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Original Article

STUDIES ON ANTIOXIDANT ACTIVITY, PHENOL AND FLAVONOID CONTENT OF *PISONIA ALBA* SPAN.

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ABSTRACT

Pisonia alba leaf were collected from Five different biodiversity of Tamilnadu to Analysis the Antioxidant activity, Total Phenol and Flavonoid content, leaf extraction from various solvents(Aqueous, Ethanol, Petroleum ether, Chloroform, and acetone). Butylated Hydroxy Toluene (BHT), Gallic acid (GA) and Quercetin (Q) were taken as standard in case of antioxidant activity, phenol and flavonoid content respectively. The leaf extracts were evaluated for antioxidant activities by DPPH (1,1– diphenyl -2- picryl - hydrazyl) radical scavenging assay. The Maximum antioxidant activity was found in ethanolic leaf extract (65.7%) from Thiruvannamalai as compare to other accessions. Total phenol and flavonoid contents were quantitatively estimated. Total phenolic content measured by Folin-Ciocalteau method varied from 9.64 to 29.72 mg.Gallic Acid Equivalents (GAE)/g and the total flavonoid contents as measured by aluminum chloride method varied from 6.32 to 12.36 mg Quercetin Equivalents (QE)/g.The ethanolic leaf extract of *pisonia alba* was found maximum in total phenol and flavonoid contents were 12.6 mg GAE /g and 7.5 mg QE /g respectively.

Keywords: ***Pisonia alba,*** antioxidant activity, DPPH, phenol and flavonoid.

INTRODUCTION

Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness.. Antioxidants also have many industrial uses, such as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Many phyto chemical studies of Medicinal plants have revealed the presence of numerous chemicals including alkaloids, flavonoids, steroids, phenols, glycosides and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Hagerman *et al.,* 2008). A number of studies have focused on the biological activities of phenolic compounds which are antioxidants and free radical scavengers (Evans *et al.,* 1995; Cespedes, 2008; Reddy *et al.,* 2008). The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders (Ito *et al.,* 1983; Evans *et al.,* 1997; Tiwari, 2001). In addition, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities,

Anti-inflammatory, anti-carcinogenic etc. Flavonoid has antioxidant activity in *in vivo* studies with rats, protecting their gastrointestinal mucosa against the reactive oxygen species generated by acute and chronic stress. Protection against oxidative stress in the human gastrointestinal tract, direct antibacterial activity, synergistic activity with antibiotics.

Pisonia alba span (*Nyctaginaceae*) is widely distributed throughout India and it is a evergreen commonly grown lettuce tree. Leaves, stem and root of this species are extensively used by the tribal's in the preparation of several folk medicines. It has been extensively used in Indian traditional medicine as an ant diabetic, anti-inflammatory agent, and used in the treatment of ulcer, dysentery and snake bite. The present study aims to investigate the antioxidant activity, total

Phenol and total flavonoid content from leaf extract of *pisonia alba*.

MATERIALS AND METHODS

Collection of *Pisonia alba* : The healthy plants of *Pisonia alba* were collected from five different

Biodiversity of Tamil Nadu namely Kancipuram, Thiruvanamalai, Chengalpet, Madhavaram and Chennai.. The collected plants were brought to the laboratory and maintained at Poonga Biotech Research Centre, Plant biotechnology division, Chennai - 600 113, Tamil Nadu, India.

Preparation of the plant extract

Preparation of the extracts was done according to a combination of the methods used by Pizzale *et al.,* (2002) and Lu and Foo, (2001). About 1g of dried *Pisonia alba* plant materials were extracted with 15 ml aqueous, ethanol (75 %), chloroform, petroleum ether and acetone extract for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40 °C to a constant weight and then dissolved in aqueous, ethanol (75 %), chloroform, petroleum ether and acetone. The solution was stored at 18 °C until use.

Qualitative analysis of antioxidant activity of *Pisonia alba* :

The antioxidant activity of leaf extracts of ***Pisonia alba*** was determined by following the method as described by George *et al.,* (1996); Samundeeswari *et al.,* (2013). 50µl of leaf extracts of *Pisonia alba* were taken in the micro titer plate. 100µl of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of free radical scavenging activity of *Pisonia alba*

The antioxidant activities were determined using DPPH (Sigma-Aldrich) as a free radical. 100µl of leaf extracts were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.,* 2005). Subsequently, at every 5 min interval, the absorption maxima of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the following formula:

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance of control)] x 100

Estimation of Total phenolic content (TPC)

The Folin–Ciocalteau reagent method has been used for the estimation of total phenolic extracts quantities according to Lister and Wilson (2001). Five concentrations of crude extracts of the plant have been prepared and then 100 µl have been taken from each concentration and mixed with 0.5 ml of Folin–Ciocalteau reagent (1/10 dilution) and 1.5 ml of Na_2CO_3 2% (w/v). The blend was incubated in the dark at room temperature for 15 min. The absorbance of blue-colored solution of all samples was measured at 765 nm. The results were expressed in mg of gallic acid equivalent (GAE) per g of dry weight of plant powders.

Estimation of Total Flavonoid Content

The 107aluminium chloride colorimetric method was modified from the procedure reported by Woisky and Salatino. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/ml. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 ml of ethanol extracts or 15 flavonoid standard solutions (100 ppm) were reacted with 107aluminium chloride for determination of flavonoid content as described above.

RESULTS AND DISCUSSION

The Antioxidant shows an important Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picryl hydrazyl) is widely used in pathogenesis of many diseases. The usage of synthetic antioxidant components may shows many side effects like toxicity and mutagenic effects, it made an alternative search of naturally occurring antioxidants (Galvez and Cordero, 2005; Tepe, *et al.,* 2005; Mammadov 2011). Different accessions of *Pisonia alba* leaf samples were used for antioxidant studies. Analysis on different extraction of ethanol (75%), petroleum ether, chloroform, Acetone and aqueous extract showed the presence of antioxidants. 50µl of leaf extracts (ethanol, petroleum ether, chloroform, Acetone and aqueous) of *pisonia alba* were estimated for free radical scavenging activity using Diphenyl-2-picryl hydrazyl (DPPH) assay. The samples observed for its bleaching from purple to yellow and pale pink were considered as strong positive and weak positive respectively (Table 1;Fig.1). Among the five accessions and five different solvent extracts of *pisonia alba* the ethanolic leaf extract collected from Thrivannamalai recorded the most effective DPPH radical scavenging activity (65.7%) followed by Kancipuram (64.2 %), Chengalpet (51.4%),Madhavaram(47.1%) and Chennai (44.2%) In each case, ethanolic leaf extracts recorded higher percentage of free radical scavenging activity than Petroleum ether extractions followed by aqueous, Acetone and chloroform extract. Phenolics are the most widespread secondary metabolite in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as radical scavengers. Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992).

In our study, total phenol content (TPC) of *Pisonia alba* leaf extract was estimated by using Folin-

Ciocalteau colorimetric method and represented in terms of gallic acid equivalent (GAE). The result of the present study showed that the phenol contents of the ethanolic leaf extracts in terms of Gallic acid equivalent were between 9.64 mg GAE/ g to 29.72 mg GAE /g. Total phenol content of *Pisonia alba* ethanolic leaf extract shows 12.6 mg GAE/g).

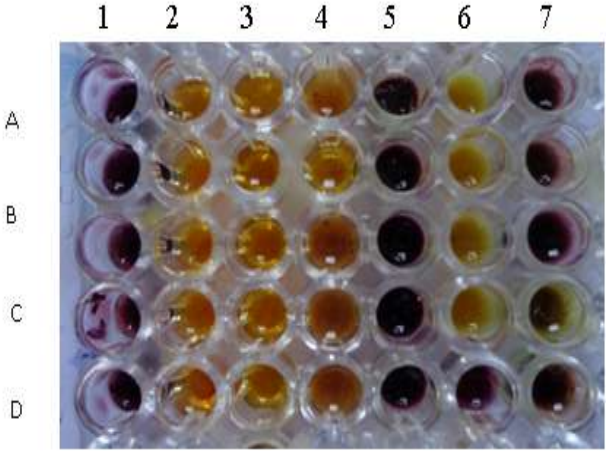
The flavonoid shows an important role in antioxidant activity and their effects in human nutrition. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler *et al.,* 2003, Cook and Samman, 1996). The result of the present study showed that the flavonoid contents of the ethanolic leaf extracts in terms of quercetin equivalent were between 6.32 to 12.37 mg QE /g. The flavonoid content *Pisonia alba is* (7.5 mg QE/g)

In the conclusions, the presence of antioxidant activity, total phenol and total flavonoid content of many medicinal plants is identifying as a new sources of therapeutical and industrial utilization.In this research work it is an attempts to assess the importance of antioxidant activity, total phenol and total flavonoid properties in leaves of *pisonia alba* to use in nutraceutical products of commercial importance. The results indicate that the plant material may become an important source of compounds with health protective potential.

Table 1: Qualitative analysis of antioxidant activity from the leaf extract of *Pisonia alba*

S. No	Extractions	*Pisonia alba* (Kancipuram)	*Pisonia alba* (Thiruvannamalai)	*Pisonia alba* (Chengalpet)	*Pisonia alba* (Madhavaram)	*Pisonia alba* (Chennai)
	BHT (standard)	+++	+++	+++	+++	+++
S1	Aqueous	++	+++	++	++	++
S2	Ethanol	+	++	+	+	+
S3	Chloroform	-	-	-	-	-
S4	Petroleum ether	+	+	+	+	-
S5	Acetone	-	-	-	-	-

+++ = very strong positive, ++ = strong positive, + = positive, - = Negative



A1: Control; A2: Standard (BHT), A3: Aqueous; A4: Ethanol; A5: Chloroform; A6: petroleum ether;
A7: Acetone –Accession I (**Kanchipuram)**

B1: Control; B2: Standard (BHT), B3: Aqueous; B4: Ethanol; B5: Chloroform; B6: petroleum ether;
B7: Acetone –Accession II **(Thiruvannamalai)**

C1: Control; C2: Standard (BHT), C3: Aqueous; C4: Ethanol; C5: Chloroform; C6: petroleum ether;
C7: Acetone –Accession III **(Chengalpet)**

D1: Control; D2: Standard (BHT), D3: Aqueous; D4: Ethanol; D5: Chloroform; D6: petroleum ether;
D7: Acetone –Accession IV **(Madhavaram)**

E1: Control; E2: Standard (BHT), E3: Aqueous; E4: Ethanol; E5: Chloroform; E6: petroleum ether;
E7: Acetone –Accession V **(Chennai)**

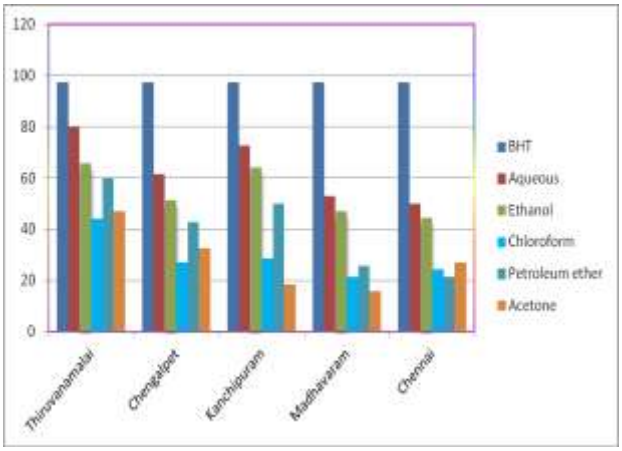
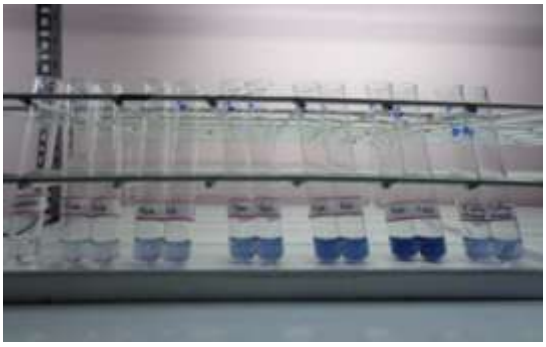


Figure.1 Quantitative analysis of antioxidant activity from the leaf extract of *Pisonia alba*

Accession	BHT	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Kancipuram	97.2	72.8	64.2	28.5	50	18.5
Thiruvannamalai	97.2	80	65.7	44.2	60	47.1
Chengalpet	97.2	61.4	51.4	27.1	42.8	32.7
Madhavaram	97.2	52.8	47.1	21.4	25.7	15.7
Chennai	97.2	50	44.2	24.2	21.4	27.1



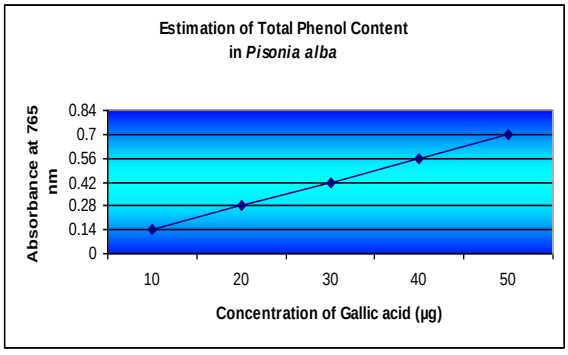
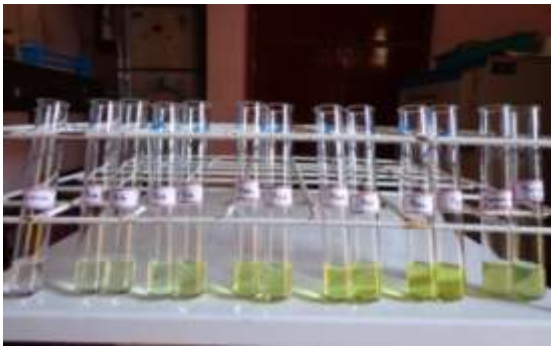


Fig.2: Estimation of Total Phenol Content in *Pisonia alba*



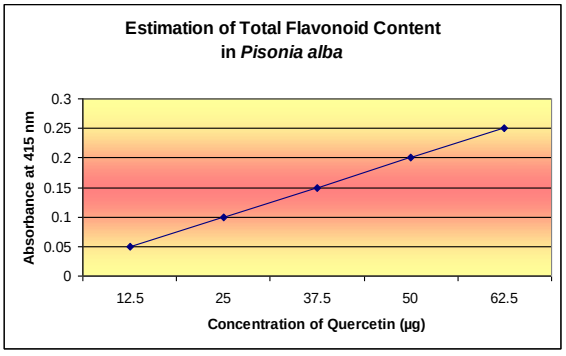


Fig.3: Estimation of Total Flavonoid Content in *Pisonia alba* :

Determination of Total phenol content (TP)

Sample	Total phenolic content (mg GAE/g dry material)
Pisonia alba	**12.6**

Sample	Total Flavonoid content (mg QE/g dry material)
Pisonia alba	**7.5**

REFERENCE

1. Sabrina ilham1, mohammad s. ali1, choudhury m. hasan2, mohammad a. kaisar2, sitesh c. bachar3 (2012) antinociceptive and hypoglycemic activity of methanolic extract of *phlogacanthus thyrsiflorus* nees, Asian journal of pharmaceutical and clinical research (vol 5) suppl 2,2012 ISSN - 0974-2441.
2. Shubashini K Sripathi, Subashree M & Lalitha P(*2011)* Cyclic Voltammetric Behaviour of The Ethanol Extracts of PisoniaGrandis(R.Br)Asian Journal of Biochemical and Pharmaceutical Research *Issue 3 (Vol. 1)* ISSN: 2231-2560
3. D Jayakumar, S Jhancy Mary and R Jaya Santhi(2010) Evaluation of antioxidant potential and antibacterial activity of

Calotropis gigantea andVinca rosea using invitro model, International Journal of pharmacology 895 ISSN 1811 - 7775.

4. Woisky R and Salatino A (1998) Analysis of propolis: some parameters and procedures for chemical quality control. J. Apic. Res. 37: 99-105.
5. Lister E and Wilson P (2001) Measurement of Total Phenolics and ABTS Assay for Antioxidant Activity (Personal Communication). Crop Research Institute Lincoln, New Zealand.
6. P Jagadeesan, D Arun prasad,P Panid kumar, s Ignacimuthu (2011) Antioxidant and free radical scavenging activities of common green from Tiruvallular district of Tamilnadu, A Indian Journal of natural product and Resources156 -163.
7. Cakir A, Maci A, Yyldyrym A, Durub ME, Harmandar M and Kazaz V (2003) Isolation and characterization of antioxidant phenolic compounds from the aerial parts of Hypericum hyssopifolium L.J. Ethnopharmacol. 87, 73-83.
8. Estevinho L, Pereira AP, Moreira L and Luis G (2008) Antioxidant and Antimicrobial effects of phenolic compounds extracts of northeast Portugal honey. Food Chemical Toxicol. 49, 3774-3779.
9. Sabir SM and Rocha JBT (2008) Antioxidant and hepatoprotective activity of aqueous extract of Solanum fastigiatum (false" Jurubebe") against paracetamol induced liver damage in mice. J. Ethnopharmacol. 120,226- 232.
10. Gavani U & Paarakh PM (2008) Antioxidant Activity of *Hyptis suaveolens* Poit. Int J Pharmacol 4. 227-229.