid and tannins. Hence it may be concluded that *Pisonia alba* can play an important role in the reduction as well as prevention of free radical induced pathogenesis. The observations of the present investigation thus apparently pointed out the neutraceutical value of this plant.

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References

1. Aseervatham GS, Sivasudha T, Jeevadevi R, Anandi AD. Environmental factors and unhealthy lifestyles influence oxidative stress in human – An overview. Environmental Science and Pollution Research International. 2013; 20(7):4356-4369.
2. Gutteridge JM, Mitchell J. Redox imbalance in the critically ill patients. British Medical Bulletin. 1999; 55(1):49-75.
3. Kuhn MA. Oxygen free radicals & antioxidants. American Journal of Nursing. 2003; 103(4):58-62.
4. Devasagayam PA, Tilak JC, Boloor KK, Sane KS, Saroj S, Lele RD. Free radicals and antioxidants in human health - Current status and future prospects. Journal of the Association of Physicians of India. 2004; 52:794-804.
5. Lunec J, Holloway KA, Cooke MS, Faux S, Griffiths HR, Evans MD. Urinary 2-deoxyguanosine: Redox regulation of DNA repair *in vivo*. Free Radical Biology and Medicine. 2002; 33(7):875-885.
6. Percival M. Antioxidants. Clinical Nutrition Insights Review. Journal of Scientific Review. 1998; 10:1-4.
7. Logani MK, Davis RE. Lipid peroxidation and biological effects of antioxidants - A review. Lipids. 1979; 15:485-492.
8. Goel HC, Prasad J, Sharma A. Anti-tumor and radio-protective action of *P. hexandrium*. Indian Journal of Experimental Biology, 1998; 36:583-585.
9. 9. Gupta VK, Sharma SK. Plants as natural antioxidants. Natural Product Radiance. 2006; 5(4):326-334.
10. Saritha B, Sumathi T. Studies on antioxidant activity, phenol and flavonoid content of *Pisoniaalba*. Asian Journal of Pharmcological and Clinical Research. 2014; 7(3):106-109.
11. Yen GH, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenesity. Journal of Agriculture and Food Chemistry. 1995; 43:27-32.
12. Blois MS. Antioxidant determination by the use of a stable free radical. Nature. 1958; 18:99-110.
13. Green LC, Wagner DA, Glowski J, Skipper PL, Wishnak JS, Tannenburm SR Analysis of nitrate, nitrite and 15^N in biological fluids. Annals Biochemistry. 1982; 126:131-136.
14. Lakenbrink C, Lapczynski S, Naiwald B, Engelhandt OH. Flavanoids and other polyphenols in consumer brews of tea and other caffeinated beverages. Journal of Agriculture and food Chemistry. 2000; 48:2848-2872.
15. Odukoya A, Olukeni A, Olukemi O, Oluwatoyin MS, Austin OA, Manusura B. Antioxidant activity of Nigerian dietary species. European Journal of Chemistry. 2005; 4(6):1086-1093.
16. Ray G, Husain SA. Oxidant, antioxidant and carciagenesis. Indian Journal of Experimental Biology. 2002; 40:1213-1232.
17. Bravo L. Polyphenolis: Chemistry, Dietary sources, Metabolism and Nutritional Significance. Nutrition Review. 1998; 56: 317-333.
18. Osawa T. Noval natural antioxidants for utilization in good and biological systems. Japan Scientific Societies Press, Japan, 1994.
19. Palanivel MG, Balasubramanian R, Kumar RS, Einstein JW, Kumar EP, Kumar MR et al. Hepatoprotective and antioxidant effect of *Pisonia aculeata* L. against CCl_4-induced hepatic damage in rats. Science and Pharmacology. 2008; 76:203-215.
20. Pradheesh G, Suresh S, Alexramani V. Phytochemical and GC-MS analysis of methanolic extract of *Pisonia grandis* R. Br. International Journal of Chemical Science. 2015; 13(3):1295-1304.