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CELLULAR AND MOLECULAR BIOLOGY

The bioprospecting potential of *Clusia fluminensis* Planch. & Triana: a scoping review

GISELLE M. FARIA, ANA PATRICIA A. LEMOS, MARIA C. ANHOLETI, SELMA R. PAIVA & LIDIA M.F. AMORIM

Abstract: Many biological activities are described for the Clusiaceae family. *Clusia fluminensis*, a species from Brazilian flora, is mainly employed for ornamental purposes. This review aimed to depict the current knowledge of *C. fluminensis* from a bioprospecting standpoint. "*Clusia fluminensis*" search term was applied in Scopus, Web of Science, PubMed and Bireme databases according to PRISMA-ScR statement. Selected papers on Phytochemistry or Bioactivity followed hand searching procedures. Bioactivity preclinical studies considered *in vitro* or *in vivo* biological systems, treated with plant extracts or isolated compounds. The outcomes were compared with standard or no treatment control groups. Critical appraisal of individual trials considered completeness in the research fields. Our results showed that 81% of the selected papers presented high level of completeness, 69% revealed phytochemical parameters and 31% biological applications of plant extracts and isolated compounds. Polyisoprenylated benzophenones, terpenoids, sterols and phenolic compounds were identified. Antiviral, insecticidal and snake antivenom activities were reported. In conclusion, the phytochemical data reinforce the reported activities. Potential applications in personal care, nutritional supplementation and pharmaceutical, food, chemical or textile industries were also identified. Toxicological and phytochemical complementary studies may be required.

Key words: Clusiaceae, natural products, phytosteroids, polyisoprenylated benzophenones, terpenoids.

INTRODUCTION

The broad diversity, structural elegance and expected effects of the organic compounds found in natural products from plants, animals or marine organisms are long recognized. The bioprospecting potential that relies on such products, when properly and consciously developed, can support many biotechnological applications. Indeed, in a context of a high biodiversity, as depicted in Brazil, potential opportunities may be verified in nutrition, cosmetics, agricultural and pharmaceutical fields. However, despite such promising scenarios, a huge field of possibilities remains unexplored, as reflected in the low number of herbal medicines authorized by the Brazilian Health Surveillance Agency (ANVISA) compared to other countries (Carvalho et al. 2018). Efforts are being made to improve this situation, based on the collaborative work of ANVISA and society, to support an appropriate and competitive regulatory framework, aligned with those established internationally (Carvalho et al. 2014, 2018).

The tropical Clusiaceae family is composed of 800 species with very distinct habits (Anholeti et al. 2017, Gustafsson et al. 2007, Stevens 2001 onwards), but sharing common classes of secondary metabolites with well

described biological and pharmacological properties. Examples of such metabolites include polyisoprenylated benzophenones, phenolic compounds, terpenoids and sterols (Piccinelli et al. 2005, Marín et al. 2018, Ribeiro et al. 2019). Such phytochemical studies may corroborate traditional folk practices, as verified for *Clusia minor* employed for the treatment of warts and sores (Marín et al. 2018); *Garcinia lucida* for digestive disorders, infectious diseases, snake bites and as aphrodisiac (Guedje et al. 2017) and with *Garcinia gardneriana* for inflammation, pain and urinary tract infection management (Demenciano et al. 2020), among many others.

Clusia fluminensis Planch. & Triana belongs to the Clusiaceae Lindley (= Guttiferae Juss.) family, is endemic in the Brazilian flora and is found in the states of Rio de Janeiro, Espírito Santo and Bahia (Mazza et al. 2019). The *Clusia* genus is unique with trees performing photosynthesis based on crassulacean acid metabolism (CAM) and well adapted to areas of high luminosity and water scarcity (Lüttge 2006, Niechayev et al. 2019). The ornamental purpose is described as the major use of *C. fluminensis* (Silva & Paiva 2012) and solid residues are generated from plant pruning, that if not properly managed, can lead to environmental and health hazards (Araújo et al. 2018). The accessibility of this residue represents a sustainable source for screening, chemical elucidation and biotechnological investigation of the potentially valuable bioactive ingredients.

Additionally, no reviews were identified regarding the bioprospecting potential of *C. fluminensis*, although a relevant paper on the anti-inflammatory properties of the Clusiaceae family in preclinical models was retrieved (de Melo et al. 2014), without, however, any specific mention to *C. fluminensis*.

In this scoping review, the current knowledge provided by a wider domain uncovered by phytochemical, *in vitro* and preclinical trials with crude extracts and isolated compounds from *C. fluminensis* is depicted. In addition, an overview of the bioprospecting potential of *C. fluminensis* derivatives is also highlighted.

MATERIALS AND METHODS

Search strategy and study selection

This scope review followed the PRISMA extension for Scoping Reviews, PRISMA-ScR (Tricco et al. 2018). The search term [*Clusia fluminensis*] was applied in Scopus, Web of Science, PubMed and Bireme (Latin American and Caribbean Center on Health Sciences) bibliographic electronic databases and concluded on March 2021. No temporal or language restrictions were specified. Two authors (APAL, GMF) independently developed the search and selection procedures and dissonances were solved by consensus. After excluding duplicate entries, any studies unrelated to *C. fluminensis*, as well as reviews were excluded. Studies that followed the inclusion criteria of belonging to the main research fields of Phytochemistry and Bioactivity were selected. Hand searching was also used to identify any additional relevant publications. The experimental preclinical bioactivity trials considered *in vitro* or *in vivo* biological systems, submitted to treatment with *C. fluminensis* extracts or isolated compounds. For comparison purposes of the biological outcome, control groups were considered, with standard or no treatment.

Critical appraisal of individual sources of evidence

In order to better identify literature gaps, the critical appraisal of the included studies was performed. For this purpose, peer-reviewed papers were scored into moderate or high levels, based on the completeness according to the research field. In this context, those studies

uncovering solely qualitative phytochemical elucidation or *in vitro* biological trials were categorized as moderate level. Phytochemical studies with structural or quantitative evaluation and biological studies based on *in vivo* trials or any alternative experimental method (Gutiérrez et al. 2021) were considered as high level records.

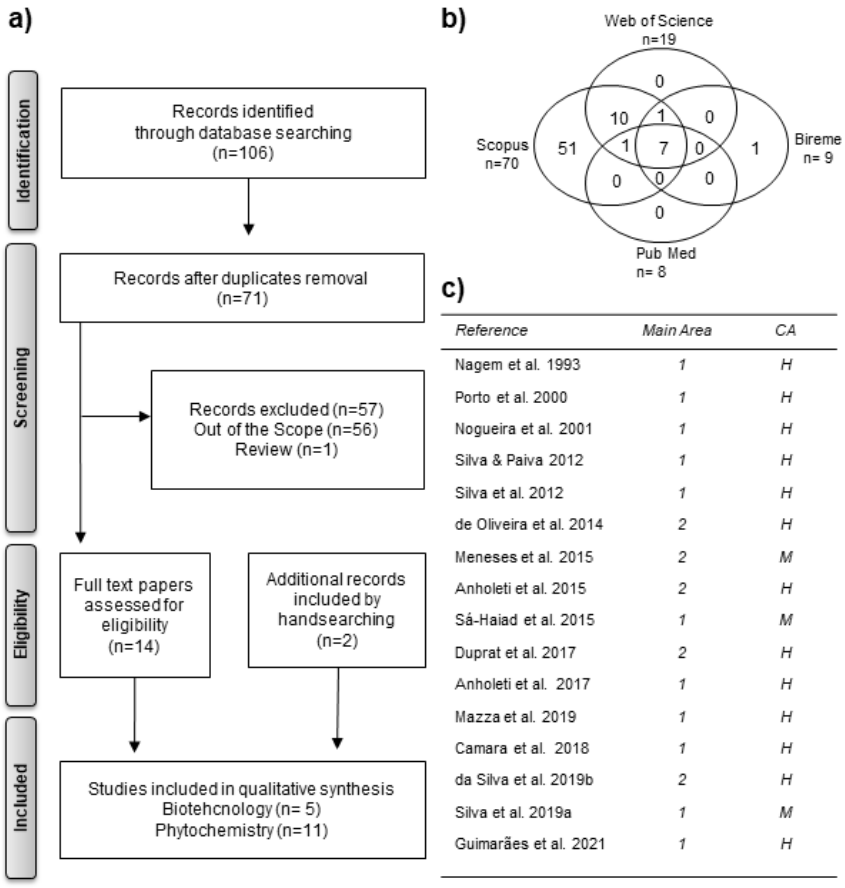
Data extraction

All records captured by the searching procedure were gathered on a Microsoft Excel document. The selected studies underwent full text reading and relevant bioactivity or phytochemical findings were summarized in figures and tables. Mendeley Desktop software (version 1.8) was chosen as the reference management package.

RESULTS

Selected studies

According to the adopted search procedure and after superposition exclusion, a total of seventy-one peer-reviewed papers were retrieved from the four selected databases (Figure 1a, b). Fourteen studies remained after the exclusion and during the data extraction, two additional eligible ones were identified. In the end, sixteen works fulfilled the inclusion criteria of classification in the Phytochemistry and Bioactivity fields. Amongst the selected records from 1993 onwards, 69% were related to qualitative and quantitative phytochemical aspects of *C. fluminensis*, the remaining 31% explored biological applications. High level studies accounted for 81% of the included papers (Figure 1c).



c)

Reference	*Main Area*	*CA*
Nagem et al. 1993	*1*	*H*
Porto et al. 2000	*1*	*H*
Nogueira et al. 2001	*1*	*H*
Silva & Paiva 2012	*1*	*H*
Silva et al. 2012	*1*	*H*
de Oliveira et al. 2014	*2*	*H*
Meneses et al. 2015	*2*	*M*
Anholeti et al. 2015	*2*	*H*
Sá-Haiad et al. 2015	*1*	*M*
Duprat et al. 2017	*2*	*H*
Anholeti et al. 2017	*1*	*H*
Mazza et al. 2019	*1*	*H*
Camara et al. 2018	*1*	*H*
da Silva et al. 2019b	*2*	*H*
Silva et al. 2019a	*1*	*M*
Guimarães et al. 2021	*1*	*H*

Figure 1. Flow diagram of literature search results and its critical appraisal. The flowchart a) shows the steps adopted on records selection. The superpositions of the captured records from four databases are depicted in diagram b). The critical appraisal (CA; H: high, M: moderate) of the selected studies according to the main research field (1: Phytochemistry, 2: Bioactivity) is shown in c).

Phytochemical studies

Previous anatomical and ultrastructural studies conducted on flowers and leaves of *C. fluminensis* revealed secretory structures (Sá-Haiad et al. 2015, Silva et al. 2019a) and subsequent histochemical investigation contributed to a qualitative phytochemical analysis. Resin, lipids, pectic substances, proteins and phenolic compounds were reported in the flower secretory structures (Sá-Haiad et al. 2015). In the same manner, mucopolysaccharides, phenolic compounds and lipids were described in the leaves (Silva et al. 2019a). Another work using histochemical analysis showed that galled and non-galled leaves, both in staminate or pistillate individuals, had no differences in the presence of triterpenes, steroids, tannins, flavonoids and quinones, being alkaloids and saponins absent. The same study also demonstrated phenolic compounds, flavanones, flavonols, flavones, xanthones, aurones, chalcones and leucoanthocianydins in leaves (Guimarães et al. 2021). Phenolic compounds, steroids (sitosterol and stigmasterol), triterpenes (α- and β-amyrin, lupenone, friedelin, epifriedelinol) and calcium oxalate were also observed in leaf wax (Anholeti et al. 2017). Additionally, the qualitative analysis of aqueous extracts of the leaves (EL), stems (ES), and fruit (EF) demonstrated the presence of tannins (EL, ES, EF), triterpenes (EL, ES, EF), flavonoids (EL, ES), coumarins (EL, ES) and saponins (EL, EF) (da Silva et al. 2019b).

Complementary data in Table I provide results regarding *C. fluminensis* identification and quantification phytochemical profile. Terpenoids are the main compounds of the essential oil of flowers (94.1%) (Nogueira et al. 2001) and fruit latex of *C. fluminensis* (91.73%) (Camara et al. 2018), with sesquiterpenes the most common. Compared to the fruit latex, the flower essential oil demonstrated a higher assorted nature of terpenoid compounds, with more than fifty different substances described (Nogueira et al. 2001, Camara et al. 2018). In agreement, the tetracyclic triterpenoid lanosterol and its isomers were identified in the hexanic extracts of fruits (40.6%) and flowers (9.74%) of *C. fluminensis* (Anholeti et al. 2015, Meneses et al. 2015, Duprat et al. 2017). The analysis of epicuticular waxes of the leaves detected friedelin (58.37%) and epifriedelinol as the major pentacyclic triterpenes, with the latter found in varying amounts (from 24 to approximately 70%), probably due to environmental conditions or ontogenetic development (Anholeti et al. 2017). Likewise, pentacyclic triterpenes (lupenone, amyrin, friedelin, α and β friedelinol) were the main constituent found in leaves of *C. fluminensis*, with sitosterol, n-octacosanol and tricosane (Nagem et al. 1993) (Table I).

The presence of the tautomeric pair of clusianone (54.85%) was reported in the hexanic extract of flowers (Anholeti et al. 2015) and was isolated with 90.32% purity by high speed counter-current chromatography (HSCCC) (Silva et al. 2012). Additionally, significant amounts (37%) of this polyisoprenylated benzophenone was also described in the flower resin (Porto et al. 2000) (Table I).

Regarding the amounts of phenolic compounds (Table I), the leaves of pistillate individuals of *C. fluminensis* had a higher content compared to the staminate forms (16.29 ± 0.66 vs 10.15 ± 0.31 mg/g, respectively), as was similarly observed for flavonoids (7.54 ± 0.09 vs 6.75 ± 0.04 mg/g, respectively) (Guimarães et al. 2021). Flavonoids were also found in the methanolic and acetonic extracts of fruits, stems and leaves of *C. fluminensis*, ranging from 8 to almost 14%. The organic extracts of fruits had the highest flavonoid content (Silva & Paiva 2012). Ascorbic acid, phenolic acids, carotenoids, fatty acids derivatives, terpenoids and benzenoids were also identified in fruits of *C. fluminensis* (Klump

Table I. ***Clusia fluminensis*** **phytochemical profile.**

Reference	Plant Organ	Solvent used on Extraction	Chemical Class*	Compound	Content	Unit	Identification / Quantification method**
Guimarães et al. (2021)	Leaves	Methanol	Total phenolics	-	16.29 ± 0.66 (pistillate individuals)	mg/g	Spectrophotometry (760 nm)
					10.15 ± 0.31 (staminate individuals)	mg/g	
			Flavonoids	-	7.54 ± 0.09 (pistillate individuals)	mg/g	Spectrophotometry (760 nm)
					6.75 ± 0.04 (staminate individuals)	mg/g	
Mazza et al. (2019)	Arils of the fruits with seeds	Sulfuric acid 0.05 M	Vitamin [a]	Ascorbic acid	3.77 ± 0.82	mg/100 g	HPLC (ion exchange chromatography) / Spectrophotometry (242.6 nm)
		Methanol : Acetic Acid	Phenolic acids [b]	Protocatechuic acid	115.14 ± 58.23	µg / g	HPLC-DAD (C18) / Spectrophotometry (270 - 310 nm)
				p-hydroxybenzoic acid	11.43 ± 0.32	µg / g	
				p-hydroxycinnamic acid	5.97 ± 0.41	µg / g	
		Methanol : Acetic Acid and alkaline or acid hydrolysis	Total phenolic acids [b]	Protocatechuic acid	55.82 ± 12.23	µg / g	
				p-hydroxybenzoic acid	16.76 ± 1.06	µg / g	
				p-hydroxycinnamic acid	49.03 ± 12.69	µg / g	
		Methanol : H_2O	Flavonoids [c]	Rhamnetin and rhamnetin derivative	-		HPLC-DAD (C18) / Spectrophotometry (270 - 310 nm)
		Acetone : Petroleum ether	Carotenoids [d]	Zeaxanthin	823	µg / g	HPLC UV-VIS / Spectrophotometry (450 nm)
				β-cryptoxanthin	-		
				Lutein	-		
Camara et al. (2018)	Fruit latex	Methanolic latex collection from fresh cut fruits	Fatty acid derivative	Tetradecane	7.82	%	GC - FID / GC - MS
			Terpenoids	α-copaene	8.69	%	GC - FID / GC - MS
				β-caryophyllene	35.61	%	
				α-humulene	2.11	%	
				Germacrene D	8.78	%	
				β-selinene	12.44	%	
				α-selinene	6.56	%	
				trans-β-guaiene	2.18	%	
				Spathulenol	6.37	%	
				Caryophyllene Oxide	3.45	%	
				10-epi-γ-eudesmol	5.54	%	
			Benzenoids	p-anysaldehyde	0.36	%	GC - FID / GC - MS
				Acetophenone	traces	-	
Anholeti et al. (2017)	Leaves wax	Chloroform	Terpenoids	Epifriedelinol	23.98 to 70	%	GC-MS
				Friedelin	58.37	%	
				α a- β amyrin	-	-	
				Lupenone	-	-	
			Steroids	Sitosterol	-	-	GC-MS
				Stigmasterol	-	-	

Table I. Continuation.

Duprat et al. (2017)	Fruit	Hexane	Terpenoids	Lanosterol	40.6	%	GC - MS
				Lanosterol isomers	10.73	%	
			Fatty acids	Palmitic acid	-	-	GC - MS
				Oleic acid	-	-	
Anholeti et al. (2015)	Flower	Hexane	Polyisoprenylated benzophenone	Clusianone tautomeric pair	54.85	%	GC-MS
			Terpenoid	Lanosterol	9.74	%	GC-MS
Meneses et al. (2015)	Flower	Hexane	Terpenoids	Lanosterol and isomers	-	-	GC-MS
Oliveira et al. (2014)	Fruit	Hexane	Terpenoid	Lanosterol	-	-	GC-MS
Silva & Paiva (2012)	Fruit	Acetone	Flavonoids	-	13.93 ± 0.21	%	Colorimetric (415 nm)
	Stem			-	9.12 ± 1.14	%	
	Fruit	Methanol		-	10.68 ± 0.58	%	
	Stem			-	9.82 ± 0.53	%	
	Leaves			-	8.07 ± 1.70	%	
Silva et al. (2012)	Flower	Hexane	Polyisoprenylated benzophenone	Clusianone tautomeric pair	6.9 yield	%	GC/MS
Porto et al. (2001)	Flower resin	Fresh material methylation with diazomethane a-diethyl ether	Polyisoprenylated benzophenones	Clusianone	37	%	RP-HPLC
				Spiritone	10	%	
				Weddellianone/ Lanceolatone	6.7	%	
Nogueira et al. (2001)	Flower petals essential oils	Fresh material distillation water with dichloromethane	Terpenoids	Sesquiterpenes	64.1	%	GC-MS
				Oxygen-containng sesquiterpenes	29.4	%	
				Monoterpenes	0.5	%	
				Oxygen-containng monoterpenes	0.1	%	
			Carboxylic acids	Carboxylic acids	2	%	GC-MS
			Long chain hydrocarbons	Oxygen-containing long chain hydrocarbons	0.2	%	GC-MS
				Long chain hydrocarbons	0.1	%	
Nagem et al. (1993)	Leaves	-	Triterpenoids	Lupenone	640	µg / g	-
				Amyrin	617.14	µg / g	
				α and- β friedelinol	328.57	µg / g	
				Friedelin	71.42	µg / g	
			Steroid	Sitosterol	457.14	µg / g	
			Alcohol of fatty acid	n-octacosanol	257.14	µg / g	
			Long chain hydrocarbons	Tricosane	642.86	µg / g	

- unavailable in the reference. * Method of analysis described by (a) Rosa et al. (2007); (b) Mattila & Kumpulainen (2002); (c) Klump et al. (2001); (d) Rodriguez-Amaya (1999); Pacheco et al. (2014). ** DAD (diode-array detection); FID (Flame Ionization Detector); GC (gas-chromatography); HPLC (High performance liquid chromatography); HSCCC (High-speed counter current chromatography); MS (Mass-spectrometry); RP (Reverse Phase); UV-VIS (ultraviolet- visible).

et al. 2001, Rodriguez-Amaya 2001, Mattila & Kumpulainen 2002, Rosa et al. 2007, Pacheco et al. 2014, Duprat et al. 2017, Camara et al. 2018, Mazza et al. 2019).

Bioactivity studies

Five studies (31%) described biological applications of the *C. fluminensis* extracts and isolated compounds, lanosterol and clusianone. An overview of such applications is provided in Table II.

Anti-viral properties

In vitro antiviral properties against HSV-1 replication and HIV-1-RT enzyme activity were explored using isolated compounds and nine organic extracts (hexane, acetone, methanol) from *C. fluminensis* (leaves, flowers, fruits, stems). The experimentally established non-cytotoxic concentration of 50 µg/ml was selected for such trials (Meneses et al. 2015) (Table II).

Four extracts had inhibitory effect on HSV-1 replication with activities close or equal (81.4 to 100%) to the control drug acyclovir (100%). Furthermore, when compared to acyclovir (CC_{50} = 216 µg/ml), lower cytotoxic profiles were achieved with methanolic extracts of leaves (CC_{50} = 325 µg/ml; 100%) or fruits (CC_{50} = 304 µg/ml; 81.4%), similarly to the hexanic extract of fruits (CC_{50} = 303 µg/ml; 95.5%), but not with the hexanic extracts of flowers (CC_{50} = 78 µg/ml; 100%). The isolated compounds lanosterol (CC_{50} = 74 µM; 100%) and clusianone (CC_{50} = 121 µM; 100%) demonstrated a higher cytotoxic profile compared to acyclovir (CC_{50} = 960 µM; 100%). Inhibition of HIV-1-RT activity was verified for methanolic extracts of leaves (41.75 ± 11.19%) or stems (20.24 ± 6.24%), lanosterol (77.31 ± 10.74%) and clusianone (37.6 ± 1.73%), but at lower extents compared to efavirenz (92.16 ± 2.34%), the widely prescribed non-nucleoside reverse transcriptase inhibitor (Kryst et al. 2015). Stems (hexanic, acetonic), fruits (hexanic, methanolic) and leaves hexanic extracts of *C. fluminensis* did not inhibit HIV-1-RT enzyme (Meneses et al. 2015).

Table II. Overview of the biological activity of *Clusia fluminensis* extracts and isolated compounds.

Biological Effect		Hexane				Dichloromethane	Acetone		Methanol			Water			Isolated compounds	
		Leaves	Flower	Fruit	Stem	Flower	Fruit	Stem	Leaves	Fruit	Stem	Leaves	Fruit	Stem	Clusianone	Lanosterol
Anti-viral*	HSV-1 Inhibition	√	√	√	√	-	√	√	√	√	√	-	-	-	√	√
	HIV-RT Inhibition	Ø	-	Ø	Ø	-	-	Ø	√	Ø	√	-	-	-	√	√
Insecticidal**	*Aedes aegypti*	-	√	Ø	-	-	-	-	-	-	-	-	-	-	√	Ø
	Dysdercus peruvianus	-	√	√	-	-	-	-	-	-	-	-	-	-	√	√
	Oncopeltus fasciatus	-	√	√		-	-	-	-	-	-	-	-	-	√	√
Snake venom antidote ***	*Bothrops jararaca*	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	Bothrops jararacussu	-	-	-	-	-	-	-	-	-	-	√	√	√	-	-

√ (active); Ø (inactive); - (not tested); * Meneses et al. (2015); ** Anholeti et al. (2015) and Duprat et al. (2017); * de Oliveira et al. (2014) and da Silva et al. (2019b).**
HSV-1 (Herpes simplex virus type 1); HIV-RT (Human Immunodeficiency virus transcriptase reverse enzyme).

Insecticidal activity

The insecticide properties of *C. fluminensis* extracts from the flowers and fruits in hexane and isolated clusianone and lanosterol were tested against *Aedes aegypti* mosquitoes and the hemipterans *Dysdercus peruvianus* and *Oncopeltus fasciatus* (Table II). Survival rates and development profiles of these holometabolous and hemimetabolous insects were assayed (Anholeti et al. 2015, Duprat et al. 2017).

Only clusianone and the hexanic extract of flowers showed biocontrol effects (survival and/or development) against *A. aegypti* (Table II). Compared to the controls, the mosquitoes treated with the *C. fluminensis* flowers extract exhibited significant delays in development from larvae to pupae and from pupae to adults, but no effect was recorded for *A. aegypti* survival rates. Interestingly, clusianone showed remarkable effects not only in the delay of insect development, but also on survival rates. On the first day of treatment, 20% of larvae died compared to the controls, with no molting of the remaining survivors to pupae or adult forms (Anholeti et al. 2015).

Different survival profiles were presented by hemipterans depending on the intervention (Duprat et al. 2017). On the last day of the experiments, the flower-based preparation led to ca. 20% reduction in survival rates for both species. A similar result was observed for the survival of *O. fasciatus* treated with the fruit extract, while in *D. peruvianus* there was only ca. 11% survival decrease. Furthermore, while the isolated compound lanosterol decreased survival rates close to 30% for both hemipterans, clusianone recorded a similar 30% survival decrease of *D. peruvianus,* and only *ca* 3% for *O. fasciatus.*

Similarly, according to the treatment selected, distinct profiles of hemipteran development were also observed (Duprat et al. 2017). Both plant extracts delayed the natural life cycle of the insects, resulting in an increased proportion of nymphs and a reduction in adults following extract-treatment. In contrast, clusianone did not affect *D. peruvianus* development but increased numbers of *O. fasciatus* adults. Lanosterol, however, delayed both *D. peruvianus* and *O. fasciatus* development (nymphs and adult stages), and also resulted in malformed insects.

Anti-bothropic venom properties

Anti-venom activities of thirteen extracts of *C. fluminensis* were explored by *in vitro* (proteolysis, hemolysis, clotting) and *in vivo* (hemorrhage, edema, myotoxicity and lethality) studies with *Bothrops jararaca* or *B. jararacussu* venom (Table III). Isolated compounds from *C. fluminensis* were tested by the pre-incubation (*in vitro; in vivo*) protocols against *B. jararaca* envenomation. Pre-incubation, treatment or prevention protocols were conducted with Swiss mice as the experimental model using appropriate routes of administration (de Oliveira et al. 2014, da Silva et al. 2019b) (Figure 2). Briefly, the pre-incubation protocols, for both *in vitro* and *in vivo* assays utilize the prior mixture of the plant extracts or isolated molecules with the snake venoms, for further biological evaluation. Second, treatment protocol administers the putative antivenom following experimental envenomation and then assesses the outcome. Lastly, the prevention protocol first pre-administers the antivenom test candidate, followed by the subsequent exposure to the snake venom. Since the studies presented distinct venom:extract ratios (*w:w*), the most common were selected (1:1, 1:2, 1:5, 1:10, 1:20 or 1:50), for comparative purposes; otherwise, the unique venon:plant ratio with a given antibothropic activity was considered.

Table III. Anti-Bothrops snake venom activity of organic and aqueous extracts of *Clusia fluminensis.*

Protocol	Biological Assay (*)	Model	Administration Route / Venom:plant ratio (w:w)(**)	Leaf			Stem				Fruit				Flower	
				Hex	MeOH	H_2O	Hex	Acet	MeOH	H_2O	Hex	Acet	MeOH	H_2O	Hex	CH_2Cl_2
Pre-incubation Protocol	Proteolysis Reduction (%)	*Bja*	NA / 1:20	44	52	90	34	78	91	40	19	100	100	100	75	47
		Bju	NA / 1:20	-	-	58	-	-	-	28	-	-	-	100	-	-
	Hemolysis Reduction (%)	*Bja*	NA / 1:20	74	71	50	41	93	99	22	24	32	59	100	42	9
	Clotting Times (sec)	*Bja*	NA / 1:20	84	85	60	73	171	150	89	89	107	100	100	107	96
		Bju	NA/ 1:5	-	-	70	-	-	-	76	-	-	-	82	-	-
	Hemorrhage Reduction (%)	*Bja*	i.d. / 1:2, 1:5 or 1:20(c)	Ø	Ø	58	Ø	Ø	Ø	24	50	100	Ø	100	Ø	Ø
		Bju	i.d. / 1:20	-	-	74	-	-	-	100	-	-	-	100	-	-
	Edema Reduction (%)	*Bja*	s.c. / 1:10	-	-	84	-	-	-	80	-	-	-	80	-	-
		Bju	s.c. / 1:10	-	-	7	-	-	-	63	-	-	-	100	-	-
	Miotoxicity Reduction (CK Units)(a)	*Bju*	i.m. / 1:10	-	-	1400	-	-	-	920	-	-	-	1360	-	-
	Survival Time (min)(b)	*Bja*	i.p.; i.v./ 1:1	-	-	360 ± 3	-	-	-	360 ± 5	-	-	-	360 ± 4	-	-
		Bju	i.v. / 1:1	-	-	145 ± 4	-	-	-	72 ± 7	-	-	-	360 ± 2	-	-
Treatment Protocol	Edema Reduction (%)	*Bja*	p.o. / 1:10	-	-	0	-	-	-	40	-	-	-	25	-	-
		Bju	p.o. / 1:10	-	-	16	-	-	-	39	-	-	-	32	-	-
	Survival Time (min)	*Bja*	i.p./ 1:1 or 1:10(d)	-	-	75 ± 0.7	-	900	900	180 ± 0.9	-	-	-	70 ± 0.6	-	-
		Bju	iv / 1:1	-	-	70 ± 9	-	-	-	70 ± 6	-	-	-	360 ± 4	-	-
	Hemorrhage Reduction (%)	*Bja*	i.v. / 1:20	-	-	51	-	-	-	60	-	-	-	41	-	-
			s.c or i.d./ 1:2 or 1:20(e)	-	-	51	-	-	-	60	10	10	-	41	-	-
			topical	-	-	-	-	-	-	-	-	-	-	50	-	-
		Bju	i.v. / 1:20	-	-	35	-	-	-	45	-	-	-	36	-	-
			s.c. / 1:20	-	-	35	-	-	-	45	-	-	-	36	-	-
			topical	-	-	-	-	-	-	-	-	-	-	55	-	-
Prevention Protocol	Hemorrhage Reduction (%)	*Bja*	p.o. / 1:20	-	-	42	-	-	-	Ø	-	-	-	43	-	-
		Bju	p.o. / 1:20	-	-	46	-	-	-	50	-	-	-	35	-	-

Data extracted from graphics and tables from de Oliveira et al. (2014) and da Silva et al. (2019b); Grayish background highlights the *in vitro* trials; Ø (inactive).
***Referred values for control as (a) ca. 1500 CK units and (b) survival times of 120 minutes for organic extracts (de Oliveira et al. 2014) or 60 minutes (da Silva et al. 2019b).**
**** Venom:plant ratios (c) 1:2 (organic extracts), 1:5 (fruit aqueous); 1:20 (leaf, stem aqueous). (d) 1:10 (organic extracts), 1:1 (aqueous extracts); (e) 1:2; i.d (organic extracts), 1:20; s.c. (aqueous extracts).**
Abbreviations: *Bja* (*Bothrops jararaca*); *Bju* (*Botrops jararacussu*); Acet (acetone); Hex (hexane); MeOH (methanol); H_2O (water); p.o. (oral), s.c. (subcutaneous), i.v. (intravenous), i.m. (intramuscular), i.p. (intraperitoneal), i.d. (intradermal).

Anti-proteolytic activity

Both organic and aqueous extracts of *C. fluminensis* inhibited, to different extents, *in vitro* azocasein degradation induced by *B. jararaca* venom. The fruit extracts (aqueous, methanolic and acetonic) had the most prominent anti-proteolytic activity, followed by extracts of the stems (methanolic) and leaves (aqueous). Regarding the isolated compounds, clusianone

exhibited 46% inhibition at the concentration of 1:50, while lanosterol (720 µg/ml) showed no inhibitory effect on proteolysis induced by *B. jararaca* venom (de Oliveira et al. 2014). With the *B. jararacussu* venom, the aqueous extract of the fruits recorded the highest inhibition of the proteolysis (da Silva et al. 2019b).

Anti-hemolytic activity

In the *in vitro* indirect hemolytic tests, *B. jararaca* venom-induced hemolysis was reduced by all the plant extracts, with distinct potencies (de Oliveira et al. 2014, da Silva et al. 2019b). Aqueous extract of fruits completely inhibited hemolytic activity, followed by methanolic and

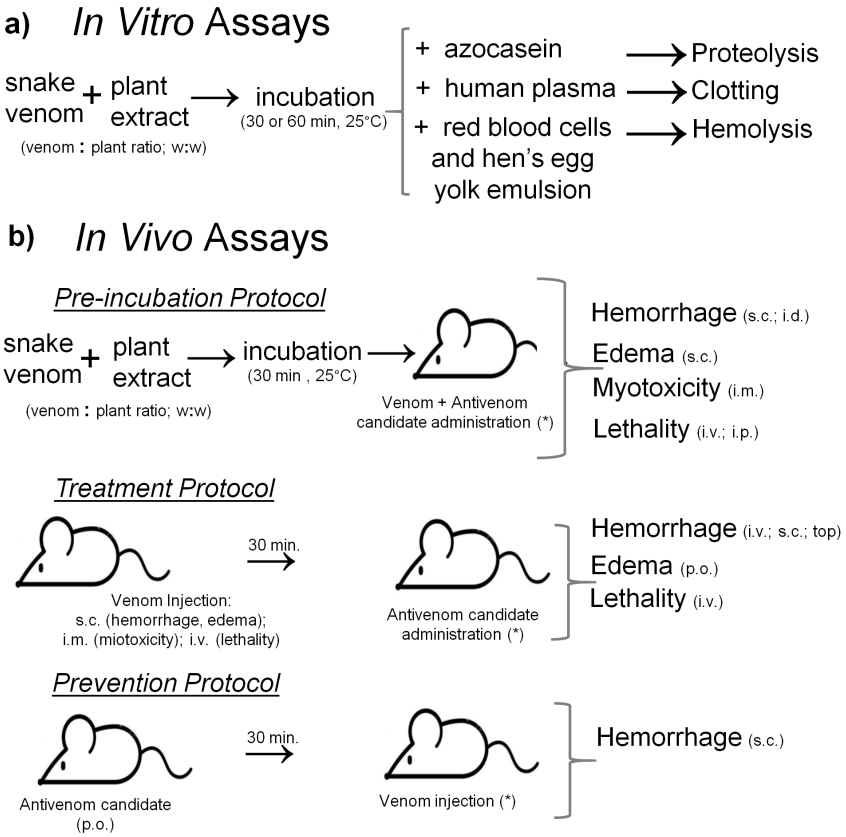


Figure 2. Antibothropic activity of *Clusia fluminensis* extracts assayed *in vitro* and *in vivo*. a) *In vitro* assays were performed in which the venoms of *Bothrops jararaca* or *B. jararacussu* were pre-incubated with plant extracts or isolated molecules and then with: (i) azocasein, (ii) plasma or (iii) a mixture of human red blood cells and hen's egg yolk emulsion to determine, respectively (i) proteolytic inhibition, (ii) clotting time and (iii) antihemolytic activity. Hemolysis induced by the venom of *B. jararacussu* was not tested, since this venom has low hemolytic activity. b) *In vivo* antibothropic evaluation was performed using pre-incubation, treatment or prevention protocols using distinct routes of administration (*). As with the *in vitro* assays, in the *in vivo* incubation protocol, venom was pre-incubated with the plant extracts before injection in mice. For the treatment protocol, the mice were pre-injected with venom and then treated with each extract. For the prevention protocol, mice were fed with the extracts before the venom injection. Distinct routes of administration for snake venoms and plant extracts were as follows: top (topical), p.o. (oral), s.c. (subcutaneous), i.v. (intravenous), i.m. (intramuscular), i.p. (intraperitoneal), i.d. (intradermal). Positive control groups received snake venom mixed with saline, and negative controls received plant extracts alone. Myotoxicity assay was only performed for *B. jararacussu* envenomation, due to its marked effect compared to *B. jararaca*.

acetonic extracts of the stems. In addition, only lanosterol (1:20), isolated from the fruit extracts, exhibited anti-hemolytic properties (65%) when incubated with *B. jararaca* venom. The *B. jararacussu* venom was not tested due to the low hemolytic activity reported (da Silva et al. 2019b).

Anti-clotting activity

In vitro clotting effects induced by *B. jararaca* venom were attenuated by all *C. fluminensis* crude extracts (Table III), by different degrees, except for the aqueous extract of leaves with clotting times identical to controls (60 seconds). The highest anti-clotting activities were recorded for acetonic and methanolic extracts of the stems (2.8-fold and 2.5-fold increase, respectively). The fruit (acetonic, methanolic, aqueous) and flower (hexanic) extracts had similar anticlotting properties (1.7-fold increase). No anticlotting properties were identified for clusianone (500 µg/ml) and lanosterol (800 µg/ml) against *B. jararaca* venom (de Oliveira et al. 2014, da Silva et al. 2019b). Regarding the *B. jararacussu* venom-induced coagulation, aqueous extracts of leaves, stems and fruits of *C. fluminensis* demonstrated ca.1.3-fold increase on clotting times compared to the venom-controls (da Silva et al. 2019b).

Antihemorrhagic activity

According to the *in vivo* pre-incubation protocol for evaluation of hemorrhagic damages induced by *B. jararaca* envenomation, acetonic extract of fruits showed complete inhibition (100%) at a 1:2 venom:plant ratio (Table III). Such activity was not observed for lanosterol or clusianone nor for other organic extracts at 1:2 ratio (de Oliveira et al. 2014). According to this same protocol, aqueous extract of fruits (1:5) also presented a remarkable antivenom effect (100%) for both *Bothrops* venoms, a similar effect observed for aqueous extract of stems (1:20) upon incubation with *B. jararacussu* venom (da Silva et al. 2019b). Aqueous extract of leaves (1:20), as well as hexanic extract of fruits (1:2) also demonstrated antihemorrhagic properties by incubation with *B. jararaca* venom, but to a lesser extent (ca. 50%) (de Oliveira et al. 2014, da Silva et al. 2019b).

Regarding the treatment protocol, antihemorrhagic properties were recorded for aqueous extracts of stems, fruits and leaves (1:20), upon envenomation, with both *Bothrops* spp, regardless of the routes of administration. A slightly better biological performance occurred for the aqueous stem extracts, against both venoms. In addition, a topical preparation of the aqueous extract of fruits (50 mg/ml) resulted in ca.50% inhibition of hemorrhage with both *Bothrops* envenomations (Table III). No significant anti-hemorrhagic effects were observed for acetonic and hexanic extracts of fruits (1:2; i.d.) (de Oliveira et al. 2014). According to the preventive protocol, a range of 35 to 50% hemorrhage inhibition resulted for aqueous extracts of *C. fluminensis*, except for aqueous extract of the stems, which were devoid of any activity against hemorrhage induced by *B. jararaca* venom (da Silva et al. 2019b).

Antiedematogenic activity

As demonstrated by *in vivo* pre-incubation protocol, aqueous extract of fruits from *C. fluminensis* showed the best antiedematogenic profile (100%) against *B. jararacussu* envenomation (Table III). Under the same experimental conditions, aqueous extracts of *C. fluminensis* stems, leaves and fruits demonstrated effects close to 80% of edema inhibition after *B. jararaca*-induced envenomation. Observing the treatment protocol, the oral administration of aqueous extract of stems or fruits was active upon edematogenic damage induced by both serpent venoms, reaching from 25 to 40% of inhibitory effect (da Silva et al. 2019b).

Antimyotoxic activity

Aqueous extract of stems of *C. fluminensis* was effective on reducing creatine kinase (CK) serum levels after experimentally induced *B. jararacussu* envenomation, when compared to aqueous extracts of fruits and leaves (da Silva et al. 2019b), as verified by the *in vivo* incubation protocol (Table III). Antimyotoxic effects were not evaluated against *B. jararaca* venom, due to its low toxicity at muscular level compared to *B. jararacussu* (Moura-da-Silva et al. 1991, da Silva et al. 2019b).

Antilethal activity

According to the incubation protocol, a 6-fold increase on mice survival times was observed for aqueous extracts of stems, leaves and fruits (1:1) after the experimentally induced *B. jararaca* envenomation (Table III). The same increase on survival times was noticed for aqueous extract of fruits for both, incubation and treatment protocols, for *B. jararacussu* poisoning. Such biological effect is compatible with those achieved by the antibothropic serum treatment under the same experimental conditions (da Silva et al. 2019b). Additionally, under the treatment protocol for *B. jararaca* envenomation, aqueous extract of stems (1:1) demonstrated 3-fold increase on survival times (da Silva et al. 2019b), while the methanolic and acetonic extracts of stems (1:10) presented a 7.5-fold increase (de Oliveira et al. 2014).

DISCUSSION

According to the records captured by this review and as the best of our knowledge, there are few studies dealing with the biological activities of *C. fluminensis* (Figure 1), most papers (67%) having been devoted to the phytochemical aspects of different parts of the plant. The remainder describes the antiviral, insecticidal and antivenom properties of the plant extracts and isolated compounds (Table II).

HSV-1 viral infections lead mostly to oral herpes, but genital herpes is also reported. This is a globally widespread, endemic, highly prevalent and incurable disease. Current available antiherpetic treatment relies on acyclovir or synthetic nucleoside analogs, presenting untoward side effects, drug resistance and no effect upon viral latent infection (Smith et al. 2001, Treml et al. 2020). Anti-HIV multidrug cocktails for disease control result in similar issues (Wu et al. 2020). Therefore, novel therapeutic options from natural origin are a promising alternative for achieving the maximal desired effects with minimal collateral damage to treat viral diseases (Ben-Shabat et al. 2020, Mohan et al. 2020, Ninfali et al. 2020).

The antiviral role of phenolic compounds, polyprenylated benzophenones, steroids and terpenes, found both in *C. fluminensis* (Table I) and also in other members of Clusiaceae family, has already been described for both DNA and RNA viruses (Gustafson et al. 1992, Akihisa et al. 2001, Rezanka et al. 2009, Shamsabadipour et al. 2013, Hisham Shady et al. 2020, Loaiza-Cano et al. 2020). The results reported for the effects of *C. fluminensis* derivates on HSV-1 are very promising (Meneses et al. 2015). The inhibition of HSV-1 replication (81.4 to 100%) was observed by *in vitro* tests with Vero cells, despite the polarity of the solvent extract (hexane, acetone, methanol), plant organ (leaf, stem, fruit, flower) or isolated compound (clusianone or lanosterol) (Meneses et al. 2015). The findings, demonstrated by a recognized experimental model adopted by many scientific trials (Treml et al. 2020) may suggest that, isolated or combined, the phytochemicals found in *C. fluminensis* could exert a multitarget effect upon the HSV-1 viral cycle and probably at the host response to the

infection. For example, the phenolic compounds identified in distinct organs from *C. fluminensis* (Table I) and the antioxidant properties demonstrated for different plant extracts (leaves, fruits and stems in acetone or methanol) (Silva & Paiva 2012) could favor the recovery of the host intracellular redox equilibrium, alleviating the promoter effect of reactive oxygen species (ROS) on the replication of such neurotropic virus (Di Sotto et al. 2018). Additionally, it is also recognized the role of phenolic compounds on suppressing key pro-inflammatory signaling pathways on host cells as those mediated by toll-like receptors (TLR) (Newton & Dixit 2012, Pérez-Cano et al. 2014). Apart from the anti-HSV-1 properties, fruits (hexanic and methanolic) and leaves methanolic extracts, also had lower cytotoxic profiles (around 1.4-fold decrease) compared to acyclovir (Meneses et al. 2015). This suggests an enhanced tolerability performance of relevance to gaining approval for treatment, as suggested for herbal extracts with antiviral application (Ni et al. 2020).

Regarding the inhibitory effect of *C. fluminensis* derivatives upon HIV-1 RT activity, the discrete effects observed for methanolic extracts of leaves (close to 40%) and stems (around 20%), with a concomitant absence of such enzymatic inhibition for the hexanic herbal fractions, suggest the presence of potential applied candidates in higher polarity fractions, such as the polyphenols, tannins, saponins and terpenoids (Table I) (Azmir et al. 2013). Indeed, anti-HIV-1 RT inhibition has already been described for such phytochemicals, as confirmed for phenolic compounds isolated from other herbal sources (Tamayose et al. 2019). Additionally, recent studies based on molecular docking also corroborates the relevance of polyphenolic derivatives as anti-HIV-1 RT inhibitors, in which structural modifications related to the water solubility, bioavailability, toxicity and efficacy are considered (El Alaoui et al. 2019). Anti-HIV properties were previously identified for lanosterol-related molecules obtained from crude alcoholic fractions of herbal sources, namely a lanostane-type triterpene from *Polyalthia suberosa* (Roxb.) Thwaites (Annonaceae) (Li et al. 1993) and protostane triterpenes from *Garcinia speciosa* Wall. *(= G. celebica* L.*)*, a member of Clusiaceae family (Rukachaisirikul et al. 2003). Such findings are corroborated by the recognized effects of triterpenes and steroids upon HSV and HIV virus (Akihisa et al. 2001, Rezanka et al. 2009, Shamsabadipour et al. 2013, Hisham Shady et al. 2020).

In addition, an anti-HIV-1 role is also suggested for clusianone (37%, HIV-RT inhibition) (Meneses et al. 2015) possibly by an interference on the virus adhesion to its host cell receptor. The virus infection is prevented at the initial stages, as verified by studies with clusianone isolated from the hexanic extract of fruits of *C. torresii* Standl., in a C8166 human T lymphoblastoid cell model for HIV-1 infection (Piccinelli et al. 2005). Additionally, a low therapeutic index was reported for clusianone, since the high activity at low dosage was accompanied by a prominent cytotoxic impact (Piccinelli et al. 2005). Such findings support future prospection of new derivatives synthesis, emphasizing selectivity and specificity aimed at a preventive approach for virus infection (Piccinelli et al. 2005). Interestingly, lanosterol treatment of infected cells showed ca. 70% of HIV-RT inhibitory effect, with a concomitant and complete inhibition on HSV-1 replication (Meneses et al. 2015). Such findings reinforce the antiviral potential of natural tetracyclic triterpenoids, especially for immunocompromised patients, in which HSV-1 complications are demonstrated to be more severe (Meyding-Lamadé & Strank 2012, Duarte et al. 2019, James et al. 2020). However, the

issues regarding cytotoxicity profile remains to be better understood and properly managed, since a preliminary trial found a 13-fold increase of lanosterol compared to acyclovir (CC_{50} = 74μM versus CC_{50} = 960 μM, respectively) (Meneses et al. 2015). Inversely, according to the same trial, the methanolic extract of leaves of *C. fluminensis* showed a cytotoxic profile 1.5-times lower than acyclovir (CC_{50} = 325 μg/ml versus CC_{50} = 216 μg/ml, respectively), associated to a partial inactivation of HIV-1 RT (ca. 40%) and a complete inhibition of HSV-1 replication (Meneses et al. 2015). Such findings highlight potential benefits of this extract for future prospection, considering an adjuvant approach for immunocompromised HIV patients.

The control of *Aedes aegypti* mosquitoes represents the most important strategy to manage public health issues related to these costly widespread viral diseases (Benelli et al. 2016, Silvério et al. 2020). Many efforts have been made to identify naturally-occurring candidates for insect vector control (da Silva et al. 2013, Pavela 2016, Gosset et al. 2017).

This present review identifies *C. fluminensis* as a very attractive source for such biotechnological development. For example, the hexanic extract of flowers markedly delayed the development of different stages of *A. aegypti* (Anholeti et al. 2015). Additionally, its main component, clusianone, exhibited larvicidal effects by the first day of treatment (20% of reduction), with no evolution of the remaining survivors to adult stage (Anholeti et al. 2015). Interestingly, similar results were not observed for the hexanic extract of fruits and for its major compound lanosterol (Anholeti et al. 2015). The insecticidal role of naturally occurring bezophenones, such as clusianone, against *A. aegypti* is reinforced by a recent study with hexanic extracts and isolated compounds from *Vismia gracilis* Hieron (Hypericaceae), inducing to toxic damage and behavioral effects in mosquitoes (Magalhaes et al. 2022). In addition, larvicidal activity against *A. aegypti* was identified for the hexanic extracts of *Hypericum carinatum* Griseb (Hypericaceae), containing as main compounds cariphenones A and B, that are benzophenones structurally related to benzopyrans and precocenes (da Silva et al. 2013). These synthetic larvicidal compounds have an inhibitory effect upon the juvenile hormones (JH) of many insects, that are involved in physiologic processes such as development and reproduction (Ayoade et al. 1996, da Silva et al. 2013, Qu et al. 2018). Indeed, the phytochemical profile of other *C. fluminensis* derivatives (Table I) suggest potential candidates for *A. aegypti* control. Firstly, the essential oil of petals (Nogueira et al. 2001), considering the larvicidal, adulticidal, repellent and oviposition effects reported for many essential oils for this purpose (Silvério et al. 2020). This activity was also demonstrated by the volatiles from leaves and fruits of *Garcinia gardneriana* (Planch. & Triana) Zappi, a member of Clusiaceae family (Fernandez et al. 2021). Secondly, the fruit latex, taking into account the larvicidal effects of β-caryophyllene and β-caryophyllene oxide (Senthil-Nathan 2020), which also reinforces a possible activity of the volatiles from *C. fluminensis*. Thirdly, leaves extracts of *C. fluminensis,* in which pentacyclic triterpenes (such as epifriedelinol and friedelin), steroids (such as sitosterol and stigmasterol) and phenolic compounds (including flavonoids and flavones) were identified (Sá-Haiad et al. 2015, Silva et al. 2019a, Guimarães et al. 2021). Similar phytochemical profile was detected in extracts of *Waltheria viscosissima* A.St.-Hil. (Malvaceae), which demonstrated larvicidal effect on *A. aegypti (*Ferreira et al. 2019, Senthil-Nathan 2020).

D. peruvianus and *O. fasciatus* also emerge as vectors of economic interest, especially for

agrobusiness (Georghiou & Mellon 1983, Alves e Silva et al. 2013). Synthetic compounds that act as insect growth regulators are employed for pest control purposes, affecting different stages of insects development, body size regulation, reproduction, longevity and behavior (Bellés et al. 2005, Qu et al. 2018, Hu et al. 2019). However, the impact in ecosystems, pest resistance and toxicity to non-target organism are issues reported for such compounds (Bellés et al. 2005, Hu et al. 2019, Fine & Corby-Harris 2021). Given this reality, the search for more selective and eco-friendly agents for pests control gains substantial relevance.

A differential insecticidal effect of *C. fluminensis* extracts and isolated compounds occurred with the two hemipteran models (Duprat et al. 2017). Firstly, clusianone recorded 30% survival decrease for *D. peruvianus* with no effects in its development, but for *O. fasciatus* this reduction was only ca. 3% and was also accompanied by a promoter effect of malformed insects (Duprat et al. 2017). Such findings are in agreement with the action of synthetic benzophenones on ecdysteroid hormone pathway in insects (Ozáez et al. 2014, 2016). Secondly, the hexanic extract of fruits reduced survival of *O. fasciatus* by ca. 20% but that of *D. peruvianus* by only ca. 11%. These findings highlight the relevance of the plant secondary metabolites as toxic, nonspecific and multitarget candidates on distinct cellular and molecular pathways related to key physiological process in insects, including, but not restricted to, hormonal imbalance (Ozáez et al. 2014, 2016, Senthil-Nathan 2020). Indeed, plant extracts enriched with phenolic compounds demonstrated toxicity and antifeedant properties to larvae of holometabolous insects (Oulebsir-Mohandkaci et al. 2018). Phenolic compounds were found in different plant organs of *C. fluminensis* (Silva & Paiva 2012, Sá-Haiad et al. 2015, Mazza et al. 2019, Silva et al. 2019a, Guimarães et al. 2021), which are also recognized to exert their toxic role on insect gut as precursors of tannins, compounds responsible for increasing the reactive species of oxygen (ROS) levels, both by autoxidation or by enzymatic catalysis producing quinone or semiquinone species (Barbehenn & Peter Constabel 2011). In addition, sesquiterpenoids are also described as antifeedant agents, which also reinforces further evaluation of essential oil of *C. fluminensis* for pest control purposes (Nogueira et al. 2001, Koul 2008). Importantly, caution should be taken regarding the extrapolation of these findings, taking into account a promoter effect of the extract solvent upon bugs development, remaining the need of further confirmatory trials.

Snakebite envenoming is a neglected public health subject that affects tropical and subtropical developing countries, accounting for marked social and financial impact due to deaths, amputations and permanent disabilities (Chippaux 2017, Magalhães et al. 2020). In Brazil, the genus *Bothrops* (Viperidae) is responsible for most snake poisoning events (de Moura et al. 2015). *B. jararaca* and *B. jararacussu* are of particular medical relevance, due to their occurrence in highly populated regions and bite severity (Araujo et al. 2017). The heterogeneous mixture of toxins present in *Bothrops* venoms leads to coagulation imbalance (hemorrhages, thrombocytopenia), local (edema, pain, erythema, ecchymosis, necrosis) and systemic effects (nausea, vomiting, hypotension, shock, neurologic, renal and cardiac abnormalities). Additionally, myotoxicity, low immunogenicity and poorly neutralization by the antibothropic treatment raises as hallmarks of *B. jararacussu* envenomation, leading to necrosis and delayed tissue repair (da Silva et al. 2007, Luna et al. 2011, Bochner et al. 2014, da Silva Aguiar et al. 2020). Key components of snake venom

include metalloproteases, serine proteases, phospholipase A_2 (PLA_2), C-type lectins (CTL), L-amino acid oxidases (LAAO), 5´-nucleotidases and hyaluronidase, playing crucial roles in prey domination (Gutierrez & Lomonte 1989, Saad et al. 2012, Gutiérrez et al. 2017, Gren et al. 2019).

Specific antiserum therapy is the current treatment for snakebite envenomation, which requires fast administration, since poor prognosis is associated with delayed medical assistance. Additionally, the antivenom fails to treat local tissular damage (Mise et al. 2018). Therefore, there is a need for ancillary therapeutic options to treat local complications of snakebites, especially in children of rural, indigenous and riverside population (de Moura et al. 2015, da Silva Souza et al. 2018, Cristino et al. 2021, Le Geyt et al. 2021). Traditionally, plant preparations have been widely described in many countries as part of common practices in folk medicine, including management of snakebite accidents. However, the proper scientific validation of such popular knowledge is needed, considering the risks associated to the worsening of clinical complications of the victims (de Moura et al. 2015, Upasani et al. 2017, da Silva et al. 2019a).

Experimentally, incubation protocol consists of commonly adopted procedure for laboratory evaluation of antivenoms. It is also recognized by regulatory agencies and employed routinely for quality control purposes by antidote manufacturers (Gutiérrez et al. 2021). In this protocol, a mixture of the venom with the neutralizing candidate is previously prepared for further *in vitro* or *in vivo* tests (Figure 2) (Theakston & Reid 1983, de Moura et al. 2014). Such assays are very useful for providing insights into mechanisms of action and interactions between toxins and potential antidotes (Sells 2003). Caution, however, is needed to avoid overestimation of the findings during data interpretation and extrapolation, since the incubation study design does not reflect the actual dynamics of envenomation, or pharmacokinetics parameters defining the antidote candidate performance (da Silva et al. 2007, de Moura et al. 2014, 2015, de Souza et al. 2020). Additional experimental supporting procedures adopted in snakebites envenomation are the prevention and treatment protocols (Figure 2) (de Moura et al. 2014). The prevention protocol considers the antidote administration before the contact with the venom, mimicking popular practices based on the ingestion of homemade plant preparations previously to activities in arboreous areas (de Moura et al. 2014). Additionally, the therapeutic administration of the antivenom candidate after the envenomation is related to self-care procedures of local application of poultices and ingestion of teas (de Moura et al. 2014, Cristino et al. 2021).

In this context, and in agreement with antivenom properties that have been already described for Clusiaceae species (Castro et al. 1999), similar findings were identified for *C. fluminensis* (Table III). Compared to the plant extracts, the isolated compounds, showed discrete neutralizing effects on *B. jararaca* envenomation by *in vitro* incubation protocol, since 46% of proteolysis and 65% of hemolysis inhibition occurred, respectively, for clusianone (1240 µg/ml) and lanosterol (600 µg/ml) (de Oliveira et al. 2014), however no effects were detected by *in vivo* incubation protocol for hemorrhage and survival values. The highest biological effects of the extracts may result from synergism between distinct bioactive molecules present in a specific extract fraction or due to other bioactive molecules different of clusianone or lanosterol (Azmir et al. 2013). For example, neutralizing properties of snake venoms have been described for the pentacyclic triterpene lupeol and also for the steroids

sitosterol and stigmasterol (Strauch et al. 2013, dos Santos et al. 2021), compounds previously identified in *C. fluminensis* organs (Nagem et al. 1993, Anholeti et al. 2017).

Taking into account the *in vitro* screening provided by two original papers, as well as any related bias (de Oliveira et al. 2014, da Silva et al. 2019b), it seems feasible that plant extracts obtained by the use of solvents with higher polarity had more promising neutralizing effects against bothropic toxins. This can be verified by comparing those obtained by extraction with water, methanol and acetone with those in which hexane or dichloromethane. Accordingly, out of thirteen *C. fluminensis* extracts explored, six stand out as follows: fruits aqueous, stems methanolic (both presenting higher rates of inhibition for proteolysis, hemolysis and coagulation induced by *Bothrops* spp.), stems acetonic (at least two neutralizing properties with ca. 100% or more against *Bothrops* spp.), fruits methanolic or acetonic (100% proteolysis inhibition against *B. jararaca*) and aqueous extracts of leaves (ca. 90% of proteolysis inhibition for *B. jararaca* venom) (Table III). Additionally, similar findings were observed for the *in vivo* incubation protocol. For example, higher rates of hemorrhage inhibition after the poisoning by *B. jararaca* (fruits aqueous and acetonic extracts) or *B. jararacussu* (aqueous extracts of fruits and stems) were achieved, and also suggested for the aqueous extracts of stems, fruits and leaves potential effect against edema, myotoxicity and increased survival times after the envenomation by both or one of the *Bothrops* species (Table III). Such findings corroborate previous studies regarding neutralizing effects on snake venoms of polar plant extracts, in which phenolic compounds were identified and recognized (Fernandes et al. 2016, Félix-Silva et al. 2017, Sachetto et al. 2018).

Finally, treatment and prevention protocols highlight remarkable antitoxin properties of each aqueous extract, as well as increases in survival times for acetonic and methanolic extracts of stems after *B. jararaca* envenomation. For example, the aqueous extract of fruits of *C. fluminensis* demonstrated a reduction close to 50% for hemorrhage induced by both *Bothrops* poisoning, despite the routes of administration (s.c., i.v., topical), as well as around 30% of edema reduction (p.o.) after both snake species envenomation. Additionally, a 6-fold increase on survival times was achieved (360 minutes) when the latter extract was given (i.v.) (Table III) just after *B. jararacussu* envenomation, demonstrating a magnitude of effect comparable to the current antibothropic serum efficacy. Additionally, this extract also demonstrated a preventive profile (p.o.) one hemorrhage induced by both *Bothrops* toxins, close to 40% inhibition (Table III).

Similarly, the aqueous extract of stems in both treatment and prevention protocols, inhibited hemorrhage by close to 60% with *B. jararaca* for the i.v. route of administration and edema by 40% reduction p.o. treatment for both *Bothrops*. Although the preventive protocol (p.o) has only demonstrated to be effective against the hemorrhage induced by *B. jararacussu* envenomation, the magnitude of 50% of inhibition seems potentially relevant, being also demonstrated a 3-fold increase on survival times after *B. jararaca* envenomation in the absence of antibothropic serum (Table III).

Future perspectives

The presence of secondary metabolites with recognized biological relevance in *C. fluminensis* (Table I) suggests future developments of biological trials. For example, the detection of zeaxanthin (823 µg/g, Table I) in seeds arils of *C. fluminensis*, reaching levels 206-fold higher than those described in corn (Mazza et al. 2019)

points to a naturally occurring source of an ingredient with potential applications in skin care preparations, dietary supplements and functional foods (Gale et al. 2003, Chew et al. 2014, Dini & Laneri 2019). Such finding gains especial relevance when considering that human beings are not able to synthesize such compound with recognized photooxidative protection role (Sandmann 2014, Jia et al. 2017, Renzi-Hammond et al. 2017). The body of evidences regarding the presence of saponins in members of Clusiaceae family (Azebaze et al. 2006, Noudogbessi et al. 2013, Omeh et al. 2014) suggests confirmatory phytochemical studies in *C. fluminensis,* having into account the antioxidant, anti-inflammatory, antiparasitic, antitumoral, antiviral and antidiabetic properties of such metabolites (Cheok et al. 2014, Omeh et al. 2014). Similarly, future exploratory trials might be considered regarding malic acid, the product accumulated by the CAM photosynthetic metabolism (Lüttge 2006). Such compound consists of a raw material with wide range of application, such as food (preservative, flavor enhancer), pharmaceutical (blood-pressure reducing purposes), cosmetic (skin care, toothpastes), chemical (metal cleaning) and textile (acrylic fiber production) (Bharathiraja et al. 2020).

Other potential activities are suggested for extracts or isolated molecules for Clusiaceae family such as anticancer, antimicrobial, trypanocidal, anticholinesterase anti-inflammatory and antileishmanial (Acuna et al. 2009, Cheok et al. 2014, de Melo et al. 2014). Although anticancer activity was not evaluated for *C. fluminensis* plant extracts, the *in vitro* cytotoxic profiles of lanosterol and clusianone from *C. fluminensis* (13-fold and 8-fold increase compared to acyclovir, respectively) (Meneses et al. 2015), could suggest further speculation of a potential effect of such compounds for anticancer purposes. Indeed, the anticancer role of polyprenylated benzophenones from Clusiaceae family (Kumar et al. 2013, Taylor et al. 2019) and clusianone (Simpkins et al. 2012, Reis et al. 2014) have already been recognized by preclinical trials, as evaluated for different solid tumors. Such effects may rely on the pleiotropic target of polyprenylated benzophenones on crucial cellular processes that support tumor progression, as cell growth inhibition, apoptosis induction, angiogenesis and cell migration inhibition (Taylor et al. 2019). In the same manner, the tetracyclic triterpene lanosterol could also be investigated, having into account the anticancer properties of its stereoisomer euphol (Gascoigne & Simes 1955). Indeed, euphol, referred as a possible chemotaxonomic marker of Clusiaceae family (Ribeiro et al. 2019), exerted antitumoral effect upon cell lines from 15 different solid tumors being also identified a chemo-sensitization with current chemotherapy for glioblastoma, thus suggesting lower doses of the chemotherapeutic agent (Silva et al. 2018, 2019b).

The comprehensive chemical characterization of plant extracts with potential biotechnological relevance also remains to be clarified, representing an important step to define the identity and quality requirements of such derivatives. In addition, when dry plant extracts are considered as the final biotechnological product, parameters related to the vegetal material processing deserve careful consideration, to retain the naturally occurring synergism exerted by the phytochemical mix of a given plant organ. Importantly, complementary toxicological evaluation is also desirable. Safety assessment represents a pivotal step in the process of accumulation of scientific evidence, mainly when considering therapeutic, cosmetic or edible prospection of natural products.

Finally, the chemical clarification of such natural products can also contribute as basis for

subsequent synthesis and modification, leading to new candidate compounds to overcome issues related to solubility, bioavailability and stability reported to phytochemicals.

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GISELLE M. FARIA[1]
https://orcid.org/0000-0002-4287-1651

ANA PATRICIA A. LEMOS[1]
https://orcid.org/0000-0002-4119-9656

MARIA C. ANHOLETI[2]
https://orcid.org/0000-0003-1552-9161

SELMA R. PAIVA[3,4]
https://orcid.org/0000-0002-8295-7011

LIDIA M.F. AMORIM[1]
https://orcid.org/0000-0001-7282-9748

[1]Universidade Federal Fluminense, Instituto de Biologia, Departamento de Biologia Celular e Molecular, Rua Prof. Marcos Waldemar de Freitas Reis, Bloco M, 311, São Domingos, 24210-201 Niterói, RJ, Brazil

[2]Universidade Federal Fluminense, Faculdade de Farmácia, Departamento de Farmácia e Administração Farmacêutica, Rua Dr. Mario Vianna, 523, Santa Rosa, 24241-001 Niterói, RJ, Brazil

[3]Universidade Federal Fluminense, Instituto de Biologia, Departamento de Biologia Geral, Rua Prof. Marcos Waldemar de Freitas Reis, Bloco M, 111, São Domingos, 24210-201 Niterói, RJ, Brazil

[4]Programa de Pós-Graduação em Ciências Aplicadas a Produtos para Saúde, Universidade Federal Fluminense, Faculdade de Farmácia, Rua Dr. Mario Vianna, 523, Santa Rosa, 24241-001 Niterói, RJ, Brazil

Correspondence to: **Lídia Amorim**
E-mail: amorimlidia@id.uff.br

Author contributions

Giselle M. Faria: search and selection of the papers, categorization by bibliographic databases, writing of original draft, data analysis and organization of the results. Ana Patricia A. Lemos: search and selection of the papers, categorization by bibliographic databases, figures design. Maria Carolina Anholeti: reviewing and editing. Selma R. Paiva: reviewing and editing, project administration, funding acquisition. Lidia M.F. Amorim: conceptualization, reviewing and editing, data analysis, supervision, project administration, funding acquisition.