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ANTI-DIABETIC AND ANTI-OXIDANT ACTIVITIES OF THE METHANOL EXTRACT AND FRACTIONS OF Cassytha filiformis Linn.

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Abstract

Cassytha filiformis aqueous decoction is used by herbalists in Mbaitoli Local Government Area of Imo State, Nigeria in the management of high sugar complications. In this study, methanol extract and bioassay-guided fractions of aerial parts of *Cassytha filiformis* were investigated for anti-diabetic and anti-oxidant activities. The anti-diabetic, total cholesterol and lipid peroxidation activities of crude extract were compared with Glibenclamide in mice while in vitro anti-oxidant activities (DPPH and FRAP) of crude extract and isolated fractions were compared with ascorbic acid. Crude extract at highest dose (600 mg/kg) reduced fasting blood sugar (FBS) by 8.39, 6.33 and 4.83 mmol/L at 7 days, 14 days and 21 days respectively while Glibenclamide reduced FBS by 7.78, 6.02 and 4.98 mmol/L at 7 days, 14 days and 21 days respectively. F3 and F4 showed synergistic effects and reduced FBS by 7.32 and 21.35 mmol/L at 1h and 15.66 and 6.31 mmol/L at 6h respectively. Serum malondialdehyde (MDA) and total cholesterol levels were lowered in the crude extract-treated animals after 21 days. Separation techniques used were thin layer chromatography (analytical and preparative) and column chromatography. DPPH anti-oxidant assay showed increasing % anti-oxidant activities at 10, 50, 100, 200 and 400 µg/ml respectively as compared to ascorbic acid. FRAP experiment gave 0.73, 1.13, 1.19, 1.41, and 1.61 µM at 10, 50, 100, 200 and 400 µg/ml respectively. Ascorbic acid has a FRAP value of 2.00 at concentrations of 100-1000 µg/ml. These results show the methanol extract and fractions of *C. filiformis* possess anti-diabetic potential and may be a source of novel drug for managing diabetes

Keywords: *Cassytha filiformis, diabetes, alloxan monohydrate, chromatography, anti-oxidants.*

Introduction

Diabetes is a chronic disorder that is affecting the world population on an epidemic scale. It results from the abnormal metabolism of glucose where insulin action is impaired; or absolute insulin deficiency results in imbalance of glucose metabolism and leads to the syndrome, diabetes mellitus [1].

The insulin deficiency causes changes in glucose metabolism and biochemical processes thereby increasing fasting (sugar) glucose level, decreasing hepatic and skeletal glycogen content and decreasing the activity of glucose-6-phosphate dehydrogenase (G6PD) [2].

Many oral anti-diabetic drugs used today fail to give a long-term glycaemic control [3]. Herbal extracts which are effective in lowering blood glucose with minimal or no side effects are known to be used as anti-diabetic remedies and as such, active compounds have been isolated from them [4].

Cassytha filiformis Linn. is a leafless, climbing, twining, vine-like, auto parasitic seed-bearing plant in the family Lauraceae [5]. It infests a wide variety of coastal plants throughout Hawaii, the Pacific, and the tropics worldwide. Indigenous to Hawaii, it is one of many higher flowering plant species that have, through evolutionary divergence, become parasitic on various organs of other higher plants. Having long ago lost certain metabolic processes and physical structures to support it and remain independent, *C. filiformis* clings to other, mainly woody plants for physical support, nutrition, and water. Its common names are kauna'oapehu, kauna'oamalo-lo, kauna'oauka, kauna'oa, malolo, pololo (in Hawaiian), dodder laurel (or laurel dodder), woe vine, and love vine [6]. This species was formerly named Cassytha senegalensis A. Chev. and *Cassytha guineensis* Schumach & Thonn. The genus name derives from "kesatha", Aramaic for "a tangled wisp of hair." *Cassytha species* are parasitic vines with small haustoria (infectious, adhesive structures used to withdraw nutrients from host organs through host cell membranes). Its stems are filiform, containing chlorophyll [6]. The leaves are reduced to minute scales.

In the Pacific region, *C. filiformis* is widely used as a medicinal plant. It is used to treat jellyfish stings in Fiji. *C. filiformis* is purported to be used by several different Polynesian cultures for treatment of cancers [7]. The plant is also used traditionally for treatment of some human birthing issues. Modern midwives recommend taking the juice made from the crushed stem for 4 weeks before the expected date of birth in order to ease labour pains and to quicken labour time and lubricate the birth canal [8]. In Palau, bark of *Terminalia cata* (scarlet macaw, aramacao) is mixed well with a whole plant of *C. filiformis* and copra, crushed together, and the juice which is squeezed out is drank for gonorrhoea. Herbalists in Ogwa, Mbaitoli Local Government Area, Imo state in the South Eastern Nigeria use *C. filiformis* aqueous extract in treating diabetes and liver disorders.

In modern medical research, *C. filiformis* has a number of biologically active chemical compounds with potential human health applications. For instance, ocoteine, a compound isolated from *C. filiformis*, was found to be an alpha 1-adrenoceptor blocking agent in rat thoracic aorta [8]. This type of chemistry has potential applications for inhibiting certain carcinomas such as prostate cancer. A number of compounds in *C. filiformis* have anti-platelet aggregation activity. *Cassytha filiformis* is medicinally used as vasorelaxant [9] and anti-trypnosomal [10]. Some of the isolated compounds from this plant are aporphine alkaloid, oxo-aporphine alkaloid, cassyformine, filiformine, cathaformine, lignan, actinodophine, and octenine [11]. This study was carried out to investigate the acclaimed anti-diabetic activity of *C. filiformis*, isolate the active compound and also screen its anti-oxidant potentials.

Methods

Plant collection

The aerial parts of *C. filiformis* were collected from the South-eastern area (Owerri) of Nigeria in April, 2019 and identified by Mr Ozioko, a taxonomist with International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu State, Nigeria. They were dried at room temperature in the absence of direct sunlight.

Preparation of the Extract

The aerial parts (1 kg) of *C. filiformis* were pulverized with a laboratory mill. This was soaked in 80 % methanol for 48 h after which it was filtered. The filtrate was concentrated with a rotary evaporator at 40 °C. The dry crude extract was stored in a refrigerator at 4 °C.

Laboratory animals

The conduct of this research was approved by the Faculty of Veterinary Medicine, University of Nigeria, Nsukka and is in accordance with the approved research guidelines on laboratory animal use of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, where the animal study was carried out. All animals were humanely handled and their welfare respected throughout this study as stipulated in the 1964 Helsinki Declaration, as amended [12]. Albino mice (21-28 g) were housed in standard stainless-steel cages (five animals per cage), maintained under standard laboratory conditions (i.e. 12:12 hour light and dark cycle; at an ambient temperature of 25 °C; 35-60 % of relative humidity); the animals were fed with standard mice pellet diet and water ad libitum [13].

Acute toxicity test

Doses of 250, 500, 1000 and 2000 mg/kg were administered to the mice in different groups (n=6) *per os*. They were monitored for 48 h for signs of toxicity [14].

Fractionation

This was performed in Prof G. O. Igile's Laboratory at the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria. The methanol crude extract (15 g) mixed with silica gel (1:4 respectively) was dried and subjected to chromatography over a silica gel 60Fglass column of length and diameter of 55 cm and 6 cm respectively. Elution was with solvent mixtures of increasing polarity from n-hexane, chloroform, ethyl acetate to methanol. Aliquots were collected in 10 ml portions with test tubes and monitored with TLC, silica gel GF254 as adsorbent and a solvent system of chloroform, ethyl acetate and methanol. UV fluorescence at 254 - 365 nm and vanillin-sulphuric acid spray (1 g vanillin dissolved in 100 ml conc. Sulphuric acid) and heated at 120 °C were used for visualization [15]. The aliquots showing similar spots were pooled together as a fraction. After anti-diabetic bioassay in mice of the F2, F3 and F4, F3 and F4 having the highest anti-diabetic activity were subjected to preparative thin layer chromatography over silica gel 60 GF254 with chloroform: ethyl acetate: methanol (2:1:2) to afford 3 pure fractions with Rf 0.636 (CFA) 2.1 mg, 0.780 (CFB1) 1.7 mg and 0.760 (CFB2) 1.4 mg respectively.

Anti-diabetic Activity with crude extract

Albino mice were made diabetic by a single intraperitoneal injection of freshly prepared alloxan monohydrate at a dose of 160 mg/kg. It was dissolved in distilled water immediately before use and administered to overnight fasted (with water available) albino mice. After 8 days, animals with fasting blood glucose of 8.0 mmol/L or more were considered diabetic and used in the study. The crude extract was given as follows: Group I served as negative control receiving the vehicle, distilled water (10 ml/kg, *per os*); groups II-IV received the test crude extract of *C. filiformis* at doses of 150, 300 and 600 mg/kg respectively and group V received Glibenclamide (2 mg/kg, *per os*). The animals were treated for 21 days and fasting blood glucose level was measured on Days 7, 14 and 21. Samples were collected by a snip-cut at the tip of the tail under mild anaesthesia and blood sugar level was measured with an auto analyser using Accu-Check Advantage II glucose kit. The sera were used to measure lipid peroxidation using malondialdehyde (MDA) levels as markers [16] and total cholesterol concentrations were evaluated by the methods described by Allain et al. [17] after 21 days.

Anti-diabetic Activity with fractions

Fractions F2, F3 and F4 were tested for anti-diabetic activity using the method described above. They were given at a dose of 5 mg/kg while Glibenclamide was given at 2 mg/kg. All treatments were *per os* on day 8 after challenge with freshly prepared alloxan monohydrate intraperitoneally at 160 mg/kg. The animals were treated once and fasting blood glucose level was measured at 1 h and 6 h.

Anti-oxidant analysis

The free radical scavenging activity of the extract was analysed by the 1, 1–diphenyl-2-picryl-hydrazyl (DPPH) assay described by Iwalewa *et al*. [18]. While the Ferric reducing antioxidant power (FRAP) Assay Method was done using the method described by Benzie and Strain [19].

Data analysis

Results from the antidiabetic studies were converted to percentages. Data for serum cholesterol and MDA were expressed as mean ± SEM and subjected to one-way analysis of variance (ANOVA) followed by Dunnett's t-test. P values <0.05 were considered significant

Results

Acute Toxicity Test

No death was recorded even at an oral dose of 2000 mg/kg after 48 h. The extract was said to be safe.

Anti-diabetic studies

The crude methanol extract of *C. filiformis* produced a dose-dependent reduction of blood glucose level in diabetic mice. The percentage blood glucose reduction was also time-related. The highest reduction in blood glucose was achieved on Day 21 post treatment. The lowest (4.83 mmol/L) of blood glucose was achieved by administering 600 mg/kg (Table 1). Glibenclamide (2 mg/kg) gave 4.98 mmol/L on Day 21. The results of MDA showed a dose-dependent decrease in MDA levels with the highest dose of the extract (600 mg/kg) giving the highest reduction which was comparable to Glibenclamide (Table 3). There was also a significant decrease ($p<0.05$) in total cholesterol level in the groups treated with the crude extract and Glibenclamide when compared with the group treated with distilled water (negative control)

Fractions F3 and F4 at 5 mg/kg also produced a reduction in fasting blood glucose levels in mice by 7.32 and 21.35 mmol/L respectively at 1 h. At 6 h post treatment, F3 and F4 produced 15.66 and 6.31 mmol/L respectively (Table 2).

Isolation of active fractions/compounds

Isolated pure fractions from F4 had Rf values of 0.760 and 0.780 respectively. F3 had a single band at 0.636. The solvent mixture for the separation was 2:1:2 (chloroform, ethyl acetate and methanol respectively). These were submitted for characterization and identification.

In vitro antioxidant assay

The methanol crude extract of *C. filiformis* showed concentration-related anti-oxidant effect in the DPPH assay. The anti-oxidant activity increased with the concentration up to a maximum at 400 µg/ml (Fig. 1). The anti-oxidant activity was comparable with that of ascorbic acid at 200 and 400 µg/ml. The FRAP assay also showed a concentration-dependent anti-oxidant activity of the crude extract. The highest anti-oxidant activity was achieved at 400 µg/ml (Fig 2).

Discussion

The crude extract of *C. filiformis* was tolerated by experimental animals up to 2000 mg/kg with no complications showing that the LD50 for the extract is quite high and thus, the extract has a wide safe range orally.

In the present study, the methanol crude extract from *C. filiformis* was able to reduce FBS levels in diabetic mice significantly ($p<0.05$) when compared with untreated mice on the one hand and much better than those treated with glibenclamide (a known antidiabetic agent) on the other hand (Table 1) from Day 7 to Day 21. Fractions F3 and F4 from the results in Table 2 showed a dose-dependent reduction of fasting blood glucose levels within 6h. Most reported antidiabetic and hypoglycaemic agents are known to achieve their mechanisms through this pathway in addition to possessing free radical scavenging abilities [20]. Total cholesterol levels were also shown to reduce with increasing doses of crude extract alongside glibenclamide (Table 1).

The mechanism by which *C. filiformis* and its fractions exert their hypoglycaemic effects at this point is uncertain although from the results of the in vitro antioxidant assays (Figure 1 and 2), we postulate that one of the mechanisms could be by scavenging free radicals. Alloxan, a diabetogenic agent is known to induce increase in blood sugar

levels in laboratory animals by increasing oxidative stress through generation of reactive free radicals [20]. Previous phytochemical studies with the crude have shown the presence of saponins and flavonoids which are very good sources of antioxidants that are capable of mopping up free radicals produced by alloxan in the process of pancreatic destruction mimicking the natural way in which the pancreas is impaired in Type 1 diabetes [9, 21].

The protective role of lipids and cholesterol in diabetes has been suggested to occur via very many different pathways. HDL have been shown to exert part of its anti-atherogenic effects by counteracting lipid peroxidation, promoting reverse cholesterol transport pathway by inducing the efflux of accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL [22].

Conclusion

In conclusion, *C. filiformis* and its fractions have shown significant anti-diabetic effects in alloxan-induced diabetic rats. It probably produced this effect by its anti-oxidant activities shown in DPPH and FRAP in vitro assays, and its effect on total cholesterol and MDA in vivo

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Conflict of interest

None.

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Table 1. Effect of the methanol crude extract of *C. filiformis* on fasting blood glucose level of alloxan-induced diabetic mice.

Dose (mg/kg)	Fasting blood glucose level (mmol/L)			Total cholesterol (mg/dl)	MDA (nM of MDA/ml serum)
	DAY 7	DAY 14	DAY 21		
GP1 (negative control)	23.20	18.16	16.17	187.33±2.33	3.40±0.03
GP 2 (150)	15.10*	10.33*	7.33*	90.21±2.11*	1.44±0.02*
GP 3 (300)	11.67*	8.11*	5.72*	84.43±1.23*	1.38±0.02*
GP 4 (600)	8.39*	6.33*	4.83*	73.51±1.57*	1.31±0.01*
GP 5 (Glibenclamide, 2 mg/kg)	7.78*	6.02*	4.98*	70.33±1.20*	1.26±0.03*

*significantly different (P<0.05) from the negative control

Table 2. Anti-diabetic activities of fractions F2, F3 and F4 on alloxan-induced diabetic mice at 1h and 6h post treatment.

Dose (5 mg/kg)	Fasting Blood Sugar (mmol/L)	
	1h	6h
F_2	15.81	19.71
F_3	7.32*	15.66
F_4	21.35	6.31*
Glibenclamide (2 mg/kg)	7.78	4.98

*Not significantly different (P<0.05) from the standard drug (Glibenclamide)

Figure 1. DPPH anti-oxidant activity of *C. filiformis* methanol crude extract

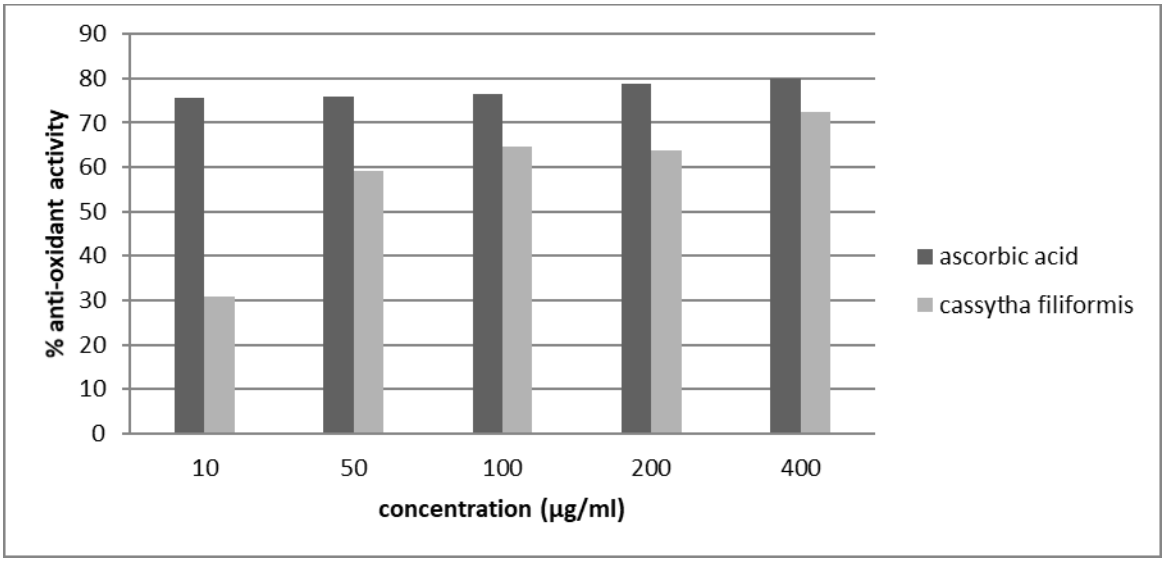

Figure 1. The anti-oxidant activity of *C. filiformis* methanol crude extract using the FRAP method.