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RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY IN TWO SPECIES OF URGINEA STEINHILL. HYACINTHACEAE

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ABSTRACT

Urginea steinhill, belongs to the family Hyacinthaceae is a well known medicinal plant. The present study was focused on the Antioxidant activity along with assessment of total Phenol, Vitamin C content of methanolic extract of two species of *Urginea*, *U.indica* and *U.wightii* bulbs collected from two different regions of South India, Tamilnadu and Karnataka using DPPH and ABTS method. Ascorbic acid and phenols are the major contributors of antioxidant activity in *U. indica* bulb which possesses significant antioxidant activity. Among the two bulb samples collected from different states of India, Sittampundi sample from Tamil Nadu(*Urginea indica*) reported more DPPH scavenging activity about 92.24 % than Yedyur sample from Karnataka (*Urginea wightii*) which is about 35.80 %. Similarly ABTS scavenging activity also more in *U.indica* (71.82 %) than *U.wightii* (9.95 %) in higher concentration respectively. The potential antioxidant activity may be due to the presence of more Vitamin C and the plant is capable of offering protection against free radical mediated damages.

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INTRODUCTION

Plants are commonly used in healing or preventing specific ailment and are considered to play a beneficial role in health care. It can synthesize a large variety of chemical substances such as Phenols, Flavonoids, Alkaloids, Tannins, Vitamins, Carotenoids etc. that are of Physiological importance, which provide raw material for pharmaceutical, cosmetic, fragrance and flavor industries (Kretovich,2005). These active compounds protect cell against the damaging effect of reactive oxygen species (ROS), reduce the oxidative stress in cell and useful in the treatment of many diseases such as cancer, arthritis, aging process, neurodegenerative disorder, cardiovascular disease and diabetic (Sowndharajan *et al.*, 2010). Therefore the study of plant as a source of medicine has become more important in the context of present global scenario, where oxidative stress is found to be one of the major causes of health hazards. Medicinal plants are considered to play a key role in Health care (Padmaja *et al.*, 2010).

Urginea steinhill is an interesting and polytypic genus with hundreds of species found mostly in India, Africa and Mediterranean regions (Airy Shaw, 1966). Among which about nine species were seen in India (Deb and Das gupta, 1987). *U. indica* is a unique, perennial geophyte with fibrous roots of 6-10 inches in length. The bulb is rounded, conical or pear shape

with transparent outer scale and usually three fourth immersed in sand. Leaves are long, linear, lanceolate and dark green in color. Flowers are bisexual, hypogynous and drooping (Shivakameshwari *et al* 2012). The species *U. indica* is commonly called as Indian Squill used in curing of edema, cough, cold, fever, hypertension, heart disease, asthma and Jaundice (Kreig 1966). Crushed or sliced bulbs are also applied at feet sole to prevent burning sensation (Kapoor, 1990, Usmanghaniet *et al.*, 1997) and externally used for removing corns and warts (prajapati *et al.*, 2003). Many scientists have reported on the primary, secondary metabolites and Pharmacological evaluation of *U. indica* bulb. (Sanjay *et al.*, 2014, Abbas *et al.*, 2012, Pandurana murthy 2011, Shenoy *et al.*, 2006, Deepak and Sailmath 2006). Based on these informations, the present study was focused on the assessment of total phenol, vitamin C content and Antioxidant status of methanolic extract of *U.indica* and *U.wightii* bulb collected from two different regions of South India, using DPPH and ABTS method.

MATERIALS AND METHODS

Extraction of Plant material

The whole plant materials were collected from Yedyur from Karnataka state and Sittampundi, from Tamil Nadu state, India

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(Fig-1 A,B,C,D).The bulbs of *Urginea* were washed, shade dried, and made into a fine powder. Fifty grams of powdered material was extracted in 500 ml methanol by Soxhlet extractor. The methanolic extract was concentrated over vacuum desiccators and solvent was removed. This extract was dissolved in methanol and used for further analysis.

Estimation of Total Phenolic Content

The total phenolic content of methanolic extract of *Urginea* bulbs were determined by Folin-ciocalteu method (Slinkard and Singleton 1977), using Catechol as standard. One milliliter of plant extract was mixed with 1ml of Folin-ciocalteu reagent and 3 ml of sodium carbonate solution (20%). The mixture was allowed to stand for 45 min. at room temperature and absorbance was measured at 725 nm by spectrophotometer. The values were expressed in terms of Catechol equivalent.

Estimation of Vitamin C

The vitamin C content of bulb extracts was determined according to the method described by Bajaj and Kaur, (1981). One milliliter of sample extract was mixed with 0.05M oxalic acid. Later 1 ml of orthophosphoric acid, 5% sulphuric acid and 5 % Ammonium molybdate were added with 3 ml of distill water and incubate for 15 min at 60 ° c in water bath. The Absorbance was measured at 760 nm. Vitamin C content was estimated and it was calculated with the help of standard curve of Ascorbic acid.

Determination of antioxidant activity

DPPH assay

The radical scavenging activity of *Urginea* bulbs were studied using 1-1-diphenyl-2-picrylhydrazyl according to Bluos (1958). One milliliter of various dilutions of bulb extract were mixed with 3ml of 0.1mM methanolic DPPH solution and incubated at 37 ° C for 30min. The wavelength of maximum absorbance of DPPH was measured at 517 nm using spectrophotometer. A decrease in DPPH solution absorbance indicates an increase in radical scavenging activity. The percent of DPPH radical scavenging activity of the sample was calculated.

ABTS radical scavenging assay

2, 2, Azino Bis-3-ethylbenzothiazoline-6-sulphonate radical cation decoloration assay was determined as described by Re et al., 1999. ABTS was prepared by mixing 7mM of ABTS with 2.5 mM of potassium persulfate and incubated in dark for 18 hours at room temperature before use. The mixture was diluted with methanol to give absorbance of 0.7± 0.02 units at 734nm, using spectrophotometer. 1 ml of diluted ABTS solution was added to sample extract of different aliquots. Absorbance was measured after 30min. of incubation at room temperature.

The percentage of antioxidant activity of tested samples was calculated by determining the decrease in absorbance at different concentration by using the equation: E = (Ac-At)/Ac) x100 where At and Ac is the respective absorbance of tested samples and control DPPH or ABTS and IC₅₀ value of the extract i.e., concentration of the extract necessary to decrease

the initial concentration of DPPH and ABTS by 50% was calculated. The result was expressed as mg of Catechol and Ascorbic acid equivalents/gm. All assays were performed in triplicate for each sample and at each concentration.

RESULT AND DISCUSSION

Phenolic compounds are major plant secondary metabolite which has several biological functions. According to the result obtained, the total Phenol content of methanolic extract of *Urginea* bulbs were found to be 0.14mg/gm and 0.40mg/gm in Yedyur and Sittampundi samples respectively and Vitamim C content was found to be 0.22mg/gm in Yedyur sample and 0.45 mg/gm in Sittampundi sample. The result indicated that the concentration of vitamin C was found to be higher than total phenols. Vitamin C is water soluble natural antioxidant and probably one of the most widely used nutrient in food industry, also used in processed foods (Jose Luis et al. 2013). It is well known that antioxidant property of plant could depends on the presence of these active compounds (Lu, and Foo 2000) The result of total phenols and vitamin C concentration obtained from the methanolic extracts of *Urginea* bulbs collected from two location were shown in Table 1.

Table 1Quantification of Phenol and Vitamin C of methanolic extracts of *U.indica* bulb

SL No.	Sample	Phenol (mg/gm)	Vitamim C (mg/gm)
1.	<i>U. indica</i> (Yedyiyur sample)	0.14	0.40
2.	<i>U. indica</i> (Sittampundi sample)	0.22	0.45

DPPH has been widely used to evaluate the antioxidant activity of plant extracts (Soares et al., 1997). DPPH undergo reduction by an antioxidant is measured in terms of decrease in its absorbency at 517 nm. As DPPH radical reacts with a suitable reducing agent, the electron becomes paired and the solution changes from purple to yellow color (Rachael Ceballos, 2013). Radical scavenging activity of methanolic extract of Yedyiyur and Sittampundi samples along with the standards (phenol and Vitamim C) are shown in Graph 1. IC₅₀ value of methanolic extracts of *U. indica* bulb samples from Yedyiyur and Sittampundi represents 166.35µg/ml and 81.10µg/ml activity respectively. The lower value of IC₅₀ indicates a higher antioxidant activity. Linear regression analysis was found to calculate IC₅₀ values (Pratt 1992).The scavenging activity of DPPH, from Yedyiyur sample was observed ranging from 0.75% to 35.80% and 46.00% to 92.24% from Sittampundi sample (Table-2). The comparative analysis indicated that *Urginea* bulb from Sittampundi has more activity than Yedyiyur sample, may be due to the higher concentration free radical inhibitor compounds, which may act as primary antioxidants.

Table2 Percentage Antioxidant activity in *U.indica* and *U.congesta*

SL No.	Plant material	DPPH Lower conc. in %	DPPH Higher conc.in %	ABTS Lower conc. in %	ABTS Higher conc. in%
1.	<i>Urginea indica</i> (Sittampundi)	46	92.24	49.17	71.82
2	<i>Urginea congesta</i> (Yedyiyur)	0.75	30.27	4.18	8.36

The decolorization of ABTS radical reflects the capacity of an antioxidant species to donate electron of hydrogen atoms to inactivate the radical species. ABTS assay is based on the inhibition of the absorbance of the radical cation.

ABTS is converted to its radical cation which is blue in color by addition of potassium persulfate and absorbs light at 734nm. During the reaction, blue colored ABTS radical cation change to colorless when the free radicals were scavenged by antioxidant (Li XC *et al.* 2011). In the present study *U.indica* bulb samples showed decrease in absorbance with moderate scavenging activity. Phenolic compounds play a vital role in the scavenging of ABTS (Pietta *et al.*, 1998). The percentage of inhibition varies from 4.18 % to 8.36 % and 49.17% to 71.82% from Yedyur and Sittampundi samples respectively with different concentration (100µg to 600µg) shown in Table-2.

Fig1 Vegetative and reproductive phases of *Urginea* Steinhill.



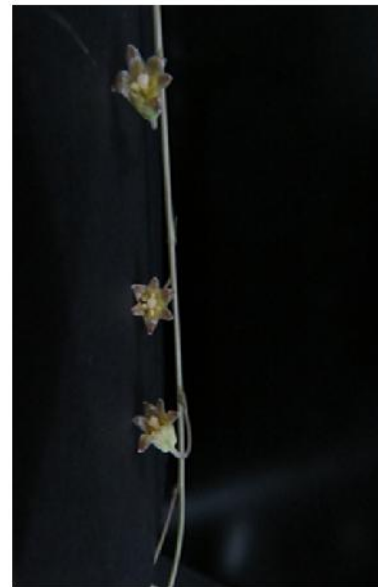
A Vegetative phase



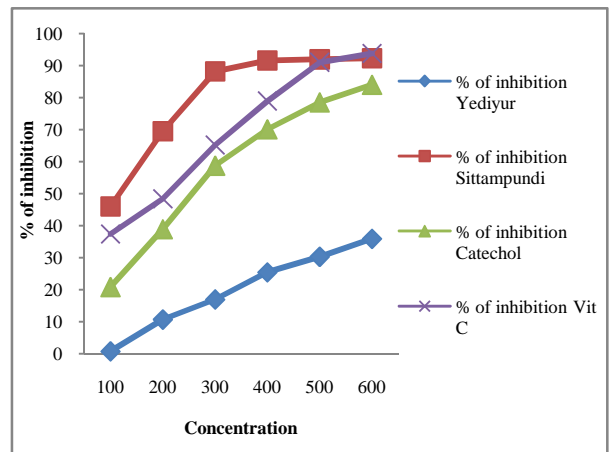
B Reproductive phase
Urginea wightii



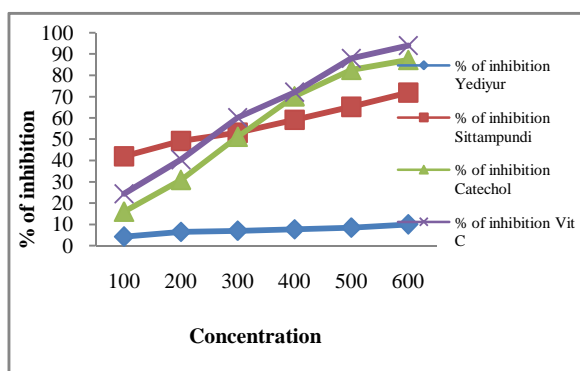
A Vegetative phase



B Reproductive phase
Urginea indica



Graph 1 DPPH radical scavenging activity



Graph 2 ABTS radical scavenging activity

Among the two extracts, the methanol extract of Sittampundi registered highest scavenging activity when compared to Yedyur sample (Graph 2). Sanjay *et al.* (2014) has reported DPPH anti scavenging activity as 38.37% from *U. indica* bulb collected from Ratnagiri regions of Maharashtra. The radical scavenging activity increases with the increase in concentration of methanolic fraction of wild onion species (Panduranga murthy 2011). According to the present study, the methanolic extract of *Urginea* bulbs collected from two different States indicated the presence of higher concentration of vitamin C than phenol. DPPH and ABTS assay gave comparable result for antioxidant activity measured in methanolic extract of *Urginea* bulb. Among the two bulb samples collected from different states of India, Sittampundi sample from Tamil Nadu reported more scavenging activity than Yedyur sample. Ascorbic acid and phenols are the major contributors of antioxidant activity in *U. indica* bulb. Hence, it is possible to conclude that methanolic extracts of *Urginea indica* bulbs have significant level of antioxidant activity due to the presence of bioactive compounds and can be a potential source of new useful drug. However, the further studies on the isolation, characterization and evaluation of active principle and the bioactive compounds necessary for the antioxidant activity has to be studied.

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