

WORLD JOURNAL OF PHARMACEUTICAL RESEARCH

SJIF Impact Factor 6.805

Volume 5, Issue 8, 1130-1142.

Research Article

ISSN 2277-7105

PHYTOCHEMICAL STUDIES IN *URGINEA INDICA*, (*KUNTH*) HYACINTHACEAE

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Article Received on 10 June 2016,

Revised on 30 June 2016, Accepted on 20 July 2016

DOI: 10.20959/wjpr20168-6794

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ABSTRACT

Secondary metabolites of plants are prooved to be highly useful in human therapy, veternary, agriculture and scientific research. The usefulness of plant material is due to the presence of alkaloids, tannins, flavanoids and phenolic compounds. In the present study there is an effort to screen these bioactive compounds in wild plant *Urginea indica* where there is really any investigations have taken place. This is prooved to be one of the great medicinal tribal plant that definitely help the mankind to resolve various health issues.

KEYWORDS: *Urginea indica*, secondary metabolites, Alkaloids, Phenols and flavonoids.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999, 2001).

Already the message "green medicine" is safe and more dependable than the costly synthetic drugs many of which have adverse side effects has passed on to many parts of the World. Characterization of extracts of medicinal plants is necessary, due to its numerous benefits to science and society. The information obtained, makes pharmacological studies possible. It also enabled structure-related activity studies to be carried out, leading to the possible synthesis of more potent drug with reduced toxicity. The use of phytochemicals as natural

antimicrobial agents, commonly called "biocides" is gaining popularity. The most essential of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. *Urginea indica* (Roxb.) Kunth is an attractive, succulent medicinal plant of the family Hyacinthaciea The most essential of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food. Traditionally herbal extracts were known to be effective against microorganisms as antimicrobial chemicals.

Further the phytochemical study and antioxidant activity of the bulbs extracts of *Urginea indica* were evaluated and Phytochemical screening indicated that bulbs are rich in a variety of primary and secondary metabolites such as carbohydrates, alkaloids, vitamin C, vitamin E, flavonoids, phenols, glycosides and saponins.

Phytochemical studies in Urginea indica

In the present study there is an effort to screen these bioactive compounds in wild plant *Urginea indica* where there is really any investigations have taken place. This is prooved to be one of the great medicinal tribal plant that definitely help the mankind to resolve various health issues.

The plant has been reported to contain glycosides including scillaren-A and scillaren-B (Prajapati et al., 2003). *Urginea indica* has been studied for its medicinal effect as an antifungal (Shenoy et al., 2006), antiangiogenic and pro-apoptotic (Deepak and Salimath, 2006).

MATERIALS AND METHODS

Fresh *Urginea indica* bulbs were collected during June-July of 2011 in Nagamangala, Magadi, Pillali. The plants were authenticated by Department of Botany, Bangalore University. The voucher specimens were kept in the Department of Botany, Jnana Bharati campus, Bangalore. Karnataka, India.

Urginea indica Kunth. extract preparation

The work include to test the effectiveness of organic solvents, their secondary metabolites and their concentration in three different accessions of *Urginea indica*.

All three accession bulbs are about 10 to 15 cm long and 12 to 15 cm in diameter. They were

collected in August when flowers and aerial leaves withers away. The fibrous roots are removed and dry outer scales are stripped off. The bulbs are longitudinally cut into thin slices. It is later dried in shade or by artificial heat.

Dried scales are translucent and brittle in dried condition. The drug is highly hygroscopic and readily absorbs moisture and becomes tough and flexible. The powder of *Urginea* may form cake and develop moulds in damp weather. The drug should be stored in tightly closed decicator with dehydrating agent in a moisture free atmosphere.

All the laboratory works are done in Department of Botany, Bangalore University and Azyme Biosciences, Jayanagar, Bangalore. On drying bulb pieces were fine powdered in mixer grinder and stored for further analysis. Then this powdered samples (20g/200ml) were soaked in hot water, ethanol, methanol, chloroform, Ethyl acetate, Petroleum ether, hexane and acetone extracts for Overnight at room temperature. For few tests directly the dried powdered bulbs were used.

Some part of dried bulbs extracts are done with soxhlet apparatus as below.

The crude samples were subjected to phytochemical screening for the presence of amino acids, proteins, saponins, triterpenoids, flavonoids, carbohydrates, alkaloids, phytosterols, glycosidal sugars, protein, tannins and phenols.

Chemical tests were carried out on the in all extract and powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973). Ref: Azyme biosciences.

Types of screening tests conducted were as given below

	Sec metabolites	Name of the test
1.	Alkaloids	Wagners test, Mayers test, Dragondroff's test
2.	Flavanoids	Lead acetate test, Alkaline Reagent Test, Ferric chloride test
3.	Saponins	Foam test
4.	Carbohydrates	Molish test
5.	Phenols	Ferric chloride test
6.	Steroids	Salkowski test
7.	Tannins	Ferric chloride test, Gelatin test
8.	Glycosides	Bornatangers test, Legal test, Liebermann's test

9.	Cardioglycosides	Bornatangers test, Keller-Killiani test, Legal test,
10.	Antraquanons	Bornatangers test

1. Test for Alkaloid

Mayers test: 3 ml aqueous extract mixed with a drop of Mayers reaget added to the sides of the test tube.

A white creamy precipitate indicate the presence of Alkaloids.

Wagners test: To few ml of the extract is mixed with few drops of Wagners reagent added at the side of the test tube.

Reddish brown precipitate indicate the presence of Alkaloids.

Positive results with **ethanol** and **water** extracts.

Dragendroff's Test

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

2. Test for flavonoids

These methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harborne, 1973).

Alkaline Reagent Test: 5 ml of dilute ammonia solution were added to a portion of the ethanol extract of each plant extract followed by addition of concentrated H₂SO₄. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Ferric chloride test – Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids

3. Test for saponin: Foam test

About 2 gms of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously

for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

4. Test for Carbohydrate: Molish test

To 2ml of extract add 10 ml of water. To this mixture add ethanolic extract of Alpha-Naphtol (20%), slowely add concentrated from the sides of test tube. It was confirmed only in **water** extract.

Red – Violet ring appears at the junction of extract indicating the presence of Carbohydrates.

5. Test for Phenols : Ferric Chloride Test: To 2 ml of test solution, a few drops of ferric chloride solution were added. Bluish green or red colour indicates the presence of phenol. The result was found in **petrolium ether and acetone.**

6. Test for steriods: Salkowski Reaction

Two ml of acetic anhydride was added to 0.5 gms **ethanolic and water** extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

- **7. Test for Tannins: Gelatin Test:** To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.
- **8. Test for glycosides**: Extracts were hydrolysed with dil. HCl and then subjected to test for glycosides.

Modified Borntrager's Test

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Borntrager's test

For 2 ml of extract add 2 ml of hydrolysate, add 3ml of chloroform and shake it thoroughly. Cloroform layer seperates and to this 10% ammonia is added.

Legal test

For 50 mg of the extract is dissolved in Pyridine sodium nitraprysside solution and make it alkaline adding.

The extract of drug is dissolved in pyridine, sodium nitroprusside solution is added to it and made alkaline by adding 10% Sodium hydroxide. Pink colur indicate the presence of Glycosided.

Liebermann's test: 2 ml of the organic extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in to it. The solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside). Positive result with **Petrolium ether, chloroform, ethanol and water.**

9. Test for cardiac glycosides (Keller-Killiani test) To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

10. Test for anthraquinone glycoside

Borntrager Test

Powdered drug is mixed with ether, which is filtered and to the filtrate add caustic soda and aqueous ammonia. Red, pink or violet colour produced indicates the presence of anthraquinone glycoside.

RESULTS

Concentration of metabolites (Table -1)

Alkaloids answered only in Ehanol and water in all accessions

Flavonoids dissolved in all type of solvents

Saponins were detected only with water

Carbohydrates were screened in acetone and water

Phenols were screened in all types of solvents indicating U.indica is rich in Phenols.

Steroids were found only in Petrolium ether and Ethanol

Tannins were seen in acetone and water extracts

Glycosides were screened in petrolium ether, chloroform, ethanol and water

Cardioglycosided were also sceened in petrolium ether, chloroform, ethanol and water

Anthraquinones were screened in all solvent extracts.

The above results show that *Urginea indica* is rich in Flavanoids, Phenols, Glycosides, Cardioglycosides and Anthraquinones.

Effectiveness of Solvents: (Table – 2)

Phytochemical screening of *Urginea indica* Kunth showed the presence of Alkaloids, Carbohydrates, Phenolic compounds, flavonoids, Saponins, steroids, Tannins, Glycosides, cardioglycosides and Anthraquones in almost all sovent extracts. The effectiveness of each solvent is as below.

- 1) Alkaloid are present in extract of Distilled water and ethanol and absent in extracts of Petroleum ether, chloroform, acetone, extracts.
- 2) Favonoids are present in all extract, Petroleum ether, chloroform, acetone, ethanol and Distilled water.
- 3) Saponins were found only with distilled water and in other extracts it is absent
- 4). Carboyhydrates were actively seen in Acetone and Aquous extract
- 5) Phenols were found in Petrolium ether, acetone, ethanol and water extracts
- 6) Steroids were answered in Petrolium ether and ethanol extract
- 7) Tannins were present in acetone and water extracts
- 8) Glycosides were found in Petrolium ether, chloroform, ethanol and water extracts
- 9) Cardioglycosides were found in Petrolium ether, chloroform, ethanol and water. Absent in Acetone
- 10) Anthraquonenes were found in all extracts.

Among all the solvents Ethanol and water were found to be effective solvents.

Natural therapeutic products play a vital role in the development of drugs for treatments of several diseases.

In the present study the *Urginea indica* bulb samples were successfully extracted using solvents based on their polarity index. The bulbs were used for phytochemical screening of secondary metabolites. It revealed that bulb extracts contain huge amount of mucilage and percentage yield was found to be highest in Ethanol In ethanol highest percentage yield were noticed indicating that ethanol acts as a universal solvent and extracts most of the compounds in *U.indica*.

The above study clearly indicates the presence of many primary metabolites like carbohydrates and secondary metabolites like Alkaloids, Phenolic compounds, flavonoids and Saponins in bulbs of *Urginea indica* accessions and it also shows the affinity or dissolving capacity in various solvents according to their polarity index.

Table – 1 – Screning Reactions and results in three populations of *Urginea indica*

Metabolites	Name of the test	Petrolium ether			Chloroform			Acetone		Ethanol			Water			
		Pop1	Pop2	Pop3	Pop1	Pop2	Pop3	Pop1	Pop2	Pop3	Pop1	Pop2	Pop3	Pop1	Pop2	Pop3
Alkaloids	Hagners test	-	-	-		-	+	-	-	+	+	+	+	+	+	+
Flavanoids	Lead acetate test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	Foam test	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Carbohydrates	Molish test	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+
Phenols	Ferric chloride test	+	+	+	-	-	+	-	+	+	+	+	-	+	+	-
Steroids	Salkowski test	+	+	+	-	-	+	-	-	-	+	+	+	-	-	-
Tannins	Ferric chloride test	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+
Glycosides	Bornatangers test	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+
Cardioglycosides	Bornatangers test	+	+	+	+	+	-	+	-	+	+	+	_	+	+	-
Antraquanons	Bornatangers test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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Table – 2: Effectiveness of solvents

Sl.no.	Metabolite	Petrolium ether	Chloroform	Acetone	Ethanol	Water
1.	Alkaloids	+	-	-	+	+
2.	Flavanoids	+	+	+	+	+
3.	Saponins	-	-	-	-	+
4.	Carbohydrates	-	-	+	-	+
5.	Phenols	+	-	+	+	+
6.	Steroids	+	-	-	+	-
7.	Tannins	-	-	+	-	+
8.	Glycosides	+	+	1	+	+
9.	Cardioglycosides	+	+	_	+	+
10.	Antraquanons	+	+	+	+	+

DISCUSSIONS

Secondary metabolites may be highly useful in human therapy, veternary, agriculture and scientific research. The usefulness of plant material is due to the presence of alcoloids, tannins, flavanoids and phenolic compounds.

Alkaloids help in controled development in living system and involved in protective functions. Flavonoids inhibit the initiation, development and growth of tumors. Flavonoids and phenolic compounds help as antioxidant activity, free radicle scavenging activity, anti inflamatory and anticancer activity (Thamaraiselvi et, al. 2012).

In this present work screening various secondary metabolites in *Urginea indica* prooves its medical use in treating humans.

Similar studies were made and documented by Dhananjay Pandey et.al in 2014 and Arokiyaraj et.al in 2009 and Kumar et.al in 2010.

Taye Tenikotan (2013) have shown the relevance of phytochemicals to plant protection. The chemical constituents of plants have been of immense importance to humans. The use of plant extracts in control of pests and diseases as well as in preservations and controlling microbial growth. There is a search for antimicrobial agents from plants (Ogunleye-2008).

The increasing Multidrug resistance of microbes to antibiotic therapy and the efficacies of these plant extracts given by Levy and Marshal (2004). The value of plant in drug discovery for medicinal purposes were discussed by Fabricant and Farus Worth (2001) and more over

plant extracts have little side effects compared to synthetic chemical drugs. These metabolites do not alter the crop quality (Droby 2006). Medicinal plants are the bio- resources for the discovery of novel bio-active compounds. These have triggered immense interest in the search of new antimicrobial drugs of plant origin (Dhananjay Pandey 2014).

Anthraquinone glycosides: The sugar residue facilitates translocation and absorption of aglycone at the site of action. These compounds are stimulant, cathartic or purgation and they exert their action by increasing the tone of smooth muscle in the wall of large intestine. Fresh mucilaginous juice of the leaves is used for the treatment of skin burns, skin abrasion and other skin irritations. Used in compound Benzoin tinctures.

Urginea indica has answered for Anthraquinone glycosides in almost all solvent extracts and in all accessions. Thus it can be used in medicine as purgative and also skin burns.

The constituents such as Alkaloids, Flavonoids, Saponines were able to show antimicrobial activity and alkaloids causing toxicity against cells of foreign organisms (Nobori et.al 1994). Saponines showed antimicrobial activity and has ability to cause leakage of certain enzymes and proteins from the cell (Zablotowicz et.al 2004).

Flavonoids to microbial infection and acts against bacterial cell walls (Marjorie 1999).

The presence of alkaloids Bufotinin which is toxic in small doses causes hallucinations. This shows that Urginoideae members can be used as Rodenticides (Taye Tenikotan 2013). The alkaloids of *Urginea* can be used in microbial inhibition according to Asl et.al (2008).

Saponins are used on injection, for which it has a pharmacological reputation. It results in the lysis of the blood cells haemolysis, like all detergents, and is therefore highly toxic. Saponins base are the basic of many arrow poisons. The best interesting part to be noted is that, saponins have always been toxic to cold-blooded creatures like snake and/or fish. Saponins show effect is on the respiratory system, by reflex stimulation of the stomach wall brought about by a stimulating expectoration. One example of an emetic-expectorant with a saponin constituent *Urginea indica*. And this present experiment prooves the presence of saponins mainly with water extract in all the three populations of *U.Indica*.

In nutshell *U.indica* bioactive compounds that are useful in plant protection as well as have potential use in medicines. Therefore the present study offers a scientific validation for the

usage of *U.indica* in curing several ailments by traditional healers. Traditional knowledge forms the strong basic system of medicine and exists in the forms of Ayurveda, Unani, Siddha and Swa-riga (Tibetan) systems of medicine. The flora and fauna are used for medicinal purposes and they have important cultural roles and as well as vital roles in forest ecology, such as pollination, seed predation and dispersal, seed germination, herbivory and predation on potential pest species.

It has been established in this study that *Urginea indica* exhibit antifungal, antibacterial and anticancer properties. Hence *U.indica* plant shows significance in plant protection, ecofriendly bio-active compounds that can be synthesised on a commercial scale from this plant to reduce dependance on synthetic drugs.

The secondary metabolites of *Urginea spp* are responsible for caryying out research work and investigate the anti- bacterial, anti- inflamatory and anti- cancerous studies in the species and accessions of *Urginea*.

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