Antidiabetic Activity of Fractions of the N-Hexane Extract of Leaves of Eriosema Psoraleoides (Lam) G.don (Leguminosae) on Alloxan-Induced Diabetic Albino Rats

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ABSTRACT
Diabetes is a chronic disease that is raising health concerns. Eriosema psoraleoides is one of the medicinal plants reported to be used in managing this condition, in Eastern Nigeria. This study investigated the effect of four fractions [(n-hexane; n-hexane: dichloromethane (50:50); dichloromethane; and dichloromethane: methanol (95:5)] of the n-hexane leaf extract of this plant on the blood glucose levels of alloxan-induced diabetic rats. The n-hexane extract was obtained by maceration, filtration, and evaporation using a rotary evaporator. Phytochemical screening of the extracts was carried out using standard methods. The extract was fractionated using column chromatography, and ten fractions were obtained. The fractions were pooled together into four fractions based on the similarity in their Rf values. A dose of 200mg/kg of each of the fractions was given orally for 7 days to the alloxan-induced diabetic rats, and plasma blood glucose concentrations determined. Preliminary screening of the extract revealed the presence of flavonoids, steroids, reducing sugars, tannins, and combined anthraquinones. Two fractions, the n-hexane: dichloromethane (50:50) and the dichloromethane, produced significant (p<0.05) decrease in blood glucose concentrations in their treated groups compared to that of glibenclamide (standard drug) treated, and the diabetic untreated groups of the diabetic rats. However, the n-hexane and the dichloromethane: methanol (95:5) fractions produced no significant (p<0.05) decrease in blood glucose concentrations. The observed antidiabetic property of the n-hexane: dichloromethane (50:50) and the dichloromethane (100%) fractions suggests that Eriosema psoraleoides could be used in the management of diabetes and this gives credence to its use in ethnomedicine for this purpose.

INTRODUCTION
Diabetes is a chronic insulin metabolic disease that is raising health concerns around the world. Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by hyperglycemia often accompanied by glycosuria, polydipsia, and polyuria [1]. Complications of this condition include retinopathy, nephropathy, and neuropathy with features of autonomic dysfunction. The global prevalence of diabetes among adults 18 years of age and above has risen from 4.7% in 1980 to 8.5% in 2014 [2]. Diabetes doubles a person’s risk of death. From 2012 to 2014, diabetes is estimated to have resulted in 1.5 to 4.9 million deaths each year.[2,3] Experts have projected that the incidence of diabetes is set to increase by 64% by 2025.[4] Medicinal plants have found use in ethnomedicine in the management of this condition. The World Health Organisation [5], advocates the use of safe and efficacious herbal medicines in Traditional Medicine Practice. Eriosema psoraleoides (Leguminosae) is one of the medicinal plants reported to be used in the management of diabetes, in Eastern Nigeria. This plant is an erect herb. The leaves are 3-foliolate, with elliptic or oblong leaflets. The flowers are golden-yellow...
and hairy. The plant is commonly called Canary Pea in West Africa. Its synonyms include Crotalaria psoraleoides Lam, and Eriosema cajanoides (Guill. & Perr.) Hook.f. Some of its local uses include the treatment of venereal diseases, diarrhoea and use as an oxytocic. Biological activities have been reported for some other species of the Eriosema genus. Five pyrano-isoflavones have been isolated from the roots of Eriosema kraussianum with penile relaxant activity [6]. Chromones and phenolic compounds with antifungal activity have been isolated from the roots of E. tuberosum [7]. An isoflavonoid glycoside with antiviral activity has also been isolated from E. tuberosum [8]. The aqueous ethanol extracts of E. kraussianum have been reported to exhibit hypoglycaemic and vasorelaxant effects on laboratory rats [9]. The purpose of this study is to investigate the antidiabetic potentials of the leaves of Eriosema psoraleoides in an effort to justify its use in ethnobotany.

Materials
The plant material was collected from Nsukka, Nigeria, and identified as Eriosema psoraleoides (Leguminosae) by Mr. A. Ozioko of the Biodiversity and Conservation Organisation (BDC), Nsukka, with Herbarium number: INTERCEDD 968. Healthy Wistar Albino rats of both sexes with a weight range of 120-200g were selected for this study. They were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Port Harcourt. The rats were fed with standard feed and water throughout the duration of the study.

Methods
Phytochemical screening of the powdered leaves was carried out using standard methods [10-12].

Extraction of Plant Materials: A 2kg of the dried and powdered leaves of E. psoraleoides was extracted with n-hexane in a maceration jar, for a period of 3 days. The solution was then filtered, and the filtrate evaporated to dryness on a rotary evaporator. A black n-hexane extract was obtained to be used for the study. For the fractionation of the n-hexane extract, the column chromatographic method used was dry packing, with silica gel as the stationary phase. The four mobile phases used were n-hexane; n-hexane: dichloromethane (50:50); dichloromethane; dichloromethane: methanol (95:5). The eluents were spotted on TLC plates and those with similar spots were bulked together and dried as different fractions.

Alloxan-induced Diabetic Assay: Diabetes was induced in the albino rats through intraperitoneal administration of 120mg of alloxan monohydrate/kg body weight of the animals [13]. After 4 days, the animals that showed blood glucose levels above 200mg/dl were considered diabetic, and used for the study. The diabetic animals were arranged into 6 groups of five animals each. A 7th group consisted of normal rats not induced with alloxan, to serve as normal control.

Treatment schedule: Rats in group 1 were treated with glibenclamide (4mg/kg body weight), group 2 were diabetic untreated, group 3 were normal control. Rats in groups 4-7 were each treated with 200mg/kg b.w. of the four fractions, respectively. Animals were fasted overnight but allowed access to water prior to oral treatment. Basal blood glucose levels at zero time were determined (with a glucometer) before oral treatment. On day 1, the blood glucose levels were determined at intervals of 60 minutes for the next 240 minutes [14]. On days 2-7, the blood glucose levels were determined 2 hours after the treatment, daily. But, rats in groups 2 (diabetic untreated) and 3 (normal control) received no treatment throughout the duration of the study. However, they had their blood glucose levels determined daily also. This study was carried out in accordance with research ethics guidelines.

Results
Results of phytochemical tests showed presence of flavonoids, combined anthraquinones, tannins, carbohydrates, reducing sugar, and steroidal nucleus. However, saponins, alkaloids, unsaturated lactone ring and deoxy sugar were absent. Figures 1 and 2, below, show the mean blood glucose levels on Day 1 and Days 2-7, respectively. Tables 1 and 2 show the corresponding percent reductions in glucose levels.
Fig. 1: Mean blood glucose levels (mg/dl) in hours on Day 1

Group 1 = Glibenclamide (4mg/kg); Group 2 = Diabetic untreated; Group 3 = Normal control; Group 4 = n-Hexane (200mg/kg); Group 5 = Hexane: DCM, 50:50 (200mg/kg); Group 6 = DCM; and Group 7 = DCM: Methanol, 95:5 (200mg/kg)

Figure 2: Graph showing the mean glucose values (mg/dl) in days

Fig. 2: Mean blood glucose levels (mg/dl) on Days 2-7

Group 1 = Glibenclamide (4mg/kg); Group 2 = Diabetic untreated; Group 3 = Normal control; Group 4 = n-Hexane (200mg/kg); Group 5 = Hexane: DCM, 50:50 (200mg/kg); Group 6 = DCM; and Group 7 = DCM: Methanol, 95:5 (200mg/kg)
Table 1: Percentage reduction in blood sugar on day 1

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>1 hour</th>
<th>2 hours</th>
<th>3 hours</th>
<th>4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glibenclamide (4mg/kg) body weight</td>
<td>9.23%*</td>
<td>13.71%*</td>
<td>20.26%*</td>
<td>22.05%*</td>
</tr>
<tr>
<td>Normal control</td>
<td>3.67%</td>
<td>13.17%*</td>
<td>-4.97%*</td>
<td>8.86%*</td>
</tr>
<tr>
<td>N-hexane (100%)</td>
<td>-59.17%</td>
<td>-53.57%</td>
<td>-47.56%</td>
<td>-43.11%</td>
</tr>
<tr>
<td>N-hexane: dichloromethane (50:50)</td>
<td>-0.68%</td>
<td>-4.20%</td>
<td>-9.30%</td>
<td>-18.62%</td>
</tr>
<tr>
<td>Dichloromethane (100%)</td>
<td>49.28%*</td>
<td>53.81%*</td>
<td>51.94%*</td>
<td>51.42%*</td>
</tr>
<tr>
<td>Dichloromethane: methanol (95:5)</td>
<td>-20.77%</td>
<td>-5.39%</td>
<td>-18.38%</td>
<td>-17.52%</td>
</tr>
</tbody>
</table>

*Significant percentage reduction (p< 0.05), compared to the diabetic untreated group.

Table 2: Percentage reduction in blood sugar levels from day 2 to day 7

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glibenclamide</td>
<td>55.5%*</td>
<td>57.8%*</td>
<td>59.1%*</td>
<td>65.2%*</td>
<td>71.2%*</td>
<td>74.8%*</td>
</tr>
<tr>
<td>Normal control</td>
<td>31.7%*</td>
<td>11.8%</td>
<td>-17.0%</td>
<td>-42.9%</td>
<td>-6.26%</td>
<td>9.50%</td>
</tr>
<tr>
<td>N-hexane (100%)</td>
<td>-29.7%</td>
<td>-47.8%</td>
<td>-17.2%</td>
<td>-21.7%</td>
<td>-6.83%</td>
<td>4.77%</td>
</tr>
<tr>
<td>N-hexane: dichloromethane (50:50)</td>
<td>15.4%</td>
<td>31.1%*</td>
<td>38.4%*</td>
<td>48.8%*</td>
<td>50.0%*</td>
<td>51.4%*</td>
</tr>
<tr>
<td>Dichloromethane (100%)</td>
<td>60.0%*</td>
<td>61.59%*</td>
<td>64.51%*</td>
<td>67.18%*</td>
<td>67.69%*</td>
<td>68.50%*</td>
</tr>
<tr>
<td>Dichloromethane: methanol (95:5)</td>
<td>-17.01%</td>
<td>-17.95%</td>
<td>-14.36%</td>
<td>-12.48%</td>
<td>-12.39%</td>
<td>-23.00%</td>
</tr>
</tbody>
</table>

*Significant percentage reduction (p< 0.05), compared to the diabetic untreated group.

Discussion
It could be seen from Figure 1 and Table 1 that the hexane: dichloromethane [DCM] (50:50), and the DCM fractions caused slight decreases in the blood glucose levels, compared with the diabetic untreated group.

In Fig.2 and Table 2, showing glucose levels from Day 2-7, the decreases caused by these two fractions were significant (p<0.05), and were comparable to that of glibenclamide. However, the n-hexane, and then the DCM:Methanol (95:5) fractions produced no significant (p < 0.05) reductions in glucose levels. Medicinal plants are used throughout the world for a range of diabetic complications. Several investigations have been conducted and many plants have shown positive activity [16]. Secondary plant metabolites like coumarins flavonoids and terpenoids have been reported to have hypoglycaemic effects in various experimental animal models [17], [18]. Effect of flavonoids on pancreatic β-cells leading to their proliferation and secretion of more insulin has been proposed.[19]. Synthesis, release, increase of insulin sensitivity or the insulin-like activity of the plant extract have also been proposed [20]. The presence of similar phytochemicals in this plant, and any of the previously proposed mechanisms could be responsible for its observed antidiabetic activity.

Conclusion
The results of this study have shown that the n-hexane: dichloromethane (50:50), and the dichloromethane fractions of the n-hexane leaf extract of Eriosema psoraleoides possess antidiabetic activities. This tends to lend credence to its use in ethnomedicine for the management of diabetes mellitus.

Further Work
Further work is on-going to isolate and characterize the active principles responsible for this reported activity.

References
7. Ma WG, Fuzzati N, Lu SL, Gu DS, Hostettmann K. Further chro...