



# Evaluation of phytochemical variations in medicinal plants of Rajasthan

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## Abstract

The present study deals with evaluation of phytochemical variations in terms of primary and secondary metabolites in 4 important medicinal plants, viz. *Arisaema tortuosum* (Whipcord lilly), *Corbichonia decumbens* (Stone plant), *Dipcadi erythraeum* (Piazi) and *Withania coagulans* (Paneer bandth) of the Rajasthan. The primary metabolites such as leaf pigments (Chl. a, Chl. b, total chlorophylls and carotenoids), proline, osmotic potential, total sugars, crude protein, phosphorus and secondary ones like total alkaloids and phenols were estimated during different growth stages/months/seasons in selected plants. Results revealed that in *D. erythraeum*, except osmotic potential and total sugars, all parameters were showed significant variations. The peak concentrations of total alkaloids and phenols in *A. tortuosum* were observed during August and October, respectively. In *C. decumbens*, maximum values of total chlorophylls were recorded during vegetative whereas total sugars and phosphorus in fruiting stage. The maximum values of total chlorophylls in *W. coagulans* were observed during winter followed by summer and minimum in rainy season. Phosphorus content was reported to be maximum during rainy season.

**Keywords:** Leaf pigments, Sugars, Alkaloids, Phenols, medicinal plants, Rajasthan

## Introduction

The Thar desert also known as Great Indian desert is considered as the world's tenth biggest desert and forms an important segment of western India and inhabits an area of about 278 330 km<sup>2</sup>. 70% of this area (196 150 km<sup>2</sup>) lies in Rajasthan, 23% (62 180 km<sup>2</sup>) in Gujarat and 7% (about 20 000 km<sup>2</sup>) in Punjab and Haryana States. The desert extends into Pakistan and called as the Cholistan desert. The whole desert in the Indian subcontinent (India and Pakistan) inhabits an area of almost half of the Arabian desert and 1/7th of the Sahara desert.<sup>1</sup>

Plants have been serving human race since the dawn of the history of human civilization. Currently 30% to 40% of the available drugs are based on the medicinal and therapeutic properties of various plants and are used as herbal supplements, botanicals and nutraceuticals. Most of the developing countries relied on starch-based food products as primary source of energy and proteins. Medicinal plants are of great importance not only for their biologically active secondary metabolites but also for their primary metabolites such as carbohydrates, proteins and lipids. These primary metabolites are essential for the growth and development of a plant and are carriers of chemical energy to the successive trophic levels of the food

chain. They make the major portion of diet of herbivores and omnivores. On the other hand, secondary metabolites are formed as by-products of metabolic pathways and though not essential for the survival of the plants but are essential in their defence system. Their medicinal properties have been utilized since long and research is still in progress to explore their applications as medicines.<sup>2</sup>

*Arisaema tortuosum* (Wall.) Schott (Family: Araceae) is a tuberous herb and is widely known for its therapeutic properties. Paste of the tuber is applied over the wound caused by snake-bite. The tubers are useful in constipation, indigestion, abdominal pain, dysentery, rheumatism, stomachache, contraceptive, piles, etc.<sup>3</sup>

*Corbichonia decumbens* (Forssk.) Exell (Family: Lophocarpaceae) is a prostrate annual herb with succulent leaves and found almost throughout India in rocky or sandy places and in dry hot areas up to 1000 m altitude. This plant is used in kidney stone problems, gonorrhoea and also as tonic. In tribals, the plant leaves are crushed and taken orally in kidney stone problems.<sup>4</sup>

*Dipcadi erythraeum* Webb. & Berth is bulbous flowering plants, belonging to the subfamily Scilloideae of family Asparagaceae. Its bulb and capsule are edible especially in Pakistan. The leaves are used as a laxative and as an



ointment for wounds.<sup>5</sup>

*Withania coagulans* (Stocks) Dunal (Family: Solanaceae) is used in Unani system, known as Tukm-e-Hayath, which is a perennial shrub and popularly known as Indian cheese maker. The fruits are used in the treatment of ulcers, rheumatism, dropsy, constipation and sexual ability.<sup>6</sup>

India is blessed with one of world's richest medicinal plant heritages. Medicinal plants constitute a resource, which has been used traditionally by people of this country for the last two millennia. The flora is recognized thus far for their medicinal use not only in terms of the number of unique species but also in terms of the wonderful traditional knowledge about such uses for human and domestic animal health and also for agriculture. For thousands of years, Indian plants have been attracting interests of foreign countries. People like China, Cambodia, Indonesia and Baghdad used to visit ancient universities of India like Takshila (700 BC) and Nalanda (500 BC) to learn health science of India. This knowledge has been traced to have extended to Egypt, Mesopotamia and to the Eastern Mediterranean and Persia also to Armenia, ancient Greece, then to ancient Europe.<sup>7</sup>

Primary metabolites are of prime importance and essentially required for growth of plants. Many of these are precursors of pharmacologically active metabolites in pharmaceutical compounds such as antipsychotic drugs.<sup>8</sup> Considering the extensive research on medicinal plants, the present study was conducted to evaluate the availability of primary and secondary metabolites in the selected 4 important medicinal plants and also to determine which month/season/growth stage is most suitable for production of these metabolites so as to obtain maximum amount of commercially important these products.

### Materials and Methods

For phytochemical analyses, leaves of *A. tortuosum* are collected randomly from natural habitat nearby Sitamata Wildlife Sanctuary (334 km away in south-eastern direction from the University Campus), Paratagarh district Rajasthan during June-October 2014 to 2016. *D. erythreinum* are periodically surveyed and collected in the field at Bhimbhadak, Jodhpur (15 km away north-west of the University Campus) during 2013 to 2015. Plant samples of *C. decumbens* were collected from Beriganga (35 km away in north-west direction from the University Campus) during rainy season, while *W. coagulans* from the Botanical Garden of J.N.V. University Campus on monthly basis. Leaves were washed with running tap water to remove the adherent foreign particles, air-dried and used for chemical analyses. Proline, osmotic potential and leaf pigments were estimated in fresh leaf samples, while other parameters from oven-dried leaves. Fresh leaves were extracted with 80% acetone for estimation of leaf pigments as per Arnon.<sup>9</sup> Free proline was estimated as per Bates et al.<sup>10</sup> Osmotic potential was estimated as suggested by Janardhan et al.<sup>11</sup> Total sugars were estimated

as per standard methods given by Plummer.<sup>12</sup> Nitrogen content was estimated by micro-Kjeldahl apparatus as suggested by Peach and Tracey<sup>13</sup> and phosphorus as per Allen et al.<sup>14</sup> Total alkaloid<sup>15</sup> and phenol<sup>16</sup> contents were estimated by acid-based titrimetric method using methyl red as an indicator and Folin-Ciocalteu's method using catechol as standard, respectively.

### Results

In *A. tortuosum*, the total alkaloid and phenol contents ranged from 2.43% to 5.04% and 11.29 to 15.74, mg g<sup>-1</sup> d. wt., being maximum during August and October, respectively (Figure 1).

Data on various primary metabolic products such as leaf pigments, proline, osmotic potential, total sugars, crude protein and phosphorus in leaves of *C. decumbens* are presented in Figures 2 to 5. It is evident from Figure 2 that the highest values of total chlorophylls were observed during vegetative whereas carotenoids in flowering stage. Values for total chlorophylls and carotenoids ranged from 1.055 to 1.319 and 0.00069 to 0.00114 mg g<sup>-1</sup> f. wt., respectively. The proline values ranged from 3.315 to 6.259 µg g<sup>-1</sup> f. wt. during 3 stages, being maximum in fruiting stage. Values for osmotic potential ranged from 0.246 to 0.442 -MPa (Figure 3). Total sugar values ranged from 16.33 to 44.626 mg g<sup>-1</sup> d. wt. during three phases, being maximum in fruiting stage (Figure 4). Crude protein was observed highest (7.160% d. wt.) during flowering phase followed by fruiting (6.231) and minimum at vegetative phase (3.580% d. wt.). The phosphorus content was highest

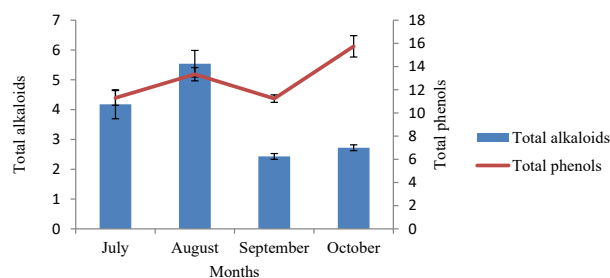


Figure 1. Total alkaloid (%) and phenol contents (mg g<sup>-1</sup> d. wt.) in *A. tortuosum* during different months (Source: Soni and Kasera<sup>17</sup>).

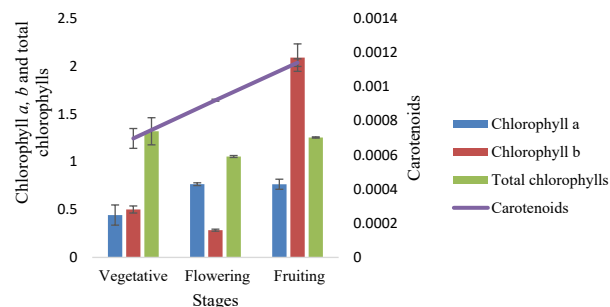
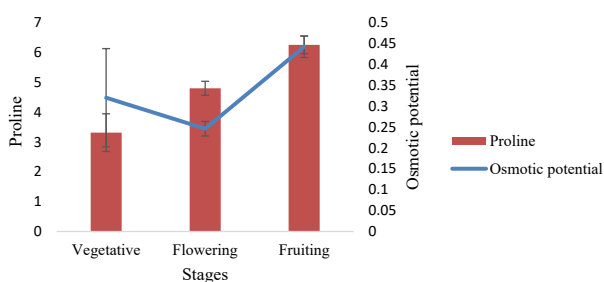
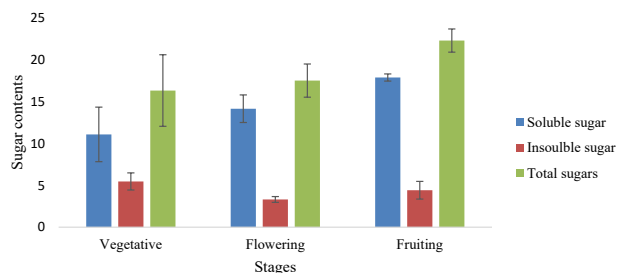


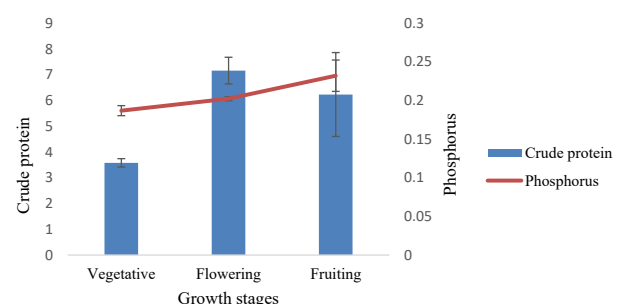
Figure 2. Leaf pigment (mg g<sup>-1</sup> f. wt.) contents in *C. decumbens* leaves during different growth stages (Mean values and standard error are presented).



**Figure 3.** Proline (mg g<sup>-1</sup> f. wt.) and osmotic potential (-MPa) values in *C. decumbens* leaves during different growth stages (Mean values and standard error are presented).



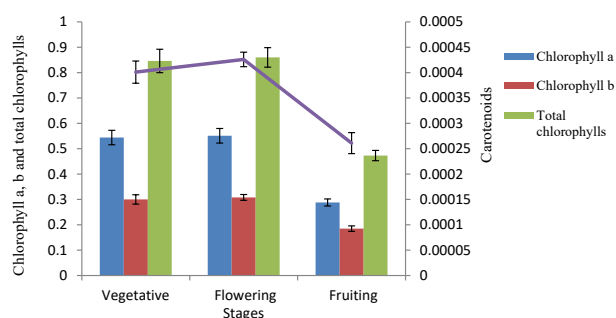
**Figure 4.** Sugar (mg g<sup>-1</sup> d. wt.) contents in *C. decumbens* leaves during different growth stages (Mean values and standard error are presented).



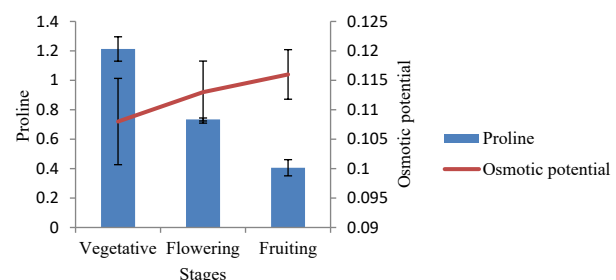
**Figure 5.** Crude protein and phosphorus contents (% d. wt.) in *C. decumbens* leaves during different growth stages (Mean values and standard error are presented).

during fruiting stage followed by flowering and minimum at vegetative stage (Figure 5).

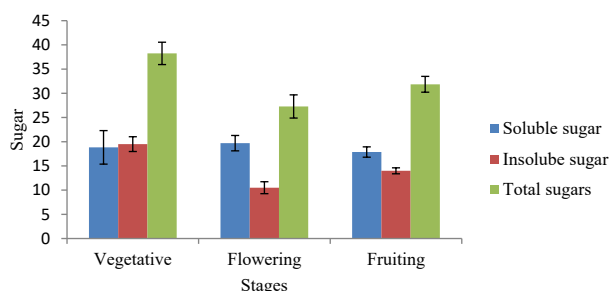
In the leaves of *D. erythraeum*, the highest values of total chlorophylls and carotenoids were observed during flowering stage. Values for total chlorophylls and carotenoids ranged from 0.473 to 0.860 and 0.000261 to 0.000426 mg g<sup>-1</sup> f. wt., respectively (Figure 6). The proline values ranged from 0.406 to 1.213 µg g<sup>-1</sup> f. wt. during three stages, being maximum in vegetative stage. Values for osmotic potential ranged from 0.108 to 0.116 -MPa. (Figure 7). Total sugar values ranged from 27.28 to 38.24 mg g<sup>-1</sup> d. wt. during three phases, being maximum in vegetative stage (Figure 8). It is evident from Figure 9 that crude protein was observed highest (4.882% d. wt.) during vegetative phase followed by flowering (4.650) and lowest at fruiting phase (3.022% d. wt.). The phosphorus content was highest during vegetative stage followed by flowering



**Figure 6.** Leaf pigment (mg g<sup>-1</sup> f. wt.) contents in *D. erythraeum* during different growth stages (Mean values and standard error are presented - Source: Sagar and Kasera<sup>18</sup>).



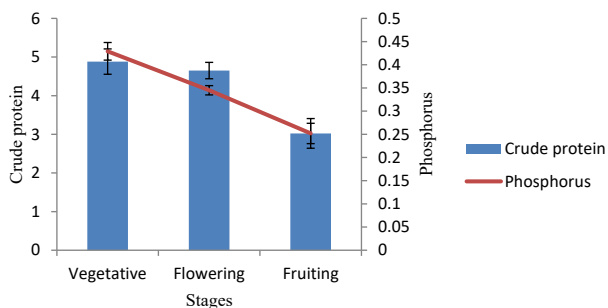
**Figure 7.** Proline (µg g<sup>-1</sup> f. wt.) and osmotic potential (-MPa) values in *D. erythraeum* leaves during different growth stages (Mean values and standard error are presented - Source: Sagar and Kasera<sup>18</sup>).



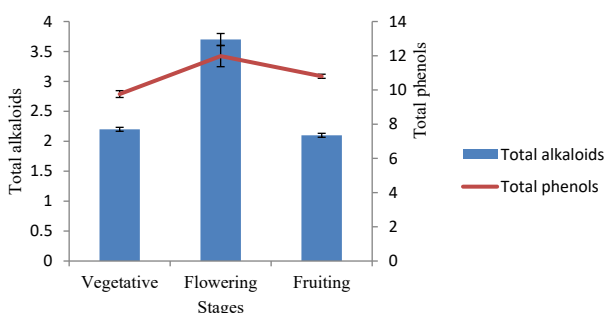
**Figure 8.** Sugar (mg g<sup>-1</sup> d. wt.) contents in *D. erythraeum* leaves during different growth stages (Mean values and standard error are presented - Source: Sagar and Kasera<sup>18</sup>).

and minimum at fruiting stage. Total alkaloid and phenol contents were found to be maximum during flowering stage and values ranged from 2.1 to 3.7 % d. wt. and 976.66 to 1198 mg 100 g<sup>-1</sup> d. wt., respectively (Figure 10).

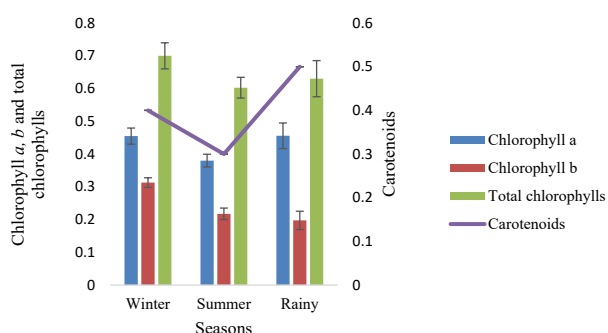
Data on various primary metabolic products in *W. coagulans* are presented in Figures 11-14. It is evident from Figure 11 that the highest values for total chlorophylls were observed during winter whereas carotenoids during rainy season. The OP values ranged from -0.274 to -0.320 MPa, being highest during winter. Maximum amount of proline was accumulated during winter, i.e. 5.336 µg g<sup>-1</sup> f. wt. (Figure 12). Total sugars ranged from 17.96 to 31.40 mg g<sup>-1</sup> d. wt. during three seasons, being maximum in summer (Figure 13). Crude protein and phosphorus were maximum in rainy and their values ranged from 3.789 to 5.777% d. wt. and 0.254% to 0.385 %, respectively (Figure 14).



**Figure 9.** Crude protein and phosphorus contents (% d. wt.) in *D. erythraeum* leaves during different growth stages (Mean values and standard error are presented - Source: Sagar and Kasera<sup>19</sup>).



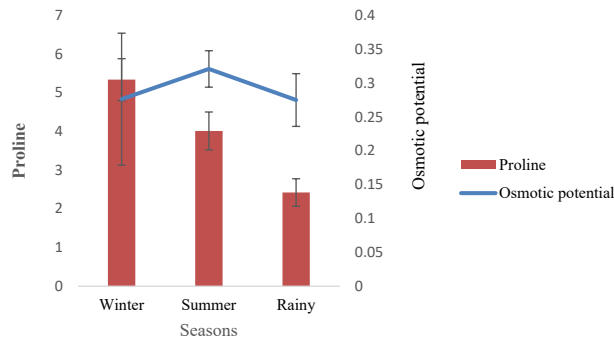
**Figure 10.** Total alkaloid (% d. wt.) and phenol (mg g<sup>-1</sup> d. wt.) contents in *D. erythraeum* leaves during different growth stages (Mean values and standard error are presented).



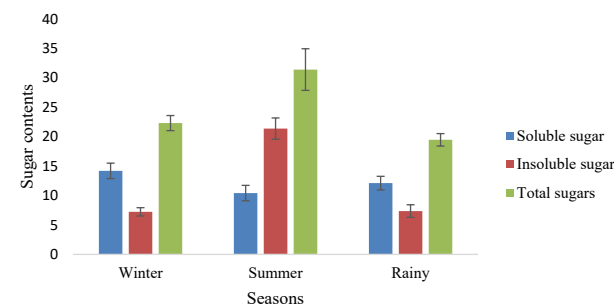
**Figure 11.** Leaf pigment (mg g<sup>-1</sup> f. wt.) contents in *W. coagulans* leaves during different months (Mean values and standard error are presented).

## Discussion

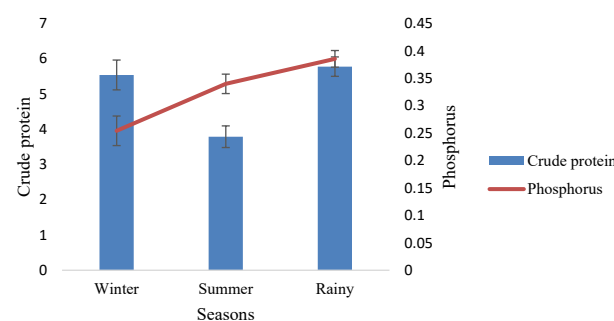
Green pigments are important sources of protective food which are highly beneficial for the maintenance of good health and prevention of diseases.<sup>19</sup> Mohammed and Sen<sup>20</sup> and Kedia et al<sup>21</sup> reported higher values of leaf pigments during flowering stage in *Trianthema portulacastrum* and *Phyllanthus fraternus*, respectively, which is in agreement with present investigations in *D. erythraeum*. Total chlorophylls in *C. decumbens* were highest at vegetative, while carotenoids in fruiting stage, may be due to sprouting of new leaves. The highest values for total chlorophylls and carotenoids in *W. coagulans* were observed during winter and rainy seasons, respectively. Kedia et al<sup>21</sup> observed maximum values of leaf pigments



**Figure 12.** Proline (mg g<sup>-1</sup> f. wt.) and osmotic potential (-MPa) values in *W. coagulans* leaves during different months (Mean values and standard error are presented).



**Figure 13.** Sugar (mg g<sup>-1</sup> d. wt.) contents in *W. coagulans* leaves during different months (Mean values and standard error are presented).



**Figure 14.** Crude protein and phosphorus (% d. wt.) contents in *W. coagulans* leaves during different months (Mean values and standard error are presented).

and carotenoids in *Phyllanthus fraternus* during winter season. In the present study, the highest values of total chlorophylls were reported in winter might be due to the appearance of new leaves.

Proline may also be helpful in maintaining the cell membrane integrity under stress.<sup>22</sup> Proline acts as a reserve source of carbon, nitrogen and energy during recovery from stress.<sup>23</sup> Shukla et al<sup>24</sup> reported highest values of osmotic potential when proline content was at peak in *Prosopis cineraria* and similar results were reported in *D. erythraeum*. Saharan et al<sup>25</sup> documented highest proline content in *Evolvulus alsinoides* during flowering phase with lowest values of osmotic potential and *vice-versa*. Sen et al<sup>26</sup> reported that proline accumulation in desert plants

was accompanied by a decrease in osmotic potential, which is in accordance with present investigation in *C. decumbens*. In the present study, maximum amount of proline was documented during winter season along with OP in *W. coagulans*. Kasera and Shukla<sup>27</sup> reported the highest OP in winter, while lowest during summer in *Leptadaenia reticulata*. Sen et al<sup>26</sup> observed that *Trianthema triquetra* exhibited maximum proline in winter season. The higher value of both parameters in *W. coagulans* during winter season can be correlated with its tolerance toward environmental stresses.

Plant sugars can be used as artificial sweeteners and they can even help diabetics by supporting the body in its rebuilding.<sup>28</sup> According to Mishra and Bhatt<sup>29</sup> the sugar content in leaves increased with age and growth of plants. Kedia et al<sup>30</sup> observed maximum values of total sugars in rainy season during vegetative stage in *Peganum harmala*, which is in accordance with *D. erythraeum*. Mohammed et al<sup>31</sup> reported lowest values of total sugars during July in *T. triquetra*. *Phyllanthus fraternus* accumulated maximum total sugars during rainy season.<sup>21</sup> The present finding also support above observations. In *C. decumbens*, maximum amount of total sugars was observed during fruiting stage followed by flowering and minimum in vegetative stage. Increase in total sugars may be due to stress conditions during fruiting and flowering stages as compared to vegetative ones. Saharan et al<sup>25</sup> reported maximum sugar content during flowering phase in *E. alsinoides*. In *W. coagulans*, maximum amount of total sugars was observed in winter whereas minimum in summer season. Kasera and Shukla<sup>27</sup> and Gehlot et al<sup>32</sup> reported maximum values of total sugars during winter season in *Leptadenia reticulata* and *Withania coagulans*, respectively. The decline in carbohydrate content during summer season may result from an imbalance between carbon production in photosynthesis and consumption in respiration as stated by Liu and Huang.<sup>33</sup>

Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future.<sup>34</sup> Total proteins increased till the maturity of plant and thereafter decreased remarkably in *Moringa oleifera* as reported by Khatun et al.<sup>35</sup> The present findings in *D. erythraeum* also support the above observations. *L. reticulata* exhibited maximum crude protein in August followed by September.<sup>27</sup> In *C. decumbens*, crude protein was observed highest during flowering phase and minimum at vegetative phase. Saharan et al<sup>25</sup> and Kedia et al<sup>21</sup> reported maximum values of crude protein during flowering stage in *E. alsinoides* and *Phyllanthus fraternus*, respectively. The present finding also supports the above observations. The crude protein contents of plants have been used as indicators of their nutritional value.<sup>36</sup> In *W. coagulans*, the maximum amount of crude protein was observed during rainy season and similar results were

reported by Mohammad et al<sup>31</sup> in *T. triquetra*.

The amount of phosphorus was more at the time of new foliage formation followed by a gradual decrease with advancement of growing season up to leaf fall in *Terminalia arjuna*.<sup>37</sup> In the present studies, it also decreased with advancement of growth phases in *D. erythraeum*. In *C. decumbens*, the phosphorus content was highest during fruiting (maturity) stage followed by flowering and minimum at vegetative stage. Khatun et al<sup>35</sup> observed that phosphorus content increased gradually with the advancement of maturity level in *M. oleifera*, which confirms our findings. Gehlot and Kasera<sup>38</sup> reported maximum phosphorus content during rainy season in *Phyllanthus amarus*, which supports our results in *W. coagulans*.

The biological function of alkaloids and their derivatives are very important and are used in analgesic, anti-spasmodic and anti-bacterial activities.<sup>39</sup> In the present studies, the maximum total alkaloid contents in *A. tortuosum* were reported during August. Verma and Kasera<sup>40</sup> observed that maximum values of total alkaloids in *A. racemosus* during rainy seasons, which is in accordance with our present studies. Kurian and Sankar<sup>41</sup> reported maximum amount of alkaloid contents in *Adhatoda vasica* during 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. The maximum amount of alkaloids was observed in roots of *Rouwolfia serpentina* and *Catharanthus roseus* and in leaves of *Datura innoxia* and *Atropa belladonna* during reproductive phase (flowering).<sup>42</sup> In the present studies, total alkaloid contents in *D. erythraeum* was maximum during reproductive stages, i.e. flowering and fruiting, which is in accordance with above findings. In younger plants, the alkaloids content is low, gradually increasing with age upto a certain period following by a decreasing tendency.<sup>43</sup>

Phenolic compounds are high level antioxidants because they have the ability to absorb and neutralize free radicals.<sup>44</sup> The maximum phenolic contents in *A. tortuosum* were observed during October. Similar results were also reported in *Tribulus rajasthanensis* by Gehlot and Kasera.<sup>45</sup> According to Tavarini and Angelini<sup>46</sup> 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. In the present study, total phenols were maximum in *D. erythraeum* leaves during flowering, whereas Verma and Kasera<sup>40</sup> reported higher amounts of during vegetative stage in *A. racemosus*.

## Conclusions

By estimating plant metabolites during different stages/ months/seasons, it was concluded that production of primary and secondary metabolic products is closely

correlated with different stages/months/seasons and environmental conditions. In *A. tortuosum*, total alkaloid and phenol contents were found to be maximum during August and October, respectively. In *D. erythraeum* total alkaloids and phenols were found to be maximum during flowering stage. In *D. erythraeum*, maximum leaf pigments were accumulated during flowering phase, while remaining primary metabolic parameters during vegetative stage. In *C. decumbens* vegetative stage was found to be most favourable for obtaining maximum production of leaf pigments, while total sugar and phosphorus during fruiting stage. In case of *W. coagulans*, winter is most favourable season for maximum accumulation of leaf pigments, proline and total sugars. Thus, the present findings might be useful to research efforts made towards enhancing the concentrations of these valuable chemical compounds and find most suitable developmental growth stage of plant for harvesting them to obtain maximum quantities of these chemicals.

### Competing Interests

None.

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