

# Hematological and Histological changes induced on the Selected Organs of (Wistar) Rats by leaf extracts from herbs of ethno medicinal application

Sharif B Hauwa<sup>1</sup> \* Gabi Baba<sup>1</sup>, Habila Yusuf<sup>1</sup>

<sup>1</sup>Department of Applied Science Kaduna Polytechnic, Kaduna - Nigeria

email: [alhasbaba@yahoo.co.uk](mailto:alhasbaba@yahoo.co.uk)

## ABSTRACT

*This study focused on the adverse effects of the extract of a mixture of 11 different herbs on the hematology and histology of liver, and kidney of rats. 20 rats were grouped into four different groups of 5 and were administered concentrations of 100mgkg<sup>-1</sup>, 200mgkg<sup>-1</sup>, 400mgkg<sup>-1</sup> and the last group as control for 29 days. The Rats were sacrificed and blood collected for hematology test. The platelet level of group treated with 200mgkg<sup>-1</sup> and 400mgkg<sup>-1</sup> decreased with values (22.36±4.88 and 21.90±26.30) as control (31.20 ± 12.70), slight increase in the level of the mean capsular volume in group treated with 200mgkg<sup>-1</sup> (97.33±19.40) and decrease in mean capsular Hemoglobin in animals treated with 400mgkg<sup>-1</sup> (10.20±2.48, 23.75± 10.71). There was a significant increase in the white blood cell of animals treated with 400mgkg<sup>-1</sup> (71.57±3.56) and control group (3.66±3.06). No pathological changes were observed with the group treated with 100 mg extract. Significant pathological changes were observed in liver and kidney at 200 and 400 mg which is suggestive of degenerative or apoptotic effects.*

## INTRODUCTION

Medicinal plants have been used by man since ages in traditional medicine as a result of their therapeutic potential. The researches on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases<sup>1,2,3,4</sup>. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs<sup>5,6</sup>.

This therefore brings about the increasing recognition of herbal medicine as an alternative form of health care. The screening of medicinal plants for their active

compounds has become very significant as that may serve as talented sources of data bank for antibiotic and other ailment prototypes<sup>7,8</sup>.

The Herbal prescriptions and natural remedies is therefore a common practice in developing countries for the treatment of various diseases and this practice is an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy<sup>9,10</sup>.

Traditionally the practice of drug combination is known; this is believed to bring about synergy. One of Such a

combination is that which includes combination from the following plants: *Annona senegalensis*, *Moringa oleifera*, *Syzyguim goineense*, *Mitragyna inermis*, *Securidaca longipedunculata*, *Dichrostacyes chinerea*, *Ximenia Americana*, *Detarium mciroparpum*, *Nauclea diderrichii*, *Cassia Arereh*, and *Jatropha curcas*. Each of these plants is said to be employed in the treatment of different ailments, Ranging from fever to anti-hypertensive<sup>11, 12,13,14,15,16,17,18,19,20,21,22</sup>. Their combination is expected to be more portent when used as remedies. The increase demand for herbal products coupled with the erroneous impression by the people that herbal products are natural and thus less harmful to the body<sup>23</sup>, has brought concern and fear over the quality, efficiency and safety of some of the available natural heals.

More over the toxicities of the most of the commonly used herbal medicine has not been reported and the once reported are said to be aggressive in behavioral changes or hypertension in chronic users<sup>24</sup>. There have been confirmed' cases of renal failure and liver diseases associated with herbal medicine consumption in some country Nigeria inclusive<sup>25, 26</sup>.

Due to the limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies there is the need to design works that looks into the safety of the commonly used plant in our immediate society to further expose the possible associated effects of the continuous use the herbs. This work however looks into the sub chronic effect of the extract of the mixture with

special attention to hematological and histological changes induced on the selected organs of the experimental model.

## MATERIALS AND METHODS

### *Plant materials*

Root and bark of each of the 11 *plants* which include *Annona senegalensis*, *Moringa oleifera*, *Syzyguim goineense*, *Mitragyna inermis*, *Securidaca longipedunculata*, *Dichrostacyes chinerea*, *Ximenia Americana*, *Detarium mcirop arpum*, *Nauclea diderrichii*, *Cassia Arereh*, *Jatropha curcas* were purchased at Kasuwan Barchi market Tudun wada Kaduna state. The plants samples were duly authenticated by a Botanist at applied science department, Kaduna Polytechnic, Kaduna. The roots and barks were collected and washed thoroughly with water and air dried in a shady area at room temperature (25<sup>0</sup>C) for about two weeks. The dried plant parts were pulverized using pestle and mortar. The processed parts were then stored in a brown bottles away from sunlight until required.

### *Extraction*

The 110g powdered plant root and bark combination of the 11 plants was macerated in 500mls of distilled water for 48 hours with an occasional shaking. It was then filtered by the use of cheese cloth, after which it was properly suction filtered and concentrated by the use of water bath at

60°C<sup>27</sup>. The extract was collected in a flask with air tight cover and kept for further tests. The percentage yield of the extract was calculated as follows:

$$\text{Percentage yield} = \frac{\text{weight of N-hexane extract obtained} \times 100}{\text{Total Weight of Sample}}$$

### ***Experimental Animals grouping***

20 Albino Swiss rats of weight 120 -225g of the same sex were obtained from Nigeria Institute for Trypanosomiasis and Onconsosiasis Research (NITR) Kaduna State Nigeria. The animals were kept in constant 12 hours light and dark cycle and maintained at a temperature of 35°C ± 2°C for 14 days for acclimatization. They were maintained on standard animal pellets with enough water. The animals were randomly grouped into four groups of 5 rats each identified as group 1, 2, 3 and 4.

### ***Sub-chronic toxicity test procedure***

#### ***Experimental procedure***

The rats were weighed just before the administration of syrup and just before they were killed. The administration of the extract was performed via oral route of administration as follows:

. Group 1 was the control group and was administer distilled water,

Group 2 was administered the syrup of 100mgkg<sup>-1</sup>, body weight

Group 3 was administered the syrup of 200mgkg<sup>-1</sup> body weight

Group 4 was administered the syrup of 400mgkg<sup>-1</sup> body weight

Over a period of 28days and at the end of the 29<sup>th</sup> day the rats were fasted overnight. The animals were subject to Deep anesthesia with chloroform on the 30<sup>th</sup> day and the organs liver, spleen lung and kidney were obtained and immediately fixed in formaldehyde solution.

### ***Tissue processing***

The liver and kidneys were obtained and trimmed of any adherent tissue and the tissue was preserved in 10% formalin for subsequent histo-pathological examination. Tissue sections of the organs were produced via normal histo-chemical methods of fixation, dehydration, impregnation, embedding, sectioning and staining (with haematoxylin and eosin)<sup>29</sup>

## **RESULTS AND DISCUSSION**

### ***Percentage yield***

The cold maceration give the percentage yield of 3.36% using aqueous as solvent.

### ***Hematological Parameters***

The results showed the hematological profile of the animals treated with 400mgkg<sup>-1</sup> had a decrease in Haemoglobin, Mean capsular Haemoglobin (10.02 2.48 and 23.75 ± 10.71) compared to the controlled group (14.50 ± 1.94 and 30.9 ± 6.31) and in animals treated with 200mgkg<sup>-1</sup> and 400mgkg<sup>-1</sup> there is a significant decrease in the

platelets ( $22.36 \pm 4.88$  and  $21.90 \pm 26.03$ ) compared to animals on control group ( $31.2 \pm 12.70$ ) (table 2). Animals treated with  $200\text{mgkg}^{-1}$  and  $400\text{mgkg}^{-1}$  have a decrease in platelet ( $22.36 \pm 4.88$  and  $21.90 \pm 26.03$ ) compared

to animals on control ( $31.20 \pm 12.70$ ) and also animals treated with  $400\text{mgkg}^{-1}$  have an elevated white blood cell count  $171.57 \pm 3.56$  compared to the control group ( $3.66 \pm 3.06$ ) (table 2),

Table 1. The percentage yield of the extracts

Extract	Percentage Yield
11 different Plant	3.63%

Table 2: The effect of plant extracts on the hematological parameters of the rats after 29 days of treatment.

Parameters	Control (std)	$100\text{mg/kg}^{-1}$	$200\text{mg/kg}^{-1}$	$400\text{mg/kg}^{-1}$
WBC ( $10^3 \text{ UI}^{-1}$ )	$3.66 \pm 3.06$	$4.31 \pm 4.31$	$4.03 \pm 0.50$	$71.51 \pm 3.50$
RBC (!) UI)	$4.25 \pm 0.14$	$14.70 \pm 14.70$	$3.70 \pm 0.63$	$3.97 \pm 2.13$
Haemoglobin(Gdl)	$14.50 \pm 1.94$	$14.70 \pm 14.70$	$11.56 \pm 0.84$	$10.20 \pm 2.48$
Heamatocrit (%)	$42.5 \pm 4.57$	$100.70 \pm 1.70$	$35.6 \pm 1.02$	$36.02 \pm 2.23$
MCV (FL)	$80.86 \pm 1.99$	$34.10 \pm 34.10$	$97.33 \pm 19.40$	$79.40 \pm 24.0$
MCH (pg)	$30.90 \pm 6.31$	$33.90 \pm 33.90$	$31.96 \pm 8.75$	$23.75 \pm 10.71$
MCHC (%)	$33.73 \pm 2.19$	$17.10 \pm 17$	$32.40 \pm 1.90$	$28.82 \pm 7.19$
Platletes	$31.20 \pm 12.70$	$3.80 \pm 3.80$	$22.36 \pm 4.88$	$21.90 \pm 20.03$

Table 3: The mean body weight of the treated and control group after 29days of treatment with the preparation

Treatment	Day 0	Day 14	Day 28	% increase body weight
Control (std)	$95.36 \pm 52.58$	$150.35 \pm 3.8$	$189.33 \pm 34.94$	74.57
$100\text{mg/kg}$	$148.41 \pm 19.56$	$161.10 \pm 18.16$	$217.8 \pm 217.80$	27.65
$200\text{mg/kg}$	$180.02 \pm 14.75$	$190.86 \pm 111.70$	$221.2 \pm 44.98$	14.45
$400\text{mg/kg}$	$206.12 \pm 25.06$	$209.14 \pm 60.85$	$208.6 \pm 87.19$	1.38

The result are in mean  $\pm$  sem.

Table 4: The mean relative organ weight of liver and kidney after 29 days of treatment with the herbal preparation

Organs(Ref Val)	Control (std)	100mg/kg <sup>-1</sup>	200mg/kg <sup>-1</sup>	400mg/kg <sup>-1</sup>
Liver (0.82)	0.39± 0.10	0.37±0.37	0.38±0.05	0.49±0.21
Kidney(0.76)	0.46 ±0.36	0.51±0.51	0.38±0.24	0.81±0.35

The results are in mean ± SEM.n NO: of rats/group

***The effect of the syrup on the percentage average weight of the treated and control group after 29days of treatment with the preparation***

The results showed progressive increase in the average body weight of the animals in the control groups up to 74.57%. While the treated groups showed inverse proportion in the average body weight between 27.65% for group treated with 100 mg/kg to 1.3% for group treated with 400mg/kg(Table 3).

***Mean relative organ weight (MROW)***

The mean relative organ weight of the control for both kidney and liver were not significantly different except for the group treated with 400 mg/kg which are both significantly higher than the normal group (table 4)

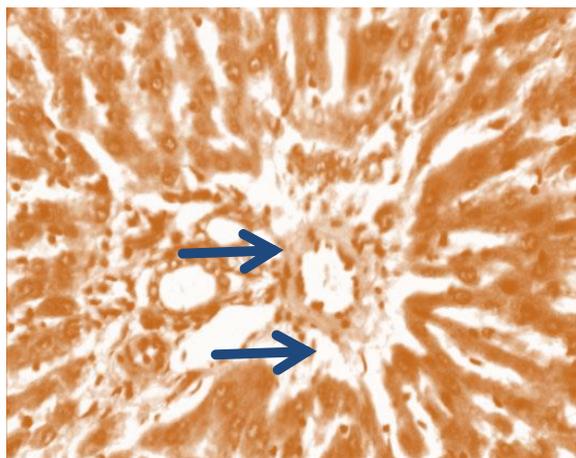
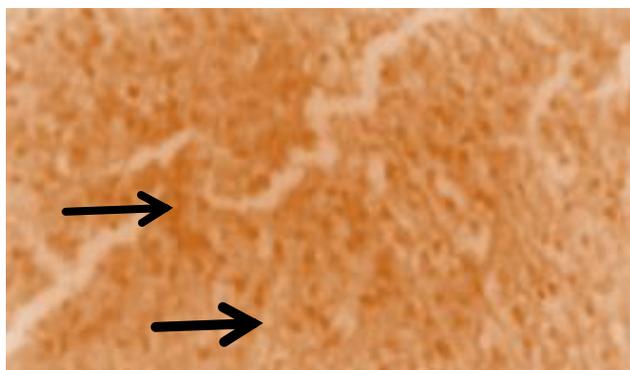


Fig. 1: Micrograph of the liver of a control rat, the central vein, sinusoid, portal triad and hepatocytes. x 400



Fig 2 Micrograph of the liver of a control rat showing normal and the central vein, sinusoid, portal triad and hepatocytes. x 160



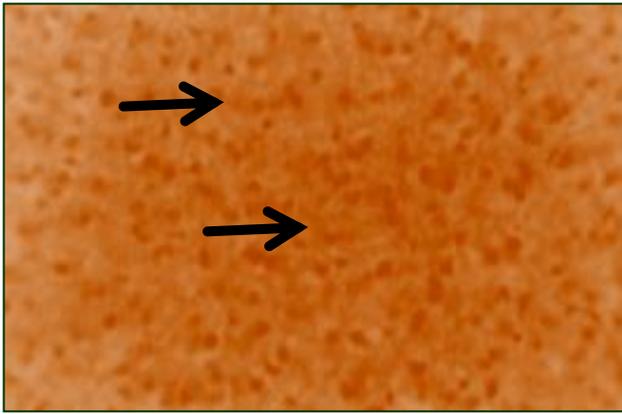


Fig 3 Micrograph of the liver of a rat treated with 100mg/kg body weight of the extract showing Mild- ballooning degeneration. x 160

Fig 4 Micrograph of the liver of rat showing Mild ballooning degeneration of hepatocytes. x 160

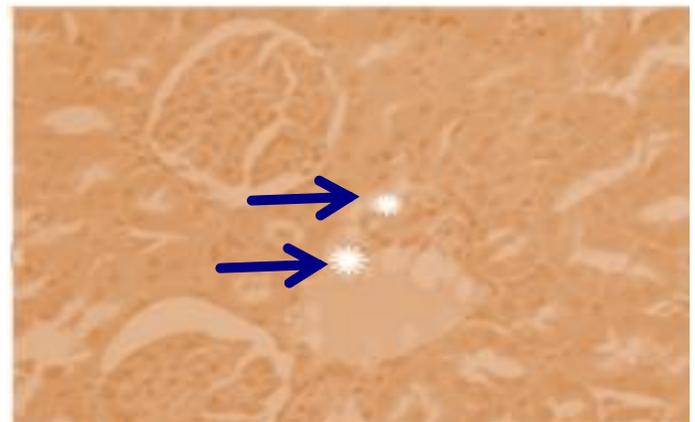


Fig. 6 Micrograph of the Kidney sections of control rat showing normal histology x400

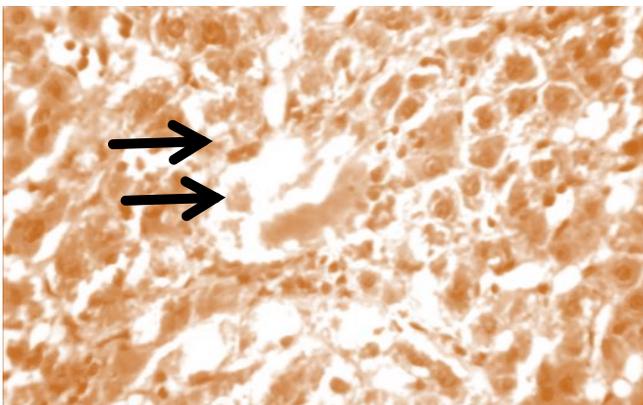


Fig 5. Micrograph of the liver of rat showing Mild ballooning degeneration of hepatocytes. x 200

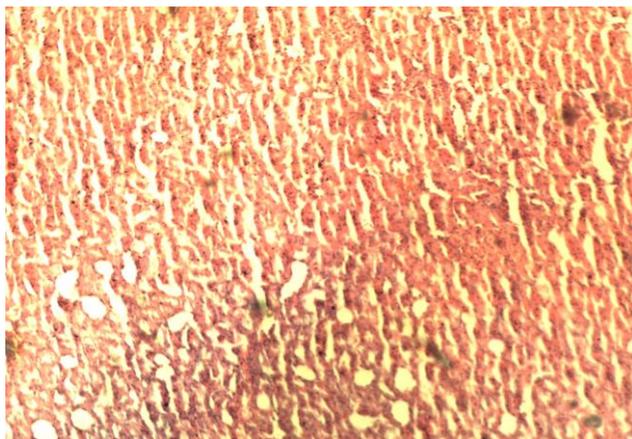


Fig 7 Kidney sections showing severe changes and death of the epithelial cells lining the convoluted tubules. Desquamated epithelium of lumen of tubule

Toxicity studies are considered a vital integral part of drug development considering the fact that herbal medicines are often used erratically without due consideration for the potential adverse effects that could possibly be associated to the use of such herbs<sup>33,34</sup>. After 28 days of treatment with the syrup, there were treatment-related changes in haematological parameters between control and treated groups, the hematological profile of the animals treated with 400mgkg<sup>-1</sup> had a decrease this could possibly lead to interference with the production of both red blood cells and the platelets. According to some researches<sup>29, 30, 31, 32</sup> the hematopoietic system is one of the most sensitive targets of toxic compounds and it is an important index of physiological and pathological status of man and other related animals. This studies revealed decreased effect on the platelets, Mean capsular Haemoglobin and have an elevated white blood cell count when compared to the control group (Tables 1

in Haemoglobin, Mean capsular Haemoglobin (10.02 ± 2.48 and 23.75 ± 10.71) compared to the controlled group (14.50 ± 1.94 and 30.9 ± 6.31) and in animals treated with 200mgkg<sup>-1</sup> and 400mgkg<sup>-1</sup> there is a significant decrease in the platelets (22.36 ± 4.88 and 21.90 ± 26.03) compared to animals on control group (31.2 ± 12.70). Indicating that the extract has some toxic impacts on the circulating red cells, and and 2). Although not much toxicity work had been conducted on most of the plant it can be speculated additional or synergetic effect of some continents herbs might have altered the physiological and pathological state of the animals which results in these changes. *Securidaca longipedunculata* have the ability of altering some of the haemopoietic systems according to acute and sub chronic toxicity analysis carried out by<sup>35</sup>. The present study has shown that treatment of rats with

control and 100mg/kg of aqueous extract of the plant combination did not show significant change on the kidney and but mild ballooning degeneration of the liver (Figure 4 and 5). .But there is significant change on the histology of the liver and kidney such as mild and moderate triaditis in group of rats administered with extracts containing 200 and 400mg/kg/body weight respectively (Fig 3, 5 and 6). It also includes mild lymphocytic infiltrates of the kidney (Fig 7).

## CONCLUSION

From the results it is indicative that consumption of this mixture at high concentrations could jeopardize the physiological state of both liver and kidney when consumed over a long period. The effect is not concentration dependent but speculative time dependent.

## RECOMMENDATION

These scientific evidences show that the extract under study possesses some chemical and pharmacological properties similar to the classes of drugs that are capable of inducing liver and kidney damage and thus, explains their capability to effect the histological changes observed. Therefore, based on the findings of this study, it is recommended that excessive consumption of combination of the herbal mixtures most especially over a long period, is capable of inducing liver and kidney damage and so should be avoided. .Further pharmacokinetic studies are also recommended for better elucidation.

## REFERENCES

1. Evans WC, Trease, Evan's 1997 pharmacognosy 14th ed, UK: WB Saunders Company Limited.
- 2.. Anbuselvi S, Jeyanthi Rebecca L, Sathish Kumar M, Senthilvelan T 2012. GC-MS study of phytochemicals in black gram using two different organic manures. *Journal of Chemical and Pharmaceutical Research.*; 4(2): 1246-1250.
- 3.Makky EA, Mashitah M, Yusoff MM, Ibrahim 2012. Impact of Medicinal Plants Phytocomponents against Antibiotic Resistant Bacteria. *Journal of Chemical and Pharmaceutical Research.*; 4(1): 881-893.
- 4.Kavitha R, Kamalakannan P, Deepa T, Elamathi R, Sridhar S, Suresh Kumar J. *In vitro* Antimicrobial Activity and Phytochemical Analysis of Indian Medicinal Plant *Couroupita guianensis* Aubl. *Journal of Chemical and Pharmaceutical Research.* 2011; 3(6): 115-121.
5. Pierangeli G, Vital G and Rivera W: Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L. f) King and Robinson and *Uncaria perrottetii* (A. Rich) Merr Extracts Medicinal Plants Res 2009; 3(7): 511-518
6. Ammara, H., Salma R., Farah, D. & Shahid, M. (2009). Antimicrobial activity of some plant extracts having hepatoprotective effects. *Journal of Medicinal Plant Research* 3(1):020-023
7. Meurer-Grimes B, Mcbeth DL, Hallihan B, Delph S. 1996; Antimicrobial activity in medicinal plants of the Scrophulariaceae and Acanthaceae.

8. Koduru S, Grierson DS, Afolayan AJ. 2006; Antimicrobial activity of *Solanum aculeastrum*. *Pharmaceutical Biology* 44: 283-286.
9. Sofowora A. 1993. Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd; . Screening Plants for Bioactive Agents 134–156 p.
10. Zhu M, Lew KT, Lieung P. 2002 Protective effect of plant formula on the ethanol induced gastric lesions in rats phytother. Res 16 : 276 - 280
11. Fuglie LJ (1999) The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Tropics. Church World Service, Dakar. 68 pp.; revised in 2001 and published as The Miracle Tree: The Multiple Attributes of Moringa, 172 pp. [http://www.echotech.org/bookstore/advanced\\_search\\_result.php?keywords=Miracle+Tree](http://www.echotech.org/bookstore/advanced_search_result.php?keywords=Miracle+Tree)
12. Shaw BP, and P Jana (1982) Clinical assessment of Sigrú (*Moringa oelifera* Lam) on Mutrakrichra (lower urinary tract infection) *NAGARJUN* 231-235.
13. Tsakala, T.M., Penge, O., John, K., 1996. Screening in vitro antibacterial activity from *Syzygium guineense* (Willd) hydrosoluble dry extract. *Annales Pharmaceutiques Franc,aises* 54, 276–279.
14. Hamil, F.A., Apio, S., Mubiru, N.K., Mosango, M., Bukenya-Ziraba, R., Maganyi, O.W., Soejarto, D.D., 2000. Traditional herbal drugs of southern Uganda, I. *Journal of Ethnopharmacology* 70, 281–300.
15. Oluwole, O.G.A., Pricilla, S.D., Jerome, D.M., Lydia, P.M., 2002. Some herbal remedies from Manzini region of Swaziland. *Journal of Ethnopharmacology* 79, 109–112..
16. Sahpaz, S., Bories, C. H., Loiseau, P. M., Cartes, D., Hocquemiller, R., Laurens, A. and Cave, A. (1994) Cytotoxic and antiparasitic activity from *Annona senegalensis* seeds. *Planta Medica* , 60, 538 – 540.
17. Stevenson, Philip C., Thamara K. Dayarathna, Steven R. Belmain, and Nigel C. Veitch. "Bisdesmosidic Saponins From Roots: Evaluation of Deterrence and Toxicity to Coleopteran Storage Pests." *Journal of Agricultural and Food Chemistry* (2009)
18. Bekele-Tesemma A, Birnie A, Tengnas B. 1993. Useful trees and shrubs for Ethiopia. Regional Soil Conservation Unit (RSCU), Swedish International Development Authority (SIDA)
19. Mishra SB, Mukerjee A, VijyaKumar M. Pharmacognostic and phytochemical evaluation of leaves extract of *Jatropha curcas* *Pharmacognosy Journal*. 2(15); 2010:9-14
20. Musa SM, Abdelrasoo FE, Elsheikh EA, Ahmed LAMN, Mahmoud AEE, Yagi SM. Ethnobotanical study of medicinal plants in the Blue Nile State, South-eastern Sudan. *J Med Plants Res*. 2011;5(17):4287-4297.
21. Chenu J. 1992. *Plantes Medicinales tropicales et camerounaises*. Ed. Berrebi Rene-Rouche Veronique. Tome 1. 214p.
22. Tanda J., 1995. La phytothérapie du diabète au Cameroun. Mémoire D.I.P.E.S. II, E.N.S., Université de Yaoundé I.
23. Raskin Ilya, David M. Ribnicky, Slavko Komarnytsky, Nebojsa Ilic, Alexander Poulev, Nikolai Borisjuk, Anita Brinker, Diego A. Moreno,

- Christophe Ripoll, Nir Yakoby, Joseph M.O'Neal, Teresa Cornwell, Ira Pastor and Bertold Fridlender, 2002 Plants and human health in the twenty-first century *TRENDS in Biotechnology* 20 (12) pp 522- 531
24. Kennedy, D.O. and Scholey, A.B. (2003) Ginseng: potential for the enhancement of cognitive performance and mood. *Pharmacology Biochemistry and Behavior* **75**, 687-700.
  25. Calixto, J.B., 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicine (phytotherapeutic agents). *Braz. J. Med. Biol. Res.*, 33: 179-189..
  26. Etuk E.U., Igbokwe V., Ajagbonna O.P and Egua M.O., 2009. Toxicological Studies of a Nigerian Commercial Polyherbal Product in Albino Rats. *Research Journal of Medicinal Plant*, 3: 52-60..
  27. Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa*, 2nd Edn. New York, NY: John Wiley and Sons.
  28. Danladi, A. U. Dahiru, Mairiga. A.A. and K. B. Abayomi 2012 Comparative Study of the Hepatoprotective Effect of Ethanolic Extract of *Telfairia Occidentalis* (Ugu) Leaves and Silymarin on Paracetamol Induced Liver Damage in Wistar Rats *Int. J. Anim. Veter. Adv.*, 4(4): 235-239,
  29. Adeneye, A.A., Ajagbonna, O.P., Adeleke, T.I., and Bello, S.O. (2006). Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. *J. Ethnopharmacol.* 105, 374-379. doi: 10.1016.
  30. Diallo, B; Vanhaelen- Fastre R, Vanhaelen M, Fiegel C, Joyeux M, Roland A 2010. Further studies on the hepatoprotective effects of *Cochlospermum tinctorium* rhizomes
  31. Diallo, B., Vanhaelen, M., Kiso, Y. and Hikino, H. (1987). Antihepatotoxic actions of *Cochlospermum tinctorium* rhizomes. *Journal of Ethnopharmacology*. 20(3):239-43
  32. Harper HA. Review of Physiological Chemistry, 14th ed. Lange Medical Publications, California, 1973.
  33. World Health Organization (1987). Principles for the Safety Assessment of Food Additives and Contaminants in Food. IPCS Environmental Health Criteria 70. Geneva: World Health Organization
  34. World Health Organization (2000). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva: WHO Press, World Health Organization
  35. Etuk, E.U., Ugwah, M.O., Ajagbonna, O.P. and Onyeyilli, P.A. (2009) Ethnobotanical survey and preliminary evaluation of medicinal plants with anti-diarrhea properties in Sokoto State, Nigeria. *Journal of Medicinal Plants Research*. 3(10):763-766