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Qualitative and Quantitative Analysis of Phytochemicals in two different species of Urginea

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Abstract

Urginea a medicinal herb belongs to family Hyacinthaceae. In the present study, the phytochemical analysis of petroleum ether and methanol extracts of two species of U*rginea* viz *Urginea indica* and *Urginea wightii* bulbs collected from Karnataka and Tamil Nadu states were carried out to find the various chemical constituents. The secondary metabolites produced by this medicinal plant are reported to have therapeutic values. The bioactive constituents like alkaloid, carbohydrate, glycoside, flavonoid, phenol, saponin, tannin and terpenoid have been analysed qualitatively in both the species. The quantitative estimation has revealed the highest concentration of terpenoids in methanolic extract of *Urginea indica* bulb collected from Seethampoondi region, where as flavonoid and alkaloids were found to be high in bulbs collected from Udupi. The petroleum ether extract of *Urginea indica* from Udupi region showed more flavonoid and alkaloid, while phenol stands next in its concentration. In *Urginea wightii*, terpenoid was present in higher concentration in methanol extract followed by alkaloids but in petroleum ether extract the alkaloid was present in higher concentration, followed by terpenoids.

Key words: *Urginea indica*, *Urginea wightii*, phytochemicals, methanol, petroleum ether.

Introduction

Plants are essential component of the universe which provides the most important sources of medicines which dates back to prehistoric period. Approximately 9000 plant species are known to have medicinal properties in India. (Farnsworth, et al., 1991). According to WHO, over 80% of the world's population rely upon such traditional plant based system of medicine to provide them with primary health care (Attisso, et al., 1983). Screening of the active compounds from plants such as phenol, flavonoid, alkaloid, terpenoids, glycosides, tannin etc. has lead to the discovery of new drugs, which are capable of exerting protective effect and treatment role against various diseases (Ness, et al., 1997). Today the traditional knowledge on constituents of medicinal plants plays a significant role in the establishment of pharmaceutical industries (Priyanga, et al.,2004). The present study was focused on qualitative and quantitative analysis of secondary metabolites like phenol, flavonoid, alkaloid and terpenoids, extracted from *Urginea indica* and *Urgenia wightii* bulbs using petroleum ether and methanol as solvents.

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The genus *Urginea* is a member of the family Hyacinthaceace and commonly called as wild onions. It comprises of about hundred species and found distributed in certain floristic regions of the world. In India it is distributed in Southern and Peninsular part including the coastal belt as well as temperate regions of Himalayas. The useful parts are bulbs which are excellent source of medicine with pharmaceutical and therauptical applications mainly as anticancer, expectorant, cardiac stimulant, used in hypertension, dyspepsia, arterio- sclerosis (Louria et al.,1985; Kendler,1987 and Dorant et al.,1996), in treatment of asthma (Marx et al.,2006), rheumatism, edema, dropsy, allergies (Brodnitz et al.,1971), gout and to treat various other ailments (Benkeblia,2004; Deepak and Salimath,2006; Shivakameshwari et al., 2006).

Material and Methods

PLANT MATERIAL

The fresh and mature bulb of two different species of genus *Urginea* such as *Urginea indica* and *Urginea wightii* were collected from different locations like the two accessions of *Urginea indica* were collected from Sithampoondi (Tamil Nadu) and Udupi (Karnataka), and the bulbs of *Urginea wightii* were collected from yediyur (Karnataka). The collected bulbs were washed,

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shade dried and cut it small pieces and finally ground to fine powder.

PREPARATION OF EXTRACT

The powdered material was separately extracted in soxhlet apparatus with petroleum ether and methanol. The extracts were subsequently concentrated under reduced pressure and the resulting residue was then filtered and stored at 4°C. All extracts were dissolved in respective solvents prior to analysis and subjected to qualitative and quantitative determination of the chemical constituents like phenols, flavonoids, alkaloids and terpenoids.

QUALITATIVE ANALYSIS OF PHYTOCONSTITUENTS

The extracts of *Urginea indica* and *Urginea wightii* bulbs were subjected to the preliminary phytochemical analysis following standard methods as described by Harborne (1973) and Trease and Evans (1989) to screen the presence of the various active principles in a qualitative way.

Quantification of total alkaloid content

The total alkaloid content in methanolic and petroleum ether extracts of *Urginea indica*, *Urginea wightii* bulbs were determined according to method employed by Singh et al 2004, using colchicine as standard. The total alkaloid content of the samples was measured using 1, 10-phenanthroline. The reaction mixture contained 1ml plant extract, 1ml of 0.025M Ferric chloride in 0.5M hydrochloric acid and 1ml of 0.05M of 1, 10-phenantroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70ºc.The absorbance of red coloured complex was measured at 510nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of colchicine.

Quantification of flavonoid content

Aluminium chloride colorimetric method was used to determine the flavonoid content as described by Chang et al., 2002. One milliliter of plant extracts was mixed with 0.5ml of aluminum chloride (1.2%) and 0.5ml of 120 mM potassium acetate. The mixture was allowed to stand for 30 minutes at room temperature. The absorbance was measured at 415nm and flavonoid content was expressed in terms of Rutin equivalent.

Quantification of total phenol content

The total phenol was estimated using catechol as standard. To l ml of plant extracts, 1ml of Folin-Ciocalteau reagent and 3ml of 20% sodium chloride solution was added. The mixture was incubated for 40 minutes at room temperature and absorbance was measured at 760nm (Singleton et al., 1999).

Quantification of terpenoid content

The terpenoid was determined calorimetrically by using linalool as standard. To 1ml of plant extracts, 1.5ml of chloroform was added and incubated for 5 minutes. Later, 0.5ml of sulphuric acid was added and cooled for 15 minutes. The mixture was incubated at room temperature for 1-2 hr in dark condition and colour changed to reddish brown to which 95% methanol and 5% distilled water was added. The absorbance was measured at 538nm.The standard graph was prepared by using standard linalool.

STATISTICAL ANALYSIS

The experiments were conducted in triplicates and data were expressed as mean ±SD and analyzed using mega stat model.

Results and Conclusion

The therapeutic effect of plants is because of the presence of active compounds. In the present study preliminary screening of methanol extract of *Urginea indica* bulb collected from Seethampoondi region revealed the presence of carbohydrates and tannins in moderate level. Alkaloid, glycoside, flavonoid, phenol, saponin, terpenoid were found to be in high concentration. In petroleum ether extract phenol and tannin was present in low levels, Glycoside, saponin and terpenoid were present in moderate level where as alkaloid, carbohydrate and flavonoid were present in high concentration.

According to the result, the methanol extract of *Urginea indica* bulb collected from Udupi region showed the higher concentration of alkaloid, carbohydrate, flavonoids, phenol and saponin, and moderate concentration of glycoside, tannin and terpenoids. The petroleum ether extract revealed the low level of alkaloid, glycoside, flavonoids and tannin, where as carbohydrate and saponin are present in moderate level, phenol and terpenoids were present in higher level.

The result also showed the presence of alkaloid, carbohydrate, flavonoids, phenol, tannin and terpenoids in higher level in methanol extract of *Urginea wightii* bulbs where as glycoside and saponin were found in moderate level. The petroleum ether extract showed glycoside and phenol in lower concentration. The alkaloid, carbohydrate flavonoid, tannin and saponin were present moderate concentration whereas terpeniod was present in higher concentration. The results of preliminary phytochemical analysis obtained are reported in the Table.1.

Table 2 summarized the results of quantitative assessment of phytochemical composition of petroleum ether and methanolic extracts of *Urginea indica* (Seethampoondi and Udupi) and *Urginea wightii* with

a focus on alkaloid, flavonoid, phenol and terpenoids. The biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and antibacterial activities (Iqbal et al., 2011). According to the present result, the total alkaloid content was found to be maximum in *Urginea wightii* and *Urginea indica* (Udupi) both in methanol (3.101± 0.056 µg/ml and 2.897± 0.319 µg/ml) and petroleum ether (2.779 ± 0.079 µg/ml and 2.279± 0.684 µg/ml) extracts. But *Urginea indica* (Seethampoondi) showed minimum concentration of alkaloid in both methanol (0.413± 0.033 µg/ml) and petroleum ether (0.460± 0.051 µg/ml) extracts.

Among the two species of *Urginea*, *Urginea indica* collected from Udupi found to be a good source of flavonoids both in methanol and petroleum ether as 2.947 ± 0.389 µg/ml and 2.576 ± 0.582 µg/ml extracts respectively, where as in *Urginea wightii* and *Urginea indica* (Seethampoondi) the flavonoids content was recorded as 0.400±0.144 µg/ml and 0.360± 0.085 µg/ml in methanol extract and 0.298±0.114 µg/ml and 0.045± 0.013 µg/ml in petroleum ether extracts. Flavonoids are the potent water soluble antioxidant and free radical scavenger which prevent oxidative cell damage and have strong anti cancerous activity (Stauth 2007).

Phenolic compounds are the major plant secondary metabolite which has several biological functions and greatly used in skin and wound treatment (Okwu et al., 2001). The total phenolic content of the studied *Urginea* species were calculated with a standard curve using Catechol. The results indicated that the phenol content is higher in methanol extract of *Urginea indica* bulb collected from Udupi and Seethampoondi (0.779±0.179 µg/ml and 0.643±0.022 µg/ml) compared to petroleum ether extract (0.133 ± 0.093 µg/ml and 0.028 ± 0.017 µg/ml). But the petroleum ether extract of *Urginea wightii* showed higher concentration of phenols as 0.630±0.094 µg/ml where as methanol extract showed lower concentration of phenols as 0.073±0.015 µg/ml. The higher concentration of terpenoid was obtained in both methanol and petroleum ether extracts of *Urginea wightii* and recorded as 5.419±1.054 µg/ml and 1.615±0.495 µg/ml respectively. The moderate concentration was found in methanol extract of *Urginea indica* from Seethampoondi (1.500±0.435 µg/ml) sample and low concentration of terpenoids was found in *Urginea indica* bulb extract collected from Udupi. Plant terpenoids play an important role in traditional herbal remedies such as dermatological diseases and also used in food, pharmaceutical and chemical industry (Dorotheca, 2015). Graph 1 and 2 determine the proportion of alkaloid, flavonoid, phenol and terpenoids in different species and their accessions in methanol and petroleum ether extract.

The present study reveals that the species of genus *Urginea* are the good source of secondary metabolites i.e., alkaloids, flavonoids, phenols and terpenoids. These plants play a vital role in preventing various diseases. The screening of medicinal plants for chemical constituents is used as the valuable sources of raw material for traditional medicine with immense therapeutical applications. The previous phytochemical screening of methanol extract from *Urginea indica* bulb from Tumkur district, Karnataka and Western Ghats of Maharashtra revealed the presence of carbohydrate, proteins, alkaloids, glycosides, saponins, tannins, quinones and phenolic compounds (Panduranga et al 2011; Sanjay et al., 2014 and Rathabai et al 2012). These results are in accordance with the present study of the phytochemical constituents. Thus, *Urginea indica* and *Urginea wightii* are promising medicinal plants and have commercial and interest in both research and pharmaceuticals companies for manufacturing of the new drugs for treatment of various diseases. Further scientific studies of these plants are necessary for proper validation of these plants.

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Table 1: Qualitative estimation of phytochemicals

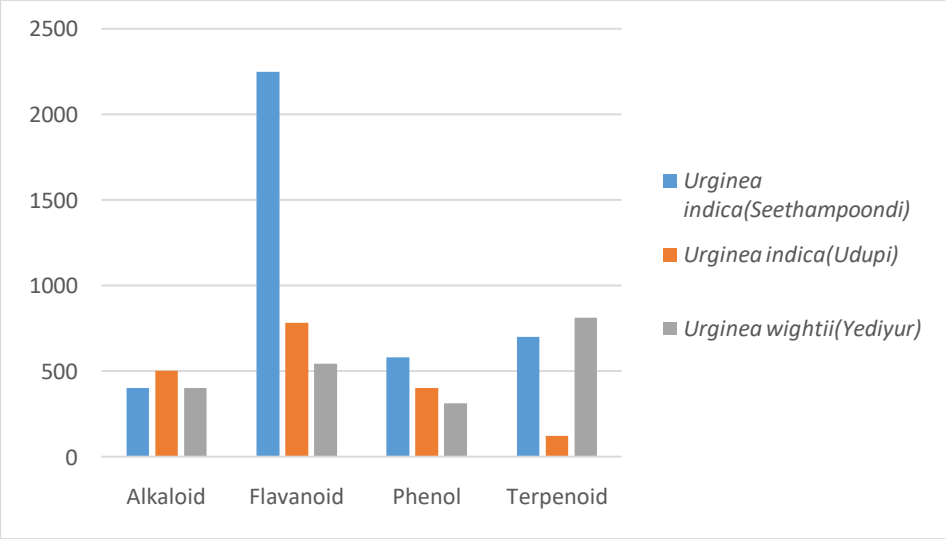
		ALKALOIDS	CARBOHYDRATES	GLYCOSIDES	FLAVANOIDS	PHENOLS	TANNIN	SAPONIN	TERPENOID
1	URGINEA INDICA METHANOL (SEETHAMPOONDI)	+++	++	+++	+++	+++	++	+++	+++

2	URGINEA INDICA PETROIEUM ETHER (SEETHAMPOONDI)	+++	+++	++	+++	+	+	++	++
3	URGINEA INDICA METHANOL (UDUPI)	+++	+++	++	+++	+++	++	+++	++
4	URGINEA INDICA PETROIEUM ETHER (UDUPI)	+	++	+	+	+++	+	++	+++
5	URGINEA WIGHTII METHANOL (YEDIYUR)	+++	+++	++	+++	+++	+++	++	+++
6	URGINEA WIGHTII PETROIEUM ETHER (YEDIYUR)	++	++	+	++	+	++	++	+++

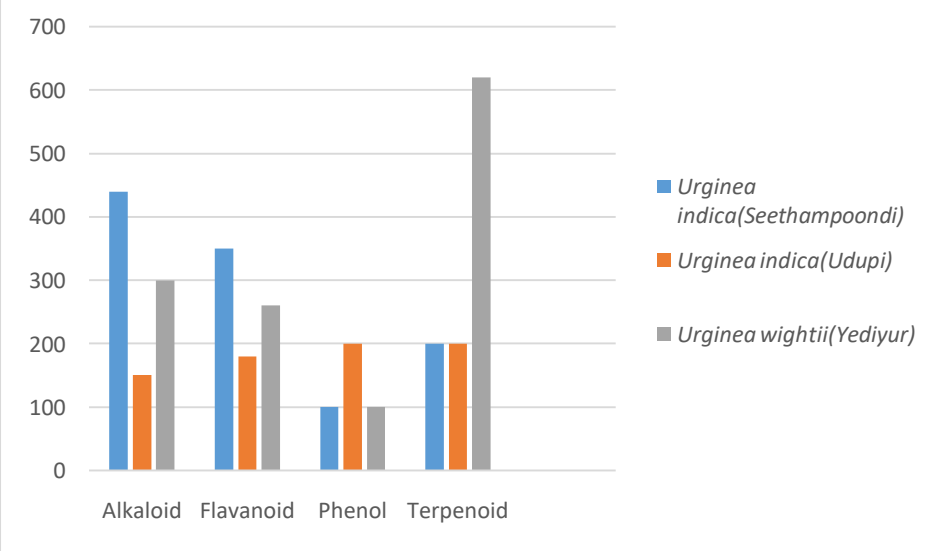
+ - Low colouration**, ++ -** Moderate colouration**, +++ -** High colouration

Table 2: Quantitative estimation of *Urginea indica* and *Urginea wightii*

	PHYTOCHEMICALS	*Urginea indica* (Seethampoondi)		*Urginea indica* (Udupi)		*Urginea Wightii* (Yediyur)	
		METHANOL µg/ml	PETROLEUM ETHER µg/ml	METHANOL µg/ml	PETROLEUM ETHER µg/ml	METHANOL µg/ml	PETROLEUM ETHER µg/ml
1	ALKALOID	0.413±0.033	0.460±0.051	2.897±0.319	2.279±0.684	3.101±0.056	2.779±0.079
2	FLAVANOD	0.360±0.085	0.045±0.013	2.947±0.389	2.576±0.582	0.400±0.144	0.298±0.114
3	PHENOL	0.643±0.022	0.028±0.017	0.779±0.179	0.133±0.093	0.073±0.015	0.630±0.094
4	TERPENOID	1.500±0.435	0.445±0.150	0.397±0.078	0.677±0.099	5.419±1.054	1.615±0.495



Graph 1: Comparison of phytochemicals in methanol extract of *Urginea indica* and *Urginea wightii* bulbs



Graph 2: Comparision of phytochemicals in petroleum ether extracts of *Urginea indica* and *Urginea wightii* bulbs

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