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Letter to the Editor

Evaluation of quantitative variation of secondary metabolites in *Bergenia ciliata* (Haw.) using high performance thin layer chromatography

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Dear Editor:

Therapeutically active metabolite contents in a medicinal plant vary in nature, which may impact on its therapeutic efficacy. *Bergenia* (*Saxifragaceae*) is an evergreen perennial herb widely distributed in Central and East Asia with about 30 species reported worldwide. It grows at a range of altitudes from the Khasia hills at 400 feet to the temperate Himalayas from Kashmir to Bhutan at 7,000–10,000 feet[1]. Its distribution over a wide range of altitudinal zones makes it a good candidate for studying variations in its metabolic profiles under different climatic conditions. Bergenin (*C*-glycoside of 4-*O*-methyl gallic acid) has been identified as a potent active secondary metabolite in *Bergenia* and other therapeutically active constituents including, among others, gallic acid (3,4,5 trihydroxybenzoic acid), (+) catechin, and gallicin (***Fig. 1***). Here, we analyzed three accessions of *Bergenia ciliata* collected from different altitudes of Uttrakhand, India, to study the quantitative variation in bergenin, gallic acid, (+) catechin, and gallicin by high performance thin layer chromatography (HPTLC). For accurate quantification, different hydrolysis conditions were employed and their effects on individual compound were assessed.

We found significant variations in the content of these compounds in the collected rhizomes of *Bergenia ciliata* ranging from 0.756 ± 0.3 to 0.85 ± 0.2 for bergenin, 0.145 ± 0.2 to 0.27 ± 0.2 for (+) catechin, 0.027 ± 0.2 to 0.166 ± 0.3 for gallic acid and 0.135 ± 0.3 to 0.186 ± 0.1 for gallicin. Such variability is expected due to variation in environmental conditions which varies on varying altitudes. ***Fig. 2*** illustrates the effect of climatic and altitudinal variations on the distribution of these four compounds in *Bergenia* rhizomes, showing that even a small altitudinal variation is capable of inducing quantitative variation in the contents of these compounds. It has been established that biosynthesis of polyphenolic compounds increases on higher altitude in response to lower environmental stress. But the present study revealed that it was not the case for every phenolic compound. Bergenin content showed no significant positive correlation with altitude while gallic acid showed significant correlation with altitude. However, it is difficult to judge whether this is due to a significant shift in the biosynthetic pathways or whether it is merely a mathematical artifact resulting from the fact that one variable varies with altitude, whereas another does not.

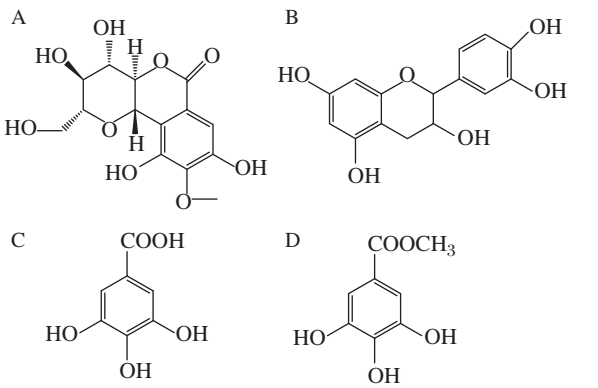

Fig. 1 Chemical structure of A: Bergenin; B: (+)Catechin; C: Gallic acid; D: Gallicin.

Phenolic compounds exist in glycoside and aglycone forms[2]. In most cases, the aglycone form is absorbed at a greater rate and has higher antioxidant activity than the glycoside form[3–6]. Hydrolysis of flavonol glycosides to their corresponding aglycones offer a practical method for the quantification of flavonoids in foods[7]. Hydrolysis is also used for quantification of phenolic acids in fruits or other plant part[8]. We exposed *Bergenia* extract to different hydrolysis conditions.

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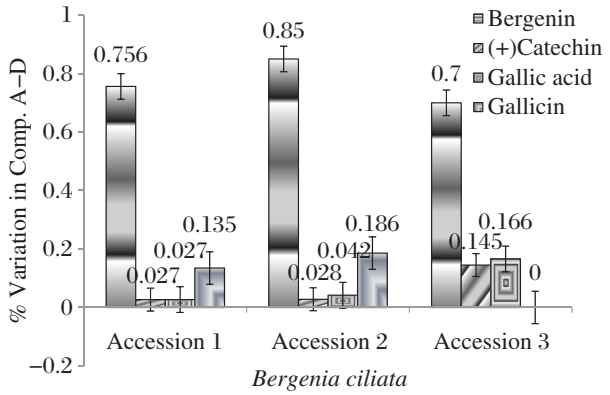

Fig. 2 Altitudinal variation in bergenin, (+) catechin, gallic acid and gallicin

Fig. 3 shows concentration variations of the four compounds under the different hydrolysis conditions. (+) Catechins are unglycosylated and naturally occur in the aglycone form. Catechin hydrolysis is pH dependent and it decomposes very rapidly in alkaline and neutral solution while remaining stable in acidic condition[9]. Processing methods of catechin rich food and beverages cause catechin degradation; acid hydrolysis may be recommended. Hydrolysis contributes not only to the cleavage of glycosides into their corresponding aglycones but also prevents their decomposition into other moieties. Bergenin is enriched in the hydrolyzed extract, suggesting that bergenin may be present in the *O*-glycoside form. These glycosides are cleaved into aglycone and aglycone moiety in a pH dependent manner. Free aglycone moiety enhances the content of naturally occurring bergenin aglycone content. Hydrolysis is also important in enrichment of biologically active aglycone from their natural glycosides at the industrial level. Gallic acid is simultaneously converted into gallicin (methyl ester of gallic acid) and gallicin into gallic acid. Among all hydrolysis conditions, acid hydrolysis is overall valuable; it prevents decomposition of (+) catechin and cleavage of bergenin glycosides into their bergenin aglycone and simultaneously esterifies gallic acid into gallicin.

In conclusion, understanding variations of therapeutically active metabolite contents in medicinal plants in nature are for better utilization of natural resources. High bergenin, (+) catechin, gallic acid and gallicin bearing rhizome environmental conditions, which accelerate the biosynthesis of these compounds, need to be conserved for better utilization. Altitudinal varia–

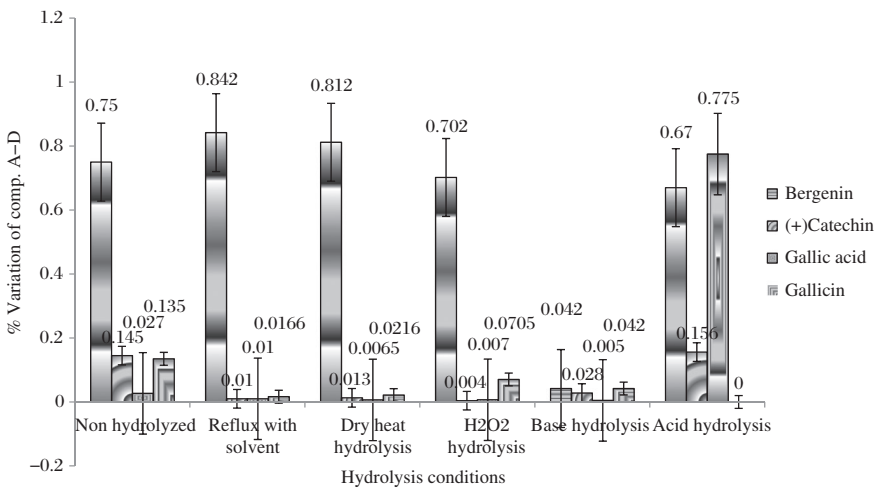

Fig. 3 Variation in bergenin, (+) catechin, gallic acid and gallicin quantity induced by different hydrolysis conditions

Table 1 **Geographical details of *Bergenia ciliata* collection**

Accession	Region explored	Collection stage	GPS information	Material
1	Taluka and Osla Uttrakhand	Pre-flowering	2355 m, 31°06′ 15.04″N, 78° 18′11.00″ E	Whole plant
2	Taluka and Osla Uttrakhand	Pre-flowering	2360 m, 31°05′ 56.36^{Nn}, 78°17′ 35.94 E	Whole plant
3	Taluka and Osla Uttrakhand	Pre-flowering	2366 m, 31°06′ 22.6″N, 78.18′ 45.4° E	Whole plant

Table 2 **Statistical analysis of calibration curves in HPTLC determination of bergenin, gallic acid, (+) catechin, and gallicin**

Parameters	Bergenin	Gallic acid	(+) Catechin	Gallicin
Accuracy	100.030 ± 0.323	99.96 ± 2.13	100.06 ± 4.10	99.54 ± 3.64
Rf value	0.42	0.78	0.84	0.91
Slope	18.34	17.69	11.04	4.439
Intercept	-8052	-3268	-739.3	282.5
Linearity range	600–800 ng/mL	300–550 ng/mL	350–550 ng/mL	300–500 ng/mL
Correlation coefficient (r)	0.998	0.993	0.991	0.996
95% confidence limits of intercept	-6786.247294	-1234.77789	266.55	569.69
Correlation coefficient (r)	0.998	0.993	0.991	0.996
LOD	128.43	176.31	105.02	99.24
LOQ	389.2	267.15	318.25	300
SE of intercept	356.9	472.59	233.91	66.74
SD of intercept	713.8	945.18	467.83	133.49
P-value	0.0013	0.0202	0.0202	0.0515

tion correlation patterns in more phenolic compounds in a larger population of *Bergenia* samples needs to be further studied. Neutral hydrolysis is the optimum condition to increase the aglycone content of bergenin in *Bergenia ciliata*, but this will lead to decomposition of (+) catechin. On the other hand, acid hydrolysis is the best hydrolysis method as it not only increases aglycone content but also prevents decomposition of (+) catechin.

Collection conditions of rhizomes of *Bergenia ciliata* are shown in ***Table 1*** and HPTLC data are provided in ***Table 2***. Detailed methods are available from authors upon request.

Yours Sincerely,

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