# EVALUATION OF PHYTOCHEMICAL, HAEMATOLOGICAL AND TOXICOLOGICAL PROPERTIES OF *Malacantha alnifolia* (P. Rous) STEM-BARK EXTRACT IN RATS

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## Abstract

Evaluation of some phytochemical, haematological and toxicological properties of the methanolic stem-bark extract of *Malacantha alnifolia* was investigated in rats in this study. The plant extract was found to contain flavonoids, saponins and reducing sugars. The extract at the highest concentration (400 mg/kg) administered caused a significant ($p<0.05$) increased in white blood cell (WBC) count compared to other groups. The activities of the antioxidant enzymes were elevated (though not statistically significant) in the treated rats compared to the control. Histopathological examination of the liver of the treated rats (400 mg/kg body weight) revealed an enlarged fatty and friable liver with diffused necrotic area as well as enlarged kidney with mild anemia in the group 5 rats. This suggests that further analytical studies should be carried out and caution should be exercised in using the stem bark extract of *Malacantha alnifolia* to alleviate diahorrea and stomach ache as acclaimed traditionally.

**Keywords:** phytochemical, haematological, toxicological, *Malacantha alnifolia*, rats.

## Introduction

Since prehistoric times man has used crude plant extract to heal and to kill (Onocha *et al,* 2003). In fact, the plant kingdom has long served as a prolific source of useful drug, foods, addictives, flavouring agents, colourants, binders and lubricants (Gamaniel, 2000). A critical look had been taken at the advantages of ethnobotanicals surveys over other approaches of studying medicinal plants for the isolation of new drugs (Wanbebe, 1998).

The ethnobotanical uses of the plant are diverse in both traditional and veterinary medical practices and the discovery of useful drug from plants in the 21st century has been one of the major challenge to Nigerian chemist (Ekpendu *et al* 2000). *Malacantha alnifolia* (*Sapotataeae*) is a perennial plant commonly found in the tropical rain forest especially in Ghana, Togo Republic and Nigeria. The leaves are tomentose becoming more or less glabrescent obviate elliptic, up to 20-25 cm long, 6-12 cm broad with 15-120 pairs of very prominent lateral nerves. The flowers are sessile, clustered with light brown-rusty calyx. It is called "Akala" by Yoruba natives of south-western Nigeria. The cotyledons from the seeds are used in the treatment of vaginal and dermatological infections in western Nigeria. The decoction of the stem-bark extract of *M..alnifolia*, apart from its usage in folk medicine for the treatment of conjunctivitis of the eye, is also used against diarrhea and stomach ache in traditional folk medicine (personal communication). The present study was therefore conducted to evaluate some phytochemical components of the stem-bark extract of *M. alnifolia* and determine whether or not its methanolic extract could produce undesirable effects in rats and by implication its numerous users.

## Materials and methods

### Animals

Twenty (20) albino rats of both sexes weighing between 100-150 g were purchased from the Department of Veterinary Anatomy, University of Agriculture, Abeokuta, and kept in well ventilated rat cages with free access to water and feeds *ad labium (Ladokun and Son Ltd Feeds)*. They were allowed to acclimatize for two weeks.

## Plant material

The stem-bark of *M. alinofolia* plant was obtained from Olokemeji Forest reserve in Eruwa, Ibarapa Local Government Area, Oyo State, Nigeria. It was authenticated by Mr T.K Odewo, a taxonomist in the herbarium section, Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan. It was allowed to air-dry for 72 hours and later pulverized into powder using mechanical pulverizer at the Wood Extraction Laboratory, University of Ibadan, Ibadan, Nigeria. The powdered sample was kept in air-tight container and stored at $0^0$C until used

## Extraction

The methanolic extraction of *M.alnifolia* stem-bark in a soxhlet extractor was carried out according to the AOAC (1990). The pulverized sample was initially defatted with distilled n-hexane (b.pt 68°C). Thereafter, the residue was allowed to dry and re-extracted with distilled methanol. The extraction was carried out for 72 hour until the solvent became colourless in the thimble of the soxhlet extractor. The extraction was concentrated in vacuo at reduced pressure to give a dark brown gummy solid.

## Phytochemical screening

The powdered sample *of Malnifolia* stem-bark was tested for the presence of secondary metabolites such as saponins, alkaloids, glycosides, flavonoids, phlobatannins and cardiac glycosides following the methods described by Trease and Evans (1978).

## Test for saponins

0.2 g of the crude extract of M.*alnifolia* stem bark was shaken with 5 ml of distilled water. The mixture was heated to boil. Frothing which persist for 40 minutes shows the presence of saponins.

## Test for alkaloids

0.2 g of crude extract was added to 10 ml of 2% $H_2S0_4$. The mixture was filtered and few drops of Dragendoff reagent was added. An orange red precipitate indicates the presence of alkaloids

## Test for glycoside

0.5 g of the crude extract was added to 10 ml of 2% HCl solution and neutralized with 5% NaOH solution. A drop of Fehling solutions A and B were added. Formation of red precipitate indicates the presence of glycoside

## Test for flavonoids

0.5 g of the crude extract was dissolved in 5% NaOH and 2% HCl was added. A change from yellow solution to colorless indicates the presence of flavonoids.

## Test for phlobatanins

0.5 g of crude extract was dissolved in distilled water and the filtered. The filtrate was boiled with 2% HCl solution. Formation of red precipitate indicate positive test.

## Test for cardiac glycosides

0.5 g of the crude extract was added to 2 ml of acetic anhydride. The mixture was then cooled in ice followed by the addition of concentrated $H_2SO_4$. A color change from violet to blue-green coloration indicates the presence of a steroidal nucleus (i.e aglycone portion of the cardiac glycosides.)

## Animal treatment

The rats were divided into five (5) groups of four animals each. Groups 2, 3, 4 and 5 were treated daily by oral administration of stem-bark methanolic extract of *M.alnifolia* using orogastric tube at 100 mg/kg, 200 mg/kg, 300 mg/kg and 400 mg/kg body weight respectively for three weeks. Group 1 served as control and received only distilled water corresponding to the highest volume of extract administered by the same route. The weights of the animal were taken prior to the administration of the plant extract and subsequently on weekly basis for 3 weeks. Blood sample were collected from the tail vein prior to the treatment and then at the end of third week following initiation of the plant extract administration.

### Blood analysis

Blood samples collected into heparinised glass capillary tubes were used for the determination of packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) count and white blood cells (WBC) count. The Hb concentration was determined by cyanomethaemoglobin method using Beckman model spectrophotometer, PCV by micro-haematocrit method while RBC and WBC count was determined using improved Neubauer Haematocytometer counting chamber (Dacie and Lewis, 1991).

### Antioxidant enzyme assays

The activities of catalase (CAT), peroxidase (POD), glutathione-s-transferase (GST), alanine glutamine transferase (ALP), alanine aspartate transferase (AST) and alkaline phosphatase (ALP) in the whole blood collected from each rat was determined according to the methods described by Aebi (1984); Addy and Goodman (1972); Habig and Jakoby (1981); and Sigma Diagnostic Kit (1985, 1987) respectively.

### Histopathology

Histopathological examination of the liver (site of biotransformation) from the rats was carried thus: Thin (5µl) cryostat sections of 10% formalin-fixed tissue were stained with hematoxylin and eosin, periodic and Schiff reagent with and without diastase, respectively. The sections were examined under light microscope at high (x400) objective power magnification.

### Statistical analysis

The data generated were subjected to Analysis of Variance (ANOVA) as outlined by Steel and Torrie (1980). Values are expressed as means ±S.D (Standard Deviation) of four rats' observations.

## Results

The results of the phytochemical screening of the stem-bark extract are shown in Table 1. The result revealed that the extract contained flavonoids, saponin and reducing sugar while alkaloids, phlobatanins, glycosides and cardiac glycoside are absent (or if present could not be detected by the methods used in this study).

**Table 1:** Results of the phytochemical screening of the stem-bark methanolic extract of *M. alnifolia.*

| Component | Results |
|---|---|
| Alkaloids | – |
| Flavonoids | + |
| Phlobatanins | – |
| Saponins | + |
| Reducing sugar | + |
| Glycoside | – |
| Cardiac glycoside | – |

Key: + = Present . – = Absent.

The effects of 21 day administration of the aqueous stem-bark extract of *M.alnifolia* on the hematological parameter of rats are reported in Table 2. A decrease (though not statistical significant) in the values of Hb, PCV and RBC levels in the treated rats compared to the control were observed. There was a significant increase ($p<0.05$) in the values of WBC of the rats in group 4 (40 mg/kg) compared to the other groups.

A significant difference in the WBC levels was observed among the treated groups compared to the control. There was no statistically significant difference in the WBC count of the control compared to group 2 rats. However, a statistically significant difference exists among the control group and those of groups 3, 4 and 5. The changes in the activities of some antioxidant enzymes and markers of hepatotoxicity assayed are reported in Table 3.

**Table 2:** Effect of 21 day administration of aqueous stem-bark methanolic extract of *M.alnifolia* on the hematological parameters in rats

| Parameters ($n = 4$) | Groups | | | | |
|---|---|---|---|---|---|
| | 1<br>0.00 mg/kg | 2<br>00 mg/kg | 3<br>200 mg/kg | 4<br>300mg/Kg | 5<br>400mg/Kg |
| PCV (%) | 48.5±4.51 | 42.0±1.63 | 44.25±1.71 | 41.5±8.19 | 44.0±1.83 |
| Hb (gm/dl) | 16.23±1.47 | 14.10±1.42 | 14.85±1.51 | 13.95±2.63 | 14.75±0.66 |
| RBC ($X10^{12}$/l) | 4.91±1.22 | 4.08±1.39 | 4.68±0.78 | 4.28±1.38 | 4.04±0.82 |
| WBC(/$mm^3$) | 9150±1121.0[a] | 10400±1416[a] | 7550±500[b] | 6950±1749[c] | 15872±1678[d] |

means ±S.D (Standard Deviation) $n = 4$.
a,b,c Means within the same row but without common superscript are significantly different ($p<0.05$).

**Table 3:** Antioxidants and biomarkers of hepatotoxicity enzymes in *M.alnifolia* extract administered rats.

| Parameters ($n = 4$) | Groups | | | | |
|---|---|---|---|---|---|
| | 1<br>0.00 (mg/kg) | 2<br>100 (mg/kg) | 3<br>200 (mg/kg) | 4<br>300 (mg/kg) | 5<br>400 (mg/kg) |
| Catalase (units/mg) | 22.2± 2.1[b] | 23.6±1.3[b] | 25.0±2.6 | 28.9±2.5 | 33.1±2.9[a] |
| Peroxidase(units/mg) | 10.2+0.9[c] | 12.6±0.9[c] | 15.8±1.1 | 15.2±1.2 | 20.4±1.5[d] |
| Glutathione transferase(nmol/mg) | 1.70±0.09 | 2.3±0.1 | 2.3±0.2 | 1.7±0.1 | 0.8±0.2 |
| ALT(I.U/L) | 100±1.7[a] | 105±1.8 | 98±1.3 | 114±2.1 | 130±1.8[b] |
| AST(I.U/L) | 75±0.7[c] | 83±0.5 | 95±0.6 | 105±0.7 | 113±0.7[d] |
| ALPI.U/L) | 92±0.5[e] | 95±0.5 | 98±0.5 | 100±0.6 | 107±0.8[f] |

Values in the same row with different superscripts are statistically significant ($p<0.05$)

### Histopathology

Pathologically, the rats in group 5 (treated with 400mg/Kg b.w) had enlarged fatty and friable liver with diffused necrotic area, kidney congested and enlarged with mild anemia. There was no evidence of pathologic index in the liver sample of the control rats.

## Discussion

The results of the phytochemical screening revealed that the stem-bark methanolic extract of *Malacantha alnifolia* contained flavonoids, saponin and reducing sugars. This implies that the extract might possess anti nutritional property since saponin, for instance, have been reported to be an anti-nutritional factor (Danmalam and Abdulrahman, 2003). This is further buttressed by the observable decreased in the body weight gain among the treated rat. However, we cannot rule out the pharmacological and nutritional importance of the flavonoids and reducing sugars that are present therein in the extract. For instance, Aletor (1993) reported that some antinutritional phytochemicals exhibit protective effects thus making them to serve a dual purpose of reducing some essential nutrients and protecting the body against a number of biochemical, physiological and metabolic disorders. More so, tannins, apart from been an antinutrient compound by way of forming insoluble complexes with proteins, is also a polyphenolic compound capable of scavenging free radicals in biological system (Oboh and Akindahunsi, 2004).The decrease in the values of WBC in the treated rats prior to an increase at 400 mg/kg extract indicate that the rats were fighting against some forms of infection probably induced as a result of administration of this plant extract.

The activities of ALT and AST are used as a sensitive indicator of acute hepatic necrosis while that of AP was an indicator of hepatobiliary disease (if any). In the present study, *M.alnifolia* fed rats (at 400 mg/kg b.w) was found to have a significantly increase in the activities of ALT, AST and AP, thereby suggesting a degenerative changes and hypofunction of the liver and kidney. This was further buttressed by the enlarged fatty friable liver with diffused necrotic areas and congested kidney as revealed by the histopathological examination of these organs. The increase in the activities of some antioxidant enzymes assayed signifies the possibility in an inherent potential of this plant to generate free radicals, the effect(s) of which these enzymes are trying to neutralize. However, the mechanism by which it does is not yet known.

These results clearly showed that *M.alnifolia* (at 400 mg/kg b.w) has harmful and stressful influence in the hepatic and renal tissues of the rats. It was therefore suggested that care and cautions should be applied in administration of this plant extract for treating human disease since our present traditional practice lack specified dosage in drug/concoction administration against ailment.

To our knowledge, this is first photochemical and biochemical investigation on this "virgin" plant, it is therefore necessary to carry out further studies on this plant in order to access its level of safety for human consumption and usage. However, further laboratory efforts are going on to isolate the active phytochemical constituents of the stem-bark methanolic extract on *M. alnifolia.*

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