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Research Article

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Antioxidative properties of *Mucuna nivea* (Roxb.) DC by DPPH Assay

Sunil Tayade[1], Kamlakar More[2*], Prashant Gawande[3]

[1]Research Scholar, Department of Botany, Sant Gadge Baba Amravati University, Amravati (M.S) India
[2]Assistant Professor, Department of Botany, Sant Gadge Baba Amravati University, Amravati (M.S) India
[3]Associate Professor, Department of Botany, Sant Gadge Baba Amravati University, Amravati (M.S) India

***Address for Correspondence:** Dr. Kamlakar More, Assistant Professor, Department of Botany, Sant Gadge Baba Amravati University, Amravati-444602, Maharashtra, India
E-mail: kamlakarmore@sgbau.ac.in

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ABSTRACT

Background: The present study was conducted to evaluate the antioxidative potential of leaves and seeds of *Mucuna nivea* due to presence of various bioactive phytoconstituents observed in preliminary phytochemical investigation.

Method: A stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was used as a standard oxidant to evaluate the antioxidant capacity of leaves and seeds extracted with five different solvents.

Result: The qualitative data were obtained by confirming the presence of phytoconstituents, the five different solvents *viz* petroleum ether, chloroform, acetone, ethanol, and methanol were chosen by increasing order of their polarity. The significant percentage of inhibition of DPPH was observed in seed extracts of petroleum ether and methanol showing 46.33% and 45.76% respectively. However, the leaves extracted in petroleum ether reveals 30.91% of inhibition followed by 29.72% of inhibition were recorded in methanolic extract of leaves. Moreover, the ascorbic acid as a standard antioxidant shows 81.46% and 80.05% inhibition of DPPH in leaves and seed extracts of solvents respectively.

Conclusion: From the observation, it can be concluded that the seeds and leaves are the good sources for antioxidants, for the further exploration of bioactive potential of *M. nivea,* the present investigation creates a basic platform for the future investigators.

Key-words: Antioxidants, Ascorbic Acid, *Mucuna nivea*, DPPH, Free radicals, Phytoconstituents

INTRODUCTION

From ancient time, medicinal plants act as a boon for human life in the world [1]. About 75% of the global population of developed and developing countries depends on plants derived medicines for the treatment of various ailments, it has been estimated that the 70,000 different pants considerably using as a medicine [2]. Most of the pharmacological studies are based on the investigation of uses of plants and its production for novel therapeutic drugs.

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Numerous health benefits have been identified like antimicrobial, anti-inflammatory, anti-diabetic, cancer preventive and antihypertensive properties [3]. To cop-up with the oxidative stress the plants synthesize various secondary metabolites as defence molecules [4]. The oxidative environment acting as a signal for the synthesis of antioxidants in plants as a defence molecule, the impaired antioxidant system of an organism resulting in a diseased condition [5]. The free radicals like superoxide radical ($O_2\bullet^-$), hydroxyl radical (•OH), peroxide radical (ROO•), and nitric oxide radical causing various pathological implications including heart diseases, reperfusion injury, inflammation, diabetes, drug toxicity, carcinogenesis and neurodegenerative diseases such as Parkinson and Alzheimer diseases [6]. The biomolecules can get oxidized by interacting with free radicals [7]. The structural alternation in nucleic acids occurs due to free

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radicals attacks [8]. The harmful effect of free radicals or oxidants can be neutralized by increasing the use of antioxidants derived food in daily life, an adequate amount of antioxidants in the body can maintain healthy status due to its free radical scavenging activity, the food processing and preservation industries continuously use the synthetic antioxidants but also reported their side effects and proves to carcinogenic [9]. Phytochemicals have a great impact on different pharmaceutical products with definite therapeutic effect [10]. Polyphenols are strong antioxidants with tremendous free radical foragers and inhibitors of lipid peroxidation. Terpenoids are useful for curing obesity-induced metabolic disorders [11]. Awareness and popularization of phytoconstituents are of prime importance for developing new drug products from medicinal plants [12]. Various medicinal plants have been documented with antioxidants potential [13]. By considering the medicinal importance of genus *Mucuna* based on the earlier reports on *M. pruriens.* The investigation on the antioxidant potential of *M. nivea* was conducted.

MATERIALS AND METHODS

Collection of samples- Plant material leaves and dry pods of *M. nivea* (Roxb.) DC was collected from the area near Maltekdi congress Nagar road, Amravati Maharashtra (India) in December 2015 and according to the phonological calendar, the frequent visits were taken to collect the samples. The assessment of antioxidative properties was performed in the Department of Botany, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India.

Identification of plant material- Identification of plant material was done with the help of standard floras; the flora of British India, Flora of Amravati District [14]. The herbarium specimen was prepared for individual plant and submitted to the Department of Botany, Sant Gadge Baba Amravati University.

Sample preparation- The collected plant parts i.e. leaves were firstly cleaned with tap water to remove dust and other contaminants followed by semi-hot water treatment. The cleaned plant material then subjected to the shade drying for about 10 days. After 10 days the plant material i.e. leaves were transferred to the oven at 40^0C for 2 hours to remove the moisture content. Dried plant material was converted to powder by using electric mixture grinder and passed through the sieve to get the similar size particles of powder. Prepared leaves powder was stored in an airtight plastic container and preserved in refrigerator for further experimentation. Seeds were removed from dry pods, the immature and infected or having diseased seeds were sorted out. The fresh and clean seeds were cut into small pieces by using mortar and pestle and small pieces of seeds were converted into a fine powder with the help of electric mixture grinder. Prepared seeds powder was stored in an airtight plastic container and preserved in refrigerator for further experimentation.

Extraction of phytochemicals- Total 10 gm powder was filled in the thimble (made up of filter paper) and extracted successively with petroleum ether, chloroform, acetone, ethanol, and methanol in 180 ml for 24 hours using soxhlet extraction assembly. The temperature of the apparatus maintained at the boiling point for each solvent. The extractions were carried out using the above mentioned different solvents with specific characteristics of increasing values of their polarity. The obtained extracts were filtered through Whatman filter paper no.42 for free and clear extract. This extract then evaporated and concentrated up to 10 ml. Resultant10 ml extract again filtered and stored in small sterile airtight bottles at -4^0C temperature.

Preliminary phytochemical analysis- The preliminary phytochemical analysis was performed for all the extracts as per standard method [15] for testing the different chemical groups such as alkaloids, flavonoids, phenol, tannins, glycosides, saponins, terpenoids and steroids present in petroleum ether, chloroform, acetone, ethanol and methanol. For every chemical group, two tests were selected for confirming the presence of phytochemicals.

Evaluation of antioxidant activity by DPPH- A stable free radical DPPH (1, 1-diphenyl-2-picrylhydrazyl) was used to calculate the antioxidant activity, the effect of test samples on DPPH radical was estimated according to the procedure described by [16]. Two ml of 6 $\times 10^{-5}$ M methanolic solution of DPPH was added to 50 µl of a methanolic solution (10 mg ml^{-1}) of the sample. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 minutes at room

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temperature. Methanolic solutions of standard ascorbic acid were tested at 10 mg/ml concentration. The percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 min duration. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula given in [17].

IP = [{AC (0)- AA (t)/ AC(0)}] × 100

Where,
AC (0) is the absorbance of the control at t= 0 min
AA (t) is the absorbance of the antioxidants at t= 16 min

Plant under study- *M. nivea* belongs to family Fabaceae and commonly called as Kuyari. Cultivated in India and fruiting observed from December to January.

Taxonomic Classification

Kingdom:	Plantae
Division:	Phanerogams
Subdivision:	Angiosperms
Class:	Dicotyledones
Subclass:	Polypetalae
Series:	Calyciflorae
Order:	Rosales
Family:	Fabaceae
Genus:	*Mucuna*
Species*:*	*Nivea*
Local Name:	Kuyari

A) Habitat of *M. nivea*

B)Twig of *M. nivea* showing Flowers

C) Green pod of *M. nivea*

D) Mature pods and Seeds of *M. nivea*

Fig. 1: A) Habit of *M. nivea*; B) Twig of *M. nivea;* C) Green pod of *M. nivea*; D) Mature pods and seeds of *M. nivea*

RESULTS

Table 1 shows the inhibition percentage of DPPH radical scavenging activity of leaves samples of *M. nivea* extracted successively with petroleum ether, chloroform, acetone, ethanol and methanol.

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Table 1: Inhibition percentage of DPPH radical scavenging activity in five different solvents extract of *M. nivea* leaves

S.No.	Inhibition percentage of different extracts														
	Pet. ether			Chloroform			Acetone			Ethanol			Methanol		
	Absorbance		%	Absorbance		%	Absorbance		%	Absorbance		%	Absorbance		%
	C	E		C	E		C	E		C	E		C	E	
1	0.436	0.340	22.01	0.658	0.516	21.58	0.141	0.120	14.89	0.219	0.170	23.37	0.142	0.122	14.08
2	0.556	0.329	40.82	0.679	0.498	26.65	0.117	0.120	32.20	0.263	0.240	08.74	0.163	0.098	39.87
3	0.515	0.361	29.90	0.661	0.473	28.44	0.154	0.140	09.09	0.258	0.152	41.08	0.159	0.103	35.22
Std.	0.192	0.038	80.20	0.190	0.035	81.57	0.194	0.053	72.68	0.187	0.032	82.88	0.193	0.029	84.97

C= Control, E= Extract

The inhibition percentage of DPPH radical scavenging activity was found to be variable in different solvents, indicating that the solvent dependant extraction of bioactive compounds from the plant parts under study.

Table 2 shows the inhibition percentage of DPPH radical scavenging activity of seed samples of *M. nivea* extracted successively with petroleum ether, chloroform, acetone, ethanol and methanol.

Table 2: Inhibition percentage of DPPH radical scavenging activity in a different extract of *M. nivea* seeds

S.No.	Inhibition percentage of different extracts														
	Pet. ether			chloroform			Acetone			Ethanol			Methanol		
	Absorbance		%	Absorbance		%	Absorbance		%	Absorbance		%	Absorbance		%
	C	E		C	E		C	E		C	E		C	E	
1	0.213	0.122	42.72	0.256	0.216	15.62	0.253	0.191	24.50	0.245	0.129	47.34	0.256	0.125	51.17
2	0.209	0.177	44.01	0.255	0.226	11.37	0.267	0.186	30.33	0.248	0.135	45.56	0.237	0.127	46.41
3	0.176	0.084	52.27	0.286	0.235	17.83	0.176	0.114	35.22	0.257	0.157	39.68	0.209	0.126	39.71
Std.	0.189	0.027	85.71	0.177	0.032	81.92	0.179	0.069	74.41	0.195	0.032	83.59	0.190	0.033	82.63

C= Control, E= Extract

Fig. 2 shows the comparative account on the anti-oxidative potential of leaves and seeds of *M.nivea* extracted in five different solvents. This comparative data on the determination of the anti-oxidative potential of plants under research creates attention to the researchers for further investigations.

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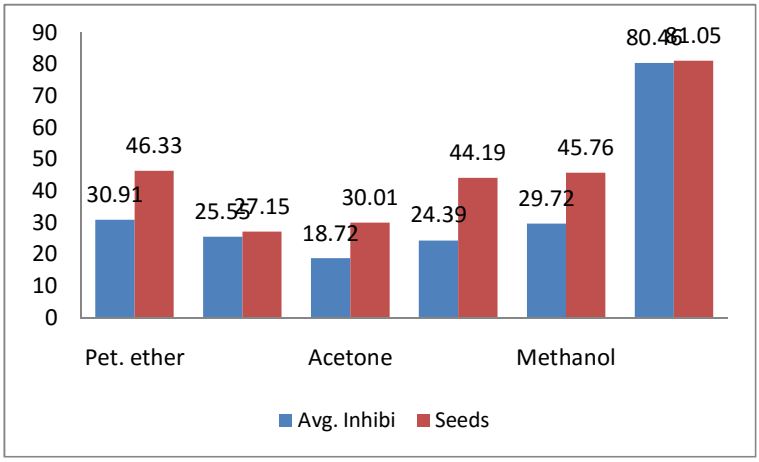

Fig. 2: Inhibition percentage of DPPH radical scavenging activity of different extracts of leaves and seeds of *M. nivea*

DISCUSSION

It has been observed that various methods are used by researchers to investigate the antioxidants potential of plants. DPPH radical scavenging activity is one of the most reliable methods to determine the antioxidants activity of samples under investigations [18] for the assessment of the antioxidant potential of leaves and seeds of *M. nivea,* the five different solvents *viz* petroleum ether, chloroform, acetone, ethanol, and methanol were chosen by increasing order of their polarity [19]. For obtaining the basic level phytochemical status, the qualitative assessment was performed which reveals the presence of alkaloids, flavonoids, phenols, tannins, saponins, glycosides, steroids and terpenoids. The antioxidant potential of *M. pruriens* seeds and leaves extracts was determined by using the solvents mentioned above. DPPH was used as a stable free radical; a freshly prepared DPPH shows a deep purple colure with absorption maxima at 517 nm [20]. During the interaction with antioxidants, the deep purple colour is converted into colourless (i.e. 2, 2-diphenyl-1-hydrazine, or substituted analogous hydrazine), resulting in a decrease in absorption at 517 nm [21]. For the comparative assessment of antioxidant potential, the Ascorbic acid was used as a standard antioxidant. Inhibition percentage of DPPH radical scavenging activity of different extracts of leaves and seeds were shown in Table 1 and 2 and the mean value of the percentage of inhibition was shown in Fig 2. The significant mean value of percentage of inhibition was recorded in seed extracts of petroleum ether and methanol i. e 46.33% and 45.76% respectively. The ascorbic acid as ac standard antioxidant shows 81.46% and 80.05% inhibition of DPPH in leaves and seed extracts of solvents respectively. However, the leaves of petroleum ether extract reveal 30.91% of inhibitions followed by 29.72% of inhibition in methanolic extract of leaves were observed. Most of the researchers have characterized *M. pruriens* in earlier studies [22]. Moreover, in the acidic extract of *M. pruriens* contains high phenolic compounds with antioxidant and hepatoprotective activity [23]. Moreover, the least percentage of inhibition was observed in leaves acetonic extracts.

CONCLUSIONS

Plants are a rich source of antioxidants creating prime attention to redevelop the ethnomedicine because they contain phenols, flavonoids, alkaloids, tannins, vitamins, terpenoids, and many more phytochemicals responsible for different pharmacological activities. From the present study, it can be concluded that the seeds and leaves of *M. nivea* are the good sources for antioxidants, and might have a good impact on neutralization of oxidative stresses. However, the compounds responsible for this activity are currently unclear.

The plant's understudy needs to be further explored to reach the identification of specific compounds responsible for bioactivity; the present investigation creates a basic platform for the future investigators, the crude idea about antioxidants potential of plants will be helpful for new researchers to formulate their hypothesis based on present demands in the field of herbal medicine.

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CONTRIBUTION OF AUTHORS

Research concept-Prashant Gawande, Kamlakar More
Research design- Sunil Tayade, Kamlakar More
Supervision-Prashant Gawande**,**
Materials- Sunil Tayade, Kamlakar More
Data collection- Sunil Tayade, Kamlakar More, Prashant Gawande
Data analysis and interpretation- Sunil Tayade, Kamlakar More, Prashant Gawande
Literature search- Sunil Tayade, Kamlakar More
Writing article- Kamlakar More, Sunil Tayade
Critical review- Kamlakar More, Prashant Gawande
Article editing- Kamlakar More
Final approval-Prashant Gawande

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