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Research Article

IN VITRO ANTIOXIDANT ACTIVITIES OF ETHANOLIC SEED EXTRACT OF *MILLETTIA PINNATA* AND LEAF EXTRACT OF *BASELLA ALBA*

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ABSTRACT

The present study deals with an antioxidant potential of ethanolic seed extract of *Millettia Pinnata* (Fabaceae) and ethanolic leaf extract of *Basella alba* (Basellaceae). The ethanolic seed extract of *Millettia Pinnata* and leaf extract *Basella alba* were investigated employing three different established invitro methods, free radical scavenging activity using 2,2-di phenyl-1-picryl hydrazyl (DPPH), reducing power assay, and Phosphomolybdenum assay. The results of the assays were compared with reference standard Gallicacid. The ethanolic seed and leaf extracts are good source of compounds with antioxidant properties and the both extracts exhibited significant free radical scavenging activity, reducing power activity and antioxidant activity.

Keywords: *Millettia Pinnata, Basella alba,* Antioxidant activity.

INTRODUCTION

Free radicals are type of reactive oxygen species (ROS), which includes superoxide anion radicals (O_2), hydroxyl radicals (OH) and non free radical species such as H_2 O_2 and singlet oxygen (O_2) are various forms of activated oxygen[1]. All these free radicals are capable of reacting with membrane lipids, nucleic proteins and enzymes and other small molecules, acids, resulting in cellular damage[2].

Antioxidants prevent the oxidation of substrate even if the compound present in a significantly lower concentration than the oxidized substrate and can be recycled in the cells (or) irreversibly damaged[3]. The main characteristic of an antioxidant is its ability to trap free radicals. Plants are rich source of free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, tannins, flavanoids., alkaloids, amines and other metabolites which are rich in antioxidant activity[4].

Millettia Pinnata is known as Indian beech. The Seed extract of this plant inhibits growth of herpes simplex virus and also posses hypoglycemic, antioxidative, anti-ulcerogenic, anti-Inflammatory and analgesic properties[5,6]. The seed oil contains 5-6% Flavonoids[7].

Basella alba is known as Malabar spinach. The leaves are thick, rugose, succulent and green to purple colour[8], it is known for being rich in β-Carotene and VitaminA[9].The Plant is reported to treat against laxative, inflammation, rubefacient, haemorrhages, skin diseases, burns. Ulcers, diarrhoea, diuretic and cancer. The present study was under taken to evaluate invitro antioxidant property of ethanolic seed extracts of *Millettia pinnata* and leaf extract of *Basella alba* using DPPH, reducing power and Phosphomolybdenum assay.

MATERIALS AND METHODS

Plant Materials

The seeds of Indian beech and leaves of Malabar spinach were collected from surrounding areas in Kakinada A.P. The Plant was identified and Authenticated by Dr. T.Raghuram, Taxonomist, Peddapuram.

Preparation of Extracts

The seeds were collected and dried under shade, then seed coat was removed and the seed pulp was powered coarsely the freshly collected leaves

of the plant were cleared from dirt and dried under shade and then coarsely powdered manually. The dried seeds and leaf powdered was macerated individually in ethanol for a period of 7 days and later subjected to hot Percolation for 8 hrs individually. The extracts obtained were Subjected to solvent evaporation for complete drying.

Chemicals and Instruments

All the Chemicals used are of Analytical grade. DPPH (2,2-di-Phenyl-1-picryl hydrazyl) has been procured from Research lab fine chemical Industries, Mumbai, Gallic acid was a gifted sample. Trichloro acetic acid, sodium phosphate, Ammonium molybdate and sulphuric acid were used for this study are of analytical grade. The Instruments UV-Visible double beam spectrophotometer (ELICO- SL-210), PH Meter, Centrifuge machine and electronic balance were used for the analysis.

IN-VITRO ANTIOXIDANT STUDY

DPPH Free Radical Scavenging Activity[10]

The free radical scavenging activity was followed by the DPPH method 0.1 mM solution of DPPH in methanol was Prepared. Gallic acid was taken as reference standard. Different concentrations of both the extracts (50,100,300 & 500 µg/ml) and standard drug (1,2.5,5µg/ml) were prepared using methanol. 1 ml of 0.1mM of DPPH solution was mixed with 3 ml of all the concentrations of both extracts and standard separately. 0.1mM DPPH and methanol mixture was blank. These mixture are kept in dark about 30 min and the optical density was measured at 517nm. The experiment was repeated triplicate. Finally the percentage inhibition of the DPPH activity was calculated using the formula.

$$\text{DPPH Scavenged (\%)} = [(A_0 - A_1)/(A_0) \times 100$$

Where A_0 is the absorbance of control reaction and A_1 is the absorbance of the sample extracts. The antioxidant activity of the ethanolic seed and leaf extract was expressed as IC_{50} and compared with standard. The IC_{50} Value was defined as the concentration (in ig/ml) of extracts that Scavenges the DPPH radicals by 50%

Reducing Power Method[11]

Different concentrations of both extracts (50,100,300 and 500 µg /ml) and standard drug (1,2.5,5 µg/ml) were prepared using distilled water. 1% potassium ferricyanide, 10% Tri chloro acetic acid, 0.1% ferric chloride and 0.2M Phosphate buffer (pH 6.6) were prepared using distilled water. Gallic acid was taken as the reference standard. Then 1ml of each concentration of both extracts and Standard were taken separately and mixed with 1ml of 0.2M phosphate buffer (pH 6.6) and 1ml of potassium ferricyanide. Incubate all these samples at 50^{0}c for 20 min. Then add 1ml of 10% Tri chloro acetic acid and centrifuge at 3000rpm for 10 min. Now separate the upper layer (2.5ml) and then add (2.5ml) distilled water, 0.5ml of freshly prepared ferric chloride. Finally the absorbances were measured at 700nm.

Phosphomolybdenum Reduction[12]

The antioxidant activity of both extracts was evaluated by the phosphomolybdenum method. The assay is based on the reduction of MO(VI)-MO(V) by the extracts and subsequent formation of green phosphate/MO(V) complex at acid pH. 0.3ml of each concentration of both extracts and standard were taken separately and mixed with 3ml of reagent solution (0.6M sulphuric acid 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95^{0}c for 90 min. Then the absorbance of the solution was measured at 695nm using spectro photometer against blank after cooling to room temperature. Methanol (0.3ml) in the place of extract was used as blank.

RESULTS AND DISCUSSION

DPPH Free Radical Scavenging Assay

The DPPH radical scavenging assay is an sensitive method for the antioxidant screening of Plant extract[13]. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517nm, which is induced by antioxidants. **Table 1** Shows the percentage of DPPH radical scavenged by Gallic acid and ethanolic seed extract *Millettia pinnata* and leaf extract *Basella alba* at various Concentrations (µg/ml). **Figure 1a, 1b,1c** illustrates a decrease in the concentration of DPPH radicals due to the scavenging ability of the soluble constituents in the ethanolic seed extract of *Millettia pinnata* and leaf extract of *Basella alba* and the standard Gallic acid as a reference compound. The IC_{50} value were found to be 100.3, 6.14, 3.13µg/ml for ethanolic seed extract *Millettia Pinnata*, leaf extract of *basella alba* and Gallic acid respectively.

Reducing Power Assay

Reducing power assay is based on the principle

that substances, which have reduction potential, react with potassium ferricyanide (Fe^{+3}) to form potassium ferrocyanide (Fe^{+2}), which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[14]. **Table 2** Shows the reducing power of ethanolic seed extract of *Millettia pinnata* and ethanolic leaf extract of *Basella alba*, and standard Gallic acid from **figure 2a,2b,2c** it was found that the absorbance of the both extracts increased with increase in concentrations.

Phosphomolybdenum Assay

Phosphomolybdenum assay is based on the reduction of MO(VI)-MO(V) and forms green colour Phospomolydenum (V) complex, which shows maximum absorbance at 695nm. **Table 3** shows the antioxidant capacity of ethanolic seed extract of *Milletta pinnata* and ethanolic leaf extract of *Basella alba* and standard Gallic acid. From **figure 3a,3b,3c** it was found that the absorbance of both extracts increased with increase in concentrations.

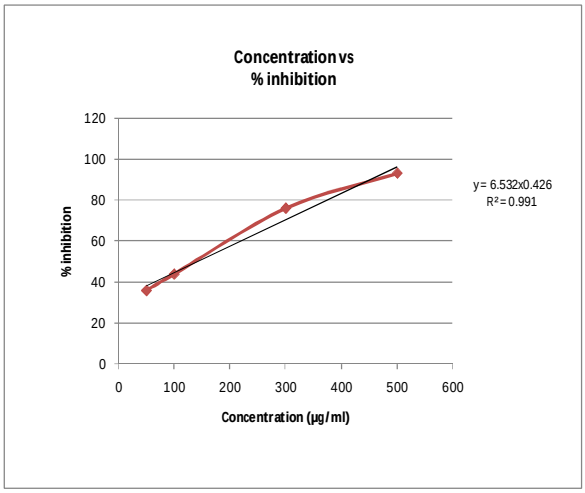

Fig 1a: Free radical scavenging activity of ethanolic Seed extract of *Millettia Pinnata*.

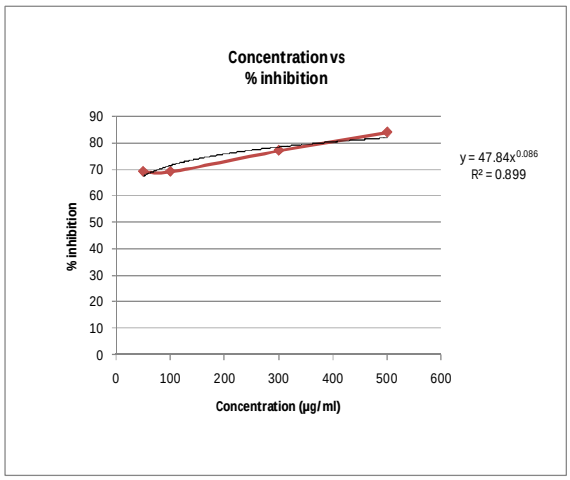

Fig 1b: Free radical scavenging activity of ethanolic Leaf extract of Basella alba.

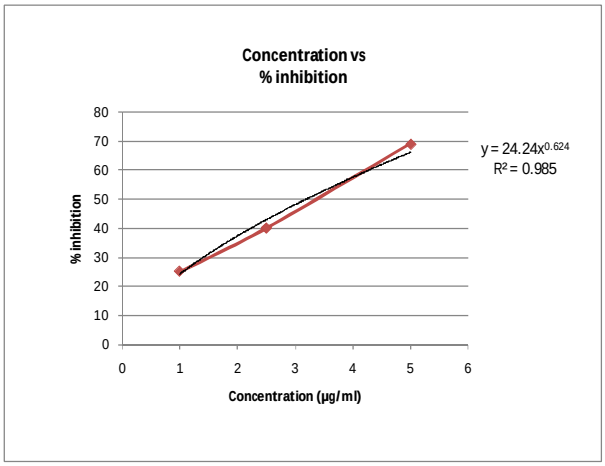

Fig 1c: Free radical scavenging activity of standard Gallic acid

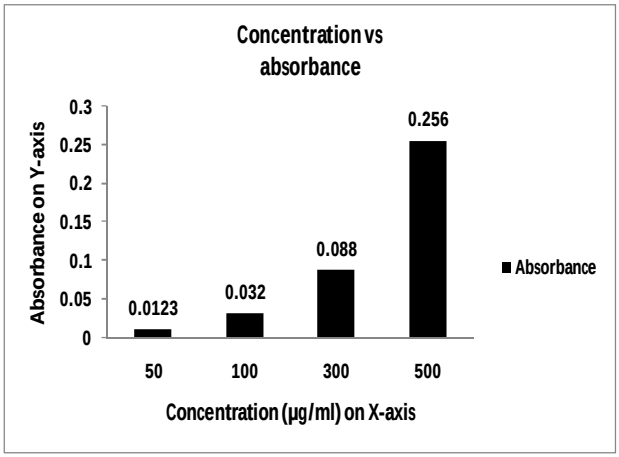

Fig 2a: Reducing power of ethanolic seed extract of Millettia Pinnata

Table 1: DPPH radical scavenging activities of ethanolic seed extract .*Millettia pinnata* and ethanolic leaf extract of *Basella alba*

Tested Materials	Concentration (µg /ml)	% inhibition mean ± SEM	$IC_{50\mu g/ml}$
Ethanolic seed extract of *Millettia pinnata*	50	38.6 ± 2.94	100.3
	100	46.45 ± 1.94	
	300	75.23 ± 0.26	
	500	93.25 ± 0.03	
Ethanolic leaf extract of *Basella alba*	50	68.45 ± 0.671	6.14
	100	69.35 ± 0.035	
	300	77.6 ± 0.311	
	500	83.9 ± 0318	
Standard Gallic acid	1	24.3 ± 1.09	3.03
	2.5	40.67 ± 0.04	
	5	66.14 ± 0.41	

Values are expressed as mean + SEM, n=3.

Table 2: Reducing power of Ethanolic seed extract of *Millettia pinnata* and Ethanolic leaf extract of *Basella alba.*

Tested Materials	Concentration (µg/ml)	Absorbance ± SEM
Ethanolic seed extract of *Millettia Pinnata*	50	0.0123 ± 0.005
	100	0.032 ± 0.004
	300	0.0882 ± 0.003
	500	0.2568 ± 0.002
Ethanolic leaf extract of *Basella alba*	50	0.0042 ± 0.006
	100	0.0098 ± 0.002
	300	0.0348 ± 0.001
	500	0.0648 ± 0.003
Standard Gallic acid	1	0.0088 ± 0.002
	2.5	0.033 ± 0.003
	5	0.0708 ± 0.005

Values are expressed as mean + SEM, n=3.

Table 3: Antioxidant capacity of ethanolic seed extract of *Millettia pinnata* and ethanolic leaf extract of *Basella alba*.

Tested Materials	Concentration (µg /ml)	Absorbance ± SEM
Ethanolic seed extract of *Millettia Pinnata*	50	0.0172 ±0.003
	100	0.0319 ± 0.003
	300	0.0625 ± 0.007
	500	0.202 ± 0.001
Ethanolic leaf extract of *Basella alba*	50	0.022 ± 0.002
	100	0.030 ± 0.007
	300	0.0604 ± 0.002
	500	0.082 ± 0.003
Standard Gallic acid	1	0.039 ± 0.003
	2.5	0.128 ± 0.002
	5	0.2 ± 0.001

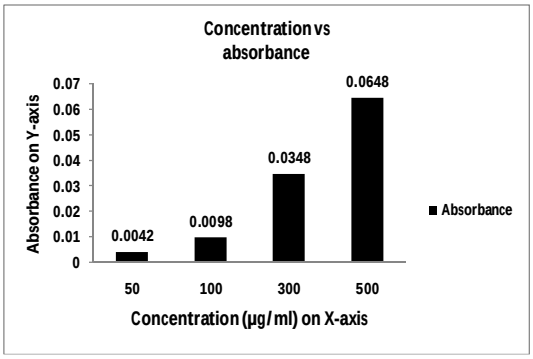

Fig 2b: Reducing power of ethanolic leaf extract of Basella alba.

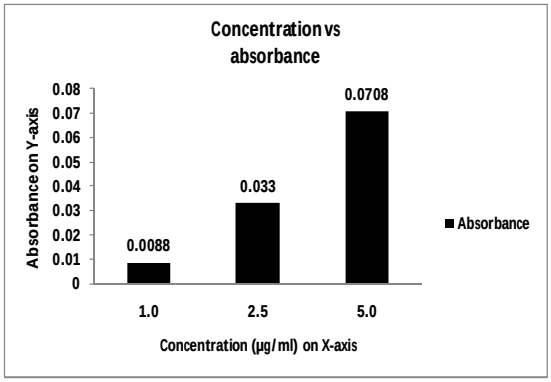

Fig 2c: Reducing power of standard Gallic acid

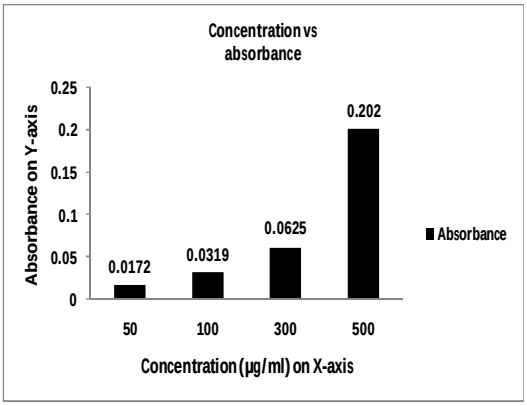

Fig 3a: Antioxidant capacity of ethanolic seed extract of Millettia Pinnata.

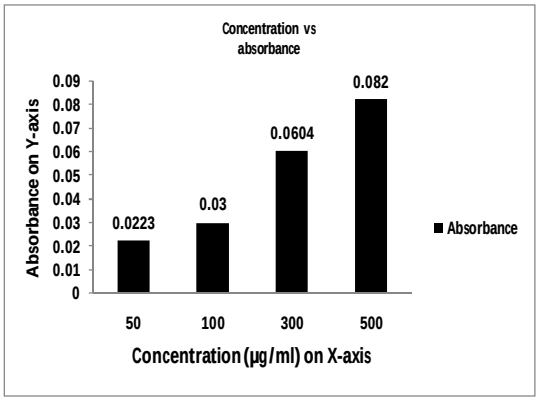

Fig 3b: Antioxidant capacity of ethanolic leaf extract of Basella alba.

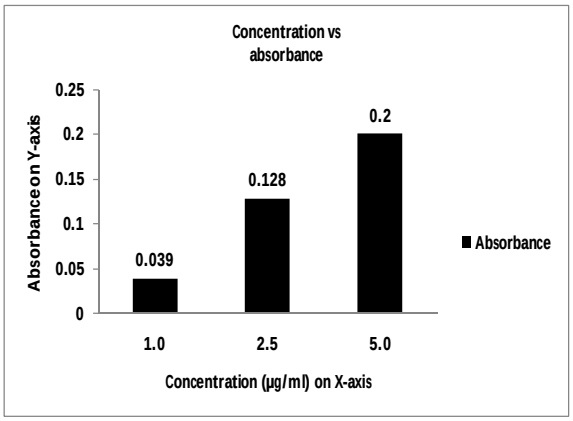

Fig 3c: Antioxidant capacity of standard Gallic acid.

CONCLUSION

The study was performed to evaluate the invitro antioxidant activity of ethanolic seed extract of Millettia pinnata and ethanolic leaf extract of Basella alba. The results obtained indicates the significant antioxidant activities in all the three established methods and the results were compared with standard Gallic acid. Further investigations may be carried out to isolate the actual phytochemical constituents responsible for the activity.

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