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Cytotoxic effect of various solvent extracts of *Acacia nilotica* pods on human erythrocyte cells

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ABSTRACT

Acacia nilotica is a multipurpose medicinal plant. Different parts of this plant are used to alleviate or treat various diseases. In the current study, an attempt was made to study the cytotoxicity of *Acacia nilotica* pod extracts towards normal human erythrocytes. The pods of *A. nilotica* were ground, then three different solvents were used in different ratios to prepare crude extracts, which were screened for *in vitro* hemolytic activity. UV-visible spectrophotometric method was used for the quantification of the hemolytic effect. The hemolysis percentages of the different extracts were found to be very minimal (Methanol extract: 4.65±0.24%; Ethanol extract: 3.77±0.48% and Aqueous extract: 2.69±0.51%), while Triton X-100 produced total hemolysis at the lowest concentration. These findings suggested that this plant could be considered as safe to human erythrocytes. However, further studies through *in vivo* toxicological tests should be considered in the future in order to best justify its safety.

Keywords: *Acacia nilotica*, Erythrocytes, Hemolytic activity, Safety.

INTRODUCTION

Medicinal plants constitute a rich source of various secondary metabolites with possible health benefits (Dai and Russell, 2010; Moutari et al., 2018; Ramatu et al., 2022). Plant remedies have been widely used for the alleviation or treatment of various human diseases (Amonkan et al., 2010; Boye et al., 2014). *Acacia nilotica*, commonly known as 'Bagaroua' in Hausa , a principal local language in Niger republic, is an economically and medicinally important plant. It is especially found in clay-sandy sites flooded by ponds (Raphael et al., 2012). The leaves, flowers, stembark, root, and pods of *Acacia nilotica* have been prescribed by traditional healers for ages for the treatment of many infectious diseases. The plant is the best source of bioactive substances such as gallic acid, ellagic acid, isoquercetin, leucocyanidin, kaempferol-7-diglucoside, naringenin-7-O-β-D-(60- O-galloyl) glucopyranoside, rutin, apigenin-6,8-bisC-glucopyranoside, m-catechol and their derivatives, as well as galloylated derivatives of (+)-catechin and (+)-gallocatechin (Malan, 1991; Singh et al., 2008; Maldini et al., 2011). Particularly, in our previous research work, we found the pods of the plant to be very rich in tannins and saponins (Manzo et al., 2019). Since the pods of *Acacia nilotica* are under

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intensive use by the community, the present study aimed to investigate the cytotoxicity effect of various organic extracts of the pods. This research would draw attention to the rational use of the plant as folk medicine as well as the understanding of the bioactive compounds in *Acacia nilotica* pods.

MATERIALS AND METHODS

Plant sample

A properly packed and well conserved powder of *Acacia nilotica* pods was purchased from the Laboratory of Natural Substances and Organic Synthesis, Department of Chemistry, University Abdou Moumouni (UAM), Niamey, Niger Republic. Procedures for the pod collection and its further laboratory authentication were highlighted in our previous published research paper (Manzo et al., 2017).

Preparation of plant extract

Twenty grams (20 g) of air-dried powder of *Acacia nilotica* pods was taken in 100 ml of each solvent (methanol, ethanol, water) in a conical flask, left for 24 hours on a rotary shaker. After filtration with a cotton wool, the extracts were evaporated using a rotary evaporator.

Preparation of erythrocytes suspension

The preparation of erythrocytes suspension was performed as described by Lazcano-Pérez et al. (2018) with some modifications. Blood freshly collected from a healthy individual (blood group B positive) in a tube containing heparin is centrifuged at 4000 rpm for five minutes in a laboratory centrifuge. After elimination of the supernatant, the pellet was washed three times with sterile phosphate buffer saline (PBS) solution by centrifugation at 4000 rpm for five minutes. The cells were resuspended in PBS to 0.5%.

Hemolytic activity

The method described by Malagoli (2007) with a slight modification was used for the test of hemolytic activity. After the incubation, a sample of 0.5 ml of the cell suspension was mixed with 0.5 ml of the plant extracts (125, 250, 500 and 1000 µg/ml concentrations in phosphate buffer saline). The mixtures were incubated for 30 min at 37°C in an incubator followed by occasional (maximum once per h) resuspension by inversion. The tubes are then centrifuged at 4000 rpm for 5 min and the supernatant is carefully taken up and used to quantify the hemolysis by measuring the absorbance at a wavelength of 540 nm using UV-Vis spectrophotometer (Thermo Scientific, Evolution 300 UV-vis spectrophotometer, Cambridge UK). For the positive control, total hemolysis is obtained by suspending the red blood cells with Triton X-100 (Sigma-Aldrich Chemical Co., Saint Louis, MO, USA). Buffer alone is used as a blank. Each experiment was performed in triplicates at each concentration. For each sample, the percentage of maximum hemolytic activity was determined by the following formula:

$$Hemolysis\ rate\ (\%) = \frac{(\text{Abs. of test sample} - \text{Abs. of blank})}{\text{Abs. of positive control}} \text{ x } 100$$

Statistical Analysis

The data obtained were analysed using the statistical software Graph Pad Prism (version 6.01). All experiments were performed in triplicate. Results are expressed as mean ±SD. The results were submitted to two-way analysis of variance (ANOVA), followed by the Tukey test for multiple comparisons and determination of significance levels. Difference in *p*-values of 0.05 or less was considered significant.

RESULTS

The hemolytic effects induced by the different concentrations of the plant extracts (Figure 1) indicated that the aqueous, ethanol and methanol extracts exhibited very weak hemolytic activity in a dose dependent manner. However, maximum rate of hemolysis was exhibited by the methanol extract (4.65±0.24%) at 1000 µg/ml, followed by ethanol extract (3.77±0.48%) and aqueous extract (2.69±0.51%). Lysis of erythrocytes was found to increase with an increase in the concentration of extracts. There were statistical differences between aqueous and ethanol extracts (p=0.01), between aqueous and methanol extracts (p=0.0001) and between ethanol and methanol extracts (p=0.001).

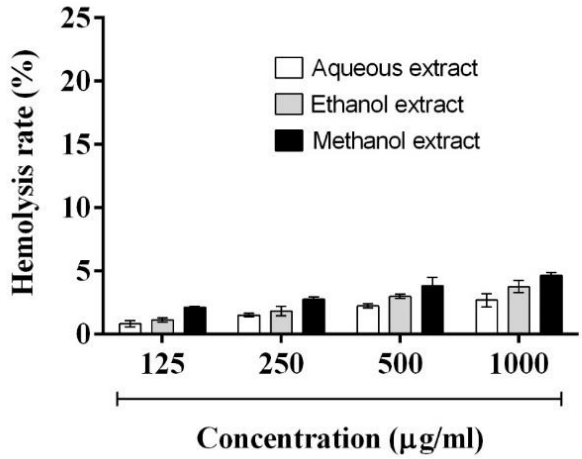

Figure 1: Hemolytic activity of *Acacia nilotica* pod extracts.

DISCUSSION

The present results showed that the plant extract exhibited very low hemolytic activity toward human erythrocytes. This hemolytic activity of the plant extracts is related to their phytochemical composition. In our previous study, the preliminary phytochemical screening revealed that *Acacia nilotica* pods extracts contain saponins which are known to have hemolytic effect (Urbanska et al., 2009). Based on their biochemical nature with a presence of a lipid-soluble sapogenin moiety linked to water soluble sugar chains, this enable saponins to easily interact with cell membrane and cause their disruption which may end up causing rupture and release of hemoglobin pigments. Several antimicrobial peptides (AMPs) have been reported to exhibit cytotoxic activity (immunotoxicity, cytotoxicity and hemotoxicity) against eukaryotic cells (Hamamoto et al., 2002; Megha and Kalpesh, 2020). Majority of plant species are recorded being an important source of AMPs and over 300 sequences have been described and classified on the basis of their identity as amino acid sequence (Wang et al., 2009). However, many of these AMPs were reported to damage erythrocytes (Evans et al., 1989; Hamamoto et al., 2002). *Acacia nilotica* amongst other valuable medicinal plants was reported to contains amino acids such as cystine, methionine, threonine, lysine and tryptophan (Patrick and Chandralal, 2010; Ndamitso et al., 2017). Therefore, *Acacia nilotica* pods extracts in which peptides were expected to be present were tested for *in vitro* hemolytic activity. Overall, the results obtained made it possible to determine the level of hemolytic effect of the main chemical groups present in the studied plant extracts. Our observations, according to which the evaluation of the hemolytic activity of medicinal plants against erythrocytes is essential for an adaptation of traditional therapy have been supported by several authors (Sulaiman et al., 2013; Kumar et al., 2020; Tabassum et al., 2022).

Conclusion

The present study investigated the *in vitro* cytotoxicity effect of various solvent extracts of *Acacia nilotica* pods. The results revealed that the aqueous extract possesses very less cytotoxic effect, followed by the ethanol and methanol extracts. This study constitutes an essential step for possible development of standardised herbal medicines ‘Médicaments Traditionnels Améliorés’ (MTAs, or improved traditional medicines). However, further studies through *in vivo*

toxicological studies should be considered in the future in order to best justify its safety.

COMPETING INTERESTS

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

LMM conducted main experiment and proposed the first draft of the manuscript under the supervision of KI and IM assisted in reorientation and other advices. All authors read and approved the final manuscript before submission.

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