Evaluation of secondary metabolites in three tuberous medicinal plants during different months from south-eastern part of Rajasthan

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Abstract
The present paper deals with variations in total alkaloid and phenol contents during different months (June-October) in three medicinally important tuberous plants, i.e. Arisaema tortuosum, Chlorophytum tuberosum and Curculigo orchioides from south-eastern part of Rajasthan. Secondary metabolites are organic compounds that are not are known to play a major role in the adaptation of plants to their environment, but represent an important source of active pharmaceuticals. Results revealed that peak concentrations of total alkaloids in A. tortuosum and C. tuberosum were observed during August, whereas during September in C. orchioides. However, total phenols in A. tortuosum, C. orchioides and C. tuberosum were reported during October, September and June, respectively.

Keywords: Sitamata wildlife sanctuary, total alkaloids, total phenols, tuberous medicinal plants

Introduction
Herbal medicine plays an important role in rural areas and various locally produced drugs are still being used as household remedies for different ailments. The increasing use of traditional therapies demands more scientifically sound evidence for the principles behind therapies and for effectiveness of medicines. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects

Kossel [2] was the first to introduce the term secondary metabolite, stating that 'whereas primary metabolites are present in every plant cell that is capable of division, whereas secondary metabolites are present only "accidentally" and are not essential for plant life'. Secondary products involved in plant defense through cytotoxicity towards microbial pathogens could prove useful as antimicrobial medicines in humans, if not too toxic. Secondary products involved in defense against herbivores through neurotoxin activity could have beneficial effects in humans, i.e. as anti-depressants, sedatives, muscle relaxants, or anesthetics through their action on the central nervous system [3].

Arisaema tortuosum (Wall.) Schott (Family: Araceae) is a tuberous herb and is widely known for its therapeutic properties. It is widely distributed in Rhododendron forest, scrub and alpine meadows in the Himalayas, Western China, Southern India and some parts of Myanmar. Paste of the tuber is applied over the wound caused by snake-bite to check poisonous effect. Its tubers are useful in constipation, indigestion, abdominal pain, dysentery, rheumatism, stomachache, contraceptive, piles, etc, [4]. In case of abscess in the seat, dried powder of tuber is applied over the seat. The tubers are used as anti-oxidant, anti-inflammatory and anti-proliferative agent [5].

Safed musli (Chlorophytum species) holds an important position in Indian herbal medicine and their role in medicinal world dates back to classical Ayurveda references of 10th and 11th centuries where its particular properties such as an aphrodisiac, digestive power, rejuvenator and immunomodulator are well known [6]. C. tuberosum Baker. belongs to family Asparagaceae, is found in rainfed areas. The plant generally grows (between 1300 and 2800 m) along the forest margins, grassy slopes and rocky places along valleys [7]. It is being sold in the market under the common name Safed musali. The white tuberous roots are the medicinally useful parts. The tuberous roots of other species of Chlorophytum, Asparagus, Bombax and Orchids are also sometimes called Safed musali leading to confusion [8].

Curculigo orchioides Gaertn, a small herbal plant belonging to the family Amaryllidaceae has been traditionally used in folk medicine as the tonic, alterative, demulcent, diuretic and restorative. It is widely distributed in China, India, Malaya, Japan and Australia. Curculigoside, a phenolic glycoside, is the major bioactive compound present in C. orchioides.
Curculigoside has a wide spectrum of pharmacological activities such as an anti-immunostimulant, anti-oxidant, anti-ischemia injury agent, etc. [19]. In many parts of India, due to its over-exploitation, it is becoming rare in occurrence. In Ayurvedic preparations, roots are considered as 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 sexual debility [10]. An amelioration of the damage caused by sustained hyperglycemia, confirming the claims of its use in traditional medicine for curing diabetes, inducing sexual dysfunction and compromised sexual potency [11]. Considering the extensive research on medicinal plants, the present study was conducted to evaluate the availability of secondary metabolites in the selected three important tuberous medicinal plants and also to determine which month is most suitable for production of secondary metabolites so to obtain maximum amount of commercially important these products.

Materials and Methods
For chemical analyses, leaves of selected plants were collected randomly from natural habitat nearby Sitamata Wildlife Sanctuary, Paratapgarh district Rajasthan during June-October during 2014 -2016. For plant analyses, fresh leaf samples were randomly collected, washed with distilled water to remove adherent soil particles and allowed to dry in oven at 80°C for minimum 24 h for further chemical analyses. Total alkaloids were estimated by acid-based titrimetric method using methyl red as an indicator and observing a faint yellow end point [12]. Total phenols were estimated by Folin-Ciocalteu’s method using catechol as standard [13]. 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 (molybdenum blue).

The mean values for six replicates of all selected plants during 2014-2016 were subjected to analysis of variance (ANOVA) as suggested by Gomez and Gomez [14].

Results and Discussions
Traditionally, wild plant parts are used as a source of herbal preparation for treatment of various ailments. They are novel source of medicines as they have a reservoir of chemical agents with therapeutic properties [15]. Secondary metabolites are found expressed in various combinations in different parts of the plant (leaves, roots, shoots, bark) at different stages of growth (seedling, seed, plantlet, mature tree) under different environmental pressures (invasive microbes, herbivores) and in numerous combinations of ways by different classes of plants [16].

The alkaloids are naturally occurring nitrogen-containing pharmacologically active organic compounds present in plant kingdom. These have made major impact on plant medicine because of its vast application. In the present studies, Fig. 2 show that the total alkaloid contents in C. orchioides varied from 5.05 to 6.22%, being maximum during September; Verma and Kasera [17] observed that maximum values of total alkaloids in A. racemosus during rainy seasons, which is in accordance with presents studies. In A. tortuosum and C. tuberosum it ranged from 2.43 to 5.04 and 2.52 to 5.73%, respectively and maximum values were obtained during August (Figs. 1 & 3). Kurian and Sankar [18] reported that in Adhatoda vasica, alkaloids contents varied during different months, being maximum in August. The rates at which alkaloids accumulate vary with the environmental and nutritional conditions under which the plant grown and often also the stage of plant development. In younger plants, the alkaloids content is low, gradually increasing with age up to a certain period following by a decreasing tendency [19].

![Fig 1: Total alkaloid and phenol contents in A. tortuosum during different months.](image1)

![Fig 2: Total alkaloid and phenol contents in C. orchioides during different months.](image2)
Phenols are a large class of secondary metabolites. Phenolics are among most active anti-oxidants as well as most important stabilization factors of the oxidative processes [20]. Phenolic compounds are produced in plants to serve a diverse range of purposes such as defence against pathogens and different forms of environmental stresses [21]. It is for this reason that the observed differences between different months could be explained on the basis of the differences in the climatic, biotic and environmental conditions experienced in different months. It is evident from Figs. 1-3 that total phenols ranged from 11.29 to 15.74, 12.11-20.87 and 8.76-11.27 mg g⁻¹ d. wt. in A. tortuosum, C. orchioides and C. tuberosum, respectively. The maximum phenolic contents in A. tortuosum were observed during October. Similar results were also reported in Tribulus rajasthanensis by Gehlot and Kasera [21]. Neube et al. [22] reported maximum total phenols during September in bulb and leaf extracts of Tulbaghia violacea and Mervilla plumbea. Similar results were also observed in case of C. orchioides. According to Tavarini and Angelini [23] as vegetative growth declines and the physiological state moves towards flowering, the secondary metabolites may be reallocated to the reproductive phase with a consequent decrease at leaf level. C. tuberosum exhibited highest amount of total phenols during June and lowest during August. Sahoo et al. [24] observed similar observation in Boerhavia diffusa and B. prioritis during summer season. Hypericum perforatum exhibited maximum phenolic contents during June, whereas in H. pruinatum during August [25], which confirm our results. Water stress can stimulate the accumulation of phenolic compounds. Deshmukh and Dhumal [26] observed that an increase in water stress caused significant increase in polyphenol oxidase activity in Sorghum bicolor. The ANOVA showed that monthly variations were significant (p>0.01) for total alkaloids and phenols in all three selected plants except for total alkaloids in C. orchioides.

Conclusions

By estimating secondary metabolites during different months, it was concluded that total alkaloid and phenolics contents are closely correlated with different months and environmental conditions. In A. tortuosum and C. tuberosum, alkaloid contents were found to be maximum during August, whereas in C. orchioides both parameters during September. C. orchioides accumulated higher amount of total alkaloids and phenols as compared to A. tortuosum and C. tuberosum. Thus, the present findings might be useful to research efforts made towards enhancing the concentrations of these valuable chemical compounds.

Acknowledgements

The authors are thankful to Professor & Head, Department of Botany, Centre of Advanced Study, J. N. Vyas University, Jodhpur for providing necessary facilities. Grateful thanks to the CSIR, New Delhi for the financial assistance in the form of NET-SRF fellowship [09/098/0126/2013-EMR-I] to the first author. Financial support received from the UGC, New Delhi in the form of UGC-(SAP-II)-CAS (No. F. 5-1/2013(SAP-II) dated 03.01.2014) is also grateful acknowledged. Thanks to the authority of Forest Department of Sitamata Wildlife Sanctuary for granting permission to collect plant materials.

References


