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Tannic Acid Equivalent and Cytotoxic Activity of Selected Medicinal Plants

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Abstrait

L'Equivalent d'Acide Tannique (EAT) des extraits aqueux des feuilles de Dialium guineense Wild (Leguminosae) et Phyllantus amarus (Schum et Thonn) Eurphorbiaceae est déterminé par l'haemanalyse. L'activité cytotoxique de ces extraits fut aussi déterminées par l'emploi de crevette de l'eau salée (BSL) bioassaie. L'EAT de D. guineense était 7.6, tandis que P. amarus était 4.2. LC_{50} pour D. guineense était 260ppm, et 370ppm pour P. amarus. Il est proposé que la cytotoxicité puisse avoir une relation directe avec le EAT.

Abstract

The Tannic Acid Equivalent (TAE) of the aqueous extracts of the leaves of Dialium guineense Wild (Leguminosae) and Phyllantus amarus (Schum & Thonn) Eurphorbiaceae was determined by haemanalysis. The cytotoxic activity of these extracts was also determined using the brine shrimp lethality (BSL) bioassay. TAE of D. guineense was 7.6, while P. amarus was 4.2. LC_{50} for D. guineense was 260ppm and 370ppm for P. amarus. It is proposed that cytotoxicity may have a direct relationship with TAE.

Key words: **Tannic Acid Equivalent (TAE), Relative Astrigency (RA), Cytotoxicity.**

Introduction

Tannins are the most frequently analysed group of phenolics in ecological studies. Haemanalysis of tannins and the concept of relative astringency was first introduced by (1). Haemanalysis was the term used for the estimation of tannins by measuring their ability to precipitate haemoglobin. The chromophore of haemoglobin allowed for its direct spectrophotometric assay in the red region of the visible spectrum, where few other substances present in plant extracts are likely to interfere. The critical requirement in this technique is for the haemolysis of red blood cells. Preserved human blood was not found suitable because of added anti-coagulants and commercial freeze dried blood products are unsuitable due to denaturation. A reliable source of fresh blood is however required. This requires the use of blood collected from a slaughter house. This was centrifuged to remove cell debris after the haemolysis step. The leaves of *D. guineense* are used for the treatment of stomach cramps, sore throat, various skin diseases and inhbition of tumours along the West African coast (2). *P. amarus* are applied for treating external furuncles and abscesses (3). Since the treatment of skin disorders can be considered for astrigency and when searching for anti-tumour compounds because they reflect disease states bearing some relevance to cancer symptoms, the cytotoxicity of these plants were tested. *D. guineense* leaves contain molluscicidal triterpenoid saponins (4) and *P. amarus* leaves contain phyllantin, phyllantidine and saponins (5). In addition, both plants contain tannins. The work with tannins has focused on digestibility reduction in the gut, phenolics may however be absorbed into the body and disrupt other physiological processes. A key issue here has been when and whether absorption into the body is possible. With tannins, this has rarely been considered because of their large size and polar nature, nevertheless, the realisation that hydrolysable tannins may hydrolyse after ingestion has led to studies of their toxicity.

Materials and Methods

D. guineense was collected in May 1989 at Ile-Ife,

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Nigeria, along Oba Aderemi Road, Obafemi Awolowo University and identified by Prof. Z. Gbile by comparison with herbarium specemens FHI 6756, FHI 24636, FHI 32225 and FHI 2819 of the herbarium of the Forestry Research Institute of Nigeria, Ibadan. Voucher specimens PHB 204, 205 and 206 are deposited in the harbarium of the Pharmacognosy Department, University of Benin, Nigeria, while *P. amarus* was collected in April, 1998.

Extraction

The powdered samples 10g each were weighed accurately into a conical flask, 100ml of distilled water was added and heated over a boiling water bath for 30 minutes. The extract was filtered with cotton wool and filtrate clarified by centrifugation. Extracts were preserved in sealed flasks at 4°C.

Determination of TAE

Fresh blood was collected from a cow at slaughter and diluted with several volumes of ice water to haemolyse the cells. The haemoglobin solution was returned to the laboratory on ice and centrifuged free of debris. 1ml of the plant extract was added to a centrifuge tube, 1ml of distilled water was added and mixed. 1ml of the diluted haemoglobin solution was also added, mixed for 15s and then centrifuged at 7500g for 30 minutes. A blank was prepared without any plant extract. The supernatant solution was poured into a spectrophotometer cuvette and absorbance recorded at 578nm. This was repeated for various concentrations of the two extracts. The extracts were replaced with solutions of known concentration of tannic acid and absorbances also recorded at 578nm. The standard curve obtained from this data was used to determine the equivalent amount of tannic acid present in the plant extracts. Results were reported as TAE. Measurements were done in triplicate for all the determinations.

Determination of RA

This represents the astringency of the tannin present, relative to tannic acid, weight per weight. The amount of tannin resent was determined using the protein tannin precipitation method in the African Pharmacopoeia 1986 (6).

Hatching the Shrimps

Brine shrimp eggs (Aquaculture products of distinction 1989 Artemia Inc.) were hatched in a shallow rectangular dish (210mm x 40mm) filled with artificial sea water (prepared by adding 3.8g sea salt per litre of dechlorinated water). An artificial perforated dam was made to divide the tank into two unequal compartments. The eggs were sprinkled into a corner of the larger compartment while the other side was placed to face the window ledge to provide natural white light. This is to attract the naupli (larvae) through perforations in the dam. The phototropic naupli were collected after 4 hours from the lighted side.

Bioassay

Then (10) naupli were transferred to each crucible using disposable pipettes and artificial sea water added to make 5ml. 3 replicas were made per concentration (30 naupli per dilution). Control groups of naupli were placed in the same volume of sea water without the extracts. The naupli were kept at room temperature (27–32°C). The number of survivors were counted after 24-hour period using a magnifying glass and also examined for the presence of motility under the microscope. Another 24-hour period was allowed for recovery and then re-examined. The % mortality was calculated with reference to the initial num-ber of naupli. The data was analysed using probit analy-sis (7) within 95% confidence interval. The response data was transformed into a straight line logarithm transformation curve and LD_{50} was derived from the best fit line obtained by linear regression analysis.

Results and Conclusion

From Table 1 TAE value for *D. guineense* was 7.6 while *P. amarus* was 4.2. Relative astringency (RA) for *D. guineense* was 0.62 while it was 0.38 for *P. amarus*. LC_{50} estimated within 95% confidence intervals for sta-

Table 1: **Analytical profile of extracts**

Extract	Tannic Acid Equivalent (TAE)	Relative Astrigency (RA)
D. guineense	7.6	0.62
P. amarus	4.2	0.38

Table 2: **Cytotoxicity profile of extracts**

Extract	% Mortality at 100ppm	200ppm	300ppm	400ppm	500ppm	1000ppm
D. guineense	10	25	65	100	100	100
P. amarus	—	—	20	50	75	100

tistically significant potencies was 260ppm for *D. guineense* and 370ppm for *P. amarus* as shown in Table 2. It is apparent from the results that there exists a direct relationship between the TAE, RA and cytotoxicity.

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