Quantification of Phytochemicals and Antioxidant Activity of Indian Squill

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ABSTRACT

Indigenous drugs with their base in medicinal plants and their origins in ancient Indian history are being recognized as the major alternative and complementary medicines. The Indian indigenous drugs have great importance both from the professional and economic points of view. Indian squill, that is *Urginea indica* (Kunth.) is an important indigenous plant belongs to the family Liliaceae. In present study the total phenolic and flavonoid compounds were estimated from the alcoholic extract of whole plant of *Urginea indica*. The alcoholic extract of whole plant of *Urginea indica* were tested for their antioxidant activity using DPPH, Phosphomolybdenum reducing power assay, nitric oxide assay, ABTS assay are Fe^{3+} reducing power assay. The quantitative analysis results of total phenol content (TPC) is 336.77 GAE/g and the total amount of flavonoid content (TFC) is 16.71 QE/g.

Key words : Indigenous drugs, *Urginea indica*, DPPH, phosphomolybdenum, nitric oxide assay, ABTS, Fe^{3+} reducing power assay, total phenol content, total flavonoid content.

Introduction :

The history of medicine in India can be traced to the remote past. The earliest mention of the medicinal use of plants is to be found in Rigveda, which is one of the oldest, if not the oldest, repositories of human knowledge, having been written between 4500 and 1600 B.C. (Chopra *et al*., 1994).

Indian squill is obtained from *Urginea indica* Kunth, family Liliaceae, a plant resembling the European squill but producing a smaller tunicate bulb and is found in sandy soil regions of the sea throughout India.

The bulbs are collected soon after the plants have flowered, divested of their dry, outer, membranous coats, cut into slices, usually longitudinally, and dried. (Wallis, 2005).

Calculii, dropsy and leprosy, antiparalytic, antirheumatic, deobstruent, digestive, cathartic and emetic, useful in haemorrhages from kidney and uterus, jaundice, skin diseases, alcoholic extract of *Urginea indica* is used to cure hypoglycaemic, remove warts (Asima Chatterjee *et al*., 2001).

Notwithstanding the wonderful advances found in modern medicine in latest decades, plants still make a major contribution to health care. Much interest, in medicinal plants however, emanates from their lengthy use in folk drug treatments in addition to their prophylactic properties, specifically in developing countries. Large variety of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very powerful to prevent the destructive processes caused by oxidative stress. (Zengin *et al*., 2011).

Free radicals are recognised to play a specific position in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. (Umamaheswari *et al*., 2008)

Phenolic compounds and Flavonoids are widely distributed in plants which have been reported to exert multiple, biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. (Miller, 1996). Thus, in this context the present study has been carried out to analyse the total phenolic content, total flavonoids content and antioxidant activity of *Urginea indica*.

MATERIALS AND METHODS

Collection of Plant specimen

Urginea indica was collected from Kancheepuram district of Tamil Nadu. The specimen was identified and authenticated by Prof. Dr. P. Jayaraman, Director, PARC, Tambaram, Chennai-45. The Reference number for *Urginea indica* (Roxb) Kunth is PARC/2013/3225. The specimen was sent to CSIR, National Institute of Science, Communication and Information Source (CSIR – NISCIR) for authentication. The Reference Number for *Urginea indica* (Roxb) Kunth is NISCAIR/RHMD/Consult/ 2014/2533/112.

Quantitative Phytochemical Analysis

Determination of Flavonoids - Aluminium chloride test (Yadav*et al.*, 2011**)**

To 1 ml of varying concentrations of extract, 3 ml of methanol, 0.2 ml of 1 M potassium acetate, 0.2 ml of 10% aluminium chloride and 5.6 ml of distilled water was added and left at room 25 temperature for 30 minutes. Absorbance of the mixture was read at 415 nm using UV–VIS spectrophotometer. Calibration curve was prepared using Quercetin as standard.

Determination of Phenolic compound – Folin Ciocaltaeu's method (McDonald *et al.*, 2001)

The total phenol content of the extract was measured at 765 nm by Folin-Ciocalteu reagent (McDonald *et al.*, 2001). The dilute methanolic extract (0.5 ml of 1:10 g ml-1) and or gallic acid (standard phenolic compound) was mixed with 5ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and 4 ml of aqueous sodium carbonate (1 M). The mixture was allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg l-1 solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg per gm of dry mass), which is a common reference compound.

Antioxidant Activity

DPPH Assay:

The Radical Scavenging Activity of test sample was determined by using DPPH assay according to Chang *et al*., (2008) with small modification. The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 1 ml of 0.1mM methanolic DPPH solution mixed with varying concentration of test sample and vortexed thoroughly. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. Ascorbic acid was been used as standard. The ability of the test sample to scavenge DPPH radical was calculated by the following equation:

$$\% \text{ of DPPH Radical Scavenging Activity (\% RSA)} = \frac{\text{Abs. control} - \text{Abs. sample} \times 100}{\text{Abs. control}}$$

Abs. control is the absorbance of DPPH radical + methanol; Abs. sample is the absorbance of DPPH radical + test sample.

Nitric Oxide Radical Scavenging Assay:

2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with sample. Various concentration of sample was taken and the mixture was incubated at 25 degree for 150 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. (Garrat, 1964)

The nitric oxide radical scavenging activity was calculated as:

%Nitric oxide scavenging potential = [(Control OD-Sample OD)/Control OD] X100.

Phosphomolymbdenum Assay:

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto *et al.*, (1999). Various concentration of the test sample was combined with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The reduction activity was calculated as:

%Phosphomolybdenum reducing potential =[(Sample OD-Control OD)/Sample OD] X100.

ABTS Assay

This assay was performed according to the method of Arnao *et al.*, (2001). The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours at room

temperature in the dark. Fresh ABTS solution was prepared for each assay. Test sample of varying concentration were allowed to react with 500µL of the ABTS solution for 15 minutes in dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer.

Reducing Power Assay

Reducing power assay the reducing power of the extracts was evaluated according to Oyaizu, 1986. Different amounts of aqueous extracts were perched in aqueous solvent and diverse with 2.5 ml of 0.2M phosphate buffer (pH 6.6), and 2.5 ml of 1% K3Fe (CN)6. This mixture was incubated at 50^{o}C for 20 min, 2.5 ml of 10% TCA was added to the blend and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was assorted with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicates increased reducing power. All the tests were performed in triplicates and the results were pooled and expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION:

Quantitative Phytochemical Analysis

The total phenolic and flavonoid compounds were estimated from the alcoholic extract of whole plant of *Urginea indica* (Table 1). The terms of the gallic acid equivalents (GAE) of phenolic compound was given for the following sample. The amount of phenolic compound found in *Urginea indica* was 336.77mg GAE/gm. In previous report the phenol content of methanolic extract of *Urginea indica* bulb was 0.22 µg/ml. (Banani Misra *et al.*, 2015).

The term of quercetin equivalent (QE) of flavonoid is given for the following sample. The amount of flavonoid found in *Urginea indica* was 16.71 QE/gm.

Table 1. Quantification of Phytochemicals

Phytochemicals	*Urginea indica*
Total Phenol Content mg GAE/g	336.77
Total Flavonoid content mg QE/g	16.71

DPPH Radical Scavenging activity

The alcohol extract of *Urginea indica* with varying concentrations ranging from 150, 300, 450, 600, 750 and 900µg/ml were tested against the free radical DPPH. Minimum inhibition percent 10.27±0.71% in *Urginea indica* was recorded with initial concentration 150 µg/mL. The results of this assay show that prominent antioxidant activity is recorded at 900µg/ml concentration with inhibition percent of 43.78 ± 3.06% for *Urginea indica*. (Table 2)

The aqueous extract of bulb of *Urginea indica* for DPPH assay was 62.41% in methanolic extracts of bulbs of *Urginea indica*. (Sanjay Jagtap *et al.*, 2014).

The methanolic extracts of bulb of *Urginea indica* with varying concentration ranging from 20, 40, 60, 80, 100, 120, 140, 160 µg/ml were tested against the DPPH assay but the maximum inhibition percentage at 150 µg/ml was higher in 99.14 ± 98.07. (Pandurangamurthy *et al.*, 2011).

From Sittampundi, the methanolic extracts of bulb of *Urginea indica* antioxidant activity using DPPH. The results show that the DPPH are higher in 92.24 at 600 µg/ml. (Banani Misra *et al.*, 2015).

Table 2. DPPH assay of *Urginea indica*

S.No	Concentration µg/mL	*Urginea indica* (%)
	150	10.27 ± 0.71
2	300	18.37 ± 1.28
3	450	22.70 ± 1.58
4	600	25.94 ± 1.81
5	750	32.97 ± 2.30
6	900	43.78 ± 3.06

Phosphomolybdate assay

The alcohol extract of *Urginea indica* with varying concentrations ranging from 200, 400, 600, 800, 1000, 1200µg/mL were tested against the phosphomolybdate assay. Minimum inhibition percentages 0.467 ±0.032% for *Urginea indica* was recorded with the initial concentration of 200 µg/mL. The results showed that the antioxidant activity is dose dependent manner of concentration 200 to 1200 µg / mL. The maximum inhibition percentage was 1.488 ± 0.059 µg/ml in *Urginea indica*. (Table 3)

Table 3. Phosphomolybdenum Reducing Power Assay of *Urginea indica*

S.No	Concentration µg/mL	*Urginea indica* (%)
1	200	0.467 ± 0.032
2	400	0.576 ± 0.040
3	600	0.765 ± 0.053
4	800	0.976 ± 0.048
5	1000	1.302 ± 0.031
6	1200	1.488 ± 0.059

Nitric oxide assay

The alcohol extracts of *Urginea indica* with varying concentrations ranging from 100, 200, 350, 400, 550, 600 µg/ml were tested against the Nitric oxide assay.

The results exhibited that the scavenging of Nitric oxide by the extract was increased in concentration dependent manner. Minimum inhibition percent of 09.07 ±0.63% for *Urginea indica* was recorded with the initial concentration of 100µg/ml. The alcoholic extracts of *Urginea indica* showed maximum activity of inhibition percentages 50.98 ± 3.56% respectively at 600 µg/mL concentration. (Table 4)

Table 4. Nitric oxide assay of *Urginea indica*

S.No	Concentration µg/mL	*Urginea indica* (%)
1	100	09.07 ± 0.63
2	200	18.47 ± 1.29
3	350	26.58 ± 1.86
4	400	34.06 ± 2.38
5	550	43.03 ± 3.01
6	600	50.98 ± 3.56

ABTS Assay

The alcohol extracts of *Urginea indica* with varying concentration ranging from 10, 20, 30, 40, 50 µg/ml were tested against the ABTS Assay. Minimum inhibition percentage of 43.51 ±3.04% for *Urginea indica* was recorded with the initial concentration of 10µg/ml.

The alcoholic extracts of *Urginea indica* showed maximum activity of inhibition percentages 90.07 ± 4.83 at 50 µg/mL concentration. (Table 5)

In previous literature, from Sittampundi, the methanolic extracts of bulb of *Urginea indica* antioxidant activity using ABTS assay. The results showed that ABTS assay was higher in 71.82 at 600 µg/ml. (Banani Misra *et al*., 2015).

Table 5. ABTS assay of *Urginea indica*

S.No	Concentration µg/mL	*Urginea indica* (%)
1	10	43.51 ± 3.04
2	20	57.25 ± 4.00
3	30	66.41 ± 4.64
4	40	73.28 ± 4.50
5	50	90.07 ± 4.83

Fe^{3+} Reducing Power Assay

The alcohol extracts of *Urginea indica* with varying concentration ranging from 50, 100, 150, 200, 250, 300 µg/ml were tested against the Fe^{3+} reducing power assay. Minimum inhibition percentage of 0.215 ± 0.010 for *Urginea indica* was recorded with the initial concentration of 50µg/ml.

The maximum Ferric reducing power assay is recorded with 0.794 ± 0.039 for *Urginea indica* respectively (Table 6).

In previous study free radical scavenging activity of bulb of *Urginea indica* with varying concentrations ranging from 10, 20, 40, 60 µg/ml. The results revealed that, methanolic extract was higher in 97.57 at 60 µg/ml. The results revealed the chloroform extract was higher in 95.91 at 60 µg/ml. (Soni *et al*., 2015).

Table 6. Fe^{3+} Reducing power assay of *Urginea indica*

S.No	Concentration µg/mL	*Urginea indica* (%)
1	50	0.215 ± 0.010
2	100	0.266 ± 0.013
3	150	0.422 ± 0.020
4	200	0.552 ± 0.027
5	250	0.660 ± 0.032
6	300	0.794 ± 0.039

Conclusion

In the present study, alcohol extract of the whole plant of *Urginea indica* were examined by DPPH assay, Phosphomolybdenum reducing power assay, Nitric oxide assay, ABTS assay, Fe^{3+} Reducing assay. Usually the phenolic compounds are effective hydrogen donors, making them good antioxidant. This result can also be correlated with the result quantitative analysis of Total Phenol Content (TPC), where the total phenol content is significant in *Urginea indica*. This may be the reason for its maximum antioxidant activity.

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