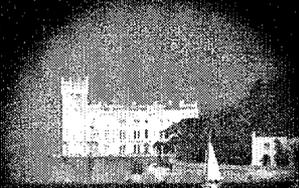




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**HAEMOLYTIC EFFECT OF SAPONIN EXTRACT
FROM *VERNONIA AMYGDALINA* (BITTER LEAF)
ON HUMAN ERYTHROCYTE**

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ABSTRACT

Leaves of *Vernonia amygdalina* were extracted using ethanol and aqueous extraction respectively. The physico-chemical analysis of the extracts revealed that both extract had darkish brown colour, sweetish bitter taste, pungent smell, positive froth and haemolytic test, this indicated the presence of saponin in both extracts. The result of the haemolytic assay revealed that blood group-O had the highest susceptibility to the saponin-induced haemolysis, while blood group-A had the least susceptibility to haemolysis among the blood group tested. Genotype-AA had the highest resistant to haemolysis by *Vernonia amygdalina* saponin induced haemolysis, while genotype-SS had the least resistant to haemolysis among the genotype tested. Furthermore the ethanol extract had a higher haemolytic activity than the aqueous extract on the various human erythrocyte analysed. This study revealed that *Vernonia amygdalina* had haemolytic substance, this substance had a high haemolytic effect on blood group-O and genotype-SS. The active haemolytic substance in both extracts was identified to be saponin.

INTRODUCTION

Vernonia amygdalina is a shrub or small tree of 6 – 15ft tall, the genus *Vernonia* includes sixty different species in areas covered by flora of West Tropical Africa [1]. They are known for their sesquiterpene lactones and over 500 compounds of this group are known. A preliminary phytochemical screening of *Vernonia amygdalina* indicated the presence of saponins, tannins, terpenes and steroids [2]. Kupchan *et. al.* [3] discovered and isolated tumor inhibitor XLVII vernodalin and vernomygdin, which are two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina*.

It is well known for its anti-diabetes and anti-hypertensive properties. Leaves of *Vernonia amygdalina* are used for curing headache while the young leafy shoots are popularly used by the Igbos and Yorubas (Southern Nigeria) as vegetable in traditional soup preparation [4]. Although a great deal had been reported on the phytochemical constituents and medicinal value of *Vernonia amygdalina*, however, there is a dearth of information on the safety of the *Vernonia amygdalina* which is consumed at least once a day by most people in the south eastern part of Nigeria as their traditional soup. The present study therefore seeks to provide information on the safety of *Vernonia amygdalina* with regard to haemolysis of human erythrocytes (In – vitro).

MATERIALS AND METHODS

Materials

Vernonia amygdalina were collected from the research farm of Federal University of Technology, Akure, Nigeria. The microtitration were carried out using microtitre plates, 25 μ l microdiluters and droppers. The chemical used were analer grades, while the water used for the analysis were glass distilled. The fresh human blood (grouped genotypes and blood groups) were collected from state specialist hospital, Akure.

Methods

Phytochemical screening for saponins

Phytochemical analysis of the samples for saponins content was carried out using Gunatilaka *et al.*, [5] methods as stated below.

Froth test

To 100mg of chopped *Vernonia amygdalina* leaf, 5ml water was added in a test-tube and shaken. Froth formation was observed, the length of the froth was measured, and time taken for the froth to disappear was equally determined.

Extraction of saponins

The *Vernonia amygdalina* leaf (0.5kg) which showed positive froth test were ground and soaked in 2 litres of ethanol (95%) in a water bath with shaker at 60°C, for 72hrs, the extracts obtained were evaporated to dryness. Thereafter the extracts were defatted using petroleum ether (60 –80°) in a soxhlet extractor for 4 hrs. The partially purified extracts were subsequently tested for saponins using haemolytic test, as described by Sodipo *et al.*, [6].

Haemolytic assay

Fresh human bloods were centrifuged (x2000g) for 10min. The supernatant was discarded and the pellets (packed cells) were washed with isotonic phosphate buffer (pH 7.2) several times, until there were clear supernatants that were used in the subsequent experiments. Haemolytic assays were conducted by two-fold serial dilution of the saponin extracts using microtitre plates with 2% (V/V) treated erythrocytes suspension from human red blood cells. The sedimentation pattern of the erythrocyte suspension in the unperturbed plates was read after 2 hrs at room temperature to determine the titre [7].

RESULTS AND DISCUSSION

Phytochemical properties of both the aqueous and ethanol extracts of bitter leaf (*Vernonia amygdalina*), as shown in Table 1, revealed that both extracts were darkish brown crystalline solid with sweetish bitter taste and pungent odour. They have positive froth test with foam length ranging from 1.5 – 1.8cm and time of foam disappearance of 6hrs 20min to 6hrs 30min. The extracts had positive haemolysis in the sense that it lysed blood. [8,9]. This characteristic of the extracts agrees with Merck Index [10] description of saponins.

Table 1. Phytochemical properties of ethanol and aqueous extracts of Bitter leaf (*Vernonia amygdalina*)

Property	Ethanol	Aqueous
Colour	Darkish brown	Darkish brown
Taste	Sweetish bitter	Sweetish bitter
Odour	Pungent	Pungent
Froth test	Positive	Positive
-Foam length	6hrs 20mins	6hrs 30mins
-Time of foam disappearance	1.5cm	1.8cm
Haemolytic test	Positive	Positive

The result of the haemolytic assay indicated that both extracts had haemolytic activity as stated earlier [7,9]. From the result shown in figures 1, ethanol extract (16 – 1024) appeared to have a more haemolytic activity on human erythrocyte than the aqueous extract, which is a possible indication that ethanol extract may have a higher concentration of the saponin, which is the active haemolytic substance in the extract. The finding agrees with earlier report by Oboh *et.al.* [9] that ethanol extract of soyabean has higher haemolytic activity than the aqueous extract. This possibly explains why saponin is usually extracted using ethanol [8,9]

The extracts showed some variation in their haemolytic activity on the different erythrocytes tested. The various blood group tested did not show a particular trend in their haemolytic activity, but it was obvious from the results that blood group A [ethanol (16), aqueous (64)] appeared to be more resistant to haemolysis than either blood group B [ethanol

(256), aqueous (256)] and O [ethanol (256), aqueous (512)]. Of all the genotype tested, genotype AA [ethanol (512), aqueous (256)] appeared to be more resistant to haemolysis than either AS [ethanol (512), aqueous (512)], or SS [ethanol (1024), aqueous (512)], which had the highest susceptibility to haemolysis.

A positive pattern, indicating full haemolysis (FH) appeared as a circular big spot of red solution surrounded by a small zone (if any), while a negative pattern, indicating no haemolysis appeared as a uniform small spot of erythrocyte at the bottom of the well, surrounded by a clear zone. In some microtitrations, where no positive pattern was observed, the clump of erythrocytes appeared as a rather large and non-uniform spot. In such instances, partial haemolysis was recorded [7].

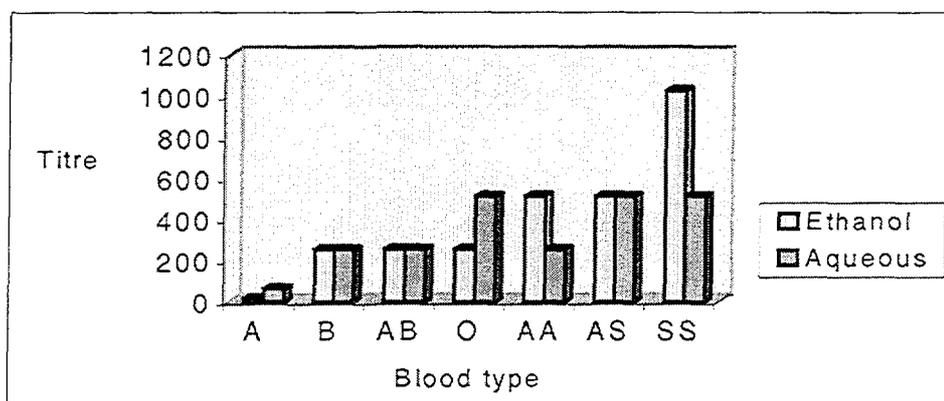


Figure 1: Haemolytic activity of ethanol and aqueous extracts on some human erythrocytes

These results agree with that of Sodipo *et.al.* [7] and Oboh *et.al* [9] on the effect of saponin extracts from *Garcinia kola* and soyabean on some human erythrocytes. To the extent that Blood group A, O, AB and B and genotype AA are less susceptible to saponin induced haemolysis, while genotype AS and SS are more susceptible to saponin induced haemolysis. This high susceptibility of genotype AS and SS can be attributed to the altered geometry of the genotype AS and SS [6].

However, bitter leaf is popularly consumed in Nigeria, either for its medicinal potential, or food values. But the fact remains that bitter leaf contains saponins. This saponin can be either beneficial or deleterious. Part of the nutritional significance of saponins stems largely from their ability to reduce blood cholesterol [11]. However, saponins have strong haemolytic properties, when ingested orally, although they usually remain in the gastro-intestinal tract, but

can sometimes leak into the blood during intestinal mucosa erosion, or damage [12]. Following such a leakage, a number of pathological abnormalities have been identified among which we may include liver damage, haemolysis, respiratory failure, convulsion and coma [10]. This study revealed that *Vernonia amygdalina* had haemolytic substance, this substance had a high haemolytic effect on blood group-O and genotype-SS. The active haemolytic substance in both extracts was identified to be saponin.

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