Evaluation of the toxic effect of aqueous extract of the bark of *Morinda morindoides* root in male Wistar rats

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Abstract

The toxic effect of the aqueous extract of the bark of the root of Morinda morindoides was studied in 24 sexually matured, male Wistar rats weighing between 150-200g. The rats were randomly divided into four groups (I-IV). Rats in groups I, II and III received 400 mg/kg, 800 mg/kg and I,600 mg/kg of 50mg/ml of the aqueous extract respectively once daily for 28 days while the control group (Group IV) was given distilled water (5ml/kg) once daily for 28 days after which the rats were euthanized. Following euthanasia, about 3 ml of blood was collected and was divided into 1.5ml each for haematology and serum chemistry. In addition, samples of the kidney, liver, heart, lungs and spleen were also harvested for histopathology. Haematological and serum biochemical values were expressed as mean ± standard error of mean and were analyzed using One-way Analysis of Variance (ANOVA) followed by Duncan's multiple range test. Lesions observed in histopathology were scored as mild, moderate or severe. Results were considered statistically significant at 95% confidence interval (P<0.05). In this study, there was no significant difference in the haematological parameters, alanine aminotransferase and aspartate aminotransferase between the treated groups and the control. Histopathologically, the extract caused mild, diffuse degeneration of the liver, mild tubular nephrosis; mononuclear cellular infiltration in the heart and mild hypoplasia of the lymphoid nodules in the spleen of rats to which 1,600 mg/kg of the extract was administered. It was therefore concluded that aqueous extract of the root of Morinda morindoides may produce subchronic toxicity at the dosage of 1600mg/kg.

Keywords: Toxicity, Haematology, Serum, Biochemistry, Histopathology, Morinda morindoides.

Introduction

People in all continents have used hundreds to thousands of indigenous plants for the treatment of ailments since pre-historic times (Huffman, 2003). Sick herbivorous animals have been observed to change their food preferences to nibble at bitter herbs they would normally reject if healthy (Huffman, 2003). Field biologists have provided evidence that lowland gorillas take 90% of their diet from the fruits of *Aframomum melegueta* (a relative of the ginger plant) which is a potent antimicrobial that keeps shigellosis and similar infections at bay (Cindy, 2002)

Many plants synthesize substances such as phenols, tannins, glycosides and alkaloids as part of their normal metabolic processes and they have played important roles in the formulation of many medications and dietary supplements such as Vitamin C. The

use of, and search for drugs and dietary supplements derived from plants have accelerated in recent years (Meite *et al*., 2009). Hence, pharmacologists, microbiologists, botanists and chemists in different countries are continuously researching on phytochemicals that could be developed for the treatment of various diseases (Meite *et al*., 2009) and to integrate them with modern orthodox medicine (Kamboj, 2000).

Nigeria is known for high prevalence of malaria and it is a leading cause of morbidity and mortality in the country (Federal Ministry of Health, 2001). Available records show that at least 50 percent of the population of Nigeria suffers from at least one episode of malaria each year and also accounts for 25 per cent of infant mortality and 30 per cent of childhood mortality in Nigeria (Ejezie *et al*., 1991). Since 75% of the population of Nigeria live in rural areas and do not have access to conventional drugs and even when they do, the drugs are expensive and not within their reach, malaria is often treated in Nigeria by self-medication using local herbs such as *Morinda morindoides* and some rural farmers also use the *Morinda morindoides* extract in the management of their animal diarrhea (Meite *et al*., 2009).

Morinda morindoides belongs to the family Rubiaceae, a family of about 450 genera and 5,500 species, distributed mainly in tropical regions with a small number also seen in temperate areas (Cimanga *et al*., 2006). The plant has long been used in traditional medicine practice of many African countries including Nigeria and is commonly called 'oju ologbo' by the Yorubas of Southwest Nigeria. The decoction of the leaves is used for the treatment of malaria, diarrhoea, intestinal worms, amoebiasis, haemorrhoids, gonorrhoea and rheumatic pains (Kambu, 1990; Tona *et al*., 1999).

Previous studies showed that the ethanolic extract of *Morinda morindoides* exhibited good in-vitro antimalarial activity against chloroquine resistant FcB1/Columbia strain of *Plasmodium falciparum* (Zirihi *et al*., 2005). *In-vivo* studies have also showed the antimalarial activity of *Morinda morindoides* plant (Tona *et al*., 2001). Other documented activities of the plant include antibacterial (Moroh *et al*., 2008), antiamoebic (Tona *et al*., 1999), spasmolytic (Cimanga *et al*., 2009), antifungal (Toure *et al*., 2010), and antidiarrhoea (Meite *et al*., 2009).

Phytochemical investigations on the butanol and ethyl acetate fractions of the leaves of *Morinda morindoides* by Cimanga *et al*., 1995, led to the identification of ten flavonoids which are quercetin, quercetin-7, quercetin-3-0-rutinoside, quercetin-3-0rhamnoside, kaempferol-3-O-rhamnoside, kaempferol-3-0-rutinoside, kaempferol-7-Orhamnosylsophoroside, chrysoeriol-7-Oneohesperodoside, apigenin-7-0-glucoside and luteolin-7-0-glucoside. Also further elucidated were the structures of eight iridoid glycosides among which are gaertneroside, gaertneric acid, methoxy-gaertneroside and epoxygaertneroside (Cimanga *et al*., 2006).

Similar to prescription drugs, a number of herbs are thought to likely cause adverse effects (Talalay and Talalay, 2001). In the previous studies demonstrating the *in-vivo* and *in-vitro* antimalarial activities of *M. morindoides* (Tona *et al*., 2001; Zirihi *et al*., 2005), the possible adverse effects were not elucidated. This study was therefore carried out to find out the toxicological effects of the administration of the aqueous extract of the bark of the root of *M. morindoides* in

Wistar rats.

Materials and Methods

Plant material

The root of *Morinda morindoides* was purchased from Kuto Market in Abeokuta, Ogun State, South-West Nigeria. Identification and authentication was done at the Forestry Research Institute of Nigeria (FRIN), Oyo State, Nigeria, where a voucher specimen of the plant was deposited.

Plant extraction

The plant extraction was done as described by (Iwu and Igboko, 1982 and Iwu 1985). Briefly, the bark of the root of *Morinda morindoides* was washed with clean water to remove extraneous matter and air dried at room temperature for 7 weeks. The plant material was then ground into fine powder and weighed. 500g of the powdered plant material was soaked in 1litre of distilled water with constant stirring using a magnetic stirrer for 24 hours. Subsequently, it was filtered twice using Whatman filter paper (No.1) and the resulting filtrate evaporated to dryness using a rotary evaporator (Stuart 302) and then stored at -4^{o}C until used. A gramme of the dried filtrate was dissolved in 20mls of distilled water to obtain a concentration of 50mg/ml for use on each day of the experiment.

Animals

Twenty four, sexually matured, male Wistar rats (*Rattus norvegicus*) weighing between 150-200g were housed in the Experimental Animal Unit of the College of Veterinary Medicine, University of Agriculture, Abeokuta, Ogun State, Nigeria in well-ventilated metal cages. They were allowed a one-week acclimatization period prior to the study, fed standard ration (Vital feed Limited, Ibadan, Nigeria) and clean water *ad-libitum*. A period of 12 hour light and 12 hour darkness was maintained.

Experimental procedure

Experimental Design

The rats were randomly divided into four groups (I-IV). Aqueous extract of *Morinda morindoides* root bark at doses of (400 mg/kg, 800 mg/kg and 1,600 mg/kg body weight) were administered orally to groups I, II and III respectively for 28 days. The fourth group (Group IV) received distilled water (5ml/kg) orally for 28 days and then euthanized. Prior to euthanasia, about 3ml of blood was collected from the medial canthus of the right eye of each rat into EDTA and plain sample bottles for the determination of complete blood count and serum chemistry respectively. Thereafter, the rats were euthanized by placing them in a glass chamber containing cotton wool soaked in di-ethyl-ether till they lost consciousness. A ventral midline abdominal incision was then made using scalpel blade size 14 to access the internal organs. The kidneys, liver, heart and spleen of each rat were identified, carefully removed and stored in Bouins fluid for histopathological evaluation.

Haematological and Serum Biochemical Examination

For the haematological studies, the PCV was done according to the method described by Schalm et.al (1975), RBC count was determined by the haematocytometry method as described by Jain (1986) and the erythrocyte indices were obtained by calculation. For serum biochemical parameters, total protein (TP) was analysed by the Biuret reaction as described by Gornall *et al.,* (1949), serum bilirubin by diazo reaction as described by Michealson (1961). Alkaline Phosphatase (ALP), aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined according to the improved

methods by Sigma Diagnostics (1985) . Albumin, globulin, urea, and creatinine were done as described by Liberati *et al*., 2004. All other assessments were as described by Varley *et al*., 1991.

Statistical Analysis

Haematological and serum biochemical values were expressed as mean ± standard error of mean and were analyzed using One-way Analysis of Variance (ANOVA) followed by Duncan's multiple range test. Lesions observed in histopathology were scored as mild, moderate or severe. Results were considered statistically significant at 95% confidence interval (P<0.05).

Results

Effect of different doses of aqueous extract of *Morinda morindoides* root bark on the haematological parameters of male Wistar rats

Administration of the aqueous extract of *M. morindoides* at 400 mg/kg, 800 mg/kg and 1600 mg/kg did not produce statistically significant changes (P<0.05) in the haematological parameters of the Wistar rats when compared with the control (Table I).

Effect of different doses of aqueous extract of *Morinda morindoides* root bark extract on the selected serum biochemical parameters of male Wistar rats

Similarly, rats dosed with 400 mg/kg and 800 mg/kg of the aqueous extract of *M. morindoides* root bark showed no statistically significant (P<0.05) changes in the values of the biochemical parameters evaluated except the group III rats dosed with 1,600mg/kg where there were significant increases observed in the values of creatinine, AST, ALT and ALP (1.10±0.2; 69.22 ±11.74; 68.76±6.5 and 161.28±5.9) respectively when compared with the control values (Table II).

There were no significant pathological lesion observed in the slides of the tissues of rats dosed with 400mg/kg and 800mg/kg aqueous extract of *M. mroindoides* but histopathology of the tissues of rats

Table I: Effect of aqueous extract of *Morinda morindoides* root bark on the haematology of male wistar rats.

GROUPS N=6	PCV (%)	RBC Count ($x10^6/mm^3$)	HB (g/dl)	WBC Count ($x10^3/mm^3$)
Group I (400 mg/kg)	29.2±2.7	4.60±0.4	9.90±0.9	3.76 ± 0.4
Group II (800 mg/kg)	26.6±0.6	4.16±1.0	9.06±0.2	3.08±0.4
Group III (1,600 mg/kg)	24.2±1.8*	3.78±0.3	8.28±0.6	3.98±0.1
Group IV(Control)	27.0± 1.5	4.24±0.2	9.22±0.4	3.68±0.4

Values are mean ± standard error of mean
*Superscripted items are statistically significant at P<0.05
PCV = Packed Cell volume
Hb = Haemoglobin Concentration
RBC = Red blood cell
WBC = White Blood Cell

Table II: Effect of aqueous extract of *Morinda morindoides* root bark on the serum biochemistry of male wistar rats

GROUPS N=6	TOTAL PROTEIN (g/l)	UREA (mg/dl)	CREATINE (mg/dl)	ALBUMIN (g/l)	GLOBULIN (g/l)	AST (u/l)	ALP (u/l)	ALT (u/l)	TOTAL BILIRUBIN (mg/dl)
Group I (400 mg/kg)	74.10±4.8	36.64±3.8	0.58±0.0	42.85±2.7	28.08±3.7	31.23±1.4	41.97±8.2	107.30±8.1	0.77±0.2
Group II (800 mg/kg)	74.22± 5.6	31.02±3.9	0.64±0.1	45.09±2.0	29.13±4.9	45.85±6.5	52.44±1.8	127.32±12.5	0.44±0.1
Group III (1,600 mg/kg)	73.55±5.3	34.70±5.7	1.10±0.2*	46.01±2.3	30.70±4.0	69.22 ±11.74*	68.76±6.5*	161.28±5.9*	0.56±0.3
Group IV(Control)	83.50±4.9	36.23±3.8	0.86±010[a]	40.97±1.7	42.14±5.7	37.27±4.3	47.92±9.5	124.00±6.4	1.08±0.3

Values are mean ± standard error of mean
*Superscripted items are statistically significant at P<0.05
AST = Aspartate amino transferase
ALT = Alanine aminotransferase
ALP = Alkaline phosphatase

administered with 1600mg/kg dose of the extract revealed mild, diffuse degeneration and necrosis of the hepatocytes, mild tubular nephrosis of the kidney and thickened epicardium and mononuclear cells infilteration of the heart (Table III)

Discussion and Conclusion

In this study, there was no significant difference in the haematological parameters and serum biochemical parameters between the treated groups and the control excepts for the group of rats treated with 1,600mg/kg dose. Histopathologically, the extract caused mild, diffuse degeneration of the liver (Fig. I), mild tubular nephrosis (Fig. II), mononuclear cellular infilteration (Fig. III) in the heart and mild hypoplasia of the lymphoid nodules in the spleen of rats to which 1,600 mg/kg of the extract was administered. The high dose of 1,600mg/kg may have elicited the significant increase in the serum enzymes of the liver ALT and ALT, AST for the cardiac muscle and also significantly increase the renal biochemical parameter creatinine. These increases in the biochemical parameters may have culminated in the observed pathology of the liver, kidney, spleen and the heart muscle reported in this study.

In the *in-vivo* assessment of the antimalarial

Table III: Effect of aqueous extract of *Morinda morindoides* root bark on the histopathology of some organs in male wistar rats

Organs	GROUP I (400 mg/kg)	GROUP II(800 mg/kg)	GROUP III (1,600 mg/kg)	GROUP IV (control)
Liver	NVL	NVL	mild, diffuse degeneration and necrosis of the hepatocytes	NVL
Kidney	NVL	NVL	mild tubular nephrosis	NVL
Spleen	NVL	NVL	NVL	NVL
Heart	NVL	NVL	thickened epicardium and mononuclear cells infilteration	NVL

NVL = No visible lesion

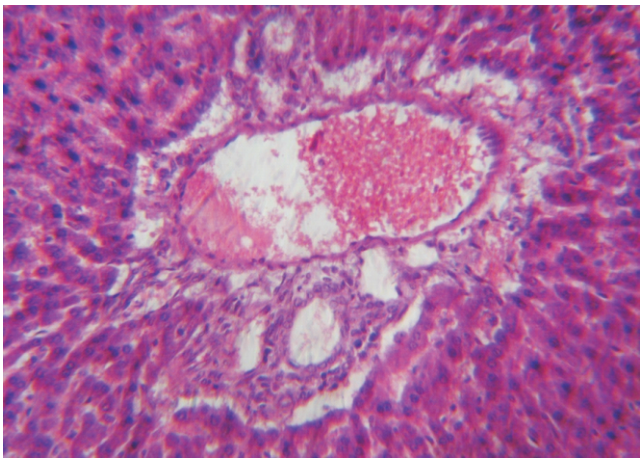

Fig I: showing section of liver with moderate periportal oedema, mild fibrosis with slight bile duct hyperplasia in Group III rats. H & E.X300

activity of *Morinda morindoides* carried out by Tona *et al*., 2001, no mortality was observed in mice treated orally with the extract as a single dose of 500 mg/kg given twice daily for 4 weeks. In another study, the ethyl acetate extract of *M. morindoides* (250, 500 and 1000 mg/kg) was administered orally to three groups of rats to evaluate the activity of the extract against castor oil-induced diarrhoea model in rats (Meite *et al*., 2009). No mortality and visible signs of weakness were observed in the rats following the extract administration of up to a dose of 6000mg/kg. Though, the above studies authenticated the use of the plant in the treatment of malaria as claimed by folklore but did not study possible toxic

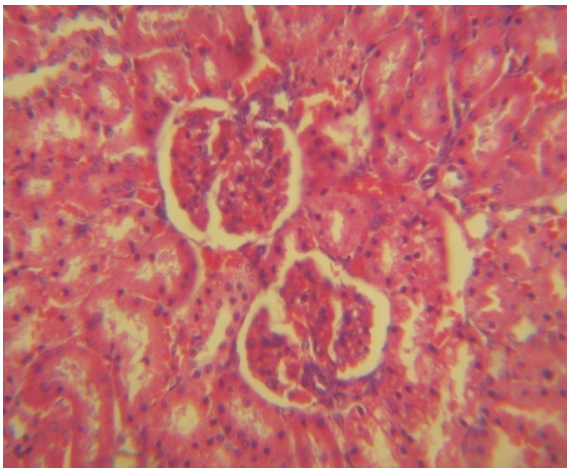

Fig II: showing section of kidney with moderate diffuse areas of nephrosis in Group III rats. H & E X 250.

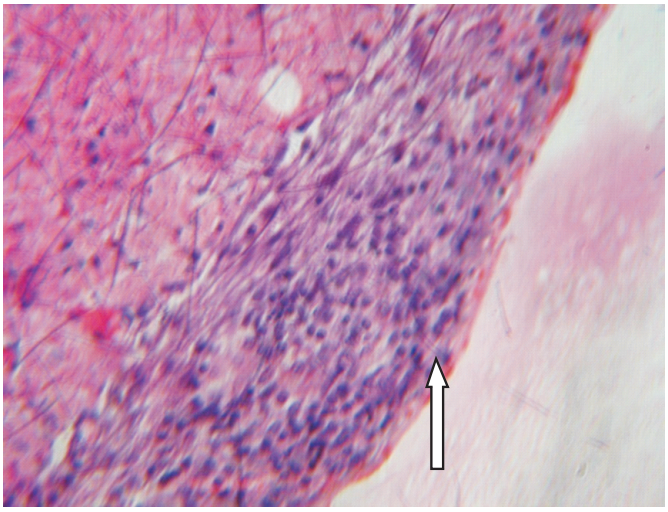

Fig III: Showing section of the heart with thickened epicardium and mononuclear cells infiltration. H & E X200.

effects for the duration of 4 weeks hence the dose ranging from 400 mg/kg to 1,600 mg/kg was used for this study for 4 weeks (28 days).

Liver enzymes (alanine aminotransferase and aspartate aminotransferase) are liberated into the blood whenever liver cells are damaged and enzyme activity in the blood is increased (Edwards *et al*., 1995). The level of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) in the blood were increased significantly at 1,600mg/kg which may indicate that the extract of *M. morindoides* could be hepatotoxic at high doses when administered for a prolonged period of time.

M. morindoides extract has been shown to contain flavonoids. Flavonoids are reported to have antioxidant activity (Ramanathan *et al*., 1989) and are effective scavengers of superoxide anions (Robak and Gryglewski, 1988). The extract may have not been hepatotoxic at low doses due to its antioxidant properties attributable to flavonoids but exhibited toxicity as dose multiplied.

Creatinine level was also increased at 1,600mg/kg dose leading to the observed mild lesion in the kidneys of rats in that group but the tissue sections of the kidneys in the groups of rats dosed with 400 and 800 mg/kg did not show any visible histopathological lesion which implies that the extract may be safe for use at these doses.

In conclusion, at the dose of 1,600 mg/kg, the extract caused mild, diffuse degeneration and necrosis of the hepatocytes of the liver, mild tubular nephrosis in the kidneys; thickened epicardium and mononuclear cells infilteration in the heart and mild hypoplasia of the lymphoid nodules in the spleen of the rats when compared with the control group. This implies that the aqueous extract of the root bark of *Morinda morindoides* has significant toxic potential at the dose of 1600mg/kg, though doses up to 6000mg/kg have been reported not to

cause mortality, toxic effects still manifest which indicate the need for caution in the use of this extract at high doses.

References

Cimanga K., De Bruyne T., Lasure A., Van Poel B., Pieters L., Vanden Berghe D., Vietnck A., Kambu K., Tona L. 1995. In vitro anticomplementary activity of constituents from *Morinda morindoides. J. Nat. Prod.* 58(3): 372-378.

Cimanga R.K, Kambu K., Tona L., Hermans, N., Apers, S., Totte, J., Pieters L., Vlietinck A.J. 2006. Citotoxicity and in-vitro susceptibility of *Entamoeba histolytica* to *Morinda morindoides* leaf extract and its isolated constituents. *Journal of Ethnopharmacology* 107: 83-90.

Cimanga R.K, Mukenyi, P.N.K, Kambu O.K., Tona G.L., Apers, S., Totte, J., Pieters L., and Vlietinck, A.J. 2009. The spasmolytic activity of extracts and some isolated compounds from the leaves of *Morinda morindoides* (Baker) Milne-Redh. (Rubiaceae). *Journal of Ethnopharmacology* 127(2): 215-220.

Cindy E. 2002. Wild Health: How animals keep themselves well and what we can larn from them. Published by Houghton Mifflin Harcourt. Pp 288.

Edwards C.R.W., Bouchier, I.A.D., Haslet, C. and Chilvers, E.R. 1995. Davidson's Principles and practice of Medicine, 17th edn. Churchill Livingstone. 488 – 490.

Ejezie G.C., Ezednachi E.N., Usanga E.A., Gemade E.I., Ikpatt N.W., Alaribe A.A. 1991. Malaria and its treatment in rural villages of Aboh Mbaise, Imo State Nigeria. *Acta Tropica.***48**:17–24.

Federal Ministry of Health 2001. Federal Ministry of Health National Strategic Plan for Roll Back Malaria in Nigeria**.**

Gornall, A.G.; Bardawill, C.J. and David, M.M. 1949. Determination of Serum protein by means of biuret reagent. J. Biol. Chem. (1949)177:751-755.

Huffman M.A. 2003. "Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants". *Proc. Nutr. Soc.* **62**(2): 371–381.

Jain, N.C. 1986. Schalm's Veterinary Haematology. 4th Ed. Lea and Febiger, Philadelphia. p 564-575.

Kamboj V.P. 2000. Herbal medicine. *Current Science.* 78(1): 35-43.

Kambu K. 1990. Elements de phytotherapic compance plants medicinales Africanes CRP-Kinshasa 20-22, 38-39, 40-41, 51-62.

Liberati T.A., Sansone, S.R. and Feuston, M.H. 2004. Hematology and clinical chemistry values in pregnant Wistar Hannover rats compared with nonmated controls. *Veterinary Clinical Pathology*. 33(2):68-73.

Meite S., N'guessan J.D., Bahi, C., Yapi, H.F., Djaman, A.J and Guede Guina, F. 2009. Antidiarrheal activity of the ethyl acetate extract of *Morinda morindoides* in rats. *Tropical Journal of Pharmaceutical Research.* 8: 201-207.

Michealson, M. 1961 Bilirubin determination in serum and Urine. *Scand. J. Clin Lab. Invest*. (Suppl. 56) 13:1

Moroh J., Bahi C., Dje K and Loukou G.F. 2008. Study of the antibacterial activity of the extract acetate (EAC) of *Morinda morindoides* (Baker) milne-redheat (rubiaceae) on in vitro growth of *Escherichia coli. Bulletin de la*

Societe Royale des Sciences de Liege. 77: 44-61.

Ramanathan R., Lau, K.K. and Das, N.P. 1989. Antiperoxidative action of flavonoids and related products in ground pork. Proceedings of 111 international symposium on flavonoids in biology and medicine, Singapore 56.

Robak K. and Gryglewski, R.J. 1988. Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol.* 37:837 – 841.

Schalm, O.W.; Jain, N.C., and Carroll, E.J. 1975. Veterinary Haematology 3rd Ed. Lea and Febiger, Philadelphia p 221-227.

Sigma Diagnostics 1985. Quantitative Colorimetric Determination in Serum, Plasma or Cerebrospinal Fluid (Procedure No. 505)

Talalay P. and Talalay P. 2001. The Importance of Using Scientific Principles in the Development of Medicinal Agents from Plants. *Academic Medicine.* 76(3):238.

Tona L., Kambu K., Mesia, K., Cimanga, R.K, Apres, S., De Bruyne, T., Pieters, L. Totte J., Vlietnick A.J. 1999. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. *Phytomedicine*. 6(1):59-66.

Tona L., Mesia, K., Ngimbi, N.P., Chrimwami Okond'Ahoka B., Cimanga, K, De Bruyne T., Hermans, N., Totte, J., Pieters, L. and Vlietnick A.J. 2001. In-vivo antimalarial activity of *Cassia occidentalis, Morinda morindoides and Phyllanthus niruri. Annals of Tropical Medicine and Parasitology.* 95(1): 45-57.

Toure A., Bahi, C., Bagre, I., N'Guessan, J.D., Djaman, A.J. and Coulibaly, A. 2010. In vitro antifungal activity of the soap formulation of the hexane leaf extract of *Morinda morindoides (Morinda;* Rubiaceae). *Tropical Journal of Pharmaceutical Research.* 9(3): 237-241.

Varley H., Gewenlock,A.H and Bell M. 1991. Practical clinical biochemistry, vol.1, 5th edition. CBS publisher and distributors., Delhi. 741-742.

Zirihi G.N., Mambu, L., Guede-Guina, F., Bodo, B., Grellier P. 2005. In vitro antiplasmodial activity of 33 West African Plants used for treatment of malaria. *Journal of Ethnopharmacology.* 98:281-28

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