RELATIVE NON-TOXICITY OF THREE LOCAL PLANT APHRODISIACS IN NIGERIA’S NORTHERN GUINEA SAVANNA

Hamza K. Yusuf

Department of Basic and Applied Science, Hassan Usman Katsina Polytechnic, Katsina
E-mail: yusufhamzak@yahoo.in

Abstract
This study on three medicinal plants namely; Nauclea latifolia S.M., Amblygonocarpus andongensis, and Bridelia africana (collected from Tafashiya and Fakuwa villages, Kankia Local Government Area, Katsina state) was carried out to determine their toxicity, using 30 albino rats in groups of 5 (2 males & 3 females) plus a control group making a total of six (6) groups. Fairly uniform conditions of age, weight, temperature, humidity, photoperiod, rat feed and water were maintained before administering the extracts in doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg, according to the procedure of Lorke. The extracts were found to be practically non-toxic (LD50 = 0 mg/kg) via qualitative analysis of the powdered samples, aqueous and ethanolic extracts based on standard procedures of Sofowora, Harborne, Trease and Evans as adopted by Edeoga et al (2005) and Ganesan and Bhatt (2008). The active ingredients were saponins, flavonoids, terpenoids, alkaloids, cardiac and steroid glycosides. However, flavonoids were not detected in B. africana. Nevertheless the detected ingredients all constitute a synergistic aggregate that is most likely responsible for the observed non-toxicity characteristic.

Keywords: Plant aphrodisiacs, phyto-chemical screening, non-toxicity, synergism

Introduction
Medicinal plant research is very vital because most of the people of the world rely heavily on traditional medicine for their health care. Maridass and De Britto (2008) estimated that about 80% of the population of the world living in vast rural areas of developing and under-developed countries still rely on medicinal plants for their health care delivery. The World Health Organization (WHO) is said to have declared that there are more traditional medicine providers than allopathic providers, especially in the rural areas (Shanmugan, Manikandan and Rajendran, 2009). Since Nigeria is a haven for traditional medicine, with abundant unexploited resources in plant products, medicinal plant research should be encouraged and enhanced in order to achieve the desired objectives. There is therefore a need to refocus medicinal plant research towards the qualitative and quantitative analysis of these natural products to enable researchers to determine their efficacy, dosage, toxicity levels and safety as well as bring about an understanding of their mode of action. This study aims at determining the dosage and toxicity levels of the plant extracts as well as determining their acute toxicity, screening for flavonoids, terpenoids, tannins, phlobatannins, saponins, alkaloids, glycosides and steroids, and at the same time calculating the median lethal dose (LD50) for each extract.

Materials and method
The collected plants were taxonomically authenticated at the Department of Plant Science Bayero University Kano. The plant samples are:
1. Nauclea latifolia S.M. Voucher or Acc. No. 137/plant No.8 Common names: African peach; Pin cushion tree. Vernacular (Hausa) name: “Tafashiya” N. latifolia, a shrub or small spreading tree widely distributed in the savanna. The leaves are broadly elliptic, reaching up to 20 cm long, more or less hairless but a pair of triangular stipules between each leaf pair abound. The flowers are in globose heads of about 5cm diameter. Whole roots of this sample were used.
2. Amblygonocarpus andongensis. Voucher or Acc. No. 91/Plant No.15, Vernacular (Hausa) name: “Kolo”. Family: Fabaceae. The plant is widespread in tropical Africa, mostly in the Savanna areas. It is a tree usually up to 9 m high, but reaching up to 18m and 1.5m girth in moist areas, with a wide flat open crown and a clean,
straight bole. The bark is grey to brown, rough and flaking off in irregular patches leaving reddish scars; slash dark brown, crumbly lighter beneath. The leaves are mostly at the ends of the erect twig and entirely glabrous with pale blue to green leaflets 12–20 per pinna. Pinnae are 2–7 pairs; opposite or subopposite and obovate, up to 2.5 cm long. Stalks carrying leaflets are very short and twisted. The flowers in the spike are white or yellowish and sweetly-scented. The fruits or pods are four-sided, woody, dark brown and 10–13 cm long by about 2.5 cm across, hanging on thick stalks 5–8 cm long. Whole roots of the sample were used.

3. *Bridelia africana*. Voucher or Acc. No. 86/Plant No. 71. Vernacular (Hausa) name: “Kizni”. The plant has sparse spines, elliptic to narrowly-elliptic leaves; usually 7–10 cm long, leaf stalk 6 mm long whilst leaf margin is scalloped or wavy and the main nerves run directly to distinct marginal nerve. Whereas floral clusters are axillary, the fruit is a drupe which is globose and about 9 mm in diameter. Stem bark of the sample were used. The three samples were all air-dried and ground into pulp or powder before preparing aqueous and ethanolic extracts of them.

Aqueous extracts:
Aqueous extract of each sample was prepared by soaking 100 g of the dried powdered sample in 200 cm$^3$ of distilled water for 12 hours. Each extract was filtered using Whatman No. 42 (125 mm) filter paper folded into a funnel on a separate conical flask, thereby removing solids and cellular materials.

Ethanolic extracts:
Ethanolic extract of each sample was prepared by mixing 500 g of the powdered plant material with 1000 cm$^3$ of the solvent. The solvent comprised of 10% Acetic acid in ethanol. The set ups were sealed with polythene to prevent fungal contamination, and left at room temperature for seven days to form percolates. Each percolate was filtered using Whatman No.1 filter paper. The filtrates were evaporated or concentrated to dryness on rotary evaporators. Both aqueous and ethanolic extracts were weighed and labelled and together with the powdered samples, stored at 4°C for the next stage i.e. Phytochemical screening.

Phytochemical screening
Chemical tests (qualitative analyses) were carried out on the aqueous and ethanolic extracts as well as the powdered samples using standard procedures of Sofowora, Harborne, Trease and Evans as adopted by Edeoga et al (2005) and Ganesan and Bhatt (2008):

Test for tannins
About 0.5 g of the dried, powdered samples weighed out using a Cent-o-gram balance, boiled in 20 cm$^3$ of distilled water in a test tube and filtered. Then a few drops of 0.1% ferric chloride solution were added and observed for brownish green or blue-black colouration.

Test for phlobatannins
An aqueous extract of each sample was boiled with 1% aqueous Hydrochloric acid (Hcl) in a boiling tube. A red precipitate showed the presence of phlobatannins.

Test for saponins
2g of the powdered sample was boiled in 20 cm$^3$ of distilled water and shaken vigorously for a stable persistent froth; to which was added 3 drops of olive oil and shaken vigorously until an emulsion was formed.

Test for flavonoids
Three methods were used to determine the presence of flavonoids:
(a) 5 cm$^3$ of dilute ammonia solution was measured out using a 10 cm$^3$ measuring cylinder and added to a portion of the aqueous filtrate of each plant extract followed by concentrated sulphuric acid or tetraoxosulphate (VI) acid ($\text{H}_2\text{SO}_4$). A yellow colouration which disappeared on standing confirmed flavonoids.
(b) A few drops of 1%aluminium solution were added to a portion of each filtrate in a test tube. A yellow colouration confirmed flavonoids.
(c) A portion of the powdered plant sample was heated with 10 cm$^3$ of ethyl acetate in a 100 cm$^3$ beaker over a steam bath for 3 minutes. The mixture was filtered using Whatman No. 1 filter paper and 4cm$^3$ of the filtrate in a test tube was shaken with 1cm$^3$ of dilute ammonia solution. A yellow colouration confirmed flavonoids.
Test for steroids / steroid glycosides
Ciulei’s procedure:
2 cm$^3$ of the ethanolic extract was taken into a test tube and evaporated to dryness. The residue was dissolved in acetic anhydride, and chloroform was then added. By means of a pipette, concentrated sulphuric acid was added by the side of the test tube. A brownish ring at the interface of the two liquids or the appearance of violet colour in the supernatant layer indicated the presence of steroids.

Test for Terpenoids (Salkowski’s test)
5 cm$^3$ of each extract was mixed with 2 cm$^3$ of chloroform in a test tube and 3 cm$^3$ of concentrated H$_2$SO$_4$, carefully added to form a layer. A reddish-brown colouration at the interface positively confirmed terpenoids.

Test for cardiac glycosides (Keller-Killiani’s test)
5 cm$^3$ of each plant extract in a small beaker (50 cm$^3$) was treated with 2 cm$^3$ of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayed with 1 cm$^3$ of concentrated sulphuric acid (H$_2$SO$_4$). A brown ring at the interface indicated a deoxyribose sugar characteristic of cardenolides. Both a violet ring and a greenish one (in the thin acetic acid layer) would indicate presence of glycosides.

Test for alkaloids
Procedure of Ganesan and Bhatt (2008):
2 grams of each of the powdered samples were weighed out using a digital precision balance and warmed with 20 cm$^3$ of 1% H$_2$SO$_4$ in a 50 cm$^3$ conical flask on a water bath. The mixture was shaken intermittently before centrifuging. The supernatant was pipetted off into a small conical flask. 0.1 cm$^3$ was taken and treated with Meyer’s reagent. A cream precipitate confirmed alkaloids.

Toxicity tests
Thirty healthy male and female white or albino rats (Rattus norvegicus) weighing averagely 140 g were used for the acute toxicity test. They kept in well ventilated cages and allowed to aclimatize with the conditions; ambient temperature, photoperiod and humidity as well as free access to rat pellets and water. They were put into three groups of five rats according to the samples each of which doses were administered orally (using special syringes) based on the procedure of Lorke i.e. 10 mg/kg, 100 mg/kg and 1,000 mg/Kg body weight. A fourth group of five rats was used as control, i.e. only given feed and water. This is the first phase of administration which was survived by the rats (no death occurred) thus paving the way for the second phase of doses; 1,600 mg/kg; 2,900 mg/kg and 5,000 mg/kg. The correct volume (cm$^3$) for each extract corresponding to each dose was determined by calculation as ‘the product of the average weight of rat (g/kg) and the ratio of the known dose (mg/kg) and the concentration of the extract (g/cm$^3$)’; V (cm$^3$) = Average wt. of rat (g/kg) x dose (mg/kg) / concentration of extract (g/cm$^3$). The Median Lethal Dose or LD$_{50}$ (i.e. the dose required to kill half the members of a tested population after a specified test duration) was determined mathematically as the Geometric mean of the Minimum dose with full mortality and the Maximum dose without mortality; LD$_{50}$=[Min.Dose(f.m.) Max.Dose(w.m.)]$^{1/2}$. Like the period for acclimatization the experiment with the animals lasted 7 days. After the administration of each dose, the animals were also observed for 3 hours in order to determine the aphrodisiac effect of each sample via a Penile Erection Index (PEI).

PEI = (Mean episodes of sucking and mounting) x (% Rats showing at least 1 episode)(El-Thaher,2001)

Results
Various phytochemical constituents from the ethanolic and aqueous extracts were detected as shown in Table 1. Table 2 gives the LD$_{50}$ values for the plant extracts orally administered on the albino rats.
Table 1: Qualitative analysis for the plant extracts

<table>
<thead>
<tr>
<th>Compounds</th>
<th>N. latifolia</th>
<th>A. andongensis</th>
<th>B. africana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroid Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + = present. - = absent.

Table 2: LD_{50} values for plant extracts orally-administered on albino rats

<table>
<thead>
<tr>
<th>Test Regimes</th>
<th>Doses (mg/kg)</th>
<th>N. latifolia</th>
<th>A. andongensis</th>
<th>B. Africana</th>
</tr>
</thead>
<tbody>
<tr>
<td>First phase</td>
<td>10</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Second phase</td>
<td>1,600</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>2,900</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>LD_{50} (mg/kg)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Key: 0/5 = No. of dead rats/ No. of rats used

Discussion
Results from the qualitative analysis of the crude drugs show that they contain alkaloids, saponins and glycosides (Table 1) A related study by Nworgu, Owolabi and Atomah (2010) had also detected sugars, saponins, flavonoids and indole alkaloids in the roots of N. latifolia, but other studies had used leaf extracts of *Lepidium sativum* rather than shoot extract (used in this study). Najeebur- Rehman et al (2011) detected alkaloids, saponins and anthraquinones while Abuelqasim (2008) showed the presence of triterpenes, alkaloids, flavonoids, tannins, coumarins and saponins. The roots of *N. Latifolia* were reported to be used as analgesic and aphrodisiac for sexual asthenia (loss of strength ) in the Congo (T.B.S, 2011) Acute toxicity test results show that all the three plant sample extracts (aqueous and ethanolic ) are practically non-toxic because there is no record of mortality in each case. (LD_{50} = 0 mg/kg). They may therefore be said to be therapeutically safe for the rats, but may not necessarily be so for humans. Although Nwinyi et al (2006) had implicated acute toxicity in a study on *Amblygonocarpus andongensis* with an LD_{50} of 547.7 mg/kg i.p, it was a stem bark extract that was used. This study used the root extract with differing levels of toxicity. The relative non-toxicity of the three crude drugs is most likely due to a synergism between the various active ingredients , possibly causing a decrease in potential toxicity of each individual active ingredient.(Birdi, Brijesh and Daswani, 2008).
Conclusion
The three plant extracts *N. latifolia, A. andongensis* and *B. africana* are practically non-toxic and so therapeutically safe to the rats, and possibly humans (LD$_{50}$ = 0). This study supports folk use of the medicinal plants.

Recommendation
Further studies are required to find out whether the samples studied will be therapeutically safe for humans, despite local belief that they are safe for human consumption. Qualitative analysis of the local drugs should be extended in order to isolate, identify, characterize and possibly determine the structure of their bioactive ingredients.

References


