

Full Length Research Paper

Effect of ethanol leaf extract of *Millettia aboensis* on selected haematological indices of Wistar albino rats

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Phytochemical screening of crude leaf extract of *Millettia aboensis* showed the presence of reducing sugar, alkaloids, flavonoids, saponins, tannins, Phlobatannins and cardiac glycosides and the absence of cyanogenic glycoside and anthraquinone. Effect of ethanol leaf extracts of *M. aboensis* on haematological parameters was evaluated in thirty Wistar albino rats consisting of six groups (5 rats per group). Groups were administered with 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of the extract and group 6 (positive control) was given normal food and water. The results showed that the haematological parameters of packed cell volume (PCV), haemoglobin concentration of the cell (Hb), total white blood cell counts (WBC) and platelets count of the rats administered with ethanol leaf extract of *M. aboensis* increased significantly ($p < 0.05$) in groups 5 (5000 mg/kg body weight of the extract), group 4 (4000 mg/kg body weight of the extract) and group 3 (3000 mg/kg body weight of the extract) compared to group 6 (positive control). Group 2 (2000 mg/kg body weight of the extract), group 1 (1000 mg/kg body weight of the extract) showed no significant difference ($p > 0.05$) in haematological parameters of packed cell volume (PCV), haemoglobin concentration of the cell (Hb), total white blood cell counts (WBC) and platelets count of the rats administered with ethanol leaf extract of *M. aboensis* compared to group 6 (positive control).

Keywords: *Millettia aboensis*, Phytochemical screening, haematological parameters.

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. They are a source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. Plants have been known to relieve various diseases in a system of traditional medicine native to the Indian subcontinent and a form of alternative medicine known as Ayurveda (Prashant et al., 2011).

Medicinal plants are the richest bio-resource of drugs of traditional medicine, orthodox medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube et al., 2008). Globally, the study of medicinal plants is of great concern in many health care systems. It has been reported by the World Health Organization that approximately 80% of the global population still depend on traditional plant-based medicines for their basic health care needs (Fransworth, 1993). The interest in traditional knowledge is more widely recognized in development policies, the media and scientific literature. In Africa, traditional healers and remedies made from plants play

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Figure 1. *Millettia aboensis* plant



Figure 2. *Millettia aboensis* plant showing its roots

an important role in the health of millions of people.

Few plants and their phytochemical constituents have been shown through pharmacological research to have medicinal effects (Okwu, 1999, 2001). The most important of the bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants for nutritional and health care purposes. They are also referred to as secondary plant metabolites. A few of them are responsible for the rich colours and aromas in plants and some act as antioxidants which protect cells against damage and may reduce the risk of developing certain types of cancer (Okwu, 1999, 2001). Antioxidants protect the body against some diseases and also protect a plant from various assaults in its natural environment. Though these bioactive compounds derived from medicinal plants can be useful but might have serious dose- related side effects (Taylor *et al.*, 2001).

The present study aims to investigate the phytochemicals present in *M. aboensis* - a medicinal plant with little documented literature, used extensively by tribal people in the Southern part of Nigeria and claimed to possess laxative, anti-malaria, anti-inflammatory and antibiotic properties in the traditional system.

Millettia is a genus of legume in the Fabaceae (Pea) family. It consists of about 150 species, and has its geographical location in Asia, Western and Central African continent. It occurs mostly in the forest zones of Nigeria, Cameroun and Equatorial Guinea.

Plant Taxonomy

The Specie *Millettia aboensis* is of the plant kingdom and the phylum is Magnoliophyta (flowering plants). It is classified as a Magnoliophyta and of the order Fabales (legumes, milkworts, and snakeroot) and belongs to the family known as Fabaceae (Pea family) and the Genus *Millettia*. They are mostly shrubs that have streaked dark reddish or chocolate coloured wood (Figure 1). The leaves are usually alternate and compound; they have margins that are seriated. They have stipule which can be leaf-like (example, pisum), thorn-like (example, robinia) or inconspicuous. Leaf margins are entire or occasionally, seriate. Both the leaves and the leaflets often have wrinkled pulvini to permit mastic movements. Most species have leaves with structures which attract ants that protect the plant from attack by herbivore insects. The plants have indeterminate inflorescences which are sometimes reduced to a single flower. They have short hypanthium and a single purple-coloured carpel with short gynophores and after fertilization produces fruits that are legumes and the common name for this type of fruit is a 'pod'. Most of the fabaceae host bacteria in their root nodules which have the ability to take up nitrogen from air for nitrogen fixation (Figure 2). The family name fabaceae was coined from Latin word 'Faba' which means 'bean'. Leguminosae is an older name still considered valid.

Information in literature on the phytochemistry of the leaves of *Millettia aboensis* is scanty. The present study

was therefore conducted to determine the effect of ethanol leaf extract of the plant on selected haematological parameters of Wistar albino rats.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh leaves of *M. aboensis* were collected from Omuihuechi, Aluu Community- a neighbouring village close to the University of Port Harcourt, Rivers State, Nigeria. The plant was identified and authenticated by a Biotechnologist Dr Edwin Nwosu of the Department of Plant Science and Biotechnology, University of Port Harcourt. The voucher specimen number UPH 587 was kept in the Pharmacognosy and Phytotherapy Laboratory of the University of Port Harcourt, Rivers State.

Animals

The experimental animals used for this study were Wistar albino rats of both sexes weighing 132-163g. The rats were 4-5 months old and were obtained from the Animal House of the Department of Biochemistry, Faculty of Chemical Sciences, College of Natural and Applied Sciences, University of Port Harcourt, Rivers State, Nigeria.

Chemicals/Reagents

All chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstand, Germany. Reagents used for the assays were products of Radox Commercial Kits.

Extraction of plant materials

The leaves of *M. aboensis* were air-dried at room temperature ($29\pm 1^\circ\text{C}$) for 2 weeks, after which it was ground to a uniform powder. The ethanol extracts were prepared by soaking 1000 g each of the dry powdered plant materials in 2.5 L of ethanol at room temperature for 72 h with intermittent shaking. The extract was filtered after 72 h, first through a Whatmann (No.42) filter paper (125mm) and then through cotton wool. The extract was concentrated using a rotary evaporator with the water-bath set at 40°C .

Determination of percentage yield of extract

The percentage yield of the extract was determined by weighing the coarse *M. aboensis* leaf before extraction

and the *M. aboensis* ethanol leaf extract after concentration and then calculated. The percentage yield of the extract was 19.2%w/w

$$\text{Percentage (\%) yield} = \frac{\text{Weight (g) of concentrated extract} \times 100}{\text{Weight (g) of ground } M. aboensis \text{ leaves}}$$

Preparation of Reagents

Fehling's Test

Fehling's Solution "A" was prepared by dissolving 7g of Copper sulphate in 100 ml of distilled water containing 2 drops of dilute sulphuric acid and Fehling's Solution "B" was prepared by dissolving 35g of Potassium sodium tartrate and 12g of NaOH in 100 ml of distilled water. These two solutions were stoppered and stored until needed.

Phytochemical screening

Phytochemical screenings were carried out on the aqueous leaf extract and on the powdered samples using standard procedures to identify the constituents as described (Sofowara 1993; Trease and Evans 2002).

Test for reducing sugars (Fehling's test)

One mL Fehling's A solution and 1 ml. of Fehling's B solution were mixed and boiled for one minute. Then 2 ml of test solution (ethanolic extract) was added to the above mixture. The solution was heated in boiling water-bath for 5 min. First a yellow, then brick-red precipitate which indicated the presence of sugar was observed (Sofowara 1993).

Test for saponins

Two grams of the powdered sample were warmed in 20ml of distilled water in a water bath (55°C) for 5 mins and filtered using a white filter paper and a glass funnel. Then 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. To the froth was added 3 drops of olive oil and shaken vigorously. The formation of a stable emulsion indicated the presence of saponins (Sofowara 1993).

Test for flavonoids

Flavonoids were determined by Mg-HCl reduction test. A piece of magnesium ribbon (powder) and 3 drops of concentrated hydrochloric acid were added to 3ml of the test extract. A red colouration indicated the presence of

flavonoids. Five millilitres of dilute ammonia solution were added to 5 ml of the aqueous filtrate of leaf extract followed by the addition of 1 ml concentrated H₂SO₄. A yellow colouration indicated the presence of flavonoids. The yellow colour disappeared on standing (Trease and Evans, 2002).

Test for cardiac glycosides

Five millilitres of extract was treated with 2ml of concentrated glacial acetic acid containing one drop of 0.10% Ferric chloride (FeCl₃) solution. This was underlaid with 1ml of concentrated sulphuric acid (H₂SO₄). A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides and a violet ring which appeared below the brown ring (Trease and Evans, 2002).

Test for tannins

Half a gramme of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. Three drops of 0.1% ferric chloride was added and observed. A brownish green colouration indicated the presence of tannins in the extract (Trease and Evans, 2002).

Test for phlobatannins

To 5 ml of the aqueous extract solution was added 5 ml of 1% aqueous hydrochloric acid (HCl) and boiled in a water bath for 5 min. Deposition of red precipitate indicated the presence of phlobatannins (Sofowora, 1993; Trease and Evans, 2002).

Test for anthraquinone

To 3ml of the aqueous solution of the extract was added 10ml of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for 5 min. It was allowed to stand for 30 min and observed. Honey comb froth was observed which indicated the presence of anthraquinone (Sofowora, 1993; Trease and Evans, 2002).

Test for alkaloids

To 1ml of 1% HCl was added 3ml of the extract in a test-tube. The mixture was heated for 20 min, cooled and filtered. Then 1 ml of the filtrate was tested with 0.5 ml Dragendorff's, Wagner's, Hager's and Mayer's reagents. Formation of reddish brown precipitate for Dragendorff's and Wagner's reagents, yellow precipitate for Hager's

and cream precipitate for Mayer's indicated the presence of alkaloids (Sofowora, 1993; Trease and Evans, 2002).

Experimental design

Thirty Wistar albino rats of both sexes weighing 132 – 163kg were housed in separate plastic cages and acclimatized for seven days on conventional rat feed. The rats were divided into six groups of five rats per group.

Treatment of the rats (Groups 1-5) with the *M. aboensis* ethanol leaf extracts was through oral intubation tube. Rats in Groups 1-5 were fed ethanol leaf extracts of concentrations 1000, 2000, 3000, 4000 and 5000 mg Kg⁻¹ body weight respectively for seven (7) days. Each rat group was also adapted to the commercial feed and water ad libitum for the seven-day study period. Rats in Group 6 (control) were given commercial feed and water ad libitum without the ethanol leaf extract.

Analysis of haematological parameters was carried out 24 hours prior to the administration of the extract to determine the baseline parameter in all the groups. The administration of the extract lasted for 7 days and analyses were done on day 7 and 14 days post treatment.

Determination of haematological parameters

The determination of haematological parameters (haemoglobin concentration, packed cell volume, total white blood cell count, platelet count, neutrophils and lymphocytes) was carried out according to the method of Dacie and Lewis (2000).

RESULTS

Phytochemical screening of *M. aboensis*

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as alkaloids, tannins, flavonoids, saponins, cardiac glycoside and phlobatannins were present in the sample screened while anthraquinone and cyanogenic glycoside were absent (Table 1).

Effect of ethanol leaf extract of *M. aboensis* on haematological indices

Normal baseline result of haematological indices haematological indices involving haemoglobin, concentration, packed cell volume, platelets count, total white blood cell count and white cell differentials was obtained on Day 1 before the administration of the

Table 1: Phytochemicals of the aqueous leaf extracts of *M. aboensis*

TEST	PRESENT	ABSENT
Reducing Sugar	+++	
Alkaloid	+	
Tannins	++	
Flavonoid	+	
Saponins	++	
Anthraquinone		-
Cardiac glycoside	+	
Cyanogenic glycoside		-
Phlobatannins	++	

Key +: Weak presence, ++: moderate presence, +++: strong presence, and -: Absent.

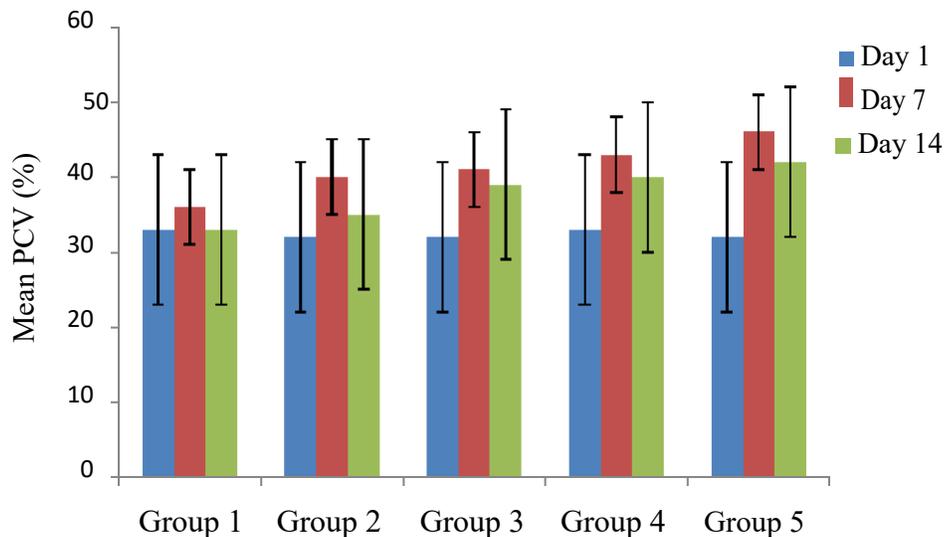


Figure 3.1: Effect of Ethanol Leaf Extract of *M. aboensis* on Haemoglobin Concentration

extract. The extract was administered for 7 days and blood samples were taken on day 7. Effect of ethanol leaf extract of *M. aboensis* on Hb concentration and PCV (figures 3.1 and 3.2) showed a significant increase ($p < 0.05$) in groups 5 (5000 mg/kg body weight of the extract), group 4 (4000 mg/kg body weight of the extract) and group 3 (3000 mg/kg body weight of the extract) on day 7 and day 14. However, Group 2 (2000 mg/kg body weight of the extract), group 1 (1000 mg/kg body weight of the extract) showed no significant difference ($p > 0.05$) in Hb concentration and PCV.

Effect of ethanol leaf extract of *M. aboensis* on mean total WBC count (figure 3.3) showed a significant increase ($p < 0.05$) in groups 5 (5000 mg/kg body weight of the extract), group 4 (4000 mg/kg body weight of the extract) and group 3 (3000 mg/kg body weight of the extract) on day 7 and day 14. However, Group 2 (2000

mg/kg body weight of the extract), group 1 (1000 mg/kg body weight of the extract) showed no significant difference ($p > 0.05$) in mean total WBC count.

A significant increase ($p < 0.05$) in platelets count (figure 3.4) was observed on day 7 and day 14 in groups 3, 4 and 5 fed respectively with 3000, 4000 and 5000 mg Kg⁻¹ body weight of ethanol leaf extract of *M. aboensis*.

Effect of ethanol leaf extract of *M. aboensis* on neutrophils (figure 3.5) showed a significant decrease ($p < 0.05$) in all the groups on day 7 and day 14 when compared to the baseline result of day 1 before they were fed with the extract.

Effect of ethanol leaf extract of *M. aboensis* on lymphocytes (figure 3.6) showed a significant increase ($p < 0.05$) in all the groups on day 7 and day 14 when compared to the baseline result of day 1 before they were fed with the extract.

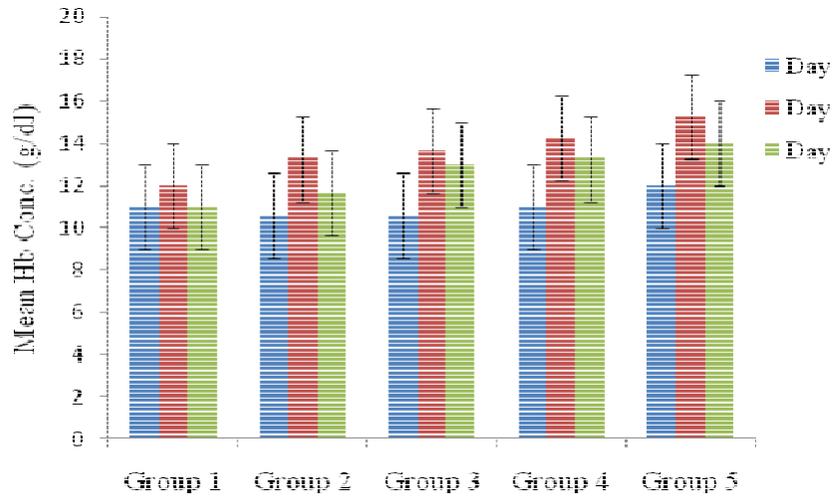


Figure 3.2: Effect of Ethanol Leaf Extract of *M. aboensis* on Packed Cell Volume of Rats

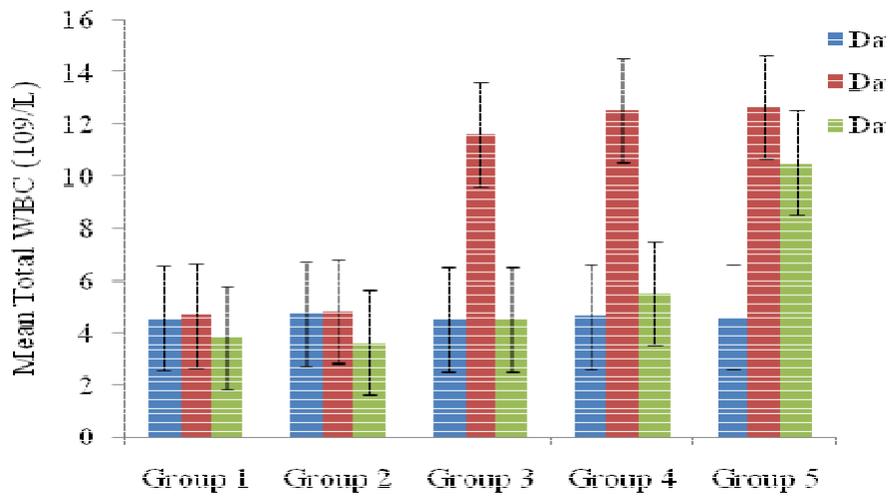


Figure 3.3: Effect of Ethanol Leaf Extract of *M. aboensis* on Total White Blood Cell Count of Rats

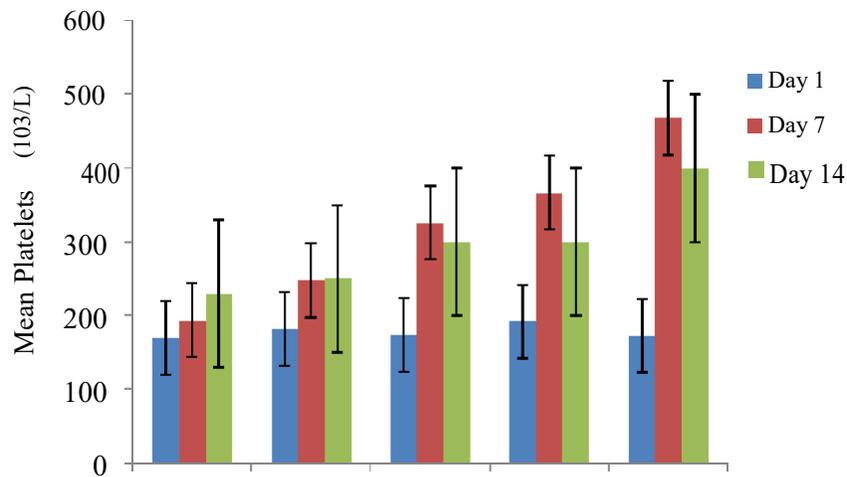


Figure 3.4: Effect of Ethanol Leaf Extract of *M. aboensis* on Platelets Count of Rats

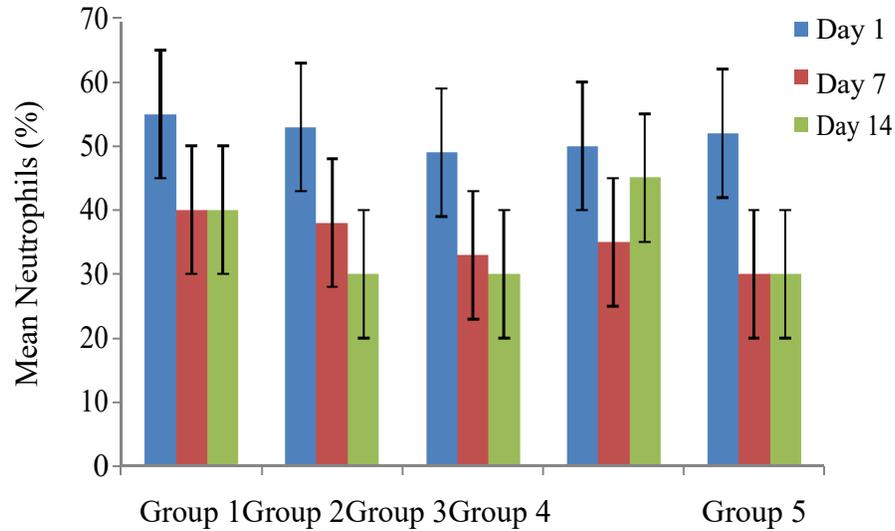


Figure 3.5: Effect of Ethanol Leaf Extract of *M. aboensis* on Neutrophils of Rats

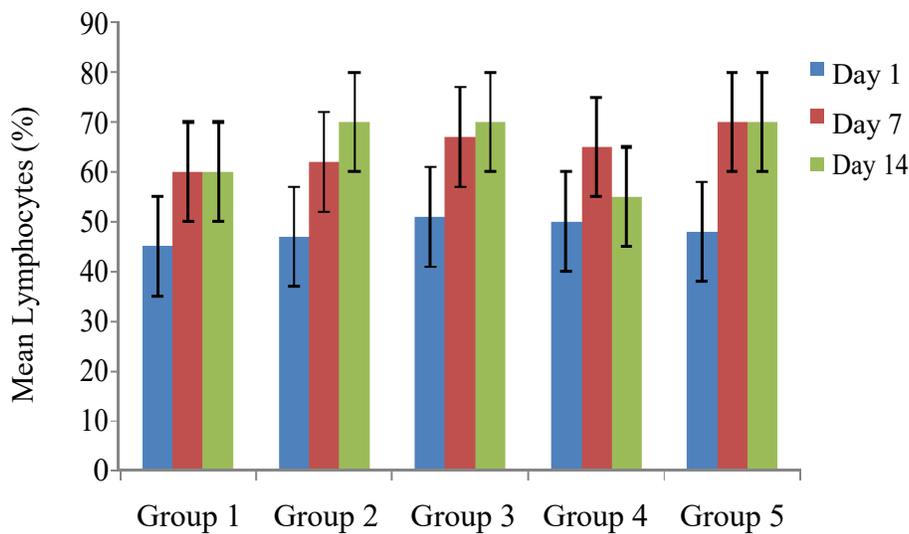


Figure 3.6: Effect of Ethanol Leaf Extract of *M. aboensis* on Lymphocytes of Rats

DISCUSSION AND CONCLUSION

Result of the phytochemical screening of the crude extract of *M. aboensis* showed that the plant is rich in flavonoids, tannins, saponins, and cardiac glycosides. According to Sofowara (1993), a plant containing all these constituents is known to show medicinal activity as well as exhibiting physiological activity. Brune *et al.*, (1989) stated that the anti-inflammatory effect of tannins helps to control all indications of gastritis, oesophagitis, enteritis and irritating bowel disorder. The result may suggest the claimed use of the leaves of this plant as a laxative. Price *et al.*, (1987) reported that saponins have anti-inflammatory and immune stimulating activities and also demonstrate antimicrobial properties particularly

against fungi, bacteria and protozoa. The presence of saponins in *M. aboensis* may be the reason why it is used traditionally for antibacterial and anti-malarial therapy. Flavonoids protect the body from inflammation, allergic reaction and viral infections. They are important in human diet as antioxidants (Buhler and Miranda 2000; Soetan 2008), *M. aboensis* is claimed to be used traditionally for the management of oedema in pregnant women.

The result of the effect of ethanol leaf extract of *M. aboensis* on the haematological parameters showed a significant increase in the Hb concentration and PCV ($p < 0.05$) in groups 5, 4, 3 and 2 (Administered with 5000, 4000, 3000 and 2000mg/Kg body weight of the extract) after seven days of administration of the extract when

compared to the baseline parameters obtained on day 1 in all the groups and with group 6 (Positive control). But, a non-significant difference ($p > 0.05$) in Hb and PCV was observed in group 1 (Administered with 1000mg/Kg body weight of the extract) when compared to group 6 (positive control). A complete blood count is used to assess symptoms such as weakness, fatigue, anaemia, infection and other disorders. Haemoglobin molecule of the red blood cells transports oxygen and gives the blood cell its red colour. The higher the haemoglobin concentration, the higher its ability to transport oxygen in the body. The effect of ethanol leaf extract of *M. aboensis* on total white blood cell count showed a significant increase ($p < 0.05$) in total white blood cell count in groups 5, 4 and 3, when compared to group 6 (positive control). The *M. aboensis* ethanol leaf extract increased the total white blood cell and platelets count in group 5 (5000 mg/kg body weight of the extract), group 4 (4000 mg/kg body weight of the extract) and group 3 (3000 mg/kg body weight of the extract) when compared to group 6 (positive control). But, group 2 (2000mg/kg body weight of the extract) and group 1 (1000 mg/kg body weight of the extract) showed no significant difference ($p > 0.05$) in total white blood cell and platelets count when compared to group 6 (positive control). This is also consistent with the work of Ambi *et al.*, (2006) that showed the potency of *Moringa oleifera* leaf extract in increasing white blood cell counts in rat.

Following plant extract administration, the level of Hb concentration and its related indices were appreciably improved especially from 2000 mg/kg. This gives an indication that the plant extract may contain some phytochemicals that can stimulate the formation of secretion or erythropoietin in the stem cells of the animals. This study suggests that ethanolic leaf extract of *M. aboensis* may increase the rate of Erythropoiesis. This could possibly mean that the extract possesses stimulatory effect on red blood cell production and could probably be used as a therapeutic agent in the treatment of anaemia.

Results from this study suggest that the ethanolic leaf extract of *M. aboensis* altered the activities of the haemopoietic system of group 5, 4 and 3 of the rats, which had the highest dose administered but did not alter much of the activities in groups 2 and 1 which received the lowest doses.

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