

Estimation of Total Phenol and Flavanoid Content and Antioxidant Activity of *Urena lobata* Leaf Extract

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ABSTRACT: *Urena lobata* is a traditionally important plant and used for the treatment of many diseases such as leaf parts of the plants were used to cure septic ulcer and sores. The present study was undertaken to estimate the total phenol, total flavanoids content and antioxidant activity of *Urena lobata* leaf extracts. The total phenol and flavanoids content was determined by using standard protocol. Phenolic content shows the highest value of 25.00 ± 0.008 mg/g in methanolic leaf extract as compared to the flavanoids content of 8.66 ± 0.005 mg/g. Different concentration of methanolic extract were subjected to antioxidant assay by using DPPH (1, 1-diphenyl-2-picrylhydrazyl) method and H_2O_2 (Hydroxyl) method. In DPPH model IC_{50} value for methanolic extract was found to be 719.1 ± 1.2 μ g/ml in comparison to the Ascorbic acid as standard with IC_{50} value of 600.83 ± 1.12 μ g/ml. In H_2O_2 model IC_{50} value for methanolic extract was found to be 440.03 ± 2.12 μ g/ml in comparison to the Ascorbic acid as standard with IC_{50} value of 600.00 ± 1.24 μ g/ml. Hence the plant is proved as a valuable plant from pharmaceutical point of view due to presence of useful secondary metabolites like antioxidant components, anticancer elements like flavanoids.

KEYWORDS: *Urena lobata*, Phenol, Flavanoid, Antioxidant activity.

I. INTRODUCTION

India is rich in sources of medicinal plants. More than 2500 plant species have been recognized in India which has important medicinal values (Kirtikar K.R. and Basu B.D., 1995). About 70 to 80% of the people worldwide largely believe and use herbal medicines in treatment of many diseases (Shanley P. and Luz L., 2003). A characteristic of plant life is the production of a vast number of natural compounds, often called secondary metabolites. Secondary metabolites are the basic sources for the establishment of several pharmaceutical industries since they have enormous medicinal properties (Lingarao M. and Savithamma N., 2011). The most important secondary metabolites are alkaloids, tannins, flavonoids, tannins, saponin and cardiac glycosides. All secondary metabolites have specific function as like saponins have antifungal activity (Sodipo *et al.*, 1991), some alkaloids may be useful against HIV infection (Mc Mahon *et al.*, 1995), flavonoids have strong anticancer activity and tannin have antimicrobial activity (Noble, 1990). Natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity. This activity is mainly due to the presence of phenolic compounds such as flavonoids, phenols and proanthocyanidins (Rice-Evans *et al.*, 1995). Phenolics are especially common in leaves, flowering tissues and woody parts, such as stems and barks. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases. This activity is due to the ability of antioxidants to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation (Erkan *et al.*, 2008). *Urena lobata* Linneous belongs to the family Malvaceae, leaves and roots are the main parts that are widely used to treat stomach-ache, diarrhoea and dysentery and also treat to gonorrhoea and persistent fever caused by malaria. Leaves are used to treat inflammation of the intestines and bladder. However, the roots are used to treat rheumatism and lumbago. In the present study estimation of total phenol and flavonoid content and antioxidant activity were carried out in leaf extract of *Urena lobata*.

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II. MATERIALS AND METHODS

- 1. Collection of Plant material:** The plant *Urena lobata* was collected from the D.H.S.K College campus of Dibrugarh district, ASSAM. The plant material was identified and authenticated by Mr. Rajib Lochon Borah, Assistant professor, Department of Botany, D.H.S.K College, Dibrugarh. The leaves were dried under shade condition and grinded into fine powder with mortar and pestle.
- 2. Extraction Process:** About 60g of powdered samples was taken and the crude extract was prepared in a soxhlet apparatus using different solvents such as Methanol, Ethanol, Chloroform, and Petroleum ether.
- 3. Quantitative Phytochemical Assays:** Quantitative Phytochemical analysis was performed after the confirmation of the presence of Phenol, Flavanoid and Saponin by qualitative phytochemical assays.

Determination of Total Phenolic Content: Total Phenolic content was analyzed by using standard method with little modifications according to Hagerman *et al.*, 2000; Siddhuraju P. and Becker K. (2003); Gulcin *et al.*, 2003. 10mg of the methanolic extract of the sample was weighed accurately and dissolved in 10 ml of distilled water. 1 ml of this solution was transferred to a test tube, then 0.5 ml of Folin-Ciocalteu reagent was added and vortexed. After 5 minutes, 1.5 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with distilled water and finally incubated for 90 minutes at room temperature. After Incubation, the absorbance against the reagent blank was determined at 765 nm. A reagent blank was prepared using distilled water instead of the plant extract. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid (20, 40, 60, 80 and 100 $\mu\text{g/ml}$). The total phenolic content of the plant was expressed as Gallic acid equivalent (GAE)/100g dry weight. Samples were analyzed in triplicates.

Determination of Total Flavanoid content: Total flavanoid content was analyzed by using standard method with slight modifications according to Zhishen *et al.*, 1999. 1ml of methanolic leaf extract of the sample (2mg/2ml) was diluted with 4 ml of water. Then, 0.3ml of 5% NaNO_2 solution was added to the mixture. After 5 min, 10% of 0.3 ml AlCl_3 was added and allowed to stand for 6 min. 2 ml of 1M NaOH was added. 2.4 ml of water was then added to the reaction flask and mixed well. Absorbance of the mixture was determined at 510 nm against water as blank. The analysis was performed in triplicate and Total Flavonoid Content was determined as quercetin equivalents (g/100g of dry weight).

Determination of DPPH Radical Scavenging Activity: 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was carried out according to Mensor *et al.*, 2001; Koleva *et al.*, 2002; Hsu *et al.*, 2007. A stock solution of methanolic leaf extract and standard ascorbic acid were prepared in the concentration of 10mg/100ml (100 $\mu\text{g/ml}$). Different concentrations (20, 40, 60, 80 and 100 $\mu\text{g/ml}$) of leaf extracts were prepared from the stock solution. One ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract and allowed to react at room temperature for 30 min. The absorbance was then recorded at 517 nm. L-Ascorbic acid was taken as standard control.

The capability of scavenging DPPH radical was calculated using the following equation:
 $\text{DPPH Scavenged (\%)} = (\text{A control} - \text{A test}) / \text{A control} \times 100$

Where “A control” is the absorbance of the control reaction and “A test” is the absorbance of the sample of the extracts. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Determination of H_2O_2 Radical Scavenging Activity: Scavenging activity of hydrogen peroxide by the plant extract was determined by the modified method of Ruch *et al.*, (1989). 4 ml of Plant extract was prepared in distilled water at various concentrations and mixed with 0.6 ml of 4 Mm H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min and its concentration was determined by measuring the absorbance at 230 nm using UV spectrophotometer against blank solution containing the phosphate buffer without H_2O_2 . L-Ascorbic acid was taken as standard control. The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated using the given formula:

H_2O_2 radical scavenging activity = $(\text{A control} - \text{A test}) / \text{A control} \times 100$

Where “A control” is the absorbance of H_2O_2 radical + methanol and “A test” is the absorbance of H_2O_2 radical + sample extract or standard. The experiment was repeated in triplicate.

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III. RESULTS AND DISCUSSIONS

Urena lobata is a traditionally important plant and used for the treatment of many diseases. Mostly the leaf parts of the plants were used to cure septic ulcer and in the treatment of sores (Buragohain J. and Konwar B.K., 2007).

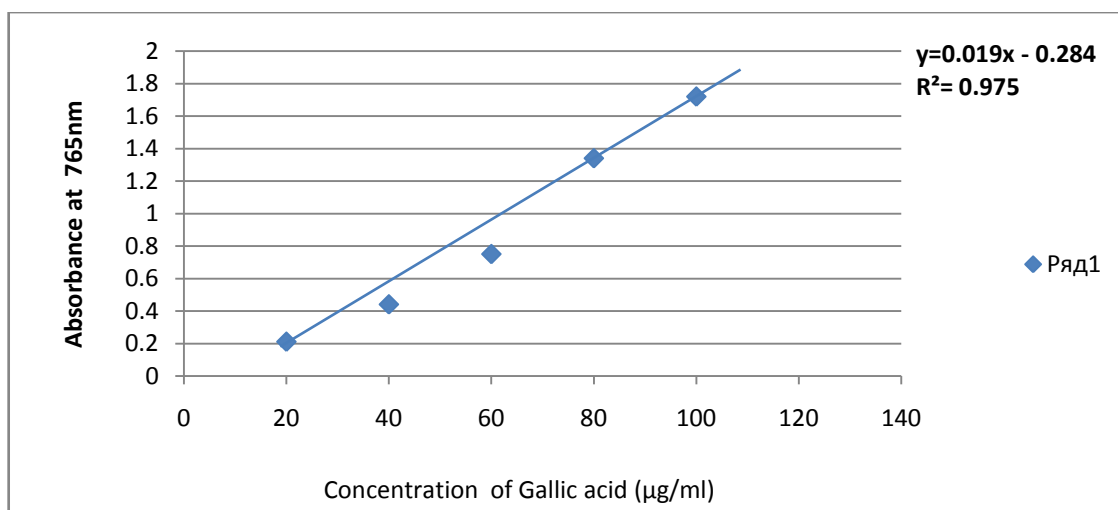


Figure1: Standard curve of Gallic acid

Figure 1 shows the quantitative estimation of total phenols done on the basis of a standard curve of gallic acid and the curve of absorbance versus concentration was described by the equation $y = 0.019x - 0.284$ ($R^2 = 0.975$), where, y = absorbance and x = concentration.

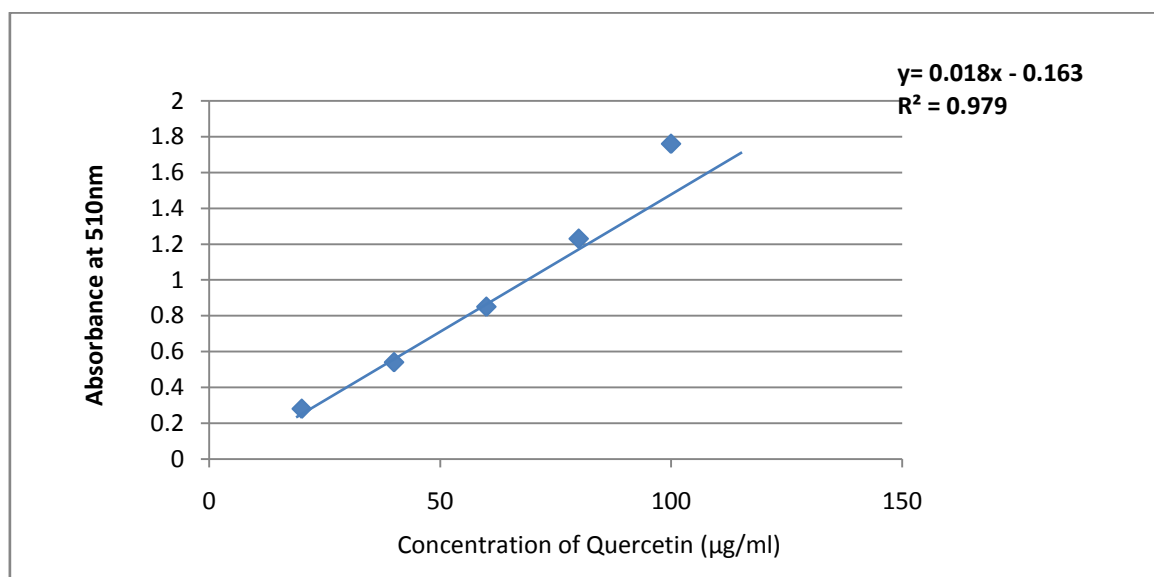


Figure 2: Standard curve of Quercetin

Figure 2 shows the quantitative estimation of total flavanoids done on the basis of a standard curve of quercetin and the curve of absorbance versus concentration was described by the equation $y = 0.018x - 0.163$ ($R^2 = 0.979$) where, y = absorbance and x = concentration.

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Constituents	Percentage (w/w %)
Phenol	25.00 $\pm$ 0.008 mg/g
Flavonoid	8.66 $\pm$ 0.005 mg/g

Table 1: Quantitative Estimation of Phytoconstituents:

Table 1 shows the values obtained for the concentration of total phenols and flavanoid expressed in terms of gallic acid equivalent (mg of GA/g of extract) and quercetin equivalent (mg of quercetin /g of extract) respectively. The total phenolic content of the methanolic leaf extract is 25.00 $\pm$ 0.008 mg/g. The total flavonoid contents of the leaf extract is found to be 8.66 $\pm$ 0.005 mg/g.

Table 2.

Test	IC ₅₀ in μ g/ml	
DPPH radical scavenging activity	Methanolic extract of <i>Urena lobata</i> leaf (Mean $\pm$ SD)	Ascorbic acid (Standard) (Mean $\pm$ SD)
	719.1 $\pm$ 1.2	600.83 $\pm$ 1.12

Table 2: IC₅₀ value of Methanolic extract of *Urena lobata* leaf.

Table 2 shows IC₅₀ values of the methanolic extracts of *Urena lobata* leaf and Ascorbic acid (standard) in DPPH radical scavenging activity. Each value represents mean $\pm$ SD (n=3)

Table 3.

Test	IC ₅₀ in μ g/ml	
H ₂ O ₂ radical scavenging activity	Methanolic extract of <i>Urena lobata</i> leaf (Mean $\pm$ SD)	Ascorbic acid (Standard) (Mean $\pm$ SD)
	440.03 $\pm$ 2.12	600.00 $\pm$ 1.24

Table 3: IC₅₀ value of Methanolic extract of *Urena lobata* leaf

Table 3: IC₅₀ values of the methanolic extracts of *Urena lobata* leaf and Ascorbic acid (standard) in H₂O₂ radical scavenging activity. Each value represents Mean $\pm$ SD (n=3)

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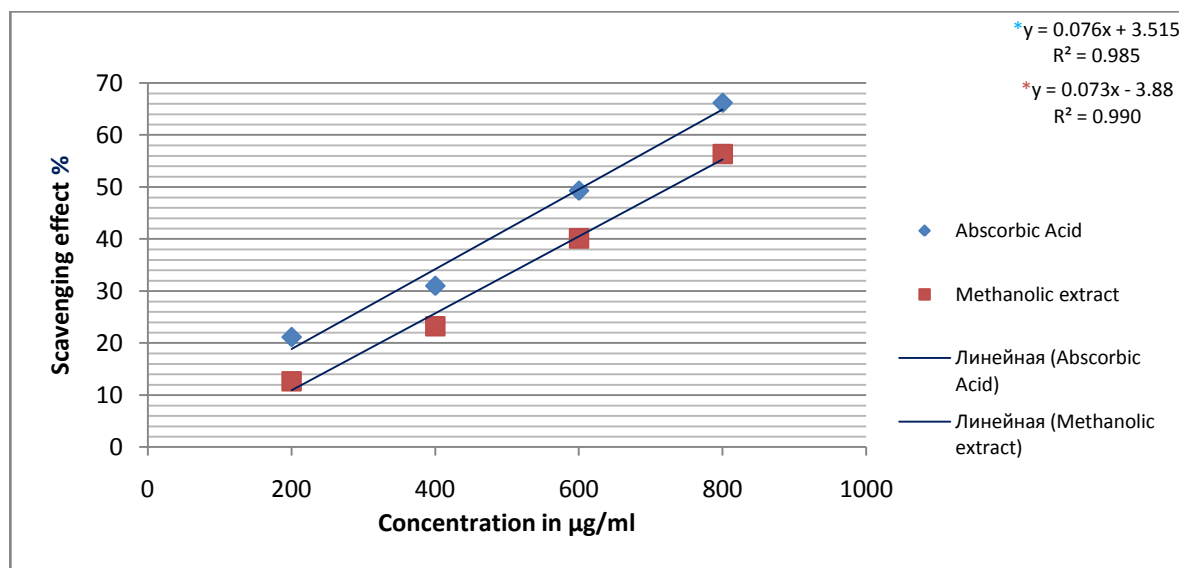


Figure 3: Antioxidant scavenging activity of Ascorbic acid (standard) and methanolic extract of *Urena lobata* leaf on DPPH.

Figure 3 shows the dose-response curve of DPPH radical scavenging activity of the methanolic extract compared with ascorbic acid, as standard. At a concentration of 600 µg/ml, the scavenging activity of methanol extract of *Urena lobata* leaves was 40.12%, while at the same concentration, that of the standard ascorbic acid was 50.00%. The IC_{50} (µg/ml) values are defined as the concentration of the extracts causing 50% inhibition of absorbance; a lower value would reflect greater antioxidant activity of the sample. The IC_{50} value of methanolic extract of *Urena lobata* was found as 719.1 ± 1.2 µg/ml and IC_{50} value of Ascorbic acid was found 600.83 ± 1.12 µg/ml.

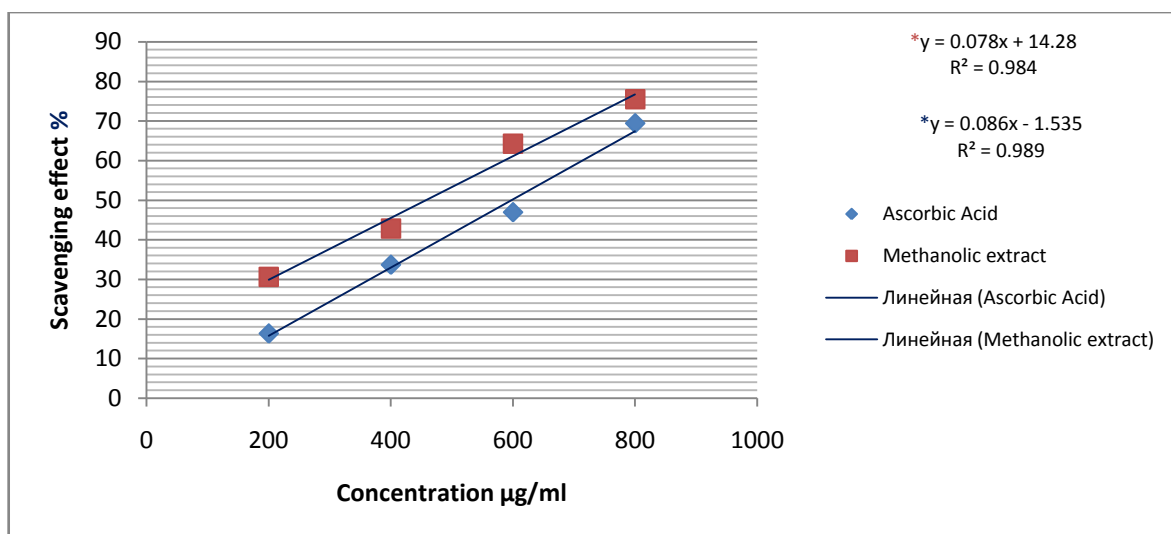


Figure 4: Antioxidant scavenging activity of Ascorbic acid (standard) and methanolic extract of *Urena lobata* leaf on H_2O_2

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Thus as Figure 4 shows the dose-response curve of H_2O_2 radical scavenging activity of the methanolic extract compared with ascorbic acid, as standard. At a concentration of 400 $\mu\text{g/ml}$, the scavenging activity of methanol extract of *Urena lobata* leaves was 43.75 %, while at the same concentration, that of the standard ascorbic acid was 32.95 %. The IC_{50} value of methanolic extract of *Urena lobata* for scavenging of H_2O_2 was found as $440.03 \pm 2.12 \mu\text{g/ml}$ and IC_{50} value of Ascorbic acid was found $600.00 \pm 1.24 \mu\text{g/ml}$.

IV. CONCLUSION

It could be concluded that the leaf extract of the *Urena lobata* (Linn) plant contains useful secondary metabolites like flavanoids, phenolic compounds in good quantity. Hence the plant extract may be considered from pharmaceutical point of view as it may contribute to the field of investigation of anti-cancer, anti-malarial, anti-stress and anti-oxidant drugs. It is important that it shows a good result in scavenging activity so that it may become useful in antifungal drug designing. More over we have to mention that this plant (leaf part) is used as medicines to treat skin lesions of live stocks, may be due to fungal, bacterial infection by an ethnic tribe, the Sonowal Kacharis of Assam. The crude extract may contain some secondary metabolites/compounds which may cause harm to livestock's as well as to human being. Hence research works has to be undertaken to ascertain the actual useful bioactive compounds which are to be separated and formulated to produce new useful drugs.

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