# *Bryophyllum pinnatum* and Related Species Used in Anthroposophic Medicine: Constituents, Pharmacological Activities, and Clinical Efficacy*

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## Abstract

*Bryophyllum pinnatum* (syn. *Kalanchoe pinnata*) is a succulent perennial plant native to Madagascar that was introduced in anthroposophic medicine in the early 20th century. In recent years, we conducted a large collaborative project to provide reliable data on the chemical composition, pharmacological properties, and clinical efficacy of *Bryophyllum*. Here, we comprehensively review the phytochemistry, as well as the pharmacological and clinical data. As to the pharmacology, special emphasis is given to properties related to the use in anthroposophic medicine as a treatment for "hyperactivity diseases", such as preterm labor, restlessness, and sleep disorders. Studies suggesting that *B. pinnatum* may become a new treatment option for overactive bladder syndrome are also reviewed. Tolerability is addressed, and toxicological data are discussed in conjunction with the presence of potentially toxic bufadienolides in *Bryophyllum* species. The few data available on two related species with medicinal uses, *Bryophyllum daigremontianum* and *Bryophyllum delagoense*, have also been included. Taken together, current data support the use of *B. pinnatum* for the mentioned indications, but further studies are needed to fully understand the modes of action, and to identify the pharmacologically active constituents.

## Abbreviations

| | |
|---|---|
| AM: | anthroposophic medicine |
| AUC: | area under the curve |
| GABA: | γ-aminobutyric acid |
| i.v.: | intravenous |
| i.p.: | intraperitoneal |
| NO: | nitric oxide |
| OAB: | overactive bladder |
| p.o.: | *per os* (oral administration) |

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## Introduction

*Bryophyllum pinnatum* (Crassulaceae) is a perennial succulent herb originating from Madagascar with a long tradition of use in tropical countries. In Europe, its utilization is more recent and almost exclusively restricted to anthroposophic medicine (AM). Introduced in 1921 by Rudolf Steiner initially for the treatment of what at that time was called "hysteria", *Bryophyllum* is now used for a variety of hyperactivity disorders. Until very recently, only a few experimental and clinical data were available to support the use of *B. pinnatum* from the perspective of rational phytotherapy. Some years ago we therefore embarked within the *Bryophyllum* Study Group (see acknowledgments) in a large collaborative project on *B. pinnatum* with the aim to provide reliable clinical, pharmacological, and chemical data on this plant. We review here the current state of knowledge on the phytochemistry, pharmacological properties, and clinical data of *B. pinnatum*. With respect to pharmacology, an emphasis is put on properties related to the use in AM, but other bioactivities are also briefly reviewed.

Some reviews have been previously published on the constituents and pharmacological activities of *B. pinnatum* [1–3] or, very recently, on the entire genus *Kalanchoe*, which includes, according to some botanical authors, species of the genus

* Dedicated to Professor Dr. Dr. h.c. mult. Kurt Hostettmann in recognition of his outstanding contribution to natural product research.

*Bryophyllum* (see below) [4]. While these reviews describe various compounds and bioactivities, none of them addresses the pharmacological and clinical data that support the therapeutic use of *Bryophyllum* preparations in European countries.

The present review focuses on *B. pinnatum*, but the few data available on *Bryophyllum daigremontianum, Bryophyllum delagoense*, and the hybrid *Bryophyllum daigremontianum* x *tubiflorum* have also been included. The German homeopathic pharmacopeia (HAB) 2014 [5] lists the two species *B. pinnatum* and *B. daigremontianum* as officinal in its monography "Bryophyllum Rh", and both have been used in AM. *B. delagoense* was introduced in the 1980s as an anthroposophic medicinal product in Germany, primarily for sedative purposes (Personal communication, Dr. med. Siegward-M. Elsas, see Acknowledgments).

## Botany

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The genus *Bryophyllum* comprises approximately 25 perennial succulent species that are native to Madagascar [6]. Meanwhile, many of them have been introduced in other tropical areas where they have sometimes become invasive plants. The genus has an intricate taxonomy, with a variable number of species and numerous synonyms, and is regarded by some authors as one of three sections (Kitchingia, Bryophyllum, and Eukalanchoe) of the genus *Kalanchoe* [6–8]. *Bryophyllum* species have a unique mode of vegetative reproduction whereby young plantlets develop on the edges of leaves before being shed for propagation. *Bryophyllum pinnatum* (Lam.) Oken, originally described by Lamarck as *Cotyledon pinnata* Lam. according to The Plant List [9], possesses approx. 20 synonyms including *Bryophyllum calycinum* Salisb. and *Kalanchoe pinnata* (Lam.) Pers., a name that is very frequently used in the literature (For the full list of synonyms, see **Table 1 S**, Supporting Information). *B. pinnatum* grows up to 1.5 m in height and is known by numerous vernacular names, such as life plant, air plant, love plant, miracle leaf, cathedral bells, and Goethe plant. The latter refers to the detailed observations written down by Wolfgang von Goethe (1749–1832) about this plant. *Bryophyllum daigremontianum* (Raym.-Hamet & Perrier) A.Berger (syn. *Kalanchoe daigremontiana* Raym.-Hamet & H. Perrier) is somewhat smaller (up to 1 m) and commonly known as mother of millions, or devil's backbone. *Bryophyllum delagoense* (Eckl. & Zeyh.) Druce (syn. *Bryophyllum tubiflorum* Harv., *Bryophyllum verticillatum* (Eliott) A. Berger, *Kalanchoe delagoensis* Eckl. & Zeyh., *Kalanchoe tubiflora* (Harv.) Raym.-Hamet, *Kalanchoe tuberosa* H. Perrier, and *Kalanchoe verticillata* Scott-Eliot [9]) grows up to 1.5 m in height and possesses narrow leaves. Vernacular names include mother of millions, devil's backbone, or chandelier plant. The accepted names of The Plant List, *B. pinnatum, B. daigremontianum,* and *B. delagoense*, are consistently used throughout this manuscript, regardless of the names used in the respective original publications.

## Constituents

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Various secondary metabolites have been reported from *Bryophyllum* species. Particular attention has been paid to the bufadienolides, owing to their toxicological relevance for grazing animals [10], and their various other bioactivities. Beside these, a large number of flavonoids have been reported in *B. pinnatum*. Further constituents of *B. pinnatum* include triterpenes, various steroids, phenanthrenes, and some ubiquitous compounds. Data on the composition of *B. daigremontianum* and *B. delagoense* are almost exclusively limited to bufadienolides.

### Bufadienolides

Thirteen bufadienolides (**1**–**13**), including three glycosides, have been reported from *B. pinnatum, B. delagoense, B. daigremontianum*, and the hybrid *B. daigremontianum* x *tubiflorum* (▶ **Table 1**, **Fig. 1**), mostly in conjunction with various bioactivities such as insecticidal and cytotoxic properties [11–23]. A characteristic structural feature is the 1,3,5-orthoacetate function, which is present in about half of these compounds. Bersaldegenin-1-acetate (**1**), bersaldegenin-1,3,5-orthoacetate (**3**), the glycoside bryotoxin A (**7**), and bryotoxin C (= bryophyllin A, **4**) have been found in all species. In contrast, bryophyllins B (**5**) and C (**6**) have been reported only in *B. pinnatum*, the glycosides kalantubosides A (**11**) and B (**12**) only in *B. delagoense*, and daigremontianin (**9**), daigredorigenin-3-acetate (**10**), and methyl daigremonate (**13**) only in *B. daigremontianum* and/or *B. daigremontianum* x *tubiflorum*. The latter compound is listed here on the basis of biogenetic considerations even though it is not a bufadienolide but rather a congener with an opened lactone ring. Bufadienolides were recently quantified in different batches of leaves and press juices of *B. pinnatum* with the aid of UHPLC-MS/MS. Bryophyllin A (**4**), bersaldegenin-1-acetate (**1**), bersaldegenin-3-acetate (**2**), and bersaldegenin 1,3,5-orthoacetate (**3**) were found to be the main bufadienolides in the analyzed plants, with the total contents in the leaves ranging from 3.78 to 40.50 mg/100 g dry weight. Interestingly, when single leaves from individual plants were analyzed, the content was found to be significantly higher in younger leaves. In the same study, the four compounds were also quantified in leaves of *B. daigremontianum* and *B. delagoense* [24].

### Flavonoids

A number of flavonoids have been identified in *B. pinnatum*. They include numerous flavonol derivatives (**14**–**35**), mainly quercetin and kaempferol glycosides, as well as a few flavone glycosides, such as acacetin, luteolin, and diosmetin glycosides (**36**–**40**) (▶ **Table 2**, **Fig. 2**) [13, 25–36]. In addition, epigallocatechin-3-O-syringate (**41**) [36] and an ethenylamino-substituted anthocyanidin with a biogenetically unlikely structure [37] have been reported.

To our knowledge, no flavonoids have been reported from *B. daigremontianum*, but quercetin (**27**), 4′-O-methylherbacetin (**42**), and 3,5,7,8,4′-pentahydroxy-3′-methoxyflavone (**43**) have been isolated from the whole plants of *B. delagoense* [38] (▶ **Fig. 2**).

### Further isoprenoids

Several triterpenes, including $\alpha$-amyrin, $\beta$-amyrin [39], $\alpha$-amyrin-$\beta$-D-glucopyranoside [40], bryophollone (**44**), bryophynol (**45**), 18$\alpha$-oleanane, $\Psi$-taraxasterol [41], taraxerone, glut-5(6)-en-3-one, and 3$\beta$-friedelanol [35], were reported from *B. pinnatum*. The plant was also shown to contain various phytosterols, such as bryophyllol (**46**) [41], stigmast-24-enol, (24 S)-stigmast-25-enol, 25-methylergost-24(28)-enol, clerosterol, 24-epiclerosterol, $\beta$-sitosterol, 22-dihydrobrassicasterol, stigmasterol, campesterol, isofucosterol, codisterol, 24-ethyl-desmosterol, ergosta-5,24(28)-dienol, 25-methylergosta-5,24(28)-dienol [42], 24-ethyl-25-hydroxycholesterol [41], peposterol, avenasterol, (24R)-stigmasta-7,25-dienol, (24 S)-stigmasta-7,25-dienol [42], and stigmast-4,20(21),23-trien-3-one (**47**) [40].

**Table 1** Bufadienolides isolated from *B. pinnatum, B. delagoense, B. daigremontianum*, and the hybrid *B. daigremontianum* x *tubiflorum*.

| Compound | Species | Plant part | References |
|---|---|---|---|
| Bersaldegenin-1-acetate (**1**) | *B. daigremontianum* | Leaves, aerial parts | [13, 20, 23] |
| | *B. daigremontianum* x *tubiflorum* | Leaves | [17] |
| | *B. pinnatum* | Leaves | [13] |
| | *B. delagoense* | Whole plant | [14] |
| Bersaldegenin-3-acetate (**2**) | *B. daigremontianum* | Leaves, aerial parts | [13, 20, 23] |
| | *B. pinnatum* | Leaves, whole plant | [13, 16] |
| Bersaldegenin-1,3,5-orthoacetate (**3**) | *B. daigremontianum* | Leaves, aerial parts | [13, 19, 20, 23] |
| | *B. daigremontianum* x *tubiflorum* | Leaves | [17, 18] |
| | *B. pinnatum* | Leaves | [13] |
| | *B. delagoense* | Flowers | [14] |
| Bryophyllin A (= bryotoxin C) (**4**) | *B. daigremontianum* | Flowers, leaves/stems | [13] |
| | *B. pinnatum* | Leaves | [13, 16] |
| | *B. pinnatum* | Flowers, leaves/stems, roots | [15, 22] |
| | *B. delagoense* | Flowers, leaves/stems, roots | [12, 14, 15] |
| Bryophyllin B (**5**) | *B. pinnatum* | Leaves, whole plant | [21] |
| Bryophyllin C (**6**) | *B. pinnatum* | Leaves | [16] |
| Bryotoxin A (**7**) | *B. daigremontianum* x *tubiflorum* | Roots | [15] |
| | *B. pinnatum* | Roots | [15] |
| | *B. delagoense* | Flowers, leaves/stems, roots | [11, 14, 15] |
| Bryotoxin B (**8**) | *B. daigremontianum* | Flowers, leaves/stems | [15] |
| | *B. daigremontianum* x *tubiflorum* | Flowers, leaves/stems, roots | [15] |
| | *B. pinnatum* | Flowers, leaves/stems, roots | [15] |
| | *B. delagoense* | Flowers, leaves/stems, roots | [12, 15] |
| Daigremontianin (**9**) | *B. daigremontianum* | Aerial parts | [19, 20, 23] |
| | *B. daigremontianum* x *tubiflorum* | Leaves | [17, 18] |
| Daigredorigenin-3-acetate (**10**) | *B. daigremontianum* | Aerial parts | [20, 23] |
| Kalantuboside A (**11**) | *B. delagoense* | Whole plant | [14] |
| Kalantuboside B (**12**) | *B. delagoense* | Whole plant | [14] |
| Methyl daigremonate (**13**) | *B. daigremontianum* x *tubiflorum* | Leaves | [17] |

From *B. daigremontianum*, glutin-5(6)-en-3*β*-ol, stigmasterol, and a mixture of *α*- and *β*-amyrin have been isolated [43]. Glutinol and friedelin have been identified by mass spectrometry as the major triterpenoids in the leaf wax [44]. The wax further contains glutanol, glutinol acetate, epifriedelanol, germanicol, and *β*-amyrin [45]. Finally, *β*-sitosterol-3-O-glucoside, stigmasterol-3-O-glucoside, a megastigmane sesquiterpene, tubiflorone (**48**) [38], and two cardenolides, kalantubolides A (**49**) and B (**50**) [14], were isolated from *B. delagoense*. The latter compounds possess a 1,3,5-orthoacetate function that is also found in many bufadienolides of the genus *Bryophyllum*. Kalantuboside B differs from kalantuboside A by a biogenetically unusual location of the carbonyl group in the lactone ring (**Fig. 3**).

## Miscellaneous metabolites

2(9-Decenyl)-phenanthrene and 2-(9-undecenyl)-phenanthrene [41] were obtained as a mixture from the leaves of *B. pinnatum* and identified by GC-MS. More recently, a nitrogen containing derivative (6-[8-(ethenylamino)phenanthren-2-yl]hex-5-yn-2-one) was also obtained [46]. However, it must be pointed out that the structure reported for the latter compound is biogenetically not plausible.

A lignan glycoside, bryophylluside (**51**), was isolated from the herbs of *B. pinnatum* [26]. Phenolic acid derivatives identified in *B. pinnatum* include gallic acid [36], syringic acid *β*-D-glucopyranosyl ester, 4′-O-*β*-D-glucopyranosyl-*cis*-*p*-coumaric acid [13], and, according to a previous review, ferulic acid, syringic acid, caffeic acid, *p*-coumaric acid, protocatechuic acid, 4-hydroxy-3-methoxy-cinnamic acid, and *p*-hydroxybenzoic acid [2]. 4-O-Ethylgallic acid, syringic acid, vanillic acid, methyl gallate, 3,4-dimethoxyphenol, phloroglucinol, and 3,4-dihydroxyallylbenzene were reported in *B. delagoense* [38].

The presence of *n*-alkanols and *n*-alkanes, such as *n*-hentriacontane and *n*-tritriacontane [39], bryophollenone (**52**) [41], and a minor constituent, 1-octen-3-ol-O-*α*-L-arabinopyranosyl-(1 → 6)-*β*-D-glucopyranoside [47], have been reported in *B. pinnatum*. In *B. daigremontianum*, ferulate esters of $C_{22}$-$C_{30}$ alcohols were found in the roots, and triacontanol was detected in the leaves [48]. Taurolipid C (**53**) was found in *B. delagoense* [14] (**Fig. 3**).

*B. pinnatum* was shown to contain large amounts of malic acid [13]. Other carboxylic acids include, according to a previous review, oxalic acid, citric acid, isocitric acid, cinnamic acid, succinic acid, oxaloacetate, and phosphoenolpyruvate [2]. In addition, the fatty acids palmitic, stearic, arachidic, and behenic acids [49], as well as various vitamins (ascorbic acid, riboflavin, thiamine, niacin, pyridoxine), were reported [2]. The rare vitamin E congeners *β*-, *γ*-, and *δ*-tocomonoenols were identified in the leaves of *B. daigremontianum*, together with the widespread *α*-, *γ*- and *δ*-tocopherols [50].

## Ethnomedical Uses

*B. pinnatum* has been widely used in traditional medicine of tropical regions where the plants grow spontaneously, such as Madagascar, Nigeria, Trinidad and Tobago, India, Indonesia, Philippines, Indo-China, and Brazil. Leaves and stems taste bitter and, due to their astringent effects, are effective against diarrhea, flatulence, and vomiting [2]. In Trinidad and Tobago, they are used to

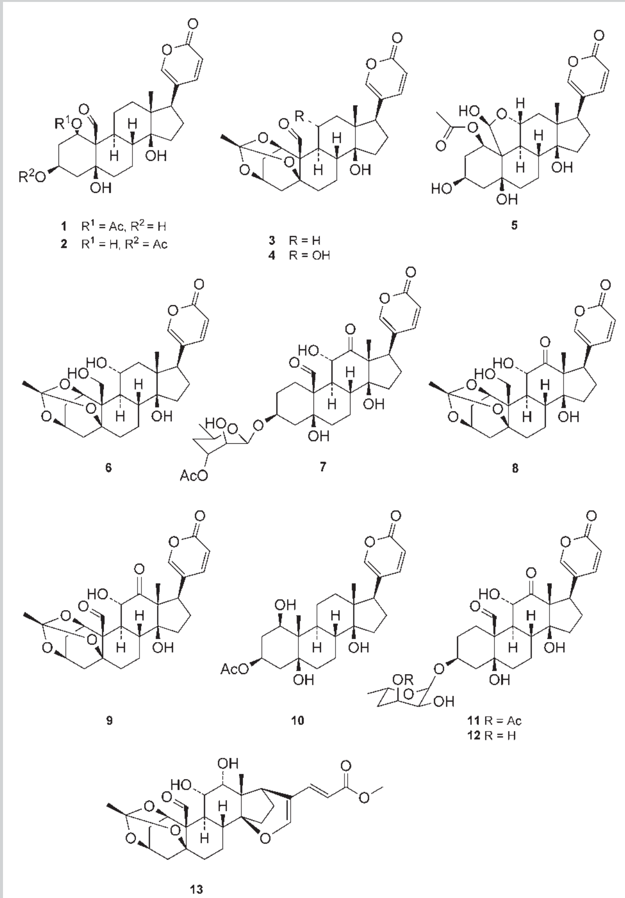

**Fig. 1** Structures of bufadienolides from *B. pinnatum*, *B. daigremontianum*, *B. delagoense*, and *B. daigremontianum* x *tubiflorum*.

treat several diseases and afflictions, such as hypertension and kidney and urinary disorders [51]. Herbalists in Nigeria use an aqueous leaf extract for the treatment of cough and in the prophylaxis of asthma [52]. In India, the leaves are employed as a hepatoprotective herb to treat jaundice [53], and have also been recommended for the treatment of wounds, bruises, and insect bites [54, 55]. Leaf preparations are used as an antipyretic and for treatment of malaria in Africa, Asia, and Latin America [56]. Further traditional uses with the corresponding countries have been listed in [3].

## *Bryophyllum pinnatum* in Anthroposophic Medicine

AM is an integrative multimodal medical system that was developed by the Austrian philosopher Rudolf Steiner (1861–1925) and the Dutch medical doctor Ita Wegman (1876–1943). AM is currently practiced in 80 countries worldwide.

In 1921, the first anthroposophic hospital in Arlesheim, Switzerland, was established by Ita Wegman. AM is practiced by physicians who are fully trained and qualified in university medicine by integrating conventional skills and methods with a holistic understanding of man and nature. From this point of view, the understanding of the human being in his entirety means the acceptance of a three-system organization with a physical body, the soul, and the spirit. This holistic view of the human being leads to an understanding of health and illness that differs from conventional medicine, and to treatments that are specifically adapted to each individual.

*Bryophyllum* plants have the unique ability to let new plantlets grow from their leaves, thereby suggesting, according to anthroposophic concepts, a strong vegetative force and great vitality. Additionally, flower formation is somehow displaced to the leaves, as demonstrated by the distribution of anthocyanins, which are normally present in flowers, and are responsible for the reddish and purple patterns on the leaves. Furthermore, the plant does flower under favorable conditions only.

From the perspective of AM, *B. pinnatum* is therapeutically indicated if the so-called astral and etheric bodies separate too much from each other. This means that the processes linked to psychic qualities, such as emotions, and the physiological processes are not well-balanced, which disturbs the basis for the healthy state of a patient. *B. pinnatum* is supposed to reunite these two parts of the human organization, thereby restoring holistic balance. Based on this principle, *B. pinnatum* has historically been used to treat inner restlessness and anxiety, and, therefore, was also called "herbal valium" due to its sedative properties [57, 58].

In 1921, *B. pinnatum* preparations were initially recommended by Rudolf Steiner as AMs to treat "hysteria" [59]. Steiner described hysteria as the condition in which the spiritual and emotional energy is not capable anymore to fully regulate normal physical functions [60]. Later, in 1970, Dr. Werner Hassauer (1928–1993), a German gynecologist, introduced *B. pinnatum* as a tocolytic agent to prevent premature labor in an AM hospital (see below) [61].

### Preparations and application

A large multicenter observational study was performed involving 38 German physicians collaborating in the Evaluation of Anthroposophic Medicine (EvaMed) network. Over 6 years, a total of 4038 prescriptions were recorded in the EvaMed data bank and showed a broad indication range [62]. *B. pinnatum* preparations are produced by Weleda AG, while WALA Heilmittel GmbH focuses on *B. daigremontianum*.

The use of *B. pinnatum* preparations is described in the German Commission C monographs. In Switzerland, *B. pinnatum* preparations are authorized by the Swiss Agency for therapeutic products (Swissmedic) as a medicinal product without any indication. *B. pinnatum* is currently used for the treatment of premature labor and for some other medical conditions, such as sleep disorders induced by restlessness, anxiety, pain induced by vital weakness, and recurrent inflammation in the metabolic system [63]. Complex anthroposophic preparations, such as *B. pinnatum* Mercurio cultum or Argento cultum contain plants which were fertilized with the corresponding homeopathic diluted metal (quicksilver or silver) [62]. These preparations are primarily used to regulate metabolic processes with or without concomitant psychological symptoms (e.g., restlessness and sleep disorders). The combination of *B. pinnatum* and Conchae (calcium carbonate from oyster shell) is also used to harmonize rhythm and is prescribed to patients suffering from difficulty falling asleep, restlessness, excitation, and exhaustion [64]. *B. pinnatum* preparations from Weleda AG are available in different galenical forms such as powder, tablets, drops, and ampoules. Globuli velati (sucrose globules coated with a syrup containing homeopathic dilutions) are commercialized by Wala Heilmittel GmbH.

Very recently, an online survey in Switzerland showed that in gynecology and obstetrics, *B. pinnatum* (50% tablets) is being

**Table 2** Flavonols and flavones isolated from *B. pinnatum*.

| | | |
|---|---|---|
| Kaempferol (**14**) | | [25, 26] |
| Kapinnatoside (kaempferol 3-O-α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside (**15**) | Leaves | [13, 27] |
| Kaempferol 3-O-β-D-xylopyranosyl-(1 → 2)-α-L-rhamnopyranoside (**16**) | Leaves | [13] |
| Kaempferitrin (kaempferol 3-O,7-O-di-α-L-rhamnopyranoside, **17**) | Whole plant | [28] |
| Kaempferol 3-O-α-L-(2-O-acetyl)rhamnopyranoside 7-O-α-L-rhamnopyranoside (**18**) | Whole plant | [28] |
| Kaempferol 3-O-α-L-(3-O-acetyl)rhamnopyranoside 7-O-α-L-rhamnopyranoside (**19**) | Whole plant | [28] |
| Kaempferol 3-O-α-L-(4-O-acetyl)rhamnopyranoside 7-O-α-L-rhamnopyranoside (**20**) | Whole plant | [28] |
| Kaempferol 3-O-α-D-glucopyranoside 7-O-α-L-rhamnopyranoside (**21**) | Whole plant | [28] |
| Afzelin (kaempferol 3-O-α-L-rhamnopyranoside, **22**) | Whole plant | [28] |
| α-Rhamnoisorobin (kaempferol 7-O-α-L-rhamnopyranoside, **23**) | Whole plant | [28] |
| Astragalin (kaempferol-3-O-β-D-glucopyranoside, **24**) | Leaves | [29] |
| Myricitrin (myricetin-3-O-α-L-rhamnopyranoside, **25**) | Leaves | [13] |
| Myricetin 3-O-α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside (**26**) | Leaves | [13] |
| Quercetin (**27**) | Leaves | [30] |
| 3′,4′-Di-O-methylquercetin (**28**) | Leaves | [31] |
| Quercetin 3-O-α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside (**29**) | Leaves, flowers | [13, 26, 27, 32, 33] |
| Quercitrin (quercetin 3-O-α-L-rhamnopyranoside, **30**) | Leaves, flowers | [13, 26, 32–34] |
| Quercetin 3-O-α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside 7-O-β-D-glucopyranoside (**31**) | Leaves | [13] |
| Isoquercitrin (Quercetin 3-O-β-D-glucopyranoside, **32**) | Flowers | [33] |
| Miquelianin (Quercetin 3-O-β-D-glucuronopyranoside, **33**) | Flowers | [33] |
| Quercetin 3-O-diarabinoside (interglycosidic linkage not reported) | Leaves | [29] |
| Rutin (quercetin 3-O-rutinoside, **34**) | Leaves | [26, 30] |
| 3,5,7,3′,5′-Pentahydroxyflavone (**35**) | Whole plant | [35] |
| Luteolin (**36**) | Leaves | [30, 36] |
| Luteolin 7-O-β-D-glucopyranoside (**37**) | Leaves | [30] |
| Diosmine (diosmetin 7-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside, **38**) | Leaves | [13] |
| Acacetin 7-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**39**) | Leaves | [13] |
| 4′,5–7-Trihydroxy-3′,8-dimethoxyflavone 7-O-β-D-glucopyranoside (**40**) | Leaves | [27] |

prescribed for pregnant women in the case of premature labor, and against restlessness and hyperactive bladder. With two-thirds of the patients being treated at the University Hospital of Zurich, this work showed that *B. pinnatum* is a herbal product whose use is no longer confined to the AM, but has been integrated in conventional settings [65].

## Pharmacological and Clinical Activities Related to Anthroposophic Medicine

*B. pinnatum* has been used in AM to treat various disorders caused by hyperactive conditions. *In vitro* and *in vivo* studies that have been performed are summarized below. More detailed information about the respective studies is provided as Supporting Information (**Table 2 S**, Supporting Information).

### Tocolysis

*B. pinnatum* preparations have been used as a tocolytic agent since years, and several studies have been performed. The German gynecologist Dr. Hassauer showed that the tocolysis with *B. pinnatum* 5% i.v. infusion and 50% trituration orally was well tolerated, and successful in 84% of the women. The treatment allowed him also to decrease the dosage of the beta-agonist fenoterol, or even replace it [61]. In a retrospective study with 170 pregnant women, the tocolytic effect of *B. pinnatum* was investigated. Group A was treated with *B. pinnatum* 5% infusion followed by the oral treatment with 50% trituration. The treatment in group B also started with *B. pinnatum* 5% infusion and due to an inadequate effect after 2 h, women additionally received fenoterol i.v. or p.o. followed by oral treatment with *B. pinnatum* 50% trituration. *B. pinnatum* showed a positive outcome comparable

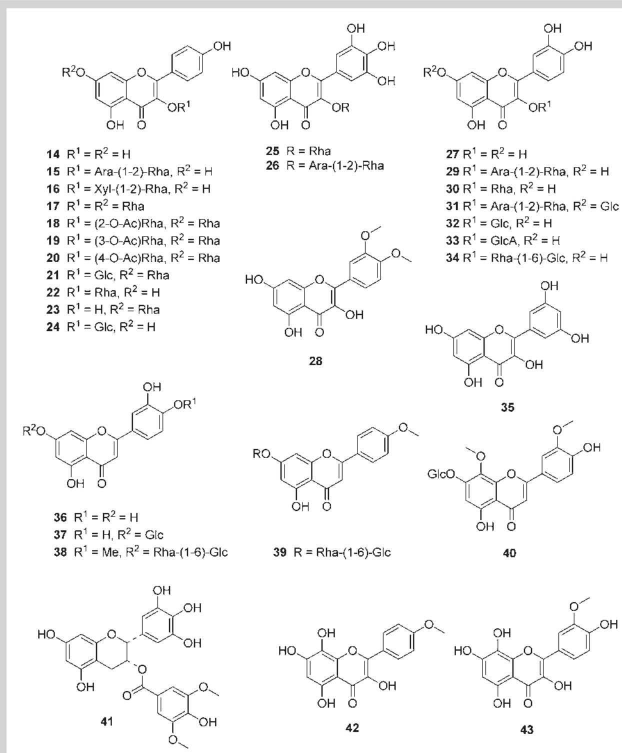


**Fig. 2** Structures of flavonoids from *B. pinnatum* and *B. delagoense*.

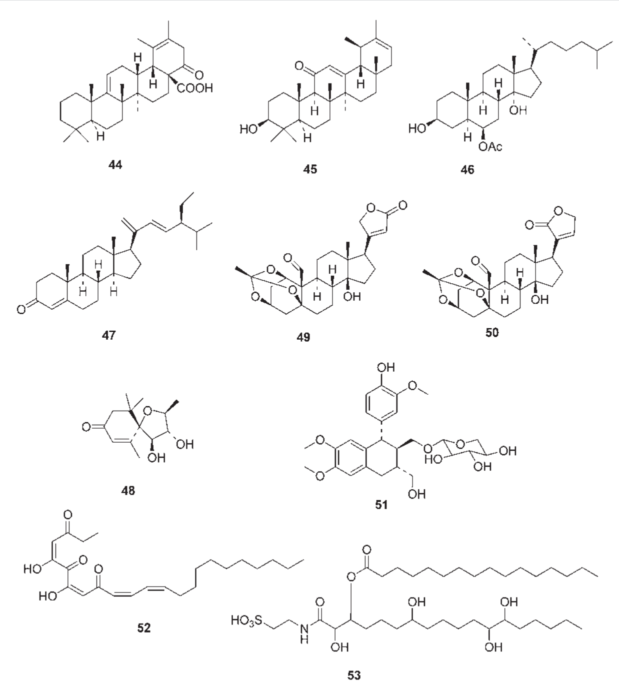

**Fig. 3** Structures of miscellaneous compounds from *B. pinnatum, B. daigremontianum*, and *B. delagoense*.

to fenoterol, and no undesired effects were recorded [66]. From 1977 to 2000, a total of 1622 deliveries were documented and evaluated by Dr. Istvan Vilàghy, a gynecologist who practiced in Switzerland. In this study, data from 253 pregnant women who needed a tocolytic therapy, and 29 premature deliveries were analyzed. In the period from 1977 to 1983, fenoterol was used to prevent premature labor resulting in an incidence of premature deliveries of 6.2%. In the following years, Dr. Vilàghi integrated *B. pinnatum* in the treatment of premature labor. From 1990 to 2000, he treated pregnant women almost exclusively with *B. pinnatum* 33% dilution. When *B. pinnatum* was used for the treatment of premature labor, the incidence of premature deliveries decreased to 1.1% [67].

In a retrospective matched-pairs study, the tolerability and tocolytic activity of i.v. administered *B. pinnatum* 5% was compared with beta-agonists (fenoterol or hexoprenaline) in a total of 67 pregnant women. This study demonstrated similar maternal and neonatal outcomes in both treatment groups. However, maternal adverse effects (palpitation, dyspnea) were significantly reduced, and the use of corticosteroids and antibiotics was lower in the group treated with *B. pinnatum*. Neonatal outcomes and morbidity rates were similar or superior in the *B. pinnatum* treatment group [68]. In addition, a prospective, randomized clinical trial assessed the efficacy and safety of *B. pinnatum* 50% chewable tablets versus a currently used calcium antagonist, nifedipine, for the treatment of premature contractions. A total of 27 pregnant women were included before the study was interrupted due to slow recruitment. Nevertheless, data showed that treatment with *B. pinnatum* as well as treatment with nifedipine led to significant decreases in the number of contractions/h. The neonatal outcome did not differ between the two groups [69].

The tocolytic activity of *B. pinnatum* was confirmed in an *in vitro* study in comparison to fenoterol. *B. pinnatum* aqueous leaf extract ($10^4$ mg/L) led to a dose-dependent inhibition of spontaneous human myometrium contractions, although the contraction frequency increased. The extract also showed a relaxant effect on oxytocin-induced contractions. Fenoterol decreased myometrial contractions and frequency [70]. Subsequently, the *in vitro* effects on spontaneous contractions in myometrial strips was investigated with *B. pinnatum* leaf press juice, and with three fractions of *B. pinnatum* extract, labelled as flavonoid, bufadienolide, and cinnamic acid fractions. After five spontaneous muscle contractions, 2 µL of the leaf press juice (undiluted) and fractions (1%, 2%, 5%, 10%, undiluted) were added to the organ bath chamber. The effects on the AUC, amplitude, and frequency of the contractions were measured. The leaf press juice significantly reduced the AUC to 82% and rapidly increased the frequency to 128% after the first application. Reduction of the amplitude to 78% was statistically significant after the second application. The fraction enriched in flavonoids (undiluted) significantly reduced the AUC to 51% and caused a rapid and large increase in frequency to 557% after the first application. The amplitude was reduced to 70% after the second application. The two other fractions did not significantly affect the AUC and the amplitude, but increased the contraction frequency more than the control [71].

The mechanism behind the tocolytic effect of *B. pinnatum* was further investigated using human myometrial cells. In hTERT-C3 cells, leaf press juice led to a dose-dependent inhibition of the oxytocin-induced increase of intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$, $IC_{50}$ = 0.94%). Comparable data were obtained in M11 cells. Furthermore, in hTERT-C3 cells kept under $Ca^{2+}$-free conditions, press juice significantly inhibited the oxytocin-induced increase of intracellular $[Ca^{2+}]$. Hence, the observed inhibitory effect was assumed to be independent of the extracellular calcium concentration. In addition, the effect of leaf press juice was investigated in SH-SY5Y human neuroblastoma cells, which are known to express voltage-dependent L-type $Ca^{2+}$ channels. The $[Ca^{2+}]_i$ response to KCl was not reduced by the press juice, but delayed, suggesting that the voltage-dependent calcium influx through the channels was restricted [72].

## Overactive bladder (OAB) syndrome

OAB is a symptomatic diagnosis that has been defined by the International Continence Society (ICS) as urinary urgency, with or without urge incontinence, usually with frequency and nocturia, after the exclusion of urinary tract infection (UTI) or other obvious pathologies [73]. Current pharmacotherapy of OAB includes drugs with various modes of action. Muscarinic receptor antagonists are the first-line pharmacotherapy for OAB and urinary incontinence. A beta3-agonist with proven clinical benefits was recently registered in several countries, but only limited data on long-term efficacy and safety are currently available. Besides, other drugs including calcium antagonists, serotonin and noradrenaline reuptake inhibitors, and estrogens as well as intravesical injection of botulinum toxin are used to mitigate symptoms of OAB.

In a prospective, randomized, double-blind, placebo-controlled study, 20 postmenopausal women suffering from OAB or urgency-dominant mixed urinary incontinence (MUI) were treated with *B. pinnatum* 50% chewable tablets or placebo. The women took 3 × 2 blinded capsules daily during 8 weeks. In a total of 15 weeks, they had 5 visits and were ask to fill out 2-day bladder diaries and answer 2 questionnaires, the King's Health Questionnaire (KHQ) and the International Consultation on Incontinence Modular Questionnaire for OAB (ICIQ-OAB). After treatment, a positive trend for *B. pinnatum* was observed relatively to the placebo. The primary endpoint, the micturition frequency/24 h, was reduced from 9.5 before to 7.8 after the treatment ($p = 0.064$). The quality of life (QoL) was comparable in the *B. pinnatum* and placebo group [74].

The effect of *B. pinnatum* leaf press juice on porcine detrusor muscle contractility was investigated in an organ bath chamber, in comparison with oxybutynin as a reference drug. Press juice (5% in the chamber) significantly inhibited detrusor contractility induced by electrical field stimulation (EFS) by 74.6% compared to the control. In addition, the press juice (10%) had a significant relaxant effect (18.7%) on carbachol-induced contractions. The leaf press juice showed good activity, although oxybutynin had a more potent inhibitory and relaxing effect on the detrusor muscle [75].

The effect of leaf press juice and different fractions of *B. pinnatum* on electrically induced porcine detrusor contractility was investigated in further experiments. The inhibitory properties of leaf press juice were confirmed, even though an initial stimulatory effect was observed. A flavonoid-enriched fraction reduced muscle contractility in a concentration- and time-dependent manner, resulting in a maximum inhibition of 78.7% at a test concentration of 1 mg/mL after 77 min. The lipophilic fraction containing the bufadienolides had no inhibitory effect on contractility at the investigated concentrations. However, due to a poor solubility of the lipophilic fraction test concentrations were significantly lower. An unexpected inhibitory effect was found in the polar fraction that contained substances that were not retained on a HP-20 column. Finally, this effect could be explained by a decreased pH in the organ bath chamber due to the presence of a large amount (17.8%) of L-malic acid in this fraction [76].

### Sleep and neurological disorders

In a prospective, multicenter, observational study, 49 pregnant women suffering from sleep disorders were treated with *B. pinnatum* 50% chewable tablets. The women took 3–8 tablets per day and were asked to complete questionnaires before and after the 14-day treatment. The number of wake-ups and the subjective quality of sleep were significantly improved, and women felt less sleepy during the day. However, a prolongation of sleep duration and reduction in the time to fall asleep was not achieved [77]. In a further study, the effect of *B. pinnatum* on sleep quality was assessed in 20 cancer patients. Treatment (3 weeks) with chewable tablets (mostly 2 × 2 tablets per day) resulted in a decrease of the Pittsburg Sleep Quality Index (PSQI) from 12.2 to 9.1, and sleepiness was slightly reduced [78].

The behavioral neuropharmacology of *B. pinnatum* aqueous leaf extract was investigated in mice, and neurosedative, CNS depressant, and anxiolytic activities were found. Furthermore, a dose-dependent muscle relaxant effect of the aqueous extract was observed, which was comparable to diazepam. Therefore, a GABAergic activity has been suggested for *B. pinnatum* [79]. Some of these neuropharmacological effects were tested in Swiss mice applying a methanolic fraction. The GABA content in the brain was estimated after i. p. administration, and the methanolic fraction led to an increase in brain GABA concentration [80].

The compounds responsible for the neurological activity are not definitively known, but a neurosedative effect of bersaldegenin-1,3,5-orthoacetate (**3**) was demonstrated in mice, whereby strongly sedative activity was observed with doses of 0.1–0.5 mg/kg b.w. However, higher doses resulted in paralysis and muscle contractions [23].

## Other Biological and Pharmacological Activities

Additional activities have been investigated both *in vitro* and *in vivo*. Some of the described activities correspond to uses in traditional medicine, but only a few studies in humans have been reported. A detailed description of the settings and outcomes can be found in **Table 3 S**, Supporting Information.

### Antimicrobial activity

*In vitro* experiments using the agar-well diffusion method demonstrated the sensitivity of several bacteria and fungi to hot water and methanolic extracts as well as to flavonoids of *B. pinnatum* [81, 82]. Several compounds, including a phenanthrene [46], α-rhamnoisorobin (**23**), and further kaempferol rhamnosides (**17, 19**–**22**) [28], showed antimicrobial activity.

### Antileishmanial activity

Leishmaniasis comprises diseases that are caused by protozoan parasites belonging to over 20 *Leishmania* species. The protozoa are transmitted by the bite of female phlebotomine sandflies. Treatment of a cutaneous leishmaniasis patient with an aqueous leaf extract of *B. pinnatum* stopped growth and led to a slight decrease of the active lesion. At the end of the 14-day treatment period, the toxicological parameters of the patient's serum were within the reference range [83]. In mice, the effect of an aqueous *B. pinnatum* extract was investigated after oral (by intragastric intubation), i. v., i. p., and topical (by rubbing the lesion site) administration. The oral treatment was most effective and was able to prevent or delay the onset of lesions in a sustained manner. Additionally, after oral, i. p., or topical application, titers of a parasite-specific antibody (IgG) were reduced to 20% when compared with untreated mice [84]. Interestingly, the activity was abolished *in vitro* and *in vivo* by cotreatment with N-monomethyl-arginine, an inhibitor of inducible NO synthase and, hence, of NO production. The authors concluded that the protective activity was possibly not due to a direct effect on the parasite, but rather to the increase of NO production of macrophages [85]. Subsequent investigations revealed that flavonoids were involved in the antileishmanial activity of the aqueous extract. Quercitrin (**30**) and a quercetin diglycoside (**29**) had the highest *in vitro* antileishmanial activity and low cytotoxicity. It has been suggested that the aglycone quercetin is relevant for the antileishmanial activity, since the corresponding kaempferol glycosides (**15** and **22**) were significantly less active [27, 34]. Orally administered quercetin and quercetin glycosides were able to stop the growth of lesions in mice. An explanation for the comparable *in vivo* activity of aglycone and glycosides could be that the same active metabolites are produced upon oral administration [86].

### Insecticidal activity

Several bufadienolides isolated from *B. pinnatum* and *B. daigremontianum* x *tubiflorum* were tested in an *in vitro* assay using 3rd instar larvae of the silkworm. Larvae were cultured on an artificial diet and further put into petri dishes containing the test samples (added to 1 g of the diet). The mortality rate was determined after 24 h. Daigremontianin (**9**), bryophyllin A (**4**), bryophyllin C (**6**), and bersaldegenin-1,3,5-orthoacetate (**3**) showed $LD_{50}$ values of 0.9, 3, 5, and 16 µg/g of diet, respectively, whereas bersaldegenin-1-acetate (**1**) and bersaldegenin-3-acetate (**2**) showed no insecticidal activity. These results suggest that the 1,3,5-orthoacetate moiety is essential for the insecticidal effect [16, 17].

### Cytotoxic and antitumor promoting activity

An *in vitro* study demonstrated a concentration-dependent inhibition of human cervical cancer cell growth when *B. pinnatum* chloroform extract, and a fraction containing steroidal glycosides, alkaloids, and steroids were tested. The fraction was more potent than the extract and showed proapoptotic activity. In contrast, higher activity was observed for the extract against human papillomavirus (HPV), which plays a pivotal role in the development of cervical cancer [87]. The butanol-soluble fraction of an ethanolic extract of fresh *B. delagoense* plants showed antiproliferative activity [88] in several cell lines via a modulation of the mitotic cell division. More recently, the water-soluble fraction of the same extract was shown to cause cell cycle arrest, and to induce senescence in lung cancer A549 cells. At doses of 10 mg and 100 mg/kg b.w. 5 times per week for 6 weeks, the fraction also reduced tumor growth in nude mice [89].

In several studies, a cytotoxic effect of bufadienolides from *Bryophyllum* species was found. Bryophyllin A (**4**) showed potent cytotoxicity in human lung carcinoma A-549 cells, KB cells, and colon HCT-8 tumor cells with $ED_{50}$ values of 10, 14, and 30 ng/mL, respectively. Bersaldegenin-3-acetate (**2**) mainly demonstrated an effect against HCT-8 cells ($ED_{50}$ = 10 ng/mL) and bryophyllin B showed cytotoxicity against KB cells with an $ED_{50}$ value of < 80 ng/mL [21, 22]. A series of five bufadienolides and two cardenolides isolated from *B. delagoense* showed significant cytotoxic activity against A549, Cal-27, A2058, and HL 60 cancer cell lines. Kalantuboside B (**12**) and bersaldegenin-1,3,5-orthoacetate (**3**) were the most potent compounds against A2058 and HL-60 cells with $IC_{50}$s of 0.01 µM [14].

Besides cytotoxic properties, bufadienolides also showed antitumor promoting activity. Five compounds isolated from *B. pinnatum* and *B. daigremontianum* x *tubiflorum* inhibited Epstein-Barr virus early antigen (EBV-EA) activation. The 1,3,5-orthoacetate moiety appeared to be important for the chemopreventive activity [18].

### Antioxidant activity

Extracts and flavonoids of *B. pinnatum* showed free radical scavenging activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. Particularly active were α-rhamnoisorobin (**23**) ($IC_{50}$ = 0.71 µg/mL) [28] and quercetin 3-O-α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranoside (**29**) ($EC_{50}$ = 1.41 µg/mL) [90].

### Immunomodulatory activity and antiallergic effects

*B. pinnatum* leaf press juice produced an *in vitro* antihistaminic effect in the guinea pig ileum and prevented histamine-induced bronchoconstriction in guinea pigs *in vivo*. Flavonoids were thought to be responsible for the selective and competitive inhibition of the $H_1$ receptor [91]. In addition, aqueous leaf extracts possessed an antiasthmatic effect by successfully protecting guinea pigs from histamine-induced preconvulsive dyspnea. The reduction of coughing bouts in guinea pigs treated with this extract confirmed its antitussive properties [52].

Mice receiving daily oral treatment with *B. pinnatum* aqueous leaf extracts during a 14-day sensitization (ovalbumin, OVA) period were protected from fatal anaphylactic shock. Quercitrin (**30**) had a protective effect in 75% of the animals and appeared to be important for the antianaphylactic effect of the extract. Furthermore, the aqueous leaf extract reduced eosinophilia as well as IL-5, IL-10, and TNF-α cytokine production [92]. In addition, the aqueous extract of *B. pinnatum* and quercetin, but not quercitrin, inhibited the development of allergic airway inflammation and airway hyperresponsiveness in mice. It is assumed that the inhibition of mast cell degranulation and reduction of TNF-α levels were involved in the antiallergic effect [93].

### Anti-inflammatory activity

In Wistar rats, oral administration of 400 mg/kg b.w. of an aqueous leaf extract of *B. pinnatum* significantly reduced paw edema [94]. This result was confirmed in another independent study that showed a significant reduction of acute inflammation by the aqueous extract (400 mg/kg b.w.; p.o.) and a new constituent, stigmast-4,20(21),23-trien-3-one (**47**) (300 mg/kg b.w.; p.o.), of 87.3% and 84.5%, respectively. The authors concluded that this steroidal compound was mainly responsible for the anti-inflammatory activity [40]. However, no data were shown to demonstrate that this steroid (isolated from a 95% ethanolic extract) was also present in the water extract. In a recent study, the inhibitory effect of a *B. pinnatum* ethanolic extract was investigated in Swiss albino mice on ear edema induced by various irritant agents such as croton oil, capsaicin, and phenol. Depending on the irritant agent, extract doses of 0.1 mg/ear or 0.5 mg/ear showed significant inhibition [30]. Also, an aqueous extract of the flowers and the quercetin diglycoside **29** inhibited croton oil-induced ear edema and reduced leucocyte migration in carrageenan-induced pleurisy in mice. The extract and **29** both reduced the TNF-α concentration in the pleural exudate. Compound **29** also showed cyclooxygenase (COX) inhibition in an enzymatic assay [95].

### Antiulcer activity

The pretreatment with leaf press juice did not prevent the development of histamine-induced ulcerations in guinea pigs [91]. However, a methanolic fraction of *B. pinnatum* possessed antiulcer activity in rats. The development of different types of acute gastric ulcers was significantly inhibited after i.p. pretreatment at doses of 100 or 300 mg/kg b.w. Additionally, the healing of acetic acid-induced gastric ulcers was improved [54]. In another study with lower doses (10–40 mg/kg b.w.), inhibition of indomethacin-induced gastric ulceration was also observed [96].

### Antinociceptive/analgesic activity

Antinociceptive activity of *B. pinnatum* was investigated in mice. Mice were treated with an aqueous extract prior to exposure to a heat-induced nociceptive pain stimulus (hot plate). In another assay, the abdominal contractions triggered by i.p. injection of 3% acetic acid were observed. In both experimental setups, the aqueous extract provided significant protection against the nociceptive stimulus compared to diclofenac [94]. In addition, the analgesic potential of the aqueous extract, a methanolic fraction, and a steroidal compound of *B. pinnatum* was examined. Using the chemical method described above, the aqueous extract and stigmast-4,20(21),23-trien-3-one (**47**) significantly reduced the number of contractions by 80.16% and 75.72%, respectively. The methanolic fraction also showed a significant reduction of contractions [40, 80]. At doses of 100–300 mg/kg b.w., the aqueous leaf extract increased the pain threshold in rats in the hot plate assay, and reduced phenylbenzoquinone-induced writhing in mice [97]. Antinociceptive properties together with anti-inflammatory activity have been recently reported for an aqueous extract of the flowers. The extract and the quercetin glycoside **29** significantly reduced the number of acetic acid-induced writhings in mice [95].

### Hepatoprotective activity

The juice of *B. pinnatum* has been used to treat jaundice in Indian folk medicines. The protective effect of a concentrated press juice and of an ethanolic extract of the marc (left after expressing the juice) against $CCl_4$-induced hepatotoxicity was examined *in vitro* and *in vivo*. The leaf press juice was more potent than the extract in rat hepatocytes as well as in the rat model. At a dose of 200 mg/kg b.w., significant decreases of elevated serum bilirubin (SBLN) levels by the juice (105% recovery), and of serum glutamyl pyruvate transaminase (SGPT) levels by the juice (92% recovery) and the ethanolic extract (81% recovery) were observed [53].

### Antiurolithic activity

According to [98], *B. pinnatum* is used by local people in Pakistan to expel kidney stones. The authors therefore assessed the antiurolithic activity of an ethanolic extract of *B. pinnatum*. Fresh urine from a man was mixed with different concentrations of the extract before sodium oxalate solution was added to induce crystallization. A concentration-dependent increase of the number of crystals was observed. However, the size and the number of calcium oxalate monohydrate (COM) crystals, which are injurious to epithelial cells, were significantly reduced, and their formation was totally inhibited at the highest concentration (100 mg/mL). Moreover, the formation of calcium oxalate dihydrate (COD) crystals was promoted rather than COM, which is beneficial since COD crystals are less urolithic than COM [98]. The antiurolithic effect of an aqueous leaf extract of *B. pinnatum* has also been studied in rats. Kidney stones were induced by ethylene glycol. The extract administered intraperitoneally at doses of 50 and 100 mg/kg b.w. significantly reduced the urine oxalate level, improved creatinine and blood urea levels, and reduced calcium oxalate deposition in the kidneys [99].

### Antidiabetic activity

Significant hypoglycemic effects were reported for oral treatment with an aqueous leaf extract of *B. pinnatum* (25–800 mg/kg b.w.) in normoglycemic and streptozotocin-induced diabetic Wistar rats [94].

### Antihypertensive activity

An aqueous leaf extract of *B. pinnatum* was reported to reduce salt-induced hypertension in rats. Doses of 25, 50, and 100 mg/kg b.w./day p.o. significantly prevented the increase of systolic and diastolic arterial pressures. On the other hand, no significant change was observed in the heart rate [100].

### Wound healing activity

Topical application of an ethanolic leaf extract of *B. pinnatum* (100 mg/kg b.w.) accelerated wound healing in Sprague Dawley rats. On day 11 after excision, the wound areas were reduced by 86.3%, and only by 68.0% in the control group. A significant increase in wound contractions and a decrease in edema at the wound site were also observed [101].

## Tolerability Studies

A retrospective and two randomized prospective clinical studies confirmed good tolerability of *B. pinnatum* (For details, see **Table 4 S**, Supporting Information). In tocolysis, administration of *B. pinnatum* 5% i.v. and 50% p.o. resulted in less side effects than under treatment with betamimetics. Specifically, the occurrence of palpitations and dyspnea were significantly lower due to a lacking effect on $\beta_1$-adrenoceptors [68]. In addition, the treatment of 14 pregnant women (*Bryophyllum* group) with *B. pinnatum* 50% chewable tablets showed no side effects that were attributable to the medication [69]. Another study revealed no significant difference in observed side effects. One woman treated with *B. pinnatum* 50% chewable tablets suffered from diarrhea and dysentery, possibly due to lactose intolerance, and a second woman developed exanthema of the face and upper thorax [74]. In a longitudinal, prospective, randomized, controlled animal study, the effect of the mother tincture (MT), 30% of *B. pinnatum*, in pregnant Wistar rats was investigated. From day 0 of gestation, 60 rats were treated with the *B. pinnatum* MT or pure vehicle. Two control groups, C1 and C2, received an equivalent to the usual daily dose and 25× the maximum daily dose of vehicle, respectively. Groups B1, B2, B3, and B4 received every day 1, 25, 50, and 100× the maximum daily dose of MT, respectively. After 20 days of treatment, weight gain (excluding fetal and placental weight) was higher in group B4 than in groups B1, C2, and B2. However, the perinea in group C1 were heavier than those in group B2. No maternal or fetal deaths, no differences in implantations and resorptions, and no differences in the number and weight of the fetuses and placentas were observed. External fetal abnormalities were not observed in groups B1–B4 [102].

## Toxicity Studies

*B. pinnatum* is well tolerated in patients. However, toxicity of *Bryophyllum* species has been reported to be related to bufadienolides. The cardiotoxic activity of bersaldegenin-1,3,5-orthoacetate (**3**) was investigated *in vitro* using isolated rabbit and guinea pig hearts. A strong positive inotropic effect was shown [20, 103]. Toxicity to cattle has been documented in earlier studies. A study was conducted including two calves that were treated with the flower heads of *B. pinnatum*. Clinical parameters were examined after administration of 20 g/kg b.w. by stomach tube. Five hours after dosing, the animals became depressed and suffered from rumen stasis and anorexia. The first calf died after 9 h due to dyspnea and tachycardia. The second calf had diarrhea until it died after 15.5 h. This study demonstrated a correlation between bufadienolides and the toxic effect in cattle [15].

An acute toxicity study was performed with a total of 25 rats (or mice, see below), which were given either a *B. pinnatum* methanolic extract or distilled water as a single dose. Mortality was observed after 24 h. A dose of 25 mg/kg caused neither death nor side effects, but the treatment with 200 mg/kg was lethal for 100% of the animals [96]. Unfortunately, information provided in the publication on the route of administration route (oral or intraperitoneal) and the animal species (rats or mice) is contradictory. A similar study was performed including Swiss albino mice. Intraperitoneal administration in mice of aqueous and methanolic extracts showed $LD_{50}$ values of 957 and 1159 mg/kg, respectively. Oral doses up to 3 g/kg b.w. in mice and rats led to no signs of toxicity [104]. In mice, an intraperitoneally administered methanolic fraction led to no deaths up to 2500 mg/kg b.w. in mice, but their behavior changed with concentrations > 100 mg/kg b.w. [80] (See also **Table 5 S**, Supporting Information).

## Conclusion

The introduction of *B. pinnatum* as an AM was based on concepts of anthroposophy, and not on scientific investigations. Meanwhile, several clinical studies support the use of the plant as a tocolytic. Recent pharmacological investigations confirmed effects on myometrial contraction, and also provided first insights into the mode of action, which appears to involve the oxytocin pathway. In addition, another "hyperactivity disease", the OAB syndrome, may represent a new therapeutic indication for *B. pinnatum* preparations. As to sleep disorders, observational studies showed a positive effect on restlessness in pregnant women and cancer patients. However, larger controlled studies are needed to confirm these preliminary data. Even if the tocolytic activity and inhibition of detrusor contractibility could be linked to a flavonoid-containing fraction, the phytochemicals responsible for the pharmacological properties and clinical effects of *B. pinnatum* are not yet entirely clear. In particular, the exact contribution of bufadienolides and flavonoids, the two characteristic groups of secondary metabolites, in the different effects remains to be established. In all clinical studies, *B. pinnatum* was well tolerated, and no serious side effects were observed. From a drug safety perspective, however, the quantity of bufadienolides in *Bryophyllum* preparations should be controlled, since some of these compounds have shown toxicity in animals. Taken together, current data confirm the potential of *B. pinnatum* for the treatment of "hyperactivity" disorders. Further studies are needed to fully understand the modes of action and to identify the active constituents. This will further consolidate the rational clinical use of *B. pinnatum*.

### Supporting information

The full list of botanical synonyms for *B. pinnatum*, and detailed information regarding the settings and outcomes of the pharmacological, clinical, toxicological, and tolerability studies are provided as Supporting Information.

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## Conflict of Interest

Two members of the *Bryophyllum* Study Group, M. Mennet and M. Schnelle, are employees of Weleda AG.

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