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PHYTOCHEMICAL EVALUATION AND ANTIDIABETIC ACTIVITY OF THE METHANOLIC EXTRACT OF *Lophira Lanceolata* STEM BARK

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Abstract

Antidiabetic potential of methanolic extract of Lophira lanceolata stem bark, a plant locally used in management of diabetes mellitus was evaluated using wister albino rats. The stem bark of the plant was extracted with 70% methanol and administered orally on alloxan-induced diabetic rats at doses of 100, 200 and 500 mg/kg body weight daily for two weeks. The hypoglycaemic effect of the extract was compared with a standard drug, glibenclamide, administered at 500 µg/kg body weight. The extract, at the concentrations used, demonstrated significant hypoglycemic effect ($P<0.05$) in a dose dependent pattern in the fasted diabetic rats after 14 days of treatment, compared to the diabetic control group. There was a general decrease in the weight of animals during the first week of treatment and treated group showed significant ($P<0.05$) weight gain at the end of two weeks treatment. Phytochemical screening revealed the presence alkaloids, flavonoids, tannins, saponins and glycosides in appreciable quantities. The result of this study has revealed the hypoglycemic potential of the methanolic extract of Lophira lanceolata stem bark.

Keywords: *Antidiabetic activity, phytochemical, glibenclamide, Lophira lanceolata, stem bark*

Introduction

Diabetes mellitus is a major health problem in all parts of the world. Almost 3.2 million people die of diabetes across the world every year (Wild *et al*., 2004). Management of diabetes without any side effect is still a challenge to the medical system (Marles and Farnsworth, 1995). This situation has led to an increasing demand for natural products with antidiabetic activity, that are safe, effective, less expensive and affordable with fewer side effects. The developing nations of Africa are rich in biodiversity and indigenous knowledge particularly ethnomedicinal practices (Mann *et al*., 2003). Nigeria is endowed with a rich biological heritage including lots of medical plants that are distributed throughout the country, and among this are those widely used for treatment of wide variety of diabetes.

Lophira lanceolata Van Tiegh. ex Keay (Ochnaceae) is a tall tree that grows in the woody savannah forests and widely distributed in tropical Africa including Nigeria (Mapongmetsen, 1954; Persinos and Quimby, 1968; Pegnyemb *et al*., 1998). The plant is commonly known as Ironwood with Nigerian local names such as: *Namijin kade* in Hausa, *Iponhon* in Yoruba, *Okopia* in Igbo, and *Maganchi* in Nupe (Mann *et al*., 2003). Ethnomedicinal uses of the plant include treating: pulmonary diseases, headaches, toothache, abdominal pains, menstrual pain, malaria, fevers and gastrointestinal problems. Others inclusive cough, dysentery, female infertility, liver infections, diarrhoea, diabetes, respiratory troubles, rheumatism and cardiovascular diseases (Ghogomu *et al*., 1989a; Igoli *et al*., 2005; Mann *et al*., 2003; Persinos *et al*., 1967). Pharmacological studies of the extracts of this plant revealed the following: antimicrobial activities (Gangoué-Piéboji *et al*., 2006); anti-trypanosomal activity (Abu *et al*., 2009); sexual stimulatory and fertility enhancing effects (Etuk and Muhammad, 2009); anti-plasmodial and antixiodant activities (Onyeto *et al*., 2014). *Lophira lanceolata* has been studied by various workers and a number of active constituents have been isolated. For instance, isolation of benzamide (Persinos *et al*., 1967); several dimeric chalcones, biflavonoids and tetraflavonoids (Ghogomu *et al*., 1987; Ghogomu *et al*., 1989a, b; Ghogomu *et al*., 1990; Pegnyemb *et al*., 1994); prenylated isoflavone and a benzoylglucoside (Pegnyemb *et al*., 1998); cupressuflavone (Sani *et al*., 2010) and betulinic acid (Sani *et al*., 2011) have been reported from its various parts. However, *Lophira lanceolata* popularly used for the ethnomedicinal treatment of diabetes has not been investigated for antidiabetic activity. The present research work was designed to investigate the possible antidiabetic activity of the stem bark methanolic extract of *Lophira lanceolata* using alloxan induced diabetic rats.

Materials and Methods

Plant material

The fresh stem bark of *Lophira lanceolata* was obtained from Patiswaran forest along Bida-Ushu Road, Niger State, Nigeria in the month of September. It was identified and authenticated by botanist of the Department of Biological Sciences, Ahmadu Bello University, Zaria where a voucher specimen labeled (*A002*) was deposited for identification.

Induction of experimental diabetes in albino rats

Male albino rats were fasted overnight (12-14 h) and their weights (100-180 g) and fasting blood glucose levels were recorded. The animals were then made diabetic by a single intraperitoneal injection of freshly prepared alloxan monohydrate (100 mg/kg body weight) dissolved in normal saline. Food and water were presented to the animals 30 min after drug administration (De Carvalho *et al*., 2003; Kamalakkanan and Prince, 2003; Nagappa *et al*., 2003). The animals were maintained according to the International Ethics on Animal handling (Guide for the Care and Use of Laboratory Animals, 1985).

Experimental Design

In the experiment, the rats were divided into 6 groups for the evaluation of fasting blood glucose level with five animals in each group.

Group 1 Normal control (administered distilled water).

Group 2 Diabetic control (diabetic rats administered).

Group 3 treated with standard drug (Glibenclamide, 500µmg/kg body weight per day).

Group 4 treated with methanol extract of *L. lanceolata* (100 mg/kg).

Group 5 treated with methanol extract of *L. lanceolata* (200 mg/kg).

Group 6 treated with methanol extract of *L. lanceolata* (500 mg/kg)

Administration of extracts and drug

Extracts and drug were administered orally by gavaging daily for 2 weeks (Pari and Venkateswaran, 2003) and treatment commenced 72 hrs after induction.

Withdrawal of blood samples

Blood glucose level was determined using an Achu-check glucometer with its commercial test strips based on glucose oxidase method. Blood sample were collected from the tip of tail at weekly intervals till the end of the study period of 2 weeks. Fasting blood glucose levels and body weight measurement of all the rats were recorded on day zero (0), seven (7) and fourteen (14) during the experimental period (Nagappa *et al*., 2003).The blood glucose was determined after overnight fasting and those with clinical signs of diabetes with a fasting blood glucose range above 150 mg/dl (Kumar *et al*., 2006; Gidado *et al*., 2005; Pari and Venkateswaran, 2003) were involved in the study.

Phytochemical analysis

Phytochemical screening of plant extract was carried out using the standard methods described by Sofowora (1998), Trease and Evans (2006) and Harborne (1998).

Results and Discussion

Qualitative phytochemical screening and quantitative estimation of the methanolic extract of *Lophira lanceolata* stem bark

The qualitative phytochemical screening and quantitative estimation of the phytochemicals of *Lophira lanceolata* crude extract of the stem bark are presented in Table 1 and Figure 1 respectively. Phytochemical screening of the methanol extract of the stem bark of *L. lanceolata* indicated the presence of flavonoids, alkaloids, saponins, tannins and glycosides which agrees with report of Sani *et al*. (2007) except for alkaloid that was absent in their investigation of the leaves. However, some earlier reports of qualitative phytochemical

analysis on the stem bark of this plant indicated the presence of alkaloids (Gill, 1992). Figure 1 shows the quantitative result in percentage of the various phytochemicals that are present in the stem bark extract of this plant. However, the present results compares well with the previous works with slight differences in their percentages (Gill, 1992). This difference might be due to the differences in the soil and climatic conditions under which the plant was found. These chemical constituents in medicinal plants can rationally explain the use of these plants in traditional medicine (Fansworth, 1984). They are known to possess medicinal activity through exhibition of physiological activity (Sofowara, 1998). The growing interest of researchers in traditional medicine has led to discovery and establishment of biological activity of many potent phytochemicals possessing antidiabetic activity (e.g. glycosides, flavonoids, terpenes, steroidal saponins, alkaloids, polysaccharides). Plants with hypoglycemic activity may contain one or more of these chemical constituents. These chemical compounds identified from this plant including: alkaloids, flavonoids, tannins, saponins and glycosides are well documented to have the potential to decrease the blood glucose level (Ragavan and Krishnakumari, 2006; Miura *et al.*, 2005; Oubre *et al.*, 1997). Thus, the significant antidiabetic effect of methanol extract of *L. lanceolata* could be due to the presence of the aforementioned constituents which could act synergistically or independently.

Table 1: Qualitative analysis of the methanol extract of *Lophira lanceolata* stembark

Extract	Flavonoids	Alkaloids		Saponins		Tannins	Glycosides
		Wagners	Mayers	Froth Test	Emulsion Test		
Methanol	+	+	+	+	+	+	+

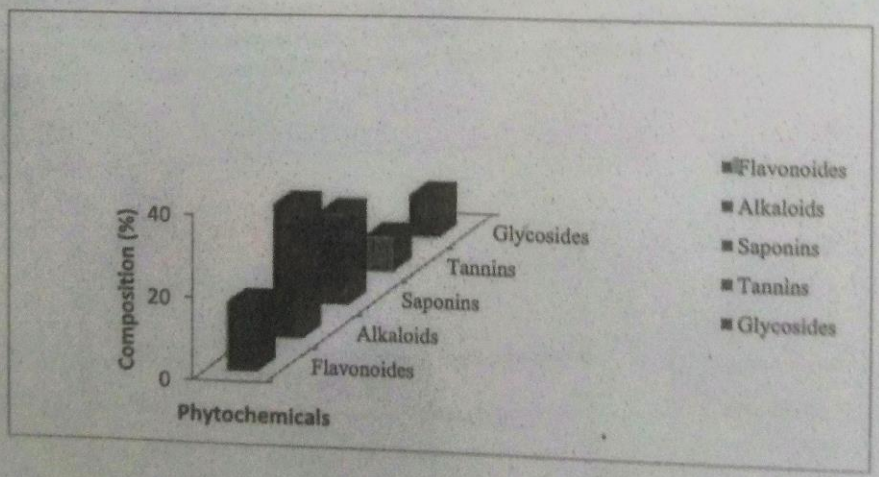

Figure 1: Quantitative analysis of the methanol extract of *Lophira lanceolata* stem bark

Effect of extract on body weight

The results of body weight and blood glucose level of normal control group, diabetic non treated group, and standard drug treated group (Glibenclamide 500µg/kg) and methanol extract treated group of *Lophira lanceolata* were summarized in figures 2 & 3. Figure 2 shows that a significant decrease was observed in the body weight of diabetic rats when compared with control rats. Treatment with extract of stem bark of *L. lanceolata* and

analysis on the stem bark of this plant indicated the presence of alkaloids (Gill, 1992). Figure 1 shows the quantitative result in percentage of the various phytochemicals that are present in the stem bark extract of this plant. However, the present results compares well with the previous works with slight differences in their percentages (Gill, 1992). This difference might be due to the differences in the soil and climatic conditions under which the plant was found. These chemical constituents in medicinal plants can rationally explain the use of these plants in traditional medicine (Fansworth, 1984). They are known to possess medicinal activity through exhibition of physiological activity (Sofowara, 1998). The growing interest of researchers in traditional medicine has led to discovery and establishment of biological activity of many potent phytochemicals possessing antidiabetic activity (e.g. glycosides, flavonoids, terpenes, steroidal saponins, alkaloids, polysaccharides). Plants with hypoglycemic activity may contain one or more of these chemical constituents. These chemical compounds identified from this plant including: alkaloids, flavonoids, tannins, saponins and glycosides are well documented to have the potential to decrease the blood glucose level (Ragavan and Krishnakumari, 2006; Miura *et al.*, 2005; Oubre *et al.*, 1997). Thus, the significant antidiabetic effect of methanol extract of *L. lanceolata* could be due to the presence of the aforementioned constituents which could act synergistically or independently.

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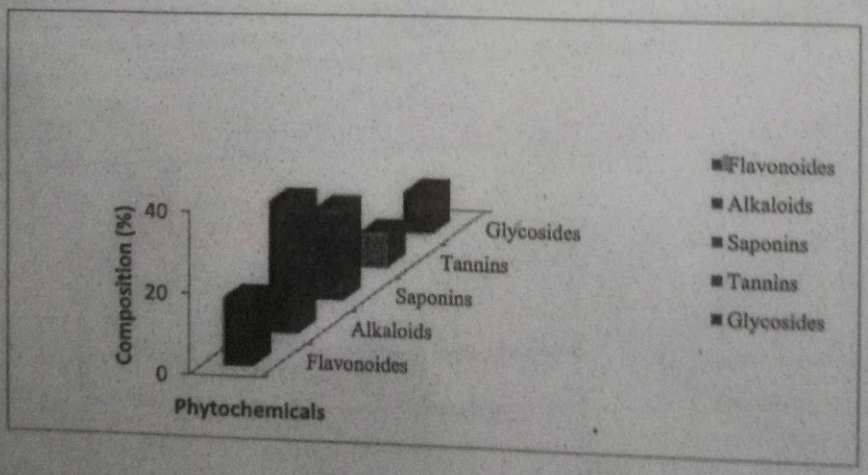

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glibenclamide, the body weight gain was increased with the effect more pronounced in 500mg/kg body weight extract of *Lophira lanceolata* treated rats than glibenclamide on 7th, 14th day of study.

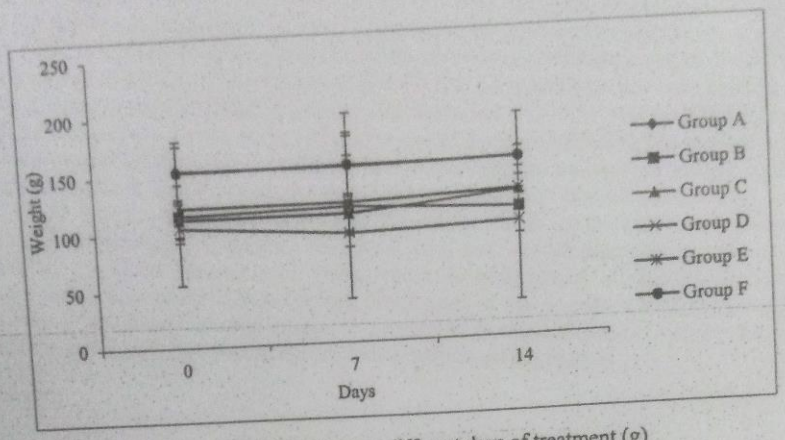
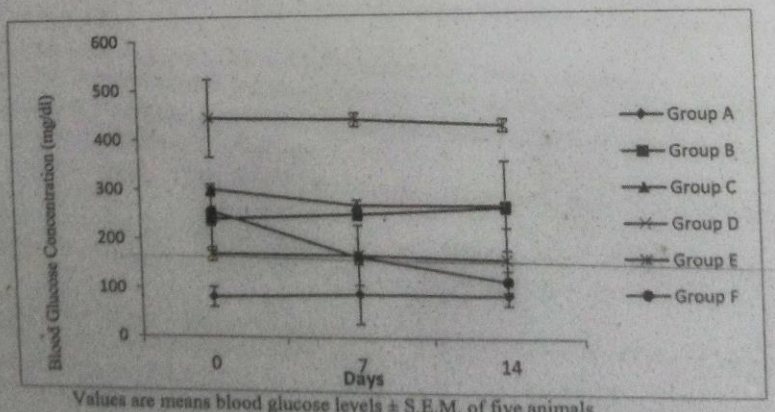


Figure 2: Weight of rats at different days of treatment (g)

Effect of extract on blood glucose level

Figure 3 showed that treatment with oral glibenclamide and other doses methanol extract of stem bark of *Lophira lanceolata* reduced blood glucose level on day 7th and 14th. The untreated diabetic control rat group showed increase in blood glucose level throughout the entire study period. Initially blood glucose level of untreated diabetic control group was 239.66±63.34 and after 14days of test period the blood glucose level was increased to 262.33±11.91. For extract concentrations of *Lophira lanceolata* (100, 200, 500 mg/kg) blood glucose were studied in three different groups of animals. All the three groups showed a significant decrease of blood glucose level on alloxan induced diabetic rats when compared to control group. The initial reading of blood glucose level of extract was 299.00±10.13. After the test period, there was marked reduction in blood glucose levels 268.00±10.58 and 266.00±88.73 in 14th days. In standard group initial blood glucose was 255.23 ±30.15 and the post test was 115.13±20.92 which showed that the standard drug produced maximum hypoglycemic effect and the statistical analysis was significant and slightly higher than that of extract group.



Values are means blood glucose levels ± S.E.M. of five animals

Figure 3: Blood glucose Level of rats at different days of treatment (mg/dl)

Antidiabetic activity of the crude extract of *Lophira lanceolata* stem bark

The result of the antidiabetic evaluation of the crude extract of *Lophira lanceolata* stem bark at three different doses (i.e.100, 200 and 500 mg/kg) revealed that the extract could not completely reduce the blood glucose level of the rats to its minimum. While those treated with standard (i.e. glibenclamide), had their blood glucose levels suppressed to its minimum. The group induced but not treated died at the 5th and 6th day of induction. The normal group that was not induced showed 100% survival. The extract also gave a promising result in the study of the activity against diabetes which is usually caused as a result of the presence of excess sugar in the blood and the inability of the body not able to break down glucose into energy. Glibenclamide has been used for many years to treat diabetes in order to stimulate insulin secretion from pancreatic β-cells. The present data indicated that the extract stem bark of *Lophira lanceolata* significantly reduced the elevated fasting blood glucose with respect to those of diabetic control animals. Based on the result obtained from the analysis carried out on the extract of *Lophira lanceolata* stem bark, it is recommended that further research work should be done using higher dosage of the extract to test for the anti-diabetic activity of the extract. From the present investigation, it can be concluded that oral administration of *Lophira lanceolata* stem bark extract produces significant antidiabetic effect in controlling the blood glucose level. Hence, the stem bark extract of *Lophira lanceolata* can be considered as a potent source of antidiabetic agents, which may be due to presence of flavanoids, alkaloids, tannins or saponins in the extract. Further studies to isolate and to characterize the active compound and to further elucidate the mechanism involved in the antidiabetic effect are in progress.

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