

Research Article

Phytochemical Evaluation and Antioxidant

Activity of *Nelumbo nucifera*, *Acorus calamus* and *Piper longum*R. Sujitha¹, B. Valentine Bhimba¹, MS. Sindhu² and P. Arumugham²¹Department of Biotechnology, Sathyabama University, Chennai, Tamil Nadu, India.²Armats Biotech Lab, Chennai, Tamil Nadu, India.**ABSTRACT**

Objective: This study was designed to investigate the antioxidant activity and presence of phytoconstituents in *Nelumbo nucifera* seeds, *Acorus calamus* and *Piper longum* by solvent extraction method. **Methods:** The total antioxidant activity was measured by DPPH radical scavenging assay. *Nelumbo nucifera* had the highest antioxidant activity followed by *Piper longum* and *Acorus calamus*. **Result:** Phytochemical analysis revealed the presence of a number of phytoconstituents such as saponins, flavonoids, phenolic compounds, carbohydrates and proteins.

1. INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs¹. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids^{2,3}. Secondary plant metabolites (phytochemicals), have been extensively investigated as a source of medicinal agents⁴. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias⁵. Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen that includes superoxide radicals, hydroxyl radicals, and hydrogen peroxide, generated as byproducts of biological reactions or from exogenous factors.

Mammalian cells possess elaborate defense mechanisms for radical detoxification such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which destroy toxic peroxides. In addition to antioxidant enzymes, non enzymatic molecules, including thioredoxin, thiols, and disulfide-bonding play important roles in antioxidant defense systems. Compounds that are known to possess antioxidant potential includes flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins, vitamins C and E, carotene, and

tocopherol⁶⁻⁸. A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported^{8,9}.

1.1. *Nelumbo nucifera*

Nelumbo nucifera Gaertn. is a monogeneric plant which belongs to family Nelumbonaceae. The whole plant serves as astringent, emollient, diuretic and sudorific and possesses antifungal, antipyretic and cardio tonic¹⁰⁻¹². Seeds of lotus are astringent and used to treat hyperdipsia, dermatopathy, halitosis, menorrhagia, leprosy and fever¹³.

1.2 *Acorus calamus*

Acorus calamus Linn. (Family: Araceae) is an aromatic semi-aquatic perennial marshy herb commonly known as sweet flag in English. The rhizome of this plant is used in the treatment of insomnia, melancholia, neurosis, epilepsy and other mental disorders either alone or as a component Ayurvedic preparations^{15,16}.

1.3 *Piper longum*

Piper longum (long pepper), is a flowering vine in the family of Piperaceae, cultivated for its fruit, which is usually dried and used as spice and has generally hotter taste. Due to multidimensional effect on various systems of body, it has been described as antipyretic, diuretic, aphrodisiac, immune-stimulant, antioxidant, hepatoprotective, digestive, rubefacient, counter irritant, antiseptic, antispasmodic¹⁷⁻²¹. Besides it is also known to enhance the bioavailability of food and drugs²².

2. MATERIALS AND METHODS**2.1 Plant selection and preparation**

The *Nelumbo nucifera* seeds, *Acorus calamus* rhizomes and *Piper longum* fruits were collected from a local market in Tambaram,

Chennai, TamilNadu. The seeds, rhizomes and fruits were shade dried for 3-4 hours and were ground into coarse powder.

2.2 Solvent Extracts

5g of each of the air dried powder was extracted in each of the three solvents: Ethyl acetate, Hexane and Methanol by Elloff's method of extraction. The above procedure is repeated thrice and the filtrate is obtained using whattman filter paper. The above solvents are selected based on their respective polarity since some of the phytochemicals present may be highly polar whereas others may be less polar or non polar²³. The above selection helps in proper extraction of phytochemicals. The solvent extracts were then condensed to obtain the crude extracts. The crude extracts were then air dried in petri plates, which were covered by perforated aluminium foil. The powder thus obtained was used for further assays.

2.3 Antioxidant activity

The seed, rhizome and fruit extracts were evaluated for antioxidant activity by DPPH assay. DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable free radical with red colour (absorbed at 517nm). Antioxidants react with DPPH, which reduces DPPH to the DPPH-H and as consequence the absorbance decreases from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant

compounds or extracts in terms of hydrogen donating ability.

2.4 Phytochemical analysis

The different phytochemical analysis was performed for establishing the profile of given extract for its chemical composition. Alkaloids, flavonoids, tannins, saponins, phenolic compounds, proteins and carbohydrates were determined according to the²³.

3. RESULTS AND DISCUSSIONS

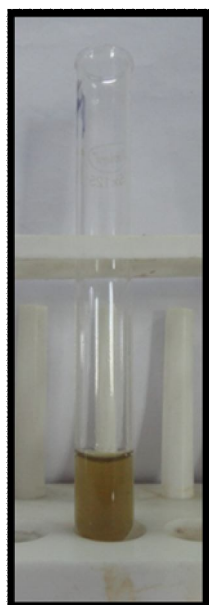
3.1. Phytochemical analysis

Phytochemicals such as flavonoids, phenolic compounds, carbohydrates and proteins were found to be present in the *N.nucifera* seeds, *A.calamus* and *P.longum* (Table 1). The above tests revealed the presence of large amount of carbohydrates in the methanolic extracts of *N.nucifera*, *A.calamus* and *P.longum*. The methanolic extract of *N.nucifera* showed the presence of proteins, phenolic compounds, flavonoids, whereas that of *A.calamus* showed the presence of saponins, phenols, and tannins. *P.longum* showed the presence of phenols, flavonoids and tannins. Protein was found only in *N.nucifera* extracts. Tannins were found in *A.calamus* and *P.longum* extracts only. Flavonoids was found in *N.nucifera* and *P.longum* extracts only. Phenolic compounds were found in all the three plant extracts. Alkaloids was present in none of the above plant extracts.

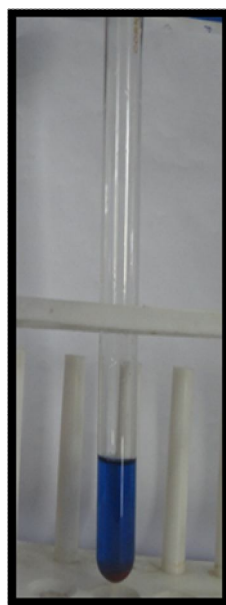
Table 1: Phytochemical analysis of herb extracts (also see illustrations)

Chemical test	<i>N. nucifera</i> (methanol)	<i>A. Calamus</i> (methanol)	<i>P. longum</i> (methanol)
Alkaloids	-	-	-
Carbohydrates	+++	+++	+++
Saponins	-	+	-
Proteins	+++	-	-
Phenolic compounds	++	+	+
Flavonoids	+	-	+
Tannins	-	+	+

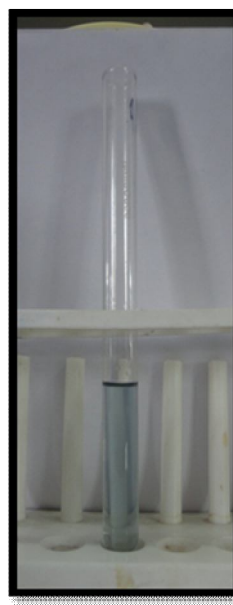
(-) denotes absent, (+) denotes mild, (++) denotes average, (+++) denotes large

Nelumbo nucifera

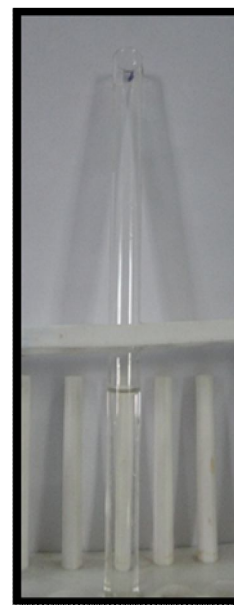
Presence of phenolic compounds



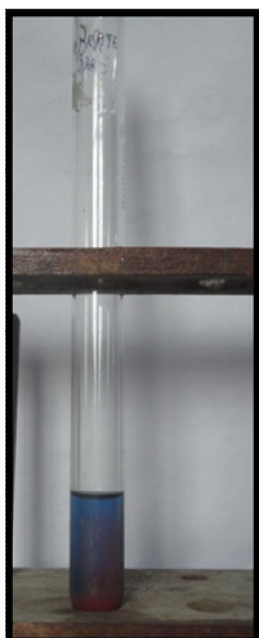
Presence of carbohydrates



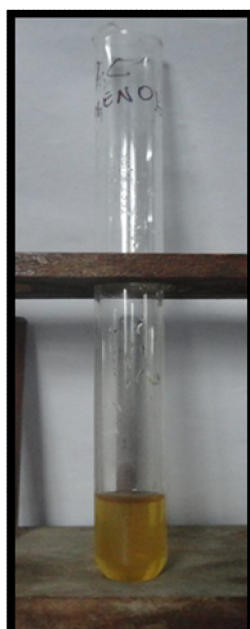
Presence of proteins



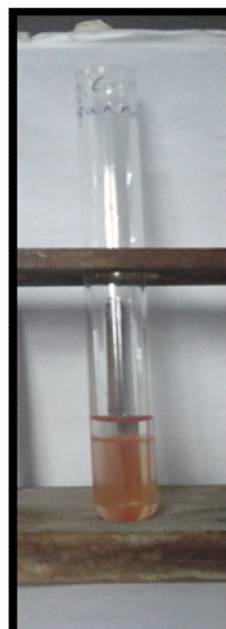
Presence of flavonoids

Acorus calamus

Presence of carbohydrates



Presence of phenolic compounds



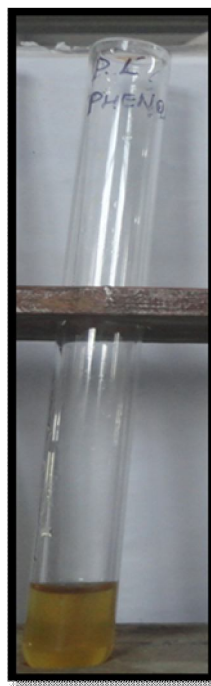
Presence of tannins



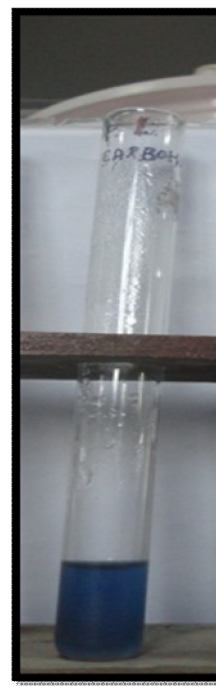
Presence of saponins

Piper longum

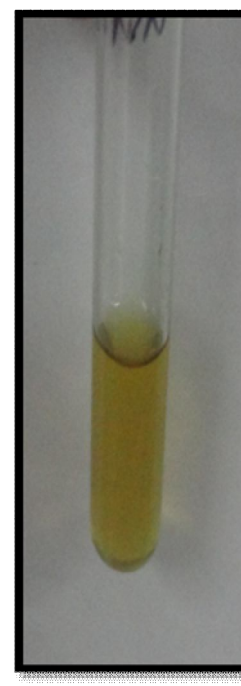
Presence of
flavonoids



Presence of phenolic
compounds



Presence of
carbohydrates



Presence of
tannins

3.2 Antioxidant assay-DPPH free radical scavenging activity

DPPH is a stable free radical, which has been widely used in phytomedicine for the assessment of scavenging activities of bioactive fractions. The scavenging activities of various plant extracts were determined

using free radicals of 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) (Table 2). Results showed that hexane fraction of *N.nucifera* exhibited the highest radical scavenging activity followed by methanolic fraction of *N.nucifera*, *P.longum* and that of *A.calamus*.

% Scavenging is calculated as follows:

$$\% \text{ Scavenging} = \frac{[(\text{Control}-\text{Test})/\text{Control}] \times 100}{}$$

Table 2: Antioxidant assay-DPPH free radical scavenging activity

S. No	Sample (µg)	DMSO (µL)	DPPH (µL)	<i>A. calamus</i>			<i>N. nucifera</i>			<i>P. longum</i>	
				M	H	E	M	H	E	M	H
1.	20	38.0	2.960	5.32	4.73	23.49	40.415	86.22	23.58	10.92	3.41
2.	40	36.0	2.960	19.85	5.11	8.73	50.115	88.39	37.78	11.20	4.92
3.	60	34.0	2.960	3.85	6.07	13.25	57.04	88.67	26.69	14.64	7.57
4.	80	32.0	2.960	10.65	3.69	9.68	67.89	89.34	29.79	15.78	7.83
5.	100	30.0	2.960	10.05	9.50	13.25	85.68	91.16	25.77	19.07	10.35
6.	120	28.0	2.960	6.51	5.99	12.95	90.53	91.24	37.84	21.59	10.85
7.	140	26.0	2.960	8.875	5.45	22.29	84.98	91.6	35.67	28.9	11.36
8.	160	24.0	2.960	10.35	9.16	40.36	90.99	92.98	39.67	31.19	15.66
9.	180	22.0	2.960	8.28	8.27	18.87	92.14	95.06	36.56	32.83	14.89
10.	200	20.0	2.960	25	18.12	20.12	92.84	96.36	42.23	34.34	17.04
11.	Control	Control	3	-	-	-	-	-	-	-	-

'H' denotes hexane extract

'M' denotes methanol extract

'E' denotes ethyl acetate extract

3.3 Thin Layer Chromatography and autobiography

Thin Layer Chromatography profile for methanol extract of *N. nucifera* seeds were thoroughly analyzed using only one solvent system.

Chloroform: Methanol = 9:1

Chloroform: Methanol=9.5:0.5

The Methanolic extract of *N.nucifera* was selected as it showed a high degree of antioxidant activity compared to that of *A.calamus* and *P.longum*. TLC was used to investigate the component present in *N.nucifera* that is responsible for the antioxidant activity. The spots were visualized with UV light at 365 nm and 254 nm. But the spots were clearly observed only after treating them with iodine. From the TLC profile it was found that methanol extract showed two

discrete bands in Chloroform: Methanol (9:1) solvent system. The bands separated better in Chloroform: Methanol (9:1) solvent system when compared to that in Chloroform: Methanol (9.5:0.5) solvent system (Fig.1). Retention factor (Rf) (Table 3) for each of the two components were calculated using the formula:

$$R_f = \frac{\text{(distance travelled by solute)}}{\text{(distance travelled by solvent)}}$$

Autobiography was performed to observe the component that is responsible for the antioxidant activity. It was done by spraying DPPH on the TLC paper containing the separated bands. Yellow colour was observed at the position of the second component. Hence the second component with Rf value 0.9412 was found to be responsible for the antioxidant activity (Fig.2)

Table 3: Retention factor for methanol extract of *N. nucifera* seed

Solvent system	Retention factor	
	Component 1	Component 2
C:M = 9:1	0.882	0.941

'C' denotes chloroform

'M' denotes methanol



Fig. 1:



Fig. 2:

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