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Antioxidant Activity of the Methanolic and Aqueous Extracts of *Urena lobata* (Linn.) by DPPH Method

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ABSTRACT

The methanol extract and aqueous extracts of *Urena lobata* were subjected to investigate its antioxidant property. The phytochemical screening demonstrated the presence of different types of compound like flavonoids, tannins, saponins and phytosterols. The methanol extract and aqueous extract of the plant were tested for antioxidant activity using scavenging activity of (1,1-diphenyl-2-picrylhydrazil) radical method .The the methanolic extract exhibited high free radical scavenging activity when compared to aqueous extract. IC_{50} was found to be 17 (µg/ml).

INTRODUCTION

Herbal medicines are now creature in modern dosage forms using modern manufacture in and processing techniques. Modern herbal research is mainly focused on activity -guided isolation (AGI) of phytoconstituents from the crude drugs. Many of the plants used in herbal medicine contain chemical constituents whose effects can be demonstrated pharmacologically. The herbal medicines/traditional medicament have therefore, been derived from rich traditions of ancient civilizations and scientific heritage [1, 2]. A free radical is considered as fragments of a molecule. They are highly reactive in nature, thus are known as reactive oxygen species and are short lived. They are continuously produced during the body's normal functions and also generated through environmental pollution, cigarette, smoke, automobile exhaust fumes, radiation, air pollutants and pesticides etc. Naturally, there is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench or scavenge them and also to protect / the body from their deleterious effect. Phenol compounds can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes [3]. Currently available synthetic antioxidants like butylatedhydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute these synthetic antioxidants with naturally occurring antioxidants even though these synthetic antioxidants show low solubility and moderate antioxidant activity [4]. Free radicals are formed mainly in three ways, (1), Free radical formation by electron transfer [2], Radical formation by hemolytic fission and [3], Ion formation by heterolytic fission.

The generation of free radicals may be accidental or deliberate. The most important reactants in free radical bio-chemistry in aerobic cells are oxygen derivatives (super oxide, hydroxyl radical), hydrogen peroxide and transition metals. Reactive free radical formed within cells can oxidize biomolecules and lead to cell death and tissue injury. Cells have developed antioxidant defences, to prevent free radical formation or limit their damaging effects. This includes enzymes to decompose peroxides, protein to sequester transition metals and range of compounds to scavenge free radicals.

Urena lobata Linn, is an erect herbaceous or semi woody, tomentose under shrub [5]. The main constituents of *Urena lobata* Linn include flavonoids, glycosides, β-sitosterol and stigmasterol furocoumarin, imperatorin, mangiferin and quercetin [6]. It also contains kaempferol, luteolin, hypolatin and gossypetin [7]. The traditional uses of the plant was found to be diuretic, febrifuge and in the treatment of rheumatism, malaria, gonorrhea, wounds and toothache. It is also used as food for animals as well as humans [3].

Though the information on the physiological properties of the *Urena lobata* was known, its antioxidant properties are not thoroughly worked out. The present study was conducted to investigate the antioxidant activity of *Urena lobata* Linn.

MATERIALS AND METHODS

Plant material collection and authentification

The plant materials of *Urena lobata* Linn were collected from the Herbal Garden Division of Kerala Ayurveda Ltd, Aluva, India and authentified. A specimen voucher was deposited in college herbarium for future references. Methanol, 1,1 diphenyl 2-picryl hydrazyl (DPPH) and ascorbic acid were procured from Sd Fine Chemicals, Bangalore, India. All other chemicals used were analytical grade.

Plant Extraction

Methanolic Extraction

The coarse material was weighed, labeled and submitted to Green Chem laboratory, Domlur, Bangalore, India for extraction. The coarsely powdered (3.0 kg) whole plant of *Urena lobata* was added to a 20 L round bottom flask which was fitted with a reflux condenser. To the above settings, 15 L of Methanol was added and the mixture refluxed at 65 °C for about 1 h. Finally the mixture was filtered and the extract was collected. The extraction process was repeated with 15 L of Methanol and combined all the extracts. The water is then evaporated under reduced pressure in a Buchi Rotary Evaporator at 95 °C, to obtain 105 g of powder extract.

Aqueous Extraction

145g of powder extract of *Urena lobata* from the aqueous extract was developed as explained under the procedure explained previously under methanolic extraction. But in this case the temperature was maintained at 100 °C for reflux the mixture [7].

Preparation of DPPH solution

From this stock solution 0.375 ml was added to each test tube.

Preparation of the test solution

Different concentrations of methanolic and aqueous extracts (5-100µg/ml) were prepared using distilled water. Different concentrations of the aliquot of the extracts were added to 1.5 ml of freshly prepared DPPH solution (3mg in 12 ml of methanol). The solution was made up to 2 ml with methanol solution (0.25 g/l), after 20 min, assayed at 515 nm, against ascorbic acid as standard. The assay protocol for aqueous extract and methanolic extract of *Urena lobata* is shown in Table 1.

Table 1: Assay protocol for aqueous extract and methanolic extract of *Urena lobata*

Test tubes	Methanol (ml)	DPPH (ml)	Drug (ml)
Control	1.625	0.375	-
Test tube 1	1.620	0.375	0.005
Test tube 2	1.615	0.375	0.010
Test tube 3	1.605	0.375	0.020
Test tube 4	1.575	0.375	0.250
Test tube 5	1.525	0.375	0.100

The radical scavenging activity (percentage inhibition and IC_{50} value) was calculated by the following formula:

$$\text{Percentage Inhibition} = (\text{A control} - \text{A sample}/\text{A control}) \times 100$$

Where, A control = the absorbance of the control (ascorbic acid), and A sample = absorbance of reaction mixture (in the presence of sample). All tests were run in triplicates ($n = 3$) and the average values were calculated.

IC_{50} Value

Inhibition Concentration (IC_{50}) was introduced by Amit Subedi and his colleagues for the interpretation of the results from DPPH method. The discoloration of sample was plotted against the sample concentration in order to calculate the IC_{50} value. It is defined as the amount of sample necessary to decrease the absorbance of DPPH by 50% [8].

RESULTS AND DISCUSSION

Plants and their products have been used for many years for human health. There are still many plants which have various medicinal values but still not explored and used. Plants contain many novel compounds with medicinal values which need scientific exploration. The free radicals are produced in aerobic cells due to consumption of oxygen in cell growth [9, 10]. Free radicals cause decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane protein leading to death [11]. These free radicals are involved in different disorders like ageing, cancer, cardiovascular disease, diabetes, rheumatoid arthritis, epilepsy & degradation of essential fatty acids [9, 10]. Antioxidant helps in treatment of above disorders. As methanol extract of this plant showed the dose dependent antioxidant activity comparable to ascorbic acid, antioxidant agent might be developed from this plant for the treatment of above disorders associated with free radicals. Antioxidant activity of methanolic extract of *Urena lobata* is shown in Table 2.

Table 2: Antioxidant activity of the methanolic extract of *Urena lobata*

Concentration (µg/ml)	Absorbance at 515 nm	Percentage inhibition (%)
Control	2.421	-
5	2.026	16.31
10	1.766	27
20	0.955	60.55
50	0.161	93.34
100	0.158	93.47

Antioxidant studies in the aqueous extract of *Urena lobata* (**Table 3)** showed very weak antioxidant activity, while methanolic extract showed a promising result as an antioxidant potential. IC_{50} of ascorbic acid is 17 (µg/ml).

Table 3: Antioxidant activity of aqueous extract of *Urena lobata*

Concentration (µg/ml)	Absorbance at 515 nm	Percentage inhibition (%)
Control	2.242	-
10	2.208	1.5
20	2.180	2.76
50	2.166	3.38
100	2.074	7.75

The statistical analysis of antioxidant study is shown in Table 4 and Fig.1.

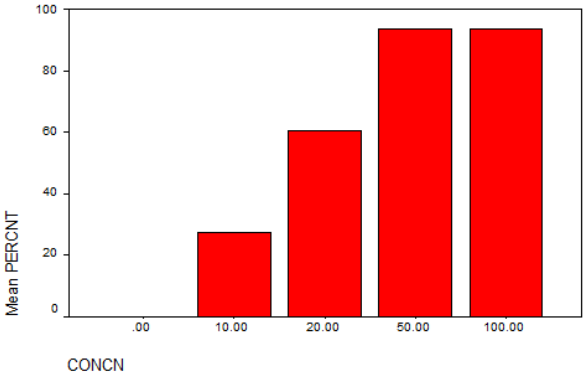
Table 4: Pearson Correlations of methanolic extract and aqueous extract of *Urena lobata*

Group	Concentrations	Absorption
1.00 CONCN Pearson Correlation, Sig.	1.000	-0.844*
(1-tailed)	.	0.036
Number	5	5
2.00 CONCN Pearson Correlation	1.000	-0.977**
Sig. (1-tailed)	.	0.002
Number	5	5

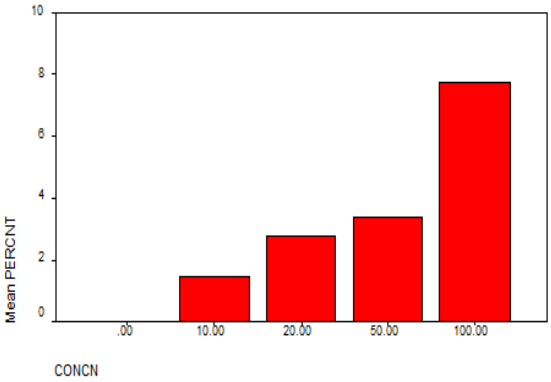
***Correlation is significant at the 0.05 level (1-tailed), ****Correlation is significant at the 0.01 level (1-tailed)

Pearson correlation (Fig 1) between concentration and absorbance increases of aqueous extract *Urena lobata* showed a -ve correlation i.e, -0.977 which has significance (1-tailed) which <0.05 and in case of the methanolic extract of *Urena lobata* when the pearson correlation was done it showed a - ve correlated of -0.844 which is significance (1-tailed) <0.05.

Figure1: Pearson Correlations of methanolic extract and aqueous extract of *Urena lobata*



Methanolic extract of *Urena lobata*



Aqueous extract of *Urena lobata*

CONCLUSION

Our findings suggest that there are still many plants in India which are not traditionally used but possess medicinal values. So, scientific studies also need to be focused on plants which are not traditionally used. For the purpose of characterizing antioxidant activity of plant extracts, it is desirable to subject it to a battery of tests that evaluates the range of activities such as DPPH, As *Urena lobata* showed the antioxidant potential, a detailed biological and phytochemical study is needed to find out the chemical constituent responsible for their activities The *in vitro* antioxidant activity of the methanol and aqueous extracts indicated the efficacy a source of natural antioxidants or nutraceuticals which will have application towards reducing oxidative stress with consequent health benefits.

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