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ANTIOXIDANT ACTIVITY OF METHANOLIC PLANT EXTRACTS OF SOME MEDICINAL PLANTS OF LEGUMINOSAE FAMILY

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ABSTRACT

Pongamiya pinnata (*L*) and *Mucuna pruriens* (*L*) are commonly used in the indian folk medicine. They are potent in treating many diseases. The lack of information about their biological activities led us to investigate the possible biological activities by determination of antioxidant activities of methanolic extracts. Antioxidant capacity was evaluated by radical scavenging method. This study suggests that *Pongamiya pinnata* (*L*) and *Mucuna pruriens* (*L*) extracts exhibit good potential for antioxidant activity and may be useful for their nutritional and medicinal functions.

KEYWORDS: *Pongamiya pinnata* (*L*) and *Mucuna pruriens* (*L*).

INTRODUCTION

India has rich diversity, good traditional & folk knowledge and developing infrastructure. Scientists of the world make good use of its natural resources and new natural resources are being explored.[1]

Different plant parts *i.e.*, root, stem, leaves, flowers, fruits may contain different genre and levels of natural chemicals or “phytochemicals”. These phytochemicals can be categorized into primary or secondary metabolites.

Primary metabolites *i.e.*, proteins, carbohydrates, vitamins, hormones and lipids are essential for plants to live and reproduce. These primary metabolites provide world with food and feed stuffs and are basis of nutrition for entire living world.[2]

Secondary metabolites are produced for purposes beyond living viz. protection and disease-prevention. Such metabolites have been proven for extra-ordinary uses of them as they show either good biological activities or have been shown to have capabilities that can be used in other purposes.[3]

Major plant compounds characterized by antioxidant activity are polyphenols. Antioxidant activity of polyphenols is accredited to their redox properties *i.e.*, adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen and decomposing peroxides.[4]

Oxidation of important biological molecules arises from daily metabolic processes and does not represent any infection. These free radical can cause oxidative damage to amino acids, lipids, proteins and DNA.[5] Such oxidation can damage cell's surface, alter its DNA or completely kill the cells. free radical and reactive oxygen species *i.e.* hydrogen peroxide, hypochloric acid and proxynitrite are produced during aerobic metabolism in the body.

Antioxidants are one such substance which has capability to neutralize free radicals or their actions. Most published chemicals with antioxidant properties have been vitamin C, Vitamin E and Beta carotene. Antioxidant abilities of phytochemicals have been shown by allyl sulphides in onion leaf, garlic, carotenoids, flavonoids which give colour and flavour to various fruits and vegetables and polyphenols etc. This is why it is long established fact for beneficial roles of fruit and vegetables in human diet providing protection against cellular damage caused by exposure to high levels of free radical.[6]

Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exert an antioxidant action. Fruits and other parts may contain many different antioxidant that serve as radical scavengers and it is relatively difficult to measure each antioxidant component separately. It is impossible to avoid free radicals, but antioxidants can minimize their effect. Radical scavengers have attracted special interest because they can protect human body from free radicals that may cause many diseases, including cancer, and contribute to the aging process.[7] Plant based antioxidants are phytochemicals that may be distributed throughout the plant. However, their distribution and quantity may vary from one plant part to another. Even with in a plant part, concentration and type of antioxidant may vary according to age and the environmental conditions of plant.[8]

MATERIAL AND METHOD

Authentication of species

Taxonomic identification of plant specimens of *Pongamia Pinnata* and *Mucuna pruriens* collected during study was confirmed from State Forest Research Institute (SFRI), Jabalpur (MP).

Collection of plant materials

Plant materials of *Pongamia Pinnata* and *Mucuna pruriens* was collected by following guidelines of good agricultural practices (GACP) for medicinal plants from Narsinghpur region of MP.

Processing of plant materials

Collected samples of *Pongamia Pinnata* root, stem bark and *Mucuna pruriens* roots, stem were cleaned, packed in jute bags and brought to Laboratory. Samples was washed thoroughly in running water to remove soil and foreign particles. Each specimen was labeled, numbered, annotated with date of collection. Fresh roots, stem bark and stem were cut into small pieces and dried at room temperature. Shade dried plant samples of above plant roots were powdered using high power grinder mill and powdered plant materials were stored in air-tight polythene bags for chemical analysis.

Antioxidant activity

In order to access Antioxidant activities associated with different parts of plant, dried, milled and sieved plant parts were extracted with solvents of decreasing polarities in order to distribute phytochemicals according to their solubility. In this process, not only much cleaner preparations of phytochemicals of choice were obtained, but also good results for Antioxidant screening could be obtained.

DPPH radical scavenging activity

A simple rapid and sensitive method for Antioxidant screening of plant extracts is free radical scavenging assay using 2,2,diphenyl-1-picrylhydrazyl (DPPH) stable free radical spectrophotometrically. DPPH is dark violet in color and in presence of an Antioxidant, DPPH radical obtains one more electron and absorbance decreases (Koleva et al, 2002). Odd electron in DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. Color turns from purple to yellow as the molar absorptivity of DPPH radical at 517 nm reduces when odd electron of DPPH radical becomes paired with hydrogen from a free

radical scavenging compound (Antioxidant) to form reduced DPPH-H. Resulting de-colorization is stoichiometric with respect to number of electrons captured.[9]

DPPH solution

2.366 mg of 2,2-diphenyal-1-picrylhydrazyl (Sigma, USA) was dissolved in 100 ml of absolute ethanol to obtain 60 µM DPPH.

Sample preparation

25mg of dried extract was dissolved in 25 ml of absolute ethanol and then was further diluted to obtain 100, 200, 400, and 600µg equivalent dry matter/millilitre.

Procedure

Scavenging effect of plant samples as well as ascorbic acid (Vit C) corresponding to quenching intensity of DPPH was carried out. Sample solution of each tested material (500µl) was mixed with the same volume of DPPH solution and allowed to stand for 1 hour at room temperature in dark (until stable absorption values were obtained). Absorbance was then measured at 517 nm using a spectrophotometer.

Percentage scavenging effect was determined by comparing absorbance of solution containing test sample to that of negative control solution EtOH (Ethanol) without test sample taking corresponding blanks. Absorbance was taken three times each after 30min. Result in mean of three measured values for each sample. Ascorbic acid in same concentration was used as positive control was known by following formula.

% antioxidant activity for DPPH= $\frac{(A-A_X)}{A} \times 100$

Where

A- Absorbance of DPPH solution with EtOH.

A_x- Absorbance of DPPH solution with test solution

RESULT AND DISCUSSION

Present study for Antioxidant activity was analysed for plant parts chosen using DPPH assay. For test, 100, 200, 400 and 600 µg/ml concentrations of plant extracts were used. Ascorbic acid in same concentrations was used as a positive control.

Percent inhibition of DPPH with increasing concentrations of extracts of *Pongamia pinnata* is shown in Table 01. With 100 µg/ml extract concentrations, ascorbic acid showed DPPH

scavenging activity as 25.93%. *Pongamia pinnata* bark extracted with MeOH (methanol) showed 22.64% DPPH free radical scavenging capability, followed by *Pongamia pinnata* root extract with MeOH. Trend was observed with increasing concentrations of extracts. Highest concentrations, i.e., 600 µg/ml produced up to 41.12% DPPH free radical scavenging activity by *Pongamia pinnata* bark MeOH extract as compared to 73.01% by ascorbic acid in same concentration. (Fig 01)

Table 01: DPPH radical scavenging activity of *Pongamia pinnata*, parts as compared to ascorbic acid control at different conc.

S.N.	Sample ID/ Conc (µg/ml)	Percentage inhibition of DPPH		
		***Pongamia pinnata* Root extract in** MeOH	***Pongamia pinnata* Bark extract in** MeOH	**Std Ascorbic Acid**
1.	100 µg /ml	20.41	22.64	25.93
2.	200 µg /ml	28.10	27.41	42.36
3.	400 µg /ml	32.16	35.16	62.80
4.	600 µg /ml	38.12	41.12	73.01

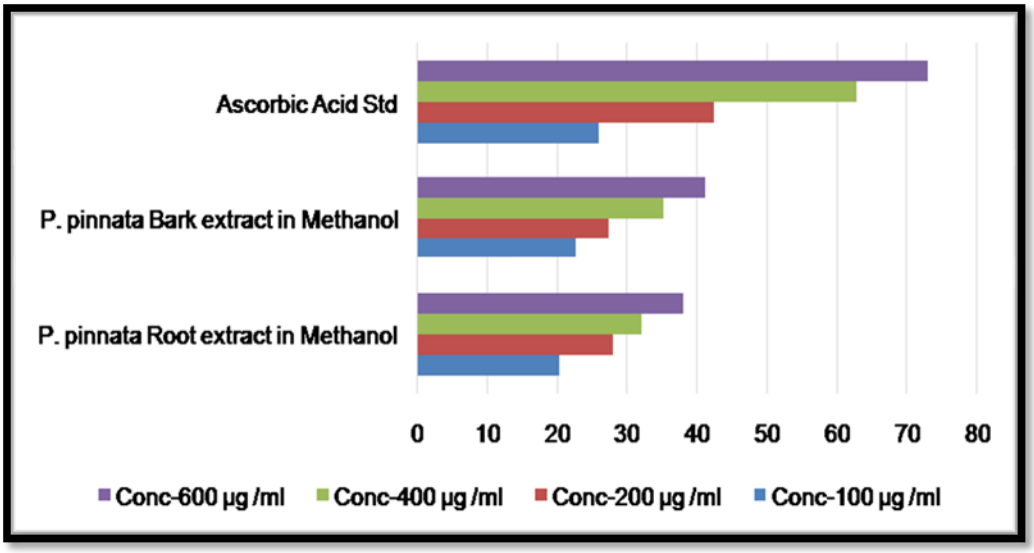

Fig 01: Comparison of antioxidant activities of different concentrations of root and bark extracts of *Pongamia pinnata* with ascorbic acid used in same concentrations.

Percent inhibition of DPPH with increasing concentrations of extracts of *Mucuna pruriens* root and stem is given in Table 02. With 100 µg/ml extract concentrations, ascorbic acid showed DPPH scavenging activity as 25.93%. *Mucuna pruriens* stem extracted with MeOH showed 24.19% DPPH free radical scavenging capability, followed by *Mucuna pruriens* root extract with MeOH.

Trend was observed with increasing concentrations of extracts. Highest concentrations, *i.e.*, 600µg/ml produced up to 39.41 % DPPH free radical scavenging activity by *Mucuna pruriens* stem MeOH extract as compared to 73.01% by ascorbic acid in same concentration. (Fig 02).

Table 02: DPPH radical scavenging activity of various plant parts of *Mucuna pruriens* (Kewanch), as compared to ascorbic acid control at different conc.

S.N.	Sample ID/ Concentration (µg /ml)	Percentage inhibition of DPPH		
		Mucuna pruriens Root extract in MeOH	*Mucuna pruriens* Stem extract in MeOH	Std Ascorbic Acid
1.	**100 µg /ml**	19.52	24.19	25.93
2.	**200 µg /ml**	22.33	29.19	42.36
3.	**400 µg /ml**	27.16	33.46	62.80
4.	600 µg /ml	31.12	39.41	73.01

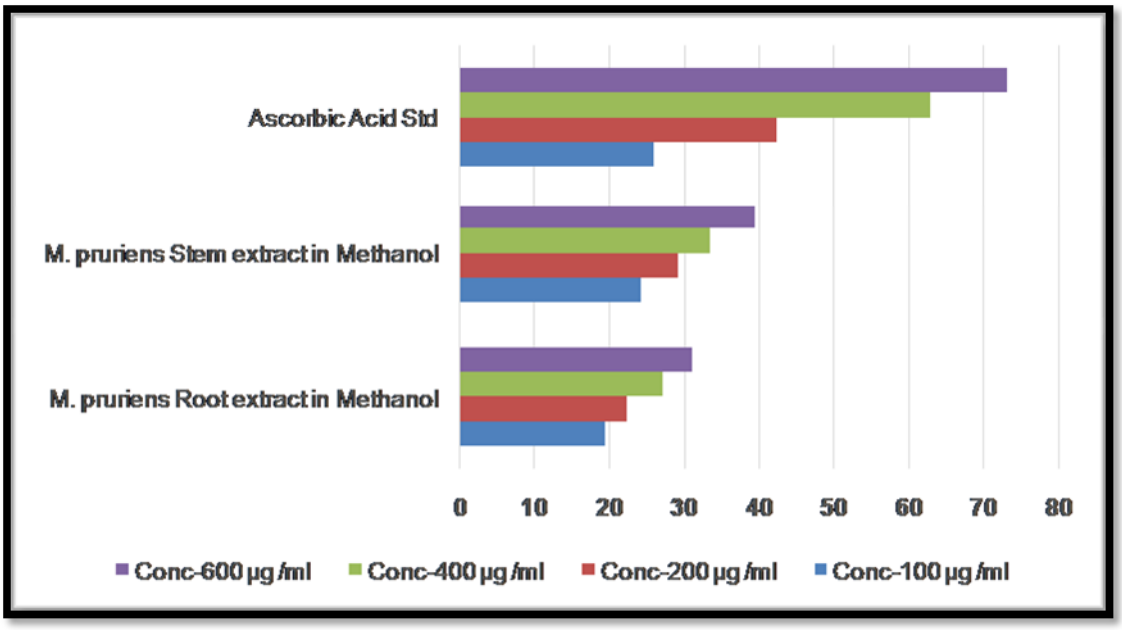

Fig 02: Comparison of antioxidant activities of different concs of *Mucuna pruriens* (Kewanch) parts with ascorbic acid used in same concs.

CONCLUSION

Plants are produce a wide range of secondary metabolites with antioxidant activities that have therapeutic potential. Medicinal plants are commonly rich in phenolic compounds such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity. Two plant species evaluated in the study demonstrated variability in antioxidant characteristics. Methanolic

Extracts of *Pongamiya pinnata* and *Mucuna pruriens* demonstrated good antioxidant potential.

Data from present results revealed that *Pongamiya pinnata* (*L*) and *Mucuna pruriens* (*L*) act as an antioxidant agent due to its free radical scavenging activity.

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