



RESEARCH ARTICLE

BIOTECHNOLOGY

PHYTOCHEMICAL ANALYSIS, IN VITRO ANTI-BACTERIAL AND ANTIOXIDANT ACTIVITIES OF WILD ONION SPS.**PANDURANGA MURTHY, G*., MAMTHARANI, D.R., TEJAS, T.S AND NIRANJAN M. SUARLIKERIMATH**

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ABSTRACT

The white bulbs of 'Wild Onion, *Urginea indica*' samples were collected from the hillocks near Vaddagere village of Tumkur district, a southern part of Karnataka. The study was performed extensively for the first time, leading to the evaluation of active constituents from wild onion bulb. The extract was prepared with Methanol and subjected for preliminary phyto-chemical and physico-chemical analysis. The total ash content, acid insoluble and water soluble ash content were evaluated along with the fluorescence characteristics of the methanolic extract of Wild onion sps. The presence of primary and secondary metabolites such as *carbohydrate, proteins, alkaloids, phenolic compounds, saponins* was confirmed through preliminary phyto-chemical analysis. The extract was found to possess anti-bacterial activity in *E. coli, S. aureus* and *P. aeruginosa* isolated from infected patients. The Minimum inhibitory concentration (MIC) was also evaluated by 'Tube dilution' method and the result was found to be considerably effective against selected pathogenic bacteria. Such an effect might contribute to explaining the traditional use of wild onion sps, *Urginea indica* in the treatment of wound healing. The antioxidant activity was estimated by using DPPH free radical scavenging assay and the activity was increased with increase in concentration of methanolic fraction of wild Onion sps. The fractions of wild onion sps are free radical scavengers and are able to react with the DPPH radical, which might be attributed to their electron donating ability and suggested that antioxidant components in this Wild Onion sps was capable of reducing oxidants and scavenging free radicals. This also indicates that, tubers of wild onion, *Urginea indica*' are of therapeutic potential due to their high free-radical scavenging activity. The presence of high amount of saponins justifies the practice of treatment for disturbances in the gastrointestinal tract by Traditional healers. The role of phyto-chemical constituents of this Wild Onion, *Urginea indica* sps in traditional medicine treatment is discussed. Hence, the formulation of extract of *Urginea indica*' needs to be purified using biophysical techniques towards development of a potential drug/ lead molecule against microbial infection, inflammation and wound healing respectively.



KEYWORDS

Liliaceae; Wild onion; *Urginea indica* Phyto-chemical, Physico-chemical analysis; anti-bacterial activity, Antioxidant activity

INTRODUCTION

Though the traditional Indian system of medicine has a long history of use, they lack adequate scientific documentation, particularly in the light of modern scientific knowledge. Wild onions are members of the onion family which grow naturally in the wild, rather than being specifically cultivated. Wild onions can be found all over the world, and several species are treated as culinary delicacies, such as the ramp, also known as *Allium tricoccum*. Gardeners sometimes find these members of the onion family irritating, because they find it difficult to eradicate from flowerbeds and lawns. Wild onion, *Urginea indica* (Roxb.) Kunth (Liliaceae), known as Indian squill, refers to an uncultivated species like other genus *Allium*, especially *Allium vineale*, *Allium canadense*, *Allium validum* which belongs to Alliaceae family. '*Urginea indica*' is recognized as one of the four species in the latest revision of Liliaceae (*Flora of West Tropical Africa*). Like cultivated onions, wild onions have a distinctive sharp flavor, and scent. Many wild onions have a very strong odour, which can sometimes make them very easy to identify when they are growing in the wild and used world wide as a cardiac drug. In general, the leaves, bulb, and flowers of wild onions can all be eaten. Most people concentrate on the leaves, rather than on the bulb. Wild onions tend to develop small bulbs with shallow roots. It is an important medicinal bulb used to cure infectious wound (Benkeblia, 2004). These species were introduced in Australia and North America, where they have become invasive species. Dry skin of wild onion is used as a yellow dye, it contains Quercetrin which is anti-allergic and is also helpful in treating inflammatory bowel disease (Brodnitz *et al*, 1971). It also enhances immune system activity like cold and fevers, mouth and pharynx

inflammation, infections, bronchitis/cough, hyper tension, dyspepsia and arterio-sclerosis (Louria *et al*, 1985; Kendler, 1987 and Dorant *et. al*, 1996). It contains sulphur compounds, carbohydrates, proteins, phenolic compounds, saponins, quercetrin (Kim, 1997). The present study is designed to explore the preliminary phyto-chemical analysis, physico-chemical analysis and finding anti-bacterial properties of Wild Onion, *Urginea indica* which are responsible for pharmacological properties.

MATERIALS AND METHODS

1. Wild Onion sample: *Urginea indica*

A survey was conducted at Vaddagere village of Koratagere taluk of Tumkur district, Karnataka during 2009-2010. The Voucher specimens were collected and identified properly, consulting a flora. The species identified was *Urginea indica* (Roxb.) Kunth (Liliaceae), known as Indian squill and the same has been deposited in the Centre for Shridevi Research Foundation, SIET, Tumkur – 572 106, Karnataka.

2. Micro organisms

Three clinical bacterial strains, *E.coli*, *S.aureus* and *Pseudomonas aeruginosa*, were obtained from the Department of Medical Microbiology, Siddartha Medical College and Hospital, Tumkur, Karnataka, India. The bacterial strains were maintained with nutrient agar and sub cultured for every three days. An inoculum's of each bacterial strain was suspended in 5ml of Mueller Hinton broth (MHB) and incubated overnight at 37°C. The overnight cultures were diluted with MHB prior to bacterial testing (Samie *et. al.*, 2005.).



3. Chemicals and Glass wares

The Chemicals used for the study are of analytical grade and general glass wares were procured from standard firms.

4. Instruments & Equipments

Soxhlets Apparatus, Spectrophotometer, Refrigerator, Laminar air flow Chamber, Hot air oven, Incubator, Pestle and Mortar etc. were the instruments and equipments used for the study.

5. Extraction and Preliminary fractionation

The *Urginea* wild bulbs were shade-dried and about 4gms of the material was extracted with 20ml each of Methanol, Petroleum ether, Benzene, Chloroform, Acetone, Ethanol and water by maceration method. This was subjected to preliminary phyto-chemical and physicochemical analysis using standard protocol (Khandelwal, 2004).

6. Preliminary physico-chemical analysis

a) Total Ash

About 4gms of air dried material was taken in crucible and ignites gradually by increasing the heat to 500°C to 600°C (until it is white). This indicates the absence of carbon followed by cooling in a desiccators and weighing.

b) Acid-insoluble Ash

To the crucible containing the total ash, add 25ml of Hydrochloric acid (nearly 70g/l) and cover with a watch glass and boil gently for 5min. Rinse the watch glass with 5ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ash-less filter paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing insoluble matter to the original crucible and further dry on a hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30min. Then weigh the sample without delay and calculate the content of acid-insoluble ash in mg/g of air-dried material.

c) Water soluble

To the crucible containing the total ash, add 25ml of water and boil for 5min. The insoluble matter is collected in a sintered glass crucible (or) on an ash-less filter paper. Wash with hot

water and ignite in a crucible for 15min at a temperature not exceeding 45°C. Subtract the weight of this residue in milligram from the weight of total ash. Finally, calculate the content of water soluble ash in mg/g of air dried material.

7. Preliminary Phyto-chemical screening

Phyto-chemical screening of the *Urginea indica* extract and fractions were carried out to identify the constituents, using standard phyto-chemical methods as described by Harborne (1973) Trease and Evans (1989).

8. Determination of anti bacterial activity

The disc diffusion method was used (Nostro *et al.*, 2000) to evaluate the anti-bacterial activity. The stock solution (100mg/ml-1) of each extract and fractions were prepared using the formulated extracts (1:1 ratio). The discs (6mm diameter) were prepared using Whatman filter paper-41 and sterilized by autoclaving. The blank sterile discs were placed on the inoculated Mueller Hinton Agar (MHA) surface and impregnated with 50mg/ml of stock solution. Further, antibiotic discs of Levofloxacin (500mg disc-1) were used as standard and methanol was used as control. The plates were incubated at 37°C for 24hrs. All tests were performed in duplicate and anti-bacterial activity was expressed as the mean diameter of inhibition zones (cm) produced by the formulated *Urginea indica*, wild Onion extract.

9. Minimum inhibitory concentration (MIC)

MIC was carried out using 'Tube dilution method' as previously reported by Lennette *et al* (1974). The dilution of concentrations (10-90mg/ml) of *Urginea* extract and fractions that exhibited sensitivity against the test organisms was prepared using test tubes containing 9ml of double strength broth. The test tubes were inoculated with the suspension of the standardized inoculums and incubated at 37°C for 24hrs. The MIC's were recorded as the lowest concentration of *Urginea indica* tuber extract showing no visible growth of the broth.

$$\text{MIC} = \frac{C \times X}{Y}$$



MIC=Minimum Inhibitory Concentration.
 C=Concentration of Antibiotic in mg/ml in total volume.
 X=Length of Microbial growth in cm.
 Y=Total length of possible growth in cm.

10. DPPH Radical Scavenging Assay

DPPH radical scavenging activities of wild onion, *Urginea indica* fractions were determined by the method of Blois (1958) with some modification. Initially, 4.3mg of DPPH was dissolved in 3.3ml of Methanol; it was protected from light by covering the test tube with Aluminium foil. 150µl of DPPH solution was added to 3ml of Methanol and Absorbance was taken immediately at 516nm for control reading. The tuber extract of *Urginea indica* with different volume *i.e.*, 30 µl, 60 µl, 90 µl, 120 µl and 150 µl were taken. The volume was made uniformly to 150µl using Methanol, each of the samples was further diluted with Methanol up to 3ml and to each 150 µl of DPPH was added. It was kept it for incubation for 15min in dark condition. The DPPH radical scavenging activity was determined by measuring the absorbance at 516nm using a UV-Spectrophotometer.

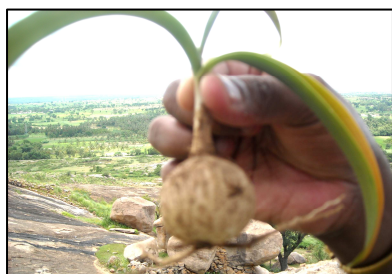


Figure-1: Showing wild Onion, *Urginea* sps collected at Vaddagere village

The DPPH radical scavenging activity (%) of the sample was calculated using the formula;
 $1 - (X/Y) \times 100$

Where, X-Absorbance of sample
 Y-Absorbance of control

RESULT AND DISCUSSION

The baseline information such as, botanical name, family, vernacular name, medicinal utility and part used of the collected wild Onion *sps*, *Urginea indica* has been represented (Fig-1& Table-1). Overall, wild Onion tuber under the study is reported to have a wide range of medicinal utilities.

The physico-chemical values and fluorescence characters of the wild onion, *Urginea indica* powder under ordinary light and UV light (UV 366nm) are determined. The Ash values of a drug give an idea of earthy matter (or) the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and water soluble ash is carried out (Table -2&3).

Botanical name-Genus	Species	Family	Vernacular name	Medicinal Utility
<i>Urginea</i>	<i>indica</i> (Roxb.) Kunth	Liliaceae	Kadu-Erulli, Indian squill	Wound healing, Pharynx inflammation, disturbances in Gastro-intestinal tract, Anti-allergic and inflammatory bowel disease etc.

Table – 2

Physico-chemical characterizations of methanol extract of Wild Onion, *U.indica*.

SL.No.	WHO parameters	Average value % w/w
01	Total ash	56.75
02	Acid insoluble ash	44.16
03	Water soluble ash	63.00

**Table – 3****Fluorescence characteristics of methanol extract in tuber of Wild Onion, *Urginea indica***

SL.No	Extract	Under ordinary light	Under UV light (366nm)
01	Methanol	white	cream

The extractive values are primarily useful for the determination of exhausted or adulterated drugs. This is in accordance with the earlier reports (Kendler, 1987 and Dorant, *et al*, 1996). Phyto-chemical analysis and antibacterial properties were determined. Preliminary phyto-chemical screening revealed the presence of Carbohydrate, Proteins, Phenolic compounds and Saponins (Table- 4&5).

Table – 4**Preliminary phyto-profile for Wild Onion *Urginea indica***

SL.No.	Solvent added	Colour	Consistency
01	Methanol	Colour less	Liquid(non-sticky)

Table – 5**Phyto-chemical analysis of methanol extracts of Wild Onion *Urginea indica***

SL. No.	Class of compound	Methanol
01.	Carbohydrates	+ve
02.	Proteins	+ve
03.	Alkaloids	- ve
04.	Phenolic compounds	+ve
05.	Saponins	+ve

The presence of primary and secondary metabolites such as *carbohydrate, proteins, alkaloids, phenolic compounds and saponins* were confirmed through preliminary phyto-chemical analysis (Table-4&5). The Methanolic extract of *Urginea indica* was found to possess anti-bacterial activity in *E. coli* (0.28), *S. aureus* (0.45) and *P. aeruginosa* (1.4). The similar result was also obtained by Kim, 1997(Table-5&6).

The Minimum inhibitory concentration (MIC) was found to be considerably effective (Graph-1) against, pathogenic bacteria (Zohri *et al*, 1995). This is because of wild onion extract can exert their effects on health system via multiple different functions, including antioxidant, anti-inflammatory, and antibacterial properties. The presence of 'organosulphur' compounds in this

species may act as scavenge oxidizing agents, inhibit the oxidation of fatty acids thereby preventing the formation of pro-inflammatory messengers and inhibit bacterial growth, via interaction with sulphur-containing enzymes (Benkeblia, N, 2004).

This indicates that, the positive effect might contribute to explaining the traditional use of wild onion, *Urginea indica* in the treatment of wound healing and presence of high amount of saponins and justifies the practice of treatment for disturbances of the gastrointestinal tract by Traditional healers.

DPPH is a kind of stable free radical and accepts an electron (or) hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging



activity (Blois, 1958). In the DPPH radical scavenging assay, antioxidants react with DPPH and exists naturally in deep violet colour to turn into a yellow coloured diphenyl--picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant. The Wild Onion sps, *Urginea indica* could be potential and rich resources of natural antioxidants. This could be developed into drug for prevention and treatment of diseases caused by oxidative stress. (Ardestani and R.Yazdanparast, 2007 and Ren-You Gan *et al*, 2010).

The DPPH radical scavenging activity of methanolic fraction from wild onion, *Urginea indica* increased with increase in fraction concentration (Graph-2). The percent scavenging ability of Methanolic fraction at 150µg/ml was higher (99.14±98.07). The results obtained in this investigation reveal that, the fractions of *Urginea indica* tuber extract are free radical scavenger and able to react with the DPPH radical, which might be attributed to their electron donating ability.

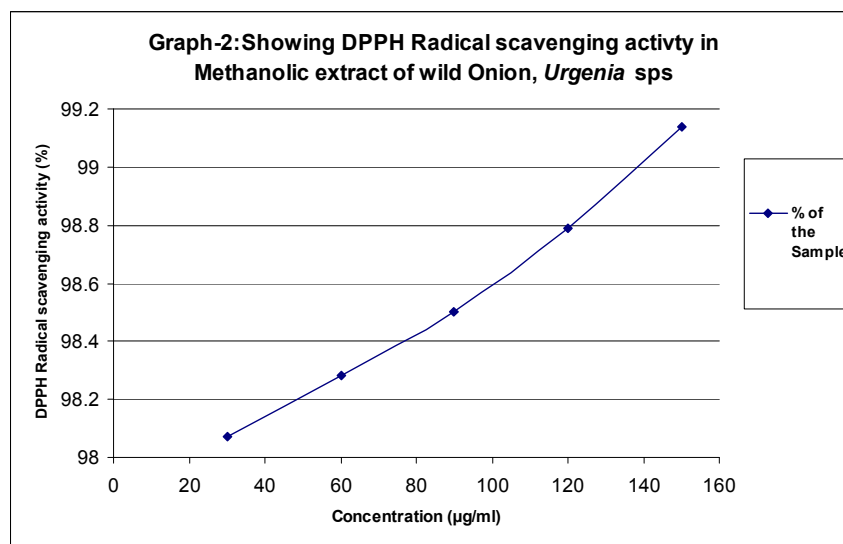
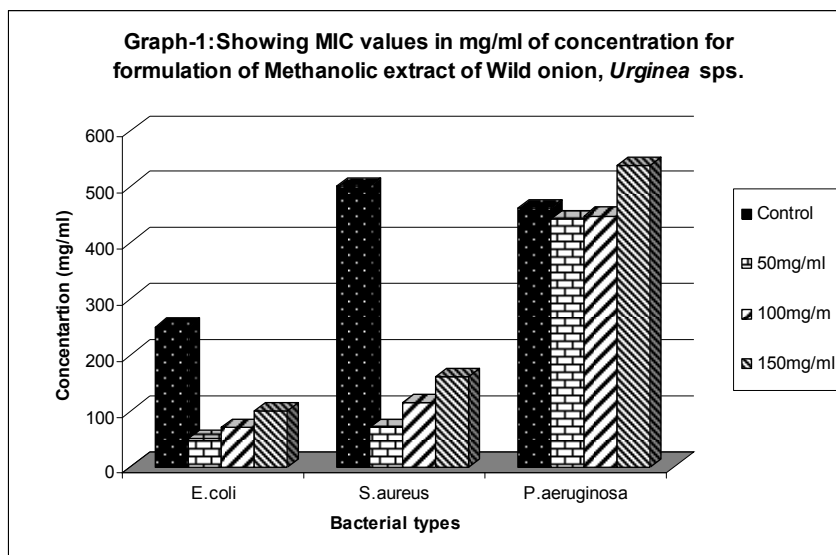
Table – 6
Anti-bacterial activity of methanol extracts of Wild Onion, *Urginea indica*

Bacterial Type	Different concentrations of Methanolic extract of Wild onion, <i>Urginea indica</i>		
	50mg/ml	100mg/ml	150mg/ml
<i>E.coli</i>	+	+	+
<i>Staphylococcus aureus</i>	+	+	+
<i>Pseudomonas aeruginosa</i>	++	++	++

+ -Average inhibition, ++ - Medium inhibition, +++ - Good inhibition

Table – 7
Zone of Inhibition bacterial by methanol extracts of Wild Onion, *Urginea indica*

SL. No	Bacterial type	Zone of inhibition in cms for formulation of Methanolic extract of wild Onion (<i>Urginea Indica</i>)				
		Std. Lavofloxacin	Control Methanol	50mg/ml	100mg/ml	150mg/ml
01.	<i>E. coli</i>	1.4	0.7	0.14	0.20	0.28
02.	<i>S. aureus</i>	-	1.4	0.20	0.32	0.45
03.	<i>P. aerogenosa</i>	1.3	1.2	1.15	1.27	1.4



CONCLUSION

In the present study, evaluation of phyto-chemical, physico-chemical, anti-bacterial and antioxidant properties of wild Onion, *Urginea indica* reveals, better efficacy against pathogenic bacteria and this might be due to presence of bio-active constituents as compared to the other existing wild Onion varieties such as *Allium vineale*, *Allium Canadense* and *Allium validum*. Hence, the wild Onion *Urginea indica* is quite interesting with respect to its distribution and morphological characteristic features. The

bioactive constituents present in the extract indicate potential source for bioactive molecules. As the wild Onion tuber extract is quite safe and its toxicity is not a problem of concern unlike those of BHT, they could be exploited as antioxidant additives or as nutritional supplements. Further, the extract of wild onion, *Urginea indica* needs to be purified using bio-physical technique to justify the practice against wound healing and inflammation by rural medicine men. In addition, the active constituents



present in this wild onion variety can be recommended to the industry towards development of drug/ lead molecule.

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