Research Article ISSN 2639-9326

Diabetes & its Complications

An Investigation into The Seasonal Variation of The Phytochemical and Anti-Diabetic Properties of The Eastern Nigerian Specie of African Mistletoe (*Loranthus Micranthus*) Sourced from *Kola Acuminata*

Osadebe P. O. and Johnson – Ajinwo O.R*

Department of Pharmaceutical, Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Department of Pharmaceutical, Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Choba.

***Correspondence:**
Johnson-Ajinwo, O.R., Department of Pharmaceutical, Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Choba.

Received: 02 April 2021; **Accepted:** 22 April 2021

Citation: Osadebe PO, Johnson – Ajinwo OR. An Investigation into The Seasonal Variation of The Phytochemical and Anti-Diabetic Properties of The Eastern Nigerian Specie of African Mistletoe (*Loranthus Micranthus*) Sourced from *Kola Acuminata*. Diabetes Complications. 2021; 5(2); 1-5.

ABSTRACT

Diabetes mellitus is the 7th killer disease worldwide and is a progressing disease with lethal consequences on the global front. Lately there have been very few new anti-diabetic agents entering the market, prompting research into medicinal plant which has been a potent source of drugs. Mistletoe; a plant synonymous with the Christmas celebration in Europe, has shown promise as an anti-diabetic plant from ethno pharmacological uses and has been validated in some researches. The eastern Nigeria species parasitic on some host trees has been researched on. However, loranthus micranthus parasitic on kola acuminata merits further investigation given its potentials as an anti-diabetic therapy. This present study was aimed at determining the anti-diabetic activities of the Crude methanol extracts of loranthus micranthus parasitic on kola acuminata sourced in the four seasons of the year; namely January (dry season), April (onset of rainy season), July (rainy season) and October (onset of dry season). The extracts were evaluated in alloxan-induced diabetic rats. The phytochemical analysis of the kola mistletoe of the four seasons was found to contain flavonoids, tannins, alkaloids, terpenoids, steroids, saponins, glycosides, reducing sugar, fats and oils. The anti-diabetic activities were determined after administration of 200mg/kg and 400mg/kg doses of the extracts at these time intervals: 1h 2h, 4h, 6h, 12h, 24h and 72h using a one touch glucometer. The April extract exhibited optimum anti-diabetic activity at both 200mg/kg and 400mg/kg dose levels. This was followed by the extracts sourced in October, July and January respectively. The phytochemical results showed that the April extract had an abundance of flavonoids, tannins and alkaloids, while the January extract which gave the least activity had lesser amounts of these photochemical constituents. This study showed that there is seasonal variation in the phytochemical constituents and anti-diabetic activities of kola mistletoes.

Keywords

Loranthus micranthus, Anti-diabetic, *Kola acuminata.*

Introduction

The human race has always been acquainted with one disease challenge or the other. Since ancient times, remedies have always been sought from natural products with much demand on medicinal plants.

Diabetes mellitus (DM) is a chronic, progressive, systemic disease characterized by dysfunction in the production of insulin resulting in an elevation of blood sugar level (hyperglycemia). DM is the seventh killer disease currently on the global front. Furthermore, DM has been implicated in other debilitating health problems, which includes; heart failure, kidney failure, blindness and amputations of lower limbs resulting from unhealed wounds [1].

Today, 422 million people are diabetic with a projected rise to 629 million by 2045. The death toll stood at 1.6 million in 2012. A recorded 5% increase in premature mortality from diabetes occurred between 2000 and 2016 [2].

The African traditional medicine is believed to be one of the oldest medicinal systems and often culturally referred to as the Cradle of Mankind [3,4]. Traditional herbal medicines have been used to treat infectious diseases for thousands of years in various parts of the world [5,6]. There has been a renewed interest in indigenous medicine worldwide because orthodox medicine is not widespread [6,7]. In poor countries, the health care has been sustained by other practices based on cultural alternatives [6]. In many developing countries, including Nigeria, 80% of patients use indigenous herbal remedies to treat various diseases [7,8]. Despite the availability of modern medicine in many cities, herbal medicines still maintain popularity for historical and cultural reasons, and also due to their efficacy and cheaper cost [7,8]. They also represent sources of potentially important new pharmaceutical substances since all parts of a plant, from roots to aerial parts, are employed in traditional remedies and can, therefore, act as sources of lead compounds [7]. There has been a boost in the use of plant remedies worldwide in recent years as well as the search for new phytochemicals aimed at obtaining more potent compounds that could be developed as therapeutic drugs for the treatment of diseases.

Nigeria boasts a unique and diverse botanical heritage with over 7,895 plant species of which about 3000 species are used therapeutically [9].

Mistletoe is one of such plants that has been used in different cultures for centuries in a variety of medicinal and non-medicinal purposes [10,11]. Mistletoe is a semi parasitic plant, which grows on a host of evergreen and deciduous trees such as *Kola Acuminata., Pentaclethra Macrophylla, Azadirachta Indica, Citrus sinensis* etc. The activities of mistletoe plant on diabetic rats, hypertensive rats, tumor development, and on the immune system have been documented [12-16]. The European Specie; *Viscum album* has been extensively studied and at present there are commercial brands available for a number of ailments which include cancer, and hypertension. In Nigeria, the two documented species *loranthus begwensis Linn* (a Northern Nigeria specie), and *loranthus micranthus (*an Eastern specie), though not extensively studied have been reported to possess, anti-microbial, anti-diabetic, anti-oxidant, anti-cancer activities [12,13,14,17]. Also, previous works have established the anti-diabetic activity of *loranthus micranthus* parasitic on *Persea americana, Baphia nitda, Kola acuminata,* and *Azadirchta indica* [12]. The researchers also established the safety of *loranthus micranthus* sourced from *kola acuminata* from toxicity studies in which the extracts had LD_{50} of 5900 mg/kg. This present work is an investigation into the seasonal variation in the phytochemical and anti-diabetic properties of *loranthus micranthus* parasitic on *Kola acuminata.*

Materials and Methods

Plant Material

Fresh leaves of *Loranthus micranthus* (L.M.) sourced in the following months: January, April, July and October representing the four seasons where obtained in Oba in Nsukka LGA of Enugu State. The plant was identified by a botanist in the Department of Botany, University of Nigeria, Nsukka, after which a voucher specimen was deposited in the department.

Extraction Procedure

The *Loranthus micranthus* leaves were dried at room temperature under a shade, and pulverized. Weighed quantities of the *Loranthus micranthus* were extracted with aqueous methanol using soxhlet extractor. The % yields were determined.

Preliminary Screening

The phytochemical constituents of both the dried leaves and the methanol extracts were determined according to the method prescribed by Trease and Evans [19].

This study was designed in line with the ethically approved experimental protocols adopted by the department of Experimental Pharmacology and Toxicology, of the Faculty of Pharmaceutical Sciences, University of Port Harcourt. Adult mixed-gender wistar rats weighing between 100-150g, bred by the animal house unit of the department of Experimental Pharmacology and Toxicology were used for the study. The rats were housed in spacious cages, to allow for free movement at room temperature, sufficient humidity and 12 hourly cycles of light and darkness. The animals, had access to standard laboratory animal feed and water prior to the commencement of the experiment.

The anti-diabetic screening of *Loranthus micranthus* (L.M) obtained from *kola acuminata* in the four seasons was carried out. Their baseline blood sugar level was determined before the experiment. The rats were fasted for 12 hours and hyperglycaemia induced by the intraperitoneal administration of 100mg/kg alloxan monolydrate. After three days, blood samples were withdrawn from the tail vein of the rats and the blood sugar levels determined using the one touch glucometer. The diabetic rats (blood sugar > 120mg/dl) were randomly divided into 10 groups (n =4). 200mg/kg dose of the crude methanol extracts of each of the four seasons was administered intraperitoneally to the first four groups of the diabetic rats separately. (NB: one seasons extract was administered to one group of rats). The next four groups received 400mg/kg dose of the extracts. The ninth group received 2ml/kg of 3% tween 80 solution (negative control), while the tenth group of rats received 10mg/kg of glibenclamide (positive control). Blood samples (<0.1ml) were collected from the tail vein of the rats for the determination of blood sugar concentration at these intervals: 0, 1, 2, 4, 12, 24, 72 hours after treatment.

Statistical Analysis

The analytical tool used was Graph pad Prism version 8 for calculation of the mean values ± standard error of mean (SEM).

The one-way ANOVA was used to determine statistical difference between means. A $p<0.05$ was considered statistically significant.

Results

Table 1: Result of percentage yield of methanol extracts of *Loranthus micranthus.*

Season	Quantity Used	% yield
January	200g	6.6
April	200g	5.8
July	200g	6.0
October	200g	5.0

Table 2: Result of Phytochemical Test on the pulverized leaves of L.M Parasitic on *Kola acuminata* harvested at different months of the seasons.

Phytochemical constituents	Months of The Seasons			
	April	July	October	January
Flavonoids	+++	++	++	+
Saponins	+	+	+	+
Tannins	++	+	++	+
Alkaloids	+++	++	+++	++
Proteins	+	+	+	+
Fats and oil	+	+	+	+
Steroids	+	+	+	++
Acidic compounds	-	+	+	+
Terpenoids	++	++	++	++
Carbohydrates	+	+	+	+
Glycosides	+	+	+	+
Reducing sugar	+	++	+	+++
Resins	Trace	+	+	+

Table 3: Result of phytochemical test on the methanol Extracts of L.M parasitic on *Kola acuminata* harvested at different months of the seasons.

Phytochemical constituents	Months of The Seasons			
	April	July	October	January
Flavonoids	+++	++	++	+
Saponins	+	+	+	+
Tannins	++	+	++	+
Alkaloids	+++	++	+++	++
Proteins	+	+	+	+
Fats and oil	+	+	+	+
Steroids	+	+	+	++
Acidic compounds	-	+	+	+
Terpenoids	++	++	++	++
Carbohydrates	+	+	+	+
Glycosides	+	+	+	+
Reducing sugar	+	++	+	+++
Resins	Trace	+	+	+

Table 4: Percentage Reduction of the Fasting Blood Glucose level of the methanol Extracts of L.M Linn harvested at different months of the seasons.

200mg/kg Dose Extract of seasons	Time Interval					
	1h	2h	4h	12h	24h	72h
April	34.27*	14.24*	25.50*	55.46*	38.08*	26.16*
July	15.72*	16.75*	16.24*	25.22*	20.90	12.09*
October	28.81*	21.88*	26.97*	46.38*	35.75*	20.96*
January	13.99	5.63	17.07*	29.18*	12.12*	7.68*
400mg/kg Dose						
April	39.29*	28.57*	46.96*	68.04*	22.32	39.46*
July	23.55*	21.82*	25.87*	31.27*	27.61*	21.04*
October	34.43*	25.74*	33.61*	58.20*	31.64*	34.43*
January	17.11*	9.23	18.96*	40.27*	22.65	19.13*
+ve Control	54.10*	51.00*	77.05*	80.51*	69.29*	71.48*
-ve control	10.37	6.48	8.15	17.78	19.07	2.59

* = $p<0.05$ refers to significance against negative control.

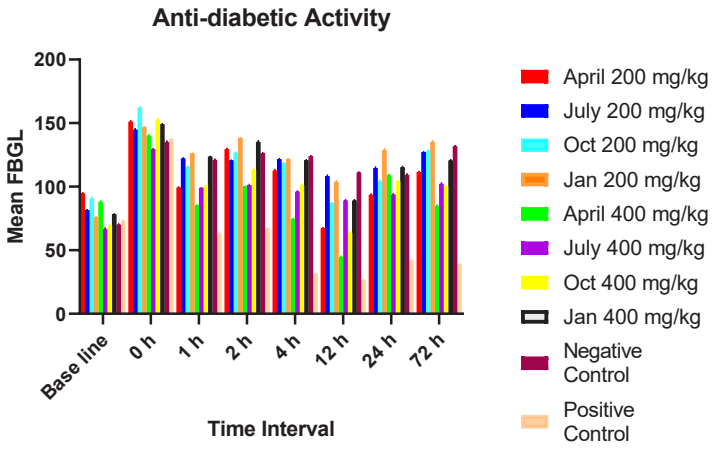

Figure 1: The mean Fasting blood glucose levels (FBGL) of the extracts at different time intervals.

Discussion

The results of the percentage yield of the methanol extracts of *loranthus micranthus* are contained in table 1 from the data presented, it was observed that there was dose conformity in the yields of the different months. An indication that there is no seasonal variation in the yield of the plant.

The results of the preliminary screening of the phytochemical constituents presented in table 2 and table 3 showed that the same phytochemical constituents were present in both the pulverized leaves and the methanol extracts of all the different months of the seasons.

Thus, the use of water and alcohol for the extraction of the active constituents of *loranthus micranthus* by indigenous communities is justified. This finding has also been observed by oguntoye and his co-workers [20] who reported anti-bacterial activities in both the water and aqueous ethanol extracts of *viscum album*. Similarly, some researchers have investigated the phytochemical constituents of *kola acuminata*. Their findings reveal these classes of compounds: saponins, tannins, alkaloid, flavonoids, glycoside, steroid, saponins glycoside and cardiac glycoside [21] were present in both the aqueous and methanol extracts of *kola acuminata*.

The results of the anti-diabetic activities of the extracts and the controls are shown in Table 4 and Figure 1 respectively. The findings are that the extracts possess anti-diabetic activities. A comparison of the anti-diabetic activities of the extracts demonstrated that the April extracts had the optimum activity both at 200mg/kg

and 400mg/kg dose levels. This was followed by the extracts of October, July and lastly January. Again the potency of the extracts demonstrated a dose-effect relationship. The results showed that at the 12h, April 400 mg/kg dose was close to that of the positive control. The extracts were significantly active (p>0.05) at most of the time intervals over the negative control. This finding strongly implies that the extracts had hypoglycemic activities. It was further observed that at 72 hrs the extracts were still active. This indicates that the extracts had a long-lasting effect. The anti-diabetic activity of *kola nitida*; a closely-related specie to *kola acuminata* has been documented [22]. This finding is consistent with a mistletoe-host tree activity relationship, previously identified by my research team [23]. A closer look at the phytochemical constituents of the April Extract revealed the presence of abundant flavonoids, alkaloids and tannins, as against the January extract which had the least quantity of flavonoids and tannins.

It is likely that due to the excessive rains in the month of July-September that there is the possibility of some phytoconstituents being leached away. Again, during the peak of the dry season (January-March), most trees experience shedding of leaves which would likely result in a decrease in the amount of phytoconstituents in the trees and may lead to a decrease in the phytoconstituents of the mistletoes which derive their nutrients from the trees.

This finding is an indication that the anti-diabetic activity of *Loranthus micranthus* parasitic on *Kola acuminata* is influenced by seasonal variation.

Similar observations has also been reported by Wendy et al, [24] who carried out an investigation into the seasonal variation in the antifungal, antibacterial and acetylcholinesterease activity in Seven South Africa sea weeds. Their results showed that the seaweeds had antibacterial activities in later winter and no activity in summer. Also, Osadebe et al. have documented the occurrence of seasonal variation in the antimicrobial activities of *loranthus micranthus* on *psidium Guajava* [25].

Conclusion

The findings of this work affirms that *Loranthus micranthus* parasitic on *Kola acuminata* has anti-diabetic activity. Also, it is observed that there is seasonal variation in the phytochemical constituents and the anti-diabetic activity of this plant. *Loranthus micranthus* parasitic on *Kola acuminata* should be preferably sourced in the month of April for use in the treatment of diabetes mellitus.

References

1. WHO, Diabetes, 8th June 2020.
2. http://www.diabetesatlas.org
3. Van Vuuren SF. Antimicrobial activity of South African medicinal plants. J Ethnopharmacol. 2008; 119: 462-472.
4. Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med. 2006; 27: 1-93.
5. Cowan M. Plant products as antimicrobial agents. Clinical Microbial Reviews. 1999; 12: 564-582.
6. Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: A review. J Ethnopharmacol. 2011; 133: 289-302.
7. Lifongo LL, Simoben CV, Ntie-Kang F. A Bioactivity Versus Ethnobotanical Survey of Medicinal Plants from Nigeria, West Africa. Nat. Prod. Bioprospect. 2014; 4: 1-19.
8. Nasir MU, Hussain S, Jabbar S, et al. A review on the nutritional content, functional properties and medicinal potential of dates. Sci Lett. 2014; 3: 17-22.
9. Usman H, Abdulrahman A, Usman A. Qualitative Phytochemical screening and in vivo Antimicrobial Effects of Mehanol Stem Bark Extract of Ficus thonningi. African Journal of Traditional Complementary and Alternative Medicine. 2009; 6: 289-295.
10. Samtleben R, Hajto T, Hostanska K, et al. Mistletoe lectins as immunostimulants (chemistry, pharmacology and clinic). In: Wagner H. (eds) Immunomodulatory Agents from Plants. Progress in Inflammation Research. Birkhäuser, Basel. 1999.
11. Hauser SP. Unproven Methods in Oncology. European J. of Cancer. 1991; 27: 1549-1551.
12. Osadebe PO, Okide GB, Akabogu LC. Study on anti-diabetic activities of crude methanolic extracts or loranthus micranthus (Linn.) sourced from five different host trees. J. Ethnopharmacology. 2004; 95: 133-138.
13. Obatomi DK, Bikomo EO, Temple VJ. Anti-diabetic properties of the African Mistletoe in Streptozotocin Induced diabetic rats. J. Ethnopharmacology. 1994; 43:13-17.
14. Obatomi DK, Aina VO, Temple VJ. Effect of African Mistletoe on blood pressure in spontaneously hypertensive rats. International J. Pharmacognosy. 1996; 34: 124-127.
15. Kuttan G, Vasudeuan DM, Kutten R. Effect of a Preparation from Viscum album on tumour development in vitro and in mice. J. Ethnopharmacol. 1990; 29: 35-41.
16. Bloksma N, Schmiermann P, De Reuver M. Stimulation of Humoral and Cellular Immunity by Viscum Preparation, Planta Medica. 1982; 46: 221-227.
17. www.paxherbals.net
18. Osadebe PO, Ukwueze SE. Comparative Study of the antimicrobial and phytochemical properties of mistletoe leaves sources from six host trees. J. of Biological Res. And Biotech. 2004; 2: 18-23.
19. Evans WC, Sanders WB. Trease and Evans Pharmacognosy. 2008.
20. Oguntoye SO, Olatunji GA, Kolawole OM, et al. Phytochemical Screening and Antibacterial Activity of viscum Album (mistletoe) extracts. Plants Science Research. 2008; 1: 44-46.
21. Omwirhiren EM, James SA, Abass AO. The Phytochemical Properties and Antimicrobial Potentials of Aqueous and Methanolic seed Extract of Cola nitida (Vent.) and Cola acuminata (Beauvoir) grown in South West, Nigeria. Saudi J. Med. Pharm. Sci. 2006; 2: 354-363.

22. Erukainure OL, Sanni O, Ijomone OM, et al. The antidiabetic properties of the hot water extract of kola nut (Cola nitida (Vent.) Schott & Endl.) in type 2 diabetic rats. J Ethnopharmacol. 2019; 242: 112033.
23. Osadebe PO, Johnson-Ajinwo OR. A Review of the Bio-Activity Relationship of Mistletoes and the Leaves of their Host Trees. Nigerian Journal of Pharmaceutical Research. 2010; 8: 145-150.
24. Wendy AS, Diana LR, Johannes VS. Seasonal variation in antifungal antibacterial and acetylcholinesterase activity in seven South African seaweeds. Journal of Applied Phycology. 2007; 19: 271-276.
25. Osadebe PO, Ukwueze SE. Investigation into the Seasonal variation in the Antimicrobial Activity and identification of the bioactive fraction of loranthus micranthus on psidium Guajava. J of Biological Res and Biotech. 2006.