



Evaluation of Haemolytic Activity of Leaves from *Acacia podalyriifolia*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FGRP and FCFDS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author APB managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Acacia podalyriifolia A. Cunn ex G. Don (Fabaceae) is a plant used in south Brazil for ornamental purposes. The leaves are used in folk medicine for the treatment of bacterial infection, diabetes, gastrointestinal disturbances, in addition to fat reduction and as an antioxidant agent. Ethnopharmacological studies reported in the literature showed the presence of coumarin, tannin, cyanogenic glycosides, alkaloids, steroids, flavonoids and saponins. The evaluation of the haemolytic activity of dry crude butanolic extract from leaves of *A. podalyriifolia* was evaluated and it revealed a low haemolytic activity when compared with the extract of *Tribullus terrestris*, herbal reference product.

Keywords: *Acacia podalyriifolia*; haemolytic activity; saponins.

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1. INTRODUCTION

A. podalyriifolia is an exotic plant, originated from Australia, widely spread in south Brazil as an ornamental plant. The leaves consisting of reduced blade and broadened petiole, which performs the photosynthetic role [1,2,3,4,5,6].

Ethnobotanical studies of *Acacia* spp. have been applied in traditional medicine for the treatment of diabetes, gastrointestinal disturbances and inflammatory diseases [7,8,9,10]. Previous studies of extracts and fractions from flowers of *A. podalyriifolia* demonstrated antibacterial and antioxidant activities [11,12].

Several biocompounds as coumarins, tannins, glycosides, cyanogenic glycosides, alkaloids, steroids, flavonoids and saponins were identified in the genre *Acacia*. Phytochemical studies on the leaves and flowers ethanol extracts of *A. podalyriifolia* identified alkaloids and phenolic flavonoids substances [13,14,11,15].

Desert bushes of *Acacia* have triterpenoid saponins that protect the seeds from predators. These compounds formed by gallic acid combined with sugars and linear monoterpenes showed anticancer activity due to the ability to induce the inhibition of the cell cycle of mammals [16]. Saponins are a group of secondary metabolites, not volatiles, surfactants that are widely spread in plant and marine animal kingdoms [17]. The ability to form complexes with steroids, proteins and phospholipids of membrane associated to amphiphilic behavior of the saponins assigns a large number of biological properties for these substances. The ability to cause a disturbance of the membrane is the most studied effect of the saponins, this property is often associated with their ability to cause lysis of erythrocytes in mammals [18].

The presence of saponins in *Acacia* genus and the use of leaves of *A. podalyriifolia* for the treatment of various pathological conditions in traditional medicine driven to identification of phytochemical profile of their extracts and evaluation of its haemolytic capacity.

2. MATERIALS AND METHODS

2.1 Plant Material

Leaves of *A. podalyriifolia* were collected in the county of Lapa, in state of Paraná, Brazil. The

samples were identified through morphological comparison with existing exsiccate in the Herbarium of the Botanical Garden of the Federal University of Rio de Janeiro. The number of voucher specimen is RB00655681.

2.2 Preparation of Extracts

The methanol extract was prepared from 14.20 g of natural leaves of *A. podalyriifolia*. The crushed leaves samples were packed on glass container and macerated with 500 mL of MeOH for 10 days. After maceration, filtered and the filtrated was concentrated in rotatory evaporator on reduced pressure, providing 2.54 g dry crude methanol extract (DCME) with 17.89% of yield. The DCME was resuspended in 30 mL of methanol to provide crude methanol extract (CME) with concentration of 84.67 mg/mL. 15 mL of CME was partitioned with 150 mL of a solution 1:1 (H₂O/BuOH). The aqueous phase was discarded and the butanolic phase was concentrated in rotatory evaporator with elevated pressure on 120°C providing 395 mg of dry crude butanol extract (DCBE) with 2.78% of income. 12.624 mg of DCBE and extract of *Tribullus terrestres* (Androsten® – Herbarium – 92 mg of extract of *Tribullus terrestres* containing 37,6 mg of Protodioscin) were added 1.2 mL of isotonic solution of NaCl 0.9% to obtain a solution with final concentration in each vial of 10.52 mg/mL of liquid crude butanol extract (LCBE). Both solutions will be used on hemolysis test.

2.3 Haemolytic Activity

Defibrinated lamb blood (Laborclin 0.6 mL-0.5%) was mixed with 0.6 mL of saline solution containing 50; 25; 13.25; 6.63; 3.31% of solutions from LCBE of *A. podalyriifolia* (10.52 mg/mL) and from reference drug *T. terrestris* (10.52 mg/mL). The mixtures were incubated for 30 min at 37°C and centrifuged at 3000rpm for 10 min. The free hemoglobin in the supernatant was measured by absorbance at 540 nm. Saline solution and distilled water were included as low and high haemolytic controls, respectively. The haemolytic percentages developed by saline solution were deducted from all groups. The concentration of *A. podalyriifolia* that induced 50% of maximal hemolysis was considered as average haemolytic dose (HD50, graphical interpolation). Each experiment was performed in triplicate for each concentration [19].

2.4 Statistical Analysis

The data obtained were expressed as mean \pm SEM and analyzed using one way ANOVA followed by Student's t-test. P values less than 0.05 ($P < 0.05$) were considered to be statistically significant.

3. RESULTS AND DISCUSSION

Plants got responsible active principles due therapeutics properties assigned to them, but also, for adverse reactions that can appear due to improper use or direct contact with it. Saponins are a group of natural compounds that show elevated toxicity because of your ability to produce hemolysis. This effect is resulted from your capacity to interact with the components of erythrocyte's cellular membrane, mainly with cholesterol's molecule, inducing a deformation of membrane as consequent overflow of intracellular content. The saponin's haemolytic activity is part of the protection system of the vegetable against predator's attack (insects, viruses, fungus and bacteria). Antimicrobial actions assigned to various plants, most of the time, are related to the presence of such compounds. The *in vitro* hemolysis test has been used routinely in toxicity studies of medicinal plants and about interest livestock being positive, specially, to species that show saponins in your constitution.

The methanolic extract of *A. podalyriifolia* was suspended on H₂O and partitioned with *n*-BuOH to concentrate saponins. Although it has sufficient data on literature that report the presence of saponins in these extracts, the

confirmation of presence in these metabolites was made by foam and Liebermann-Burchard tests.

The evaluation of capacity to cause lysis of erythrocytes from extracts of *A. podalyriifolia* was evaluated in comparison with the herbal medicine Androsten®. The drug reference shows 37,6 mg of protodioscin in each 92 mg of extract from *T. terrestres*, with the protodioscin as steroidal saponin. The haemolytic concentration (HD₅₀ in $\mu\text{g/mL}$) of extract from *A. podalyriifolia* and of drug reference capable of inducing 50% of max hemolysis was considered as medium haemolytic dose and obtained by graphical interpolation (Fig. 1).

The dry crude extract butanolic (DCEB) of *A. podalyriifolia* is 600 times less haemolytic than the extract *T. terrestris*. Therefore, can considerate that the saponin present on extract of *T. terrestris*, protodioscin, is a steroidal saponin and because this, is expected a high percentage of hemolysis due to a major interaction with membrane's cholesterol. However, literature data shows triterpenoid saponins identification on extracts of *A. podalyriifolia*, which proves, in a certain way, with a low haemolytic percentage observed in this study [18,19]. The evaluation separated from dilution of the extracts (Fig. 2) of *A. podalyriifolia* and *T. terrestres* permits to compare in each dilution your haemolytic response. The extract of *T. terrestres* presents low variation of haemolytic percentage with the reduce of concentration, however, the haemolytic activity of *A. podalyriifolia* falls abruptly with the reduce of concentration.

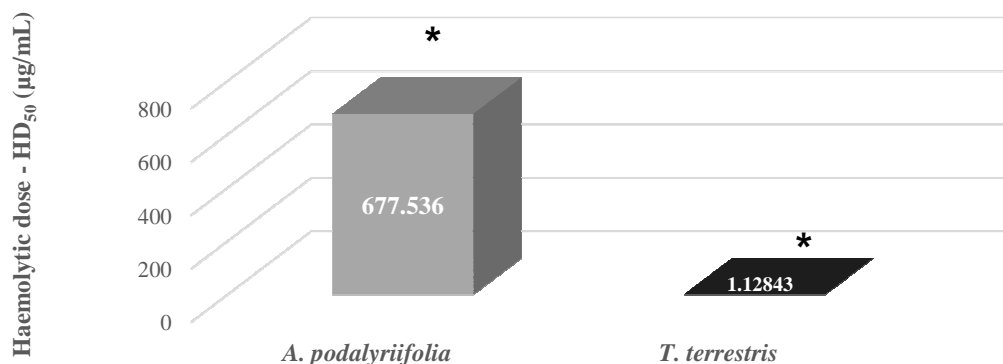


Fig. 1. Haemolytic concentration (HD₅₀) in $\mu\text{g/mL}$, the extracts of *A. podalyriifolia* and *T. terrestris*

Results are mean \pm SEM (T). * $p < 0.05$ ANOVA followed by student's T test ($n = 15$)

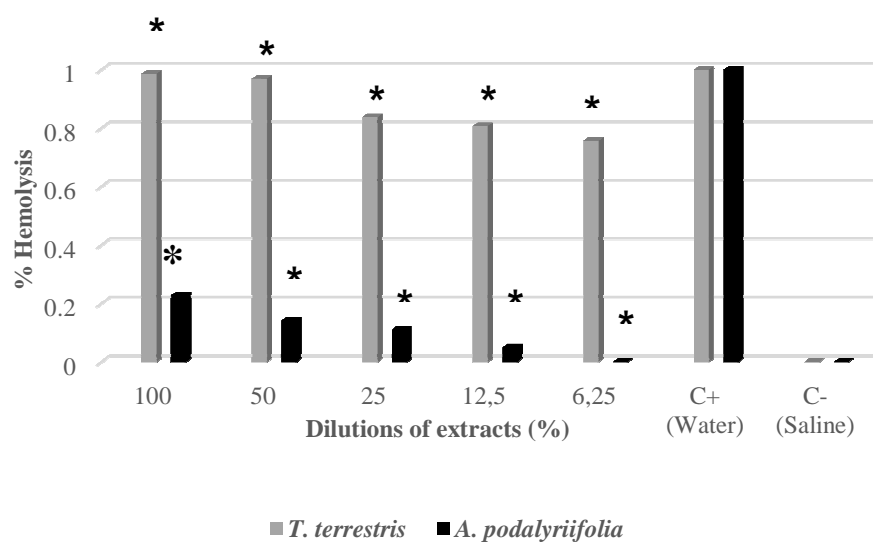


Fig. 2. Haemolytic activity, in $\mu\text{g/mL}$, of the dilutions of the *A. podalyriifolia* and *T. terrestris* extracts. The haemolytic concentration (HD_{50} , in $\mu\text{g/mL}$) of extract from *A. podalyriifolia* and of drug reference capable of inducing 50% of max hemolysis was considered as medium haemolytic dose and obtained by graphical interpolation. The experiments were performed in triplicate for each concentration

Results are mean \pm SEM (T); * $p < 0.05$ ANOVA followed by Student's T test ($n = 15$)

4. CONCLUSION

Hemolysis tests performed with dry crude extract butanolic (DCEB) of leaves from *A. podalyriifolia* showed a low percentage haemolytic when compared to extract of *T. terrestris*, herbal medicine used as reference. It is believed that the low haemolytic response observed is related to the fact of saponins present in *A. podalyriifolia* have triterpenoid skeleton. Therefore, in general, the low haemolytic activity of these extracts reflect in a low toxicity, which somehow, leads the isolation, the purification, the elucidation and pharmacological evaluation of saponins present in this extract, since there is no data in the literature that report the isolation and purification of bioactive compounds of this kind for the specie under study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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