Research Article

Phytochemical Screening and Variation Studies in Secondary Metabolite Contents in Rhizomes of *Curculigo orchioides* from Madhya Pradesh State of India

Hari Om Saxena*, Samiksha Parihar, Ganesh Pawar, G. Rajeshwar Rao, Hemant Kumar and Sant Kumar

Tropical Forest Research Institute, Jabalpur (M.P.) - 482021, India

Abstract

The phytochemicals can be broadly classified into primary and secondary metabolites. The medicinal properties of the plants are contributed due to the secondary metabolites such as phenols, tannins, alkaloids, flavonoids, saponins, cardiac glycosides etc. In the present investigation, phytochemical screening and quantification of secondary metabolites in rhizomes of *Curculigo orchioides* collected from 11 medicinal plants conservation areas (MPCAs) of Madhya Pradesh state were carried out. Preliminary phytochemical screening exhibited mainly the presence of alkaloids, flavonoids, phenols and tannins which were quantified using standard quantification procedures. The results showed the presence of alkaloids, flavonoids, phenols and tannins in the range of 3.66 - 10.82 mg CE/ g dry extract wt, 2.34 – 8.44 mg QE/ g dry extract wt, 11.73 – 54.92 mg GAE/ g dry extract wt and 8.12 – 14.83 mg TAE/ g dry extract wt respectively. The phenolic content in the samples was found to be highest amongst all the other phytochemicals reported.

Keywords: *Curculigo orchioides*, Phytochemical screening**,** Secondary metabolites, Madhya Pradesh

***Correspondence**
Author: Hari Om Saxena
Email: hariomsaxena81@gmail.com

Introduction

Among the myriad of properties that the plants possess, one of the most essential characteristics that it is known for is its medicinal properties. Plant-derived medicines have long been known to be a part of humankind especially being used in India and China. The indigenous people of the rest of the world are also known to have been using plant-based medicines to treat various kinds of diseases. Approximately 80% of the world's population prefers plant-based medicines as their first line of defence to maintain health and combat various diseases [1]. India is considered as one of the richest countries in terms of genetic diversity of medicinal plants, which is majorly contributed due to its wide topography and climate [2]. The medicinal value of these plants is contributed due to by some chemically active substances such as alkaloids, flavanoids, tannins, terpenoids, saponins, phenolic compounds, cardiac glycosides etc. which form the backbone of modern system of medicines [3]. These chemically active substances are the secondary metabolites or phytochemicals which contribute to the ethnomedicinal properties of the plants [4].

Curculigo orchioides also known as Golden eye grass or *Kali musli* is a perennial herb with tuberous rhizome belonging to the family Hypoxidaceae. *C. orchioides* has long been known for its miraculous medicinal properties such as treating bronchitis, chronic cough, asthma, and hepatitis; it also acts as an appetite stimulant and regulates gastrointestinal function [5]. The plant is the source of curculigosides which are bioactive phenolic compounds derived from *C. orchioides* that have antioxidative, anti-inflammatory, antidepressant, and antiosteoporotic actions and can be utilized to treat several disorders including osteoporosis and rheumatoid arthritis [6, 7].

Madhya Pradesh, a central Indian state, is a storehouse of vast medicinal plant resources. The state comprised of 11 agro-climatic regions, variation in phytochemical components of medicinal plants is obvious. As far as *C. orchioides* is concerned, the scientific validation and quality standardization of this prestigious species in terms of secondary metabolites concentration is still needed. The present study dealt with the variations in the secondary metabolite contents in rhizomes of *C. orchioides* collected from 11 different locations of Madhya Pradesh state through qualitative and quantitative analysis.

Experimental

Fresh, healthy and mature rhizomes of *C. orchioides were* collected from 11 MPCAs belonging to different agro-climatic regions of Madhya Pradesh state of India (**Figure 1**). To remove soil and other extraneous particles, the rhizomes were thoroughly washed in water. These were cut into small pieces, initially dried in sun and then in shade before drying in a hot air oven at 40ºC. The dried rhizomes were ground into powder and used to make extracts. The

major equipment used for the study was UV-VIS Spectrophotometer and all the chemicals used were of AR grade.

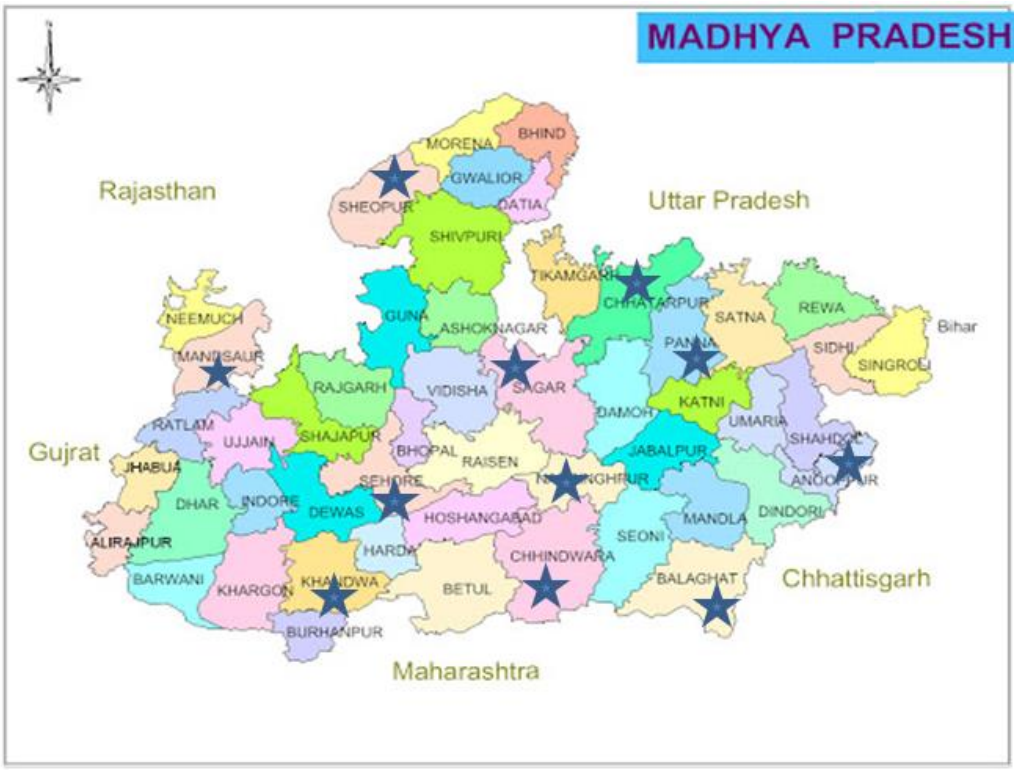

Figure 1 Collection sites of rhizomes from 11 MPCAs of Madhya Pradesh

Preparation of extracts for phytochemical screening

100 mg of dried and powdered rhizomes were soaked for overnight in 25 ml of methanol, ethanol, chloroform and ethyl acetate solvents separately. The filtrates from various extracts were employed for qualitative phytochemical analysis.

Preliminary phytochemical analysis

The preliminary phytochemical analysis of crude extracts of *C. orchioides* rhizomes was carried out according to Harborne (1998) [8] and Trease and Evans' methods (1989) [9].

Quantitative estimation of secondary metabolites

Estimation of total alkaloid content (TAC)

Total alkaloid content in the powdered samples was measured using the 1,10–phenanthroline method with minor modifications. 100 mg powdered sample was extracted in 10 ml 80% ethanol. This was then filtered through muslin cloth and centrifuged for 10 minutes at 5000 rpm. The supernatant obtained was used for further estimation of total alkaloids. The reaction mixture contained 1 ml of extract, 1 ml 0.025 molar $FeCl_3$ in 0.5 molar HCl, and 1 ml 0.05 molar 1, 10 – phenanthroline in ethanol. In a hot water bath with a temperature of 70±2°C, the mixture was incubated for 30 minutes. The absorbance of the red-colored complex was measured at 510 nm against the reagent blank. The standard curve of Colchicine (**Figure 2**) was used to determine the TAC, expressed as milligram of colchicine equivalent per gram of dry extract weight (mg CE/ g dry extract wt) [10].

Estimation of total flavanoids content (TFC)

The total flavonoids were determined using a colorimetric method. A sample of 0.5gm was weighed and maintained for 24 hours in 95% ethanol. It was then filtered and volume was made up to 25 ml with 80% ethanol. A quantity of 0.5 ml of filtrate was then mixed with 1.5 ml of 95 % ethanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of potassium acetate, and 2.8 ml of distilled water. After that, the tubes were incubated for 30 minutes at room temperature. Observations were taken at 415 nm. The standard curve of quercetin (**Figure 3**) was used to determine the TFC and was expressed as milligram of quercetin equivalent per gram of dry extract weight (mg QE/ g dry extract wt) [11].

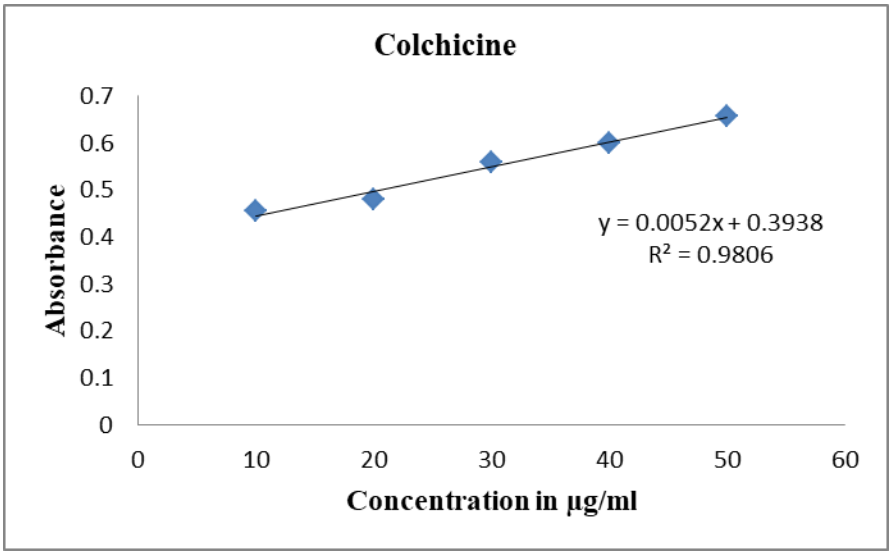

Figure 2 Standard graph of Colchicine standard

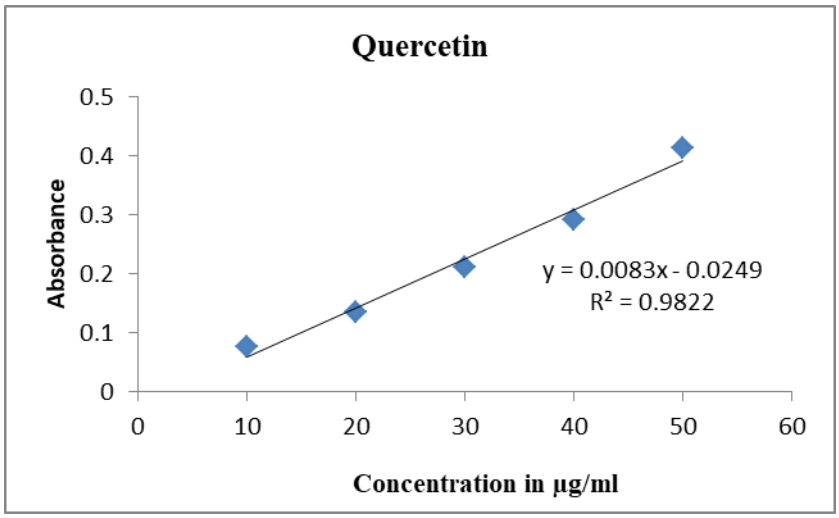

Figure 3 Standard graph of Quercetin standard

Estimation of total phenol content (TPC)

The total phenolic content of *C. orchioides* was determined using the Folin-Ciocalteau method. 0.5g of the sample was ground in mortar and pestle using 80% ethanol. The slurry was centrifuged at 10,000 rpm for 20 mins. The supernatant was then evaporated until it was completely dry. In a known volume of distilled water, the residue was dissolved. After that, 0.2 mL of sample was placed in a test tube and the volume was increased to 3 mL with distilled water. After that the Folin Ciocalteau reagent (0.5 mL) was added. After 3 minutes, each tube received 2 ml of 20% Na_2CO_3 solution, which was properly mixed before being immersed in boiling water for exactly 1 minute, cooled, and absorbance measured at 650 nm against a blank. The standard curve of gallic acid (**Figure 4**) was used to determine the TPC and was expressed as milligram of gallic acid equivalent per gram of dry extract weight (mg GAE/g dry wt) [12].

Estimation of total tanin content (TTC)

The Folin-Ciocalteu method was used to determine the tannins. A volumetric flask (10 ml) was filled with 0.1 ml of the sample extract, 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 percent sodium carbonate solution, and 10 ml of distilled water. The liquid was thoroughly mixed before being stored at room temperature for 30 minutes. The same procedure was used to generate a set of tannic acid reference standard solutions (100, 200, 300, 400, 500 ug/ml). UV/ Visible spectrophotometer was used to measure the absorbance of test and standard solutions against a blank at 700 nm. The standard curve of tannic acid (**Figure 5**) was used to determine the TTC and was expressed as milligram of tannic acid equivalent per gram of dry extract weight (mg TAE/g dry wt) [13].

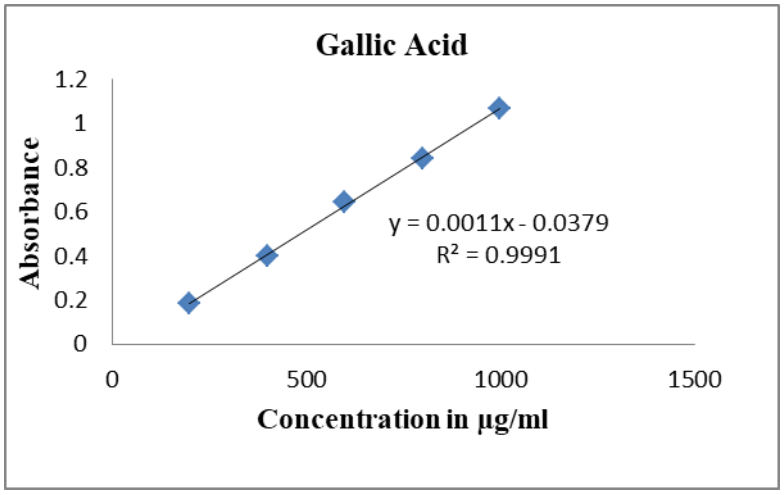

Figure 4 Standard graph of Gallic acid standard

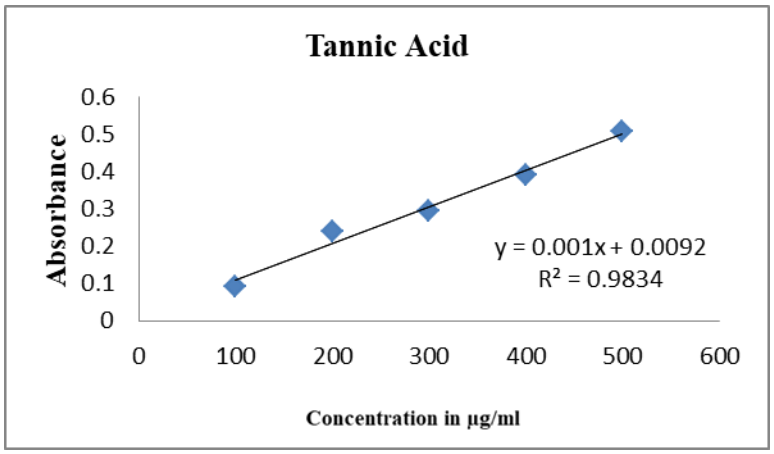

Figure 5 Standard graph of Tannic acid standard

Statistical analysis

The results are expressed as Mean±SD. One-way ANOVA was used to analyse the level of statistical significance between groups. P≤0.05 was considered statistically significant.

Results and Discussion

The rhizomes of *C. orchioides* have been long employed in India's traditional medicinal systems, therefore the study was aimed towards the quantification of secondary metabolites in these tuberous parts. The phytochemical screening of extracts helped in determining the most effective extracts and therapeutic properties of the plant. The presence of flavonoids, tannins, alkaloids, terpenoids, phenols, and cardiac glycosides indicates towards both physiological as well as therapeutic properties of the rhizomes of this plant [14]. **Table 1** summarises the preliminary phytochemical qualitative analysis of the tuberous rhizome of *C. orchioides* with different solvents used for extraction. The prime phytochemicals like, alkaloids and phenols were found in all the extracts. Flavonoids and tannins were present in the extracts of methanol, ethanol, and chloroform but were found to be absent in ethyl acetate whereas cardiac glycosides were found to be present in chloroform and ethanol extracts only. Saponins and steroids were completely absent in all the extracts. In earlier study, methanolic extract exhibited the presence of flavonoids, phenols, glycosides, saponins, terpenoids and sterols while the absence of alkaloids and tannins [15]. The absence of alkaloids and phenols is in contradiction with our results. In another study carried out Jagtap et al., presence of alkaloids in methanolic extract while absence of saponins in chloroform extract is in agreement with our results [16].

The quantitative estimation value of secondary metabolites in rhizomes is given in **Table 2** and **Figure 6**. Variability of phytochemicals was observed in different agro-climatic zones in the current study**.** Alkaloid content was estimated to be present in the highest and lowest amount from the sample of Kupi Khori (1.082±0.011) and Kalibhit (0.210±0.064) respectively. The highest content of flavonoid was found in the sample from Kupi Jatashanker (0.844±0.049) whereas the lowest was estimated from the sample of Pakka Parcha (0.191±0.053). When phenol was

taken into consideration, the samples taken from Bhaisamukunda (7.660±0.079) was noted to have the highest TPC and the lowest was found in the sample from Pakka Parcha (0.191±0.053)**.** The highest content of tannins was observed in the sample from Pakka Parcha (1.532±0.091) and the lowest from the sample from Narayanpur (0.780±0.044). The phytochemical quantification does not exhibit a particular trend, such as the rhizomes from Kupi Jatashanker reflects the highest TFC and TAC amongst all other samples but no such trend can be seen concerning TPC and TTC. Also, the sample from Pakka Parcha shows the highest TTC but also shows the lowest TFC out of all the samples. This is the first study showing the variations in secondary metabolites from different agroclimatic regions. Earlier work was confined to variations in secondary metabolite concentrations (alkaloids and phenols) with different months and environmental conditions, revealed September the best month for maximum contents of both phytochemicals [17].

Table 1 Preliminary phytochemical screening of *C. orchioides*

S. No.	Phytochemical constituents	Chloroform extract	Ethanol extract	Ethyl acetate extract	Methanol extract
1	Alkaloids	++	++	++	++
2	Cardiac glycosides	+	-	-	-
3	Flavonoids	++	++	-	++
4	Phenols	++	+++	+	+++
5	Saponins	-	-	-	-
6	Steroids	-	-	-	-
7	Tannins	++	++	-	++
8	Terpenoids	+	-	-	+

(+) = present and (-) not detected

Table 2 Quantitative phytoconstituent analysis of rhizomes in *Curculigo orchides*

Agroclimatic regions	Districts	Name of MPCAs	TAC (mg CE/ g dry wt)	TFC (mg QE/ g dry wt)	TPC (mg GAE/ g dry wt)	TTC (mg TAE/ g dry wt)
Northern Hills Region of Chhattisgarh	Anuppur	Amarkantak	4.22±0.04^{a}	5.24±0.03^{a}	50.13±1.33^{a}	12.17 ± 0.01^{a}
Central Narmada Valley	Narsinghpur	Bhaisamukunda	8.5±0.05^{b}	7.92±0.03^{b}	76.6±0.08^{b}	14.83 ± 0.13^{b}
Bundelkhand	Chhatarpur	Kupi Jatashanker	9.34 ± 0.08^{c}	8.44±0.05^{c}	54.92±0.03^{c}	11.35 ± 0.06^{c}
Satpura Plateau	Chhindwara	Kapoornala	3.69 ± 0.06^{d}	4.91±0.04^{d}	39.78±0.37^{d}	11.7 ± 0.06^{d}
Nimar Plains	Khandwa	Kalibhit	2.1 ± 0.06^{e}	3.92±0.04^{e}	49.3 ± 0.26^{e}	8.3 ± 0.03^{e}
Malwa Plateau	Mandsour	Naweli	5.71 ± 0.13^{f}	7.08 ± 0.09^{f}	50.07 ± 0.67^{f}	13.52 ± 0.38^{f}
Vindhyan Plateau	Sagar	Narayanpur	5.56 ± 0.05^{g}	3.4 ± 0.05^{g}	15.88 ± 0.19^{g}	7.8 ± 0.04^{g}
Kymore plateau & Satpura Hills	Panna	Shyamagiri	3.66 ± 0.07^{h}	2.34 ± 0.04^{h}	17.62 ± 0.26^{h}	10.52 ± 0.09^{h}
Grid region	Sheopur	Khori	10.82±0.01^{i}	3.62±0.03^{i}	12.92±0.26^{i}	8.12±0.05^{i}
Central Narmada Valley	Hoshangabad	Pakka Parcha	7.15 ± 0.15^{j}	1.91 ± 0.05^{j}	8.68 ± 0.07^{j}	15.32 ± 0.09^{j}
Chhattisgarh Plains	Balaghat	Bithli	9.08 ± 0.14^{k}	4 ± 0.04^{k}	11.73 ± 0.06^{K}	11.18 ± 0.01^{k}

Mean values within each column represented by different letters differ significantly (P≤ 0.05)

Phytochemical compounds though themselves do not provide nutrition to the plant but aid the plant with the defence mechanism and these phytochemicals when used on humans to treat various ailments produce phenomenal results. Cancer, cardiovascular problems, neurogenerative disorders, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral, and parasitic infections, psychotic diseases, spasmodic conditions, ulcers, osteoporosis, and other diseases have all been reported to be successfully treated with the use of various bioactive compounds [18]. The preliminary phytochemical screening aids in isolating and characterizing the chemical constituents present in plant extract and gives knowledge of chemical constituents of plants necessary for understanding herbal drugs and their preparation, as well as for determining the true value of folk medicines [19]. Madhya Pradesh has a great variation in the temperature and rain fall patterns in different seasons, along with other

environmental and climatic fluctuation. The variation in the phytochemical contents observed in the present study may be due to the effect of the environmental factors such as rainfall, humidity, light intensity, drought, temperature, high salinity, and minerals as reported in earlier studies [20-22]. Stressed conditions are also known to trigger the content of phytochemicals in a plant, as they help the plant to adapt and survive in distressing circumstances [23, 24].

Previous studies have also referred to such variations in phytochemicals in samples of *Solaum indiucm* [25], *Uraria picta* [26], *Gloriosa superba* [27], and *Hemidesmus indicus* [28] that were collected from varied agro-climatic regions of central India.

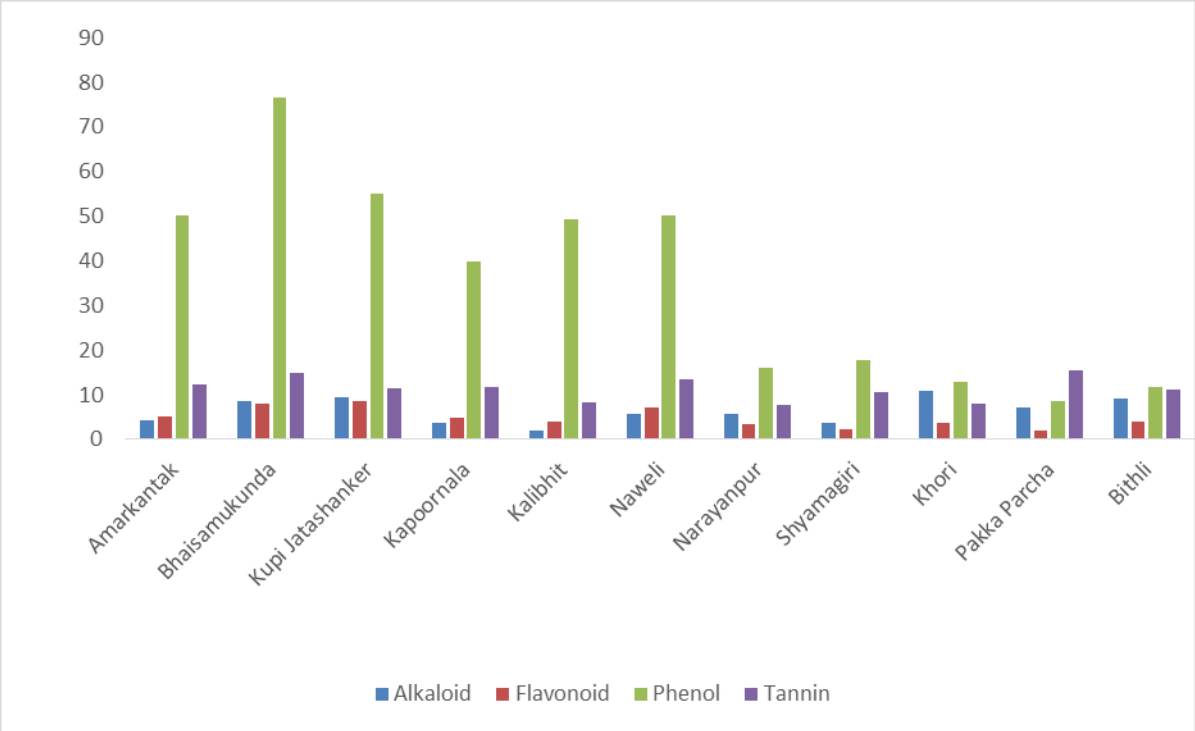

Figure 6 Representation of estimated secondary metabolites

Conclusion

Secondary metabolites are the vital plant components that contribute towards the therapeutic properties. The study reveals that total phenolic content is more abundant in the rhizomes of *C. orchioides* than the other phytochemicals. The presence of the specific group of compounds shows specific medicinal actions of this species. However, further work may be carried out to isolate, characterize and identify the particular compounds responsible for drug development.

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