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Detection of phytochemicals in the methanol extract of *Urginea indica* (R.) BULB

Bhavana R. Tambe *, Sunayana R. Vikhe and Parmeshwar M. Pathade

Department of pharmacognosy, Pravara rural college of pharmacy, pravaranagar, MH, India 413736.

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Abstract

Urginea indica R, commonly known as Jangli Pyaz, belongs to the Liliaceae family and is renowned for its medicinal properties, particularly in traditional Indian medicine. This research focuses on the extraction and detection of phytochemicals from the bulbs of *Urginea indica* R, highlighting their potential therapeutic applications. Utilizing Soxhlet extraction with methanol as a solvent, we successfully extracted a range of bioactive compounds, including flavonoids, tannins, and alkaloids. The extraction process involved grinding the dried bulbs into a coarse powder and conducting prolonged methanol extraction to enhance yield while preserving sensitive phytochemicals. Qualitative phytochemical screening confirmed the presence of significant secondary metabolites, such as alkaloids, flavonoids, phenolic compounds, tannins, proteins, carbohydrates, glycosides, saponins, terpenoids, steroids .These findings not only enhance our understanding of the health benefits associated with *Urginea indica* R. but also provide a foundational chemical database that can guide future research into its pharmacological applications. The study emphasizes the importance of exploring natural sources for developing novel therapeutic agents that can address various health challenges.

Keywords: *Urginea indica*; Methanol; Phytochemical; Flavonoids; Terpenoids

1. Introduction

The bulb of *Urginea indica* R, commonly known as Jangli Pyaz, is a member of the Liliaceae family and is recognized for its medicinal properties. This plant is native to India and has been traditionally used in folk medicine for various ailments. The bulbs contain a rich array of bioactive compounds, including bufadienolides, flavonoids, tannins, and alkaloids, which are believed to contribute to their therapeutic effects.[1] Given the increasing interest in phytochemicals derived from natural sources, the extraction and characterization of these compounds from *Urginea indica* R. are essential for understanding their potential health benefits and applications in modern medicine[2].

Soxhlet extraction is a widely employed technique for the efficient extraction of phytochemicals from plant materials. This method allows for continuous extraction by cycling the solvent through the plant material, thereby maximizing yield and minimizing solvent usage.[3] In the case of *Urginea indica* R, methanol is often used as a solvent due to its ability to dissolve a broad range of polar and non-polar compounds [4]. The process typically involves grinding the dried bulbs into a coarse powder, followed by extraction with methanol in a Soxhlet apparatus over several hours. This method not only enhances the extraction efficiency but also preserves the integrity of sensitive phytochemicals [5].

Following extraction, a comprehensive phytochemical investigation is conducted to identify in the bioactive compounds present in the methanol extract. Qualitative tests reveal the presence of various secondary metabolites such as saponins, flavonoids, tannins, and alkaloids. For instance, studies have shown that the methanol extract exhibits significant antioxidant activity, which is crucial for combating oxidative stress-related diseases [6].

* Corresponding author: Bhavana R. Tambe.

The findings from these investigations contribute to a growing body of knowledge regarding *Urginea indica* R. phytochemical study. By isolating specific bioactive compounds and understanding their mechanisms of action, researchers can pave the way for developing new therapeutic agents derived from this plant. Moreover, such studies underscore the importance of traditional medicinal plants in contemporary pharmacology and highlight their potential role in drug discovery and development.

2. Materials and methods

- **Chemicals:** Methanol
- **Equipment:** Soxhelt apparatus
- **Authentication:** The *Urginea indica* R.plant were identified and authenticated using herbarium collection at Padmashri vikhe patil college of arts, science and commerce, pravaranagar A/P –Loni kd.Tal- Rahata, Dist-Ahilyanagar.
- **Collection:** The plant *Urginea indica* R. were collected in the month of august-september(in rainy season) from Dudheshwar gadh, Nimgaon-Jali, Tal.Sangamner, Dist. Ahilyanagar.
- **Drying of sample:** Collected bulbs samples were washed thoroughly with running tap water and then with distilled water to remove all the dust and soil particles.After that bulbs were cut into small pieces and dry under shade.
- **Soxhlet extraction:** Extraction of the bulbs sample was made by using the Soxhlet apparatus with methanol solvent. In this process, firstly, 50 gm of the coarse powdered sample were taken into a thimble using filter paper and then extracted with 250 ml of methanol in the Soxhlet apparatus. This apparatus was run for 8-10 hours, not exceeding the boiling temperature for the respective solvent. After that, extract was filtered with Whatman filter paper in a flask and stored at 4°C in the refrigerator for further use [7,8,9].

2.1. Preliminary analysis of phytoconstituents

Preliminary analysis of extract was carried out to identify the presence of various phytoconstituents by employing standard protocols. The results were summarized in Table 1 after conducting the following chemical tests.

2.1.1. Tests for alkaloids

- Dragendorff 's test: By adding 1 ml of Dragendorff"s reagent to 2 ml of extract, an orange red precipitate was formed, indicating the presence of alkaloids.
- Mayer's test: Few drops of Mayer's reagent were added to 1 ml of extract. A yellowish or white precipitate was formed, indicating the presence of alkaloids.
- Hager's test: 2 ml of extract were treated with few drops of Hager's reagent. A yellow precipitate was formed, indicating the presence of alkaloids.

2.1.2. Tests for flavonoids

- Alkaline reagent test: 2-3 drops of sodium hydroxide were added to 2 ml of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.
- Shinod's test: 10 drops of dilute HCL and a piece of magnesium were added to 1 ml of extract, the resulting deep pink colour indicating the presence of flavonoids.

2.1.3. Test for phenolic compounds and tannins

- Ferric chloride test: 2 ml of 5% neutral ferric chloride solution were added to 1 ml of extract, the dark blue colour indicating the presence of phenolic compounds and tannins.
- Lead tetra acetic acid test: 1 ml of lead tetra acetate solution was treated with 0.5 ml of extract, precipitate formation indicating the presence of phenolic compounds and tannins.

2.1.4. Tests for proteins

- Biuret test: 2 drops of 3% copper sulphate and few drops of 10% sodium hydroxide were added to 1 ml of extract, violet or red colour formation indicating that proteins are present.
- Ninhydrin test: 2 drops of 0.2% freshly prepared ninhydrin solution added to 1 ml of extract formation of purple colour shows the presence of proteins.

2.1.5. Test for carbohydrates

- Molish test: Few drops of alcoholic a-naphthol solution were added to 2 ml of extract. Later, few drops of concentrated H_2SO_4 were added along the walls of test tube. At the junction of two liquids, a violet colour ring appeared, indicating that carbohydrates were present.
- Benedict's test: To 5 ml of Benedict's reagent, 8-10 drops extract were added, then heated for five minutes; the resulting dark red precipitate indicated the presence of carbohydrates.
- Fehling's test: To 2 ml of extract, an equal volume of Fehling's (A & B) solution was added and heated for five minutes, the resulting red/dark red precipitate indicating the presence of carbohydrates

2.1.6. Tests for glycosides

- Keller Killiani test: A solution of 0.5 ml, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2 ml of extract. Later, 1 ml of concentrated H_2SO_4, was added along the walls of the test tube. The appearance of deep blue colour at the junction of two liquids indicated the presence of cardiac glycosides.

2.1.7. Tests for saponins

- A drop of Na_2CO_3 solution was added to 5 ml of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponins.

2.1.8. Test for terpenoids

- Horizon test: 2 ml of trichloroacetic acid was added to 1 ml of extract. The presence of terpenoids was confirmed by the formation of a red precipitate.

2.1.9. Test for steroids

- Salkowski test: The test extract was shaken with chloroform and concentrated H2SO4 was added along the walls of a test tube; a red colour appeared, indicating the presence of steroids.

2.1.10. Test for starch

- Iodine test: 2 ml of iodine solution with potassium iodine were added to 2 ml of test extract, and the appearance of a blue colour indicated that presence of starch [10,11].

3. Results

Preliminary phytoconstituents analysis of methanol extract of *Urginea indica* R. bulbs results in the following table 1.

The preliminary phytoconstituents analysis of the methanol extract of *Urginea indica* R.bulbs reveals a rich profile of bioactive compounds.Indeed phytochemical investigations of this plant have resulted in occurrences of alkaloids, flavonoids, phenolic compounds, tannins, proteins, carbohydrates, glycosides, saponins, terpenoids, steroids. Table 1 illustrates the results of phytochemical screening of methanol the extracts of *Urginea indica* R bulbs. The qualitative analysis of alkaloids (Dragendorff 's test, Mayer's test, Hager's test) were carried out in methanol extract. There is formation of orange, white and yellow precipitate confirmed the presence of alkaloids respectively. The qualitative analysis of flavonoids (Alkaline reagent test and Shinod's test) was carried out in methanol extract. The extract solution becomes colourless and deep pink colour confirmed the presence of flavonoids respectively. The qualitative analysis of phenolic compounds and tannins (Ferric chloride test and Lead tetra acetic acid test) were carried out in methanol extract. The extract solution becomes dark blue colour and formation of precipitate confirmed presence of phenolic compounds and tannins respectively. The qualitative analysis of proteins (Biuret test and Ninhydrin test) was carried out in methanol extract. The extract solution becomes red and purple colour confirms the presence of proteins respectively. The qualitative analysis of carbohydrates (Molish test, Benedict's test and Fehling's test) was carried out in methanol extract. Appearance of violet colour ring, formation of dark red and red precipitate confirmed presence of carbohydrates respectively. The qualitative analysis of glycosides (Keller Killiani test) was carried out in methanol extract. There is appearance of deep blue colour at the junction of two liquids confirmed the presence of cardiac glycosides. The qualitative analysis of saponins were carried out in methanol extract. Formation of foam of the extract solution confirmed the presence of saponins. The qualitative analysis of terpenoids (Horizon test) were carried out in methanol extract. Formation of red precipitate confirmed the presence of terpenoids. The qualitative analysis of steroids (Salkowski test) was carried out in methanol extract. The extract solutions become red colour confirmed the presence

of steroids. The qualitative analysis of starch (Iodine test) was carried out in methanol extract. There is no appearance of blue colour indicated the absence of starch.

Table 1 Preliminary phytoconstituents analysis of methanol extract of *Urginea indica* R. bulbs

S.N	**Phytoconstituents**	**Test**	**Methanol extract**
1	Alkaloids	Dragendorff ’s test	+
		Mayer’s test	+
		Hager’s test	+
2	Flavonoids	Alkaline reagent test	+
		Shinod’s test	+
3	Phenolic compounds and tannins	Ferric chloride test	+
		Lead tetra acetic acid test	+
4	Proteins	Biuret test	+
		Ninhydrin test	+
5	Carbohydrates	Molish test	+
		Benedict’s test	+
		Fehling’s test	+
6	Glycosides	Keller Killiani test	+
7	Saponins	-	+
8	Terpenoids	Horizon test	+
9	Steroids	Salkowski test	+
10	Starch	Iodine test	-

(+) = Presence of phytoconstituents, (-) = Absence of phytoconstituents.

4. Discussion

Phytochemical investigation through Soxhlet extraction is a crucial method for isolating bioactive compounds from plant materials, allowing for comprehensive analysis of their potential therapeutic properties. This technique involves the continuous extraction of compounds using a solvent, typically methanol, which is favored for its ability to solubilize a wide range of phytochemicals due to its polarity.

The preliminary phytoconstituents analysis of the methanol extract of *Urginea indica* R. bulb has revealed a diverse array of bioactive compounds, underscoring the plant's potential medicinal applications. This discussion synthesizes the findings from qualitative analyses, emphasizing the significance of each identified phytochemical group.

4.1. Overview of Phytochemical Constituents

The analysis confirmed the presence of various phytochemicals, including alkaloids, flavonoids, phenolic compounds, tannins, proteins, carbohydrates, glycosides, saponins, terpenoids, steroids, and starch. Each of these compounds contributes to the therapeutic properties attributed to *Urginea indica* [12].

Alkaloids were identified using Dragendorff’s test, Mayer’s test, and Hager’s test. The formation of orange, white, and yellow precipitates confirmed their presence. Alkaloids are known for their pharmacological effects, including analgesic and anti-inflammatory properties. Their presence in *Urginea indica* R.suggests potential applications in pain management and inflammation-related conditions. The qualitative analysis for flavonoids involved alkaline reagent and Shinod’s tests, resulting in color changes indicative of flavonoid presence. Flavonoids are recognized for their antioxidant properties and ability to scavenge free radicals, which may contribute to the plant's protective effects against oxidative stress and related diseases [13].

The presence of phenolic compounds was confirmed through Ferric chloride tests, yielding a dark blue color. Tannins were identified via Lead tetraacetate tests with precipitate formation. Both groups are known for their antioxidant activities and potential roles in preventing chronic diseases such as cancer and cardiovascular disorders. [14,15]Proteins were detected using Biuret and Ninhydrin tests, resulting in red and purple color formations respectively. The presence of proteins indicates nutritional value and may also suggest enzymatic activities beneficial for biological processes. Carbohydrates were identified through Molish's test, Benedict's test, and Fehling's test, with violet rings and colored precipitates confirming their presence. Carbohydrates serve as energy sources and may also have roles in metabolic processes.

The Keller-Killiani test confirmed the presence of cardiac glycosides through the appearance of a deep blue color at the liquid junction. Cardiac glycosides are important for their role in treating heart conditions by enhancing cardiac contractility. Saponins were identified by foam formation in the extract solution. These compounds have been associated with various health benefits, including cholesterol-lowering effects and immune system enhancement [16].

Terpenoids were confirmed through Horizon tests with red precipitate formation, while steroids were detected via Salkowski tests showing red coloration. Both classes of compounds are known for their diverse biological activities including anti-inflammatory and antimicrobial effects [16,17]. Interestingly, starch was not detected in the extract as indicated by the iodine test's failure to produce a blue color. This absence may suggest a focus on other primary metabolites over polysaccharides in this particular extraction method.

5. Conclusion

The comprehensive phytochemical screening of *Urginea indica* R.bulb highlights its rich profile of bioactive compounds that may contribute to its traditional medicinal uses. The presence of alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, terpenoids, and steroids suggests significant therapeutic potential that warrants further investigation into their specific mechanisms of action and clinical applications. Future studies should focus on isolating these compounds to evaluate their efficacy in various pharmacological contexts while considering safety profiles for potential therapeutic use.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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