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Comparative Evaluation of the Phenolic Content, Antioxidant Properties, and Antidiabetic Effects of N-Butanol Extracts of *Ficus exasperata* Leaf and *Nigella sativa,* Oil in Diabetic *Drosophila melanogaster*

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ABSTRACT

Oxidative stress plays a key role in the pathogenesis of diabetes mellitus, and plant-based antioxidants have shown potential in diabetes management. Research has demonstrated the ethnomedicinal uses and pharmacological activities of *Ficus exasperata* and *Nigella sativa,* oil in ameliorating various health conditions. This study evaluated and compared the phenolic content, antioxidant capacity, and antidiabetic effects of n-butanol extracts of *Ficus exasperata* leaf and *Nigella sativa,* oil. The extracts *in vitro* antioxidant properties were assessed through Total Phenolic Content (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, and Ferric Reducing Antioxidant Power (FRAP) assays, while the antidiabetic effects were evaluated by measuring glucose levels and total thiol concentrations in diabetic flies treated with n-butanol extracts of *Ficus exasperata* and *Nigella sativa,* oil *in vivo*. Both extracts exhibited significant antioxidant activity and effectively reduced hyperglycemia while restoring total thiol levels with the n-butanol extract of *Ficus exasperata* offering the best effects. The *Nigella sativa,* oil showed greater ferric reducing antioxidant power. The higher phenolic contents, DPPH radical inhibition, and restoration of total thiol concentration and significant reduction of glucose level demonstrated by the n-butanol fraction of *Ficus exasperata*, makes it the recommended fraction for further therapeutic exploration. Future studies should focus on isolation and characterization of its active compounds and assess long-term efficacy and safety in more comprehensive models.

Keywords: Diabetes mellitus; Phenolic content; n-butanol extracts; *Ficus exasperata; Nigella sativa,;*n-butanol extracts; Oxidative stress; *Drosophila melanogaster*

INTRODUCTION

Diabetes is a complex endocrine and metabolic disease that is typified by hyperglycemia brought on by either insufficient or ineffective insulin secretion, action, or both. Long-term health problems and organ failure are caused by hyperglycemia, especially in the kidneys, heart, blood vessels,

nerves, and eyes [1]. The two most common types of diabetes mellitus are type 1 and type 2 [2]. Type 1 is caused by the body's inability to produce insulin and necessitates insulin injections [3]. Given the availability of insulin, fasting hyperglycemia is a disease known as type 2 diabetes mellitus [4].

According to the International Diabetic Federation (IDF), 463 million people worldwide today have diabetes, and by 2045, the prevalence is expected to rise to 700 million [5]. This rise in Type 2 diabetes is attributed to excess body weight and physical inactivity, leading to the formation of Reactive Oxygen Species (ROS) due to impaired insulin synthesis from pancreatic β-cell death by apoptosis. Enhancing the body's antioxidant system through supplements and plant compounds may reduce oxidative stress and help prevent the disease in its early stages [6]. Recent research has focused on combating oxidative stress and diabetes mellitus using medicinal plants and natural products as potential alternative treatments [7]. Medicinal plants are considered effective for health issues, offering minimal side effects, affordability, and accessibility. They are significant for discovering new therapeutic agents and have gained recognition for their bioactive compounds, including hypoglycemic, antioxidants, and hypolipidemic agents [8].

Ficus exasperata commonly known as the "SANDPAPER LEAF," a species belonging to the *Moraceae* family that is widely distributed in tropical and subtropical regions, including Africa from Mozambique, Zambia, and northern Angola to Senegal, Ethiopia and also in the southern part of the Arabian Peninsula and India, where it has been traditionally utilized for various therapeutic purposes, contains several bioactive compounds including phenolic acids, flavonoids, tannins, saponin, alkaloids, and glycosides [9-13] attributed to its wide range of pharmacological activities including antiulcer, hypotensive, hypoglycemic, hypolipidemic, anti-inflammatory, anxiolytic, oxytocin inhibiting, anticonvulsant, antinociceptive, antimicrobial, anticandidal, insecticidal and pesticidal properties [14,15]. *Nigella sativa,* (NS) is an annual herbaceous flowering plant that is primarily found in Middle Eastern nations. It is a member of the *Rununculaceae* family. It has been used extensively as a spice and condiment in food and is also referred to as "black seed" or "kalonji." Traditional medicine has used various forms of NS, including extract, oil, and powder, to treat a number of ailments, including bronchitis, fever, cough, diarrhea, and gastrointestinal disorders [16,17]. According to reports, NS has a number of therapeutic benefits, including hypolipidemic, anti-inflammatory, antidiabetic, antioxidant, and anticancer actions [18]. Previous studies have demonstrated the antioxidative and antidiabetic potentials of both botanicals [19-21]. Building on these discoveries, this study aims to evaluate and compare the phenolic content, antioxidant, and antidiabetic activities of n-butanol extracts of *Ficus exasperata* leaves and *Nigella sativa,* oil to determine their efficacy in mitigating oxidative stress in diabetic *Drosophila melanogaster*, while contributing to the growing body of evidence supporting the use of plant-based antioxidants in pharmaceutical and nutraceutical applications.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Ficus exasperata* was obtained from Akungba Akoko and black seed (Nigella. sativa) were purchased from a local market in Etioro, Ondo State of Nigeria. The plants were identified and authenticated in the herbarium of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba Akoko and voucher specimens were deposited for further references.

Preparation of plant extracts

Ficus exasperata

The preparation of extracts, extraction and the solvent-partitioned fractionation of crude extract of *Ficus exasperata* was carried out according to the method described by Shodehinde et al. [19] with slight modifications. The *Ficus exasperata* leaves were cleaned thoroughly to remove any debris or dirt, after which they were air-dried for 3 weeks at room temperature until sufficiently dried and pulverized into a coarse powder. A portion of the pulverized sample (1849.7g) was macerated in 70% methanol for 72 hrs. The mixture was ran through a separating funnel, after which the residue was left to dry and the filtrate was left to evaporate for about 2 weeks. The resulting methanol extract was dissolved in 400 mL of water and further partitioned with n-butanol, yielding n-butanol fraction. These fractions were dried and stored for further analysis.

Nigella sativa,

The *Nigella sativa,* seeds were cleaned thoroughly to remove any debris or dirt, after which they were air-dried at room temperature until sufficiently dried and pulverized into a fine powder. To extract *Nigella sativa,* oil, 1849.7g of the seed powder was thoroughly mixed with 70% ethanol in a separating funnel; the mixture was sealed and allowed to stand at room temperature for 24 hours with occasional shaking. After 24 hours, the mixture was filtered to obtain the crude extract which was then purified by further filtration. To obtain the n-butanol extract, the

oil was dissolved in a small volume of ethanol after which n-butanol was added to the solution. The mixture was thoroughly mixed to obtain a two-phase mixture and left to stand for 24hours at room temperature. After 24 hours, the n-butanol layer was separated and dried to obtain a semi solid extract which was stored in a sterile airtight container until it was ready for use.

In vitro antioxidant assays determination

Determination of total phenol content

The total phenol content was determined according to the method of Singleton et al. [22]. Briefly, appropriate dilutions of the extracts were oxidized with 2.5mL of 10% Folin-Ciocalteu's reagent (v/v) and neutralized by 2.0mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45°C and the absorbance was measured at 765nm. The total phenol content was subsequently calculated as Gallic Acid Equivalent (GAE).

Determination of Ferric Reducing Antioxidant Property (FRAP)

The reducing property of the extracts will be determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu [23]. As 2.5 mL aliquot was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then 2.5 mL of 10% trichloroacetic acid was added. The mixture was centrifuged at 650 rpm for 10 min. As 5 mL of the supernatant was measured with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric-reducing antioxidant property was subsequently calculated as Abscorbic Acid Equivalent (AAE).

DPPH free radical scavenging ability

The free radical-scavenging ability of each sample fraction against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was evaluated according to the method of Gyamfi et al. with modifications [24]. To 1 mL of 0.4 mM methanol solution of DPPH radicals, 0.1 mL of the sample extracts was added. The mixture was left in the dark for 30 min, and the absorbance was measured at 516 nm in the spectrophotometer.

In Vivo Study

Fly stock and culture

The *Drosophila melanogaster*used in this study was obtained from the Drosophila Laboratory at the Department of Biochemistry, University of Ibadan, Nigeria. The flies were maintained at a temperature of 25±2°C at Phyto-Fakts Health Product Laboratory, Akungba-Akoko, Nigeria. The diet consisted of water, yeast, corn meal, brewer's yeast, agar-agar, and Nipagin as a preservative, as well as the fly culture.

Preparation of the feed

Preparation of feed was done according to the method described by Shodehinde et al. [19]. The cornmeal was mixed thoroughly in 50 mL of water. Then 3 mL of ethanol was measured, and Nipagin was added to it, and the mixture was shaken. Then 100 mL of water was poured into the vessel to boil. After boiling the water, Agar was poured into the boiling water and mixed thoroughly using a stirring stick. Then, yeast was added and mixed immediately to avoid lumps. Then, the mixture of cornmeal was added to it and mixed thoroughly to avoid lumps. Then 50 mL of water was used to rinse the beaker, and poured it into the pot. Then, the mixture of Nipagin and ethanol was added to it and left on fire for about 3 min. The mixture was then poured into glass jars immediately before it thickened.

Acclimatization

The flies were fed for two weeks so that they could adapt to the new environment. The Drosophila's were fed with the feed as detailed above, and during the first 2 weeks, the Drosophila's were fed with the feed without the addition of extracts.

Induction of flies with diabetes using sucrose

The fruit flies were induced with type 2 diabetes according to the method of Omale et al. with slight modifications [25]. The 2.5 g sucrose/10 g diet was added to the normal diet of the fly with other diet components remaining constant (1% agar, 3.4% yeast, 8.3% cornmeal, and 1% nipagin) to induce diabetes in the Harwich strain of D. melanogaster. The flies were exposed to sucrose incorporated in the diet for 10 days and observed for diabetes symptoms such as low rate of L3 larvae emergence, decreased body size, and decreased locomotive activities.

Grouping of flies and treatment

The anti-diabetic and anti-oxidative effects of *Ficus exasperata* and *Nigella sativa,* fractions on *Drosophila melanogaster*were investigated by incorporating them in the diet prepared for the files, and metformin served as reference drug for 10 days. Fifty flies were included in each group with three replicates arranged according to the design given below:

Group 1: Basal diet

Group 2: Diabetic flies treated with 10 mg Metformin/10 g diet.

Group 3: Diabetic flies without treatment

Group 4: Diabetic flies treated with 2.0 mg of the n-butanol fraction of *Ficus exasperata* /10 g diet.

Group 5: Diabetic flies treated with 4.0 mg of the n-butanol fraction of *Ficus exasperata* /10 g diet.

Group 6: Diabetic flies treated with 2.0 mg of the n-butanol fraction of *Nigella sativa,*/10 g diet.

Group 7: Diabetic flies treated with 4.0 mg of the n-butanol fraction of *Nigella sativa,*/10 g diet.

After the treatment of flies was carried out for 10 days, the flies were anesthetized using ethanol. The weights of flies were taken at a ratio of 1 mg of flies/ 10 µL of buffer and were homogenized using 0.1 M phosphate buffer (pH 7.0). The homogenates were centrifuged for 10 min at 4000 x g and the supernatants were separated from the pellets. The supernatants were kept and used for glucose assay and determination of Total Thiol content.

Evaluation of glucose concentration

The assay for determining the concentration of glucose in the fly homogenate was conducted according to the procedure of Trinder using the Agappe LiquiCHEK Kit [26].

Estimation of total thiol content

The total thiol level was assayed according to the method of Ellman, as described by Abolaji et al, with some modifications [27]. The total reaction mixture of 600 µL containing 25 µL of the sample, 510 µL potassium phosphate buffer (pH 7.4), 30 µL DTNB, and 30 µL GSH was incubated for 30 min at 25°C, and the absorbance was read at 412 nm using a spectrophotometer. A GSH standard curve was prepared to extrapolate the total thiol content, and the results were calculated per mg protein content.

Statistical analysis

All antioxidant studies were performed in triplicate. The data was statistically analyzed using GraphPad Prism. The data was presented as a Mean ± Standard Deviation (SD). The statistical analyses employed One-way Analysis of Variance (ANOVA) with multiple comparisons. The level of significance was set at $p<0.05$.

RESULTS

Investigation of the *in vitro* total phenolic content and free radical scavenging ability of n-butanol extracts of both *Ficus exasperata* and *Nigella sativa,* oil revealed that both botanicals possessed appreciable phenolic contents and free radical scavenging potentials. The Total phenolic content and DPPH radical inhibition were significantly higher in the n-butanol extract of *Ficus exasperata* (Figures 1&2), while the *Nigella sativa,* oil demonstrated higher Ferric Reducing Antioxidant Property (FRAP) activity (Figure 3).

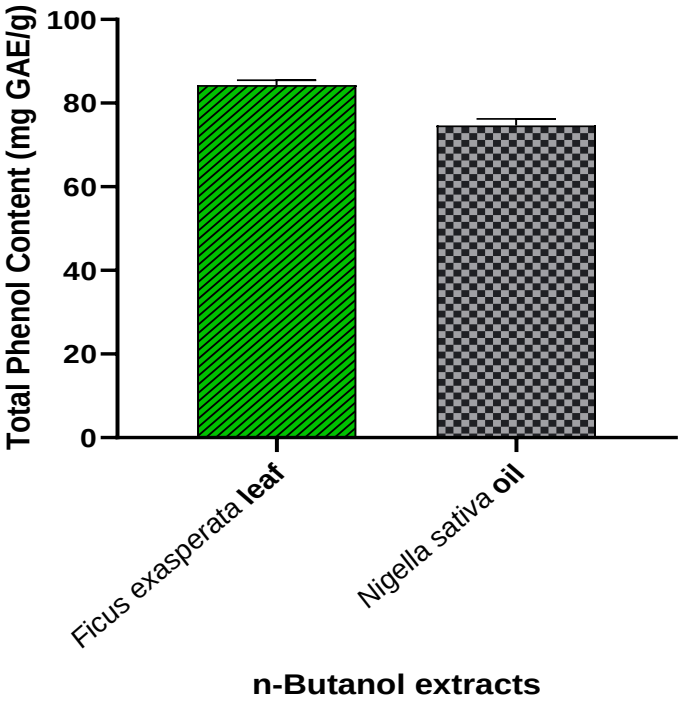

Figure 1: Total Phenol Content of N-Butanol Extracts *Ficus exasperata* leaf and *Nigella sativa,* oil.

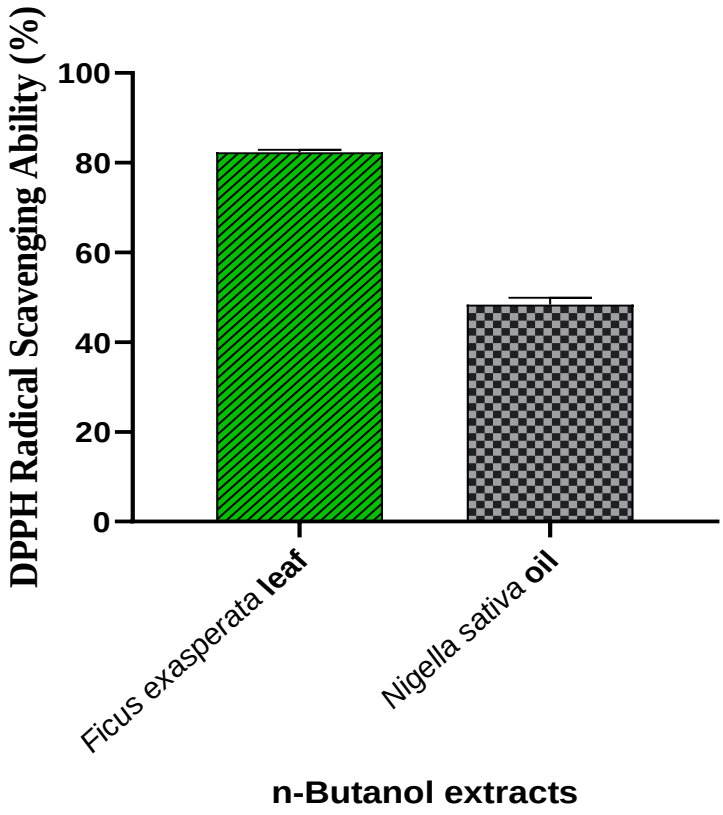

Figure 2: DPPH Radical Scavenging Ability of N-Butanol Extracts of *Ficus exasperata* Leaf and *Nigella sativa,* Oil.

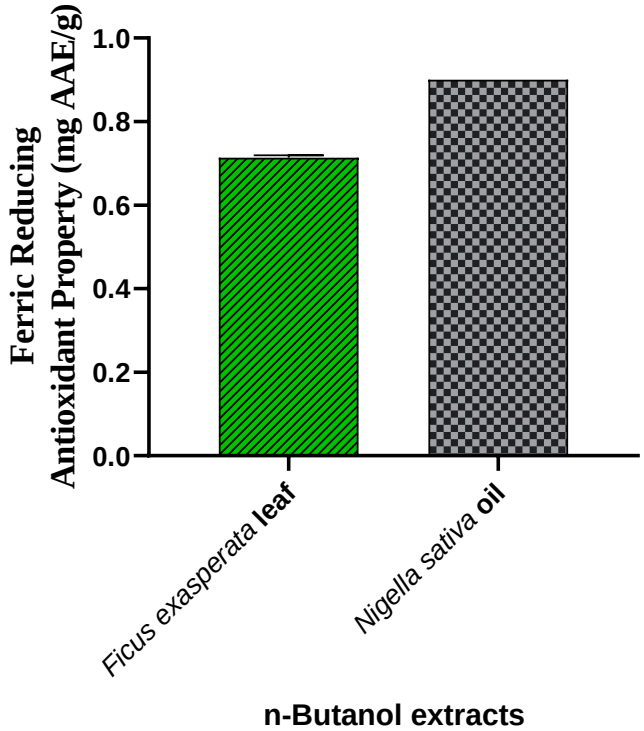

Figure 3: Ferric Reducing Antioxidant Property of N-Butanol Extracts of *Ficus exasperata* Leaf and *Nigella sativa* oil.

The glucose levels of diabetic *Drosophila melanogaster*treated with different concentrations of *Ficus exasperata* leaf and *Nigella sativa*, oil extracts are shown in Figure 4. The results showed that both extracts of *Ficus exasperata* leaf and *Nigella sativa*, oil significantly (****p<0.0001 *vs* DF+No Treatment) reduced glucose levels in diabetic *Drosophila melanogaster*, showing antidiabetic activity, particularly at a concentration of 2.0mg/10g diet with the *Ficus exasperata* ($$p<0.001) extracts offering the best effects. Notably, there was a dose-dependent increase in total thiol content of diabetic *Drosophila melanogaster*treated with *Ficus exasperata* leaf and *Nigella sativa*, oil extracts (Figures 4&5). The extract of *Ficus exasperata* leaf, particularly at a concentration of 4.0mg/10g diet significantly increased the total thiol content in diabetic *Drosophila melanogaster*compared to the *Nigella sativa*, oil ($$$$p<0.0001) (Figure 1).

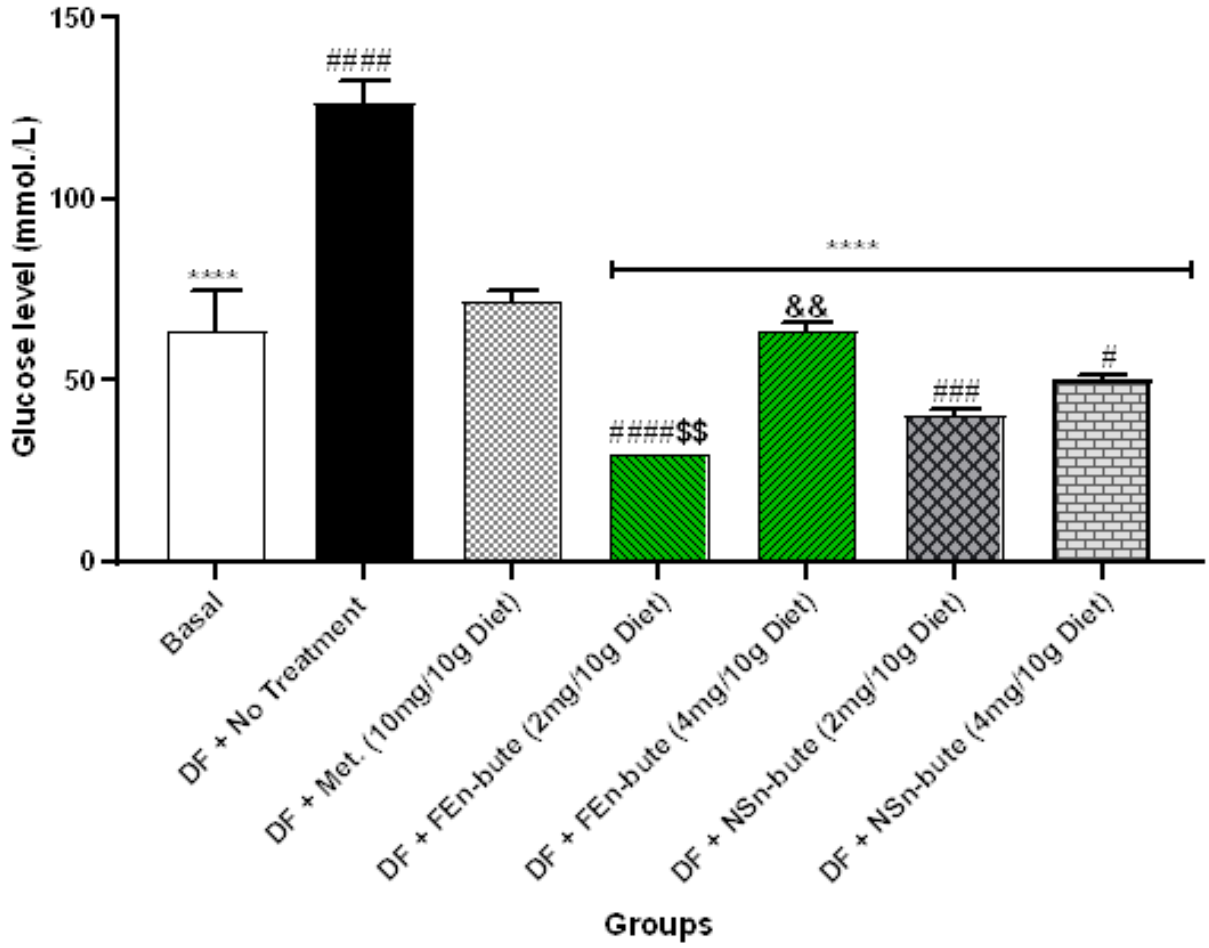

Figure 4: Effect of N-Butanol Extracts of *Ficus exasperata* Leaf and *Nigella sativa,* On Glucose Concentration in Diabetic *Drosophila melanogaster*.

Note: DF (Diabetic Fly); FEn-bute (*Ficus exasperata* leaf n-butanol extract); NSn-bute (*Nigella sativa,* oil n-butanol extract); Met (Metformin). Values are significantly different $^{\#}$p<0.05, $^{\#\#\#}$p<0.0001, $^{\#\#\#\#}$p<0.0001 compared to Basal, ****p<0.0001 compared to DF+No Treatment, $^{\$\$}$p<0.001 compared to DF+NSn-bute (4mg/10g Diet), &&p<0.001 compared to DF+NSn-bute (2mg/10g Diet).

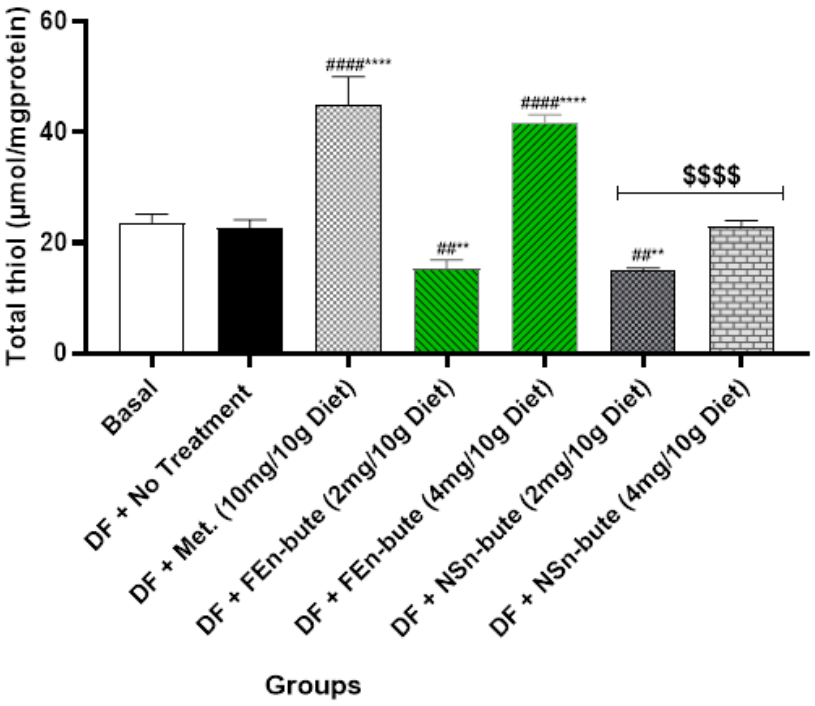

Figure 5: N-Butanol Extracts of *Ficus exasperata* Leaf and *Nigella sativa,* Oil Ameliorate Total Thiol Level in Diabetic *Drosophila melanogaster*.

Note: DF (Diabetic Fly); FEn-bute (*Ficus exasperata* leaf n-butanol extract); NSn-bute (*Nigella sativa,* oil n-butanol extract); Met (Metformin). Values are significantly different $^{\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$ compared to Basal, $^{**}p<0.001$, $^{****}p<0.0001$ compared to DF+No Treatment, $\$\$\$p<0.0001$ compared to DF+FEn-bute (4mg/10g Diet).

DISCUSSION

The present study evaluated and compared the phenolic content, antioxidant capacity, and antidiabetic effects of n-butanol extracts of *Ficus exasperata* leaf and *Nigella sativa,* oil using a diabetic *Drosophila melanogaster*model. Our results indicate that both extracts possess significant antioxidant properties and were effective in reducing hyperglycemia and restoring total thiol levels in diabetic flies. Reactive Oxygen Species (ROS), or free radicals, arise from external chemicals and metabolic processes. Excessive ROS lead to oxidative stress, disrupting the balance of oxidants and antioxidants. This imbalance can damage biomolecules such as nucleic acids, proteins, lipids, and DNA, potentially causing cancer, cardiovascular diseases, muscular degeneration, neurological disorders, and inflammation [11]. Maintaining this balance is crucial for biological health and external antioxidants can alleviate oxidative stress by acting as free radical scavengers and reducing agents, thereby minimizing associated damage [28,29]. Stress is defined at the molecular level as any strain that produces free radicals, and diabetes mellitus is a stress-related illness. Oxidative stress is hypothesized to play a role in the etiology of diabetes mellitus [30].

Plant-based materials have gained significant attention due to their versatile uses, and many plants have been explored as potential sources of natural antioxidants [31]. In this study, *Ficus exasperata* extract demonstrated higher total phenolic content and DPPH radical scavenging activity compared to the *Nigella sativa,* oil, corroborating previous phytochemical analyses reporting its richness in bioactive compounds such as phenolics, and flavonoids, which contribute to its antioxidant potential [9,11]. Phenolic compounds, known for their electron-donating properties, are key contributors to the neutralization of free radicals and oxidative stress and plants belonging to the *Ficus* species are widely recognized for their significance in traditional medicine [11]. Conversely, *Nigella sativa,* oil demonstrated a stronger ferric reducing antioxidant property (FRAP). Salehi et al, and Mohammed et al, found that *Nigella sativa,* oil had strong antioxidant activity, which was attributed to the presence of thymoquinone, a major component of the oil other constituents in its potent redox activity [32,33].

Both extracts demonstrated antihyperglycemic effects. The reduction of glucose levels in diabetic flies was more pronounced at the 2.0 mg/10 g diet concentration compared to untreated flies, with the n-butanol fraction of *Ficus exasperata* exhibiting the most potent effect. The total thiol concentration is an important parameter for assessing the antioxidant status of a biological system. Thiols are important components of the antioxidant defense system as they can directly scavenge free radicals or regenerate other antioxidants such as glutathione [34]. The decrease in total thiol concentration in diabetic flies suggests that diabetes is associated with a decrease in the antioxidant defense system, which may contribute to the development of diabetic complications [19,34]. The augmented total thiol concentration in the diabetic flies treated with n-butanol extracts of *Ficus exasperata* and *Nigella sativa,* oil compared to the untreated diabetic flies suggests that these botanicals extract have antioxidant activities and may help to restore the antioxidant defense system. The decrease in total thiol concentration of diabetic flies is in agreement with the results of Prakash et al, and Shodehinde et al, [19,20,34]. Interestingly, treatment with n-butanol fraction showed of *Ficus exasperata* demonstrated dose-dependent restoration of total thiol level corroborating previous studies showing that *Ficus exasperata* possesses significant antioxidant and antidiabetic activities, mediated through restoration of thiol defenses, reduction of oxidative stress, and modulation of insulin signaling genes [19,20].

CONCLUSION

This study demonstrates that n-butanol extracts of *Ficus exasperata* leaf and *Nigella sativa,* oil possess significant antioxidant and antidiabetic properties in a diabetic *Drosophila melanogaster*model. Both extracts effectively reduced hyperglycemia and restored antioxidant thiol levels, with the n-butanol fraction of *Ficus exasperata* exhibiting the greatest efficacy. These *in vivo* glucose-lowering effects, coupled with improved thiol status, suggest potential in managing diabetes-associated oxidative stress. The superior performance of *Ficus exasperata* underscores its potential as a lead natural fraction for further therapeutic development. Future research should prioritize the detailed characterization of its bioactive compounds and assess its long-term efficacy and safety using more comprehensive models to facilitate clinical translation.

DECLARATIONS

Conflict of interest

The authors declare no conflicts of interest relevant to this manuscript.

Funding declaration

No funding was received.

Author's contribution

S.A designed and supervised the study. O.V., L., S. D., D. O., S. O., G. D., A. J., and O. V. conducted the methodology. S. A., O. V., and L., provided the materials. L., analyzed and interpreted the results. O. V and L., writes the original draft of the manuscript. S. A., O. S., and L., reviewed and edited the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Consent to publish declaration

Not applicable.

Consent to participate declaration

Not applicable.

Data availability

All relevant data are within the paper.

Ethics declaration

Not applicable.

Clinical trial number

Not applicable.

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