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Effect of Stem Bark Ethanolic Extract of Terminalia mantaly (Umbrella Tree) on Haematological Indices, Biochemical Indices and Histopathology of Albino Rats

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Abstract- ***This study was carried out to determine the sub-chronic toxicity of stem bark ethanolic extract of Terminalia mantaly on Albino Rats. The plant extract was prepared by cold maceration of the powder in ethanol for 48 hours and filtered. Albino rats were obtained grouped into three groups of five rats each with the control group and administered the extracts for 28 days. On 29th day, blood was collected to determine the haematological and biochemical parameters. Later, the animals were sacrificed, liver and kidney were removed for histopathological analysis to determine the structural changes. The data obtained was analyzed using one way ANOVA and there was no much statistical significance difference when compared with the control. The result showed that there was 0 mortality recorded after administration of the extract from 300mg/kg to 500mg/kg on the animals after 28 days. Animals do not show any major sign of toxicity. The result on the biochemical and haematological indices determination were within the range values when compared with the control group. The histopathological examination of liver and kidney showed normal architectures with no differences in histological and cellular structures of all organs.***

Keywords: Biochemical analysis, Hamatological analysis, Histopathology, Terminalia mantaly

I. INTRODUCTION

Terminalia mantaly also known as Umbrella tree is a plant of the family Combretaceae which is used in traditional medicinal practice in Madagascar, Côte d'Ivoire and Cameroon. Its stem bark and leaves are used for the treatment of dysentery, mouth Candidiasis and postpartum care. The leaves are used in the treatment of malaria [1]. The traditional use of this plant in some African countries in treatment of some illness without undergoing toxicological examination is of major concern. Therefore, it has become profoundly important to investigate the toxicology of this plant to know whether it is safe for use. Terminalia mantaly grows 10-20m with an erect stem and neat, conspicuously layered branches. Bark is pale grey, smooth and rather mottled. Leaves are smooth, bright green when young, in terminal rosettes of 4-9 unequal leaves on short, thickened stems. The length of the stem is up to 7 cm, apex is broadly rounded, and base is much tapered. Flowers are small, greenish, in erect spikes to 5 cm long. The Fruit is small with oval seeds of about 1.5 cm long with no obvious wings [2].

The generic name comes from the Latin 'terminalis' (ending), and refers to the habit of the leaves being crowded at the ends of the shoots [2].

II. MATERIALS AND METHODS

2.1 Collection and Identification of the Experimental Plant

Fresh plant of Terminalia mantaly H. Perrier was collected from the premises of Federal University Birnin Kebbi and was authenticated at the herbarium of the Department of Biological sciences Federal University Birnin Kebbi, Kebbi State, Nigeria.

2.2 Preparation and Extraction of Plant Materials

The fresh leaves, roots and stem bark of Terminalia mantaly were shade dried in the laboratory at room temperature separately. The dried leaves, roots and stem bark were pounded to powder using mortar and pestle separately. Each sample was cold macerated in 70% ethanol at room temperature for 72 hours and then filtered using muslin cloth. The filtrates were dried in a rotary evaporator at 70oC. The extracts were stored in the freezer until required for use.

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2.3 Source of Experimental Animals and Maintenance

Twenty albino rats of both sexes (male and females were used in this study and were obtained from Animal House in Zoology Department of Ahmadu Bello University Zaria, Nigeria and were transported to the General Biology Laboratory, Federal University Birnin Kebbi, Kebbi State, Nigeria. The animals were housed in standard plastic cages at room temperature and moisture under natural environment of 12:12h dark/light cycle. The animals were fed with their feeds and water and allowed to acclimatize for two weeks.

2.4 Sub-Chronic Toxicity Study

The method of Organization for Economic and Community Development (OECD 407) was adopted for this study. Twenty rats were divided into four groups of five rats each. Plant extracts were administered to group I, II and III at 300mg/kg/bw, 400mg/kg/bw and 500mg/kg/bw respectively and group IV (control) were administered water and feed only. The experiment lasted for 28 days. They were observed weekly for changes in weakness, color of the eyes and mortality. The rats were fed with water and food throughout the period of the experiment. Observation was done on daily basis for general symptoms of toxicity such as colour of the eye, colour of the stool, weekness and mortality. Weights of the animals were taken weekly using digital weighing balance. On the 29th day, blood samples were collected for haematological and biochemical tests. The animals were sacrificed and kidney and liver were removed for histopathology.

2.5 Haematological Indices Determination

Blood samples were collected from the rats in each group using syringe and bottle containing ethylenediamine tetra acetic acid (EDTA) as anticoagulant. The haematological composition of the blood was measured using Automated MindrayHaematological Machine to determine haematological parameters such as Packed Cell Volume, Red Blood Cell, White Blood Cell, Hb, MCV, MCH and MCHC.

2.6 Biochemical Indices Determination

Biochemical analysis was carried out using Automated Chemistry Analyzer to determine biochemical indices such as AST, ALT, ALP, Albumin, Glubolin, Glucose and Total Protein. Blood samples were collected from each rat in each group. Blood was collected using syringe in plain bottles. The blood collected was centrifuged at 200 rpm to separate the serum from the blood. The collected serum was introduced into the machine and the reading was displayed between 10-20 minutes.

2.7 Histopathology of the Liver and Kidney

This was carried out using the method described by [3].The animals were subjected to cardiac perfusion with saline under anesthesia. The specimens of the liver and kidney were isolated and stored in a refrigerator. Small portions of the liver and kidney were fixed in 10% neutral buffered formalin. The fixed specimens were processed overnight for dehydration, clearing and impregnation using automatic processor (Sukura, Japan). The specimens were embedded in paraffin blocks using an embedding station and thin sections were made using microtome. The thin sections were stained with Hematoxylin and Eosin. The specimens were mounted and observed under light microscope. Photomicrographs of the samples were recorded for comparison of structural changes and abnormalities in tissue sections.

2.8 Data Analysis

The data on haemotological and biochemical analysis were analyzed using one way ANOVA. The analysis was carried out using statistical software SPSS version 20.5. All data was represented as mean ± standard deviation.

III. RESULTS

3.1 Sub-Chronic Toxicity of Leaves Ethanolic Extract Of Terminalia Mantaly on Albino Rats

The effect of the sub-chronic administration of ethanolic extract of Terminalia mantalyfor 28 days resulted in no death in all the treated and control groups. The extract at concentration of 500mg, 400mg and 300mg produce no signs of toxicity in rats after 28 days post administration. There was no decrease of body weight, no sign of weakness, changes in color of the eyes, changes in appearance of faeces and no mortality was recorded.

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3.2 Effect of Ethanolic Extract of Terminalia Mantalyon Haematological Parameters of Albino Rats For 28 Days

Result of haematological parameters (PCV, Hb, RBC, WBC, MCV, MCH and MCHC) of control and animals treated with sub-chronic doses of the ethanol extract of Terminalia mantalyare presented in Table 1. The rats in control group recorded 42.33±0.57 PCV, 15.66±0.57 Hb,15.66±0.57 WBC, 8.33±0.57 RBC, 58.66±0.57 MCV, 18.66±0.57 MCH and 33.33±0.57 MCHC.

The rats administered with 300mg/kg of the plant extract for 28 days recorded 40.33±0.57 PCV, 14.33±0.57 Hb, 16.00±0.00 WBC, 9.00±0.00 RBC, 59.33±0.57 MCV, 19.00±0.00 MCH and34.00±1.00 MCHC. The rats administered with 400mg/kg of the plant extract for 28 days recorded 42.00±0.00 PCV, 15.66±0.57 Hb, 16.66±0.57 WBC, 9.00±1.00 RBC, 59.66±0.57 MCV, 19.33±0.57 MCH and 34.33±1.52 MCHC. The rats administered with 500mg/kg of the plant extract for 28 days recorded 44.00±1.00 PCV, 15.66±0.57 Hb, 17.33±0.57 WBC, 9.66±0.57 RBC, 60.33±0.57 MCV, 19.66±0.57 MCH and 36.00±0.00 MCHC.

The result of Hb, RBC, MCH and MCHC of animals treated with 300mg/kg, 400mg/kg and 500mg/kg showed no significant difference ($p<0.05$) with the control group. However, 300mg/kg treated group showed decrease Hb count by day 28 compared to the control and other treated groups. The result of WBC and MCV of animals treated with 500mg/kg is significantly different from the control group. However, there is no significant difference ($p<0.05$) between animals treated with 300mg/kg and 400mg/kg with the control group. The result of PCV of animals treated with 300mg/kg is significantly different ($p<0.05$) from the control group but there is no significant difference between animals treated with 400mg/kg and 500mg/kg with the control group. However, 300mg/kg and 400mg/kg treated groups showed decrease PCV counts by day 28 compared to the control group (Table 1).

Table 1: Effect Of Ethanolic Extract Of Terminalia Mantalyon Haematological Parameters Of Albino Rats For 28 Days

	Concentrations (mg/kg) of *Terminalia mantaly* extract			
Blood Parameters	**Control**	**300mg/kg**	**400mg/kg**	**500mg/kg**
PCV	42.33±0.57^{b}	40.33±0.57^{a}	42.00±0.00^{b}	44.00±1.00^{c}
Hb	15.66±0.57^{b}	14.33±0.57^{a}	15.66±0.57^{b}	15.66±0.57^{b}
WBC	15.66±0.57^{a}	16.00±0.00^{ab}	16.66±0.57^{ab}	17.33±0.57^{c}
RBC	8.33±0.57^{a}	9.00±0.00^{ab}	9.00±1.00^{ab}	9.66±0.57^{b}
MCV	58.66 ±0.57^{a}	59.33±0.57^{ab}	59.66±0.57^{ab}	60.33±0.57^{b}
MCH	18.66±0.57^{a}	19.00±0.00^{a}	19.33±0.57^{a}	19.66±0.57^{a}
MCHC	33.33±0.57^{a}	34.00±1.00^{a}	34.33±1.52^{ab}	36.00±0.00^{b}

KEY: Superscripts= means followed by the same superscripts are statistically the same with the control using one-way ANOVA post hoc (Duncan) at $p< 0.05$, others had statistically significant lower antimalarial activity with the control.

3.3 Effect Of Ethanolic Extract Of Terminalia Mantaly On Biochemical Indices Of Albino Rats For 28 Days

The effect of stem bark ethanolic extract of Terminalia mantaly on some biochemical indices of rats treated with sub-chronic doses of the extract for 28 days is presented in Table 2. The rats in control group recorded 4.33±0.57 AST, 3.66±0.57 ALT, 24.66±1.52 ALP, 4.66±1.15 Albumin, 2.00±0.00 Glubolin, 23.00±1.00 Glucose and 6.33±0.57 Total protein. The rats administered with 300mg/kg of the plant extract for 28 days recorded 5.00±1.00 AST, 4.33±0.57 ALT, 25.33±0.57 ALP, 4.33±0.57 Albumin, 1.66±0.57 Glubolin, 22.33±1.52 Glucose and 5.66±0.57 Total protein. The rats administered with 400mg/kg of the plant extract for 28 days recorded 5.33±0.57 AST, 4.66±0.57 ALT, 25.66±0.57 ALP, 4.00±0.00 Albumin, 1.33±0.57 Glubolin, 22.00±1.00 Glucose and 5.33±0.57 Total protein. The rats administered with 500mg/kg of the plant extract for 28 days recorded 5.66±0.57 AST, 5.00±0.00 ALT, 26.00±0.00 ALP, 3.66±0.57 Albumin, 1.00±0.00 Glubolin, 21.66±1.54 Glucose and 5.00±0.00 Total protein.

The result of AST, ALT and ALP showed increasing values in groups treated with 300mg, 400mg and 500mg of the plantcompared to the control group. However, there is no significance difference between the AST, ALT and ALP of treated groups and the control group. The result of Albumin,

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Glubolin, Glucose and Total protein showed decreasing values i n groups treated with 300mg, 400mg and 500mg of stem bark ethanolic extract of Terminalia mantalycompared to the control group. However, there is no significance difference between the Albumin, Glubolin, Glucose and Total protein of treated groups and the control group (Table 2).

Table 2: Effect Of Ethanolic Extract of Terminalia Mantaly on Biochemical Indices of Albino Rats For 28 Days

	Concentrations (mg/kg) of *Terminalia mantaly* extract			
Blood Parameters	Control	300mg/kg	400mg/kg	500mg/kg
AST	4.33±0.57^{a}	5.00±1.00^{a}	5.33±0.57^{a}	5.66±0.57^{a}
ALT	3.66±0.57^{a}	4.33±0.57^{ab}	4.66±0.57^{b}	5.00±0.00^{b}
ALP	24.66±1.52^{a}	25.33±0.57^{a}	25.66±0.57^{a}	26.00±0.00^{a}
Albumin	4.66±1.15^{a}	4.33±0.57^{a}	4.00±0.00^{a}	3.66±0.57^{a}
Glubolin	2.00±0.00^{b}	1.66±0.57^{ab}	1.33±0.57^{ab}	1.00±0.00^{a}
Glucose	23.00±1.00^{a}	22.33±1.52^{a}	22.00±1.00^{a}	21.66±1.54^{a}
Total Protein	6.33±0.57^{b}	5.66±0.57^{ab}	5.33±0.57^{a}	5.00±0.00^{a}

KEY: Superscripts= means followed by the same superscripts are statistically the same with the control using one way ANOVA post hoc (Duncan) at $p< 0.05$, others had statistically significant lower antimalarial activity with the control (Positive).

3.4 Histopathology

Microscopic examination of the tissue sections of liver and kidney of rats that were treated with 300mg/kg, 400mg/kg and 500mg/kg of extracts of stem bark ethanol extracts of Terminalia mantaly showed normal architectures with no differences in histological and cellular structures of all organs. The hepatocytes and central vein of livers of treated groups are similar to the control group (Plate 1a-1d). The glomerular, distal, and proximal tubules of kidneys of treated groups are similar to the control group (Plate 2a-2d).

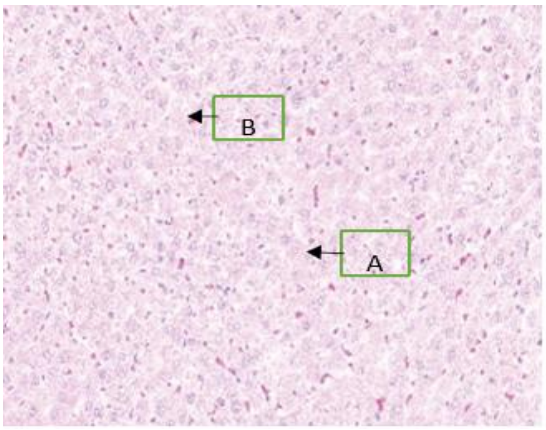

Plate 1a: Photomicrograph of liver of control (Group 4) albino rat treated with water and rat feed showing normal cellular structures of hepatocytes (A) and central vein (B) (H&Ex100).

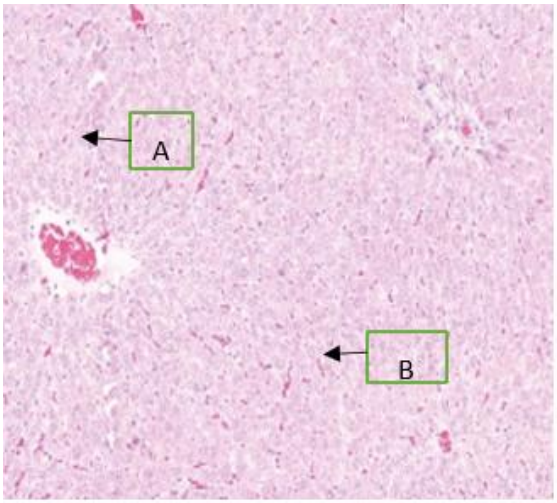

Plate 1b: Photomicrograph of liver of(Group 1)albino rat treated with 300mg/kg extract showing normal cellular structures ofF Hepatocytes (A) and central vein (B) (H& Ex100).

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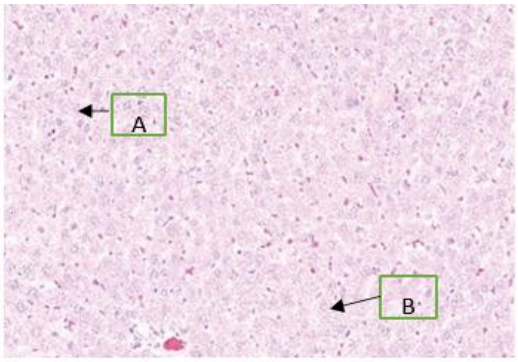

Plate 1c: Photomicrograph of liver of rat treated with 400mg/kg (Group 2) of stem bark ethanolic extract of *Terminalia mantaly* showing normal cellular structures of hepatocytes (A) and centralein (B) (H&Ex100)

vein (B) (H&Ex100).

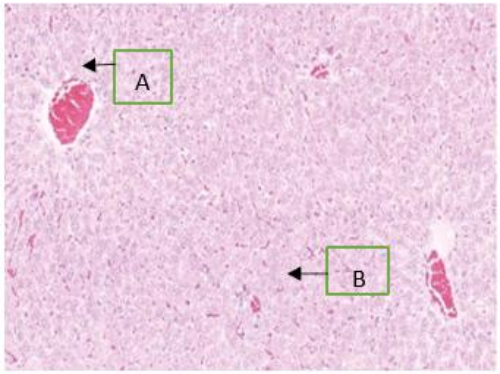

Plate 1d: Photomicrograph of liver of ra t treated with 500mg/kg (Group 3)of stem bark ethanolic extract of *Terminalia mantaly* showing normal cellular structures of hepatocytes (A) and central vein (B) (H&Ex100)

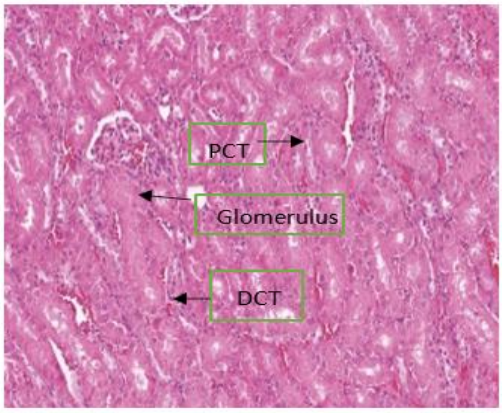

Plate 2a: Photomicrograph of kidney of Positive control (Group 4) albino rat treated with water and rat feed showing the appearance of the glomerular, distal (DCT), and proximal tubules (PCT) (H&Ex100).

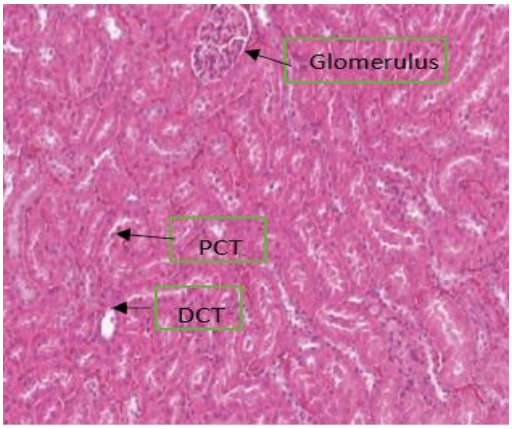

Plate 2b: Photomicrograph of kidney of rat treated with 300mg/kg (Group 1) of stem bark ethanolic extract of Terminalia mantaly showing the appearance of the glomerular, distal (DCT), and proximal tubules (PCT) (H&Ex100).

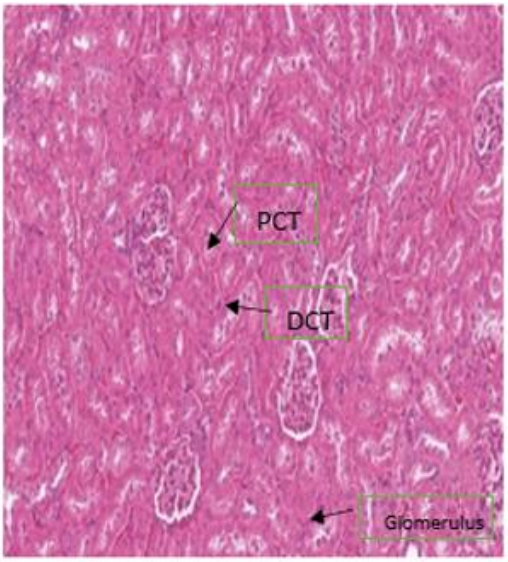

Plate 2c: Photomicrograph of kidney of tatreated with 400mg/kg (Group 2)of stem bark ethanolic extract of *T. mantaly* showing the appearance of the glomerular, distal (DCT), and proximal tubules (PCT) (H&Ex100).

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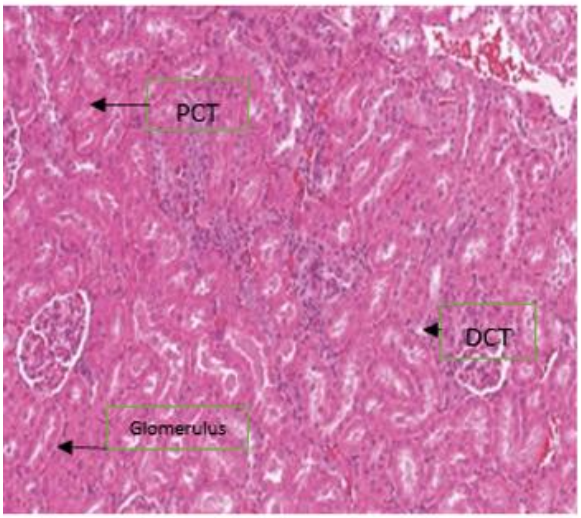

Plate 2d: Photomicrograph of kidney of rat treated with 500mg/kg (Group 3) of stem bark ethanolic extract of *Terminalia mantaly* showing the appearance of t he glomerular, distal (DCT), and proximal tubules (PCT) (H&Ex100).

IV. DISCUSSION

There was no mortality recorded after administration of the stem bark ethanoliic extract from lowest to highest dosage to the animals after 28 days. Animals do not show any decrease in body weight, changes in colour of the eyes, changes in colour of faeces and no sign of weakness among the animals. This agreed with the findings of [4] in a closely related species *Terminalia catappa* and *Terminalia superba* where they reported that there was no death and any adverse effect recorded on mice treated with 2000mg/kg of the extracts and there was no significant difference between the test groups and the control groups.

The haematological test showed that Hb, RBC, MCH and MCHC of animals in treated groups showed no significant difference with the control group but group treated with 300mg/kg showed decrease Hb count by day 28 compared to the control and other treated groups. The result of WBC and MCV of animals treated with 500mg/kg is significantly different from the control group. However, there is no significant difference between animals treated with 300mg/kg and 400mg/kg with the control group. This result showed an increase in values of RBC, WBC, MCH, MCHC and MCV. It agrees with the findings of [5] where they reported increased values of RBC and WBC in animals treated with 400mg/kg of the extract and the positive control but disagrees with the decrease in values of MCV, MCH and MCHC. The increase in WBC is an indicative of leukocytosis. Leukocytosis may be caused by benign conditions such as infections, stress and hemolytic anaemia [6].

The result of PCV of animals treated with 300mg/kg is significantly different from the control group but there is no significant difference between animals treated with 400mg/kg and 500mg/kg with the control group. However, 300mg/kg and 400mg/kg treated groups showed decrease PCV counts by day 28 compared to the control group.

The result of biochemical test showed an increasing values in AST, ALT and ALP but there was no significance difference with the control group. This may be as a result of injury caused by the infection. AST, ALT and ALP are enzymes mainly found in the liver, red blood cells, heart, pancreas, kidneys and biliary ducts of the liver. The levels of AST and ALT in serum are used to diagnose body tissues especially the heart and the liver to see whether they are injured or not [5]. On the other hand, there was a decrease in values of Albumin, Glubolin, Glucose and Total protein but there was no significance difference with the control.

V. CONCLUSION

The stem bark ethanolic extract did not show any sign of toxicity on both haematological and biochemical parameters including histological studies of the liver and kidney when animals were administered with the plant extract at different concentrations for 28 days.The sub-chronic toxicity on this study justifies the safe use of the plant to treat illnesses as no any major signs of toxicity where found on the animals.

VI. RECOMMENDATION

More researches are recommended for the use of Terminalia mantaly to treat diseases by screening the bioactive composition of the plant.

VII. ACKNOWLEDGEMENT

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