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Research Article

Protective role of *Ocimum basilicum* (Sweet Basil) and *Ocimum gratissimum* (Scent leaves) Against Oxidative Stress in Alloxan-induced Diabetes in Wistar Albino Rats

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Abstract

Oxidative stress is globally known as one of the causes of diabetic mellitus and it debilitating effects and complications. This study revealed the antioxidant potential of ethanolic leaf extracts from *Ocimum basilicum and Ocimum gratissimum* using alloxan-induced diabetic Wistar albino rats. The phytochemical profiling revealed the presence of bioactive compounds like anthraquinones, phenolic, flavonoid, tannin, saponin, and reducing sugars. Comparatively, there was a variation among the two plants screened for the phytochemistry. Furthermore, the anti-nutrient composition also, elucidate that oxalate, phytate and cyanide levels were relatively low. The findings from the in-vitro antioxidant tests revealed notable free radical scavenging activity. As expected, the reference standards quercetin showed significantly higher ($p < 0.05$) radical scavenging activity than *Ocimum basilicum and Ocimum gratissimum* extracts. Between the two, however, the *Ocimum gratissimum* extract consistently outperformed the *Ocimum basilicum* extract. Also, the in-vivo study, was conducted with fourty male Wistar albino rats divided into seven groups: a normal control, an alloxan induced diabetic group and four co-treatment groups receiving the extracts and standard drug treated group alone. Administration of alloxan was significantly ($p < 0.05$) decrease in antioxidant enzymes such as SOD, Catalase and GSH. Nevertheless, co-treatment with *Ocimum basilicum and Ocimum gratissimum* extracts revealed an increase in the endogenous antioxidant enzyme levels and decrease oxidative level. Conclusively, the study adjudges that *Ocimum basilicum and Ocimum gratissimum* extracts possess antioxidant potential for the management of diabetes mellitus caused by oxidative stress.

Keywords: *Ocimum basilicum*; *Ocimum gratissimum*; Antioxidant Activity; Oxidative Stress; Alloxan-Induced Diabetes; Phytochemicals

Introduction

The imbalance between free radical generation and antioxidant defense systems is globally recognized as a major contributor to cellular damage and disease progression [1]. Reactive oxygen species (ROS) are known to impair cellular structures, including lipids, proteins, and DNA, thereby promoting pathological conditions such as diabetes, cardiovascular disorders, and neurodegeneration [2].

The stability of biological systems is widely considered dependent on the parity between oxidation and antioxidant activity. Under healthy physiological parameters, the human antioxidant defense network which includes glutathione (GSH), glutathione peroxidase, catalase, and superoxide dismutase facilitates the removal of surplus radicals like peroxyradicals (ROO-), alkoxyl radicals (RO-), hydroxyl radicals (OH-), and superoxide anions (O2-). However, the human endogenous antioxidant defense system is often unable to completely neutralize oxidative stress under disease conditions without assistance from exogenous antioxidant agents [3].

Medicinal plants have continued to attract attention due to their rich content of bioactive compounds, commonly referred to as phytochemicals. These compounds, including flavonoids, tannins, and saponins, contribute significantly to the antioxidant potential of plants [4]. Among such plants, *Ocimum basilicum* and *Ocimum gratissimum*, belonging to the Lamiaceae family, are widely consumed and traditionally used for therapeutic purposes across tropical regions.

Previous studies have reported that species of *Ocimum* exhibit diverse pharmacological activities, including antimicrobial, antihypertensive, antidiabetic, and antioxidant effects [5]. However, comparative evaluation of their antioxidant potential under diabetic conditions remains limited.

Accordingly, the present study aims to contribute to this ongoing discourse by evaluating the antioxidant activity of *Ocimum basilicum* and *Ocimum gratissimum* in alloxan-induced oxidative stress.

Materials and Methods

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. The reagents included dichromate solution (5%), hydrogen peroxide (0.2 M), acidic acid, phosphate buffer, and carbonate buffer (0.05 M, pH 10.2). Adrenaline solution (0.3 mM), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Quercetin, and methanol were also employed.

Additional reagents comprised potassium acetate, aluminium chloride, reduced glutathione (GSH), and Ellman`s reagent (5,5-dithiobis-(2-nitrobenzonoic acid), DTNB). A precipitating solution, trichloroacetic acid (TCA, 30%), and thiobarbituric acid (0.75%) were used for lipid peroxidation assays.

Furthermore, Tris-KCl buffer (0.15 M, pH 7.4), ferric chloride ($FeCl_3$), sodium hydroxide (NaOH, 20%), Benedicts qualitative reagents, chloroform, ammonia solution (10%), hydrochloric acid (HCl 10%), acetic anhydride, and concentrated sulfuric acid (H_2SO_4) were utilized throughout the experiment.

Equipment, instrumentation and glassware

Manual blender, beaker, test tubes, wattman paper, water bath, refrigerator, centrifuge, spectrophotometer, spatula, funnels, pipette, stopwatch.

Plant collection and extraction

Fresh leaves of *Ocimum basilicum* and *Ocimum gratissimum* were collected from Anyigba Market, Dekina Local government of Kogi State. The Plant materials were subsequently taken to the department of plant Science and Biotechnology for proper identification and authentication. The collected samples were air-dried at room temperature, packed in clean paper bags, and stored under appropriate conditions until further use. The dried leaves were pulverized using a manual blender to obtain a fine power. The process was carried out through Soxhlet extraction method. After extraction, the ethanol extract was connected using a water bath at 45°C to remove excess solvent and the concentrated extract obtained was then stored for biochemical analysis.

Phytochemical screening

Standard qualitative methods were used to detect the presence of saponins, tannins, anthraquinones, and reducing sugars according to method of [6].

Antioxidant assays

DPPH radical scavenging activity, reducing power assay and Total flavonoid content was determine using the extract according to the method of [7].

Experimental design and animal handling

Adult Wistar strain albino rats with a body weight range of 160g and 210g were obtained from the animal Breeding and Care Facility of Federal University of Agriculture Makurdi, Benue State, Nigeria. The animals were kept in standard rat cages at room temperature (25 ± 4°C) with a normal 12-hour light/dark cycle and received standard commercial pelleted rat chow and water *ad libitum.* The rats were housed in the animal house facility, department of Biochemistry Kogi State University Anyigba known now as prince Audu Abubarkar University. The rats were allowed to acclimatized for a period of 14 days. A total fourty (40) albino rats were used for this study. The animals were randomly assigned into seven (7) groups, with five (5) rats in each group.

Table 1: Experimental grouping and treatment of animals.

Group	Description	Treatment administered	Dose
Group I	Normal control	No treatment	-
Group II	Diabetic control	No extract administered	-
Group 111	Diabetic rats	*Ocimum basilicum* extract	100 mg/kg b.w
Group IV	Diabetic rats	*Ocimum basilicum* extract	200 mg/kg b.w
Group V	Diabetic rats	*Ocimum gratissimum* extract	100 mg/kg b.w
Group VI	Diabetic rats	*Ocimum gratissimum* extract	200 kg/kg b.w
Group VII	Diabetic rats (standard control)	Glibenclamide	5 mg/kg b.w

Induction of experimental diabetes

Alloxan monohydrate was freshly prepared in normal saline prior to administration to ensure stability. A single dose was administered intraperitoneally to induce diabetes, a compound known to generate ROS and selectively destroy pancreatic β-cells. After giving alloxan, glucose test was carried to confirmed if the rats are diabetic.

Collection and preparation of blood sample

Blood samples (2.5 mL) were obtained from the rats through cardiac puncture. The collected blood was transferred into plain tubes for serum separation and into EDTA tubes for plasma collection, both of which were subsequently used for biochemical analyses.

Biochemical analysis

Serum biochemical parameters were determined using established laboratory methods. These included: Superoxide dismutase (SOD) activity (Misra and Fridovich method), Reduced glutathione (GSH) (Beutler., *et al*. method) and Lipid peroxidation (LPO) via TBARS assay.

Statistical analysis

The data obtained were presented as mean ± standard error of mean. Statistical differences between the treated and control groups were evaluated using one-way analysis of variance (ANOVA), followed by a one-sample t-test where appropriate. All analyses were carried out using Graphpad Prism version 7.0 (GraphPad Software, CA, USA). Statistical significance was accepted at $p < 0.05$. The results were expressed as the Mean ± Standard Error of Mean, and the differences between treated and control groups were statistically assessed using one-way ANOVA and paired with one sample T-test.

Results Discussion

Phytochemical composition

The tables above give the average concentration of anti-nutrients present in the sample. The result showed low concentrations of oxalate and phytate, and no cyanate effects on the sample.

Table 2: Result of the phytochemical screening of *Ocimum basilicum* and *Ocimum gratissimum*.

Key

Positive (+) = present

Negative (–) = negative

Both plants contained tannins and combined anthraquinones. *O. basilicum* additionally contained reducing sugars and free anthraquinones, while saponins were detected only in *O. gratissimum.*

Phytochemicals	*Ocimum basilicum*	*Ocimum gratissimum*
Saponins	+	+
Tannins	+	+
Phenolic compound	+	++
Combined anthraquinones	+	+
Free anthraquinones	_	+
Flavonoids	+	++
Alkaloids	+	+

Table 3: Anti-nutrient composition of dried *Ocimum gratissimum* leaves.

Anti-nutrient	Concentration (mg/100g)
Oxalate	0.14 ± 0.09
Phytate	0.08 ± 0.11
Cyanide	0.02 ± 0.34

Table 4: Anti-nutrient composition of dried *Ocimum basiliccum* leaves.

Anti-nutrient	Concentration (mg/100g)
Oxalate	0.01 ± 0.12
Phytate	0.21 ± 0.16
Cyanide	0.03 ± 0.01

Table 5: Results for *in vitro* antioxidant studies of *Ocimum basilicum and Ocimum gratissimum.*

Antioxidant analysis	*Ocimum basilicum*	*Ocimum gratissimum*	Quercetin standard drug
Reducing power	1.533 mg GAE/gdw	1.903 mg GAE/gdw	
DPPH screening	61.2%	88.6%	92.4%
activity in %			
Total flavonoid	0.146 mgG-1 of extracted compound	0.253 mgG-1 of extracted compound	

O. gratissimum showed slightly higher antioxidant activity: DPPH scavenging: 88.6% (*O. basilicum*) vs 61.2% (*O. gratissimum*) and Reducing power: 1.533 vs 1.903 mgGAE/gdw as compared the standard drug quercetin 92.4%

Effects on superoxide dismutase (SOD) activity

Alloxan-induced diabetic rats significantly decreased SOD activity compared to control ($p < 0.05$). Treatment with *O. basilicum* and *O. gratissimum* restored SOD activity in a dose-dependent manner. The 200 mg/kg *Ocimum gratissimum* extract produced the greatest increase, bringing SOD levels close to standard drug Glibenclamide.

Lipid peroxidation (LPO) activity

Alloxan treatment (Group II) significantly ($p < 0.05$), increased oxidative stress levels compared to the standard drug

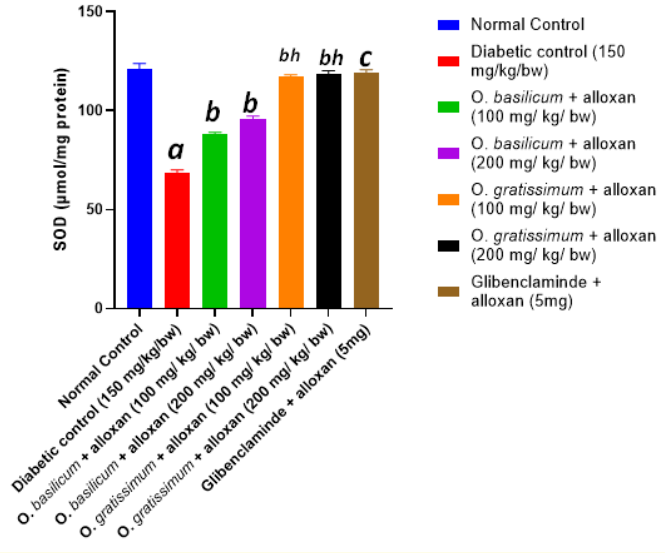

Figure 1: Effects of *O. basilicum* and *O. gratissimum* extracts on Superoxide dismutase of alloxan-induced wistar albino rats.

glibenclamide. Co-administration with all extracts significantly reduced LPO levels. The highest reduction was observed in Group VI *O. gratissimum* (200 mg/kg), followed by Group IV (*O. gratissmum* + alloxan 200 mg/kg).

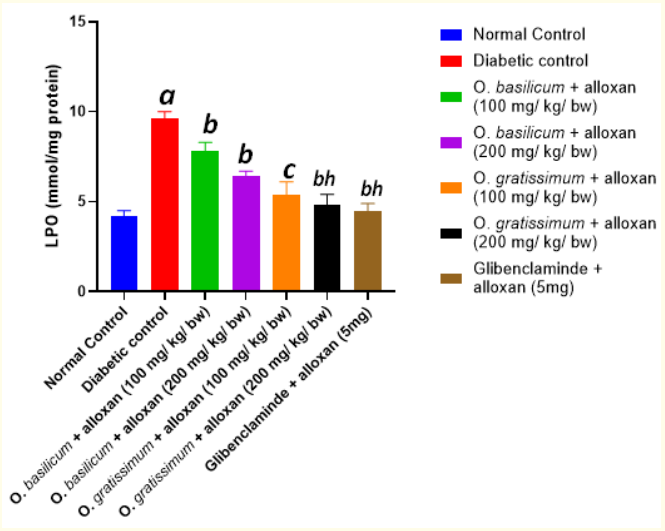

Figure 2: Effects of *O. basilicum* and *O. gratissimum* extracts on Lipid peroxidation of alloxan-induced wistar albino rats.

Reduced glutathione (GSH) activity

Similarly, alloxan administration significantly ($p < 0.05$) reduced GSH activity and all extracts significantly ($p < 0.05$) elevated GSH activity compared to diabetic control group, with the highest effect seen in Group VI *O. gratissmum* + alloxan (200 mg/kg).

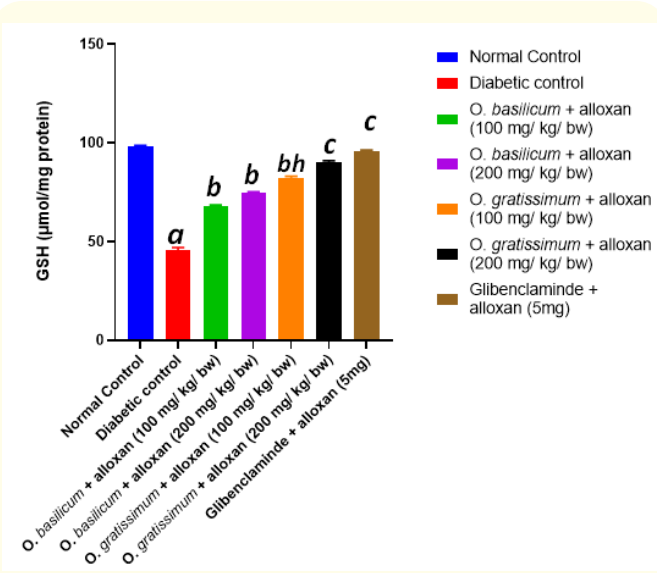

Figure 3: Effects of *O. basilicum* and *O. gratissimum* extracts on Reduced Glutathione of alloxan-induced Wistar albino rats.

As shown in Figures 1, 2 and 3, groups annotated with the same letter (e.g., both marked "a") did not differ significantly from each other, whereas groups bearing different letters (e.g., "c" versus "b") exhibited statistically significant differences. For instance, in the SOD assay, the standard drug glibenclamide and the O. *gratissmum* + alloxan (200 mg/kg) groups both carried the letter "c", indicating statistically similar values. In contrast, the diabetic control group was marked with a different letter ("a"), confirming a significant reduction in SOD activity. Likewise other LPO and GSH assays.

Discussion

The availability of bioactive phytochemicals in both *Ocimum basilicum* and *Ocimum gratissimum* reinforces their traditional use in herbal medicine. Compounds such as phenolic and flavonoid are well-recognized for their ability to scavenge free radicals and chelate metal ions, thereby mitigating oxidative damage [8]. The strong DPPH radical scavenging activity observed in this study is in line with previous findings on *Ocimum* species, highlighting their

efficiency as hydrogen donors [9]. Moreover, the enhancement of antioxidant enzyme activities, including superoxide dismutase (SOD) and reduced glutathione (GSH), following extract administration suggests that these plants may support the body's endogenous defense mechanisms [10].

In diabetic control animals, lipid peroxidation (LPO) levels were elevated, confirming that oxidative stress is a central feature of alloxan-induced diabetes, consistent with the observations reported by [11]. Treatment with the plant extracts significantly reduced LPO, indicating a protective effect against oxidative membrane damage. Between the two species, *O. gratissimum* demonstrated slightly superior antioxidant activity compared to *O. basilicum*, though both exhibited substantial therapeutic potential [12].

The phytochemical profiles of the leaves, summarized in Table 3, revealed a rich presence of bioactive constituents. Both plants tested positive for tannins and combined anthraquinones. Interestingly, phenolic and flavonoid were found highest in *O. gratissimum*, while Free anthraquinones were unique to *O. basilicum.* This diversity in phytochemicals supports the notion that both species possess biologically active molecules responsible for their medicinal properties. In table 4, the anti-nutrients constituents show a low or negligible percentage of oxalate, phytate and cyanide. Oxalate, which was seen at the low concentration, may be beneficial for reducing the risk of kidney stones and enhancing mineral bioavailability, making it a preferable choice for individuals at risk of oxalate-related health issues [13].

Phytate also contributes to reduced mineral bioavailability by chelating essential minerals like iron, zinc, and calcium. However, it has been reported that phytate has beneficial effect such as antioxidant and anticancer properties and play a protective role in the prevention of some chronic disease [14]. Likewise, the low cyanate content revealed that the plant is safe for consumption and has no effect in the body metabolism or complication in the electron transport chain. Table 5 presents the antioxidant properties of the extracts. *O. gratissimum* displayed a slightly higher total flavonoid content (0.253 mg/g) than *O. basilicum* (0.146 mg/g). Similarly, the reducing power of the ethanolic extracts was greater in *O. gratissimum* (1.903 mg GAE/gdw) compared to *O. basilicum* (1.533 mg GAE/gdw), indicating stronger electron-donating capacity. Free radical scavenging ability, assessed via the DPPH assay, showed that *O. gratissimum* had slightly higher activity (88.6%) than *O. basilicum* (61.2%), both far exceeding the catechin control (20.06%). Also, they are close to the standard Quercetin, which show an antioxidant activity of (92.4%). These results confirm the potent antioxidant potential of both species, supporting their role as natural sources of free radical scavengers. The figures above illustrate the impact of the extracts on antioxidant enzymes in alloxan-induced diabetic rats. Diabetes induction led to a significant decrease ($p < 0.05$) in SOD and GSH activities respectively, compared to normal controls and standard groups, while LPO levels increased by 38%, indicative of enhanced oxidative stress. These findings corroborate previous reports linking hyperglycemia to increased reactive oxygen species production and oxidative damage [15]. Overall, this study reinforces that *O. basilicum* and *O. gratissimum* are rich sources of phytochemicals with pronounced antioxidant activity. Previous studies have also documented the presence of biologically active compounds in these species [16,17], suggesting that their antioxidant effects are largely attributable to these constituents [18]. Nevertheless, further research is essential to evaluate the safety profile and determine effective therapeutic dosages of these plant extracts, particularly given their widespread traditional use where dosage is often not standardized [19].

Conclusion

This study demonstrates that *Ocimum basilicum* and *Ocimum gratissimum* possess notable antioxidant properties and *Ocimum gratissimum* showed more antioxidant activity than *Ocimum basilicum*, attributable to their phytochemical constituents. Their ability to restore antioxidant enzyme activity and reduce lipid peroxidation highlights their potential as natural agents in managing oxidative stress associated with diabetes. Their accessibility and low cost further support their relevance in traditional and complementary medicine.

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This study did not receive funds/grants from any agency or organization for the submitted work.

Author's Declarations

The authors state that there are no conflicts of interest.

Ethical Approval

The research was carried out in line with institutional ethical guidelines governing animal care and use.

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