PharmacologyOnLine December 30, 2016

Archives • 2016 • vol.3 • 56-60

THROMBOLYTIC ACTIVITY EVALUATION OF EXTRACTS FROM FABACEAE SPECIES *BAUHINIA VARIEGATA*, *B. RUFA,* AND *STRYPHNODENDRON ADSTRINGENS*

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Abstract

In continuing the search for natural products presenting in vitro thrombolytic activity, eleven extracts from three Fabaceae species were evaluated. Crude hexane, ethanol and aqueous extracts from *Bauhinia variegata* (leaves), *Bauhinia rufa* (leaves), and *Stryphnodendron adstringens* (leaves and stem bark), were evaluated for their ability to clot lysis in comparison to streptokinase. *Bauhinia rufa* and *S. adstringens* are Brazilian native species, while B*. variegata,* a native of Asia is a widely planted ornamentally in Brazil. The three are used as medicinal species. *Stryphnodendron adstringens* has been used for wound healing, as well as anti-inflammatory and antimicrobial activity. *Bauhinia rufa* and *B. variegata* are used to control diabetes mellitus. Four extracts promoted more than 20 % clot lysis: *S. adstringens* leaf aqueous extract (23.71%); *B. variegata* leaf hexane and ethanol extracts (23.11% and 29.72% respectively), and *B. rufa* leaf extract (26.17%). These results should be taken into account in future studies to achieve natural antithrombotic products.

Keywords: Thrombolytic activity; Medicinal plants; Cerrado

Introduction

Plant species used medicinally, are often a source of useful and pharmacologically relevant compounds. Moreover, natural products usually can act as pharmacophores and present a high degree of stereochemistry, two interesting characteristics for the discovery of new drugs (1). Several antithrombotic compounds, such as heparin, vitamin K antagonists, streptokinase, and urokinase originate from a natural source or were developed from a natural product model. In recent studies, it is possible to observe the increasing search for new natural products presenting antithrombotic activity (2-4). Recently, the *in vitro* antithrombotic potential of some Brazilian plant species was reported (5, 6). Three Fabaceae species, occurring in Cerrado, were screened *in vitro* for antithrombotic potential. Cerrado, the Brazilian Savannah, is considered the richest neotropical Savannah (7) and the second largest Brazilian biome, with over 12.000 plant species, of which 36% are woody (8).

Although accounts for 30% of biodiversity in Brazil, Cerrado suffers from the high deforestation rates caused, mainly, by monoculture commodities and pasture (9). Such loss of biological diversity implies in the extinction of several species, some of them used as medicinal. The genus *Stryphnodendron* comprises 30 neotropical species (10) of which 21 are native to Brazil (11). One of them is *Stryphnodendron* adstringens (Mart.) Coville, an endemic species, known as "barbatimão," "barbatimão verdadeiro" and "casca-de-virgindade." The stem bark is traditionally used for wound healing, as an anti-ulcer, and to treat hemorrhoid problems and vaginal infections (12). *Bauhinia* is a pantropical genus, usually presenting bilobate leaves (13, 14) and 65 native species can be found in Brazil (11). Because of their leaf shape, these plants are known as "unha-de-vaca" or "pata-de-vaca" (cow paw), and several species have been used to "treat" diabetes (15). *Bauhinia rufa* (Bong.) Steud. is a Brazilian native species and Cerrado inhabitants use the leaves for decoction or to prepare "garrafadas" (medicinal plants extracted with wine or "cachaça"). Besides remedies to treat diabetes, *B. rufa* is traditionally used as anti-hyperlipidemic, anorectic, diuretic, anti-obesity treatments (16). Although *Bauhinia variegata* L. is not a native species, is widely employed in Brazil, for ornamental purposes. This species is also called as "pata-de-vaca" and used to "treat" diabetes. Considering it is important to know about biological and pharmacological properties of medicinal plants, this paper describes the thrombolytic activity evaluation of extracts from *Bauhinia rufa*, *B. variegata*, and *Stryphnodendron adstringens* collected from the Cerrado biome.

Methods

Plant material

Bauhinia rufa, *B. variegata*, and *Stryphnodendron adstringens* were collected in Brasilia, Brazil. Voucher specimens have been deposited in the Herbarium of Universidade de Brasilia (UB) under identification numbers Fagg, CW 2185, UB 165705 andUB 139571, respectively.

The plant material was dried for about 72 hours in an oven at 38-40 °C and ground into coarse powder by knife mill. The extracts were prepared as previously described (17, 18). Briefly, the dried and powdered plant material was macerated at room temperature for seven days (repeated three times) with hexane, followed by ethanol. After filtration, the solvents were removed under reduced pressure, at a temperature below 40 °C, furnishing crude hexane and ethanol extracts. The aqueous extract was obtained by infusion, using distilled water. After filtration, the obtained infusion was lyophilized resulting in the crude aqueous extract. All the extracts were preserved under low temperature (-30 °C) for the subsequent analysis.

In vitro thrombolytic activity

The plant extracts, solubilized in propylene glycol 10% v/v, were evaluated at concentration of 1,0 mg/mL. As a positive control, streptokinase 100.000 UI, solubilized in distilled water was used. Propylene glycol 10% was also tested as a negative control. The *in vitro* thrombolytic activity of the extracts was evaluated according to the methodology proposed by Prasad et al. (2006) (19), with modifications (5). Human venous blood (500 µL), freshly obtained from volunteers, was incubated at 37 °C for 45 minutes. After the incubation time, the remained fluid was removed without disrupting the clot, and each microcentrifuge tube was weighed to determine the clot weight. After the addition of 150 mL of the sample, the tubes were incubated at 37 °C for 90 minutes, and the fluid from clot lysis was carefully aspirated. Then, the clot was again weighed and the percentage of clot lysis was calculated by the difference in the clot mass before and after the addition of the sample. The assay was performed in triplicate. The Student's t-test with independent samples was applied to evaluate the presence of significant differences ($p < 0.05$) between the extract and the positive and negative controls. The statistical analyses were realized using the software GraphPad

Prism Version 6.0. The Ethics Committee from Federal University of Espírito Santo (UFES), Brazil, approved this project, at the under protocol number 148.873.

Results

The results are showed at Table 1.

As expected, the highest percentage of lysis was achieved by streptokinase, whose value was 50,36%. Among the evaluated extracts, four were able to promote more than 20 % of clot lysis: *S. adstringens* leaf aqueous extract (23.71%); *B. variegata* leaf hexane and ethanol extracts (23.11% and 29.72% respectively), and *B. rufa* leaf extract (26.17%).

Discussion

According to WHO, approximately 31.0 % of all deaths worldwide in 2012 were due to cardiovascular diseases. Among them, an estimated 7.4 million death were due to coronary heart disease and 6.7 million were due to stroke (20). In general, heart attacks and strokes are acute events, mainly caused by a blockage preventing blood from flowing to the heart or brain. An alternative for blood flow restoration is the use of thrombolytic drugs. However, the thrombolytic drugs arsenal is still limited when compared to other therapeutic classes, such as the antiplatelet agents used in cardiovascular diseases (21). Moreover, new thrombolytic agents are needed to expand the list of drugs in this pharmacological class.

The three studied plant species showed clots lysis ability from weak to moderate, in comparison with streptokinase and the vehicle (51.36% and 5.54%, respectively). Several researchers have described similar results with other Fabaceae plant species. The methanol extract from seeds of *Vigna unguicula*ta (2 mg/mL) showed little activity (12.01%) (22). The methanol leaf extract of *Bauhinia acuminata* (10 mg/mL) showed a percentage of clot lysis equal to 10.1%, close to the value found in water (7.6%) (23). Sohaily et al. (2016) evaluated the thrombolytic activity of methanol extract of *Albizia lebbeck* bark (10 mg/mL) and found a thrombolytic activity similar to streptokinase (24). Al-Mamun et al. (2012), studying the thrombolytic activity of nine medicinal and spicy plants (10 mg/mL), available in Bangladesh, including two Fabaceae species, found results ranging from 22.1% to 62.4% of clots lysis (25).

Polyphenols present inhibitory action towards platelet aggregation (26). It is interesting to observe that the ethanol extract of *S. adstringens* leaves showed thrombolytic activity, while none of the stem bark extracts from the same species presented significant activity (percentage ranging from 3.87% to 11.67%). Flavonoids, saponins, and coumarins can be found in leaves of *S. adstringens* (27) and these compounds can explain, at least in part, the obtained result for ethanol extract from leaves. On the other hand, the high content of proanthocyanidins in stem barks of *S. adstringens* is well known (28). The tannins are responsible for the wound healing activity of crude extracts from stem bark, due to the ability of vasoconstriction, and astringent activity, increasing the number of cross-link among collagen fibers (29). *Bauhinia variegata* is one of the most studied among the *Bauhinia* species. The chemical composition of leaves is fully established, and the crude extracts are rich in triterpenes and steroidal compounds (hexane extracts) and flavonoids (ethanol and, in a minor amount, aqueous extracts) (30). Moreover, sesquiterpenes, mainly germacrene D and g-elemene are the prevalent compounds in the essential oil from leaves of this species (31).

The presence of these class of compound can explain why both hexane and ethanol extracts from leaves of *B. variegata* showed significant thrombolytic activity (32). Although *Bauhinia rufa* is often used medicinally, there is a lack of studies concerning to the chemical composition of this species. Polyphenols and epicuticular wax were reported in leaves as well as an essential oil with a predominance of the terpenes viridiflorol and spathulenol (31, 33). Also, from seeds of this species was isolated a protease inhibitor (34). Only the hexane extract from leaves of *B. rufa* showed significant thrombolytic activity (29.72%). This unexpected result can be a stimulus to the chemical study of this extract, searching for the compound responsible for the biological action.

As far we know, this is the first study to assess the in vitro thrombolytic activity of *Bauhinia rufa*, *B. variegata*, and *Stryphnodendron adstringens.* The present results can contribute to the knowledge about the potential of these three medicinal species from the Fabaceae family.

Acknowledgments

Financial support from The National Council of Technological and Scientific Development (CNPQ), The Brazilian Innovation Agency (FINEP), Coordination for the Qualification of High-Level Personnel (CAPES), and Postgraduate Program in Pharmaceutical Science from Federal University of Espirito Santo (PPGCFAR -UFES).

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Table 1. The thrombolytic activity of *Stryphnodendron adstringens*, *Bauhinia rufa*, and *Bauhinia variegata* extracts. *p value when compared to vehicle (propylene glycol 10%). **p value when compared to streptokinase 100.000 UI. ND = Not determined

Samples	*Extracts*		*Clot lysis (%)*	*p* value*	*p* value**
	Used parts	*solvent*			
Stryphnodendron adstringens	leaves	hexane	9.22	> 0.05	< 0.05
		ethanol	15.43	< 0.05	< 0.05
		water	23.71	< 0.05	< 0.05
	stem barks	hexane	3.87	> 0.05	< 0.05
		ethanol	11.67	> 0.05	< 0.05
		water	5.29	> 0.05	< 0.05
Bauhinia rufa	leaves	hexane	26.17	< 0.05	< 0.05
		ethanol	12.92	> 0.05	< 0.05
		water	7.44	> 0.05	< 0.05
Bauhinia variegata	leaves	hexane	23.11	< 0.05	< 0.05
		ethanol	29,72	< 0.05	< 0.05
		water	ND		
Streptokinase (100.000 UI)			51.36		
Propyleneglycol 10%			5.54		