# The Potential of Biologically Active Brazilian Plant Species as a Strategy to Search for Molecular Models for Mosquito Control

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## ABSTRACT

Natural products are a valuable source of biologically active compounds and continue to play an important role in modern drug discovery due to their great structural diversity and unique biological properties. Brazilian biodiversity is one of the most extensive in the world and could be an effective source of new chemical entities for drug discovery. Mosquitoes are vectors for the transmission of dengue, Zika, chikungunya, yellow fever, and many other diseases of public health importance. These diseases have a major impact on tropical and subtropical countries, and their incidence has increased dramatically in recent decades, reaching billions of people at risk worldwide. The prevention of these diseases is mainly through vector control, which is becoming more difficult because of the emergence of resistant mosquito populations to the chemical insecticides. Strategies to provide efficient and safe vector control are needed, and secondary metabolites from plant species from the Brazilian biodiversity, especially Cerrado, that are biologically active for mosquito control are herein highlighted. Also, this is a literature revision of targets as insights to promote advances in the task of developing active compounds for vector control. In view of the expansion and occurrence of arboviruses diseases worldwide, scientific reviews on bioactive natural products are important to provide molecular models for vector control and contribute with effective measures to reduce their incidence.

## Introduction

Natural products still stand out among the 1394 small-molecule approved drugs introduced in the market as pharmaceuticals during the period 1981–2019 in which more than 65% are derived from natural products or with an inspired structure [1]. Tropical ecosystems are particularly rich in chemistry diversity [2] because of the large number of different species present and the interactions among these species. Brazil has a huge biodiversity accounting for 20% of all living species [3]. Therefore, the search for natural products in plants from Brazilian biomes, a rich source of biologically active compounds [4], is an important source of new chemical entities in drug discovery. The Cerrado is a genuinely Brazilian savanna that covers about 2 million km$^2$ or 21% of the

**ABBREVIATIONS**

| | |
|---|---|
| **AaegOBP** | odorant-binding protein from *Ae. Aegypti* |
| **AaFKBP12** | FKBP12 protein from *Ae. Aegypti* |
| **aaNAT** | arylakylamine N-acetyltransferase |
| **AaPK1** | *Ae. aegypti* pyruvate kinase 1 |
| **ADP** | adenosine diphosphate |
| ***Ae.*** | *Aedes* |
| **AeHKT** | *Ae. aegypti* 3-hydroxykynurenine transferase |
| **AeKAT** | *Ae. aegypti* kynurenine aminotransferase |
| **AeSCP-2** | *Ae. aegypti* sterol carrier protein-2 |
| **AGTs** | alanine glyoxylate aminotransferases |
| **ATP** | adenosine triphosphate |
| **CHD** | chromodomain helicase DNA-binding protein |
| **CHIKV** | chikungunya virus |
| **CYD-TDV** | dengue vaccine Dengvaxia |
| **D7** | biogenic amine-binding salivary protein |
| **DDT** | dichlorodiphenyltrichloroethane |
| **DENV** | dengue virus |
| **FKBP** | FK506-binding protein |
| **GST** | glutathione S-transferases |
| **$LC_{50}$** | lethal concentration required to kill 50% of the population |
| **LTC** | cysteinyl leukotriene |
| **mJHBP** | mosquito juvenile hormone-binding protein |
| **$NuBBE_{DB}$** | database of natural products from Brazilian biodiversity |
| **OBP** | odorant-binding protein |
| **PDB** | Protein Data Bank |
| **PK** | pyruvate kinase |
| **SCP-2** | sterol carrier protein |
| **ZIKV** | Zika virus |

Brazilian territory [4] and is considered to be a hotspot of biodiversity with a high concentration of species, e.g., more than 10000 species of plants, which means high levels of biodiversity [5]. However, due to the expansion of agricultural activities as well as extensive deforestation, several species of the Cerrado are at risk of extinction [3, 6]. This is the second largest Brazilian biome, with singular characteristics of plant morphologies, soil, climate, and landscape [7]. Its flora accounts for 40% of endemic species, being the most biodiverse tropical savanna in the world [7]. Together with the Atlantic Forest, this is the most commonly reported biome in the $NuBBE_{DB}$ [8]. The accelerate loss of Cerrado biodiversity will disturb the biome, and its ecological imbalance will increase the risk of infectious diseases caused by mosquitoes. Natural products from Brazilian biomes, especially from Cerrado, could be interesting models for insecticide discovery, which will be addressed in this review.

Mosquitoes are important arbovirus vectors distributed in more than 100 genera from the order Diptera, family Culicidae. *Aedes*, *Culex*, and *Anopheles* genera are the main vectors of several arboviruses that cause diseases of great public health impact [9]. The genus *Anopheles* is responsible for transmiting the protozoa *Plasmodium falciparum* that causes malaria. This genus includes more than 400 species and approximately 70 of these are reported to transmit malaria parasites. Most common vectors of malaria by regions are *An. gambiae* in Africa, *An. Farauti* in Asia, *An. atroparvus* in Europe/Middle East, and *An. darlingi* in Brazil [10]. Mosquitoes from the *Culex* genus are vectors of arboviruses such as the agents that cause West Nile fever and Japanese encephalitis, transmitting diseases to humans, birds, and other animals. *Culex* species are spread worldwide. *Culex pipiens* and *Culex quinquefasciatus* are prevalent in urbanized areas, while *Culex tarsalis* is mostly found in rural areas. [9, 11]. The mosquito *Aedes aegypti* is the main vector for the arboviruses that cause dengue, Zika, chikungunya [12], and yellow fever [9], diseases that are of great impact on worldwide health.

Vector control is currently one of the main strategies to prevent these diseases. This review deals with plant species explored as a sustainable source for vector control of *Ae. aegypti*. This is also an extensive literature review of targets as insights to promote advances in the task of developing products for mosquito control. Such products require optimization of pharmacodynamic and pharmacokinetic properties that might be tackled by a medicinal chemistry approach and is herein discussed. Although the mosquito species main focus of this review is *Ae. aegypti*, the bioactive agents could be further explored in research for developing insecticides for many mosquito species.

*Ae. aegypti* is now distributed in almost all continents, putting billions of people at risk of the diseases transmitted by this vector [13]. Additionally, these diseases have a secondary vector and can also be transmited by *Aedes Albopictus*, which is present in Asia and parts of the Americas [13]. To contextualize the impact of the diseases transmited by these mosquitoes, dengue is caused by four serotypes of dengue virus (DENV1–4), has been identified in 128 countries, and its geographical distribution has increased in the past decades [14]. The global incidence of dengue has increased dramatically in recent decades and about half of the world's population, (est. 3.9 billion people) is at risk [14, 15]. An estimate indicates 390 million dengue infections every year worldwide [15, 16]. In Brazil, the incidence of dengue has grown considerably; in 2018, 265934 cases (155 deaths) were reported, in 2019, there were 2226914 (789 deaths) cases, and in only 16 weeks of 2020, there were 794565 cases of dengue and 181 deaths [17]. ZIKV transmission is primarily via the mosquito *Ae. aegypti*, but it is also transmitted during pregnancy, sexual contact, transfusion of blood, and organ transplantation [18]. The first recorded incidence of ZIKV disease was reported on the Island of Yap in 2007, followed by cases in several other countries. The ZIKV infection has been associated with central nervous system diseases, congenital microcephaly in children of women who had Zika, and pregnancy complications such as fetal loss, stillbirth, and preterm birth [18, 19]. CHIKV infection is an economical problem for many countries and has been identified in Asia, Africa, Europe, and the Americas, with over 2 million cases being reported since 2005 [20].

There is still no specific drug available to treat dengue, as well as neither vaccines nor drugs to prevent or treat Zika or chikungunya [13, 21]. The first dengue vaccine, Dengvaxia (CYD-TDV) developed by Sanofi Pasteur, is an attenuated tetravalent product licensed in 2015, and approved in 20 countries for use in people

aged 9–45 years living in endemic areas [22–24]. However, its use is limited because of an increased risk of more severe forms of dengue due to the vaccine [24]. The prophylaxis of these diseases is mainly through vector control [15]. Mosquito control is a challenge as resistance has developed in mosquitoes and there is a constant use of insecticides with high toxicity, which has become a major environmental concern. In addition, there is a high density of human populations and a general lack of resources in most endemic countries [25].

The most widespread method for controlling disease vectors is the application of chemical insecticides. Many of these have been successful against *Ae. aegypti* larvae and adults such as organophosphates, organochlorines, and pyrethroids. However, current chemical agents are not selective and can be toxic to non-target organisms. In addition, considering the insecticide resistance in several areas of the world, its use has been facing problems and the search for new alternatives for the control of disease vectors is needed [26]. Resistance creates yet another difficulty in vector control and raises questions about environmental safety and toxicity to other species, including humans. In Brazil, for example, the control of *Ae. aegypti* began in 1947 with the use of the organochloride DDT. In 1955, the mosquito was eradicated, but was reintroduced in Brazil in 1967, and temephos was then used for its control because it was already resistant to DDT. In 1973, the mosquito was eradicated for the second time, but reintroduced in 1976 and not eradicated since [27]. This showed that insecticides can successfully control mosquito vectors to the point of complete eradication. However, new products are constantly needed to cope with reintroductions and insecticide resistance.

## Natural Products from Brazilian Biodiversity as a Source of New Models for *Aedes aegypti* Control

Natural products are a valuable source of chemical compounds that can be used for the development of new insecticides. Plant-based insecticides have been widely used in agricultural pest control and against vectors of insects that impact public health. Plant extracts are expected to be biodegradable and pose a low risk to other living organisms. However, toxicity tests should always be conducted to ensure the safety of these natural products [26]. Considering the potential of the Brazilian biodiversity in the discovery of new environmentally safe biological secondary metabolites for mosquito control, studies on Brazilian plants are herein presented to show their potential for the development of new mosquito control innovation. ▶ **Table 1** lists the larvicidal activity ($LC_{50}$) of species from 32 Brazilian plant families against *Ae. aegypti*. The search was made in the SciFinder database (up to 2020) using the term "*Aedes*" and refined by the words "Brazil" and "plant" and the PubMed database (up to 2020) using the terms "*Aedes*", "Brazil" "plant", and "$LC_{50}$". All articles in Portuguese and English were selected and the criterion of inclusion was the report of the $LC_{50}$ values (24–48 h) of native Brazilian plant extracts against *Ae. aegypti*. When several life cycle forms were active, we report the larvicidal activity, which usually shows the lower $LC_{50}$.

Species of the Annonaceae family have insecticidal properties due to the presence of secondary metabolites compounds known as "annonaceous acetogenins". These compounds constitute C-35/C-37 natural products derived from fatty acids and show potent and varied biological activities, such as cytotoxic, antitumoral, antimalarial, antifeedant, antimicrobial, pesticidal, and immunosuppressive [45]. Five Annonaceae species collected in the Cerrado region of Mato Grosso-Brazil were extracted with methanol defatted with hexane, methanol defatted with dichloromethane, methanol, hexane, and dichloromethane and tested to evaluate the effect against *Ae. aegypti* larvae. The species *Annona crassiflora, Annona mucosa* and *Annona coriacea* presented high larvicidal activity. The methanol defatted with hexane and hexane extracts from *A. coriacea* showed 100% activity at 100 µg/mL ($LC_{50}$ = 7 µg/mL), and the dichloromethane extract had 58.75% activity. Methanol (100 µg/mL) and methanol defatted with hexane (1 mg/mL) from *A. mucosa* showed 100% activity ($LC_{50}$ = 10 µg/mL), while the hexane and dichloromethane extracts provided activity higher than 90%. The hexane extract from *A. crassiflora* (1 mg/mL) provided 91.25% larvae mortality. The phytochemical compounds of Annonaceae can differ between species and solvents used for extraction. These species had high activity larvicide against *Ae. aegypti* larvae, thus being a potential source to produce new insecticide molecules [90]. Another study tested the toxicity of the acetogenin squamocin (**1**) (▶ **Table 2**) isolated from *A. mucosa* seeds collected in Mato Grosso against *Ae. aegypti*. This compound exhibited good activity with an $LC_{50}$ = 0.01 µg/mL and $LC_{90}$ = 0.11 µg/mL [91].

Recent research using a bioguided approach with modern analytical techniques and dereplication strategies showed it was effective in obtaining natural active compounds. Dereplication strategies can identify known compounds in mixtures according to similar fragmentation profiles and masses using datasets. The detection of compounds and the evaluation of the metabolomic profile is possible prior to isolation. The ArboControl Brasil, a program of the Brazilian Ministry of Health, studied the Brazilian Cerrado Plant Extract Bank, revealing active extracts from Annonaceae taxon. Different organs of *Cardiopetalum calophyllum, Duguetia furfuracea, Xylopia aromatica*, and *Xylopia emarginata* were prepared with 34 hexane and ethanol crude extracts at a 250 µg/mL concentration in order to test against *Ae. aegypti* larvae. Only four were active: leaf hexane and root wood ethanol extracts of *X. aromatica* and root bark and root wood hexane extracts of *D. furfuracea*. Dereplication strategies were performed on ethyl acetate and methanol extracts and revealed the presence of flavonoids, diterpenes, pheophorbides, and alkaloids. Three phenylpropanoids and one diterpenoid were isolated from *D. furfuracea* and *X. aromatica: α*-asarone (**2**) (▶ **Table 2**), 2,4,5-trimethoxy-styrene (**3**) (▶ **Table 2**), 2,4,5-trimethoxybenzaldehyde (**4**) (▶ **Table 2**), and ent-labda-8(17),13(16),14-trien-18-oic acid (**5**) (▶ **Table 2**). Compound **2** showed 100% mortality of *Ae. aegypti* larvae at 250 µg/mL. A mixture of **2** and **3** at a ratio of 2:1 achieved the highest activity. Extracts from the *X. aromatica* leaf hexane extract and compound **5** showed that $LC_{50}$ values decreased (activity increased) with exposure time. These active compounds are promising for the control of *Ae. aegypti*, showing the potential of the Annonaceae family [92].

▶ **Table 1** Plant species from the Brazilian biodiversity and their lethal activity ($LC_{50}$, ppm), against *Ae. aegypti* larvae.

| Family | Species | Plant part | Extract, oil, e. o.[1] | $LC_{50}$ (24 h) ppm | Ref. |
|---|---|---|---|---|---|
| **Anacardiaceae** | *Anacardium humile* A. St.-Hil. | leaves | e. o. | 21 | [28] |
| | *Anacardium occidentalis* L. | n. a. | oil | 14[2] | [29, 30] |
| | *Myracrodruon urundeuva* Fr. Allemao | seeds | ethanol | 15 | [31] |
| | | leaves | water | 202 | [32] |
| | *Schinopsis brasiliensis* Engler | seeds | ethanol | 661 | [31, 33] |
| | | stem bark | ethanol 90 % | 345[2, 3] | [34] |
| | *Schinus terebenthifolia* Raddi | fruits | e. o. | 172–344 | [31, 33] |
| **Annonaceae** | *Annona crassiflora* Mart | root bark | ethanol | 0.71[2] | [35] |
| | | root wood | | 8.9[2] | |
| | | stem | | 16[2] | |
| | *Annona glabra* L. | seeds | ethanol | 0.06[2] | [35] |
| | | stem | | 27 | [29] |
| | *Annona muricata* L. | roots | ethanol | 42[2] | [35] |
| | | seeds | ethanol | 94 | [36, 37] |
| | *Annona squamosa* L. | roots | ethanol | 32[2] | [35] |
| | | leaves | | 169[2] | |
| | | seeds | | 5[2] | |
| **Asteraceae** | *Acmella oleracea* L. | leaves | ethanol | 145[4] | [38] |
| | *Ageratum conyzoides* L. | n. a. | e. o. | 148[2] | [29, 39] |
| | *Artemisia vulgaris* L. | leaves | e. o. | 114 | [39, 40] |
| | *Baccharis reticularia* DC. | leaves | e. o. | 118[2, 5] | [40] |
| | *Tagetes erecta* L. | stem | e. o. | 80 | [41] |
| **Bignoniaceae** | *Tabebuia avellanedae* Lorentz ex Griseb. | trunk bark | ethanol | 442 | [42] |
| | | wood | acetone | 100[2] | [43] |
| | | | ethyl acetate | 151[2] | |
| **Boraginaceae** | *Auxemma glazioviana* Taub. | heartwood | e. o. | 2–3 | [44] |
| | *Cordia curassavica* (Jacq.) Roem. & Schult. | leaves | e. o. | 97 | [45] |
| | *Cordia leucomalloides* Taroda | | | 63 | |
| | *Cordia globosa* (Jacq.) HBK. | leaves | e. o. | 28 | [46] |
| **Burseraceae** | *Commiphora leptophloeos* (Mart.) J. B. Gillett | leaves | e. o. | 99 | [47] |
| **Combretaceae** | *Terminalia fagifolia* Mart. | trunk | ethanol | 373 | [42] |
| **Connaraceae** | *Connarus detersus* Planch. | seeds | ethanol | 216[6] | [31, 48] |
| | *Rourea doniana* Baker | leaves | chloroform | 171 | [48] |
| | | stem | hexane | 12 | |
| **Convolvulaceae** | *Merremia aegyptia* (L.) Urb. | leaves | acetone | 120 | [48] |
| | | | hexane | 144 | |
| **Euphorbiaceae** | *Croton argyrophylloides* Müll. Arg. | leaves | e. o. | 95 | [49, 50] |
| | *Croton heliotropiifolius* Kunth | leaves | e. o. | 544 | [51] |
| | *Croton pulegiodorus* Baill. | | | 159 | |
| | *Croton nepetifolius* Baill. | leaves | e. o. | 66 | [49] |
| | *Croton rhamnifolioides* Pax & K. Hoffm. | leaves | e. o. | 122 | [52] |
| | *Croton regelianus* Müll. Arg. | leaves | e. o. | 24 | [53] |
| | *Croton sonderianus* Müll. Arg. | leaves | e. o. | 55 | [49] |
| | *Croton tetradenius* Baill. | leaves | e. o. | 152 | [54] |
| | *Croton zehntneri* Pax & K. Hoffm. | leaves | e. o. | 26 | [49] |

*cont.*

▶ **Table 1** *Continued*

| Family | Species | Plant part | Extract, oil, e. o.[1] | $LC_{50}$ (24 h) ppm | Ref. |
|---|---|---|---|---|---|
| **Fabaceae** | *Albizia polyantha* (A. Spreng.) G. P. Lewis | stem | ethanol | 547 | [42] |
| | *Anadenanthera macrocarpa* (Benth.) Brenan | seeds | water | 430 | [55] |
| | *Copaifera langsdorffii* Desf. | n. a. | oil | 41[2] | [29] |
| | *Copaifera multijuga* Hayne | bark | ethanol | 81 | [56] |
| | | leaves | ethanol | 166 | |
| | | oil-resin | o. e. | 18 | |
| | *Dalbergia brasiliensis* Vogel | leaves | ethanol | 24[7] | [29,57] |
| | *Derris urucu* (Killip & A. C. Sm.) J. F. Macbr. | root | ethanol | 18 | [58] |
| | *Dioclea megacarpa* Rolfe | seeds | ethanol | 20[6] | [31,42] |
| | *Enterolobium contortisiliquum* (Vell.) Morong. | seeds | ethanol | 369[6] | [31,57] |
| | *Hymenaea courbaril* L. | fruit peel | e. o. | 15 | [59] |
| | *Luetzelburgia auriculate* (Allemão) Ducke | seeds | ethanol | 8.6[6] | [31,55] |
| | *Ormosia arborea* (Vell.) Harms | leaves | ethanol | 238 | [31,60] |
| | | seeds | ethanol | 111 | |
| | *Parkia platycephala* Benth. | seeds | ethanol | 13[6] | [31] |
| | *Senna obtusifolia* (L.) H. S. Irwin & Barneby | seeds | ethanol | 19[6] | [31] |
| | *Tephrosia egregia* Sandwith | stem | hexane | 13 | [61] |
| **Lamiaceae** | *Hyptis martiusii* Benth. | leaves | e. o. | 18 | [62] |
| | *Hyptis pectinata* (L.) Poit. | leaves | e. o. | 366 | [63] |
| | *Ocimum carnosum* (Spreng.) Link & Otto ex Benth. | inflorescences | e. o. | 109 | [64] |
| | *Ocimum campechianum* Mill. | leaves | e. o. | 81 | [64] |
| **Lauraceae** | *Licaria puchury-major* (Mart.) Kosterm. | seeds | e. o. | 99 | [65] |
| | *Ocotea velloziana* (Meisn.) Mez | trunk bark | ethanol | 213 | [42] |
| **Leguminosae** | *Copaifera reticulata* Ducke | trunk | oil-resin | 8.9 | [66] |
| | *Bauhinia acuruana* (Moric) | leaves | e. o. | 56 | [67] |
| | *Derris* sp. | roots | ethanol | 4.8[2] | [35] |
| | *Erythrina mulungu* Mart. ex Benth. | stem bark | ethanol | 37[2] | [35] |
| | *Pterodon polygalaeflorus* Benth | seeds | ethanol | 20[2] | [35] |
| **Malvaceae** | *Helicteres velutina* K. Schum. | roots | ethanol | 171 | [68] |
| | | stem | ethanol | 139 | |
| | *Waltheria viscosissima* A. St.-Hil | roots | ethanol | 5 | [69] |
| **Meliaceae** | *Carapa guianensis* Aubl. | n. a. | oil | 16–57[2] | [29,70] |
| | *Guarea kunthiana* A. Juss. | seeds | ethanol | 169 | [71] |
| | *Guarea humaitensis* T. D. Penn. | leaves and branches | e. o. | 49 | [72] |
| | *Guarea scabra* A. Juss. | leaves and branches | e. o. | 99 | [72] |
| | *Guarea sylvatica* C. DC. | leaves and branches | e. o. | 118 | [72] |

*cont.*

▶ **Table 1** *Continued*

| Family | Species | Plant part | Extract, oil, e. o.[1] | $LC_{50}$ (24 h) ppm | Ref. |
|---|---|---|---|---|---|
| **Myrtaceae** | *Eugenia brejoensis* Mazine | leaves | e. o. | 214 | [73] |
| | *Eugenia candolleana* DC. | leaves | e. o. | 300 | [74] |
| | *Eugenia piauhiensis* Vellaff. | leaves | e. o. | 230 | [75] |
| | *Myrcia sylvatica* (G. Mey.) DC. | leaves | e. o. | 79[2] | [76] |
| | *Psidium guajava* L. | leaves | e. o. | 39–64 | [77] |
| | *Psidium myrsinites* Mart. ex DC. | leaves | e. o. | 292 | [75] |
| **Passifloraceae** | *Turnera ulmifolia* L. | leaves | ethanol | 242 | [60] |
| **Pinaceae** | *Pinus caribaea* Morelet | leaves | acetone | 92 | [78] |
| **Piperaceae** | *Piper aduncum* L. | leaves | e. o. | 290 | [79] [60] |
| | | | hexane | 342 | |
| | | | chloroform | 192 | |
| | *Piper corcovadensis* (Miq.) C. DC. | leaves | e. o. | 31 | [80] |
| | *Piper hispidum* Sw. | leaves | ethanol | 169 | [60] |
| | | | chloroform | 567 | |
| | *Piper klotzschianum* (Kunth) C. DC. | Seeds | e. o. | 13 | [81] |
| | | roots | | 10 | |
| | *Piper marginatum* Jacq. | leaves | e. o. | 19[6] | [82] |
| | | stem | | 14[6] | |
| | | inflorescence | | 14[6] | |
| | *Piper tuberculatum* Jacq. | leaves | e. o. | 106 | [39] |
| | *Ottonia anisum* Spreng. | seeds | e. o. | 13 | [83] |
| | | roots | e. o. | 10 | |
| **Plantaginacea** | *Scoparia dulcis* L. | leaves | ethanol | 83 | [68] |
| **Poaceae** | *Cymbopogon winterianus* Jowitt ex Bor | n. a. | e. o. | 98[2] | [29] |
| | *Cymbopogon flexuosus* (Nees ex Steud.) Wats. | leaves | e. o. | 121 | [39] |
| **Polygonaceae** | *Coccoloba mollis* Casar. | stem | hexane | 137 | [48] |
| | | stem barks | hexane | 128 | |
| | *Triplaris americana* L. | stem | hexane | 117 | [48] |
| | | roots | hexane | 97 | |
| **Rhamnaceae** | *Ziziphus joazeiro* Mart. | seeds | ethanol | 189[6] | [31] |
| **Rubiaceae** | *Guettarda grazielae* M. R. V. Barbosa | stem | ethyl acetate | 51 | [48] |
| | | stem bark | chloroform | 152 | |
| | | leaves | hexane[8] | 131 | |
| | *Spermacoce latifolia* Aubl. | leaves | hexane | 415 | [60] |
| | | | methanol | 625 | |
| | *Spermacoce verticillate* L. | stem | hexane | 115 | [48] |
| | | aerial parts | hexane | 84 | |
| **Rutaceae** | *Citrus limonia* (L.) Osbeck | fruit peel | e. o. | 519 | [84] |
| | *Citrus sinensis* (L.) Osbeck | fruit peel | e. o. | 538 | [84] |
| | *Zanthoxylum* sp. | stem | ethanol | 537 | [42] |
| | | leaves | | 435 | |
| **Sapindaceae** | *Talisia esculenta* (A. St.-Hil.) Radlk. | seeds | ethanol | 412[6] | [31] |
| **Scrophulariaceae** | *Stemodia maritima* L. | leaves | e. o. | 55 | [85] |
| | | stem | | 23 | |
| **Siparunaceae** | *Siparuna guianensis* Aubl. | leaves | e. o. | 87 | [86] |
| | *Siparuna camporum* (Tul.) A. DC. | leaves | e. o. | 251 | [75] |

*cont.*

▶ **Table 1** *Continued*

| Family | Species | Plant part | Extract, oil, e.o.[1] | $LC_{50}$ (24 h) ppm | Ref. |
|---|---|---|---|---|---|
| **Smilacaceae** | *Smilax brasiliensis* Spreng. | leaves | methanol | 986 | [87] |
| **Solanaceae** | *Solanum variabile* Mart. | leaves | methanol | 188 | [60] |
| **Verbenaceae** | *Lippia alba* (Mill.) N. E. Br. ex Britton & P. Wilson | leaves | e.o. | 37 | [75,88] |
| | *Lippia gracilis Schauer* | | | 56 | |
| | | | | 26 | |
| | *Lippia microphylla* Cham. | | | 76 | |
| | *Lippia nodiflora* Cham. | | | 107 | |
| | *Lippia sidoides* Cham. | leaves | e.o. | 19 | [49,62] |
| | *Lippia pedunculosa* Hayek | leaves | e.o. | 58 | [89] |
| | *Vitex cymose* Bertero ex Spreng. | stem | ethanol | 875 | [42] |

[1]e.o.: essential oil, [2]48 h, [3]chloroform partition, [4]hexane fraction, [5]nanoemulsion, [6]pupicidal activity, [7]ethyl acetate fraction, [8]fraction from the ethanolic extract, n.a.: not available

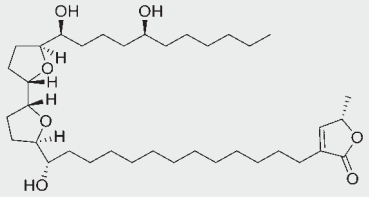
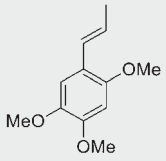
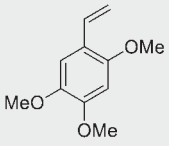
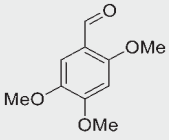
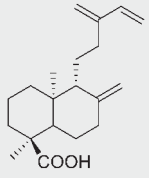
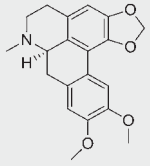
Larvicidal activity against *Ae. aegypti* was tested on a total of 42 ethanolic extracts from 30 native plants belonging to 18 different families from Pantanal and Cerrado in the region of Mato Grosso do Sul – Brazil. The larvicidal activity was found in six of these plants: *Tabebuia avellanedae, Terminalia fagifolia, Ocotea velloziana, Albizia polyantha, Zanthoxylum* sp., and *Vitex cymosa*. Among these species, the most promising result was obtained for the ethanol extract of the trunk bark of *O. velloziana* ($LC_{50}$ = 213.70 µg/mL). In the phytochemical investigation, the aporphine alkaloid (+)-dicentrine (**6**) (▶ **Table 2**) was isolated and characterized as the compound responsible for the larvicidal activity. Other alkaloids have been identified in this species but showed no larvicidal effects. There was no previous information about the activity of the plant or dicentrine against *Ae. aegypti* and this was the first report in the investigation of this active constituent using a bioassay-guided investigation. The occurrence of dicentrine in several species from neotropical areas of the world can be explored as a potential alternative strategy for the control of *Ae. aegypti* [42].

*Piper* is the largest genus of the family Piperaceae, which is known to be rich in essential oils with insecticidal activity and as insect repellent. *Piper aduncum* is widely distributed in tropical regions and its essential oil is used in folk medicine to treat respiratory and inflammatory diseases. Studies reported that the essential oil of *P. aduncum* is active as an insecticide and larvicide, but this activity depends on geographic location from where the plant was collected. This could be due the occurrence of chemotypes and differences in the chemical composition. The larvicide and insecticide action of *P. aduncum* collected in the Amazon Forest against the larvae and the adult insects of *Anopheles marajoana* and *Ae. aegypti* resulted in an $LC_{50}$ of around 50 ppm. The leaves of this species collected in the Brazilian Cerrado were evaluated and activity was aprox. 280 ppm. The main difference is that the major constituent of the specimen collected in the Amazon Forest is a dillapiole and, in this case, they considered the dillapiole responsible for the biological activity. For the Cerrado specimen, monoterpenes and sesquiterpenes were identified in the essential oil of *P. aduncum*, and the main monoterpenes, 1,8-cineole (**7**) (▶ **Table 2**), $\alpha$-pinene (**8**) (▶ **Table 2**), $\beta$-pinene (**9**) (▶ **Table 2**), and *trans*-ocimene (**10**) (▶ **Table 2**), were identified in great amounts, representing 80% of the essential oil. However, there are differences in the volatile composition of leaves from *P. aduncum* from different locations that are reported in the literature. Dilapiole was not identified in the Cerrado specimen, hence, the activity observed is due to other metabolites. The evaluation of 1,8-cineole against *Ae. aegypti* did not provid a larvicidal effect, but the combination of the other monoterpenes increased activity. The collected data suggest that the essential oil showed great larvicidal activity against *Ae. aegypti* and it can be a new possibility for combating the mosquito due to the components present in the essential oil [79].

Meliaceae is a family of the genus *Guarea*, and it is a rich source of secondary metabolites, including limonoids and protolimonoids. A collection of 36 ethanol extracts from different anatomical parts (aerial, leaves, stems, roots, fruits, and seeds) of 27 plant species, native to Cerrado from the Midwest of Brazil, were evaluated regarding their effects against *Ae. aegypti* larvae. Only the extract obtained from the seeds of *Guarea kunthiana* (Meliaceae) was shown to be active. A bioassay-guided investigation of the ethanol extract showed the larvicidal activity containing two principal constituents, the isolation and characterization of the known protolimonoids melianodiol (**11**) (▶ **Table 2**) and meliantriol (**12**) (▶ **Table 2**). In the evaluation of toxicity against *Ae. Aegypti*, only melianodiol was biologically active, with an $LC_{50}$ = 28 µM. The result revealed that this natural product could be a potential candidate for the development of new biocontrol agents against *Ae. aegypti* larvae [71].

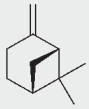
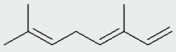
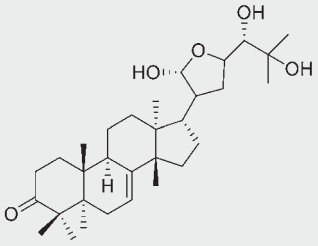
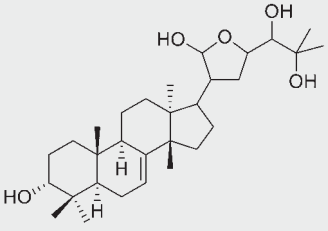
In a study with the hexane and ethanol extracts of 27 plant species of the Cerrado biome, the activity against *Ae. aegypti* larvae was tested. The species tested were prepared in five different concentrations. At 500 µg/mL, activity was observed in the ethanol extracts of *A. crassiflora, Serjania lethalis,* and *Xylopia aromatica*, and in the hexane extracts of *A. crassiflora, Duguetia furfuracea, Piptocarpha rotundifolia, Casearia sylvestris* var. *lingua*, and *Cybista*

▶ **Table 2** Biological active compounds from Brazilian Cerrado plant species against *Ae. aegypti*.

| Species | Compound structure | Stage of mosquito targeted | Ref. |
|---|---|---|---|
| *Annona mucosa* Jacq. |  squamocin, **1**, acetogenin | *Ae. aegypti* larvae | [91] |
| *Duguetia furfuracea* (A. St.-Hil.) Saff.; *Xylopia aromatica* (Lam.) Mart. |  α-asarole, **2**, diterpenoid | Third-stage *Ae. aegypti* larvae | [92] |
| |  2,4,5-trimethoxy-styrene, **3**, diterpenoid | Third-stage *Ae. aegypti* larvae | [92] |
| |  4,2,4,5-trimethoxybenzaldehyde, **4**, diterpenoid | Third-stage *Ae. aegypti* larvae | [92] |
| |  *ent*-labda-8(17),13(16),14-trien-18-oic acid, **5**, diterpenoid | Third-stage *Ae. aegypti* larvae | [92] |
| *Ocotea velloziana* (Meisn.) Mez |  (+)-dicentrine, **6**, alkaloid | *Ae. aegypti* larvae | [42] |

*cont.*

▶ **Table 2** *Continued*

| Species | Compound structure | Stage of mosquito targeted | Ref. |
|---|---|---|---|
| *Piper aduncum* L. |  1,8-cineole, **7**, monoterpenoid | *Ae. aegypti* larvae | [79] |
| |  *α*-pinene, **8**, monoterpenoid | *Ae. aegypti* larvae | [79] |
| |  *β*-pinene, **9**, monoterpenoid | *Ae. aegypti* larvae | [79] |
| |  *trans*-ocimene, **10**, monoterpenoid | *Ae. aegypti* larvae | [79] |
| *Guarea kunthiana* A. Juss. |  melianodiol, **11**, protolimonoid | *Ae. aegypti* larvae | [71] |
| |  meliantriol, **12**, protolimonoid | *Ae. aegypti* larvae | [71] |

*xantisyphilitica*. These species presented larvicidal properties against the mosquito, and the authors indicate a great potential for the future isolation of active chemical compounds. The larvicidal activity was observed with a mortality of over 65 % of larvae in 24 h. Different plant parts were tested, and the predominance of activity in this study was in the root and stem extracts, suggesting the presence of important secondary metabolites responsible for the activity that is biosynthesized in these parts of the plant. The authors do not provide information about the compounds responsible for the larvicidal activity, but further studies on the activity found that these extracts can contribute in controlling the *Ae. aegypti* vector [93].

*T. avellanedae* is a typical tree from Cerrado used in popular medicine and has been studied for its anti-inflammatory, antioxidant, and anticancer properties. It has been extensively studied for the effect of constituents, mainly quinones, which demonstrated potent larvicidal activity against the mosquito species *Ae. aegypti*. Tests with three different extracts (ethyl acetate, etha-

nol, and acetone) obtained from *T. avellanedae* wood residue indicated the presence of phenols and tannins. The larvicidal activity of acetone, ethanol, and ethyl acetate extracts against larvae of *Ae. aegypti* were tested and presented toxicity depending on the concentrations used. All extracts showed that activity increased with increasing exposure time. The ethanol extract, with an $LC_{95}$ = 412.3 µg/mL, promoted activity for a period of 96 h, while the ethyl acetate extract had an $LC_{95}$ = 319 µg/mL for a period of 48 h, and the ethanol solution reached 98.7 % mortality in the first 12 h. The oviposition deterrent activity of *T. avellanedae* against adult *Ae. aegypti* females with acetone, ethyl acetate, and ethanolic extracts were more effective at 333.3 µg/mL in comparison with controls ($p < 0.01$). The ethyl acetate was an oviposition deterred in all concentrations tested. Reports in the literature showed that chemical composition can be similar from other studies, depending on the type of plant, plant part, and different ways of extraction. Regarding the extract of the species *T. avellanedae* and the presence of bioactive compounds, further studies were carried out corroborating the substantial larvicidal and oviposition repellency against *Ae. aegypti*, with it being a potential source to control dengue vectors [43].

*Sapindus saponaria* is a plant in which the bark, root, and fruit are used in folk medicine as a tranquilizer, astringent, diuretic, expectorant, tonic, blood purifier, and against cough. A study tested the morphological changes and larvicidal activity of *S. saponaria* collected in the Cerrado region of the Brazilian city Goiânia against *Ae. aegypti* larvae. In order to analyze larvicidal activity, morphological changes, and lethal mechanisms on *Ae. aegypti* larvae with the application of botanical insecticides, a study showed that the main action of these insecticides occurs mainly at mesenteric cells [94]. The ethanol extract fruit peel from *S. saponaria* was prepared in different concentrations and tested with the larvae up to 48 h. In 100 ppm, it achieved 100 % mortality of *Ae. aegypti* larvae after 3 h, 70 % in 75 ppm and 40 % in 50 ppm. With 30 min of larvae exposure to the ethanol extract in 100 ppm and 75 ppm, they started to lose mobility, however, in larvae exposure to 50 ppm, the effect was observed after 2 h. There was a loss of larvicidal activity of the extract with increased storage time, indicating possible degradation of the active compounds. The morpho-histological study showed damages to the mesenteric cells of *Ae. aegypti*. This is indicative that the chemical components present in *S. saponaria* can be an alternative method to the control of *Ae. aegypti*. The knowledge of the mechanism of action of these compounds are important for intensification of the effects and development of insecticidal products [95].

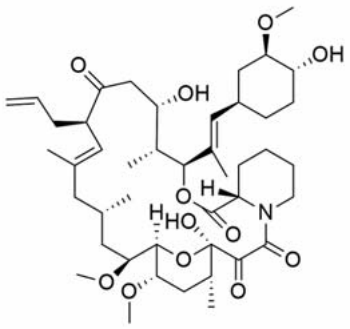
The Anacardiaceae family is characterized by the presence of flavonoids, terpenes, steroids, xanthones, phenolic, and catecholic compounds, called phenolic lipids, that usually show toxic or allergenic proprieties [96]. In the composition of this family's species, there are terpenes in which the components are widely studied due to the important biological activity against *Ae. aegypti*. *Anacardium humile* leaves were collected in the Cerrado region of Campo Grande and the hexane, ethanol, and aqueous extracts and oil from the leaves were prepared in different concentrations. No toxicity was found for hexane, ethanol, and aqueous extracts against *Ae. aegypti* larvae, however, the essential oil of the leaves presented 100 % larvae mortality in concentrations up 0.125 % ($LC_{50}$ = 20.9 ppm). Further studies about the pharmacologic profile of *A. humile* could provide more information on the favorable results for the control of *Ae. aegypti* [28].

A collection of 29 Cerrado species were collected in the Brazilian city Brasília, and insecticidal activity of 67 plant ethanol, hexane, dichloromethane, and hydroalcoholic extracts were evaluated at the 500 µg/mL concentration against *Ae. aegypti* larvae. The dichloromethane extract from *Kielmeyera coriacea* leaves provided activity greater than 90 %, with an $LC_{50}$ = 112.79 µg/mL. A few other extracts showed activity equal to or greater than 50 %: hexane extracts of stem bark from *Talauma ovata*, root wood and leaves from *Schinus terebinthifolius*, and root bark from *Matayba guianensis*; ethanol extracts of bark and stem wood of *Xylopia emarginata* and leaf dichloromethane extract from *S. terebinthifolius*. The chemical composition of *K. coriacea* identified the presence of flavonoids, steroids, terpenes, and tannins in the ethanol extract [97]. Future studies may analyze the active extracts, mainly *K. coriacea*, that showed activity against *Ae. aegypti* larvae, contributing to the search for new natural compounds with larvicidal activity [98].

## Molecular Targets for the Design of Compounds to Control *Aedes aegypti*

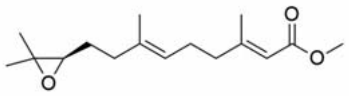
A rational strategy to address the resistance developed by *Ae. aegypti* against current insecticides is the design of compounds with different mechanisms of action from the ones currently in use. Rational design in the search for new drugs and active compounds is fundamental for the success in developing new products. Collaboration among researchers in the fields of ecology, molecular biology, chemistry, and medicine is a good strategy for the development of prototypes for the control of *Ae. aegypti*. From a natural selection point of view, it is expected that the metabolites produced by an organism have a utility for their producer, and therefore natural products are produced in order to make specific interactions with biomolecules. This is one of the reasons why natural products are the primordial source of medicinal compounds and are therefore of great interest for medicinal chemistry [99]. However, these compounds are biosynthesized and optimized for the biological system of their producer, with specific pharmacokinetic properties. Through synthetic or semisynthetic structural modifications, the chemical structure of bioactive compounds isolated from an organism can be optimized to achieve the desired bioavailability for anthropological or medicinal use. In the stages of optimization of molecular properties, the structure of the compounds can be modified with the objective of enhancing their pharmacodynamic (potency, affinity, selectivity) and pharmacokinetic properties (absorption, distribution, metabolism, excretion), and to minimize toxicity [100].

The design of active compounds is mainly based on the knowledge of the mechanisms by which bioactive molecules interact with their molecular targets [101]. Structure-based drug design by means of molecular modeling is one of the most efficient strategies used to evaluate the interaction of active compounds and its biological target, and improve pharmacodynamics [102]. This is a useful method to identify crucial intermolecular interactions in




13

▶ **Fig. 1** Immunosuppressive drug tacrolimus reported to bind AaFKBP12.




14

▶ **Fig. 2** Juvenile hormone III, an insect growth regulator reported to bind the mosquito juvenile hormone-binding protein.

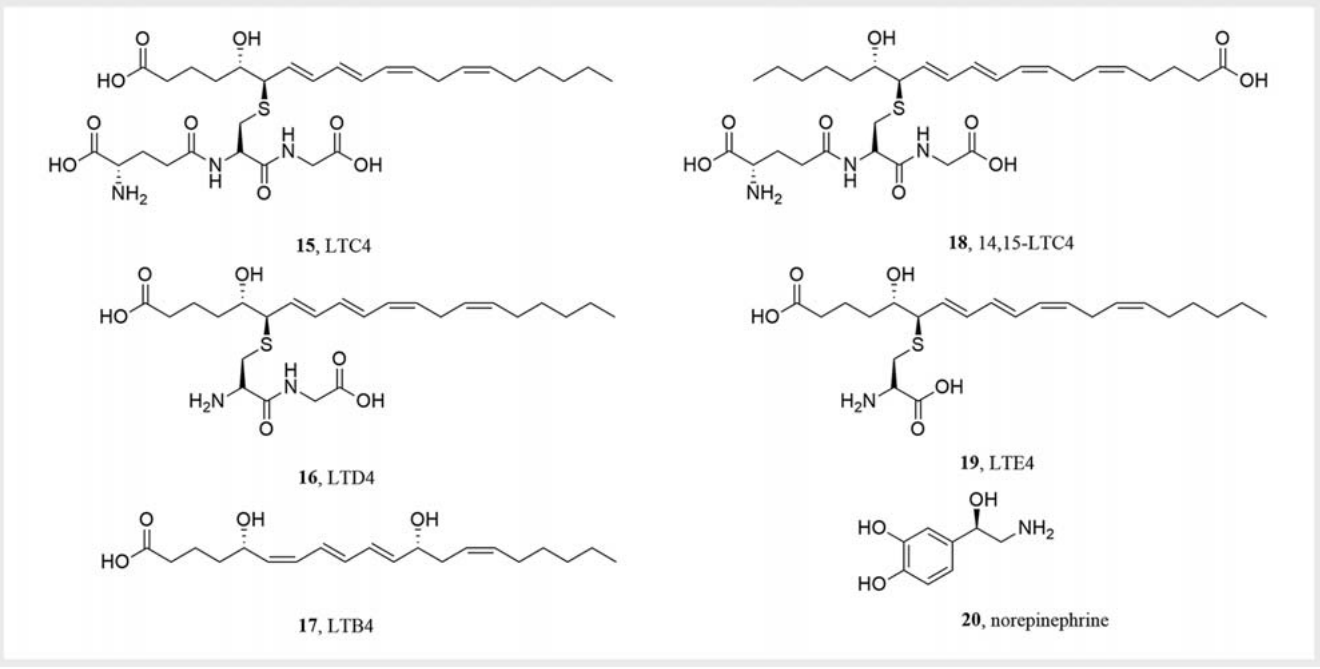
the process of molecular recognition and for the selection of hits that bind or fit to the target protein binding cavity. In the search for new compounds to be used for mosquito control, the understanding of the molecular mechanism of disease transmission by the mosquitoes is fundamental. One rational approach is to search or design compounds that interact with a biological target in order to modulate its activity and impair mosquito activity. The target is ideally a protein specific to the mosquito in order to minimize toxicity for humans and other animals. A few targets are herein described, and represented in ▶ **Fig. 1**, in order to provide molecular aspects important to consider in the design of compounds for mosquito control.

The isomerase FKBP is described as the molecular target for the immunosuppressive drug tacrolimus, strongly binding in a hydrophobic pocket (**13**) (▶ **Fig. 2**) and blocking the signaling pathway mediated by calcineurin [103]. The solution structure of the FKBP12 protein from *Ae. aegypti* (AaFKBP12, PDB ID 3UQI) was determined with 1.3 Å resolution [104]. In addition, a (3-(N-morpholino)propanesulfonic acid) buffer molecule was crystallized in the active site, which could be an interesting nucleus for designing inhibitors for AaFKBP12. Sequence similarity with HsFKBP12 is 72% and strategies should be addressed to design selective compounds to avoid toxicity to humans. The structure of AaFKBP12 might provide a useful target for the design of potential ligands to control the dengue-transmitting vector [103, 104].

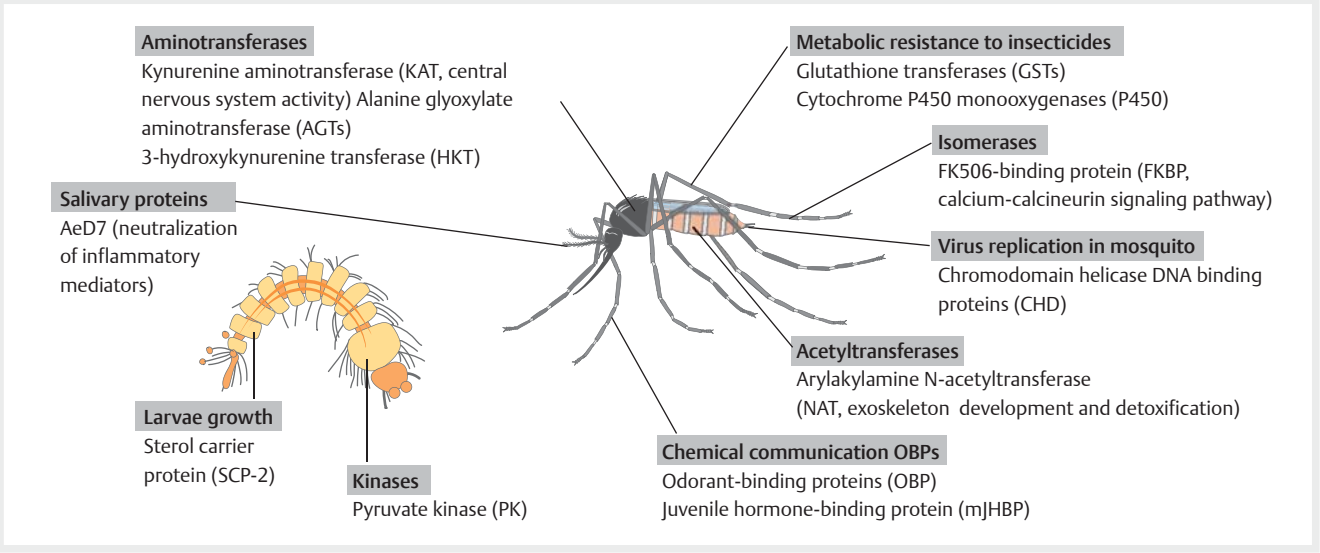
The chemical communication and olfactory system of mosquitoes is also a strategic target for the development of repellents and attractants in order to control vector populations and, thus, disease transmission. OBPs are the carriers of semiochemicals that trigger signaling to activate odorant receptors [105, 106]. A series of potential ligands was evaluated to define requirements for a good fitting, and a structure-activity relationship study demonstrated that the best ligands were compounds with two aromatic rings connected by a short rigid chain [107]. Insect OBPs differ from vertebrate OBPs and have a hydrophobic cavity that encapsulate ligands and carry OBP-semiochemical complexes through the aqueous sensillar lymph to transmembrane odor receptors [91]. A study reported the structure of the major odorant-binding protein (AaegOBP1 = AaegOBP39) from *Ae. aegypti* describing its binding pocket, a continuous hydrophobic tunnel that involves a dimer of this protein, but no low molecular weight ligand was co-crystalized in the structure determination studies. Also, it has been proposed that binding affinity and protein conformation of the binding pocket is pH dependent. The structure of the protein was deposited in the PDB (3K1E) and is available for computational studies [105]. Also, an *in vitro* assay to measure affinity of ligands is described with OBP22 using N-phenyl-1-naphtylamine as the fluorescent reporter. The probe is excited at 337 nm and emission spectra can be recorded between 380 and 450 nm. The *Ae. aegypti* complete set of olfactory genes includes 66 putative OBPs and these could be interesting targets for mosquito control agent designs [108].

Another target is the mJHBP (PDB code 5V13) belonging to the insect OBP family and related to the D7 proteins. The JH (**14**) (▶ **Fig. 3**), an insect growth regulator responsible for development, metamorphosis, reproductive development, and mating behavior, was reported to bind in the binding pocket of the N-terminal domain, and the C-terminal domain extends over the surface of the N-terminal domain and closes the entry to the binding pocket of mJHBP. The protein is selective to binding the 10*R* enantiomer of JH. The epoxy end of JH III is located deep in the core of the domain, and the methyl ester group is oriented toward its surface. There is a hydrogen bond between the epoxy group and the phenolic hydroxyl of Tyr-129, and the isoprenoid chain is surrounded by hydrophobic residues. Only methyl ester epoxides are able to bind to mJHBP [109]. The structural information on the binding mode of the natural substrate can be used to design substrate-mimetic ligands, a useful strategy to achieve good selectivity.

The blood feeding of the female mosquito is a key event for transmitting arboviruses. During this event, the mosquito release, in the saliva, anti-inflammatory agents and proteins to counteract these mediators and extend the period for feeding. One such protein is D7, a biogenic amine-binding salivary protein. Proteins like D7 are responsible to avoid host defensive behaviors, and therefore inhibiting them might interrupt feeding. The structure of the D7 from *Ae. aegypti* was determined recently, a protein that acts to neutralize two classes of inflammatory mediators. The N-terminal domain leads to the protein interior and is lined mainly with hydrophobic residues. It may accommodate bioactive lipid mediators involved in host inflammatory responses to mosquito feeding, specifically leukotrienes. A few leukotrienes (**15–19**) (▶ **Fig. 4**) were tested in binding assays, and the highest binding affinity was observed for LTCs (LTC4, LTD4, and LTE4). The lipid




▶ **Fig. 3** Leukotrienes and norepinephrine evaluated as ligands for the AeD7, an odorant-binding protein associated with blood feeding of the female mosquito.




▶ **Fig. 4** Molecular targets for mosquito vector control. Strategies include targeting larvae and adult stage including viral replication and metabolic proteins. Source: Angelika Brauner

chain is suggested to be the primary contact point with the protein and the peptide portion of the compounds may play a minor role in binding. Structure-activity relationships were determined for these structures and could be useful in the design of new active compounds. Additionally, norepinephrine (**20**) (▶ **Fig. 4**) was detected binding to the C-terminal domain. The protein structures are deposited in the PDB codes 3DXL (unliganded structure), 3DZT (LTE4 complex), and 3DYE (norepinephrine complex). These structures could provide a suitable target with two binding pockets for computational studies and design of compounds to interrupt mosquito feeding. Inhibition of these proteins would prevent feeding and reduce the efficiency of virus transmission [110].

Another target is PK, which is responsible for catalyzing the last stage of the glycolytic pathway. It catalyzes the transphosphorylation from phosphoenolpyruvate and ADP to pyruvate and ATP. Glycolysis provides energy and intermediate precursors to other

important metabolic pathways to assure limited flight performance and survival time. The activity of PK in *Ae. aegypti* mosquitoes has been shown to be elevated throughout embryogenesis, thus suggesting an increase of glycolysis in *Ae. aegypti* embryos. Thus, PK regulation becomes crucial to the metabolic needs of the mosquito. The protein AaPK1 was characterized to clarify the factors that regulate the enzyme. In order to maximize the mosquito's survival time, they seek nectar for carbohydrates, and feeding sugars increase mosquito survival. Carbohydrates are necessary for blood meal digestion and reproduction. Given these factors, this study demonstrates that AaPK1 is regulated by specific sugars, amino acids, and phosphorylated sugars that are responsible for regulating important properties [111].

aaNATs are involved in the development of the exoskeleton and detoxification process in mosquitoes. aaNATs are important for mosquito survival and pose an interesting target to impair mosquito development. The structure of mosquito aaNATs are considerably different from human ones, and inhibitors of the mosquito aaNATs are expected not to interact with mammalian ones. In *Ae. aegypti*, there are 13 aaNATs that have been identified, showing the significance of arylakylamine acetylation in these insect species. The characterization of aaNAT structures from insects is essential for investigating the chemistry and physiology of aaNATs in mosquitoes, and it is a reference to develop new research about this family of proteins, which are essential in several insects that are vectors of diseases. Crystal structures of aaNAT2, aaNAT5b, and aaNAT7 are available in the PDB and consist of possible targets for developing new insecticides [112].

AeKAT can catalyze amino acids and keto acids that are biologically relevant. They have important activities in the central nervous system, and further investigations are needed to provide more information for their catalytic mechanism and biochemical differences in mosquitoes and humans. The crystal structure of AeKAT is available in the PDB for further studies [113]. AGTs are typical of dypteran insects such as *Ae. aegypti* and are catalysts for glyoxylic acid conversion to glycine. Mosquitoes are unique in possessing two AGTs, AeAGT and AeHKT, the latter being important in tryptophan metabolism for the transamination of 3-hydroxykynurenine to xanthurenic acid. AeHKT is only active on certain mosquito stages and its substract is the precursor for mosquito eye pigments. These functional adaptation and evolution aspects might be an interesting strategy to develop aminotransferase inhibitors that could be used for mosquito control. Crystal structures of AeAGT and AeHKT are available in the PDB [114].

CHDs are a class of enzymes that contribute to the interaction between DNA and nucleosomes involved in cellular processes such as replication, transcription, recombination, repair, and development. CHDs are involved in the replication of viruses, and homologs of CHDs were identified in *Ae. aegypti*. A significant reduction of its expression has been verified in female mosquitoes infected with Wolbachia. AeCHD7 is highly expressed during dengue infection in *Ae. aegypti* and seems required for DENV replication [115]. AeCHD7 could be an interesting target for the design of compounds to reduce DENV replication in mosquitoes.

Metabolic resistance to insecticides in *Ae. aegypti* involves alterations in the expression of a complex group of enzymes, which increases the detoxification process. These enzymes are mainly GSTs, esterases, and cytochrome P450 monooxygenases (P450). *Ae. aegypti* genome project revealed a set of 160 P450 genes, 49 GSTs, and 26 esterases [116].

GSTs are responsible for catalyzing the conjugation of glutathione to xenobiotic compounds in the detoxification process [117]. This enzyme was proposed to play a role in insect chemodetection by protecting the chemosensory system. Most of the GSTs comprise a super family structure of cytosolic-dimeric enzymes grouped into six classes, namely, Delta, Epsilon, Omega, Sigma, Theta, and Zeta [118]. The two major classes, Delta and Epsilon, are insect specific and are often involved with resistance [119–121]. The insecticides observed to be metabolized by GSTs include organophosphates and organochlorides [122, 123]. It was shown that DDT dehydrochlorination mediated by the Epsilon class GST confers resistance in *Ae. aegypti* and other mosquito species [118, 124, 125]. GST contribution to pyrethroid resistance is based in two modes of action. One is GST binding and seizure of pyrethroid, inhibiting GST activity [123, 126]. The second is through the GST protective role in oxidative stress, as the oxidative stress is a consequence of pyrethroid toxicity [127]. Generally, all GSTs adopt a very similar conserved tertiary structure, and for a functional active site, a dimeric quaternary structure is essential, as the active site is formed by amino acid residues from both subunits [128, 129]. GST inhibition could be an adjuvant strategy to reduce resistance acquired by mosquitoes.

In insects, cholesterol is required as a component of cellular membranes and as a precursor of the insect molting hormone, named ecdysone. This hormone ensures normal growth, development, and reproduction [130, 131]. However, insects lack several enzymes in the cholesterol biosynthesis pathway [132], which make them unable to synthesize cholesterol *de novo* [130, 132]. Thus, it is essential that the insects obtain cholesterol through a dietary source, making cholesterol uptake and transport potential targets for the development of new mosquito larvicides [133]. In insects, SCP-2 is involved in the absorption of lipid droplets to the cytoplasmic membrane in the body fat or intracellular cholesterol transportation from the luminal side to the basal side of the midgut epithelium [134–136]. As cholesterol is highly hydrophobic, carrier proteins mediate its delivery by shielding its hydrophobic moiety from the aqueous environment of the cytoplasm [137–139]. SCP-2 belongs to the SCP-2 gene family, which contains a sterol-binding domain located at the C-terminus, identified in vertebrates, insects, plants, yeast, bacteria, and fungi [140, 141]. AeSCP-2 is expressed mainly at the cytoplasm [142–144] and has been shown to aid in the uptake of cholesterol in mosquito cells, mosquito development, and reproduction [134, 145]. AeSCP-2 is mainly expressed throughout the feeding larval stages in midgut tissue, the main site of cholesterol absorption [142]. AeSCP-2 knockdown resulted in a reduction of cholesterol uptake in larvae and high larval mortality. In adults, it caused reduced, inefficient cholesterol uptake after blood meals and a fecundity decrease [145]. However, it had little effect on palmitic acid uptake [145]. Studies also demonstrated that DENV replication in human host cells depends on both *de novo* cholesterol biosynthesis and intracellular transport. When SCP-2 function was inhibited, the production of DENV viral particles was impaired, probably because the SCP-2 mediates the cholesterol trafficking pathway, which is

critical for DENV production. AeSCP-2 is different from vertebrate SCP-2, with their hydrophobic moieties oriented at the opposite ends of the protein. Moreover, AeSCP-2 is essential for mosquito survival and development, whereas vertebrate SCP-2 is not vital for their survival and fertility genomic data have shown that *Ae. aegypti* has four genes encoding single SCP-2 domain proteins [141, 144]. AeSCP-2 and AeSCP-2-like proteins have very similar temporal and spatial expression profiles with variable affinity to different lipids [141, 142]. It was shown that AeSCP-2 contributes to the uptake of cholesterol from a blood meal in female mosquitoes and AeSCP-2-like protein affects the uptake of free fatty acids [145]. This carrier protein could be used as a target for drug design in order to selectively control mosquito populations.

## Conclusions

The impact caused by arboviruses diseases on the health systems of tropical countries is huge. Although arboviruses cause a great health impact in Brazil, there are only a few studies of the Cerrado extensive biodiversity for new insecticides to control their vector. Vector control is a useful strategy to control transmission of these diseases, with the mosquito *Ae. aegypti* being the major target in this context. Natural products from the Brazilian biome Cerrado are highlighted as a valuable source of agents with potential to be sustainably explored by our health systems and industries for the development of products for mosquito control. The development of such products requires optimization of pharmacodynamic and pharmacokinetic properties that might be tackled by a medicinal chemistry approach. The study of the mechanisms of action of active compounds and their biological targets provide important knowledge in this task. An extensive search of targets was herein presented as insights to promote advances in the highly difficult task of developing products to control *Ae. aegypti*.

### Contributors' Statement

Designed the work: M. Valli, A. D. Andricopulo, L. S. Espindola, V. S. Bolzani; collected and analysed data: M. Valli, L. C. V. Atanázio, G. C. Monteiro, R. R. Coelho, D. P. Demarque; supervised and discussed collection of data: A. D. Andricopulo, L. S. Espindola, V. S. Bolzani; drafted the manuscript: M. Valli, L. C. V. Atanázio, G. C. Monteiro, R. R. Coelho, D. P. Demarque. All authors revised the manuscript.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### Conflict of Interest

The authors declare that they have no conflict of interest.

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