

## First report on calcium is the primary target behind the destructive behavior of snail in Western Ghat region of Maharashtra, India

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### Abstract

*Calotropis gigantia* L. (Family - Apocynaceae) and *Barleria cristata* L. (Family - Acanthaceae) are an important medicinal plant commonly found in western ghat region of Maharashtra, India. In previous study, it was reported that during Monsoon season pest Snail have been widely affecting the *C. gigantia* L. and *B. cristata* L. To tracing reason behind its literature of review was done thoroughly. After literature of review, it was understood that calcium is crucial for Snail growth, reproduction and shell formation. So, focusing on calcium, Alizarin Red staining and EDTA method was used for the detection the calcium in leaves of *C. gigantia* L. and *B. cristata* L. Surprisingly, results were exhibited that *C. gigantia* L. (0.5g/g leaf tissue) and *B. cristata* L. (0.3g/g leaf tissue) are good source of calcium. Hence, this paper first time reported that calcium is the primary target behind the attack of Snail on Medicinally Important *Calotropis gigantia* (L.) and *Barleria cristata* L. in Western Ghat Region of Maharashtra, India.

**Keywords:** Snail, *calotropis gigantia*, *barleria cristata*, medicinal plant, pest, western ghat, calcium

### Introduction

Ratnagiri District is a biodiversity rich site of Konkan region situated at foot of Western Ghat region of Maharashtra, India. *Calotropis gigantia* L. (Family - Apocynaceae) and *Barleria cristata* L. (Family - Acanthaceae) are an important medicinal plant commonly found in this region. Species *B. cristata* L. (Family Acanthaceae) is a well-known perennial, Ayurvedic herb has been utilized for the treatment of a toothache, catarrhal affections, urinary infection, jaundice, fever, gastrointestinal disorders, whooping cough, inflammations, glandular swellings, and as diuretic and tonic without any toxic effects. Similarly, species *C. gigantia* L. (Family Apocynaceae) commonly called as "crown flower" used in folk medicine preparation. It shows various pharmacological activities such as analgesic activity, pregnancy prevention, CNS activity, antipyretic activity, anti-diarrheal activity, anti-inflammatory activity, procoagulant activity, free radical scavenging activity, antimicrobial activity, anti-tumor, antifungal, antitussive, and antifeedant activity (Kumar et. al. 2011).

In previous study it has been reported that during the rainy season high destructive behavior of pest snail observed on *B. cristata* L. and *C. gigantia* L. Severe leaf biomass loss was observed in both plants. Hence to understand the reason behind snail attack, review of literature was done thoroughly. After reviewing of literature, it has been understanding that calcium is crucial for Snail growth, reproduction and for shell formation. So, focusing on calcium, Alizarin Red staining method used for detection of calcium in leaves of *C. gigantia* L. and *B. cristata* L.

### Material and Method

#### Study area and Plant material

Occurrence of Snail on *B. cristata* L. and *C. gigantia* L. leaves is seasonal. Mainly rainy season is good for snail growth and development. Therefore, monsoon season (June

to September) and three districts of Maharashtra namely Ratnagiri, Raigad and Sindhudurg were selected as study area. Fresh Leaf petiole, T.S. of leaves and petiole of both plants used for calcium detection.

#### Alizarin Red Staining and EDTA method used for Calcium Detection

2% Alizarin Red Staining method (Buchholz, et.al.2023) [1] was used for staining T.S. of leaf of plants to confirm the calcium. For estimation of calcium content from the leaves of plants EDTA method was used (Harold L. Barrows, Elmer C. Simpson, 1962) [7]. With help of mortar and pestle 1gm of leaf sample was crushed into 10 ml of dil. H<sub>2</sub>O. Then crude extract was filtered and diluted into dil. H<sub>2</sub>O to make its final volume 400ml. From that 10ml was used as sample for experiment. 1ml of alkaline buffer added in flask containing 10ml of sample and mixed properly with help of glass rod. Then added tiny pinch of Eriochrome balck T as indicator and again stirred mixture with the help of glass rod to develop wine red colour. After titration of mixture with 1N Na<sub>2</sub>EDTA Wine red colour to pale blue colour considered as endpoint and burette reading was used for calculation of Calcium content. Experiment was repeated three times with same results. Each experiment was done separately for both *B. cristata* L. and *C. gigantia* L. plants.

#### Result and Discussion

In previous study, due to snail attack huge biomass loss was observed in both *B. cristata* L. and *C. gigantia* L. from three selected districts namely Ratnagiri, Raigad and Sindhudurg (Fig.1). During Monsoon season, due to heavy rain low temperature and high percentage of humidity present in the environment favours the snail growth and development. After literature of review it was understood that calcium is the basic requirement of snail for their shell development and reproduction. Marxen et.al. (2003) [9] was reported in

their study molluscan shells consist of very large amount of calcium carbonate i.e. 95-99.9% by weight. Focusing on calcium as essential element in snail diet, 2% Alizarin Red stain (Buchholz, et.al.2023) [1] and EDTA method (Harold L. Barrows, Elmer C. Simpson, 1962) [7] was used for calcium detection in both the plants. T.S of leaves of both the plants was stained with 2% Alizarin Red stain and observed under the compound microscope at 10X

magnification. Presence of Red coloured calcium oxalate at epidermal region clearly indicated that calcium was present in the leaves of both plants (Franceschi and Nakata, 2005) [4]. Parenchymatous cells also showed presence of calcium in it. Milky latex of leaf petiole of *C. gigantia* L. was also used to test the calcium. Immediate conversion of Milky latex into dark red colour confirmed presence of calcium in it (Fig.1 & Fig.2).



**Fig 1.** Alizarin red staining method use for *C. gigantia* L. leaves: A. & B. Snail feeding on *C. gigantia* L. leaf C. and D. Milky latex turns in red, E.T.S. Petiole of *C. gigantia* L, F.T.S. of leaf of *C. gigantia* L.



**Fig 2.** Alizarin red staining method use for *B. cristata* L. leaves: A. & B. Snail feeding on *B. cristata* L. leaf C. Petiole of *B. cristata* L turns in red colour, D. T.S. of leaf of *B. cristata* L

These results strongly supported calcium is the primary target behind the attack of snail on these plants. Snail mainly feed on leaves of plants only, so leaf extract of both plants were used to find the amount of calcium present in it. EDTA method was used for that. As per results, high calcium content i.e. 0.5g/g of fresh leaf tissue in *C. gigantia* L. plants while in 0.3g/g of fresh leaf tissue in *B. cristata* L. recorded. Hence, this results again strongly supports the primary aim of pest snail attack on these plants was the calcium. Several Studies on snail reported that they required large quantity of calcium for their growth and reproduction

(Wagge, 1952; Crowell, 1973) [12, 3]. Particularly, shell of snail build from calcium protects the snail's internal organs, prevent water loss, provide shelter from the cold, and protect from predators and microorganisms. Snails regularly feed on soil possibly to extract calcium (Crowell, 1973; Gomot *et al.*, 1989) [3, 5]. Tropical snails mostly grow to a larger size due to calcium-rich soils (Owen, 1965) [11]. The Calcium content in their diet influences the snails' nutritional preferences (Chevalier,2000) [2], and its availability can be constrained by shell growth (Gouveia, 2011) [6]. Calcium deficiency can retard snail growth and

render their shells more susceptible to damage. Western Ghat region of Maharashtra contain red laterite soil which is nutrient deprived soil. In laterite soil percentage of calcium ion is very low as compared to black soil and not able to fulfil the requirement of snail but plants like *C. gigantea* L. and *B. cristata* L. are the good source for calcium now become primary target of pest snail. Actually, this study is at primary level work, further there is need to work on it specially on how snail tolerate to the toxic substance of *C. gigantea* L. because many herbivores avoid feed on this plant due its toxicity.

### Conclusion

Occurrence of Snail attack on leaves of *C. gigantea* L. and *B. cristata* L. is not the coincidence because due lack of calcium content in soil as well as not enough calcium present in surrounding plants exhibit that for survival reason they are highly depends on these plants.

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