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 Yediyur 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.

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 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 Yediyur from Karnataka state and Sittampundi, from Tamil Nadu state, India...
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 1 ml 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.

**Determination of antioxidant activity**

**DPPH assay**

The radical scavenging activity of Urginea bulbs were studied using 1-1-diphenyl-2-picyrylhydrazyl according to Blois (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. 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 afte 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 IC_{50} 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 Yediyur and Sittampundi samples respectively and Vitamin C content was found to be 0.22mg/gm in Yediyur 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 1** Quantitation of Phenol and Vitamin C of methanolic extracts of U.indica bulb

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Sample</th>
<th>Phenol (mg/gm)</th>
<th>Vitamin C (mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>U. indica (Yediyur sample)</td>
<td>0.14</td>
<td>0.40</td>
</tr>
<tr>
<td>2</td>
<td>U. indica (Sittampundi sample)</td>
<td>0.22</td>
<td>0.45</td>
</tr>
</tbody>
</table>

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 Yediyur and Sittampundi samples along with the standards (phenol and Vitamin C) are shown in Graph 1. IC_{50} value of methanolic extracts of U. indica bulb samples from Yediyur and Sittampundi represents 166.35µg/ml and 81.10µg/ml activity respectively. The lower value of IC_{50} indicates a higher antioxidant activity. Linear regression analysis was found to calculate IC_{50} values (Pratt 1992).The scavenging activity of DPPH, from Yediyur 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 Yediyur sample, may be due to the higher concentration free radical inhibitor compounds, which may act as primary antioxidants.

**Table 2** Percentage Antioxidant activity in U.indica and U.congesta

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Plant material</th>
<th>DPPH Lower conc. in %</th>
<th>DPPH Higher conc. in %</th>
<th>ABTS Lower conc. in %</th>
<th>ABTS Higher conc. in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urginea indica (Sittampundi)</td>
<td>46</td>
<td>92.24</td>
<td>49.17</td>
<td>71.82</td>
</tr>
<tr>
<td>2</td>
<td>Urginea congesta (Yediyur)</td>
<td>0.75</td>
<td>30.27</td>
<td>4.18</td>
<td>8.36</td>
</tr>
</tbody>
</table>
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 734 nm. 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 Yediyur and Sittampundi samples repectively with different concentration (100µg to 600µg) shown in Table-2.

**Fig1** Vegetative and reproductive phases of Urginea Steinhill.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>% of inhibition</th>
<th>Yediyur</th>
<th>Sittampundi</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>4.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>8.36</td>
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<tr>
<td>300</td>
<td>49.17</td>
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<tr>
<td>400</td>
<td>60.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>71.82</td>
<td></td>
<td></td>
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<tr>
<td>600</td>
<td>81.23</td>
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</table>

**Graph 1** DPPH radical scavenging activity

*Urginea indica*

*Urginea wightii*
Among the two extracts, the methanol extract of Sittampundu registered highest scavenging activity when compared to Yediyur 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, Sittampundu sample from Tamil Nadu reported more scavenging activity than Yediyur 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.

**Acknowledgement**

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**Reference**

7. Jose Luis SB and Maria del SSS (2013). Antioxidant role of Ascorbic acid and its protective effect on chronic diseases. Agricultural and biological Science. DOI:10.5772/52181