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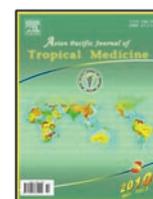
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Seasonal variation for the antidiabetic activity of *Loranthus micranthus* methanol extract

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ABSTRACT

Objective: To determine the season in which the Eastern Nigeria mistletoe, *Loranthus micranthus*, parasitic on *Persea americana* possesses optimum antidiabetic activity and to determine the seasonal variation in the constituents. **Methods:** The antidiabetic activities of the aqueous methanol extracts of the leaves of Eastern Nigeria mistletoe, *Loranthus micranthus*, harvested in two seasons of the year, the onset of rainy season (April) and the peak of rainy season (July) were compared. The tests were carried out on six (6) groups (A–F) of alloxan-induced diabetic rats. Groups A and B received 200 mg/kg and 400 mg/kg of the April sample extracts respectively while groups C and D received same doses of the July sample extracts. Group E and F which were the positive and negative controls received 10 mg/kg of glibenclamide and 2 ml/kg of 3% tween 20 respectively. The blood glucose levels of the animals were monitored hourly with a glucometer for six hours. The phytochemical analysis of the plant extracts were also carried out by standard procedures. **Results:** The results showed that group A and B exhibited significant ($P < 0.05$) percentage reduction in the fasting blood sugar (FBS) level of the animals (38.9% and 39.2% respectively) with maximum reduction observed at the 5th and 6th hour respectively compared to glibenclamide (71.3%). Group C showed no significant ($P > 0.05$) FBS reduction (15.9%) while group D exhibited highly significant ($P < 0.01$) reduction (47.5%) with the maximum reduction occurring after 6 hours. The phytochemical analysis of the crude methanol extracts revealed the presence of carbohydrates, glycosides, saponins, tannins, flavonoids, steroids, terpenoids, acidic compounds, resins and oils. These were present in different proportions in both seasons. **Conclusions:** This study shows that there is a seasonal, dose-dependent variation in the chemical composition *viz-a-viz* the antidiabetic activity of the plant under study. This activity is highest at the peak of the rainy season.

1. Introduction

Diabetes mellitus is a clinical syndrome with severe socio-economic importance characterized by hyperglycemia, due to absolute or relative lack of insulin[1]. Diabetes is a life-long disorder and the aim of therapy is to keep blood glucose level as near to normal as possible, prevent hyperglycemia and its acute and chronic complications. The management of diabetes involves both the non-pharmacological and pharmacological approaches. While non-pharmacological approach includes exercise, diet control and surgery, pharmacological approach includes the use of drugs such as insulin, and oral hypoglycemic agents.

The conventional antidiabetic drugs are not only costly but associated with lots of adverse effects[1,2].

The search for safer and affordable antidiabetic drugs has led to the testing of many plants for such activity. Interestingly, some potent antidiabetic principles have so far been isolated from plants. Among these plants which have been shown to possess antidiabetic activity is the semi-parasitic plant, *Loranthus miranthus*, which is the Eastern Nigeria species of the African mistletoe[3]. Mistletoes generally grow on a variety of host trees, which usually determine its phytochemical components and hence its bioactivity. Recently, group of researchers were able to establish a host tree dependent antidiabetic activity of this Eastern Nigeria specie of mistletoe[4–7]. However, the main active principle(s) responsible for its antidiabetic activity have not yet been isolated and unequivocally characterized. The specie of the plant has also been reported to possess other pharmacological activities including antihypertensive,

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anti-epileptic, anti-microbial, and immune stimulating effects^[8–11]. Similar host tree dependent bioactivities have been reported for mistletoes from other parts of the world^[9].

Aside the influence of host tree on the phytochemical composition *cum* potency of the biological activities of the comparative European mistletoe, *Viscum album*, other factors such as season of harvesting, soil type for cultivation and age at harvesting were reported to also influence the measured activities^[3]. Moreover, it has been shown that the phytochemical constituents of plants usually vary with seasons of the year^[12]. In Eastern Nigeria, there are four distinct seasons namely, the long rainy season which starts in April and lasts till the end of July with peak period in July; the short dry season which occurs in August for 3–4 weeks; the short rainy season which starts from early September to mid October with its peak at the end of September, and the long dry season which starts from late October and lasts till March. In consideration of these seasons of the year in Eastern Nigeria, it is worthwhile to assess the influence of these seasons on the antidiabetic potentials of *Loranthus micranthus*.

The aim of this study is therefore to determine the season in which the plant under consideration; *Loranthus micranthus*, parasitic on *Persea americana* possesses the highest antidiabetic activity and to determine the seasonal variation in the constituents. The seasons chosen for the study are the onset of the rainy season (April) and the peak of the rainy season (July)

2. Materials and Methods

2.1 . Plant materials

Loranthus micranthus leaves parasitic on *Persea americana* were collected in two different months, April and July. The plants were collected from Orba in Udenu LGA of Enugu State, Eastern Nigeria and identified by Mr Alfred Ozioko, a taxonomist of the Centre for Bioresource Development and Conservation Program (BDCCP), Nsukka. The plant leaves were air-dried under shade and were pulverized with home grinder (model No. 401513, China). They were sieved and stored in clean water-proof bags.

2.2. Chemical reagents

Chemicals and Reagents used are Methanol (Sigma Chemicals, USA), alloxan monohydrate (Sigma Chemicals, USA), glibenclamide (NGC, Nigeria), Tween 20.

2.3. Animals

Wiser rats (70–155 g), aged 3–4 months were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were maintained in normal and standard laboratory conditions and fed with commercial diet (Vital Feed Nig. Ltd.) and water, *ad libitum*.

2.4. Preparation of extract

The method of extraction used was cold maceration. Briefly, 1 kg each of ground air dried leaves from the

seasons under study were soaked in 3.5 L of 95% aqueous methanol and covered. The set up was intermittently shaken vigorously. The marc was removed firstly by clarification using muslin and later by filtration through Whatman No 2 filter paper. The filtrates were combined and dried *in-vacuo* using a rotary evaporator at (40–50) °C.

2.5. Anti-diabetic studies

Twenty four rats were used for the experiment. The animals were fasted for 12 hours before induction of hyperglycemia. The induction of hyperglycemia involves intra-peritoneal administration of freshly prepared alloxan monohydrate, at a dose of 140 mg/kg body weight. Forty eight hours later, blood was withdrawn from the tail of the rats and tested for hyperglycemia, using Bayer Contour glucose meter.

The rats were randomly divided into six groups ($n=4$) and labeled A–F. The drugs were administered intraperitoneally as follows: Group A: 200 mg/kg of April extract; Group B: 400 mg/kg of April extract; Group C: 200 mg/kg of July extract; Group D: 400 mg/kg of July extract; Group E: 10 mg/kg of glibenclamide; Group F: 0.1 mg/kg of 3% tween 20. While groups A, B, C, and D were the test groups, groups E and F served as positive and negative controls respectively. Following the administration of drugs as outlined above, blood was withdrawn from the tail veins of the rats at 0, 1, 2, 3, 4, 5, and 6 hrs. The blood samples were tested for glucose using the automated Bayer Contour glucose meter. The percentage of maximum reduction of fasting blood sugar (FBS) was calculated at the time of maximum reduction t (hour) using the formula:

% maximum reduction of FBS = $[(G_x - G_0)/G_0] \times 100$ where G_0 and G_x were the values of 0-hr and t -hr respectively^[13].

2.6. Phytochemical test

Standard procedures^[14] were followed in the tests for the phytochemical constituents of the plant and results recorded accordingly.

2.7. Statistical analysis

The results from the experiment were expressed as the mean values for each group \pm SEM (standard error in mean) and the statistical significance between the test and the control groups were evaluated, using the analysis of variance method in student's t -test^[15]. $P < 0.05$ or $P < 0.01$ were considered significant and highly significant respectively^[15].

3. Results

3.1. Antidiabetic activity of the extract from April sample

Figure 1 showed antidiabetic activity of the April sample of *Loranthus micranthus*. It showed that the plant extract for group A significantly reduced the blood sugar level in the experimental animals with maximum reduction occurring at the 5th hour (38.9%) ($P < 0.05$), while group B showed significant reduction (39.2%) at the 6th hour as compared to the controls, group E (71.3%) and group F (–3%) ($P < 0.05$).

3.2. Antidiabetic activity of the extract from July sample

The result of the effect of the extracts from this season in comparison with the controls was shown in Figure 2. Group C exhibited no significant mean FBS lowering activity (maximum reduction:15.9%)($P>0.05$) and no steady decline in glycemia; while group D showed highly significant activity (339–178 mg/dL; reduction of 47.5%) compared to glibenclamide (484.7–139.0 mg/dL; reduction of 71.3%)($P<0.01$). Both groups, D (400 mg/kg of July sample) and E (glibenclamide) caused a steady decline in glycemia after 1 hour up to the 6th hour post extract/drug administration.

3.3. Phytochemical analysis of extract

Resins were present in both samples in equal proportions. The April sample contained higher amounts of alkaloids, steroids, acidic compounds and carbohydrates than the July sample. Reducing sugars, glycosides, saponins and tannins were absent in the July sample. However, the July sample shows the presence of flavonoids and oils in higher concentrations. Comparatively, the results showed marked variation in their phytochemical constituents with the July sample having lesser constituents.

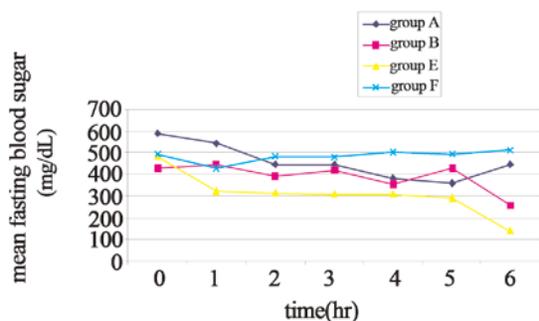


Figure 1. Effect of *Loranthus micranthus* extract from April sample, glibenclamide and 3% Tween 20 on alloxan-induced diabetic rats.

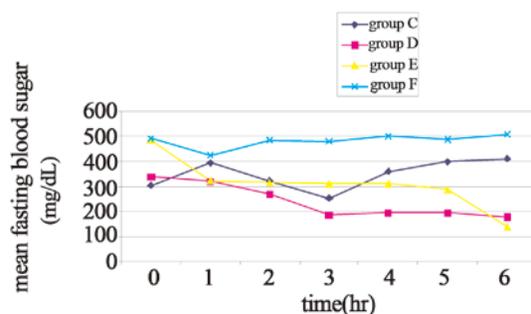


Figure 2. Effect of *Loranthus micranthus* extract from July sample, glibenclamide and 3% Tween 20 on alloxan-induced diabetic rats.

4. Discussion

The yields from the aqueous methanol extracts from the April and July samples were 13.58% and 13.85% respectively. These results are in line with previous reports for the plant^[4]. Usually, a solvent mixture like aqueous

methanol is a suitable polarity blend for the extraction of almost all possible constituents in plant leaves. This is because the methanol provides the needed organic environment while blending well the polar and inorganic properties of water. However, earlier reports with other method of extraction other than cold maceration afforded higher yield^[4]. It is expected that exhaustive cold maceration based extraction could afford higher yield.

In this study, diabetes in white Wister experimental rats was induced through the intraperitoneal administration of freshly prepared alloxan. Alloxan induces diabetes by selective necrosis of pancreatic β -cells^[13,16]. Diabetes is usually detected after 48 hrs post alloxan administration by the presence of stable hyperglycemia. All the animals used for study were confirmed to be diabetic before the commencement of drug administration. The diabetic state was confirmed by a stable fasting blood sugar higher than or equal to 126 mg/dL^[17]. Result showed that the plant extract for group A significantly reduced the blood sugar level in the experimental animals (38.9%) with maximum reduction occurring at the 5th hour while group B showed significant reduction(39.2%)($P<0.05$) at the 6th hour when both are compared to the controls, group E (71.3%) and group F (-3.0%). However, both extracts did not show appreciable steady reduction in the blood glucose level of the animals as there were a lot of fluctuations in their activities. This observation suggests that in addition to the presence of the supposed antidiabetic active principle (s) in the April sample, there could be high concentration of other constituents that could possibly antagonize the hypoglycemic effect of the extracts in this season. In fact, the result of the phytochemical analysis confirms the presence of high amount of carbohydrate and reducing sugar in April.

In contrast, the results from the July samples were slightly different. While group C exhibited no significant mean fasting blood sugar lowering activity (maximum reduction: 15.9%)($P>0.05$) and no steady decline in glycemia, group D showed highly significant activity (339–178 mg/dL; reduction of 47.5%) compared to glibenclamide (484.7–139.0 mg/dL; reduction of 71.3%)($P<0.01$). Interestingly, group D had the highest antidiabetic activity of all the test groups and the pattern of glucose lowering was quite comparable to glibenclamide as both groups, D (400 mg/kg of July sample) and E (glibenclamide) caused a steady decline in glycemia after 1 hour up to the 6th hour post extract and or drug administration. Thus the results show a dose-dependent activity and they compare favorably with those of other researchers using related species of the plant^[18,19]. In addition, aqueous extracts of other medicinal plants have been demonstrated as possessing dose dependent antidiabetic properties^[20,21].

In terms of safety, the administered doses of 200 and 400 mg/kg are quite safe as the acute toxicity (LD_{50}) of the aqueous methanol extract of the mistletoe harvested from *Persea americana* has been reported to be 5 916 mg/kg^[22]. Higher LD_{50} values were recently reported for Eastern Nigeria mistletoes harvested from other host trees^[7]. In addition, no significant biochemical changes in rats, using

therapeutic of the crude methanol extract, has been reported for the plant^[23]. This safety data on Eastern Nigeria mistletoe is an indication that it might be safer than other versions of mistletoe from different continents of the world^[24].

Comparatively, the results show marked variation in their phytochemical constituents. Resins are present in both samples in equal proportions. The April sample contains higher amounts of alkaloids, steroids, acidic compounds and carbohydrates than the July sample. Reducing sugars, glycosides, saponins and tannins are absent in the July sample. However, the July sample shows the presence of flavonoids and oils in higher concentrations.

The results showed marked variation in their phytochemical constituents with the July sample possessing lesser amount of constituents. The presence of reducing sugars, and higher proportion of carbohydrates could be the major draw-back in the antidiabetic activity of the April sample as was observed in the results. The reasons are obvious. The seasonal variation in the phytochemical constituents could explain the differences observed in the antidiabetic activities of extracts from both seasons. Interestingly, previous works linked the antidiabetic activity of *Loranthus micranthus* with its flavonoid content^[22,25]. It could be argued that the higher activity seen in the July extract could be attributed to its higher flavonoid and lower carbohydrate contents. This could also be supported by the dose dependent activity observed in the study.

Loranthus micranthus, the Eastern Nigeria mistletoe harvested from *Persea americana* showed a marked, dose and season dependent anti-diabetic activity, with higher activity observed during the peak of the rainy season (July sample). The seasonal variation in the flavonoid content could be responsible for this seasonal variation of the activity investigated. Further detailed study involving the actual isolation and characterization of the active principle(s) is recommended.

Conflict of interest statement

We declare that we have no conflict of interest.

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