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Evaluation of Phytochemical Profile and Haemolytic Activity of *Acacia mearnsii*

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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 ADPB managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Acacia mearnsii is characterized as a tree with dark green leaves and grow on any types of soil. Its bipinnate leaves with individual leaflets that are used in folk medicine in wound healing, antioxidant, anti-inflammatory and antiviral. Phytochemical tests demonstrated the presence of saponins, flavonoids and tannins. The haemolytic activity evaluation of dry crude buthanolic leaves extract of *A. mearnsii* confirming the presence of saponins as a chemical marker of this species presenting a low haemolytic content in comparison with *Tribullus terrestres*, herbal reference product.

Keywords: Acacia mearnsii; haemolytic activity; saponins.

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1. INTRODUCTION

Acacia mearnsii is a native species of Australia, popularly known like dark-acacia, introduced in south of Brazil. Since then, it was largely used on reforestation, due to its great commercial and economic significance [1]. The main attribute of this species is the capacity to fix nitrogen on soil and organic material incorporation, providing protection and productive maintenance [2].

This plant is a tree with dark green leaves of about 10 to 30 meters of height and grow on any types of soil. Its leaves are composed, bipinnate, with individual leaflets shorter in relation to its width [3]. The wood is used for manufacturing of cellulose, agglomerate and energy achievement of charcoal production, from its bark is extracted the tannin, employed on pharmaceutical and leather industries, applied like a dye, corrosion inhibitor, facilitator of liquid flow on pipes and like a flocculant agent in water reservoirs.

Tannins are group of polyphenolic chemical substances found in plant cells, it can be divided in two groups, water soluble and condensed, that composed of flavonoid units, which is the most common group found in dark-acacia extract [4,5,6]. Species rich in tannins have been used for diarrhea, hypertension, wounds, burns, kidney, gastric and inflammatory problems [7].

The antioxidant activity attributed to the flavonoids and tannins were reported to helped in healing processes, as long as the free radicals are an important factor on formation of ulcerative and erosive lesions on gastrointestinal tract [8] [9]. Flavonoids are a group of polyphenolic substances, largely distributed on plant kingdom with diversified biological activity [10].

In addition to the phenolic compounds, terpenes also group of secondary metabolites that have important pharmacological activity. Studies revealed the presence of the triterpenoid saponins in species of *Acacia* genus [11]. Saponins are glycosides from steroids or from polycyclic terpenes, it has lipophilic and hydrophilic characteristics that determines the property to reduce the superficial tension of water and its detergent actions.

Pharmaceutical interest in saponins is due to their used in formulation adjuvant, active component in herbal drugs, material for steroids synthesis and primarily haemolytic activity was due to the ability of glycoside to combine with cholesterol molecules present on the membrane of the erythrocyte, disturbing the intern-extern balance and promoting a cell rupture, with consequent hemoglobin released [12]. Some saponins in species of the gender *Acacia* demonstrated anti-inflammatory, antimicrobial, antiviral and antitumor properties [13,14].

The presence of secondary metabolites found on barks of *Acacia mearnsii* with countless pharmacological activities reported in literature and the lack of information about presence of saponins stimulated the phytochemical evaluation of its leaves extract and also to test its haemolytic capacity.

2. MATERIALS AND METHODS

2.1 Plant Material

Leaves of *Acacia mearnsii* were collected in the county of Lapa, in the State of Paraná, Brazil. Species identification was carried out at the Botanical Garden of National Museum of Federal University of Rio de Janeiro. A voucher specimen is deposited in the Botanical Garden of National Museum of Federal University of Rio de Janeiro, under the registration number R 223 878.

2.2 Preparation of the Extracts

The methanol extract was prepared from 579.8 g of natural leaves of *Acacia mearnsii*. The vegetable samples were packed in a glass container and macerated with 1765 mL of MeOH for 10 days. After maceration, the filtered fluid was concentrated in a rotary evaporator under reduced pressure, providing 131.9 g of dry crude methanol extract (DCME) with 22.75% of yield. The DCME was resuspended in 100 ml of methanol to provide the raw methanol extract (RME) with concentration of 1.319 mg/mL. 50 mL of RME was partitioned with 275 mL of a solution (H2O/BuOH 50:50). The aqueous phase was discarded and the (buthanolic or buthanolic please recheck) phase was concentrated in the rotary evaporator with elevated pressure on 100ºC providing 2.759 mg of dry crude buthanolic extract (DCBE) with 0.00047% of income. In 12.624 mg of DCBE and *Tribullus terrestres* extract (Androsten ® – Herbarium – 92 mg extract *Tribullus terrestres* containing 31.592 mg of protodioscin) were added 1.2 mL of isotonic solution of NaCl 0.9% in order to obtain a solution with a final concentration in each bottle of 10.52 mg/mL. Both solutions are subsequently used in the haemolysis assay.

2.3 Phytochemical Assays

For evaluation of different classes of secondary metabolites, precipitation reaction, color test and foam tests were undertaken by using fresh leaves and (RME) of *A. mearnsii*.

The saponins test was prepared with decoct of leaves in neutral pH, this solution was vertically stirred verifying if there was formation of persistent foam for 15 minutes in the presence of HCl. For tannins observed formation of precipitate upon addition of gelatin solution 2% in 1 mL of (RME). The presence of flavonoids proceeded (Shinoda´s or Shinoda recheck) reaction in an acid medium, adding 1 mL of RME and magnesium metal.

2.4 Haemolytic Assays

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 and 3.31% of solutions from DCBE of *A. mearnsii* (10.52 mg/mL) and from reference drug *T. terrestres* (10.52 mg/mL). The mixtures were incubated for 30 min at 37ºC and centrifuged at 3000 rpm for 10 min. The free hemoglobin in the supernatant was measured at 540 nm absorbance. Saline solution and distilled water were included as high and low haemolytic controls, respectively. The haemolytic percentages developed by saline solution were deducted from all groups. The concentration of *A. mearnsii* that induced 50% of maximal haemolysis was considered as a low haemolytic dose (HD50, graphical interpolation). Each experiment was performed in triplicate for each concentration [15].

2.5 Data Analysis

The data obtained were expressed by means of various statistical packages to indicate mean ± 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

The secondary metabolites for being factors of interaction between organisms, it often shows remarkable biological activities, many are commercially useful in pharmaceutical, food, agronomic and perfumes industries. The pharmaceutically greater interest is mainly the number of pharmacologically valuable substances. In this way the interest in knowing the main active principles of plants with potential use in the cure and treatment of various ailments has grown.

The phytochemical research aimed to know the chemical constituents of the plant species or evaluate their presence in them, when it does not have chemical studies on the species of interest. The phytochemical analysis can identify the relevant secondary metabolites groups.

The (RME) of the leaves of *A. mearnsii* indicated a positive result for the presence of tannins by precipitate formation, flavonoids by red color formation and saponins by foaming after vigorous stirring. These substances in the plant kingdom has a defensive function against predators or attracting pollinators, but also have other biological activities [16].

The tannins prevent peroxidation of lipids and degradation of nucleotides; also accelerate the wound healing process. Flavonoids have antioxidant, anti-inflammatory and anticancer properties [7,10]. Saponins have been associated with anti-inflammatory, antiviral, antimicrobial, antifungal and haemolytic activity due to the ability to interact with the steroid saponins membrane [15].

The haemolytic evaluation of (RME) of the leaves of *A. mearnsii* was performed in comparison with the herbal medicine which has Androsten ® presenting 31.592 mg of protodioscin, which is a steroidal saponin in each 94 mg of *Tribullus terrestres* extract. There are strong indications that the haemolytic activity of saponins is related to the type of aglycone present in its structure. The steroidal saponins have a higher haemolytic activity when compared to triterpenoidal saponins [17]. The haemolytic concentration (CH50, in ug/ml) of *A. mearnsii* extract and that of reference drug capable of inducing 50% of maximum hemolysis was considered low haemolytic dose and obtained by graphical interpolation (Fig. 1).

The dry crude buthanol extract (DCBE) of *A. mearnsii* is 5000 times less haemolytic than extract of *T. terrestres*. Protodioscin present in extract of *T. terrestres* is a steroidal saponin and therefore have higher haemolytic capacity when compared to the triterpenoid, the high haemolytic capacity of steroidal saponins related to its ability to interact with more intensity with the cholesterol of cellular membranes [18]. The individual

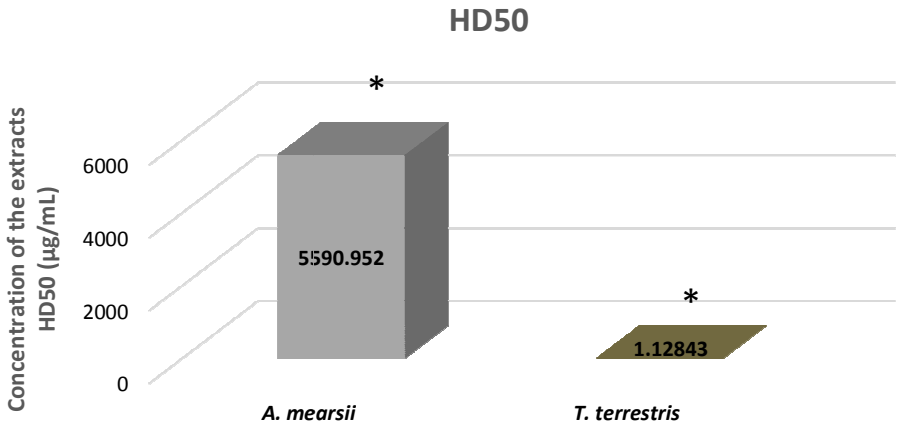

Fig. 1. Haemolytic concentration (HD50), in µg/mL, the extracts of *A. mearnsii* and *T. terrestres*
*Results as Mean ± SEM. *p<0,05 ANOVA, followed by Student's T test (n = 15)*

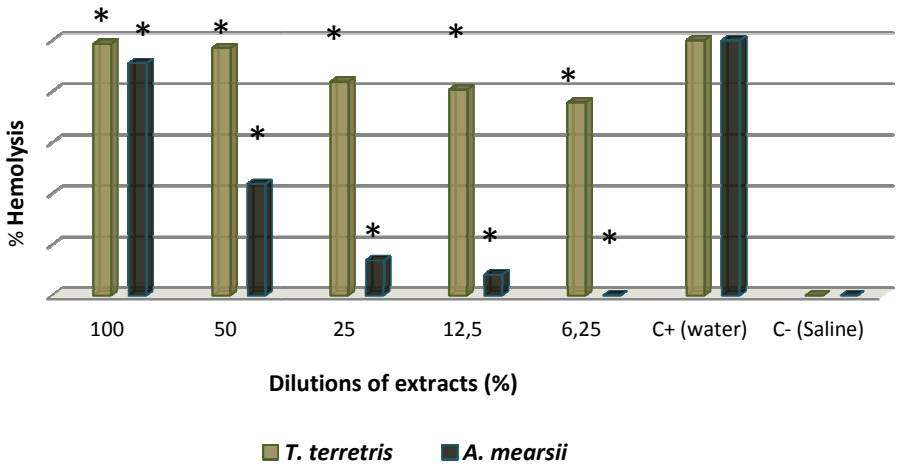

Fig. 2. Hemolytic activity in ug/mL, the dilutions of the extracts of *A. mearnsii* and T. land. The concentration hemolytic (CH50) capable of inducing 50% of maximum hemolysis was considered low hemolytic dose is obtained by interpolation. The experiments were performed in triplicate for each concentration
*Mean ± SEM. * p <0.05 ANOVA followed by Student's T test (n=15)*

assessment of dilutions of the extracts (above Fig. 2) of *A. mearnsii* and *T. terrestres* allows comparing in each dilution its haemolytic response. Dilutions of extract of *T. terrestres* have a high haemolytic percentage. Extract of *T. terrestres* shows low variation of haemolytic percentage with the decreasing of concentration, but the haemolytic activity of the extract of *A. mearnsii* shows a drop in its haemolytic percentage, as long as it reduces its concentration. The low haemolytic activity found in extract of *A. mearnsii* may be related to its triterpenoid aglycone of its saponins, so that, their extracts show low toxicity, which propels the isolation, elucidation and pharmacological evaluation of the saponins found on extracts of *A. mearnsii*, as long as there was no data found on literature on the isolation and purification of the active constituents of this species.

4. CONCLUSION

The leaves of *A. mearnsii* indicated the presence of tannins, flavonoids and saponins. The last one has been associated to the ability to interact with the steroid membrane and cause rupture of erytrocytes. The haemolytic evaluation of the leaves of *A. mearnsi* was performed in

comparison with the herbal medicine, *Tribullus terrestres*, which has Androsten® as a steroidal saponin. There are strong indications that the haemolytic activity of saponins is related to the type of aglycone present in its structure. The steroidal saponins have a higher haemolytic activity when compared to triterpenoidal saponins. The haemolytic concentration (CH50, in ug/ml) of *A. mearnsii* extract was bigger than herbal reference drug, what suggest a low haemolytic activity.

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.

REFERENCES

1. Schneider PR, Camillo SB de A, Finger CAG, Frizzo SMB. Determinação de equações da produção de tanino de acácia negra, *Acacia mearnsii* De Wild. Ci. Fl. 1999;9(1):103-113.
2. Schumacher MV, Brun EJ, Rodrigues LM, Santos EM. Retorno de nutrientes via deposição de serrapilheira em um povoamento de acácia-negra (*Acacia mearnsii* de Wild.) no estado do Rio Grande do Sul. Rev. Árv. 2003;27(6):791-798.
3. Sherry SP. The black wattle (*Acacia mearnsii*). 1971;402.
4. Peters DJ, Constabel CP. Molecular analysis of herbivore-induced condensed tannin synthesis: Cloning and expression of dihydroflavonol reductase from trembling aspen (*Populus tremuloides*). Plant. J. 2002;32(5):701-712.
5. Tanner GJ, Francki KT, Abrahams S, Watson JM, Larkim PJ, Ashton AR. Proanthocyanidin biosynthesis in plants. Purification of legume leucoanthocyanidin reductase and molecular cloning of its cDNA. J. Biol. Chem. 2003;278(34): 31647-31656.
6. Kitamura S, Shikazono N, Tanaka A. Transparent Testa 19 is involved in the accumulation of both anthocyanins and proantocyanidins in arabidopsis. Plant J. 2004;37(1):104-114.
7. Haslam E. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. J Nat Prod. 1996;59(2):205-15.
8. Borreli F, Izzo AA. The plant kingdom as a source of anti-ulcer remedies. Phytother Res. 2000;14(8):1099-1573.
9. Carbonezi CA, Hamerski L, Gunatilaka AAL, Cavalheiro A, Castro-Gamboa I, Silva DHS, Furlan M, You MCM, Lopes MN, Bolzani VS. Bioactive flavone dimers from *Ouratea multiflora* (Ochnaceae). Rev. Bras. Farmacogn. 2007;17(3):319-324.
10. Coutinho MAS, Muzitano MF, Costa SS. Flavonoides: Potenciais agentes terapêuticos para o processo inflamatório. Ver. Virt. Quim. 2009;1(3):241-256.
11. Felipe MR, Pozuelo MJ. Las leguminosas: Su valor nutritivo, medicinal y ecológico. Schironia: Rev. Cient. Coleg. Oficial Farmac. Madrid. 2006;5:50-57.
12. Paiva ARQ, Ribeiro KL, Gadelha TS, Gadelha CAA. Análise qualitativa da presença de saponinas e sua atividade hemolítica verificada nas amostras de *Acacia bonariensis e Panax ginseng.* XI Encontro de Iniciação a Docência. João Pessoa – PB, Brasil; 2007.
13. ElSohly HN, Danner S, Li XC, Nimrod AC, Clark AM. New antimycobacterial saponin from *Colubrina retusa*. J. Nat. Prod. 1999;62(9):1341-1342.
14. Xu QM, Shu Z, He WJ, Chen LY, Yang SL, Yang G, Liu YL, Li XR. Antitumor activity of *Pulsatilla chinensis* (Bunge) Regel saponins in human liver tumor 7402 cells *in vitro* and *in vivo*. Phytom. 2012;19(3-4): 293-300.
15. Santos WR, Bernardo RR, Peçanha LMT, Palatnik M, Parente JP, De Souza CBP. Haemolytic activities of plant saponins and adjuvants. Effect of *Periandra mediterranea* saponin on the humoral response to the FML antigen of Leishmania donovani. Vaccine. 1997; 15(9):1024-1029.

16. Costa JGM, Costa GM, Rodrigues FFG, Angélico EC, Silva MR, Mota ML, Santos NKA, Cardoso ALH, Lemos TLG. Estudo químico-biológico dos óleos essenciais de *Hyptis martiusii*, *Lippia sidoides* e *Syzigium aromaticum* frente a larvas do *Aedes aegypti*. Rev. Bras. Farmacog. 2005; 15(4):304-309.
17. Takechi M, Tanaka Y. Haemolytic time course differences between steroid and triterpenoid saponins. Plant Med. 1995; 61(1):76–77.
18. Glauert AM, Dingle JT, Lucy JA. Action of saponin on biological cell membranes. Nature. 1962;196:952-955.

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