



American Journal of Histology and Cytology (ISSN:2637-5117)



EVALUATION OF HEMOLYTIC ACTIVITY OF YANGAMBIN ISOLATED FROM *Ocotea duckei* VATTIMO-GIL

Maria Isabel de Assis Lima Castro^{1*}, Marta Gerusa Soares de Lucena^{1,2}, Camilla Vila Nova Soares Silva³, Eliete Cavalcanti da Silva^{1,2}

¹Postgraduate Program in Morphotechnology - Federal University of Pernambuco, ²Department of Histology and Embryology - Federal University of Pernambuco, ³Multiprofessional Residency Program in Health Surveillance - Recife Health Department

ABSTRACT

Yangambin, a lignan isolated from the leaves of *Ocotea duckei* Vattimo-Gil, has several pharmacological activities described in literature. However, few information about its toxicity has been reported. Red cells represent about 90% of blood cells and have interesting structural and molecular characteristics as experimental models in toxicological studies. Thus, the aim of this study was to evaluate the hemolytic action of yangambin in sheep blood. The hemolysis assay was performed at concentrations of 50, 25 and 12.5 µg/mL of yangambin in triplicate and hemolysis percentage was defined through the absorbance resulting from the test concentrations compared to the positive control. The results showed that yangambin did not cause hemolysis at the concentrations tested, therefore it did not cause damage to the plasma membrane of sheep erythrocytes.

Keywords: Hemolytic activity, cytotoxicity, Yangambin.

*Correspondence to Author:

Maria Isabel de Assis Lima Castro
Postgraduate Program in Morphotechnology - Federal University of Pernambuco,

How to cite this article:

Maria Isabel de Assis Lima Castro, Marta Gerusa Soares de Lucena, Camilla Vila Nova Soares Silva³, Eliete Cavalcanti da Silva. EVALUATION OF HEMOLYTIC ACTIVITY OF YANGAMBIN ISOLATED FROM *Ocotea duckei* VATTIMO-GIL. American Journal of Histology and Cytology, 2021, 4:9.

 **eSciPub**
eSciPub LLC, Houston, TX USA.
Website: <https://escipub.com/>

INTRODUCTION

Yangambin is a lignan isolated from the leaves of *Ocotea duckei* Vattimo-Gil. Several pharmacological activities have been reported in the literature, including: leishmanicidal, anxiolytic, anti-tumor and hypotensive activity. Therefore, cytotoxic evaluation is necessary to ensure the therapeutic applicability of this future phytotherapeutic^[1].

In vitro studies are important for understanding the mechanisms of interaction with living organisms and are increasingly being proposed to replace *in vivo* toxicity tests. These tests are the first step in evaluating the viability of using different materials for health, and these studies are generally carried out in cell culture (cytotoxicity)^[2].

Several types of cells are used in cytotoxicity tests and are selected according to the possible applications of the material under study. Among these cells, blood cells can be used for materials that will enter the bloodstream and have systemic distribution, constituting as hemocompatibility tests^[3].

Red cells represent about 90% of blood cells and have interesting structural and molecular characteristics as experimental models in toxicological studies. A complex structure, with a large amount of lipids and proteins on their surface with a membrane that consists of a phospholipid bilayer (intra/extracellular barriers), which represents 50% of its mass, are responsible for phenomena such as communication between cells, immunological recognition and cell adhesion^[4].

The rupture of the membrane and release of hemoglobin present in red blood cells is called hemolysis. When hemoglobin is free in the plasma it can cause damage to vital organs such as liver, kidneys and heart, being important as a form of evaluation in cytotoxicity tests^[5]. Several methods are available to assess hemolytic activity, among them the observation of ultraviolet absorbance, a technique

recommended by the Brazilian Health Regulatory Agency^[6].

Thus, knowing the pharmacological potential of yangambin, and being of fundamental importance to evaluate the toxicity of compounds with active principles, this work aimed to evaluate the cytotoxicity of yangambin through the *in vitro* hemolysis assay in erythrocytes.

METHODS

Extraction and isolation of yangambin

Yangambin was isolated from the leaves of *O. duckei* Vattimo-Gil (Lauraceae) according to the method described by Barbosa-Filho et al^[7]. Botanical material was collected in the municipality of Santa Rita, Paraíba State, Brazil (voucher Agra4309). Fifteen kilograms of the plant material were used. After drying and ball-milling, the powder was extracted with ethanol and concentrated in a rotary evaporator, yielding 6.5% crude ethanol extract in relation to the dry plant powder. For the isolation of lignoids, 300 g of ethanol extract was suspended in 10% acetic acid and then filtered, obtaining an insoluble residue and the acidic aqueous solution, which was extracted in a separator funnel with two liters of dichloromethane. The dichloromethane phase was filtered dry with anhydrous sodium sulfate and concentrated under reduced pressure, yielding the total lignoid fraction, which was separated on a silica gel 60 column (Merck-0.063-0.200 mm) and eluted with hexane, chloroform, and methanol, pure or in binary mixtures, using an increasing polarity gradient. Fractions eluted with MeOH-CHCl₃ (5-95) yielded pure yangambin. The identification of yangambin was performed by ¹H and ¹³C NMR spectral data analysis.

Hemolytic activity test

The hemolytic test was performed following the methodology described by Dacie and Lewis (1975)^[8], with modifications. Commercial sheep blood samples were purchased from Laborclin® (25 µL). Three concentrations of yangambin were analyzed: 12.5 µg/mL, 25 µg/mL and 50

$\mu\text{g/mL}$, diluted in 0.9% NaCl saline solution. A tube containing only saline was designated for the negative control and distilled water was used for the positive control. Thereafter, each tube received 25 μL of sheep blood and all samples were incubated for 30 minutes. After, the samples were centrifuged at 3500 rpm for 15 minutes. The supernatant was then analyzed in the Bioplus (BIO-200) spectrophotometer at 540nm, to obtain the resulting absorbance. The assay was performed in triplicate and the hemolysis percentage was defined with the result of the positive control being designated as 100%.

RESULTS

Yangambin did not show hemolytic activity since no hemolysis formation was observed in any of the concentrations tested (12.5, 25 and 50 $\mu\text{g/mL}$), with the saline solution remaining clear after centrifugation, that is, the red blood cells remained intact at the bottom of the tubes, with the formation of a precipitate, without cell lysis (Figure 1). Consequently, there was no statistical difference in the percentage of absorbance of the solutions with yangambin when compared to the negative control, as shown in figure 2.

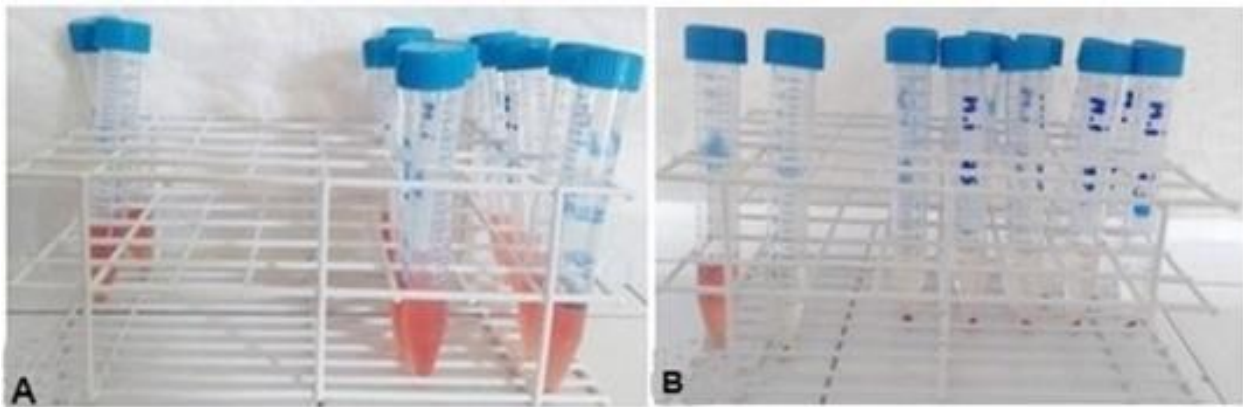


Figure 1. Hemolytic activity of yangambin at different concentrations. Solutions before (A) and after centrifugation (B)

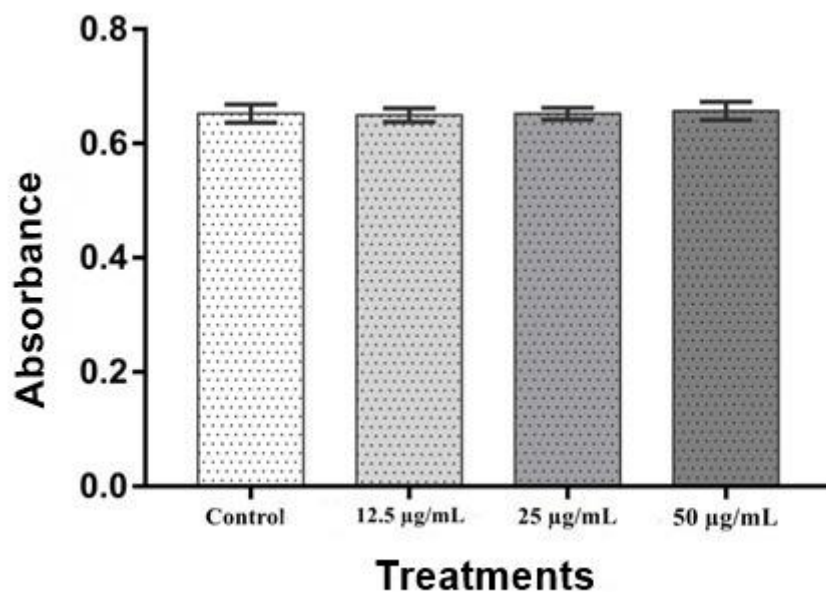


Figure 2. Mean absorbance of different concentrations of yangambin and negative control. There was no significant difference at 5% probability by the Tukey test

DISCUSSION

The hemolytic action of different toxic compounds is attributed to several non-specific mechanisms. For example, surfactant compounds, which produce their hemolytic effect through the solubilization of the erythrocyte plasma membrane, or by osmotic lysis, which promotes changes in the permeability of the red blood cell membrane^[9]. The cytotoxicity assay in sheep erythrocytes did not show hemolytic activity of yangambin, since the results found showed that there was no lysis of the erythrocyte membrane. Similar results were found with methanolic extract of Cinnamon (*Cinnamomum tamala*), genus belonging to Lauraceae^[10].

CONCLUSION

Yangambin did not show *in vitro* hemolytic activity at the concentrations tested. Thus, it seems to not damage the erythrocyte membrane.

REFERENCES

- [1]. Monte-Neto RL, Sousa LMA, Dias CS, Barbosa-Filho JM, Oliveira MR, Figueiredo RCBQ. Morphological and physiological changes in leishmania promastigotes induced by yangambin, a lignan obtained from *Ocotea duckei*. *Exp Parasitol*, 127 (2011) 215.
- [2]. Algharably EAH, Kreutz R, Gundert-Remy U. Importance of in vitro conditions for modeling the in vivo dose in humans by in vitro–in vivo extrapolation (IVIVE). *Archives of Toxicology*, 1:1-7, 2019.
- [3]. International Organization for Standardization (ISO). ISO 10993-5 Biological evaluation of medical devices – Tests for in vitro cytotoxicity. Switzerland; 2010.
- [4]. Sarkar S, Bose D, Giri RP, Mukhopadhyay MK, Chakrabarti A. Status of Membrane Asymmetry in Erythrocytes: Role of Spectrin. *Biochemical and Biophysical Roles of Cell Surface Molecules*, 3–11, 2018.
- [5]. Flood SL, Burkholder JM. *Chattonella subsalsa* (Raphidophyceae) growth and hemolytic activity in response to agriculturally-derived estuarine contaminants. *Harmful Algae*, V. 76: 66-79, 2018.
- [6]. Souza TC, Guedes AS, Santos LCS. Estudo fotoquímico e avaliação *in vitro* da atividade hemolítica de extratos aquosos do *Illicium verum* utilizado para o tratamento dos sintomas

- clínicos da dengue. *Revista Diálogos & Ciência (D&C)*, v. 2, n. 40 (17), p.141-157, 2017.
- [7]. Barbosa-Filho JM, Vargas MRW, Silva IG, França IS, Moraes LCSL, Cunha EVL, Silva MS, Souza MFV, Chaves MCO, Almeida RN, Agra MF. *Ocotea duckei*: Exceptional source of yangambin and other furofuran lignans. *Anais da Academia Brasileira de Ciencias*, 71: 231-238, 1999
- [8]. Dacie JV; Lewis SM. *Practical Haematology*. 5th edition. Churchill Livingstone: Edinburgh, London, New York, 1975.
- [9]. Aparicio RM, García-Celma MJ, Vinardell MP, Mitjans M. 2005. In vitro studies of the hemolytic activity of microemulsions in human erythrocytes. *Journal of Pharmaceutical and Biomedical Analysis*. 39: 1063-1067.
- [10]. Dandapat S, Kumar AMP; Sinha MP. Therapeutic efficacy of *Cinnamomum tamala* (Buch.- Ham.) and *Aegle marmelos* (L.) leaf. *Balneo Res J*, 5 (2014) 113.

