

RESEARCH ARTICLE

Total flavonoid concentrations of bryophytes from Tianmu Mountain, Zhejiang Province (China): Phylogeny and ecological factors

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Abstract

The flavonoids in bryophytes may have great significance in phylogeny and metabolism research. However, to date there has been little research on bryophyte metabolites, especially flavonoids. To redress this somewhat, we determined flavonoid concentrations of bryophytes from Tianmu Mountain through a colorimetric assay and considered the factors influencing the results. This is the first time that the flavonoid contents of bryophytes have been examined in detail. The results revealed a range of total flavonoid concentrations in 90 samples collected from Tianmu Mountain from 1.8 to 22.3 mg/g (w/w). The total flavonoid contents of liverworts were generally higher than those of mosses; acrocarpous mosses had generally higher values than that of pleurocarpous mosses. The total flavonoid contents of bryophytes growing at lower light levels were general higher than those growing in full-sun. The total flavonoid contents of epiphytic bryophytes were highest, while those of aquatic bryophytes were the lowest. Total flavonoid contents of species growing at low-latitudes were much higher than those at high-latitude individuals. In conclusion, total flavonoid contents of bryophytes have some connection with plant phylogeny; more flavonoids might be contained in relatively primitive bryophytes. Meanwhile, the effects of ecological factors on total flavonoid contents of bryophytes exist; light and habitat (especially tree habitat and river habitat) might be representative factor.

Introduction

Flavonoids, representing one type of plant secondary metabolites with over 10,000 known structures [1–2], are not only vital for plant growth and development [3], but also play an important role in the prevention and management of modern diseases [4–8]. Flavonoids are not restricted to vascular plants, but can also be found in bryophytes [9]. However, most flavonoid research has focused on the former, whilst bryophytes have only been sporadically studied [10–11].

Bryophytes, the oldest group of terrestrial plants [12], have experienced over 400 million years of extreme climatic change. Bryophytes are second only to angiosperms, within the kingdom Plantae, in the number of species in the group. Bryophyte flavonoids are of great significance in research on phylogeny and metabolism [13–14]. Extracts from bryophytes which contain flavonoids have been investigated extensively for their potential pharmacological applications: cytotoxic, anticancer and antitumor [15–17], antifungal [18–19], antibacterial [20–21] and antioxidant activities, and their ability to inhibit AChE activity [22].

Due to their small size, it is difficult to collect bryophyte samples in the field that are large enough for chemical experiments. Research on the chemical composition of bryophytes, especially flavonoids, is relatively rare. Globally, there are approximately 23 000 species of bryophytes, with 3021 species in China, of which about 50 species have been used in traditional Chinese medicine [23]. To date, flavonoids had been reported from only 1.4% of bryophyte species in China.

Tianmu Mountain (Tianmu Mountain National Nature Reserve, 30°18'30"-30°21'37"N, 119°24'11"-119°27'11"E) is located in Lin'an city, Zhejiang Province, China. The climate represents a transition from the mid-subtropics to the northern subtropics [24]. Sixty-seven species of liverwort, belonging to 32 genera and 24 families, and 220 species of moss, belonging to 152 genera and 65 families have been reported on Tianmu Mountain, growing under various environmental conditions [25]. Prior to this work, there had been no studies of the flavonoids in the bryophytes growing at the site.

This study specifically aims to address the following questions (1) what about the flavonoids of bryophytes from Tianmu Mountain? (2) Which factors could impact total flavonoid concentrations?

Materials and methods

Plant materials

The names of the authority who issued the permission for the two locations were Tianmu Mountain National Natural Reserve and E'erguna National Natural Reserve. Ninety samples, from 61 species and 31 families in [S1 Table](#) were collected from the Tianmu Mountain National Natural Reserve, Zhejiang Province (China) during April, June and July of 2013. Twenty-three samples, from 20 species and 9 families in [S2 Table](#) were collected from the E'erguna National Natural Reserve, Inner Mongolia (China) during July of 2013. The specimens were collected and identified by Yuhuan Wu who was familiar with bryoflora in Zhejiang Province. Voucher specimens were deposited in the College of Life & Environmental Science, Hangzhou Normal University (HTC).

Chemicals and reagents

Details were presented in our previous report [20].

Preparation of plant extracts

Fresh and undamaged plants were collected and stored for analysis. After being washed and dried in the shade, samples were further dried at 75°C for 2 days and then ground up. A portion of each dried sample (1.00 g) was extracted with 60% ethanol (25 mL) at 50°C for 2 h, followed by ultrasound-assisted extraction for 20 min. This process was performed twice. The extracts were filtered, and the volume of the solution kept constant at 50 mL.

Determination of total flavonoid content

The total flavonoid concentrations were measured as described in our previous report [20]. A colorimetric assay was used to determine the total flavonoid content. The calibration curves for rutin would give A, B and r^2 at 510 nm (OD). With the same way as rutin, total flavonoid contents of samples were determined. The formula was used as follows: total flavonoid content (mg/g) = $[(OD_1+OD_2+OD_3)/3-A]/B * 10/2 * volume/100 * 100\%$

Statistical analysis

Statistical analysis was undertaken using R software, SPSS and Origin 7.5. Datas were reported as the mean of three independent samples.

Results and discussion

Total flavonoid content in 60% ethanol extracts from bryophytes of Tianmu Mountain

The total flavonoid concentrations found in the collected bryophytes were expressed as rutin equivalents in mg/g (w/w) and summarized in S1 Table. The range of total flavonoid concentrations in the 90 samples collected from Tianmu Mountain was from 1.8 to 22.3 mg/g (w/w). *Bazzania tridens* (Reinw., Blume & Nees). exhibited the highest total flavonoid content (22.3 mg/g), and *Hypnum oldhamii* (Mitt.) Jaeg. the lowest (1.8 mg/g). Thus, the total flavonoid content of *B. tridens* was about 12 times that of *H. oldhamii*. Figs 1–3 showed the concentrations for: the 34 samples for which the total flavonoid concentration was more than 10.0 mg/g; the 20 samples with concentrations between 5.0 and 10.0 mg/g; and the 36 samples with concentrations less than 5.0 mg/g. The total flavonoid contents of 62% of bryophytes from Tianmu Mountain were less than 10.0 mg/g (Fig 4).

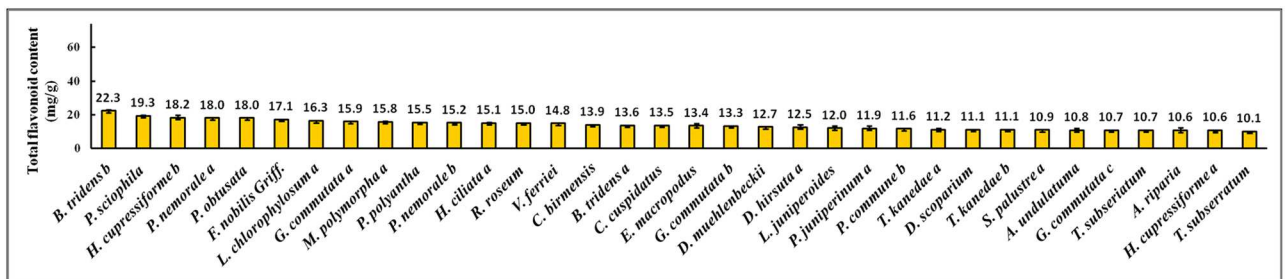


Fig 1. Bryophyte samples with total flavonoid concentration higher than 10.0 mg/g.

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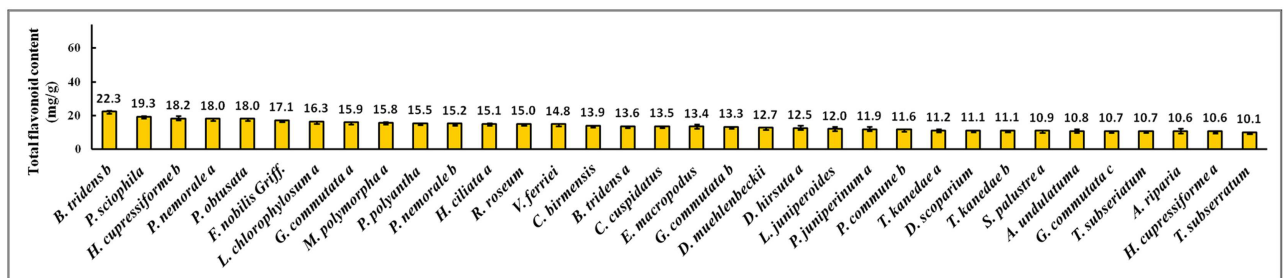


Fig 2. Bryophyte samples with total flavonoid concentration between 5.0 and 10.0 mg/g.

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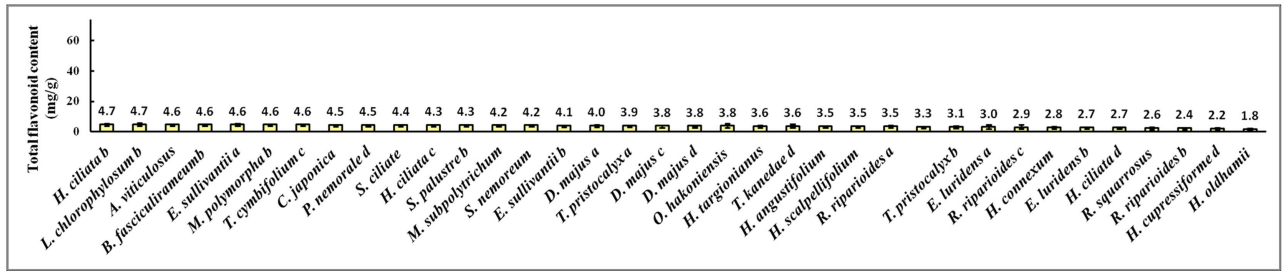


Fig 3. Bryophyte samples with total flavonoid concentration less than 5.0 mg/g.

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Reviewing the ample literature on total flavonoid concentrations in plants, the range for spermatophyte species was from around 0.095 mg/g to 25.01 mg/g [26–32], and the total flavonoid content of most pteridophytes was reportedly greater than 50.0 mg/g [33–34]. Our results were clearly different from other researched plant groups. The total flavonoid contents of bryophytes were similar to those of spermatophytes, but far less than those of pteridophytes. This may be due to the differences in evolutionary status [11].

The relationship between total flavonoid concentrations and phylogeny

Differences in the evolutionary status of bryophytes have been reported [35]. Some research suggested that liverworts were the earliest terrestrial plants [36], but other studies indicated that liverworts and mosses were combined as sister taxa [37]. Moreover, long-standing

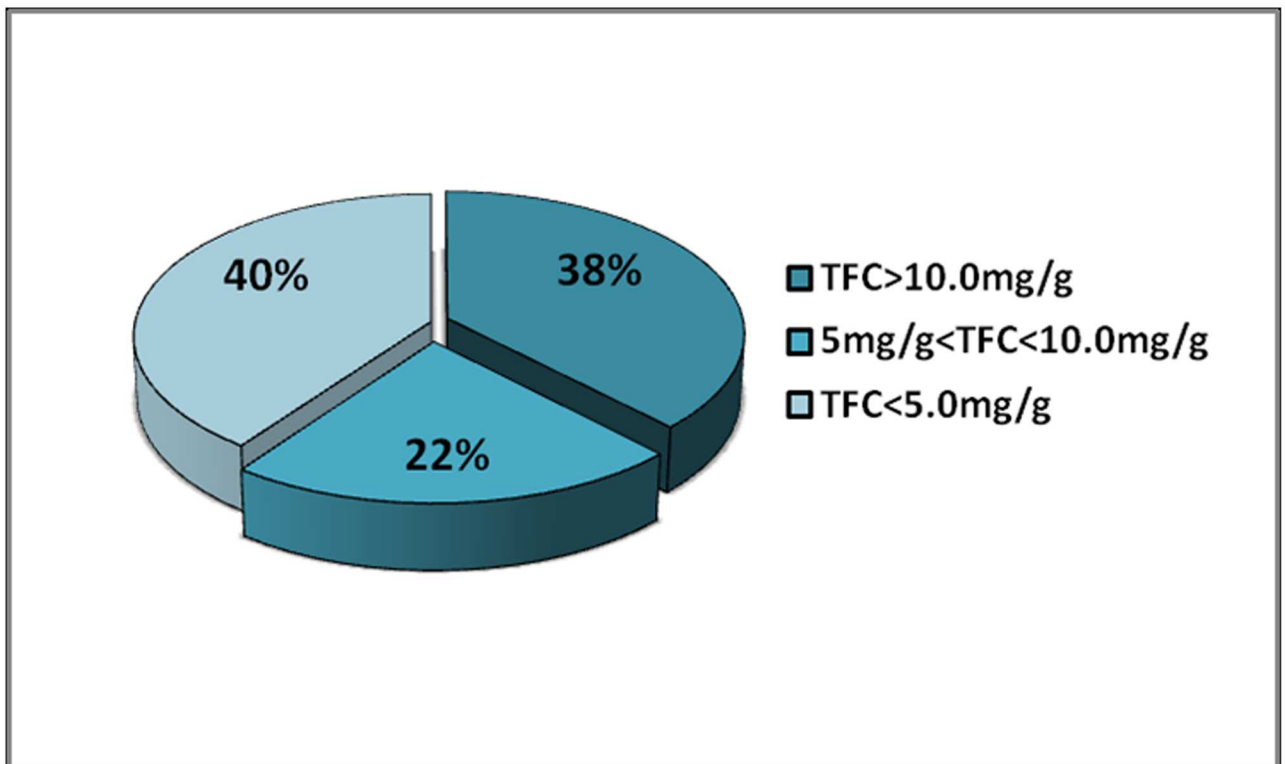


Fig 4. Total flavonoid concentration of bryophytes.

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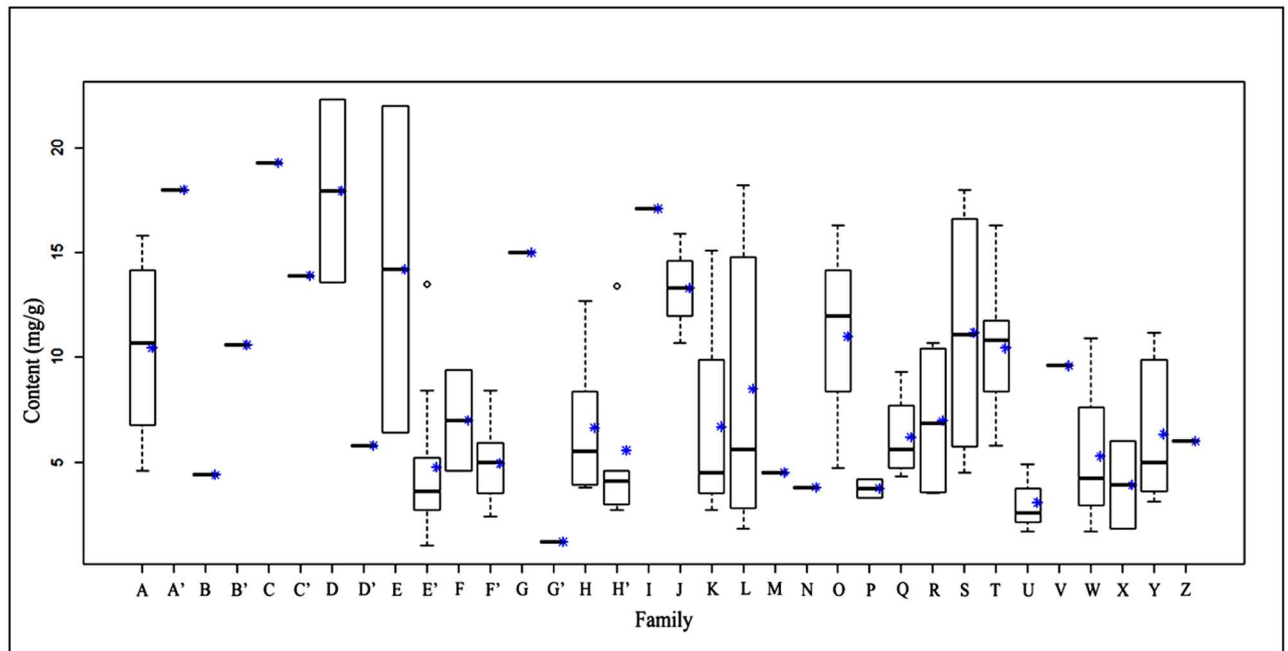


Fig 5. Relationship between flavonoid content and families of bryophytes. Liverworts: A = Marchantiaceae; A' = Porellaceae; B = Scapaniaceae; B' = Pallaviciniaceae; C = Plagiochilaceae; C' = Lophoziaceae; D = Lepidoziaceae; D' = Aytoniaceae; E = Ptilidiaceae. **Moss:** E' = Amblystegiaceae; F = Anomodontaceae; F' = Brachytheciaceae; G = Bryaceae; G' = Climaciaceae; H = Dicranaceae; H' = Entodontaceae; I = Fissidentaceae; J = Grimmiaceae; K = Hedwigiaceae; L = Hypnaceae; M = Hypopterygiaceae; N = Leskeaceae; O = Leucobryaceae; P = Meteoriaceae; Q = Mniaceae; R = Neckeraceae; S = Plagiotheciaceae; T = Polytrichaceae; U = Rhytidiaceae; V = Sematophyllaceae; W = Sphagnaceae; X = taxianke; Y = Thuidiaceae; Z = Trachypodaceae; *mean.

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hypotheses relating to liverwort evolution had been questioned [38]. The mosses, the largest of all bryophyte groups, included both acrocarpous and pleurocarpous mosses [39], but the evolutionary relationship between the two had long been controversial [40].

Herein, all samples, including the samples collected from E'erguna National Natural Reserve S2 Table, were separated into two groups, namely liverworts and mosses. The samples of mosses were also divided into two subgroups, acrocarpous mosses and pleurocarpous mosses. The comparison between liverworts and mosses with respect to total flavonoid content was shown in Fig 5, and the comparison between acrocarpous mosses and pleurocarpous mosses in Fig 6. To our knowledge, this was the first comparison of total flavonoid concentrations in bryophytes of different evolutionary status.

Since 1962 researchers have been attempting to use flavonoids to determine plant phylogenetic sequences. Although there was not a good fossil record, more and more evidences showed that flavonoid content was related to phylogeny [13–14]. Our data confirmed these results. Furthermore, we found that the total flavonoid content of liverworts was generally higher than that of mosses, and the mean total flavonoid content of Marchantiopsida (9.52 mg/g) was less than that of Jungermanniopsida (14.5 mg/g) in the liverworts. Acrocarpous mosses generally had higher concentrations than pleurocarpous ones, although interspecific differences were found.

The above results may suggest that more flavonoids might be contained in relatively primitive bryophytes. In recent report, more flavonoids were also found in primitive ferns [41]. Bryophyte and pteridophytes both belong to cryptogam, so it was speculated that primitive

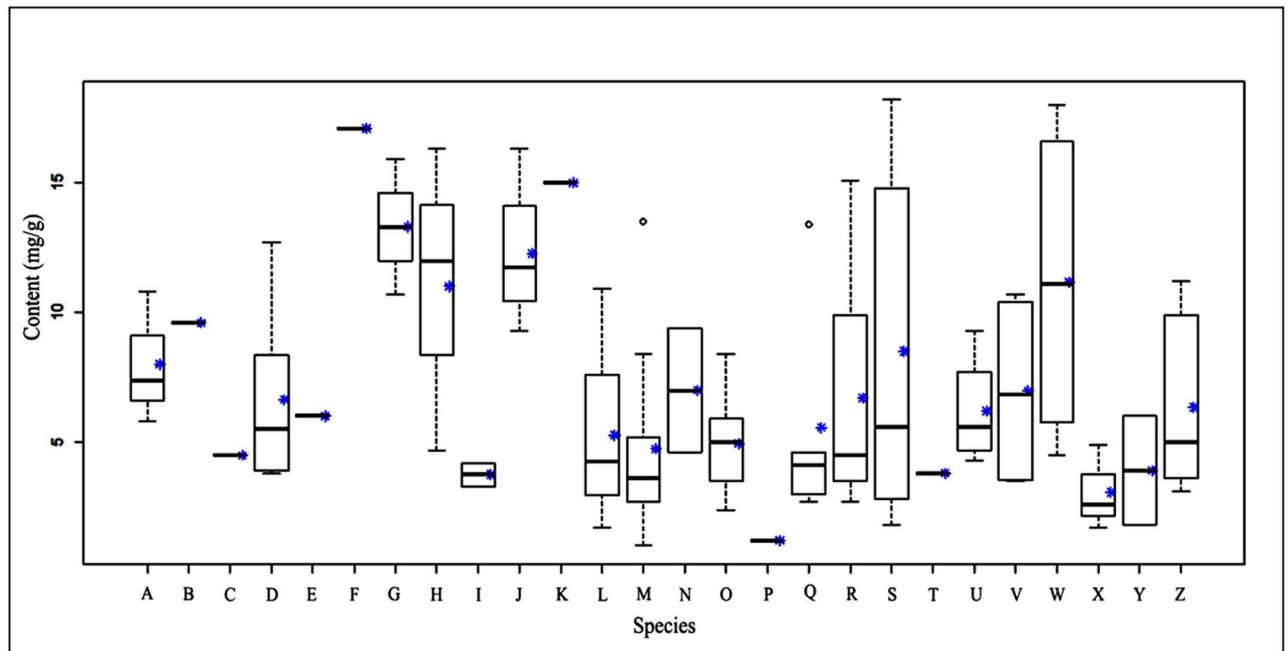


Fig 6. Relationship between flavonoid content and families of moss. **Acrocarpous:** A = Polytrichaceae; B = Sematophyllaceae; C = Hypopterygiaceae; D = Dicranaceae; E = Trachypodaceae; F = Fissidentaceae; G = Grimmiaceae; H = Leucobryaceae; I = Meteoriaceae; J = Polytrichaceae; K = Bryaceae; L = Sphagnaceae; **Pleurocarpous:** M = Amblystegiaceae; N = Anomodontaceae; O = Brachytheciaceae; P = Climaciaceae; Q = Entodontaceae; R = Hedwigiaceae; S = Hypraceae; T = Leskeaceae; U = Mniaceae; V = Neckeraceae; W = Plagiotheciaceae; X = Rhytidiaceae; Y = Taxianke; Z = Thuidiaceae; *mean.

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species might contain more flavonoids in cryptogam. In addition, the relationship between total flavonoid content and environmental factors should not be ignored.

The relationship between total flavonoid concentrations and ecological factors

Flavonoids are important secondary metabolites in plants and are regulated by the environment. Dixon et al. (1995) found that changes in ecological factors associated with both abiotic and biotic stresses could alter the flavonoid content of plants [42]. Various ecological factors have been shown to have an impact on the secondary metabolite profile in angiospermae [43–44]. Nevertheless, the effects of environmental factors on the flavonoid content of bryophytes remain unclear.

The ecological factors that we examined were light, habitat, altitude, and latitude. Fig 7 showed the relationship between total flavonoid concentrations in bryophytes (113 samples) and these factors.

The effects of light on flavonoids in angiospermae have been shown to be significant [45–46]. Our results confirm this view. Total flavonoid concentrations in bryophytes growing in less sunshine conditions were generally higher than those in bryophytes growing in full sunshine. The results were same with pteridophytes [41]. The stress environment such as dimmer sunshine would promote the synthesis of flavonoids [47]. Light quality has been found that could alter flavonoid production [48–49].

It had been reported that there was no effect of altitude on total flavonoid concentrations in *Sphagnum junghuhnianum* in tropical montane forests of Borneo [50]. Our results were in accordance with this result. Total flavonoid concentrations in bryophytes exhibited no obvious

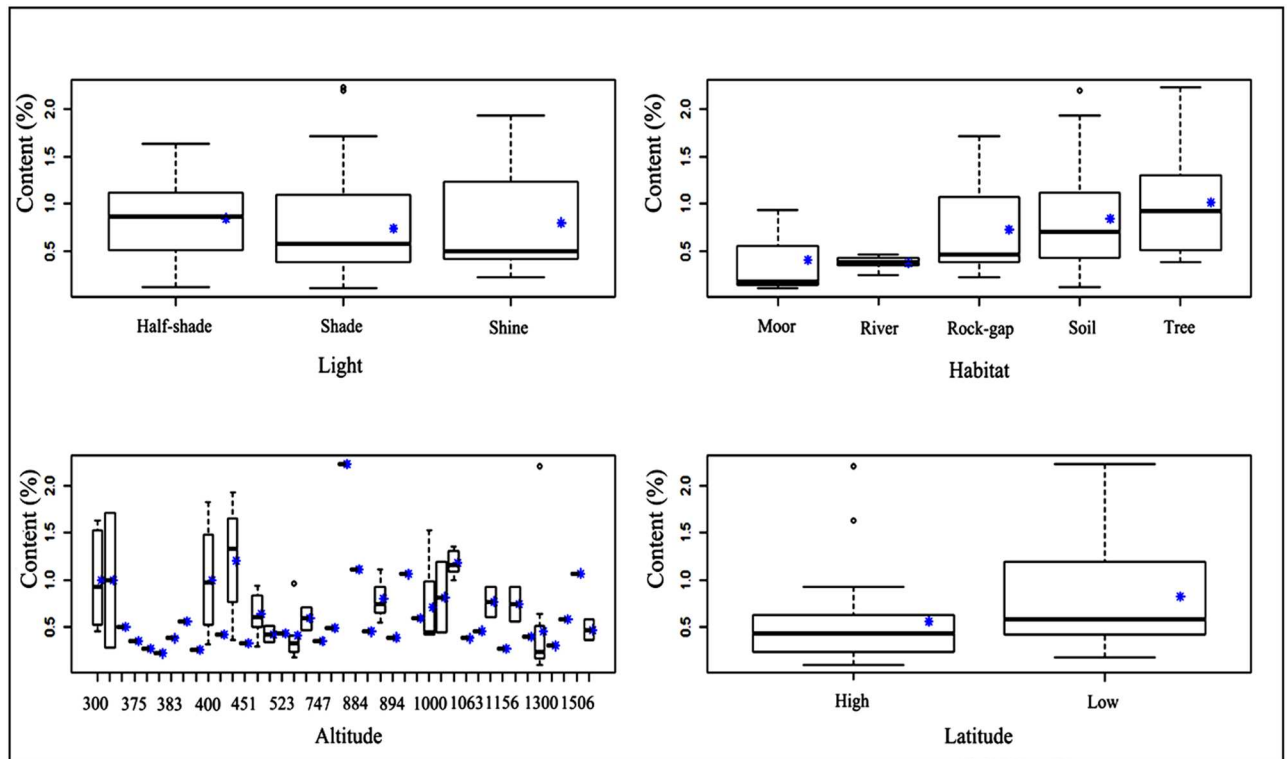


Fig 7. Relationships between ecological factors and total flavonoid content. High latitude: E'erguna National Natural Reserve; **Low latitude:** Tianmu Mountain National Natural Reserve.

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relationship with altitude, but the interspecific differences were more obvious. Temperature decreases with the increase of altitude, which could raise the flavonoid content of bryophytes [51], this may be due to the distribution diversity of bryophytes.

The habitats of bryophytes were divided into moor, river, rock crevice, soil and tree. The results showed that total flavonoid concentrations in epiphytic bryophytes were the highest, while those of aquatic bryophytes the lowest. The results were also same with pteridophytes [41]. In addition, interspecific differences of epiphytic bryophytes were significant. This might be due to the fact that epiphytic species were exposed in the air and experience complex and varied environments, requiring them to synthesize flavonoids for biochemical protection. Conversely, aquatic bryophytes growing in a relatively constant environment were less influenced by ecological factors, so the protection conferred by flavonoids was less necessary. In addition, bryophytes living in rock-gap and soil were vulnerable to sunshine as well as other ecological factors, which led to lower flavonoid content than epiphytic bryophytes.

Total flavonoid content was higher in species growing at low latitudes than those at high latitudes, and interspecific differences of species at low latitudes were clear. This may be explained by the presence of different species at different latitudes.

Our results demonstrated that ecological factors do, indeed, influenced total flavonoid concentrations of bryophytes, as it the case in pteridophyte.

In conclusion, the range of total flavonoid concentrations of bryophytes from Tianmu Mountain was 1.8 to 22.3 mg/g (w/w), which was much lower than ferns. Total flavonoid contents of bryophytes have some connection with plant phylogeny, and more flavonoids might be contained in relatively primitive bryophytes. Meanwhile, the effects of ecological factors on

total flavonoid contents of bryophytes existed; light and habitat (especially tree habitat and river habitat) might be main and representative factor.

Supporting information

S1 Table. Location, taxonomic information and total flavonoid concentrations of bryophytes from the Tianmu Mountain National Natural Reserve.

(DOCX)

S2 Table. Location, taxonomic information and total flavonoid concentrations in bryophytes from the E'erguna National Natural Reserve.

(DOCX)

Author Contributions

Conceptualization: QXW.

Data curation: XW.

Formal analysis: XW.

Funding acquisition: QXW YHW.

Investigation: XW.

Methodology: QXW XW.

Project administration: QXW YHW XW.

Resources: QXW YHW XLD JGC.

Software: XW.

Supervision: QXW.

Validation: XW.

Visualization: XW.

Writing – original draft: XW.

Writing – review & editing: QXW YHW JGC JBX.

References

1. Xiao JB, Muzashvili TS, Georgiev MI. Advances in the biotechnological glycosylation of valuable flavonoids. *Biotechnol Adv.* 2014; 32: 1145–1156. doi: [10.1016/j.biotechadv.2014.04.006](https://doi.org/10.1016/j.biotechadv.2014.04.006) PMID: [24780153](https://pubmed.ncbi.nlm.nih.gov/24780153/)
2. Hae DW, Jeongseon K. Dietary flavonoid intake and smoking-related cancer risk: a meta-analysis. *PLoS One*, 2013; 8: e75604. doi: [10.1371/journal.pone.0075604](https://doi.org/10.1371/journal.pone.0075604) PMID: [24069431](https://pubmed.ncbi.nlm.nih.gov/24069431/)
3. Manoj GS, Murugan K. Phenolic profiles, antimicrobial and antioxidant potentiality of methanolic extract of a liverwort, *Plagiochila beddomei* Steph. Indian. *J Nat Prod Resour.* 2012; 3: 173–183.
4. Andrae-Marobela K, Ghislain FW, Okatch H, Majinda RR. Polyphenols: a diverse class of multi-target anti-HIV-1 agents. *Curr Drug Metabo.* 2013; 14: 392–413.
5. Delmas D, Xiao JB. EDITORIAL (Hot Topic: Natural Polyphenols Properties: Chemopreventive and Chemosensitizing Activities). *Anti-Cancer Agent Med.* 2012; 12: 835–835.
6. Xiao JB, Högger P. Stability of Dietary Polyphenols under the Cell Culture Conditions: Avoiding Erroneous Conclusions. *J Agr Food Chem.* 2015; 63: 1547–1557.
7. Xiao JB. Natural polyphenols and diabetes: understanding their mechanism of action. *Curr Med Chem.* 2015; 22: 2–3. PMID: [25312215](https://pubmed.ncbi.nlm.nih.gov/25312215/)

8. Panickar KS. Effects of dietary polyphenols on neuroregulatory factors and pathways that mediate food intake and energy regulation in obesity. *Mol Nutr Food Res*. 2013; 57: 34–47. doi: [10.1002/mnfr.201200431](https://doi.org/10.1002/mnfr.201200431) PMID: [23125162](https://pubmed.ncbi.nlm.nih.gov/23125162/)
9. Xie CF, Lou HX. Secondary metabolites in bryophytes: an ecological aspect. *Chem Biodivers*. 2009; 6: 303–312. doi: [10.1002/cbdv.200700450](https://doi.org/10.1002/cbdv.200700450) PMID: [19319866](https://pubmed.ncbi.nlm.nih.gov/19319866/)
10. Crawford DJ. Flavonoid chemistry and angiosperm evolution. *Bot Review*. 1978; 44: 431–456.
11. Giannasi DE. Systematic aspects of flavonoid biosynthesis and evolution. *Bot Rev*. 1978; 44: 414–415.
12. Edwards D, Duckett JG, Richardson JB. Hepatic characters in the earliest land plants. *Nature*. 1995; 374: 635–636.
13. Gornall RJ, Bohm BA. Angiosperm flavonoid evolution: a reappraisal. *Syst Bot*. 1978; 353–368.
14. Noro T, Fukushima S, Saiki Y, Ueno A, Akahori Y. Studies on the constituents of *Leptorumohra miqueliana* H. Ito. II. The structure of protofarrerol. *Yakugaku Zasshi*. 1969; 89: 851–856. PMID: [5817240](https://pubmed.ncbi.nlm.nih.gov/5817240/)
15. Shi YQ, Liao YX, Qu XJ, Yuan HQ, Li S, Qu JB, et al. Marchantin C, a macrocyclic bisbibenzyl, induces apoptosis of human glioma A172 cells. *Cancer Lett*. 2008; 262: 173–182. doi: [10.1016/j.canlet.2007.12.014](https://doi.org/10.1016/j.canlet.2007.12.014) PMID: [18215458](https://pubmed.ncbi.nlm.nih.gov/18215458/)
16. Shi YQ, Zhu CJ, Yuan HQ, Li BQ, Gao J, Qu XJ, et al. Marchantin C, a novel microtubule inhibitor from liverwort with anti-tumor activity both in vivo and in vitro. *Cancer Lett*. 2009; 276: 160–170. doi: [10.1016/j.canlet.2008.11.004](https://doi.org/10.1016/j.canlet.2008.11.004) PMID: [19095349](https://pubmed.ncbi.nlm.nih.gov/19095349/)
17. Shen J, Li G, Liu Q, He Q, Gu J, Shi Y, et al. Marchantin C: a potential anti-invasion agent in glioma cells. *Cancer Biol Ther*. 2010; 9: 33–39. PMID: [19923918](https://pubmed.ncbi.nlm.nih.gov/19923918/)
18. Niu C, Qu JB, Lou HX. Antifungal bis [bibenzyls] from the Chinese liverwort *Marchantia polymorpha* L. *Chem Biodivers*. 2006; 3: 34–40. doi: [10.1002/cbdv.200690004](https://doi.org/10.1002/cbdv.200690004) PMID: [17193213](https://pubmed.ncbi.nlm.nih.gov/17193213/)
19. Veljić M, Ćirić A, Soković M, Janačković P, Marin P. Antibacterial and antifungal activity of the liverwort (*Ptilidium pulcherrimum*) methanol extract. *Arch Biol Sci*. 2010; 62: 381–395.
20. Mitre GB, Kamiya N, Bardón A, Asakawa Y. Africane-type sesquiterpenoids from the Argentine liverwort *Porella swartziana* and their antibacterial activity. *J Nat Prod*. 2004; 67: 31–36. doi: [10.1021/np030074z](https://doi.org/10.1021/np030074z) PMID: [14738381](https://pubmed.ncbi.nlm.nih.gov/14738381/)
21. Ivanova V, Kolarova M, Aleksieva K, Dornberger KJ, Haertl A, Moellmann U, et al. Sanionins: anti-inflammatory and antibacterial agents with weak cytotoxicity from the antarctic moss *Sanionia georgico-uncinata*. *Biochem Biotech*. 2007; 37: 343–352.
22. Wang X, Wu YH, Cao JG, Wang QX, Xiao JB. Flavonoids, antioxidant potential, and acetylcholinesterase inhibition activity of the extracts from the gametophyte and archegoniophore of *Marchantia polymorpha* L. *Molecules*. 2016; 21: 360; doi: [10.3390/molecules21030360](https://doi.org/10.3390/molecules21030360) PMID: [26999088](https://pubmed.ncbi.nlm.nih.gov/26999088/)
23. Wu YH, Yang HY, Luo H, Gao Q. Resources of medicinal bryophytes in north-eastern China and their exploitation. *Chin J Ecol*. 2004; 23: 218–223.
24. Niu X, Jiang DH, Zhang JM, Fang CY, Chen XF, Sun H. Characteristics of CO₂ flux in an old growth mixed forest in Tianmu Mountain, Zhejiang, China. *Chin J Appl Ecol*. 2016; 27: 1–8.
25. Wang DH, Wang YF, Zuo Q, Li M, Wei QQ, Li XQ, et al. Comparison of bryophyte diversity in West Tianmu Mountain from 1977 to 2011. *Biodivers Sci*. 2013; 21: 170–176.
26. Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem*. 2007; 101: 140–147.
27. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complem Altern M*. 2012; 12: 1.
28. Ramamoorthy PK, Bono A. Antioxidant activity, total phenolic and flavonoid content of *Morinda citrifolia* fruit extracts from various extraction processes. *JESTEC*. 2007; 2: 70–80.
29. Atanassova M, Georgieva S, Ivancheva K. Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. *J Univ Chem Technol Metallurgy*. 2011; 46: 81–88.
30. Stanojević L, Stanković M, Nikolić V, Nikolić L, Ristić D, Čanadanovic-Brunet J, et al. Antioxidant activity and total phenolic and flavonoid contents of *Hieracium pilosella* L. extracts. *Sensors*. 2009; 9: 5702–5714. doi: [10.3390/s90705702](https://doi.org/10.3390/s90705702) PMID: [22346723](https://pubmed.ncbi.nlm.nih.gov/22346723/)
31. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J Univ Chem Technol Metallurgy*. 2005; 40: 255–260.
32. Son ES, Oh SS, Han DS, Lee JM. Contents of total flavonoid and biological activities of edible plants. *J Korean Soc Food Cult*. 2001; 16: 504–514.

33. Xia X, Cao JG, Zheng YX, Wang QX, Xiao JB. Flavonoid concentrations and bioactivity of flavonoid extracts from 19 species of ferns from China. *Ind Crop Prod*. 2014; 58: 91–98.
34. Cao JG, Zheng YX, Xia X, Wang QX, Xiao JB. Total flavonoid contents, antioxidant potential and acetylcholinesterase inhibition activity of the extracts from 15 ferns in China. *Ind Crop Prod*. 2015; 75: 135–140.
35. Qiu YL, Li LB, Wang B, Chen ZD, Knoop V, Groth-Malonek M, et al. The deepest divergences in land plants inferred from phylogenomic evidence. *Natl A Sci*. 2006; 103: 15511–15516.
36. Qiu YL, Cho Y, Cox JC, Palmer JD. The gain of three mitochondrial introns identifies liverworts as the earliest land plants. *Nature*. 1998; 394: 671–674. doi: [10.1038/29286](https://doi.org/10.1038/29286) PMID: [9716129](https://pubmed.ncbi.nlm.nih.gov/9716129/)
37. Beckert S, Steinhauser S, Muhle H, Knoop V. A molecular phylogeny of bryophytes based on nucleotide sequences of the mitochondrial nad5 gene. *Pl Syst Evol*. 1999; 218: 179–192.
38. He-Nygrén X, Juslén A, Ahonen I, Gleeny D, Piippo S. Illuminating the evolutionary history of liverworts (Marchantiophyta)-towards a natural classification. *Cladistics*. 2006; 22: 1–31.
39. Hyvönen J, Koskinen S, Merrill GLS, Hedderson TA, Stenroos S. Phylogeny of the Polytrichales (Bryophyta) based on simultaneous analysis of molecular and morphological data. *Mol Phylogenet Evol*. 2004; 31: 915–928. doi: [10.1016/j.ympev.2003.11.003](https://doi.org/10.1016/j.ympev.2003.11.003) PMID: [15120390](https://pubmed.ncbi.nlm.nih.gov/15120390/)
40. Shaw AJ, Anderson LE. Peristome development in mosses in relation to systematics and evolution. II. *Tetraphis pellucida* (Tetraphidaceae). *Am J Bot*. 1988; 75: 1019–1032.
41. Wang X, Wang ML, Cao JG, Wu YH, Xiao JB, Wang QX. Analysis of flavonoids and antioxidants in extracts of ferns from Tianmu Mountain in Zhejiang Province (China). *Ind Crop Prod*. 2017; 97: 137–145.
42. Edwards D, Duckett JG, Richardson JB. Hepatic characters in the earliest land plants. *Nature*. 1995; 374: 635–636.
43. Spitaler R, Schlorhauser PD, Ellmerer EP, Merfort I, Bortenschlager S, Stuppner H, et al. Altitudinal variation of secondary metabolite profiles in flowering heads of *Arnica montana* cv. ARBO. *Phytochemistry*. 2006; 67: 409–417. doi: [10.1016/j.phytochem.2005.11.018](https://doi.org/10.1016/j.phytochem.2005.11.018) PMID: [16405933](https://pubmed.ncbi.nlm.nih.gov/16405933/)
44. Skrzypczak-Pietraszek E, Pietraszek J. Seasonal changes of flavonoid content in *Melittis melissophyllum* L. (Lamiaceae). *Chem Biodivers*. 2014; 11: 562–570. doi: [10.1002/cbdv.201300148](https://doi.org/10.1002/cbdv.201300148) PMID: [24706626](https://pubmed.ncbi.nlm.nih.gov/24706626/)
45. Neves CRSS, Procópio MC, Penna TCV. Micropropagation photoautotrophic *Kalanchoe pinnata* in water and humus with use of natural light, and determination of total flavonoids: a review. *IJSRST*. 2016; 2: 1–13.
46. Bottomley W, Smith H, Galston A. A phytochrome mediated effect of light on the hydroxylation pattern of flavonoids in *Pisum sativum* var. 'Alaska'. *Nature*. 1965; 207: 1311–1312.
47. Agati G, Galardi C, Gravano E, Romani A, Tattini M. Flavonoid distribution in tissues of *Phillyrea latifolia* L. leaves as estimated by microspectrofluorometry and multispectral fluorescence microimaging. *Photoch Photobio*. 2002; 76: 350–360.
48. Liu HK, Chen YY, Hu TT, Zhang SJ, Zhang YH, Zhao TY, et al. The influence of light-emitting diodes on the phenolic compounds and antioxidant activities in pea sprouts. *J Funct Foods*. 2016; 25: 459–465.
49. Fu B, Ji XM, Zhao MQ, He F, Wang XL, Wang YD, et al. The influence of light quality on the accumulation of flavonoids in tobacco (*Nicotiana tabacum* L.) leaves. *J Photoch Photobio B*. 2016; 162: 544–549.
50. Majuakim L, Ng SY, Abu Bakar MF, Suleiman M. Effect of altitude on total phenolics and flavonoids in *Sphagnum junghuhnianum* in tropical montane forests of Borneo. *Sepilok Bulletin*. 2014; 19&20: 23–32.
51. Caldwell CR, Britz SJ, Mirecki RM. Effect of temperature, elevated carbon dioxide, and drought during seed development on the isoflavone content of dwarf soybean [*Glycine max* (L.) Merrill] grown in controlled environments. *J Agric Food Chem*. 2005; 53: 1125–1129. doi: [10.1021/jf0355351](https://doi.org/10.1021/jf0355351) PMID: [15713029](https://pubmed.ncbi.nlm.nih.gov/15713029/)