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# Anura venom (Tetrapoda: Amphibia) with therapeutic possibilities

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## 1. Introduction

Toads, frogs, and tree frogs belong to the Phylum Chordata, Superclass Tetrapoda Class Amphibia, Order Anura, Families Bufonidae, Dendrobatidae, Eleutherodactylidae, Hylidae Leiuperidae, Leptodactylidae, Mantellidae and Myobatrachidae. Amphibians are vertebrates known for having “double lives”, as they have an aquatic and a terrestrial life phase. All amphibians are ectotherms. Amphibians have very permeable skin, which is used to carry out cutaneous and pulmonary respiration. They have a three-chambered heart [1-4].

Anura has a sac on each side of the head called a parotoid gland, with a spongy structure containing a milky fluid. Contrary to what many believe, this milky liquid is not poisonous to humans, causing at most irritation and, even then, only if placed in the mouth or eyes. They only use this liquid to defend themselves from predators (Figure 1) [5-6].

**Figure 1.** Female *Rhinella jimi* (Stevaux, 2002) (Anura: Bufonidae) sapo-cururu exhibiting the right parotoid macrogland

localized at the postorbital region. The arrows point to the glandular pores. (2) A parotoid sectioned according to a frontal plane, from which the venom was withdrawn. Notice the alveolar, honeycomb-like internal structure. (3) Higher magnification of the alveoli showing the walls (arrows) and floors (asterisks). (4) Longitudinal section of two parotoid bottle-shaped glands (G). The arrows point to the pores obstructed by a thick epithelium. D, the dermis. Paraffin, HE. (5) Venom jets squirting from the pores, after parotoid manual compression. (6) View of the pores on the parotoid surface. Note the small slit (large arrows) in the duct centre. Source: Toxicon Volume 54, Issue 3, 1 September 2009, Pages 197-207https://doi.org/10.1016/j.toxicon.2009.03.029.

They are nocturnal and males croak during the breeding season. The skin of Anura is thin and permeable and, therefore, these animals are very sensitive to changes in both the aquatic environment and the soil and air. The skin is also important in absorbing water since these animals do not drink water. They do not have strong teeth, only weak serrations, and therefore do not bite or chew the animals that make up their diet. They use their elastic tongue attached to the front of the mouth in most species to capture potential prey [7-9].

Most species live near rivers and lakes, as the female needs water to lay her eggs. Tadpoles also need water to survive. Some species have glands on their heads, where they produce a toxic poison to scare away or even kill other animals that pose some type of danger. Despite having few predators in nature, the main ones are snakes and large birds. The frogs' main source of food is insects. They catch insects using their large tongues. The toad's skin is rougher and drier than that of the Anura [7-9].

**Reproduction:** Males croak to attract a partner to mate. Mahos croak at the edge of lakes, on the ground, or in the water, and sometimes hidden among vegetation. They normally croak and reproduce in the place where they were born. Courtship for mating: males attract females through specific mating calls, which vary between species. This is the first step of your reproductive cycle [7-9].

After a female chooses a male, she enters a position called Amplexus. Here, the male holds the female from behind, preparing her for spawning. Laying spawning: The female releases the eggs, usually into the water (external fertilization). Depending on the species, these eggs are typically laid in clusters or strings [7-9].

Simultaneously, the male releases sperm into the eggs to fertilize them. This external fertilization is typical in most frog species. The fertilized eggs, now known as zygotes, turn into tadpoles. This phase is marked by significant cell growth and differentiation. After hatching, the larvae are known as tadpoles. They are aquatic and have gills, tails, and mouths for grazing. tadpoles go through a transformation process (metamorphosis) where they develop legs, lungs, and other adult characteristics [7-9].

During this phase, the tail is absorbed and eating habits change. Juvenile phase: after metamorphosis, they resemble adults, but are smaller. They continue to grow and mature at this stage. Once fully mature, the frogs reach sexual maturity and can reproduce, thus completing the cycle. This reproductive process is influenced by environmental factors such as temperature and humidity. The most common frog species found in Brazil are the cane toad *Rhinella marina* (Laurenti, 1758) (Anura: Bufonidae), the green-bellied *Scinax nasicus* (Cope, 1862) ( (Anura: Hylidae), the yellow-crowned frog

*Hypsiboas raniceps* Cope, 1862, the black toad and yellow frog Dendropsophus leucophyllatus (Beireis, 1783) (Anura: Hylidae) and the green and yellow frog *Dendropsophus minutus* (Peters, 1872) (Anura: Hylidae) [9-10].

## 2. Chemical defenses

Chemical defenses in amphibians represent a set of defensive adaptations that act to protect against predators, parasites, and microorganisms. Defense chemicals are synthesized in the organism's own body, but some animals are capable of sequestering these compounds from the diet. Among these chemical compounds are some groups of lipophilic alkaloids that are sequestered from arthropods. A wide variety of lipophilic alkaloids is observed in some groups of animals. These alkaloids represent a large defense arsenal distributed in glands present in your skin, the vast majority of them come from the ant diet and have not yet been found elsewhere in nature (Figure 2) [11-13].

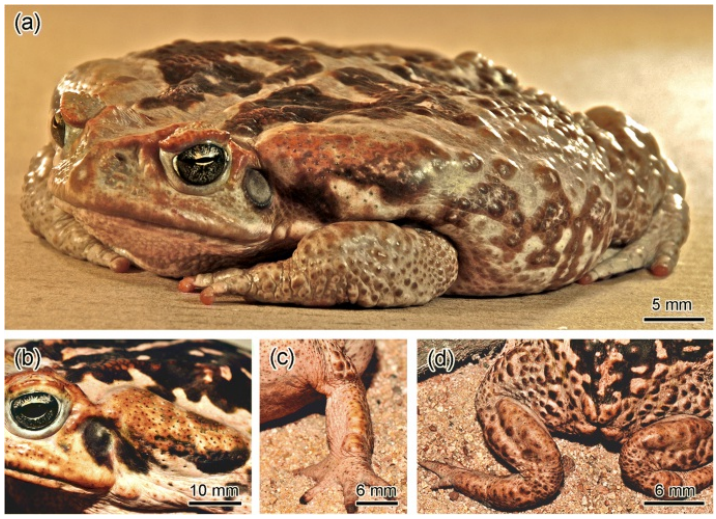


**Figure 2.** *Rhinella jimi* (Stevaux, 2002) (Anura: Bufonidae) as a representative of the group*Rhinella marina* (Laurenti, 1758) (Anura: Bufonidae). (a) Note the parotoids, the numerous dorsal warts, and the macroglands on the limbs. (b) A parotoid macrogland. (c) The radial macrogland on the forelimb. (d) The tibial (or paracnemic) macroglands on the hind limbs. Source: https://doi.org/10.1186/s12983-018-0294-5.

The toad's venom is in glands on the surface of its skin and is only released when it suffers some aggression such as when an animal attacks it. The frog has what we call passive defense: it does not attack and does not react when attacked. It only inflates its lungs to expose the glands, and the venom is only released when the predator bites or squeezes these glands. Some groups of amphibians developed under pressure from predators, and the microscopic1glands in their skin became macroscopic (Figure 3]. [14-15].

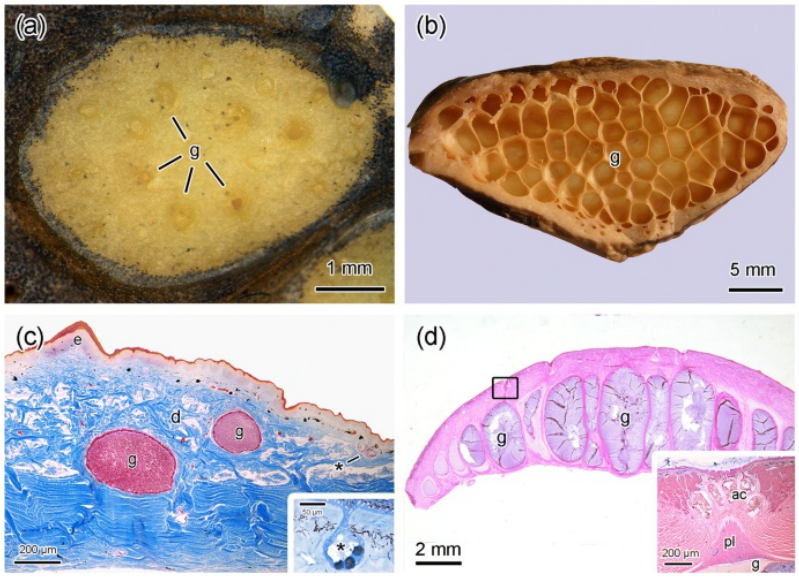


**Figure 3.** Morphological comparison between the dorsal warts and the parotoid macroglands. (a) Longitudinal section of a dorsal wart. Note the small number of poison glands (g). (b) Longitudinal section of a parotoid. The honeycomb-like structure is formed by a large number of subunits side by side, each one lodging a poison gland (g). (c) Transverse histological section of a dorsal wart, showing poison glands (g) and a mucous gland (*) highlighted in the insert. e, epidermis, d, dermis. (d) Transverse histological section of a parotoid macrogland showing the juxtaposed distribution of the poison glands (g). Note that the glands are larger in the centre of the structure and decrease towards the periphery. The insert represents a high magnification of the area delimited by the rectangle, showing a duct obstructed by an epithelial plug (pl) surrounded by accessory glands (ac). Species: Rhinella marina (a, c, d) and Rhinella icterica (b). Staining: Mallory's trichrome (c), toluidine blue-fuchsine (c, insert), hematoxylin-eosin (d), Source: https://doi.org/10.1186/s12983-018-0294-5.

Toad venom can contain hundreds of substances, divided into four main groups: alkaloids, steroids, biogenic amines, and peptides. Most of these substances are still unknown to science and have been studied much less than snake venom. What is known is that some compounds are psychoactive and, when they encounter the mucous membrane of the mouth, eyes, or nose, they can cause hallucinations and convulsions in predators, as well as respiratory and heart problems. The poison, however, can slowly corrode the mucosa and enter the bloodstream, which can be fatal. When the poison is not normally ingested in human poisoning, simply wash the area that meets the substance [15-16].

Some do not contain poison and those that are poisonous can vary in terms of degree of toxicity and effects. The toad's venom is produced in the skin glands and the paratoid gland just behind the eyes. When pressed, the glands release the toxin: a secretion with a viscous, whitish appearance, like milk [16-17].

### 3. Species/Poison/ Substances.

The cane toad *Rhaebo guttatus* (Schneider, 1799) (Anura: Bufonidae), also known as the bullfrog, is one of the types of poisonous frog that can release poison at the slightest sign of threat, without the need to squeeze the gland. The gland responsible for producing the venom is located close to the region behind the eyes, and the cane toad can expel the

venom up to two meters away [17-18].

This frog easily adapts to different types of environments and is found in different regions of Brazil, from the Atlantic Forest and the Cerrado to urbanized areas. The changes that occur in the frog's venom vary, as there are species that are more poisonous than others. We list some clinical signs here: Skin irritation; eye irritation; blindness; seizure; abdominal pain; vomiting; other neurological signs; cardiac arrhythmia; and death [18-19].

The secretion of cardiac glycosides from the paratoid glands represents a steroid nucleus, with a lactone ring in its 17 carbons, essential for its selective activity in the heart, a third carbon level produces the glycosidic bonds that read the physical properties of solubility and lipid solubility, potency and binding with plasma proteins, elimination and duration of effect. Contains toxins such as bufogenins, bufotoxins, bufotenins, epinephrine, serotonin, ergosterol, cholesterol, and 5-hydroxytryptamine [19-20].

The action of bufogenins and bufotoxins, which are cardioactive steroids, results in ventricular fibrillation, as seen in digitalis poisoning. The greatest risk of poisoning occurs in summer, spring, and autumn, at night, and after rain. Bufotoxin has an enzymatic level that inhibits the ATPase of the sodium and potassium pump of the cardiac muscle fiber, blocking activity in sodium channels, and increasing the concentration of intracellular calcium, causing increased cardiac contraction and reduced heart rate (Figure 4) [20-21].

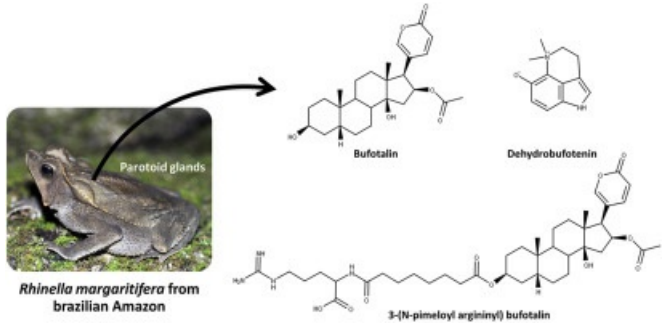


**Figure 4.** *Rhinella margaritifera* Laurenti (Laurenti, 1768) poison in bufonids (Anura: Bufonidae) includes proteins, biogenic amines, toxic bufadienolides, and alkaloids. The chemical composition of the methanolic extract of parotoid gland secretions by the Amazonian toad was evaluated in a UFLC-DAD-micrOTOF system five arginine diacids, six bufagenins (telocinobufagin, marinobufagin, bufotalin, cinobufotalin, bufalin and cinobufagin), six bufotoxins, and an alkaloid (dehydrobufotenin). Sources: https://www.researchgate.net/figure/An-adult-common-toad-with-the-length-and-width-of-the-left-parotoid-gland-shown-by_fig1_331404443 and https://doi.org/10.1016/j.toxicon.2020.04.106.

**Therapeutic possibility:** Bufonids produce very potent toxins in their skin, especially concentrated in the parotoid glands, dorsal structures located in the post-orbital region, and can be fatal to predators when ingested However, several studies have been exploring these compounds in pharmacological models for antitumor and cytotoxic, cardiotonic, antifungal, antimicrobial and antiparasitic activity [21-22].

As sufoteninas são substâncias com ação semelhante à serotonina, dentro destas estão os numerosos grupos de bufoteninas, quimicamente são bases orgânicas que contêm um anel indol; e tem efeito vasopressor. Na Adrenalina essa substâncias agonistas do sistema nervoso autónomo que atuam nos receptores alfa1, beta1 e beta2; o alfa induzem vasoconstrição na pele e nas vísceras; vasodilatação muscular e brônquica por ação sobre alfa2, por ação sobre o beta1 que produz aumento da contração cardíaca e da frequência cardíaca [21-22].

Noradrenaline is an antagonist that acts on alpha1 and beta1 receptors with the same effect as adrenaline. cholesterol, ergosterol, and Gamasistosterol (not mentioned previously), these groups of steroids do not play an important role in the toxic action of the venom. These substances cause cardiovascular problems such as fibrillation, sinus blockage, and cardiac arrest [23-24].

Although toads can be poisonous, they are extremely important to nature. They feed mainly on insects, such as crickets, grasshoppers, mosquitoes, flies, larvae, bedbugs, ants, termites, and small rodents, ensuring the balance of species and the ecosystem. By controlling some of these insects, diseases they transmit can also be avoided, such as dengue fever, zika, chikungunya, yellow fever, and Chagas disease [24-25].

Although toads can be poisonous, they are extremely important to nature. They feed mainly on insects, such as crickets, grasshoppers, mosquitoes, flies, larvae, bedbugs, ants, termites, and small rodents, ensuring the balance of species and the ecosystem. By controlling some of these insects, diseases they transmit can also be avoided, such as dengue fever, zika, yellow fever, and Chagas disease. The frog of the species *Incilius alvarius* (Girard, 1859), also known as the Colorado River frog, is found in the United States, Mexico, and Brazil and has become popular in recent years due to the psychoactive characteristics of substances in its venom. The venom is released when the animal feels threatened and occurs because of pressure on the glands in its skin [25-26].

The poison of the bufo toad is caused mainly by the presence of a substance called 5-MeO-DMT, (5-methoxy-N, N-dimethyltryptamine or O-methyl-bufotenine) is from the tryptamine class of occurrence. 5-MeO-DMT is a potent, fast-acting, and short-acting hallucinogen in humans. The effects begin three to four minutes after ingestion, peak in about thirty-five and forty minutes, and end after an hour. The effects are similar to those of other tryptamine psychedelics, such as those present in mushrooms, and may include distortions in visual, auditory, and temporal perception, emotional experiences, and memory impairment [27-36].

**Therapeutic possibility:** Scientific studies point to 5-MeO-DMT as useful in treating mental health problems when combined with psychological therapies. It can cause rapid and sustained reductions in symptoms of depression, anxiety, and stress. This substance stimulates neuroendocrine function, immunoregulation, and anti-inflammatory processes in the brain, which can contribute to mental health treatments (Figure 5). [27-36].

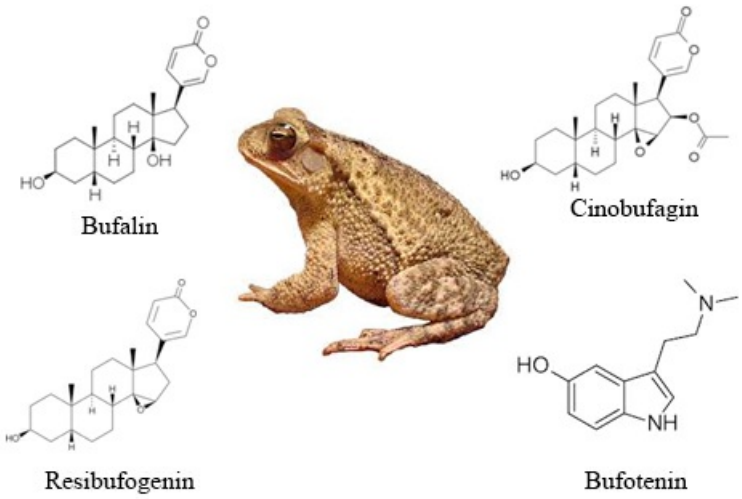


**Figure 5.** Some typical toxic amines and steroid derivatives of toads. Source: https://nifc.gov.vn/en/technical-news/prevention-of-toad-poisoning-post2057.html.

They explain that the substance binds to two serotonergic receptors, 5-HT1A and 5-HT2A, in the Central Nervous System (CNS). Serotonin is a neurotransmitter closely linked to mood and the feeling of well-being, which is why its activation provides hallucinogenic effects. The scientists write that subjective effects following the administration of 5-MeO-DMT include distortions in auditory and temporal perception, amplification of emotional states, and feelings of ego dissolution that are generally short-lived depending on the route of administration [27-36].

**Therapeutic possibility:** Different species of amphibians have already identified molecules with antimicrobial properties. **Therapeutic possibility:** One of the published studies demonstrated in pre-clinical tests that a peptide isolated from can eliminate the protozoan Trypanosoma cruzi Chagas, 1909 (Kinetoplatida: Trypanosomatidae), which causes Chagas Disease [27-36].

**Therapeutic possibility:** Other research has shown that the skin of the frog*Phyllomedusa hypochondrialis* (Daudin, 1802) (Anura: Phyllomedusidae) contains antimicrobial compounds. Amphibians are considered nature's apothecaries. they produce a multitude of substances. We identified antibiotics present in the animal's skin, which explains why it never suffers from skin infections [27-36].

According to researchers, most of the compounds present in*Incilius alvarius* venom are part of our brain metabolism, such as adrenaline and bufotenin itself. The chemical structure of bufotenine was described in 1934 and synthesized in the laboratory in 1936. In recent years, some scientific studies have already identified high levels of bufotenine in the urine of people with schizophrenia and autism spectrum disorder [asd], for example, states. Research published in 2010 found a correlation between high levels of bufotenine and the severity of ASD [27-36].

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Scientists do not know the origin of the incredible toxicity of the frog*Phyllobates terribilis* Myers, Daly, and Malkin, 1978, but these amphibians may absorb poison from plants, which is carried by their prey. *P. terribilis* frogs raised in captivity and isolated from insects naturally found in their native habitat do not develop poison. The medical research community has been exploring possible medicinal uses for *P. terribilis* toxins. **Therapeutic possibility:** A synthetic version of one of the poison's compounds has already been developed and promises to be a powerful painkiller. Active ingredient: epibatidine, an alkaloid with an analgesic effect that is not addictive but is 200 times stronger than morphine. Its therapeutic use has already been considered by doctors; it becomes lethal in doses so small that it is not worth the risk [37-42].

A species of amphibian with venom injection as a defense mechanism was found in the semiarid northeastern region of Brazil. An interesting feature of the morphology of the frog of the species *Corythomantis greeningi* Boulenger, 1896 (Anura: Hylidae) is the similarity of its skull to that of a thorny cactus. This ability to inject makes C. greening almost unique. When threatened, the species tilts its head and stings its enemies, rubbing the spines and stinging the would-be predator's body [37-42].

**Dilemma:** Frog poisoning is a dangerous and frequent phenomenon in Vietnam, although there have been many warnings about the risks of using frogs as food. However, some parts of your body contain toxins that can be deadly. **Therapeutic possibility:** In traditional medicine, frog meat is used to treat child malnutrition due to its high protein and zinc content.

The species' head not only serves defensive purposes but also to prevent desiccation. The frog prevents its body from drying out through a process known as Phragmosis, an adaptive behavior in which frogs lock themselves inside holes, trees, or rocks, using their bodies. Although frogs do urinate as a measure of self-defense, the liquid itself does not contain any toxic substances. The real poison is released by the granular glands present in its skin. This is a type of passive defense (Figure 6) [37-42].

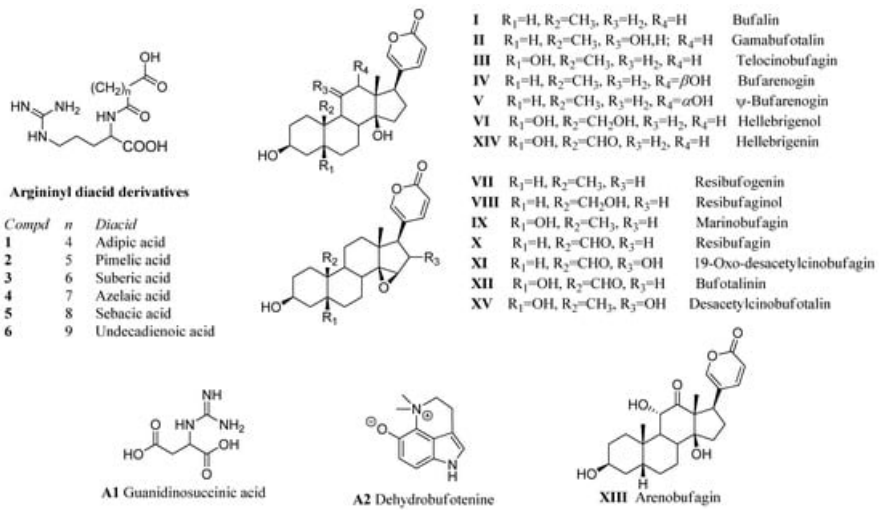


**Figure 6.** Structure of the compounds A1, A2, genines (bufadienolides) I–XV, and argininyl diacids 1–6 tentatively

identified in PGS of toad *Rhinella horribilis* (Wiegmann, 1833). Source: Toxins. 2020: 12(9): 608. https://doi.org/10.3390/toxins12090608.

**Therapeutic possibility:** The compound from the Bufadienolidae class called Marinobufagin, isolated from the glandular secretion known as venom, of the cane toad of the *R. marina* species, has the control action used in the clinic to treat many histologically different types of cancer, the drug doxorubicin, by activating apoptosis as a pathway of cell death, but with the advantage of being approximately 70 times more specific against leukemic cells than against normal leukocytes also suggest that the potent in vitro antiproliferative activity of the isolated compound is species-specific since only human tumor cells proved to be sensitive [37-42].

**References**

[1] Hickman CP. Integrated principles of Zoology. 16th ed. Rio de Janeiro: Guanabara Koogan. 2016.

[2] Pough F, et al. The life of Vertebrates. 1st ed. Rio de Janeiro: Atheneu. 2013.

[3] Toledo LF, et al. The anuran calling repertoire in the light of social context. Acta Ethologica. 2013; 18: 87–99.

[4] Frost D. Amphibian species of the world: an online reference. 1st ed. New York: American Museum of Natural History. 2009.

[5] Calanca I. Unesp researchers describe new species of frog [Internet]. Rio Claro: Jornal da UNESP; @2023 [cited 2024 Apr 18]. Available from https://jornal.unesp.br/2023/08/29/pesquisadores-da-unesp-describem-nova-especie-de-sapo/.

[6] France JMS. The composition of the ururuzinho toad poison you change according to your diet? [Internet]. Alfenas: Federal University of Alfenas; @2015 [cited 2024]. Available from file:///C:/Users/USUARIO/Downloads/Disserta%C3%A7%C3%A3o%20de%20Juceli%20Maria%20da%20Silva%20Fran%C3%A7a.pdf.

[7] Yovanovich CAM. The dual rod system of amphibians supports color discrimination at the absolute visual threshold. Philosophical Transactions of the Royal Society B: Biological Sciences. 2017; 1717: 20160066.

[8] Cruz CO. Toads [Internet]. Recife: Infoescoal; @2016 [cited 2024 Apr 18]. Available from https://www.infoescola.com/anfibios/sapo/.

[9] Kindermann C, Narayan EJ, Jean-Marc H. The Neuro-hormonal control of rapid dynamic skin color change in an amphibian during amplexus. PLoS ONE. 2014; (12): e114120.6.

[10] Bagnara T, Matsumoto J. Comparative anatomy and physiology of pigment cells in nonmammalian tissues. 1st ed. Oxford: Blackwell Publishing Ltd. 2020.

[11] Burnie D. Thematic Dictionary of Biology. 1st ed. São Paulo: Editora Scipione 1997.

[12] Canhete JLL, Ramos FF. The importance of amphibians and their characteristics: learning through a didactic

sequence Revista Insignare Scentia. 2021; 4(6): 167-186.

[13] Duellman WE, Trueb L. Biology of amphibians. 1st ed. Baltimore: Johns Hopkins University Press. 1994.

[14] Guimarães L, Pereira J, Ferraz C. Amphibians in the early years: teaching strategy involving fairy tales for teaching basic biology. Revista Insignare Scientia. 2020; 3(5): 362-371.

[15] Passive but efficient defense: understand why frogs have poison [Internet]. São Paulo: Instituto Butantan; @2022 [cited 2024 Apr 17]. Available from https://butantan.gov.br/butantan-educa/defesa-passiva-mas-efficient-entenda-por-que-os-sapos-tem-veneno.

[16] Editorial National Geographic Brazil [Internet}. São Paulo: The poison of the bufo toad: how the potent hallucinogen produced by this amphibian works; @2024 [cited 2024 Apr 17]. Available from https://www.nationalgeographicbrasil.com/animais/2023/05/o-veneno-do-sapo-bufo-como-age-o-potente-alucinogeno-produtor-por-esse-anfibio.

[17] Psychoactive substances from frog venom pose dangers to human health and are linked to psychiatric disorders [Internet]. São Paulo: Instituto Butantan; @2022 [cited 2024 Apr 17]. Available from https://butantan.gov.br/noticias/substancias-psicoativas-de-veneno-de-sapo-trazem-perigos-a-saude-humana-e-tem-relacao-com-transtornos-psiquiatricos.

[18] O Globo [Internet]. Rio de Janeiro: Frog poison what it is and what are the risks of the hallucinogenic drug seized in Goiás; @2023 [cited 2024 Apr 17]. Available from https://oglobo.globo.com/saude/noticia/2023/04/veneno-de-sapo-o-que-e-e-quais-os-riscos-da-droga -hallucinogen-seized-in-goias.ghtml.

[19] Nicholson SS. Toxicology. In: Ettinger SJ, Feldman EC. Treatise on veterinary internal medicine. 1st ed. São Paulo: Manole. 1995.

[20] Sonne L, et al. Toad venom intoxication in a dog. Rural Science. 2008; 38(6): 1787-1789.

[21] Brubacher JR, et al. Efficacy of digoxin Fab fragments (Digibind) in the treatment of toad venom poisoning. Toxicon. 1999; 37: 931-942.

[22] Godoy L, et al. Toxicity of the secretion of parotid glands in frogs (Bibliographic Update). National University of the Northeast Scientific and Technological Communications. 2005: 20.

[23] Roder JD. Toads. In: Plumlee KH, eds. Clinical veterinary toxicology. 1st ed. Jefferson City: Mosby; 2003. p. 1-113.

[24] Gowda RM, et al. Toad venom poisoning: resemblance to digoxin toxicity and therapeutic implications. Heart. 2002; 14(89): 483-485.

[25] Palumbo NE, et al. Experimental induction, and treatment of toad poisoning in the dog. Journal of the American Veterinary Medical Association. 1975; 167(11):1000-1004.

[26] Roberts BK, et al. *Bufo marinus* poisoning in dogs: 94 cases (1997-1998). Journal of the American Veterinary Medical Association. 2000; 216(12): 1941-1944.

[27] Sakate M, Oliveira PCL. Toad envenomation in dogs: effects and treatment. Journal Venomous Animals and Toxins. 2000; 1(6): 53-62.

[28] Osweiler GD. Toxicology. 1st ed. Philadelphia: Lippincott William & Wilkins. 1995.

[29] Godoy L. Ortiz L, Teibler P, Acosta O. Toxicity of the secretion of parotid glands in frogs (Bibliographic Update) [Internet]. Corrientes: Technological Scientific Communications, National University of the Northeast; @2005 [cited 2024 Apr 17]. Available from file:///C:/Users/USUARIO/Downloads/Toxicidad_Sapo%20(1).pdf.

[30] Mailho-Fontana PL., et al. Caracterização morfológica e bioquímica das glândulas venenosas cutâneas em sapos (grupo Rinella marina) de diferentes ambientes. Fronteiras em Zoologia. 2018; 15:46.

[31] Hoffman BF, Bigger JR. Digitalis and associated cardiac glycosides. In: Goodman LS, Gilman A, eds. The bases of pharmacological and therapeutic aspects. 8st ed. Rio de Janeiro: Guanabara Koogan; 1991; p. 536-552.

[32] Curtis H, Barnes MS. Biology. 6th ed. New York: Freeman. 1992.

[33] Lucas OJ, Sakate PC. Toads envenoming dogs: effects and treatment. Journal of Venomous Animals and Toxins. 2000; 6(1): 52-62.

[34] Brownlee AA, Johnson P, Mills IH. Actions of bufalin and cinobufalin two bufadienolides respectively more active and less active than ouabain, ouabain binding, and rob uptake by human erythrocytes. Clinical Science. 1990; 78: 169-174

[35] Vaiano B. Poisonous frogs can help us produce better painkillers [Internet]. São Paulo: Super Interesting; @2024 [cited 2024 Apr 17]. Available from https://super.abril.com.br/ciencia/sapos-venenosos-produzem-analgesico-200-vezes-mais-forte-que-a-morfina.

[36] Jared C. Venomous frogs use heads as weapons. Current Biology. 2015; 25: 2166–2170.

[37] Jared C, et al. Head co-ossification, phragmosis, and defense in the casque-headed tree frog*Corythomantis greeningi*. Journal of Zoology. 2005; 265: 1–8.

[38] Schmeda-Hirschmann G, et al. Antiproliferative activity and chemical composition of the venom from the Amazonian toad *Rhinella marina* (Anura: Bufonidae). Toxicon: 2016; 121:119-129.

[39] Sousa LQ. Bufadienolides from amphibians: A promising source of anticancer prototypes for radical innovation, apoptosis triggering, and Na+/K+-ATPase inhibition. Toxicon. 2017; 127: 63-76.

[40] Jared C. Parotoid macro glands in the toad (*Rhinella jimi*): Their structure and functioning in passive defense. Toxicon. 2009; 54(3): 197-207.

[41] Sinhorin AP, et al. Chemical profile of the parotoid gland secretion of the Amazonian toad (*Rhinella margaritifera*). Toxicon. 2020;182: 30-33.

[42]. Schmeda-Hirschmannn G, et al.. The parotoid gland secretion from Peruvian toad*Rhinella horribilis* (Wiegmann, 1833): Chemical composition and effect on the proliferation and migration of lung cancer cells. Toxins. 2020: 12(9): 608.