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GC-MS SCREENING, ACUTE TOXICITY AND IN VIVO ANTIDIABETIC ACTIVITY OF THE METHANOL WHOLE PLANT EXTRACT OF Plantago rugelii (Plantaginaceae)

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ABSTRACT
A metabolic disorder like Diabetes Mellitus requires serious attention to prevent its associated long-term complications. Plantago rugelii is an important medicinal plant used in South-South Nigeria for the management and treatment of diabetes mellitus amongst others. The present investigation focuses on the GC-MS profile, oral acute toxicity and the antidiabetic potential of the methanol whole plant extract of the plant. The fresh whole plant was obtained,air-dried, pulverized, cold macerated using absolute methanol and concentrated to dryness. Acute oral toxicity was conducted using standard procedure. The oral antidiabetic effect was evaluated in vivo on six groups of rats with five rats per group. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (160 mg/kg) in ice-cold 0.9% v/v normal saline. The extracts at 50, 100 and 200 mg/kg body weight reduced glycaemia by 30.43%, 43.78% and 59.54% respectively as against the glibenclamide treated rats, which has an 82.77% reduction. There was no mortality at 4 g/kg p.o. after 24 hours and no sign of delayed toxicity or mortality after 14 days of observation. The GC-MS spectra revealed twenty-one (21) phytoconstituents of which some have established antidiabetic effects. The antidiabetic effect of the plant could be attributed to the presence of the established biological phytochemicals however; bioassay-guided isolation and characterization of the phytocompound(s) should be carried out to identify the lead compound(s).

Keywords: Plantago rugelii, GC-MS, diabetes, antidiabetic, toxicity

INTRODUCTION

Mankind has long been used in different forms such as coarse, decoctions or portion and herbal drinks for healing over the past centuries (Farombi, 2003). Diabetes mellitus is a global problem, and successful treatment has not yet been discovered (Malviya et al., 2010). The increased prevalence of diabetes in Africa is threatening the developmental gains Africa has achieved (WHO, 1999). By 2015, there were more than 1.7 million diabetic sufferers in Nigeria with the prevalence of adults with diabetes in Nigeria aged between 20-79 years reported to be 2.0% (IDF, 2018). Of the two types of diabetes, type 2 diabetes mellitus that is prevalent in Nigeria is mostly due to a combination of insulin resistance and an inadequatecompensatory insulin secretory response (Kumar et al., 2012). Current therapy in use for diabetes like insulin and various oral hypoglycaemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, among others. These agents however are reported to produce serious adverse effects such as liver problems, lactic acidosis and diarrhea (Rajalakshmi et al., 2009). More than 50% of all the drugs currently in use are of natural product origin (Balandrin et al., 1993). These secondary metabolites are the compounds in plants responsible for their bioactive properties (WHO, 2020).

Plantago rugelii is commonly called ''plantain'' and locally as ''okpoatu'' by the Igbo, ''kpo kududu'' by the Yoruba and ''anahor'' by the Ika speaking tribes of Nigeria. The plant is found all over the world including Asia, Australia, New Zealand, Africa and Europe. The whole plant of P. rugelii has been utilized as topical for wounds (in vivo and invitro), microbial infections (Cyril et al., 2017), bites, stings, bronchial infection, hepatitis, and jaundice among others (David, 2006). A search at literature reviews shows that there is no scientific report on the antidiabetic activity of this plant and for this purpose, the plant is being screened scientifically for its in vivo antidiabetic activity as well as identify the phytoconstituents responsible for the biological activity via gas chromatography-mass spectrometry (GC-MS) method.

MATERIALS AND METHODS

Collection, identification and preparation of plant materials

Fresh P. rugelii whole plants with the roots, stem and leaves intact were collected in June 2019 from a forest in Ugbowo (around the premises of the University of Benin) in Benin City, Edo State Nigeria. The plant material was identified and authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan where an herbarium specimen was deposited and specimen number FHI 109775 issued. The plant was carefully washed with water to remove earthy material, air-dried before they were reduced to a fine powder with the aid of an electric milling machine.

Preparation of extract

Powdered plant material (500 g) was extracted with absolute methanol by maceration at room temperature for 72 hours. The extract was concentrated to dryness using a rotary evaporator at 40oC under reduced pressure. The concentrated extract was air-dried, weighed and stored in pre-weighed airtight glass container.

Animal procurement and treatment

Adult Swiss albino mice (21 – 32 g) and Wistar albino rats (150 – 200 g) of both sexes were obtained from the Animal House, Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University Sokoto Nigeria. The animals were handled by international principles guiding the use and handling of experimental animals.

Acute toxicity studies

Overnight-fasted Swiss albino mice were used for this study. The animals were randomly divided into five groups of five animals each and the extract was administered orally at doses of 500, 1000, 2000 and 4000 mg/kg to groups I, II, III and IV respectively while the control group V, received distilled water by the same route. General symptoms of toxicity and mortality in each group were observed within 24 h. Animals that survived after 24 hours were observed for another 14 days (no extract administration) for any sign of delayed toxicity (Cyril et al., 2017).

GC-MS analysis

The analysis of the extract was performed using GC-MS (Model: GCMS-QP 2010, Shimadzu, Tokyo, Japan) equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25mm film thickness. For GC-MS detection, the ionization energy of 70 eV was used. The carrier gas was Helium (99.99%) used at a constant flow rate of 1.51 ml/min. Injector and mass transfer line temperature were set at 200oC and 240oC respectively. The oven temperature was set from 70 to 220oC at 10oC/min. Two microlitres of the sample were injected in a split mode with a scan range of 40-1000 m/z. The total running time of GC-MS was 31 min. The relative percentage of the extract was expressed as a percentage with peak area normalization.

Identification of components

The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with the NIST08 library source (Stein, 1990).

In vivo antidiabetic assay

Induction of diabetes

Diabetes was induced by intraperitoneal administration of 160 mg/kg body weight of freshly prepared alloxan monohydrate ice-cold 0.9% v/v/ normal saline solution to rats fasted overnight. The rats were allowed access to 10% aqueous glucose as drinking water for four hours after injection of alloxan. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the lateral tail vein of each rat under mild anesthesia and the fasting blood glucose (FBG) was estimated with a glucometer (Accu-Check, Roche, Germany) (James et al., 2020). FBG greater than or equal to 200 mg/dL were considered diabetic

Experimental design

The rats were randomly distributed into 6 groups of 5 rats each. The extract and standard drug were given orally. Groups A and B were normal (vehicle control group received 2 mL of normal saline) and untreated diabetic control groups respectively. While group C (positive control received orally 600 µg/kg bw of glibenclamide), groups D, E and F received methanol extract of Plantago rugelii (MEPR) at dosages of 50, 100 and 200 mg/kg bw respectively. Blood glucose concentration was measured after 30 min, 1 hr, 3 hr and 6 hr after administration of a single dose of each of the regimens (Monjoy et al., 2012; James et al., 2020)

Statistical analysis

All quantitative data for statistical analysis were analyzed using GraphPad Prism.Results were presented as the mean and standard error of the mean (Mean ± SEM). The statistical significance between the control and each of the treated groups was determined by Dennett's post hoc test after one-way ANOVA. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of the crude methanol extract was found to be 4.71% in agreement with the report of Cyril et al., 2017.

Acute toxicity screening

The result of the acute toxicity profile is presented in table 1.

Table 1: **Acute toxicity profile of P. rugelii plant extract**

Dose (mg/kg bw)	Mortality Ratio	% Mortality
500	0/5	0
1000	0/5	0
2000	0/5	0
4000	0/5	0

The administered graded doses of the methanol extract of P. rugelli did not result in mortality over the 24 hr period. No death or delayed toxicity was observed in the animals after 14 days. This shows that the LD50 was above 4000 mg/kg in agreement with the report of Cyril et al., 2017 who as well reported no mortality at a higher dose of 8 g/kg bw.

Phytochemical analysis using Gas Chromatography-Mass Spectrometry

The active principles with their retention time and area percentage in the MEPR are presented Figures 1 and Table 2 respectively.

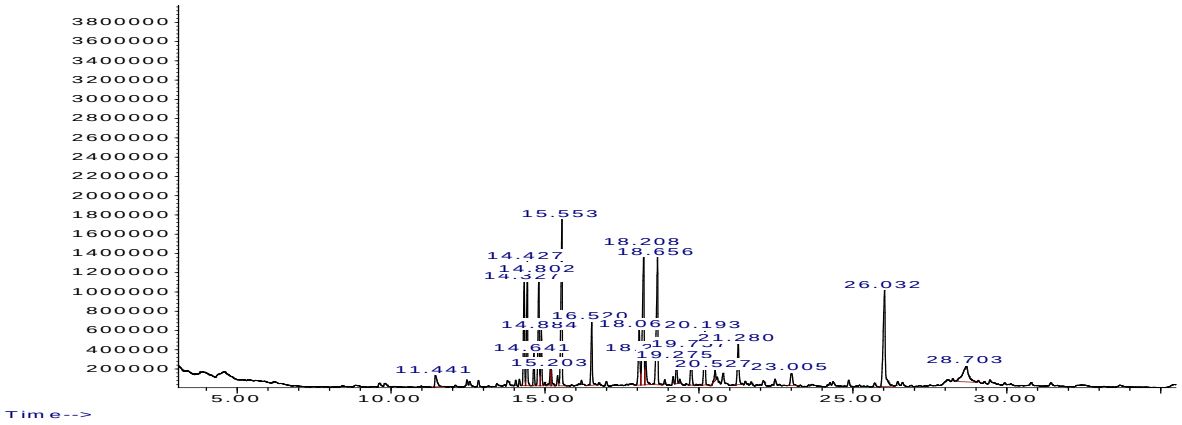

Figure I: GC-MS chromatogram of P. rugelii methanol whole plant extract

Table 2: GC-MS spectral analysis of methanol extract of P. rugelii.

S/N	Name of the compound	Area %
1	Apiol	1.331
2	Neophytadiene	6.266
3	6,10,14-trimethyl-2-pentadecanone	6.620
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol)	1.681
5	9-Heptadecanone	6.857
6	3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol)	2.945
7	2 – heptadecanone	0.895
8	methylhexadec-11-enoate	11.312
9	Methyl-18-oxidanyloctadeca-9,12-dienoate	3.988
10	cis-13-octadecanoic acid, methyl ester	4.493
11	11-octadecanoic acid, methyl ester	11.282
12	Methyl strearate	2.302
13	9-octadecanoic acid, ethyl ester	10.199
14	Octadecanoic acid, ethyl ester	1.494
15	Neophytadiene	2.596
16	methyl-9-cis-11,13-trans-octadecatrienoate	3.884
17	Cyclohexaneethanol-β-methyl -	0.656
18	Bis-(2-ethylhexyl) phthalate	3.269
19	2-acetyl-3-(1-methyl-2-pyrrolyl)1,4-benzenediol	1.413
20	Bis(2-ethylhexyl) phthalate	10.526
21	2-Acetyl-3-(1-methyl-2-pyrrolyl)-1,4-benzenediol	4.955

The GC-MS analysis of MEPR revealed the presence of 21 phytoconstituents, among which are established antidiabetic effects. Apiol as a polyvalent active natural product has been established to target different anti-diabetic receptors and reported to be the active anti-diabetic phytoconstituent present in the leaves of Steblus asper exhibiting the anti-hyperglycemic activity (Monjoy et al., 2012). Neophytadiene has been identified as an important constituent of the essential oil of Prangos gaubae responsible for its antioxidant, antidiabetic, anti-obesity, and neuroprotective properties (Mir-Babak et al., 2017). Phytol, a colourless, high boiling oil which part of chlorophyll have been established to possess antidiabetic activity especially in type-II diabetic patients (McCarty, 2001; Elmazar et al., 2013; Priyankar et al., 2015). Polyunsaturated fatty acids like cis-13-octadecanoic acid methyl ester, 11-octadecanoic acid methyl ester, 9-octadecanoic acid ethyl ester and octadecanoic acid ethyl ester are found in the lipids of cell membranes. They have been established to have antioxidant, anti-inflammatory, hepatoprotective, insectifuge, immunomodulatory, anticancer, anti-diabetic and anti-arthritic properties (Houseknecht et al., 1998; Jones, 2002). They exhibit their antidiabetic activity by possessing insulin secretion, insulin stimulation, α- glucosidase (Artanti et al., 2012; Zuraini et al., 2012; Balogun et al., 2013; Wuttke et al., 2013). Methyl stearate has been shown to complement other phytoconstituents in the Nerium oleander leaf extract in possessing hypoglycaemic potential as well as ameliorates diabetes-associated hyperlipidemic and nephropathic complications dually by improving carbohydrate metabolism and providing antioxidative protection (Priyankar et al., 2015).

Effect of whole plant methanol extract of P. rugelii on alloxan-induced wistar rats

The result of the effect of P. rugeliimethanol extract on blood glucose levels is presented in Figure 2.

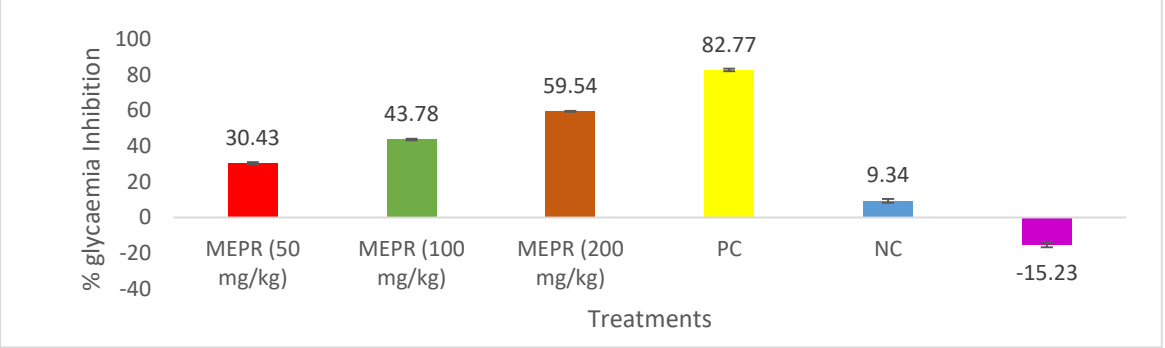

Figure 2: Percentage glycemic inhibition in FBG of normal control (NC), diabetic control (DC), positive control (PC) and alloxan-induced diabetic rats treated with methanol extract of Plantago rugelii

The results of the antidiabetic properties of the extracts as shown in figure 2 reveals a dose-dependent inhibition of hyperglycaemia thou not significant compared to the conventional standard drug (glibenclamide, 0.6 mg/kg) used for this study. The antidiabetic activities of the plant is in agreement with the folkloric usage of the plant in the

management of diabetes. The observed hypoglycaemic activity could be associated with the reported phytochemicals present in the plant (Table 2) which have established antidiabetic agents. The possible mechanisms underlying the hypoglycaemic activity mightinclude inhibition of intestinal absorption of glucose, facilitation of glucose-induced insulin release, enhancement of peripheral glucose uptake, promotion of the regeneration of β-cell of islets of Langerhans and amelioration of oxidative stress (Kibiti, and Afolayan, 2015) attributed to the presence of a variety of phytoconstituents present in this plant. The blood-glucose-lowering effect of the plant extracts may also be attributed to the presence of flavonoids, alkaloids and saponins that have been known to confer hypoglycaemic activity (Middleton et al., 2000) all of which has been previously reported by Cyril et al., 2017

CONCLUSION

The present study revealed that the plant Plantago rugelii is relatively safe up to a dose of 4 g/kg body weight. The methanols extract of the plant has in a dose-dependent manner hypoglycemic effect thou not comparable to that of the standard drug employed for the study. The observed activity could be attributed to the synergistic activities of the bioactive principles present in the plant. Further research is on the way to ascertain the actual bioactive constituent(s) responsible for this effect.

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