Research article

# Bufadienolides - natural, biologically active compounds for medicines and cosmetics. A review.

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***Abstract:*** *Toad skin secretions are a rich source of various biologically active compounds, such as alkaloids, bufadienolides, biogenic amines, or peptides. Also plants from Hyacinthaceae and Crassulaceae can be a potent source of these groups of molecules. These compounds play a crucial role in amphibians' and plants' physiology such as defense against predators or pathogenic microorganisms. Among them, bufadienolides are the focus of many researches in recent years. These molecules have a very interesting, steroidal chemical structure and have potent activity at the cellular level. They possess cardiotonic, antiviral, antibacterial, antitumor, anti-inflammatory, hemostatic, bacteriostatic, wound-healing, and antiparasitic properties. Although the structures of about 500 bufadienolides are known, it is strongly suggested that this group of compounds is still very poorly examined. Moreover, bufadienolides may be an excellent basis for the chemical synthesis of new drugs with selective bioactivity. The aim of this paper is to briefly overview bufadienolides as potent compounds for medicines and cosmetics.*

***Key words:*** *bufadienolides, Bufo, Kalanchoe, natural cosmetics, medicine, ethnomedicines*

## Introduction

Bufadienolides are a group of natural, biologically active compounds derived from both plant and animal sources. These compounds are especially interesting from a point of view of their chemical structure, high biological activity, and possibility of their chemical transformation into other structures with modified activity. Bufadienolides are C-24 polyhydroxy steroids with a six-membered lactone ring in the C-17β position (Fig. 1). In nature these substances occur mostly in glycosides form, bounded with one to three sugar residues in the C-3 position. Sugar units in bufadienolides are the most often glucose and rhamnose [1].

Although the first described bufadienolide was scillaren A (**1**), derived from the plant, Egyptian squill (*Scilla maritima*) the name originates from the genus *Bufo*, for which they are the most common. The suffix -adiene- refers to the two double bonds in the lactone ring and the ending -olide refers to the lactone structure. By the same rule, related structures with only one double bond in the lactone structure are called bufenolides, and those with a saturated lactone ring are called bufanolides [2].

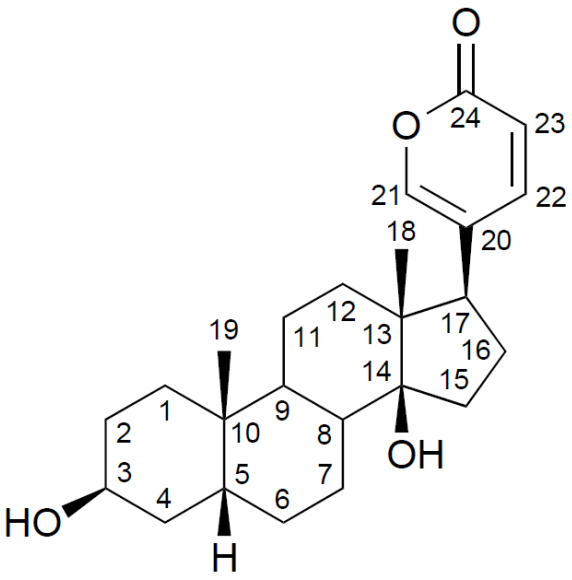



**Figure 1.** General structure of bufadienolides with carbon numbering [3].

## Occurrence

Bufadienolides are not widely distributed in nature. They are found in animals and plants from only a few families. The toads (Bufonidae) have by far the greatest variety of these compounds (Tab. 1). Among other animals, they were discovered only in four other species: in one of the reptiles of the family Colubridae, specifically tiger keelback (*Rhabdophis tigrinus*) and in three representatives of insects from the firefly family (Lampyridae) and genus *Photinus*.

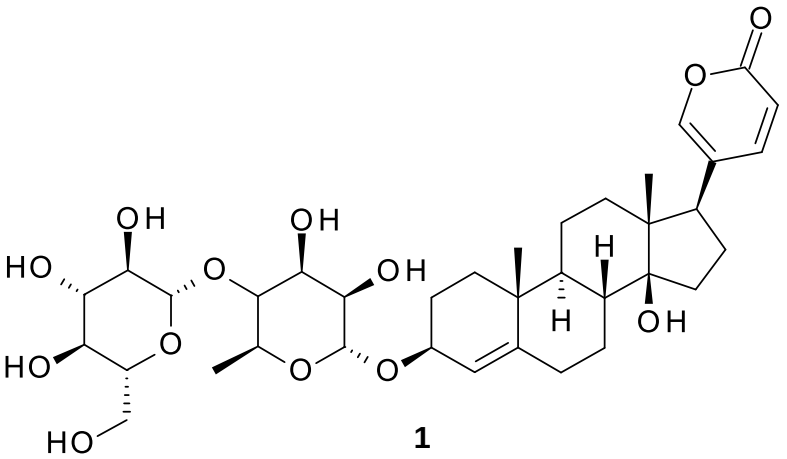
Toads inhabit ecological niches where they are extremely often exposed to contact with many pathogens. Moreover, their epidermis is exposed to numerous injuries and damages. It is no wonder that in the course of evolution they have developed innate immunity, and their genome contains numerous genes encoding peptides, enzymes, and immune proteins, which are expressed on the surface of the skin. Research shows that alkaloids, biogenic amines, or bufadienolides play

an important role in this group of animals, counteracting a wide range of pathogens [4,5].

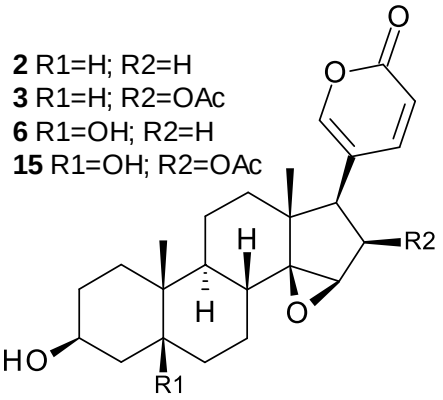
**Table 1.** Bufadienolides from selected species of Bufonidae family. Structures are shown in Fig. 2

| Species | Bufadienolides | Biological activity | References |
|---|---|---|---|
| *Bufo bufo gargarizans* | resibufogenin (**2**), cinobufagin (**3**), bufalin (**4**) | cytotoxic, antitumor | [6] |
| *Bufo marinus* | Telocinobufagin (**5**), marinobufagin (**6**), bufalin, resibufogenin | cardiotonic, analgesic, amtitumor | [7] |
| *Bufo melanostictus* | bufotalin, cinobufagin, bufalin | cytotoxic, antitumor | [6,7] |
| *Bufo rubescens* | marinobufagin, telocinobufagin | antibacterial | [8] |
| *Bufo viridis* | arenobufagin (**7**), hellebrigenin (**8**) | cardiotonic, analgesic, antitumor | [7] |
| *Rhaebo guttatus* | marinobufagin | antiproliferative | [9] |
| *Rhinella jimi* | hellebrigenin, telocinobufagin | antiparasitic | [4,9] |
| *Rhinella marina* | telocinobufagin, marinobufagin, bufalin, resibufogenin | antiproliferative | [9] |
| *Rhinella schneideri* | marinobufagin, bufalin, telocinobufagin, hellebrigenin | against skin diseases, antiproliferative, cytotoxic | [5,10] |

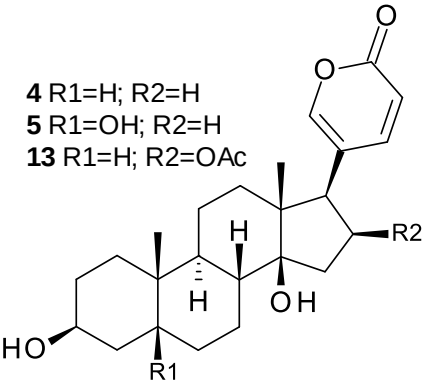
In the plant kingdom, bufadienolides are found in several families such as *Iridaceae*, *Melianthaceae*, *Ranunculaceae*, and *Santalaceae* but only within *Hyacinthaceae* and *Crassulaceae*, especially within genus *Kalanchoe*, a large variety of these compounds is observed (Tab. 2). In the rest of the families, bufadienolides are present only in a single species and their diversity is limited to a very basic level [1,5].



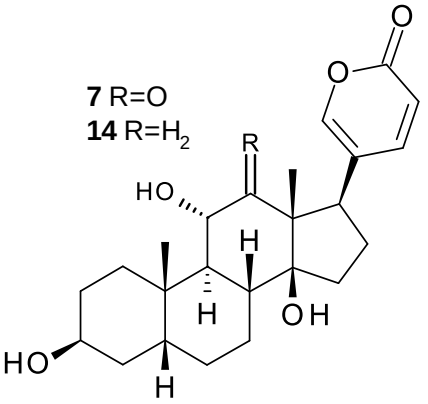
**1**



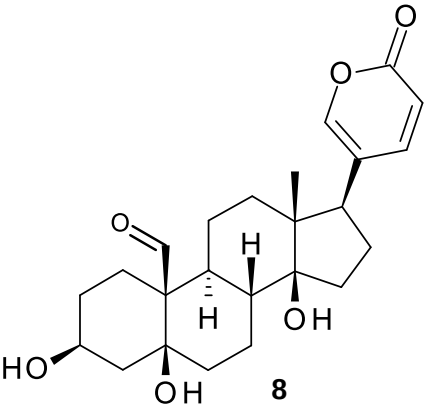
**2** R1=H; R2=H
**3** R1=H; R2=OAc
**6** R1=OH; R2=H
**15** R1=OH; R2=OAc



**4** R1=H; R2=H
**5** R1=OH; R2=H
**13** R1=H; R2=OAc



**7** R=O
**14** R=$H_2$



**8**

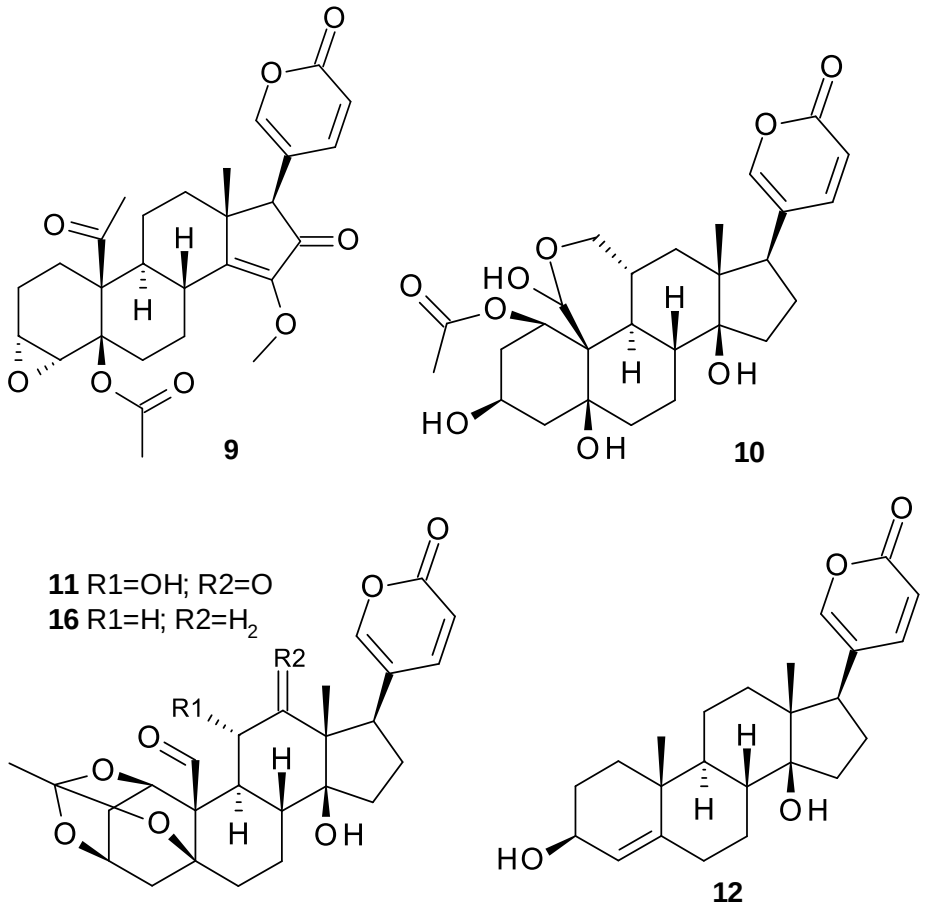



**Figure 3. Chemical structures of selected bufadienolides: scillaren A (1), resibufogenin (2), cinobufagin (3), bufalin (4), telocinobufagin (5), marinobufagin (6), arenobufagin (7), hellebrigenin (8), abyssinin (9), bryophyllin B (10), daigremontianin (11), scillarein (12), bufotalin (13),** gamabufotalin (**14**), cinobufotalin (**15**)**,** bersaldegenin-1,3,5-orthoacetate (**16**).

## Biological activity

The healing properties of bufadienolides were already used in the 16th century BC. in ancient Egypt, where the extract from sea squill (*Urginea maritima*) has been used to treat heart diseases [11]. Squill, as a medicinal plant, was cultivated as early as the 19th century AD, but it was gradually replaced by foxglove (Digitalis sp.), which, due to the more specific activities of cardiac glycosides contained within it, was better suited for this purpose.

**Table 2.** Bufadienolides from selected plants

| Species | Bufadienolides | Biological activity | References |
|---|---|---|---|
| *Cotyledon orbiculata* | tylecosides, orbicusides | neurotoxic, cardiotoxic | [12] |
| *Drimia robusta* | scillirosidines, urginin | anti-inflammatory, antibacterial | [13] |
| *Kalanchoe daigremontiana* | daigremontianines, bersaldegenines | anticancer, anti-inflammatory, cardioactive | [14] |
| *Kalanchoe gracilis* | kalanchosides | cytotoxic | [15] |
| *Kalanchoe lanceolata* | hellebrigenin, lanceotoxines | cardiotonic | [16] |
| *Kalanchoe pinnata* | bryophyllines | anti-tumor, cardiotonic | [17] |
| *Tylecodon grandiflorus* | tyledosides | neurotoxic | [18] |
| *Urginea altissima* | urginin | anti-respiratory diseases | [13] |
| *Urginea maritima* | bufotalines, scillarenes | cardiotonic, anti-inflammatory | [13] |
| *Urginea lydenburgensis* | lydenburgenin, scillarenes | anti-respiratory diseases, anti-inflammatory | [19] |

Another drug based on bufadienolides, known for centuries and used by the Indians of Chaco, Guayaki, and Guarani (Paraguay,) and Pantanal (Brazil) against a wide spectrum of diseases, from wounds and skin diseases to cancer, is a decoction of cururú toad skin (*Rhinella schneideri*) [10]. Cururú should not be confused with curare. For some reason, a frequently repeated mistake is stating that the substance that South American Indians poison their arrowheads had anything in common with amphibians. Curare is an extract of plant alkaloids, mainly from species of the Menispermaceae family [20]. Indeed, Indians sometimes use secretion from the skin of Dendrobatidae frogs to poison their arrowheads, but it is not curare [21].

Ch'an Su (also known in Japan as Senso) is a drug that is used to this day in Chinese traditional medicine and was known already during the Tang Dynasty (6th century AD). It is produced from the skin secretions of giant toads, such as *Bufo gargarizans* or *B. melanostictus*. The main biologically active ingredients of Ch'an Su are bufadienolides. The medicine may contain up to several dozen different derivatives of these compounds. Their number and composition depend on the toad species, the area of occurrence, and the method of production, but the effect on the human body is similar. Ch'an Su has cardiotonic, diuretic, soothing,

and hemostatic properties. Moreover, due to its anesthetic and cytotoxic effects, it is considered an effective anti-cancer drug [6–8].

Although most of the studies concern bufadienolides derived from toad skin secretion, some plant species are valuable herbs in ethnomedicinal, or traditional medicine of Africa and Asia and also contain a high concentration of bufadienolides. In response to growing ethical concerns about the use of drug and cosmetic ingredients of animal origin, some plants especially from *Kalanchoe* genus are increasingly subject of interest. *Kalanchoe pinnata*, *Kalanchoe daigremontiana*, *Kalanchoe tubiflora*, as well as the hybrid of these two *Kalanchoe daigremontiana* × *tubiflora*, also *Melianthus comosus* and *Bersama abyssinica* (from Melianthaceae family) are a well-known source of bufadienolides but also some other valuable compounds such as flavonoids, phenolic acids, anthocyanins, alkaloids, saponins and tannins [14].

The content of bufadienolides in plant tissues varies depending both on the plant species and the growing region [22]. K. pinnata growing in Brazil contains 3-4 times more bufadienolides than the same plant species growing in Germany. Moreover, the contents of bufadienolides in individual parts of plants vary, even within the same species. K. daigremontiana cultivated in Germany showed high levels of bufadienolides in leaves, while the one cultivated in Poland, contained them in roots and stems, but not in leaves [14]. That suggests that bufadienolides, similarly to other secondary metabolites such as polyphenols, are involved in plant protection against pathogens, and their concentration may be a non-specific response to the occurrence of infection. There are no sufficient data in the literature to show specific relationships between the content of bufadienolides and the part of the plant, the cultivation method, external stressors, or the time of harvest. Such research will be crucial for the cosmetics industry in terms of standardisation content of bioactive compounds for cosmetics formulas.

The interest in bufadienolides started in the early 1940s when the structure of the first bufadienolide, later called scillaren A (by Stoll in 1933) was determined [1]. Improvements in chromatographic and spectrometric methods in the 1960s made it possible to study many other molecules of this type. In the last two decades a dynamic increase in interest in these compounds can be observed (Fig. 3). The main reason for the special interest in bufadienolides, apart from their high biological activity, is the search for new compounds against multidrug-resistant bacterial strains, which are an increasingly serious problem. Abyssinin (**9**), telocinobufagin, or marinobufagin have strong bactericidal properties [8]. Moreover, great hopes are placed on bufadienolides as potential anti-cancer drugs. The most frequently mentioned compounds are bufalin (**4**), scillarein (**12**), bufotalin (**13**), gamabufotalin (**14**), cinobufotalin (**15**), or cinobufagin [23]. The use of the cytotoxic and immunomodulating activity of bufadienolides in the development of modern anti-cancer therapies, so-called combination therapies, where these compounds would support standard chemotherapy, radiotherapy and immunotherapy is also considered. Since bufadienolides show a synergistic effect

with known cytostatics, the possibilities of using their supporting properties in the so-called combined chemotherapy are being investigated [24]. An additional positive is the activity of bufadienolides on the central nervous system, resulting in calming and pain relief [25].

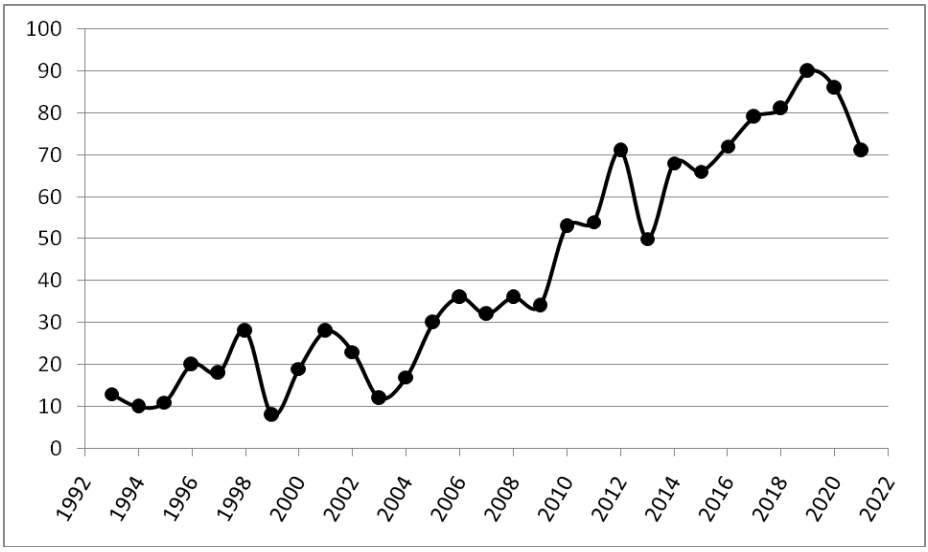



**Figure 2. Graph showing** number of papers published in recent years in PubMed database containing word "bufadienolide" in its title.

Numerous studies have proven that various naturally occurring bufadienolides are, above all, perfect cardiac glycosides - by inhibiting the Na+/K+ -ATPase enzyme, they cause a positive inotropic effect - thanks to which the strength of the heart muscle contraction is enhanced [26].

The report on the positive results of studies on telocinobufagin (**5**) and hellebrigenin (**8**) in terms of their use in combating two groups of human parasites that cause diseases whose treatment is very burdensome caused great interest. Hellebrigenin has been shown to be highly effective against trypanosomatids (family Trypanosomatidae), which cause trypanosomiasis - African sleeping sickness and Chagas disease. On the other hand, both telocinobufagin and hellebrigenin showed high activity against leishmaniasis, caused by flagellates of the genus Leishmania [4].

## The use in cosmetics and medicine

The modern cosmetics industry is increasingly focused on promoting products containing ingredients of natural origin, isolated from various parts of plants: extracts, plant oils, essential oils, or dried, raw material in powder. Natural raw materials are characterized by the presence of many bioactive compounds and thus act on a very wide spectrum of ailments. Especially there is a growing interest in searching for new active substances for cosmetics counteracting or

soothing eczemas and psoriatic skin. Most of the pharmacological and therapeutic studies on the use of bufadienolides in the literature concern compounds of animal origin. However, there is systematically increasing researches on bufadienolides contained in plant organs, mainly from the genus Kalanchoe.

The most obvious use seems to be the use of bufadienolides as compounds with antioxidant properties. The structure based on cyclic structures with numerous hydroxyl groups makes bufadienolides a good scavenger of free radicals. Research on K. daigremontiana root extract showed over four times higher antioxidant activity than epicatechin [27]. In addition, it should be mentioned that bufadienolides can also be an excellent base of compounds for obtaining their derivatives with increased antioxidant properties and reduced toxicity using the biotransformation reaction [28].

So far, the most attention has been paid to plant-derived bufadienolides as potential drugs with chemoprotective and anticancer properties. As early as 1989, described strong cytotoxic properties of bryophyllin B (**10**), isolated from K. pinnata against KB cell line [29]. Some other researchers assessed the effects of bufadienolides produced by *K. gracilis* [15]. All of the 8 tested compounds showed strong cytotoxic properties against human tumor cell lines such as nasopharyngeal (KB) and its MDR variant (KB-VIN), lung (A549), ovarian (1A9), prostate (PC-3), ileocecal (HCT-8), and epidermoid (A431) cells. In turn, extracts of K. hybrida showed a marked anti-tumor activity against human breast cancer (MCF-7), large cell lung cancer (NCI-H460), and anaplastic astrocytoma (SF-268). Among the analyzed compounds, the highest values of cytotoxicity were found for bersaldegenin 3-acetate and daigredorigenin 3-acetate [30]. Also, bufadienolide glycosides isolated from *K. tubiflora show high cytotoxic activity against* human cancer cell lines: lung (A549), oral adenosquamous carcinoma (Cal-27), melanoma (A2058) and promyelocytic leukemia (HL-60)[31].

It is especially worth paying attention to the strong activity against skin cancer cell lines that have been tested. The cytotoxic activity of bufadienolides at the level of 0.007 μg/mL or 0.01 μM respectively for epidermoid (A431) and melanoma (A2058) allow them to be considered very interesting as ingredients of cosmetic formulations applied to the skin.

Important antiviral properties of bufadienolides have also been demonstrated. Extracts from *K. pinnata* and *K. daigremontiana* × *tubiflora* have a significant ability to activate antibodies for *the* Epstein-Barr virus. Bryophyllin A showed the strongest activity ($IC_{50}$ = 0.4 μM)[32]. Bryophyllin B (**10**) was also able to inhibit the replication of the human immunodeficiency virus (HIV). In this study, the activity was at the level of $ED_{50}$ <0.25 μg/mL [31].

## Other use of bufadienolides

Another interesting application of plant extracts containing bufadienolides would be their use as potential insecticides. Research has shown that daigremontianin (**11**) and bersaldegenin-1,3,5-orthoacetate (**16**) present in the

leaves of *K. deigremotiana* can effectively inhibit the development and survival of moth larvae *Bombyx mori* [32].

It is also worth noting that research on bufadienolides is very important not only from the point of view of humans themselves but may also turn out to be important for the preservation of many species of endangered amphibians. By understanding the relationship between the structure and activity of active substances in skin secretions, it will be possible to better understand the processes of immunity and develop methods of protection against pathogens such as the fungus Batrachochytrium dendrobatidis, which decimates amphibians all over the world, and efforts to combat it do not bring any measurable results [33,34].

## Cosmetics with *Crassulaceae*

In the last years, there has been growing interest in cosmetic products containing extracts from *Crasullaceae* species, especially from the *Kalanchoe* genus. The biological and pharmacological effects of the application of skin care products with such compounds were mainly due to their soothing and regenerating properties for damaged, sensitive, and psoriatic skin. Products with Kalanchoe extracts are concerning as relieving for dull and dry skin or skin conditions like acne and breakouts. It can help increase the production of collagen in the skin so works like an anti-aging ingredient. A common advantage of the application of Kalanchoe extract is mitigation of inflammation and that it fits all types of skin [35].

Some other sources pointing that cream containing *Kalanchoe daigremotiana* extract helped cure psoriatic plaques and promote the gradual disappearance of psoriatic foci. Moreover, it does not affect the natural pH of the skin and moisturizes and strengthens the skin's hydrolipid barrier. Scientists concluded that *Kalanchoe daigremotiana* extract can be an alternative to topical steroid drugs for the treatment of various forms of plaque psoriasis [36]. Another species, *Kalanchoe pinnata* is also considered very useful in cosmetic formulations. Extract from leaves is recognized for its anti-aging and preservative properties. Moreover, it possesses interesting antibacterial and antioxidant properties and a non-cytotoxic impact on skin cells [37]. *Kalanchoe pinnata* extract was also used in formulations of antibacterial and antiseptic herbal cream. The tested cream possess better antibacterial activity than available on the market one [38]. *Kalanchoe pinnata* extract was also tested for its wound healing effectiveness. In the study, two cream formulations were tested - with crude leaves extract and with purified flavonoids. Experiments in rats showed that both formulations speed up the wound healing process. However, cream with crude extract is considered more profitable and less expensive compared to expensive and time-consuming steps for the isolation of pure flavonoid fraction [39]. *Kalanchoe pinnata* extract is also considered for its ability to retain water similarly to hyaluronic acid [40]. Cosmetic companies are increasingly interested in getting to the market with Kalanchoe extract. Its properties as an ingredient are

more and more appreciated both by consumers as well as for cosmetic formulation. It helps neutralise bacteria and prevent their growth in cosmetics and also acts as a preservative. For consumers, it helps get rid of swelling and redness associated with acne and breakouts, reduces pain, tenders and also soothes irritations, and brings wound healing effects [41].

## Conclusion

To summarise, bufadienolides are an important group of compounds with high biological activity in numerous physiological processes, a huge variety of derivatives, and even greater possibilities for their further transformation. Due to these properties, they have been the subject of numerous studies and scientific publications in recent years. Current trends in medicine and cosmetology force researchers to seek for plant-origin biologically active compounds. Plant extracts from various parts of the Crassulaceae plants offer an easily available and very rich source of compounds from the bufadienolides family and many other valuable substances. And so far, most reports confirm that at least some of these compounds may have significant effects in the future in many fields of medicine and cosmetic applications.

## References

1. Krenn L, Kopp B. Bufadienolides from animal and plant sources. Phytochemistry **1998**, 48:1-29.
2. Favre H, Giles P, Hellwich K-H, McNaught A, Moss G, Powell W. Revised Section F: Natural products and related compounds (IUPAC Recommendations 1999). Corrections and modifications Pure Appl Chem **2004**, 76:1283-1292.
3. Steyn PS, van Heerden FR. Bufadienolides of plant and animal origin. Nat Prod Rep **1998**, 15:397-413.
4. Tempone AG, Pimenta DC, Lebrun I, Sartorelli P, Taniwaki NN, de Andrade HF, *et al.* Antileishmanial and antitrypanosomal activity of bufadienolides isolated from the toad *Rhinella jimi* parotoid macrogland secretion. Toxicon **2008**, 52:13-21.
5. Cunha-Filho GA, Resck IS, Cavalcanti BC, Pessoa CÓ, Moraes MO, Ferreira JRO, *et al.* Cytotoxic profile of natural and some modified bufadienolides from toad *Rhinella schneideri* parotoid gland secretion. Toxicon **2010,** 56:339-348.
6. Zhang J, Sun Y, Liu J-H, Yu B-Y, Xu Q. Microbial transformation of three bufadienolides by Nocardia sp. and some insight for the cytotoxic structure–activity relationship (SAR). *Bioorg.* Med. Chem. Lett. **2007**, 17:6062-6065.
7. Gao H, Zehl M, Leitner A, Wu X, Wang Z, Kopp B. Comparison of toad venoms from different Bufo species by HPLC and LC-DAD-MS/MS. J Ethnopharmacol **2010**, 131:368376.
8. Cunha Filho GA, Schwartz CA, Resck IS, Murta MM, Lemos SS, Castro MS, *et al.* Antimicrobial activity of the bufadienolides marinobufagin and telocinobufagin isolated as major components from skin secretion of the toad *Bufo rubescens*. *Toxicon Off J In. So.* Toxinology **2005**, 45:77-782.
9. Ferreira PMP, Lima DJB, Debiasi BW, Soares BM, Machado K da C, Noronha J da C, *et al.* Antiproliferative activity of *Rhinella marina* and *Rhaebo guttatus* venom extracts from Southern Amazon. Toxicon **2013**, 72:43-51.

10. Schmeda-Hirschmann G, Quispe C, Theoduloz C, de Sousa PT, Parizotto C. Antiproliferative activity and new argininyl bufadienolide esters from the "cururú" toad *Rhinella* (Bufo) *schneideri*. J Ethnopharmacol **2014**, 155:1076-1085.
11. Gentry HS, Verbiscar AJ, Banigan TF. Red squill (*Urginea maritima, Liliaceae*). Econ Bot **1987**, 41:267-282.
12. Steyn PS, Heerden FR van, Vleggaar R, Anderson LAP. Bufadienolide glycosides of the *Crassulaceae.* Structure and stereochemistry of orbicusides A—C, novel toxic metabolites of *Cotyledon orbiculata*. J Chem Soc Perkin Trans 1 **1986**, 1633-1636.
13. Pohl T, Koorbanally C, Crouch NR, Mulholland DA. Bufadienolides from *Drimia robusta* and *Urginea altissima* (*Hyacinthaceae*). Phytochemistry **2001**, 58:557-561.
14. Kolodziejczyk-Czepas J, Sieradzka M, Moniuszko-Szajwaj B, Pecio Ł, Ponczek MB, Nowak P, *et al.* Bufadienolides from *Kalanchoe daigremontiana* as thrombin inhibitors-In vitro and in silico study. Int J Biol Macromol **2017**, 99:141-150.
15. Wu P-L, Hsu Y-L, Wu T-S, Bastow KF, Lee K-H. Kalanchosides A−C, New Cytotoxic Bufadienolides from the Aerial Parts of Kalanchoe gracilis. Org Lett **2006**, 8:5207-5210.
16. Anderson LAP, Steyn PS, Heerden FR van. The characterization of two novel bufadienolides, lanceotoxins A and B from *Kalanchoe lanceolata*[Forssk.] Pers *J Chem Soc Perkin Trans 1* **1984**, 1573-1575.
17. Supratman U, Fujita T, Akiyama K, Hayashi H. New insecticidal bufadienolide, bryophyllin C, from *Kalanchoe pinnata*. Biosci Biotechnol Biochem **2000**, 64:1310-1312.
18. Steyn PS, Heerden FR van, Vleggaar R, Anderson LAP. Structure elucidation and absolute configuration of the tyledosides, bufadienolide glycosides from *Tylecodon grandiflorus*. J Chem Soc Perkin Trans 1 **1986**, 429-435.
19. Crouch NR, du Toit K, Mulholland DA, Drewes SE. Bufadienolides from bulbs of *Urginea lydenburgensis* (*Hyacinthaceae: Urgineoideae*). Phytochemistry **2006**, 67:2140-2145.
20. Gray TC. The use of d-tubocurarine chloride in anaesthesia. Ann R Coll Surg Engl **1947**, 1:191-203.
21. Myers CW, Daly JW, Malkin B. A dangerously toxic new frog (*Phyllobates*) used by Emberá Indians of western Colombia, with discussion of blowgun fabrication and dart poisoning. Bulletin of the AMNH **1978**, 161, article 2.
22. Oufir M, Seiler C, Gerodetti M, Gerber J, Fürer K, Mennet-von Eiff M, *et al.* Quantification of bufadienolides in *Bryophyllum pinnatum* leaves and manufactured products by UHPLC-ESIMS/MS. Planta Med **2015**, 81:1190-1197.
23. Kamano Y, Yamashita A, Nogawa T, Morita H, Takeya K, Itokawa H, *et al.* QSAR evaluation of the Ch'an Su and related bufadienolides against the colchicine-resistant primary liver carcinoma cell line PLC/PRF/5(1). J Med Chem **2002**, 45:5440-5447.
24. Kamboj A, Rathour A, Kaur M.. Bufadienolides and their medicinal utility: A review. Int J Pharm Pharm Sci **2013**, 5:20-27.
25. Wang G, Sun G, Tang W, Pan X. The application of traditional Chinese medicine to the management of hepatic cancerous pain. J Tradit Chin Med Chung Tsa Chih Ying Wen Pan **1994**, 14:132-138.
26. Meng C, Xu D, Son Y-J, Kubota C. Simulation-based economic feasibility analysis of grafting technology for propagation operation, 62nd IIE Annual Conference and Expo 2012, pp. 1923-1932.

27. Kolodziejczyk-Czepas J, Nowak P, Wachowicz B, Piechocka J, Głowacki R, Moniuszko-Szajwaj B, *et al.* Antioxidant efficacy of *Kalanchoe daigremontiana* bufadienolide-rich fraction in blood plasma in vitro. Pharm. Biol. **2016**, 54:3182-3188.
28. Aucamp J. 2014. The antioxidant properties of bufadienolides, analogous to the orbicusides of *Cotyledon orbiculata* L. var *orbiculata* (Haw.) DC. *undefined*.
29. Yamagishi T, Haruna M, Yan XZ, Chang JJ, Lee KH. Antitumor agents, 110. Bryophyllin B, a novel potent cytotoxic bufadienolide from *Bryophyllum pinnatum*. J Nat Prod **1989**, 52:1071-1079.
30. Kuo P-C, Kuo T-H, Su C-R, Liou M-J, Wu T-S. Cytotoxic principles and α-pyrone ring-opening derivatives of bufadienolides from *Kalanchoe hybrida*. Tetrahedron **2008**, 64:3392-3396.
31. Huang H-C, Lin M-K, Yang H-L, Hseu Y-C, Liaw C-C, Tseng Y-H, *et al.* Cardenolides and bufadienolide glycosides from *Kalanchoe tubiflora* and evaluation of cytotoxicity. Planta Med **2013**, 79:1362-1369.
32. Supratman U, Fujita T, Akiyama K, Hayashi H. Insecticidal compounds from Kalanchoe daigremontiana x tubiflora. Phytochemistry **2001**, 58:311-314.
33. Holden WM, Reinert LK, Hanlon SM, Parris MJ, Rollins-Smith LA. Development of antimicrobial peptide defenses of southern leopard frogs, *Rana sphenocephala*, against the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*. Dev Comp Immunol **2015**, 48:65-75.
34. Ackleh AS, Carter J, Chellamuthu VK, Ma B. A model for the interaction of frog population dynamics with *Batrachochytrium dendrobatidis*, *Janthinobacterium lividum* and temperature and its implication for chytridiomycosis management. Ecol Model **2016**, 320:158-169.
35. What are the 3 secrets of Kalanchoe plant for anti-aging benefits? – ZALI. Available from https://zalicosmetics.com/discover-3-secrets-of-choosing-a-kalanchoe-product-for-best-anti-aging-benefits/. Accessed Jul. 21, 2022.
36. Effect of *Kalanchoe daigremontiana* on skin regeneration. Available from https://helpcosmetics-b2b.com/blogs/blog-helpcosmetics/wplyw-kalanchoe-daigremontiana-na-regeneracje-skory. Accessed Jul. 21, 2022.
37. Destandau E, Krolikiewicz-Renimel I, El Abdellaoui S, Cancellieri P, Fougère L, Toribio A, *et al.* Bio-guided targeting for preservative and anti-ageing cosmetic ingredient development. Cosmetics **2014**, 1:14-28.
38. Pattewar SV, Patil DN. Formulation of herbal antibacterial cream by using extract from *Kalanchoe pinnata* leaves. Res J Top Cosmet Sci **2014**, 5:1-4.
39. Coutinho MAS, Casanova LM, Nascimento LBDS, Leal D, Palmero C, Toma HK, *et al.* Wound healing cream formulated with *Kalanchoe pinnata* major flavonoid is as effective as the aqueous leaf extract cream in a rat model of excisional wound. Nat Prod Res **2021**, 35:6034-6039.
40. Leaf of life - quenches skin from within - clarins - clarins. Available from https://www.clarinsusa.com/en/explore-beauty-trip/leaf-of-life.html. Accessed Jul. 21, 2022.
41. The wonderful benefits of the Kalanchoe in cosmetics and how it can benefit acne – ZALI. Available from https://zalicosmetics.com/the-wonderful-benefits-of-the-kalanchoe-in-cosmetics-and-how-it-can-benefit-acne/. Accessed Jul. 21, 2022.