**International Journal of Zoology and Applied Biosciences** **ISSN: 2455-9571**
**Volume 2, Issue 6, pp: 311-314, 2017**
**https://doi.org/10.55126/ijzab.2017.v02.i06.009**
**http://www.ijzab.com**
**Research Article**

# ANTIOXIDANT PROPERTIES OF *PISONIA ALBA* PLANT LEAF EXTRACT

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**Article History:** Received 8th October 2017; Accepted 20th November 2017; 25th November 2017

## ABSTRACT

The methonolic extract of the leaves of *Pisonia alba* (PA) were subjected to phytochemical analysis by standard qualitative analysis and the *invitro* antioxidant activity was evaluated by determination of total antioxidant capacity, 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Antioxidant activity, Nitric oxide potential. The analyses revealed that the methanol extract of *Co* was able to efficiently scavenge the free radicals in a dose dependant manner. The results were compared with the standard antioxidant ascorbic acid. Thus, further research may be warranted to study active compounds of *P. alba* that confer the antioxidant activity. The findings presented here might have implications in the population disease prevention field.

**Keywords:** *Pisonia alba*, DPPH, Total antioxidant, Nitric oxide scavenge, Methanol.

## INTRODUCTION

Asia has abundant species of medicinal and aromatic plants and traditional medicines have practiced in Asia since ancient times. India has made use of medicinal plants to cure ailments of thousands of years. According to World Health Organization the goal of health for all can't be achieved without herbal medicines, while the demand for herbal medicine is growing in developing countries, there are indications that consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives in traditional medicine (Tamizhazhagan *et al.*, 2017). As much as 70% of India's population used traditional medicine (Gupta, 1993). Free radical especially reactive oxygen species (ROS), such as superoxide (O2), hydroxyl (OH) and hydrogen peroxide (H2O2) have greater brunt on human both from within the body and from their surroundings. If the body fails to eliminate, ROS can attack on biomolecules such as lipids, proteins, enzymes, DNA and RNA. Though, human body possesses many defense mechanisms through antioxidant enzymes and non-enzymatic compounds against these oxidative stresses. But when these free radicals go out of control, the organism becomes incapable to scavenge all ROS which may lead to the development of chronic diseases, such as cancer, arteriosclerosis, nephritis, diabetes mellitus, liver injury, rheumatism, ischemia, cardiovascular and neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Amel *et al.,* 2013). Similarly phenols and flavonoids are important plant secondary metabolites, that's having conjugated ring structures and hydroxyl groups, that may have the potential to function as antioxidants by scavenging the free radicals which are involved in oxidative processes via hydrogenation or complexation with oxidizing species and may resist many oxidative stresses and diseases (Thatoi *et al.,* 2014). The incessant formation of free radicals in human's body can be controlled naturally and synthetically by different value compounds known as antioxidants. Presently butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), gallic acid esters and tertiary butylated hydroquinon available synthetic antioxidants, but their use is restricted and there is a propensity to surrogate them with natural antioxidants that may be found in medicinal plants, (Barlow, 1990; Tamizhazhagan and Pugazhendy, 2017) Research has been paying attention on the biological effects of plants which are traditionally used as cholinesterase inhibitors and radical scavenging in-vitro as well as in-vivo (Moyo *et al.,* 2010). This study is also focused on and antioxidant potential of *Pisonia alba.*

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## MATERIALS AND METHODS

### Preparation of extracts

The plant was collected from Mariyappa nagar, near to Annamalai University. The plant extracts were prepared as described earlier (Aqil and Ahmad, 2007) with little modification. Hundred (100) grams of dry plant powder were soaked in 1 litre of 97% methanol for 3-5 days with intermittent shaking. At the end of extraction, it was passed through Whatman filter paper No. 1 (Whatman Ltd., England). This methanolic filtrate was concentrated under reduced pressure on a rotary evaporator at 40 C and then stored at 4ºC for further use. The filtrate was reconstituted in a known amount of DMSO to obtain methanol extract of known concentration.

### Antioxidant assay

The antioxidant activity of the plant extracts was tested using two methods: ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. The FTC method was used to measure the amount of peroxide at the beginning of the lipid peroxidation, in which peroxide reacts with ferrous chloride and form ferric ion. The ferric ion then combines with ammonium thiocyanate and produce ferric thiocyanate. The substance is red in colour. The thicker the colour was the higher the absorbance. Whereas the TBA methods measure free radicals present after peroxide oxidation.

### DPPH radical scavenging activity

The free radical scavenging activity by different plant extracts was done according to the method reported by (Gyamfi *et al*., 2002) 3cv. Fifty microliters of the plant extract in methanol, yielding 100µg/ml respectively in each reaction was mixed with 1ml of 0.1mM DPPH in methanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4). Methanol (50µl) only was used as control of experiment. After 30 min of incubation at room temperature, the reduction of the DPPH free radical was measured reading the absorbance at 517 nm. L-Ascorbic acid and BHT are used as controls. The percent inhibition was calculated from the following equation: % Inhibition = [Absorbance of control – Absorbance of test sample / Absorbance of control] 100.

### In vitro nitric oxide radical (NO) Scavenging assay

Nitric Oxide generated from sodium nitroprusside (SNP) was measured according to the method of (Marcocci *et al.*, 1994). Briefly, the reaction mixture (5.0 ml) containing SNP (5 mM) in phosphatebuffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25˚C for 180 min in front of a visible polychromatic light source (25W tungsten lamp). The nitric oxide radical thus generated interacted with oxygen to produce the nitrite ion (Nitric Oxide) which was assayed at 30 min intervals by mixing 1.0 ml of the incubation mixture with an equal amount of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride. The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm. The nitrite generated in the presence or absence of the plant extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out at least three times and the data presented as an average of three independent determinations.

## RESULTS

The total antioxidant level was highly presented in the plants extracts compared with other standard solutions fractions were analyzed three have good activity against standard BHT (Figure 1). The DPPH free radical compound has been widely used to test the free radical scavenging ability of various food samples; the antioxidant present neutralizes the DPPH by the transfer of an electron or hydrogen atom. The reduction capacity of DPPH could be determined by colour changes from purple to yellow by read at 517 nm (Figure 1).

The methanolic extract of *P. alba* demonstrated H-donor activity in our study. The DPPH radical scavenging activity of extracted material was detected and compared with standard antioxidant - vitamin C. The extract of *P. alba* tested against DPPH stable radicals spectrophotometrically which reveals that the radical scavenging activity of *P. alba* methanol extract possessed excellent antioxidant capacity by increased with the increasing concentration of the extract (Figure 2).

At a concentration of 100 µg/ml of methanol extract the percentage of inhibition was found to be 78%. However, the scavenging activity of ascorbic acid at the same concentration was 85.02%. The methanol extract of *P. alba* was found at the concentration of 50 µg/ml. The methanolic extract of *P. alba* effectively reduced the generation of nitric oxide from sodium nitroprusside. P. alba methanol extract showed nitric oxide scavenging activity at the concentration of 10 µg/ml while the standard vitamin C was showed 50 µg/ml (Figure 3). Scavenging of Nitric Oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent.

The absorbance of the chromophore was measured at 546 nm in the presence of the extract. *P. alba* extract proved to decrease in amount of nitrite generated from the decomposition of sodium nitroprusside in vitro. Methanol extract recorded maximum percentage of NO activity of 84.11% at the concentration 50 µg/ml.

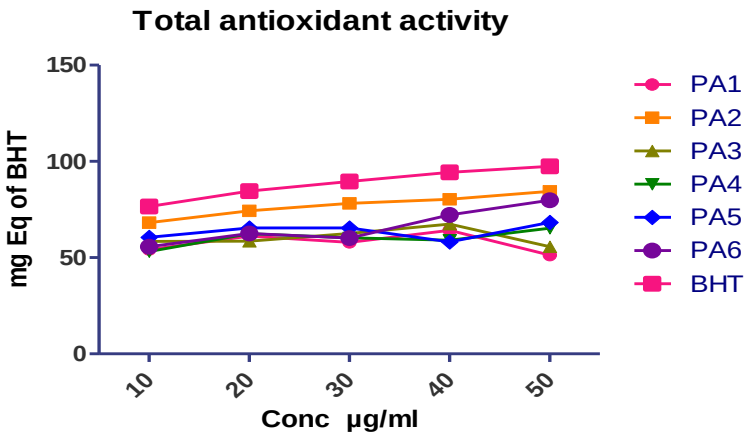


**Figure 1.** *Pisonia alba* plant extracts total antioxidant activity.

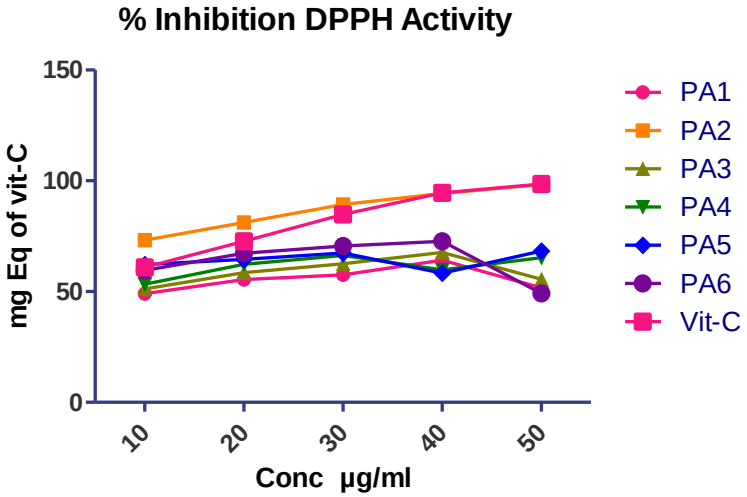


**Figure 2.** *Pisonia alba* plant extracts inhibition DPPH activity.

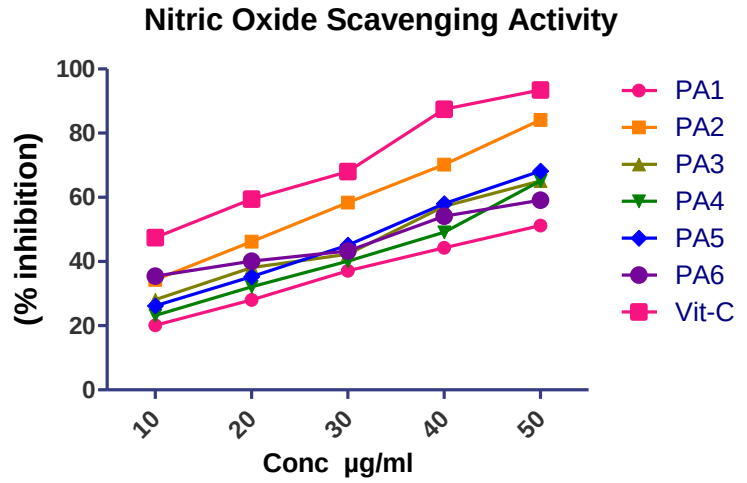


**Figure 3.** *Pisonia alba* plant extracts showed nitric oxide scavenging activity.

## DISCUSSION

Antioxidants due to their scavenging activity are useful for the management of those diseases. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts (Koleva *et al.,* 2002; Suresh *et al.*, 2008). The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful (Kanatt *et al.*, 2007). Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine (Beyer, 1994; Aqil *et al.,* 2006). These differences might be due to their different antioxidant mechanisms or variations in their ability to scavenge free radicals. A fair correlation between total phenolic content and antioxidant activity was also observed. These observations clearly indicated a cross linkage between phenolics and antioxidant activity. However, a large number of phytocompound groups are implicated forantioxidant activity. The results based on the related to other finding more efficacy have the medicinal plant of *P. alba.*

## CONCLUSION

In addition to those pharmaceutical properties of *P. alba* reported in the literature, this research showed that leaves of this plant may possess considerable antioxidant activities compared to the rest of the medicinal plants as well as BHA and ascorbic acid (as positive controls). Thus, further research may be warranted to study active compounds of *P. alba* that confer the antioxidant activity. The findings presented here might have implications in the population disease prevention field.

## ACKNOWLEDGEMENT

The authors are gratefully acknowledged the facility provides Department of Zoology Dr. R. Karuppasamy, Annamalai University, Tamilnadu, India. And Dr. Ramarajan PH environment laboratory, Vallam, Thanjavur, Funds support of this research Adi Dravidar Welfare department Tamilnadu, India

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