



Phytochemical evaluation of two plants of family liliaceae collected from Jhalawar district of Rajasthan

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Abstract

Plants seem to be the perfect candidates in this regard owing to presence of several bioactive secondary metabolites, which have been bestowed with the ability to fortify plant defense and also counteract menacious disease-causing agents. In this context, the current thesis summarizes the pharmacological attributes of 21 different plants based on a survey across different geographical regions in Jhalawar district of Rajasthan during the year 2020-21 & 2021-2022 based on information collected from local healers and experts of tribal medicine. Furthermore, the researchers continued with two different plants, namely, *Chlorophytum tuberosum* Baker and *Urginea indica* Roxb and determined the amount of primary and secondary metabolites in them.

Keywords: *Chlorophytum tuberosum* baker; *Urginea indica* Roxb, flavonoids, alkaloids, phenols

Introduction

Plants produce a diverse array of secondary or specialized metabolites, which play critical roles in plant adaptation under various environmental conditions. Furthermore, these phytochemicals are extensively employed in the fields of human nutrition and medicine. It is estimated that the plant kingdom produces around one million metabolites, while a significant number of these remain undiscovered. These specialized metabolites are derived from specific primary metabolite precursor(s), such as sugars, amino acids, nucleotides, organic acids, and fatty acids. These precursors are crucial for maintaining cellular homeostasis and sustaining the life of entire organisms. In addition to their essential role, key metabolic pathways are subject to rigorous regulation and are intricately connected to complex metabolic networks. As a result, genes that encode fundamental metabolic enzymes are under purifying selection and are typically conserved across all plants. In contrast, specialized metabolism shows a significant degree of diversity. However, certain fundamental metabolic pathways underwent changes throughout the course of plant evolution, resulting in significant effects on the overall physiology, metabolism, and adaptability of plants.

Primary metabolites are directly involved in growth, development as well as effective maintenance of plant metabolism. The primary metabolites in plants mainly comprise of carbohydrates, lipids, amino acids and proteins. In addition to primary metabolites, Secondary metabolites are compounds synthesized by plants to enhance their competitiveness within their specific ecological niche. These little chemicals have a broad spectrum of impacts on the plant itself as well as on other living species. They stimulate the process of flowering, fruit formation, and leaf shedding, while also promoting continuous growth in perennial plants or indicating the seasonal shedding of leaves in deciduous plants. They function as antimicrobials and serve as attractants or, conversely, as repellents. The plant kingdom has yielded a staggering number of over 50,000 secondary metabolites. Secondary plant metabolites are essential for the activities of medicinal herbs and many modern drugs.

Secondary metabolites have demonstrated diverse biological effects, serving as the scientific foundation for the utilization of herbs in traditional medicine among numerous ancient societies. These substances have been characterized as having antibiotic, antifungal, and antiviral properties, which enables them to safeguard plants from harmful microorganisms. In addition, they serve as significant UV-absorbing compounds, so protecting leaves from severe light-induced damage (Hussein and El-Anssary, 2019)^[6].

In this study, we focused the later analysis on two different plants, namely, *Chlorophytum tuberosum* Baker and *Urginea indica* Roxb and determined the concentration of primary and secondary metabolites in these plants.

Materials and Methods

Sample Collection and Processing:

The two chosen plants were obtained from Jhalawar, Rajasthan, India during separate occasions. All specimens exhibited no signs of microbiological contamination or physical deterioration. The specimens underwent a washing process, following which the various components of the plants were segregated. The specimens were subjected to shade drying at ambient temperature for a duration of 10 days. The dried components were pulverized into a fine powder. The powdered samples were maintained in individual airtight containers and were consistently utilized for further phytochemical investigation.

Sample Extraction

The plant pieces were subjected to a cold percolation process utilizing methanol as a solvent to extract their dry powder. A quantity of 10 grams of the desiccated powder was placed into a conical flask containing 100 milliliters of methanol. The flask was then subjected to agitation on an orbital shaker at a speed of 120 revolutions per minute for a duration of 24 hours. Following a 24-hour period, the extracts underwent filtration using Whatman filter paper no.1 in order to eliminate any remnants of peel particles. Subsequently, the extracts were subjected to evaporation under vacuum conditions.

Qualitative determination of primary metabolites

Carbohydrates were determined using Fehling's Test. Proteins were determined by Biuret Test. Tannins were confirmed by FeCl₃ Test.

Quantitative determination of determination of primary metabolites

The total amount of soluble carbohydrates was calculated using the methodology outlined by Hedge and Hofreiter (1962)^[2]. The amount of protein was evaluated using an approach developed by Lowry and colleagues (1957). Total free amino acids were measured using the ninhydrin method, which was carried out in accordance with the protocol outlined by Moore and Stein (1948)^[9]. 1gm of each dried peel sample was homogenised with 10 ml distilled water by using a mortar and pestle to determine lipid content (Jayaraman, 1981).

Qualitative determination of secondary metabolites

In each extract, alkaloids were determined using standard protocols proposed by Brain and Turner (1975) and Evans (1996). Presence of flavonoids was determined by using protocol of Sofowara (1993) and Harborne (1973). Liebermann-Burchard test was done for phytosterols. The presence of saponins was determined by foam test.

Quantitative Determination of secondary metabolites

The spectrophotometric approach was utilised in order to ascertain the level of phenolics that were present in plant extracts. The Folin-Ciocalteu test was employed as the method for determining the total phenol content of the sample. The overall phenol concentration was reported as milligrammes of gallic acid equivalents per gramme of

extract (Ghasemzadeh *et al.*, 2010; Rasool *et al.*, 2011). The aluminium chloride colorimetric test was utilised in order to determine the total flavonoid concentration. The overall flavonoid concentration was reported in milligrammes of quercetin equivalents per gramme of extract (Kaviarasan *et al.*, 2007; Hanane *et al.*, 2010). The overall alkaloid concentration was given in terms of mg of AE per gramme of extract (Fazel *et al.*, 2008; Rao *et al.*, 2012). The Folin-Ciocalteu method was utilised in order to quantify the tannins. The amount of tannin was measured in milligrammes of gallic acid equivalents per gramme of extract (Marinova *et al.*, 2005; Singh *et al.*, 2012; AfifyAel-M *et al.*, 2012). The method developed by Obadoni and Ochuko was utilised to ascertain the level of saponin (2001). The technique developed by Ferguson in 1956 was used to determine the terpenoids content (Kim *et al.*, 2003).

Results

Determination of primary metabolites in plants

Thereafter, out of a total of 21 different plants, the researchers chose two plants, namely, *Chlorophytum tuberosum* Baker and *Urginea indica* Roxb for further study based on their abundance, ease of cultivation and pharmacological usage, The results show that in *Chlorophytum tuberosum* Baker, total soluble sugar, starch, protein, lipids and Tannins were recorded as 11±0.19 mg/g.DW, 8±0.73 mg/g.DW, 25.3±1.32 mg/g.FW, 66.15±3.25 mg/g.DW and 13.67±1.36 mg/g.DW respectively. While in *Urginea indica* Roxb, those were recorded as 8±1.16 mg/g.DW, 7.6±0.44 mg/g.DW, 12.52±2.1 mg/g.FW, 30.5±2.58 mg/g.DW, and 16.72±0.8 mg/g.DW respectively (Table 1).

Table 1: Quantitative determination of primary metabolites present in the selected plants.

S.No.		Total soluble sugar (mg/g.DW)	Starch (mg/g.DW)	Protein (mg/g.FW)	Lipid (mg/g.DW)	Tannins (mg/g.DW)
1	<i>Chlorophytum</i>	11±0.19	8±0.73	25.3±1.32	66.15±3.25	13.67±1.36
2	<i>Urginea</i>	8±1.16	7.6±0.44	12.52±2.11	30.5±2.58	16.72±0.85

Determination of secondary metabolites in plants

Thereafter, both the plant species, namely, *Chlorophytum tuberosum* Baker and *Urginea indica* Roxb were qualitatively and quantitatively assayed for presence of secondary metabolites, namely, total flavonoids, alkaloids, phytosterols, saponins and tannins. In *Chlorophytum tuberosum* Baker, total flavonoids, alkaloids, phytosterols,

saponins and tannins were recorded as 2.55±0.34 mg/gdw, 7.86±0.03 mg/gdw, 14.88±0.27 mg/gdw, 2.55±0.08 mg/gdw and 13.07±0.56 mg/gdw respectively while in *Urginea indica* Roxb, those were recorded as 2.79±0.08 mg/gdw, 9.78±0.004 mg/gdw, 23.61±0.18 mg/gdw, 5.17±0.17 mg/gdw and 36.48±1.73 mg/gdw respectively. Flavonoids were found in free and bound forms (Table 2).

Table 2: Quantitative determination of secondary metabolites in the selected plants.

	Flavonoids			Alkaloids	Phytosterols	Saponins	Total phenols (mg/g.DW)
	Total flavonoids (mg/gDW)	Bound flavonoids (mg/gDW)	Free flavonoids (mg/gDW)	(mg/g DW)	(mg/g DW)	(mg/g. DW)	
<i>Chlorophytum</i>	2.55±0.34	1.03±0.06	1.52±0.03	7.86±0.03	14.88±0.27	2.55±0.08	13.07±0.56
<i>Urginea</i>	2.79±0.08	1.02±0.05	1.77±0.007	9.78±0.004	23.61±0.18	5.17±0.17	36.48±1.73

Discussion

Plants occupy the central stage in this intricately woven ecosystem threaded by different living as well non-living components that are capable of fulfilling perfectly orchestrated functions contributing to their own survival as well as perpetuation of the ecosystem as a whole. Plants have rightly been termed as the "primary producers" owing to their unparalleled ability to photosynthesize and provide much needed nutrients and oxygen to the inhabitants of this

planet; thereby forming the "lifeline" and contributing to sustenance of all forms of life on earth (Vezzani *et al.*, 2018). In addition to fulfilling the role of primary producers and oxygen furnishers; plants act as a vast reservoir of bioactive secondary metabolites and phytochemicals, which play a pivotal role in alleviating chronic inflammatory and oxidative diseased ailments inflicting torment on the mankind (Hussein *et al.*, 2019; Pant *et al.*, 2021)^[6]. It is because of these bioactive phytochemicals, that plants and

their extracts have formed a part of the traditional medicinal regime and long been used as biomedicine for treatment of several ailments by the traditional healers and local people. The ancient knowledge in Vedas and Upanishads mention the usage and applicability of several plants and their extracts in alleviation of numerous ailments. Furthermore, the recent research over the last few decades has further endorsed the medicinal and pharmacological attributes of plants, providing scientific proofs and validation to the age-old knowledge.

In the study, the researchers further narrowed down to studying the medicinal and pharmacological attributes of two different plant species, namely,:

- *Chlorophytum tuberosum* Baker
- *Urginea indica* Roxb

The results show content of primary metabolites, namely, total soluble sugar, starch, protein, lipids and Tannins; in the two plant species. The results show higher content of total soluble sugar, starch, protein and lipids in *Chlorophytum tuberosum* Baker while *Urginea indica* Roxb contained higher amount of tannins. All these primary metabolites fulfill several crucial functions in plants such as formation of plant cellular architecture, composition of DNA and RNA, aiding in fulfilling energy requirements of plants, plant defense and tolerance to abiotic stress by accumulation of amino acids such as proline (Zaynab *et al*, 2019; Sanchez *et al*, 2008)^[12].

Thereafter, the researchers determined the amount of different secondary metabolites in the two plant species. The results show higher content of total flavanoids, bound flavanoids, free flavanoids, alkaloids, phytosterols, saponins and total phenols in *Urginea indica* Roxb in comparison to *Chlorophytum tuberosum* Baker. Presence of secondary metabolites fulfill different functions, both for plants as well as humans, which include:

- Flavanoids protect plants from the effect of harmful radiations, aid in pollen development, help in detoxification of plant oxidative stress, aid in defense from attack of phytopathogens, attraction of symbiotic organisms for mutualistic relationships, nitrogen fixation, development of plant root and several other functions (Khalid *et al*, 2019).
- Plant phenolic compounds aid in several developmental functions such as seed germination, cell division, regulation of developmental processes involved in plant growth; physiological and biochemical functions such as oxidative phosphorylation in plants, biosynthesis and metabolism of hormones and photosynthetic pigments, stomatal conductance, osmoregulation, controlling membrane properties and form structural component of plant cell wall as well as protection of plants from fungal, bacterial and other phytopathogens (Rehman *et al*, 2012).
- Plant phytosterols act as hypolipidemic and reduce absorption of cholesterol owing to their structural similarity with cholesterol and hence reduce the level of cholesterol in blood (Wang *et al*, 2012).
- Plant alkaloids aid in fortification of plant defense system by preventing the plant from being attacked by pathogens and herbivores and are also cardio protective, anti-inflammatory, anaesthetic and antioxidant in nature (Ali *et al*, 2019)^[11].

- Plant saponins are also defensive in nature and aid in strengthening plant defense system from being attacked by phytopathogens and herbivores (Hussain *et al*, 2019)^[3].

The current study is an addition to the already existing repertoire of studies unveiling the numerous medicinal attributes of plants and their associated phytochemicals. Apart from highlighting the pharmacological attributes of the tested plant species; the current study conveys a very crucial message about the “latent heritage of plants” in the form of bioactive phytochemicals; which needs to be conserved along with the existing knowledge passed on from past generations and gained by the traditional healers over years of their practice. This would enable us to form a solid groundwork for the future generations and enable synergism of conventional plant phytochemicals with the modern-day antimicrobials; which would shape the future of medicine and healthcare industry in coming years.

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