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Phytochemical and Pharmacological Basis for the Ethnomedicinal Use of Root Extracts from *Anogeissus leiocarpus* as an Antidiabetic in Eastern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOO was largely responsible for the design, collection of plant materials and execution of the experiment as well as writing of the initial draft of manuscript. Author POO was responsible for the overall supervision of the work and editing of manuscript. Author WOO participated in the research design and statistical analysis while author CEU assisted with the plant identification alongside A. O. Ozioko and also formed part of the editorial team. All authors read and approved the final manuscript.

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ABSTRACT

Purpose: To obtain and validate evidence for or against the continued traditional use of *A. leiocarpus* root extract as an antidiabetic.
Methods: Aqueous methanol, ethanol, trona-treated ethanol extracts of the plant whole root, were screened for antidiabetic activity using alloxan-induced diabetic rats at three different oral dose

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levels (100-500 mg $kg^{-1}day^{-1}$) against standards. Phytochemical and acute toxicity tests were also carried out on the extracts.
Results: Tannins, saponins, carbohydrates occurred in very high amounts while steroids, terpenoids alkaloids, flavonoids and resins were moderate. The extracts exhibited a dose-dependent potent and statistically significant ($p<0.05$, ANOVA) antidiabetic activities up to the dose of 200 mg kg^{-1} compared to the untreated group with a glucose reduction values of 52.69±5.99% and 66.61±5.79%, respectively, at 12 and 24 h compared to 24.39±1.54% and 38.79±4.06% recorded for the positive control. Above 200 mg/kg dose, the extract did not produce additional activity. The calculated LD_{50} values of all the extracts were above 10,000 mg kg^{-1} in mice.
Conclusion: This study validates the traditional use of root extracts of *A. leiocarpus* as a potent, cheap and alternative antidiabetic remedy.

Keywords: A. leiocarpus; combretaceae; alloxan-induced; aqueous methanol; ethanol; trona-treated.

1. INTRODUCTION

Plants are complex systems capable of making diverse compounds that have complex chemical structures and a variety of physical and biochemical properties. Repeatedly, natural products have been proven as unmatchable sources of molecules for effective treatment and mitigation of disease burdens of man and animal [1]. In fact, Africans foresee future with a fast growing dependence on herbal medicines for the health needs of world's populations, especially with the avalanche of advancing research in science and technology- biotechnology, molecular biology, plant genomics, and recently, metabolomics. Most times, the thrust for drug development from nature is driven by several ethnomedicinal claims of the potencies of plant or animal-derived bioactive substances. The ethno botanical uses of plants are diverse in both traditional and veterinary medical practices [2], and the use of plants for medicinal purposes dates back to antiquity [3]. Therefore, the ethno-based evidences for the use of these medicinal plants must be validated through *in vitro*/*in vivo* proof of concept [4,5]. The high proportion (average of 80%) of diverse populations in several African nations depend largely on crude herbal drugs for their health needs mostly owed to economic factors, hunger, crises, violence and poor distribution of adequate health facilities [6]. These stress factors usually precipitates serious health problems amongst Africans, notable of which are hypertension and other cardiac diseases, malaria, HIV/AIDS and diabetes. Of all these, diabetes account for over 0.06% morbidity and mortality worldwide (population of approximately 7 billion people) and usually occurs with severe complications. This projection is expected to double by the year 2030 [7]. This Figure, which is far higher than that of HIV-related deaths is obviously higher in Sub-Saharan Africa where poverty encourages heavy poor dieting (high sugar-high calorie foods) resulting in a high incidence of this disease at almost an epidemic scale. According to the WHO estimates of 2000 for the African region, diabetes alone accounted for over 10% of both mortality and morbidity [8]. In Africa therefore, several plants are used by herbalists for the treatment of *diabetes mellitus* [9] and some have been scientifically validated [10-12]. Recently, in a continued effort and in collaboration with notable herbalists based in eastern Nigeria for the purpose of finding putative antidiabetic plants in Nigeria, several of the herbalists claimed that a plant which was identified and confirmed by taxonomist (Mr. Alfred Ozioko) as *A. leiocarpus* (DC.) Guill and Perr, (Combretaceae), has highly potent antidiabetic activities in humans (oral conversations; 2010). During the collection, one of the herbalists accompanied us to the site. The taxonomist also affirmed that the plant root was used as anti-diabetic agent in his locality.

A. leiocarpus, also known as 'African birch' or 'Axle-wood tree', is a tall evergreen tree native to African savannas of tropical Africa [13]. It is the sole West African species of the genus *Anogeissus*, a genus otherwise distributed from tropical central and East Africa through tropical Southeast Asia [13]. The leaves serve as a fodder to livestock and small branches with leaves are crushed to make one of the yellow dyes [14]. The gum is used to make ink more viscous or to glue leather and is used occasionally as Arabic gum replacement. The gum was used as food additives mixed with gum Arabic or as a substitute for it [15]. It is used in traditional medicine as a remedy for many ailments of livestock and man, which include: helminthosis, schistosomiasis, leprosy, diarrhea and psoriasis [13,16]. Recently, castalagin, a hydrolysable tannin, was isolated from the plant

stem bark and this compound mediated the killing of *Leishmania in vitro* [17]. *A. leiocarpus* is used medically for the treatment of ascariasis, gonorrhea, general body pain, blood clots, asthma, coughing and tuberculosis [18]. It is also used as vermifuges and the leaves decoction is used for washing and fumigation [16]. Uniquely, this plant grows only in swampy areas in Nigeria. Our thorough and intensive literature searches did not show any report of its use as an antidiabetic in folkloric medicine till date. The obvious need to validate this strong folkloric claim became the drive for the present study.

1.1 Aims and Objectives of the Study

The main aim of the present study was to investigate an ethnopharmacological claim that extracts of *A. leiocarpus* possess potent antidiabetic property. In addition, the study had an objective to verify the claim that extraction done in the presence of sodium sesquicarbonate (trona) was better in terms of its antidiabetic activity.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

A. leiocarpus roots and leaves were collected by the researchers in the month of October, 2010 from swampy area in Adani, Uzo-Uwani Local Government Area of Enugu State in south eastern Nigeria in collaboration with one of the practicing herbalist. The roots were identified and certified by Mr. Alfred O. Ozioko, a taxonomist with the Bioresources Development and Conservation Programme (BDCP) centre in Nsukka. Voucher specimens of the plant parts have been deposited at the centre and the herbarium unit of the Pharmacognosy and Environmental Medicine Department of our Institution with the numbers BDC-10-032 and PHE-07-2010, respectively. The plant parts were cleaned, shade-dried and pulverized. This was stored in a polyethylene bag at room temperature (27±3℃) prior to use.

2.2 Chemicals/Reagents Used

Glibenclamide (glanil®) and pure sample (Nigerian German Chemicals), Alloxan, Tween 80, Diphenylpicryl hydrazine (DPPH), ascorbic acid, absolute ethanol, absolute methanol, DMSO were obtained from Sigma Aldrich. Normal saline and dextrose saline were obtained from DANA, Nigeria Limited), and trona ("Akanwu") was obtained from a large market near our institution.

2.3 Animals Used

Batches of albino mice (20-33 g) or rats (120-165 g) of both sexes were procured from the animal house, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, and from the animal house, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were kept in standard laboratory conditions and fed with pelletized rodent commercial diet (vital feed Nig., Ltd) and water *ad libitum* throughout the study. They were exposed to 12 h light-dark cycle. This investigation was conducted following an approval by the relevant ethical Committee on laboratory animal use and international rules were observed. Specifically, the experimental protocols were executed in accordance with the guidelines of the Ethics Committee of the University of Nigeria as registered by the National Health Research Ethics Committee of Nigeria (as per the approved ref: NHREC/05/01/2008B). The animal care and handling was in line with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) [19].

2.4 Preparation of Crude Aqueous-Methanol Extract

The whole roots *A. leiocarpus* were cleansed and dried under shade for 8 days. They were pulverized in mechanized laboratory grinder (MANESTY-F520, England) to fine powder. A total of 200 g of the powder was extracted in batches with absolute ethanol; first wetted with 200 ml of absolute ethanol and the actual extraction carried out with another 300 ml of aqueous-methanol using Soxhlet extractor for 24 h. The resulting ethanol extract was evaporated to dryness under vacuum at 40±5℃ to afford dry extract which was weighed and its percentage yield was calculated. The trona-treated ethanol extract was obtained similarly except that 2.5% w/w (2.5 g/100 g powdered plant material) was added to the extracting mixture in the Soxhlet. The dry extract was placed in a clean plastic container and stored in a refrigerator until use. This process was repeated for all other host trees.

2.5 Acute Toxicity Tests (LD_{50}) of Extracts

Acute toxicity tests were performed using the Lorke's method (1983) with the aqueous-methanol extracts [20]. Essentially, this method involves an initial dose range determination stage in which nine mice were used (three animals per treatment group). The aqueous-methanol extract was dissolved in 3% tween 20 solution and doses of 10, 100, and 1000 mg/kg were administered intraperitoneally to the respective groups of mice. The animals were then observed for 24 h. No deaths occurred in any of the animal after 24 h and the second stage was carried out. Doses of 5000 and 10000 mg/kg were administered to two groups of one animal per group. The animals were again observed for 24 h. LD_{50} was then calculated as geometric mean of the highest dose that did not kill any animal and the largest dose that killed all the animals. This was done with all the extracts.

2.6 Phytochemical Test

This was carried out as described by Harborne [21]. All reagents for the phytochemical test were freshly prepared following standard procedures.

2.7 Anti-diabetic Evaluation

The effects of the ethanol extract of *A. leiocarpus* containing trona ("akanwu") on alloxan-induced diabetic rats was carried out following a modification of the method by [22]. Briefly, 20 rats were used for the experiment. The animals were acclimatized and fed with water and commercial animal feed (Top Feed Limited, Nigeria) pellets. They were then fasted for 12 h. The basal glucose concentration was checked before the induction of diabetes. Diabetes was induced by intra-peritoneal administration of 140 mg/kg body weight of alloxan monohydrate (90.0 % pure) freshly prepared in normal saline. The rats were then fed normally and dextrose water added to their drinking water until after 48 h of the induction of diabetes. Blood samples were collected from the tail vein of the rats and blood glucose concentration at 0 h was determined using Accu-Chek® Active glucometer. The diabetic rats with blood glucose level greater than 150 mg/dl were grouped into five groups (n=4). The five groups were then treated as follows: The first group (positive control) received 15 mg/kg body weight glibenclamide dissolved in 3% Tween 80 solution, the ethanol extract of *A. leiocarpus* containing trona dissolved in 5%. DMSO was given to the second, third and fourth groups at a dose of 100, 200, and 400 mg/kg body weight, respectively. The last group (negative control) received only 0.5 ml of 5% DMSO solution. Blood samples were collected from the tail vein of the rats and blood glucose concentrations were determined at 0, 1, 3, 5, 6, 12 and 24 h using Accu-Chek® Active glucometer. Similar procedures were repeated for the aqueous methanol and ethanol extracts. The results were recorded accordingly.

2.8 Statistical Analysis

The results obtained were recorded as were the mean values with the standard error in mean (SEM) and statistical significance between treated and control groups were evaluated by the Students' *t*-test and one way analysis of variance (ANOVA; Fischer LSD *post hoc* test). Differences between means of treated and control group and also between solvents at P=.05 was considered significant.

3. RESULTS

The extraction process by Soxhlet method afforded yields of 14.64, 15.76, and 16.80% for the ethanol, trona-treated ethanol and methanol extracts respectively. It is obvious that trona showed negligible effect on the overall yield of extract. The use of trona does not therefore, enhance yield during extraction. The result of the preliminary phytochemical test shows that all the obtained extracts of *A. leiocarpus* contain tannins, glycosides, saponins, carbohydrates, flavonoids, resins, alkaloids, terpenoids, steroids and reducing sugars. Proteins, oils and acidic compounds were generally absent in all tested extracts. These phytoconstituents were present in varying amounts. Remarkably, while tannins, glycosides, saponins, carbohydrates and reducing sugar occurred in very high amounts, steroids, terpenoids alkaloids, flavonoids and resins occurred in moderate amounts in all extracts. The acute toxicity tests of the methanol extract following oral administration and using Lorke's modified method, showed the average LD_{50} value to be above 10,000 mg/kg. Obviously, this value signifies high margin of safety in mice when administered orally. The results of the activities of the different doses (100, 200 and 400 mg/kg) of the crude ethanol, the trona-treated ethanol and the methanol extracts on the alloxan-induced diabetic rat are shown as percentage reductions in glycaemia over 12 h in Tables 1, 2 and 3 respectively.

Table 1. Antidiabetic activities of the ethanolic extract of *A. leiocarpus*

	Percentage reduction in blood glucose of rats post treatment (%)					
Treatment (mg/kg)	**1 h**	**3 h**	**5 h**	**6 h**	**12 h**	**24 h**
100	-19.36±6.02	17.95±0.20*	20.98±2.79*	27.82±1.49	52.69±5.99**	41.41±4.11
200	-13.81±7.62	25.23±4.44*	25.54±1.06*	38.00±1.02*	66.61±5.79**	69.77±6.31**
400	-25.18±3.64	-21.44±3.02	-29.35±1.01	21.51±0.30	9.06±2.38	6.62±4.99
GB (15 mg/kg)	-4.60±3.17	-2.21±2.77	6.53±1.32	23.71±1.54	24.39±1.66**	38.79±4.06**
VH (0.5 ml DMSO)	-28.18±3.21	3.59±1.25	11.24±1.03	11.01±1.01	16.06±0.83	33.41±3.72

** ** Significant reduction in hyperglycemia at p=.05 and 0.01 respectively the extract compared to the positive control; GB (Glibenclamide); VH (Vehicle)*

Table 2. Antidiabetic activities of the trona-treated ethanolic extract of *A. leiocarpus*

	Percentage reduction in blood glucose of rats post treatment (%)					
Treatment (mg/kg)	**1 h**	**3 h**	**5 h**	**6 h**	**12 h**	**24 h**
100	-21.24±4.09	18.40±1.30*	21.74±2.32*	29.91±2.18*	54.72±4.85**	40.32 ±3.23
200	-23.32±5.13	22.21±2.11*	23.34±1.09*	36.21±2.77*	65.67±3.65**	67.86±6.31**
400	-25.18±3.62	-27.54±4.15	-27.37±2.04	20.65±1.29	10.05±1.08	8.76±3.48
GB (15 mg/kg)	-15.34±3.30	-6.27±1.45	7.22±0.39*	24.83±2.35*	23.42±2.04*	43.65±4.21**
VH (0.5 ml DMSO)	-27.34±4.65	4.61±0.98	10.78±1.07	11.01±1.17	10.13± 0.72	32.35±2.64

**, ** Significant reduction in hyperglycemia at p=.05 and 0.01 respectively the extract compared to the positive control; GB (Glibenclamide); VH (Vehicle)*

Table 3. Antidiabetic activities of the methanolic extract of *A. leiocarpus*

	Percentage reduction in blood glucose of rats post treatment (%)					
Treatment (mg/kg)	**1 h**	**3 h**	**5 h**	**6 h**	**12 h**	**24 h**
100	-55.09±3.75	65.55±5.17	-55.09±3.75	-41.80±1.55	12.32±7.48**	18.02±6.76
200	-53.50±7.08	-19.07±1.22	-14.57±0.46	-10.31±0.43	10.21±3.66**	17.02±4.80
400	-15.05±0.95	13.80±1.15	19.18±0.26	17.17±0.59**	31.76±1.84	27.35±1.11
GB (15 mg/kg)	-8.18±3.22	-3.44±1.29	11.24±0.01	10.86±0.30**	16.20±0.84	33.40±3.71
VH (0.5 ml DMSO)	-24.60±3.14	-12.21±2.57	-16.53±0.03	-23.71±1.56	31.80±2.92	30.34±2.68

*** Significant reduction in hyperglycemia at p< 0.01 by the extract compared to the positive control, GB (Glibenclamide); VH (Vehicle)*

At the third hour post treatment with the ethanol extract, there was observed, a progressive and significant (p=.05) reduction in glycaemia of the rats the at 100 and 200 mg/kg doses of the aqueous ethanol extract of *A. leiocarpus* with the maximum response produced at 24 h by the 200 mg/kg dose (69.77±6.31% reduction) compared to the unstimulated control (33.41±3.72% reduction) and positive control; Glibenclamide (38.79±4.06% reduction). Similar trends were observed for the responses at the 12th h. Surprisingly, repeated experiments showed that a higher dose of 400 mg/kg did not produce reduction in glycaemia as expected. Trona-treated ethanol extract produced similar trend of activities (reduction in glycaemia) but without any obvious advantage as was claimed by the practicing herbalist (Table 2).

The use of methanol as solvent for extraction was found to produce extracts of moderate activity than the ethanol extracts even at comparatively lower doses. However, higher doses of the methanol extract produced a steady

incremental reduction in glucose level up till the 5th h (Table 3). This suggests that the herbalist's choice of ethanol as solvent for extraction is reasonable.

4. DISCUSSION

The avalanche of claims of the antidiabetic potency of *A. leiocarpus* in human subjects by several herbalists in South Eastern Nigeria and notably from a renowned middle-aged herbalist from Delta State, Nigeria but resident in Nsukka, south eastern Nigeria in 2010 spurred interest to work on this plant (*A. leiocarpus*). According to him, the extracts usually performed better in the reduction of glycaemia compared to established orthodox antidiabetic drugs. Remarkable from his several years of practice, is that, recalcitrant hyperglycemia to orthodox antidiabetic agents usually responded fastly to the very first two dosing with the extract. These were indeed claims that needed reproducible scientific validation. Spurred by these strong claims, we conducted a field study in early part of 2010 and discovered that several of such herbalists used the plant as either part or main constituent of their antidiabetic concoction. As part of evidence, we also discovered that at the site of collection of the plant parts, most of the tree stands visited had at least a root part collected from them and preliminary inquiries from locals led credence that the collections were for antidiabetic use. Furthermore, the root had very fast regenerative power as usually less than 12 weeks is needed for harvested root part to recover fully. Further evidence from blood glucose measurement showed that indeed, the ethanol extracts had potent antidiabetic activity. Placebo effects of solvent were ruled out accordingly. In response to all these interesting findings established from the field study, we set out into this investigative research work. Although this information was discovered with much excitement, the paramount and primary interest to us became that of toxicity. In response to this concern, the acute toxicity of the extracts was evaluated using the Lorke's approach and found that all the methanol and ethanol extracts were very safe with LD_{50} values generally greater than 10,000 mg/kg. The current data obtained did not suggest that the use of sodium sesquicarbonate (trona) in the extraction produced adverse effect on the safety of the extracts. This is in agreement with the practice in most parts of Nigeria; the use of trona to soften hard cereals and reduce its cooking time considerably has not produced any reported toxicity concern for many centuries. Besides, the trona is usually used in comparatively small quantity compared to the volume of extracts. These findings suggest in strong terms, that the extracts of *A. leiocarpus* have very good (wide) margin of safety. Although these data are suggestive of widely safe extracts, the sub-acute and chronic toxicity profiles as well as the cytotoxicity studies should be established in the near future. The obtained yields of 14.64%, 15.76% and 16.80% for the ethanol, trona-treated ethanol and methanol extracts are fairly high compared to other reported yields from roots of plants [23]. This fairly high yield of extracts is in tandem with the observed rich composition of phytoconstituents of *A. leiocarpus*. The result of the preliminary phytochemical test shows that all the obtained extracts of *A. leiocarpus* contain tannins, glycosides, saponins, carbohydrates, flavonoids, resins, alkaloids, terpenoids, steroids and reducing sugars. Proteins, oils and acidic compounds were generally absent in all tested extracts. These phytoconstituents were present in varying amounts. It is remarkable to note that, while tannins, glycosides, saponins, carbohydrates and reducing sugar occurred in very high amounts, steroids, terpenoids alkaloids, flavonoids and resins occurred in moderate amounts in all extracts. As part of evidence for affirmation of these, several of these constituents (saponins, flavonoids, terpenoids, steroids and alkaloids) which occurred in either high or moderate amounts have been reported to exhibit potent antidiabetic activity [24]. In terms of antidiabetic potencies, the crude ethanol extract of *A. leiocarpus* showed marked activity which was fairly dose-dependent up to the 200 mg/kg body weight dose. Above this dose, there was an aberrant reduction in activity with increasing dose level. At the third hour post treatment with the ethanol extract, there was observed, a progressive and significant ($p<0.05$) reduction in glycaemia of the rats at the 100 and 200 mg/kg doses of the aqueous ethanol extract of *A. leiocarpus* with the maximum response produced at 24 h by the 200 mg/kg dose (69.77±6.31% reduction) compared to the unstimulated control (33.41±3.72% reduction) and positive control; Glibenclamide (38.79±4.06% reduction). This shows that the herbalist claims are supported by the present data as the extracts at these doses performed even better than the positive drug, Glibenclamide. At the dose of 400 mg/kg, the ethanol extract exhibited significant activity at 3 h post treatment but became less active after 12 h. This aberrant behaviour could not be completely

explained by the present data but it is well know that certain plant metabolites usually exhibit biphasic pharmacological activities pattern. Although, the methanol extract was higher in yield, it produced a haphazard pattern in the reduction of glycaemia with the 100 mg/kg body weight dose producing the highest reduction at 3 h post treatment. At present, we are unable to explain the basis of this aberrant behavior. Nevertheless, it further supports the use of ethanol as the better extracting solvent as claimed by the herbalist. The activity spectrum of the ethanol extracts is similar to the positive drug, Glibenclamide that has established long duration of action [25]. The present data did not provide concrete evidence to support the use of trona during the extraction stage as there was no marginal advantage recorded from its use. Chemically, the basic nature of trona could enhance the extraction process by sequestering acidic constituents such as flavonoids or enhancing the solubility of basic constituents (like alkaloids) in the plant root and free them for extracting solvent. This however, is a speculation that needs further verification. In summary, the present data represents an exciting finding supporting the use of ethanol extracts of *A. leiocarpus* as a potent antidiabetic in ethnomedicinal practice of eastern Nigeria. This is in addition to other numerous activities associated with this plant root extract.

5. CONCLUSION

In conclusion, the crude ethanol extract of *A. leiocarpus* possesses potent anti-diabetic activities, and therefore can be potentially used as safe alternative in the treatment and management of *Diabetes mellitus*. The use of trona in the extraction process did not yield any statistically significant antidiabetic activity compared to the ethanol extract alone. Its use in the preparation of the extracts is therefore discouraged.

CONSENT

It is not applicable.

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COMPETING INTERESTS

The authors declare that there is no conflict and or competition of interest(s) in this research work. Furthermore, the authors have the full support of the herbalist for the present research work and any other work that may emanate from it.

REFERENCES

1. Perry LM. Medicinal plants: Attributed properties and uses. Cambridge: MIT Press. 1980;10-15.
2. Agunu A, Ibrahim NDG, Onyiloyi GA, Abdurrahman EM. Toxicity of stem bark extract of *Steganoteaenia araliacea* in rats. Nigerian J Nat Prod Med. 2003;7:65-67.
3. Ogunyemi AO. The origin of herbal cure and its spread. In: Sofowara A, editor. Proceedings of a Conference on African medicinal plants. Ile-Ife: University of Ife Press. 1979;20-22.
4. Paul C, Arnold J, Vlietinck Dirk VR, Loouis M. Anti-infective potential of natural products: How to develop a strong *in vitro* 'proof-of-concept'. J Ethnopharmacol. 2006;106:290-302.
5. Osadebe PO, Omeje EO. Comparative acute toxicities and immunomodulatory potentials of five Eastern Nigeria mistletoes. J. Ethnopharmacol. 2009;126: 287-293.
6. Bagozz D. WHO report on the use of traditional medicines in Africa; 2011. Available:http://www.allcountries.org/health/traditional_medicine.html. [cited 2011 October 11].
7. Kirigia JM. The economic burden of Diabetes mellitus in the WHO African region. African Health Monitor; 2008.
8. Obatomi DK, Oye AAA, Temple VJ. Reduction in serum glucose and cholesterol level in experimental diabetic rats treated with extracts of African Mistletoe (*Loranthus begwensis*). Med Sci Res. 1997;25:651–654.
9. Augustine KT. Therapeutic values of onions (*Allium sativum)*. Indian J Expt Biol. 1996;34:634-640.
10. Kelkar SM, Keckly GS, Bapat VA. Determination of the antidiabetic activities of Allium *cepa* (onions) on tissue cultures. Indian J Biochem Biophysiol. 2001;38: 277-279.
11. Aderibigbe AO, Emudianughe TS, Lawal BAS. Evaluation of anti-diabetic actions of

Magnifera indica in mice. J Phyto Res. 2001;15:456-458.
12. Iwu MM. Anti-diabetic and aldose reductase activities of biflavonoids of *Garcinia kola*. J Pharm Pharmacol. 1990; 42:290-292.
13. Burkill HM. The useful plants of West Africa vol.1, families A.D Royal Botanical Gardens, Kew. 1985;417.
14. Mann A, Gbate M, Umar NA. Medical and economic plants from Nupe land. Bida: Jube Evans Publishers. 2003;1-67.
15. Ahmed SE, Mohamed BE, Karamalla KA. Analytical studies of the gum exudates from *Anogeissus leiocarpus*. Pakistan J Nutr. 2010;8:782-786.
16. Onyeyili PA. Antihelmintic efficacy of some plants used in ethno veterinary practices in the arid zone of north eastern Nigeria. RGA NO 28 project Report; 2000.
17. Shuaibu MN, Pandey K, Wuyep PA, Yanagi T, Hirayama K, Ichinose A, Tanaka T. Castalagin from *Anogeissus leiocarpus* mediates the killing of *Leishmania in vitro*. Parasitol Res. 2008;6:1333-1338.
18. Ibrahim MB, Owonubi MO, Onaolapo JA. Antibacterial effect of the leaf, stem and root bark of *Anogeissus leiocarpus* on *S. aureus*, *S. pyrogens*, *E. coli, P. vulgaris*. J Pharmac Res Dev. 1997;2:20-26.
19. EEC. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Official J Eur Communities. 1986;L358:1-29.
20. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54:275-287.
21. Harborne HB. Phytochemical Methods: A Guide to Modern Technique of plant Analysis. 2nd edn; London: Chapman and Hall. 1984;282.
22. Osadebe PO, Okide GB, Akabogu IC. Study on anti-diabetic activities of crude methanol extracts of *Loranthus micranthus* (Linn) sourced from five different host trees. J Ethnopharmacol. 2004;95:133-138.
23. Ali S. Chemical constituents, antimicrobial and antioxidant activity of the hexane extract from root and seed of *Levisticum persicum* Freyn and Bornm. J Med Plant Res. 2011;5:5127-5131.
24. Cristinia H, Pedro MGB, Franklin B, Marienela V, Luis P, Sandra B. Hypoglycemic and anti-hyperglycaemic effects of *Witheringia solanaceae* in normal and alloxan-induced hyperglycaemic rats. J Ethnopharmacol. 2011;133:907-910.
25. Katzung BG, Masters SB, Trevor AJ, Katzung BG (ed). Basic and Clinical Pharmacology, 12th Edition, New York: McGraw-Hill Companies.

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