

Research Paper:

Antioxidant and Hypolipidemic Effects of Methanolic Root Extract of *Chromolaena odorata* in Alloxan-induced Diabetic Rats



Olujemisi Omotayo Omonije^{1*}, Abubakar Ndamam Saidu¹, Hadiza Lami Muhammad¹

1. Department of Biochemistry, Federal University of Technology, Minna, Nigeria.



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* Corresponding author:

Olujemisi Omonije, PhD.

Address: Department of Biochemistry,
Federal University of Technology,
Minna, Nigeria

E-mail: olujemisi.pg915840@st.futminna.edu.ng

ABSTRACT

Background: *Chromolaena odorata* (*C. odorata*) is a medicinal plant with hypoglycemic effect known in traditional medicine. The present study investigated the in vitro antioxidant and in vivo anti-hyperglycemic effect of the methanol root extract of *C. odorata* in rats.

Methods: Phytochemical compositions were evaluated, using standard procedures while the antioxidant activities were examined, using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Free Radical Scavenging and Ferric Reducing Power (FRAP) assays. Twenty albino rats were administered 120 mg/kg of body weight (bw) alloxan and were divided into four groups of five animals each. Groups A to D were treated with 2 ml/kg of normal saline, 300 mg/kg, 500 mg/kg bw extract and 5 mg/kg bw glibenclamide, respectively, through oral route for 14 days. Blood samples were collected and prepared for the analysis of serum lipids profile.

Results: The extract contained tannins (54.76±4.08 mg/100 g), saponins (322.78±17.35 mg/100 g), glycosides, alkaloids (74.34±6.08 mg/100 g), flavonoids (79.63±4.55 mg/100 g), phenols (154.32±11.21 mg/100 g), glycosides, steroids and reducing sugar. The extract promoted the inhibition of DPPH radicals with IC₅₀ values of 191.68±1.68 µg/mL. In comparison with the untreated controls, total cholesterol, Low Density Lipoprotein (LDL)-cholesterol and triglycerides were significantly reduced (P<0.05) following treatment with *C. odorata* extract while High Density Lipoprotein (HDL)-cholesterol, was significantly increased.

Conclusion: The *C. odorata* extract exhibited antioxidant and hypolipidemic effects in alloxan-induced diabetic rat, thus could be considered as a natural product in the management of dyslipidemia secondary to diabetes.

Keywords: Antioxidants; *C. odorata* extract; Diabetes mellitus; Hypolipidemia; Blood lipid profile

Introduction

Diabetes is a condition where the pancreas produces insufficient amount of insulin or when the body is unable to efficiently recognize the insulin or both [1]. Although some pathogenesis of diabetic complications are not well understood, many biochemical path-

ways associated with the hyperglycemia have been implicated [2]. Besides hyperglycaemia, hyperlipidaemia is also considered one of the major factors, which causes diabetic complications including death [3]. In diabetes, the causes and sites of intervention in biochemical process are diverse and high serum lipid profile has been implicated [4]. In the progression of diabetes, it is established that tissue damage may be due to free radicals

attacking cell membranes through peroxidation of unsaturated fatty acids, leading to extensive damage and dysfunction of cellular membranes [5].

A numbers of reactive oxygen and non-oxygen radicals may be formed in the human body during normal cellular metabolism, and in foods in the course of production and processing [6]. These reactive radicals increase peroxidation of lipids and oxidize cellular molecules, such as proteins, lipids and DNA. These radicals also cause oxidative stress or even cell death, and impair tissues and initiate or aggravate disease processes, such as atherosclerosis, uncontrolled cellular growth, damaged lungs alveoli, emphysema, or impaired liver functions (cirrhosis), arthritis and diabetes [7, 8]. Antioxidants inhibit the oxidation of cellular molecules by blocking the chain reactions involved, and thus prevent or restore the cellular damages [9]. They exert their effects by acting as a chelator of metal catalysts, inhibiting free radicals, spurring antioxidant enzymes, plummeting α -tocopherol radicals, and hindering oxidases [10].

The plant, *C. odorata*, is a perennial shrub belonging to the Asteraceae family, native to south and central America [11]. Although, it is considered as one of the world's worst weeds, *C. odorata* leaves and other parts have been used as folk medicine to treat a variety of ailments in tropical Africa, including malaria, dysentery, toothache, diarrhea, diabetes, skin diseases, fever and wounds [11-14]. This study aimed to investigate the antioxidant and anti-hyperglycemic properties of the extract of *C. odorata* whole roots.

Materials and Methods

Chemicals and Reagent: Randox Liquizyme assay and Spectrum diagnostic kits were used to determine the lipid profile. All chemicals and reagents were of analytical grade.

Sample preparation and extraction: Fresh whole root sample of *C. odorata* was collected in March 2016 at the Federal University of Technology Staff School, Minna, Niger State in Northern Nigeria. The roots were thoroughly washed under running water to remove the contaminants, were cut into pieces, dried for 2 weeks at 37°C, and powdered, using a grinder mill. A 50g sample of the root material was extracted with 200mL of methanol, using soxhlet apparatus and the resulting extract was concentrated in a rotary evaporator.

Phytochemical composition: Qualitative and quantitative phytochemical analyses were carried out on the root extract as described previously [15-17].

Determination of in vitro antioxidant activity: The antioxidant activity of the extract was determined by two in vitro methods as follow: a) using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) free radical scavenging; and b) reducing power assay methods at a varying of concentrations (2.5-100 μ g/mL), based on the method of Oyaizu [18]. All assays were carried out in triplicates and the data were subjected to statistical analyses.

Experimental animal for in vivo study: Healthy albino rats of average weight (134.87 \pm 3.23 g) were obtained from the animal holding unit, Federal University of Technology, Minna, Niger State, Nigeria. The rats were maintained at laboratory conditions of temperature and humidity, and at 12 hour light/dark cycles. They had free access to rat food pellets and water ad libitum. Approval to conduct this study was granted by the Committee on Ethics for Medical and Scientific Research, Federal University of Technology, Minna, Nigeria. Also, we observed the current internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review. Similarly, the study followed the Guidelines for the Care and the Use of Laboratory Animals issued by the American Commission of Life Sciences, National Research Council [19].

Experimental design: Twenty albino rats were administered a freshly prepared dose of alloxan monohydrate (120 mg/kg) intra-peritoneally. The animals had fasted overnight. The diabetic state was confirmed by testing the glucose level above 200 mg/kg bw [20]. The animals were divided into four groups and were treated with 2 ml/kg of normal saline, 300 mg/kg or 500 mg/kg bw extract, and 5 mg/kg bw glibenclamide, respectively. All treatments were administered daily through oral route for 14 days. Five rats were also assigned as the normal control group without any treatment. The blood glucose level was monitored and the body weight taken every three days. On the 15th day, animals in all groups were euthanized; blood samples collected and prepared to extract the serum according to the method described by previous studies [21, 22].

Serum lipid profile: Serum total cholesterol, triglyceride, high and low density lipoprotein cholesterol (HDL-c & LDL-c) were estimated, using Randox diagnostic kit (United Kingdom) as described previously [23-26].

Statistical Analyses: Data were analyzed using Statistical Analysis System (SAS) and presented as Mean±SEM. Comparisons between the groups were carried out by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The level of significance was set at $P<0.05$ [27].

Results

Phytochemical Composition: The phytochemical composition of the root extract of *C. odorata* contained tannins (54.76 ± 4.08 mg/100 g), saponins (322.78 ± 17.35 mg/100 g), alkaloids (74.34 ± 6.08 mg/100 g), flavonoids (79.63 ± 4.55 mg/100 g), phenols (154.32 ± 11.21 mg/100 g), glycosides, steroids and reducing sugar (Table 1).

In vitro Antioxidant Activities: Figure 1 shows the effect of *C. odorata* extract on DPPH radicals. The extract exhibited dose dependent DPPH radical scavenging activity with IC_{50} values of 191.68 ± 1.68 µg/mL compared to ascorbic acid, with the IC_{50} value of 45.89 ± 0.78 µg/mL. The extract also exhibited significant FRAP activities (Figure 2).

Hypoglycemic Effect of the Extract: The root extract of *C. odorata* at 300 and 600 mg/kg bw caused 64.15% and 68.27% hypoglycemic effects, respectively, while

the standard drug (gilbenclamide) at 5 mg/kg bw caused a 71.34 % hypoglycemic effect in rats.

Hypolipidemic Effect of the Extract: The effect of *C. odorata* root extract on the lipid profile in alloxan-induced diabetic rats is shown in Figure 3. There was a significant increase in the serum levels of total cholesterol, triglycerides and LDL levels ($P<0.05$) in diabetic untreated rats compared to those in the control group. The oral administration of the root extract caused a significant reduction in total cholesterol, triacylglycerol and LDL ($P<0.05$). Also, the HDL increased significantly to levels approaching that of the control rats.

Discussion

There has been increasing research on the role of African natural products in the treatment and clinical management of tropical and oxidative stress-related diseases [7, 28, 29]. The results presented in Table 1 indicate that *C. odorata* contained tannins (54.76 ± 4.08 mg/100 g), saponins (322.78 ± 17.35 mg/100 g), glycosides, alkaloids (74.34 ± 6.08 mg/100 g), flavonoids (79.63 ± 4.55 mg/100 g), phenols (154.32 ± 11.21 mg/100 g), steroids and reducing sugars. This corroborated with the report of two previous studies on *C. odorata* root extract [30].

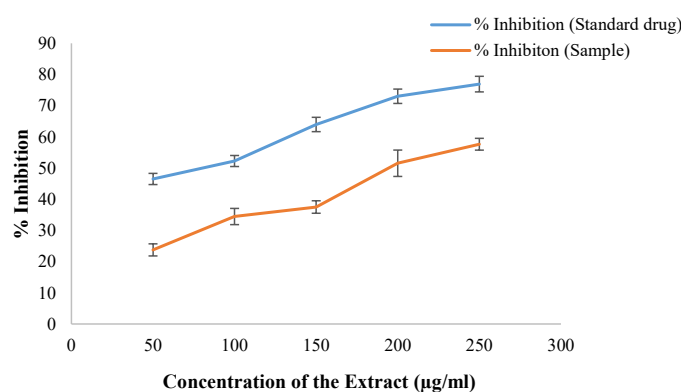


Figure 1. Free radical scavenging activity of the *C. odorata* roots extract

Table 1. Phytochemical constituents of the methanol extract of *C. odorata* root

Constituent	Inference	Composition (mg/100 g)	
Alkaloid	Balsams Saponins	+	74.34±6.08
Steroid	Flavonoids	+	322.78±17.35
	Anthraquinone Glycoside	+	79.63±4.55
Reducing Sugar	Tannins	+	54.76 ±4.08
	Phenol	+	154.32 ±11.21

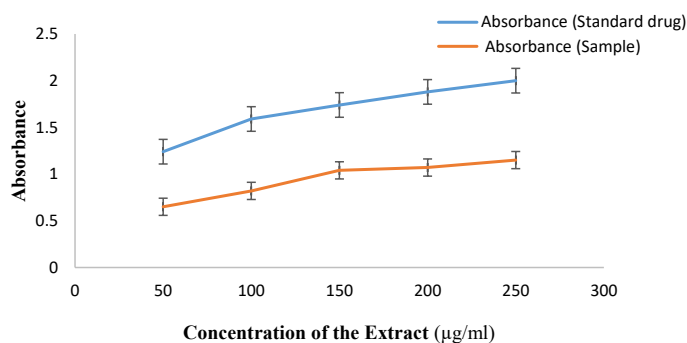


Figure 2. Ferric reducing activity of the *C. odorata* roots extract

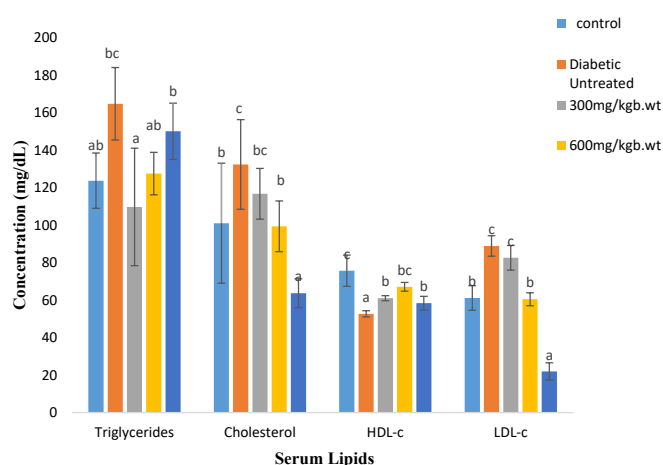


Figure 3. Effect of *C. odorata* extract on lipid profile

Each bar represents the mean and standard error of mean of triplicate determinations. Bars with different superscript alphabets are significantly different from each other ($P < 0.05$).

However, a previous study conducted by Bamisaye et al. [14] did not find alkaloids while Agaba et al. [31] did not report the presence of steroids, glycosides and reducing sugar in *C. odorata* roots. The variation in phytochemical composition of the extract could be attributed to the seasonal changes in sample collection, geographic location of the plant, and the polarity of the solvent used for the extraction process [32, 33].

The phenol content (154 ± 11.21 mg/100 g) of *C. odorata* root extract, as reported in this study, was higher than the value (143.3 ± 5.14 mg/100 g) reported by Agaba et al. [31] but lower than the level (397.48 ± 3.07 mg/100 g) as reported previously for the *Vernonia amygdalina* (Asteraceae) leaf extract [34]. Phenol has been reported to promote various physiological benefits, such as anti-tumor, antioxidant, and anti-diabetic effects. Polyphenol exerts its anti-diabetic effects by regulating insulin secretion, inhibiting

α -glucosidase and enhancing antioxidant activity [6]. Flavonoids have been reported to contain anti-diabetic properties as a result of the modulatory effects on blood sugar transporters. Specifically, this is accomplished by enhancing insulin secretion, reducing apoptosis, promoting proliferation of pancreatic β -cells, reducing insulin resistance, inhibiting inflammation and oxidative stress in muscle, and promoting translocation of GLUT4 via PI3K/AKT and AMPK pathways [10]. The alkaloids content was higher (74.0 ± 4.55 mg/100 g) compared to that (72.25 ± 7.02 mg/100 g) reported by Agaba et al. [31] for *C. odorata* leaf extract but lower than that reported for the root extract (115.8 ± 6.71 mg/100 g). Alkaloids induced relatively high glucose uptake in β -TC6 and C2C12 cells, showed ample antioxidant potential by alleviating H_2O_2 -induced oxidative damage in β -TC6 cells, and served as “insulin sensitizer” in the management of type 2 diabetes. The mean tannins content (54.0 ± 4.08 mg/100 g) was lower than the value (145.0 ± 11.33 mg/100 g) reported by Agaba et al. [31] for *C. odorata* root extract.

In this study, saponins were the most abundant phytochemical detected in the *C. odorata* root extract. The mean saponins value (322 ± 17.35 mg/10 g) was lower than that (348.8 ± 15.67 mg/10 g) reported by Agaba et al. [31]. Saponins from various plants have been reported to have hypoglycemic property. The role of saponin in the regulation of plasma glucose level and preventing diabetic complications are due to the antioxidant activity and reduction of insulin resistance in tissues, among others [10].

The protective roles of plant products against free radicals and oxidative stressors are well documented [7]. The IC_{50} value was negatively related to the antioxidant activity of the plant extract. This represents the amount of antioxidant needed to decrease the radical concentration by 50% [33]. In the present study, the extract exhibited a significant and dose-dependent inhibition of the DPPH activity, ranging from 23.76 ± 1.94 μ g/ml to 57.64 ± 1.89 μ g/ml for 50 μ g/ml to 250 μ g/ml, respectively, of the extract (Figure 1). Our result for the IC_{50} value (203 μ g/ml) was lower than that (378 μ g/ml) reported by another study [3]. The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity [9]. Our results showed that the extract promoted the transformation of Fe^{3+} to Fe^{2+} in a dose dependent manner. The phenol and flavonoid contents of the extract, as determined in this study, could be responsible for the observed free radical scavenging activity of the extract [7].

Dyslipidemia is a frequent metabolic disorder that is associated with diabetes mellitus. The hyperlipidemia is attributed to insulin-induced elevated lipolytic hormonal action on fat tissue [2]. Under normal metabolic condition, insulin activates lipoprotein lipase, which hydrolyses triacylglycerol [25]. However, in diabetes, the lipoprotein lipase is not active due to the insulin deficiency that results in hyper-triglyceridemia [25]. A number of metabolic abnormalities have been associated with the etiology of diabetic hyper-triglyceridemia and hypercholesterolemia. The decline in cholesterol and triglycerides, and increase in HDL cholesterol that were observed in this study suggest the presence of metabolites with hypolipidemic properties in the extract (Figure 3). Saponin and flavonoid contents of the extract have been associated with hypo-cholesterolemia. This effect of saponin fits with the class of direct hypoglycemic agents, as opposed to that for indirect agents, such as sulphonylurea that stimulates the release of insulin from the pancreas [25]. This finding is consistent with that of Yusufoglu et al. [2] who reported that *F. duranii* attenuated the plasma lipoproteins abnormalities in diabetic rats and significantly improved the levels approaching normal.

Conclusions

The methanolic root extract of *C. odorata* exhibited antioxidant, hypoglycemic and hypo-lipidemic effects. Therefore, it could be speculated that the observed hypoglycemic activity might be related to the presence of phytochemical constituents, such as flavonoids, saponins and tannins. These properties provide the extract with the potential for the therapeutic applications in patients with diabetes.

Ethical Considerations

Compliance with ethical guidelines

The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

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Author's contributions

All authors contributed in preparing this article.

Conflict of interest

The authors declared no conflict of interests.

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