

## TEMPORAL HETEROGENEITY IN PRIMARY AND SECONDARY METABOLIC PRODUCTS OF MEDICINAL PLANTS OF SITAMATA WILDLIFE SANCTUARY IN RAJASTHAN, INDIA

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### Abstract

Phyto-chemical investigation in three tuberous medicinal plants, viz., *Arisaema tortuosum* (Wall.) Schott, *Chlorophytum tuberosum* (Roxb.) Baker and *Curculigo orchioides* Gaertn., growing in Sitamata Wildlife Sanctuary of Rajasthan were conducted for both primary and secondary metabolites. Experiment was carried out during June to October to find out monthly variation in accumulation of both primary (total contents of sugars, crude protein and phosphorus) and secondary metabolites (total contents of alkaloids and phenols) in tubers of these three species. In *A. tortuosum* (Wall.) Schott, crude protein and total alkaloids were found maximum during September, whereas phosphorus and total phenols during July. In *C. tuberosum* (Roxb.) Baker and *C. orchioides* Gaertn. maximum values of crude protein, phosphorus and total alkaloids were found during July. It is, therefore, concluded that the maximum production of different metabolites were found to be in the month of July and it is the suitable month for harvesting tubers.

### Introduction

The practice of using traditional medicines have a very long history; it is the sum total of the practices based on the theories, beliefs and experiences of different cultures and times, often inexplicable, used in the maintenance of health, as like in the prevention, diagnosis, improvement and treatment of illnesses. Herbal medicine is the use of medicinal plants for prevention and treatment of diseases; it ranges from traditional and popular medicines of every country to the use of standardized and titrated herbal extracts. In every country traditional medicine find foundation in magical or religious beliefs, or popular experience and the WHO is engaged to establish definitive guidelines for methodology of clinical research and the appraisal of effectiveness of traditional medicine (Firenzuoli and Gori 2007).

Plant species produces various metabolic products for their growth and development. Primary metabolites are directly involved in normal growth, development and reproduction. They are of prime importance and essentially required for growth of plants such as sugar, protein, lipids, starch, etc. Many primary metabolites act as precursors of pharmacologically active metabolites (Sagwan *et al.* 2010). The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct (Wink 1999). Studies on plant secondary metabolites have been increasing over the last 50 years. These molecules are known to play a major role in the adaptation of plants to their environment, but also represent an important source of active pharmaceuticals (Bourgaud *et al.* 2001). The storage organ of plants shows great biological activities. Chemical substances that produce definite physiological actions on human bodies accumulate in storage organs of the plants. The most important of these bioactive compounds are alkaloids, flavanoids and phenolic compounds (Buwa and Staden 2006).

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*Arisaema tortuosum* (Wall.) Schott (Family: Araceae) is a tuberous herb. The paste of the tuber is applied to the wound caused by snake-bite to prevent poisonous effect. Its tubers are useful to treat constipation, indigestion, abdominal pain, dysentery, rheumatism, stomachache, piles and also used as contraceptive, (Azam *et al.* 2016). In case of abscess in the neck, dried powder of tuber is applied to it. The tubers are used as an anti-oxidant, anti-inflammatory and anti-proliferative agent (Nile and Park 2014). The plant grows in large clumps and up to 6.0 - 7.0 ft in height. It resembles with carnivorous plants, but in fact they attract flies and other insects for pollination, not as food. The fascinating "flower" a pulpit-like hooded spathe enclosing a fleshy, erect spadix usually rises in spring. Flowers and fruits appear during July-August.

*Chlorophytum tuberosum* (Roxb. ) Baker (Family Asparagaceae) is found in rainfed areas and have properties such as an aphrodisiac, digestive, rejuvenator and immunomodulator (Chaudhari *et al.* 2012). *Safed musali* is used as a galactagogue, having health promoting properties, immuno-enhancing, hepatoprotective, antioxidants activities, etc. (Govindarajan *et al.* 2005). The tubers are also used in fever and leucorrhoea (Kirtikar and Basu, 1975). The plant is an erect tuberous growing up to a height of 1.5 - 2.0 ft with sheathing leaf base, acute to acuminate with entire margin. The roots are tuberous with ellipsoid tubers hanging from them. Flowering and fruiting take place from late June-July and August-September, respectively.

*Curculigo orchioides* Gaertn., a small herbal plant belongs to the family Hypoxidaceae. Curculigoside, a phenolic glycoside, is the major bioactive compound present has a wide spectrum of pharmacological activities such as an anti-immunostimulant, anti-oxidant, anti-ischemia injury agent, etc. (Wu *et al.* 2012). In many parts of India, due to its over-exploitation, it is becoming rare in occurrence. In Ayurvedic preparations, roots are considered as an aphrodisiac, appetizer, fattening and useful in treatment of piles, biliousness, fatigue, blood related disorders, etc. In Indian medicine, powdered rhizomes with milk are taken as a restorative tonic and also for the treatment of sexual debility (Khare 2007). It is a perennial herb with thick tuberous rootstock without aerial stem. It grows in the grassy plains and on the hills. Vegetative and flowering take place simultaneously. Flowering and fruiting simultaneously starts from late July and October.

Keeping this in view the present study was initiated with an aim to identify the availability of primary and secondary metabolites in three tuberous medicinally important tuberous plants, *i.e.* *Arisaema tortuosum* (Wall.) Schott, *Chlorophytum tuberosum* (Roxb.) Baker and *Curculigo orchioides* Gaertn. from Sitamata Wildlife Sanctuary, Rajasthan during different months.

### Materials and Methods

For chemical analyses, tubers of selected plants were collected randomly from natural habitat nearby Sitamata Wildlife Sanctuary, which is located between 74°25' E and 24°04' N in Paratapgarh district of the Rajasthan State during June-October 2015 to 2017. The tubers were washed with running tap water, oven-dried 48 hrs at 80°C and ground for further chemical analyses. The total sugars including soluble and insoluble ones were estimated from oven-dried plant material using the principle of hydroxymethyl furfural reaction with anthrone to produce green colour (Plummer 1971). Tubers were acid digested with H<sub>2</sub>SO<sub>4</sub> + H<sub>2</sub>O<sub>2</sub> and crude protein was estimated as per Allen *et al.* (1976). From the digested solution, nitrogen was estimated by Micro Kjeldahl method as described by Peach and Tracey (1955). Estimation of phosphorus was based on the development of molybdenum blue in sulphuric acid system and measured by spectrophotometer at 700 nm (Allen *et al.* 1976). Total alkaloids were estimated by acid-based titrimetric method using methyl red as an indicator and observing a faint yellow end point (Debnath *et al.* 2015). Total phenols were estimated by Folin-Ciocalteu's method using catechol as standard. This estimation was based on the principle that phenols react with phosphomolybdic

acid in Folin-Ciocalteu reagent in alkaline medium and produced blue coloured complex, *i.e.* molybdenum blue (Bray and Thorpe 1954).

The data collected during the years 2015 to 2017 were subjected to ANOVA (Gomez and Gomez 1984) and mean values are presented in graphical forms.

## Results and Discussion

Biologically active compounds contain a remarkably diverse assay of organic compounds and the carbohydrates are not only the first formed organic compounds in the plants as a result of photosynthesis, but also a major source of energy. The values of total sugars ranged from 38.78 - 46.99, 40.37 - 52.99 and 30.10 - 48.74 mg/g d. wt. in *A. tortuosum* (Wall.) Schott (Fig. 1), *C. tuberosum* (Roxb.) Baker (Fig. 4) and *C. orchoides* Gaertn. (Fig.7), respectively. The tubers of *A. tortuosum* were accumulated maximum total sugars during August, whereas in *C. orchoides* and *C. tuberosum* during June and September, respectively. Seasonal phytochemical variation in *Peganum harmala* showed maximum accumulation of total sugars during rainy season (Kedia *et al.* 2009). Sagar and Kasera (2017) reported maximum total sugars during September in *Drimia indica* from the Indian Thar desert. According to Singh (2011), the sugar contents in tubers of *Costus speciosus* and *Pueraria tuberosa* were 44.51 and 52.26 mg/g d. wt. during June, respectively, which is almost similar to *C. orchoides*.

Data presented in Fig. 2 show that the crude protein contents in *A. tortuosum* varied from 26.03 to 83.29 mg/g d. wt., being maximum during September. The maximum crude protein contents in leaves of *Trianthema triquetra* during rainy season were reported by Mohammed *et al.* (2000). In *C. tuberosum* and *C. orchoides*, it ranged from 88.50 - 130.15 (Fig. 5) and 70.28 - 127.55 (Fig. 8) mg/g d. wt, respectively, being maximum during July. Sagar and Kasera (2017) also reported maximum crude protein in leaves of *D. indica* during July. The decline in crude protein was observed in August may be due to the setting of phenological development as the plant transiting from the vegetative to flowering stage.

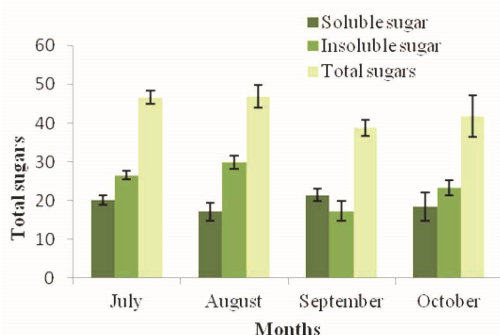


Fig. 1. Sugar contents (mg/g) in *A. tortuosum* (Wall.) Schott tubers during different months (Mean values and standard error are presented).

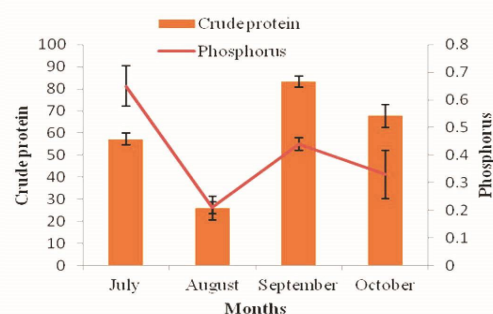


Fig. 2. Crude protein (mg/g) and phosphorus (%) contents in *A. tortuosum* (Wall.) Schott tubers during different months (Mean values and standard error are presented).

Phosphorus contents in selected plants were maximum during July when plants were in vegetative phase. Naidu and Swami (1994) and Sagar and Kasera (2017) reported highest amount of phosphorus at the time of new foliage formation in *Terminalia arjuna* and *Dipcadi erythraeum*, respectively. In here it ranged from 0.21 - 0.65 (Fig. 2), 0.27 - 0.53 (Fig. 5) and 0.15 - 0.56 (Fig. 8) in *A. tortuosum*, *C. tuberosum* and *C. orchoides*, respectively. Similar results were also reported by Sagar (2018) in bulbs of *D. erythraeum* and *D. indica* during vegetative phase. Daiya

and Kasera (2017) and Gehlot and Kasera (2013) reported maximum phosphorus contents during rainy season in *Withania coagulans* and *Phyllanthus amarus*, respectively which is accordance to present results.

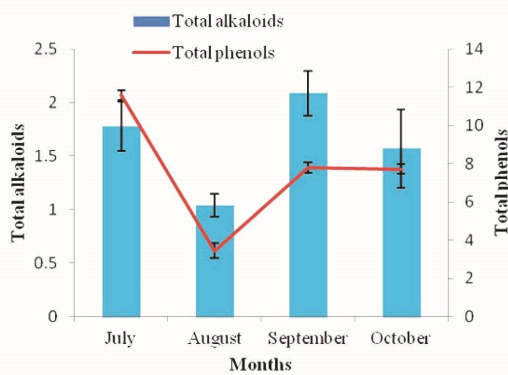


Fig. 3. Total alkaloid (%) and phenol (mg/g) contents in *A. tortuosum* (Wall.) Schott tubers during different months (Mean values and standard error are presented).

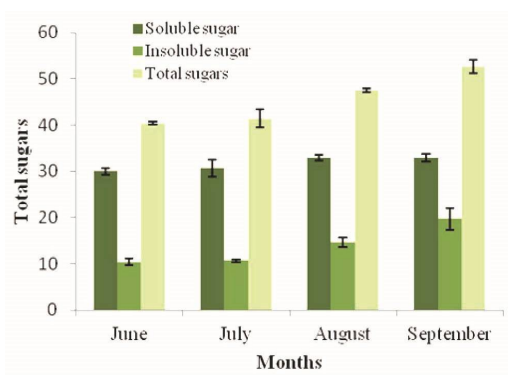


Fig. 4. Sugar contents (mg/g) in *C. tuberosum* (Roxb.) Baker tubers during different months (Mean values and standard error are presented).

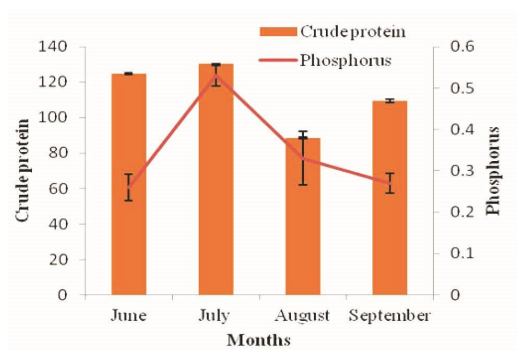


Fig. 5. Crude protein (mg/g) and phosphorus (%) contents in *C. tuberosum* (Roxb.) Baker tubers during different months (Mean values and standard error are presented).

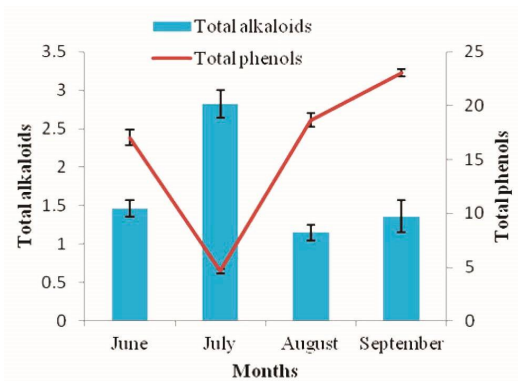


Fig. 6. Total alkaloid (%) and phenol (mg/g) contents in *C. tuberosum* (Roxb.) Baker tubers during different months (Mean values and standard error are presented).

Phenolics are groups of secondary metabolites which are synthesized by plant tubers and have indicated to several biological activities such as anti-oxidant, anti-aging, anti-diabetic, anti-mutagenic, anti-carcinogenic, anti-inflammatory and anti-microbial (Nile and Khobragade 2009). Total phenols in tubers of *A. tortuosum* ranged from 3.45 to 11.60 mg/g d. wt., being maximum during July (Fig. 3). It is evident from Fig. 9 that the maximum phenolic contents (22.26 mg/g d.wt.) in *C. orchioides* were observed during August. Similar results were also reported in *Hypericum pruinatum* by Ayan *et al.* (2006). Ncube *et al.* (2011) and Ahmad *et al.* (2017) reported maximum total phenols during September in bulbs of *Tulbaghia violacea* and roots of *Solanum incanum*, respectively. Similar results were also reported in the present studies with *C. tuberosum* (Fig. 6). There is a well-established positive relationship between the intensity of solar radiation and quantity of phenolics produced by plants (Harbowy and Balentine 1997). In relation to the

results of the present studies, high phenolic concentrations during rainy season (July - September) could notably be due to the highly fluctuating temperatures.

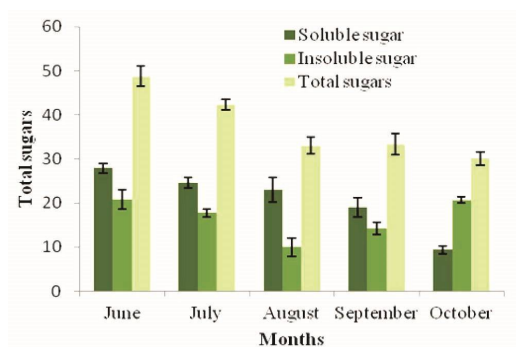


Fig. 7. Sugar contents (mg/g) in *C. orchioides* Gaertn. tubers during different months (Mean values and standard error are presented).

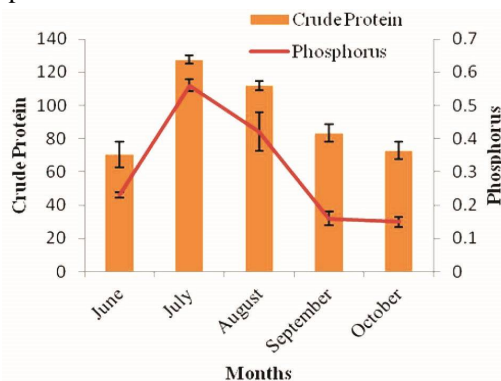


Fig. 8. Crude protein (mg/g) and phosphorus (%) contents in *C. orchioides* Gaertn. tubers during different months (Mean values and standard error are presented).

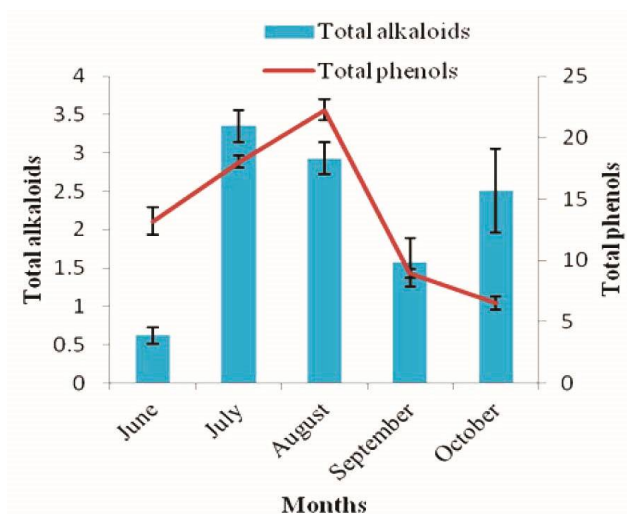


Fig. 9. Total alkaloid (%) and phenol (mg/g) contents in *C. orchioides* Gaertn. tubers during different months (Mean values and standard error are presented).

The alkaloids are naturally occurring nitrogen-containing pharmacologically active organic compounds present in plant kingdom and have made major impact on plant medicine because of its vast application. In Ayurveda, it is believed that many of Tikta (bitter) and Katu (pungent) tasted herbs are rich in alkaloids. In tubers of *A. tortuosum*, higher amount to total alkaloids were found during September (Fig. 3). Kale (2010) observed highest total alkaloids in *Datura metel* during rainy season. The leaves of *C. orchioides* showed highest total alkaloids during September (Soni and Kaseria 2018). *C. orchioides* (Fig. 6) and *C. tuberosum* (Fig. 9) exhibit peak concentration of total alkaloids during July and then gradually decreasing trend was observed in later months. In *Aconitum elwesii*, crude alkaloid content was maximum during pre-flowering seasons (July-August), whereas in roots during November (Sinam and Devi 2011).

Statistical analyses revealed that sampling month is the only factor contributing for the observed variations in total sugars, crude protein and total phenols ( $p < 0.05$  levels) in all these three plants, while phosphorus and total alkaloids varied non-significantly in *A. tortuosum*. Except phosphorus, data for all parameters were significant in *C. tuberosum*.

It can be concluded from present study that tubers of *all* these three species *showed* maximum phosphorus contents during July. Higher amount of crude protein and total alkaloids were observed during September while in two other species during July. In *A. tortuosum*, total sugars and phenols were maximum in August and July, respectively while in *C. tuberosum* during September. *C. orchioides* exhibited maximum values of total sugars and phenols in June and August, respectively. Tubers of *C. tuberosum* accumulated higher amount of total phenols as compared to other plants, however total alkaloids were maximum in *C. orchioides*. The tubers of these plant species could feasibly be harvested during rainy season (July-September) for medicinal use to cure different diseases for improving the sustainability of these medicinal plant resources. Phenolic and alkaloid compound levels appeared to be higher during rainy season, a phenomenon which implies that the periods coincide with the favourable stimuli for the production of these metabolites.

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