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Evaluation of antioxidant and anti-lipase activities of *Centrosema pubescens* Benth aqueous and ethanolic leaf extracts

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Abstract

There is a correlation between obesity and oxidative stress, which is characterized by an imbalance between reactive oxygen species (ROS) and the free radical scavengers in the body. This imbalance arises as a result of fat accumulation. The purpose of this study was to investigate the bioactive components, free radical scavenging activity in vitro, and anti-lipase activity of aqueous and ethanolic leaf extracts of *Centrosema pubescens* Benth. Both the aqueous and ethanolic leaf extracts produced a yield of 15.79% and 8.03% respectively, after being air-dried, blended to powder, extracted with solvent and dried to paste using a water bath. Both extracts contained bioactive substances such as proteins, flavonoids, tannins, phenols, and alkaloids. The ethanol extract had higher amounts of these compounds than the water extract did. The extract samples displayed modest in-vitro scavenging capabilities for 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) radicals, as well as ferric reducing antioxidant power (FRAP) and total antioxidant capacity compared to the standard compound, ascorbic acid. Furthermore, the ethanol extract displayed a more pronounced inhibitory influence on pancreatic lipase, a key enzyme in lipid digestion, suggesting its potential in obesity control through the lowering of fat absorption. The research underlines the importance of solvent selection in maximizing the extraction of bioactive components and boosting the therapeutic effectiveness of herbal remedies.

Key Words: *Centrosema pubescens*, Oxidative stress, Anti-lipase, Antioxidant, Pancreatic lipase inhibition, Bioactive, Leaf extract

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INTRODUCTION

Obesity and oxidative stress are serious health issues worldwide, contributing to a range of metabolic ailments, which includes cardiovascular diseases, diabetes, and many forms of cancer. In 2022, about 1 in 8 persons worldwide were affected by obesity. Since 1990, the rate of adult obesity has more than doubled, while youth obesity has quadrupled (WHO, 2024). Oxidative stress, which is defined by a disproportion between reactive oxygen species (ROS) and the body's free radical scavenger, exacerbates these health concerns by destroying cells and tissues (Birben *et al.*, 2012). Therefore, developing effective treatments to tackle both obesity and oxidative stress is crucial. Obesity management strategies generally focus on reducing calorie consumption, improving energy expenditure, or inhibiting lipid absorption. Pancreatic lipase is an important enzyme in the digestion of dietary fats, and its suppression is a prominent target for anti-obesity drugs. Natural inhibitors of pancreatic lipase can limit fat absorption and consequently aid in weight management (Birari and Bhutani, 2007).

Natural products, particularly medicinal plants, have garnered interest due to their probable therapeutic benefits with less bad effects compared to synthetic drugs. Medicinal plants are rich in bioactive compounds, such as flavonoids, alkaloids, tannins, and phenols, which display different pharmacological activities including antioxidant and anti-obesity actions (Pandey and Rizvi, 2009). *Centrosema pubescens* Benth (*C. pubescens* Benth) is a leguminous plant commonly utilized in numerous folk medicines for its supposed health effects. It is one of the constituents of the popular combination of herbs used for weight loss known as Aju Mbaise (Ekeke *et al.*, 2021). There are 31 plant species in total that belong to 24 different families in the Aju Mbaise wrap. These herbs are employed to remedy ailments like high blood pressure, typhoid fever, malaria, post-partum (shortly after giving birth), female infertility, diabetes, arthritis, and weight reduction (Ekeke *et al.*, 2021). *Centrosema pubescens* Benth is a plant of interest for weight loss since it has been demonstrated to have antioxidant qualities (Murugan *et al.*, 2020). Despite its traditional usage, scientific proof of its health advantages, particularly its anti-obesity potentials, remains unraveled.

Recent research studies have highlighted the significance of exploring medicinal herbs for their antioxidant and anti-obesity qualities. For instance, Kumar *et al.* (2020) underlined the potential of plant-based antioxidants in lowering oxidative damage and regulating obesity. Similarly, studies by Aziz *et al.* (2019) shows the efficacy of many plant extracts in blocking pancreatic lipase, suggesting their potential application in obesity control. These results underscore the relevance of exploring *C. pubescens* Benth for its bioactive components and therapeutic potentials. This study examined the bioactive components, in-vitro antioxidant and anti-lipase effects of *C. pubescens* Benth aqueous and ethanolic leaf extracts of by conducting comprehensive in-vitro investigations, the study seeks to support the traditional usage of this plant and expose its potential as a plant-based therapy for obesity control and its connected illnesses.

MATERIALS AND METHODS

Plant materials

Leaves of *C. pubescens* Benth were collected from a bush in Uruoka, Abraka in Delta State and the specimen was authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria, while voucher specimen (UBH-C292) was deposited in the herbarium.

Extract preparation

Preparation of aqueous and ethanolic leaf extracts of *C. pubescens* Benth was done following the modified method reported by Anigboro *et al.*, (2019). The leaves of *C. pubescens* Benth were cleaned with water before dried under shade until a steady weight was reached. They were ground to powder using an electric blender. Then, 100 g of the ground leaves were soaked in 800 ml of distilled water and ethanol for 48 hours with stirring at 24 hours. The extracts were filtered on a double-layer cheese cloth and secondly with Whatman No. 1 filter paper. The resulting filtrates were evaporated to dryness in a water bath maintained at 50 ^{o}C. The resulting extracts were weighed for yield calculation using the procedure below, and stored in the refrigerator at 4oC until when necessary for biochemical analysis.

$$\text{Yield formula (\%)} = \frac{\text{weight of dried extract}}{\text{weight of dried plant sample}} \times 100$$

Qualitative biochemical analysis

Using 20 mg/ml of the extracts' solutions, the qualitative analysis of the aqueous and ethanol

leaf extracts of *C. pubescens* Benth was carried out to check for the presence of alkaloids, saponins, phlobatanin, tannins, phenols, flavonoids, steroid, thiols, terpenes, cardiac glycosides, carbohydrates and proteins following the methods described by: Njoku and Obi (2009) and Borokini and Omotayo (2012).

Quantitative phytochemical analysis

The quantitative biochemical analysis of the leaf extracts of *C. pubescens* Benth was carried out to check for the amount of some bioactive compounds using standard protocols. The determination of total flavonoids (Jia *et al.*, 1999), reducing sugar (Miller, 1959), proteins (Gornall *et al.*, 1949), alkaloids (Shamsa *et al.*, 2008), phenols and tannins (Singleton and Rossi, 1965) were done using the respective standard methods, which were also reported by Anigboro *et al.* (2022). A calibration curve was constructed (with varying concentrations of respective standard compounds) from which the results were expressed as milligram equivalents/g of the extracts.

***In-vitro* evaluation of antioxidant activity**

The following antioxidant assays was carried out with the leaf extract samples and a standard antioxidant compound (ascorbic acid) at 0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml concentrations. In this study, DPPH (2, 2-diphenyl-1-picrylhydrazyl) and nitric oxide free radical scavenging activities of the extract samples were evaluated using the methods described by Manzocco *et al.,* (1998) and Marcocci *et al.,* (1994) respectively. Also, the reducing power and total antioxidant capacity of the extract samples was carried out using the methods described by Oyaizu (1986) and Prieto *et al.,* (1999) respectively.

***In-vitro* enzyme inhibition assay: Pancreatic lipase inhibition assay**

The impact of *C. pubescens* Benth extracts on pancreatic lipase activity was measured according to the technique described by Liu *et al.*, (2013) and Alias *et al.* (2017) with minimal adjustments. The extract solution with dilutions ranging from 0.02 to 0.50 mg/mL. (0.25 mL) was incubated with 0.5 mL of 0.6 mg/mL enzyme solution in 100mM phosphate buffer saline, pH 7.2 (150 mM sodium chloride and 0.5% Triton-X-100), 1.0 mL of buffer solution (stated above), and 0.25mL of 1.0 mg/mL para-nitrophenylbutyrate (pNPB) solution (in acetonitrile) for 30 min at 37℃. Lipase activity was measured by quantifying the breakdown of pNPB to yellow-colored para-nitrophenol at 400 nm using spectrophotometer. A reference test was conducted using the identical process indicated above but substituting the extract with distilled water. The experimental analyses were carried out in triplicates. The pancreatic lipase inhibitory activity was measured as % inhibition using Equation.

Pancreatic lipase inhibition (%) = $\frac{ABS_{ref} - ABS_{sam}}{ABS_{ref}} \times 100$

Where ABS_{ref} and ABS_{sam} denote the respective absorbances of reference and sample extract.

Statistical analysis

The SPSS-PC software (version 22.0) was used for statistical analysis. One-way Analysis of Variance (ANOVA) was used to analyze the data in the software. The data were presented as Mean±SD (standard deviation) for triple evaluation and compared at 95% confidence level. Graphical representations were generated using Excel 2019/Office 365.

RESULTS

Qualitative phytochemical screening of leaf extract of *Centrosema pubescens* Benth

The results of the qualitative phytochemical analysis of *C. pubescens* Benth is presented in Table 1. It shows the presence of bioactive compounds of tannins, phlobatannin, flavonoid, cardiac glycosides, alkaloids, phenol, protein, thiols, saponin, carbohydrates and terpenes in aqueous extract of *C. pubescens* Benth (ACP)*;* tannins, phlobatannin, flavonoid, alkaloids, phenol, steroids, protein and carbohydrates in ethanol extracts of *C. pubescens* Benth (ECP).

The findings of the quantitative analysis of aqueous and ethanolic extracts of *C. pubescens* Benth are illustrated in Figure 1. It shows that concentrations of reducing sugar and alkaloids are much higher than other phytochemicals in each extract. when both extracts are compared, it reveals that phenol and tannin has no significant difference, while flavonoid, protein, reducing sugar and alkaloids is significantly higher in ethanol when compared to aqueous extract.

Table 1: Qualitative phytochemical screening of aqueous and ethanolic extracts of *C. pubescens* Benth.

Extracts	Yield (%)	Tannin	Phlobatanin	Flavonoid	Cardiac Glycoside	Alkaloids	Phenol	Steroids	Proteins	Thiols	Saponins	Carbohydrates	Terpenes
ACP	15.79	+	+	+	+	+	+	-	+	+	+	++	+
ECP	8.03	+	++	++	-	++	++	++	++	-	-	++	-

Key: [+] = present, [++] = moderately present, [+++] = Highly present, [-] = Not detected

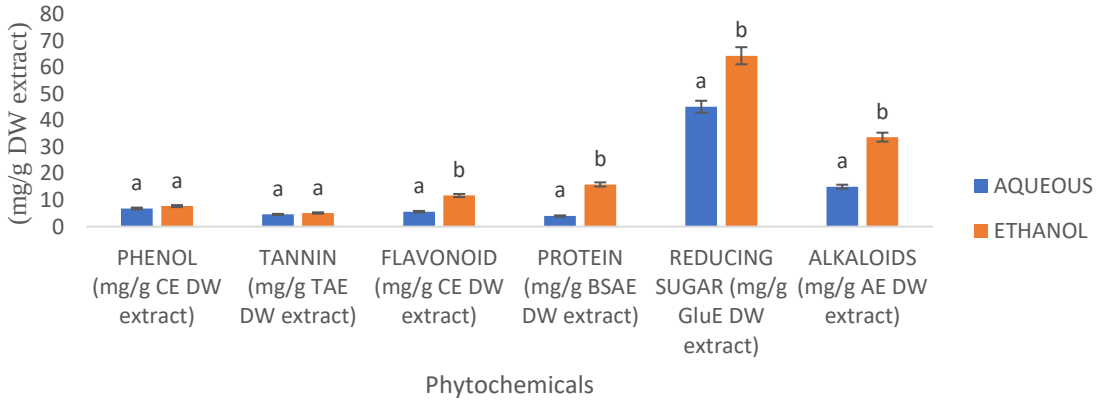

Figure 1: Quantitative phytochemical evaluation of aqueous and ethanolic leaf extracts of *C. pubescens* Benth

Key: milligram per gram (mg/g), catechin equivalent (ce), tannin equivalent (tae), bovine serum albumin equivalent (bsae), glucose equivalent (glue), alkaloid equivalent (ae), dry weight of extract (dw extract).

In-vitro antioxidant activities of leaf extracts of *C. pubescens* Benth

DPPH (2,2-diphenyl -1-picrylhydrazyl) free radical scavenging activity in leaf extracts of *C. pubescens* Benth

The results of the *in-vitro* free radical scavenging activity of 2,2-diphenyl -1-picrylhydrazyl (DPPH) in aqueous and ethanolic leaf extracts of *C. pubescens* Benth is shown in Figure 2. Results shows that the scavenging activities of the ethanol extract of *C. pubescens* Benth is significantly ($p \leq 0.05$) higher and closer to standard, when compared to aqueous extract.

Nitric Oxide (NO) free radical scavenging activity in leaf extracts of *C. pubescens* Benth

The results of the *in-vitro* free radical scavenging activity of Nitric Oxide (NO) in aqueous and ethanolic leaf extracts of *C. pubescens* Benth is shown in Figure 3. It reveals that both aqueous and ethanolic extracts have nitric oxide (NO) scavenging abilities, but the ethanolic extract may have a higher inhibitory effect on NO radicals.

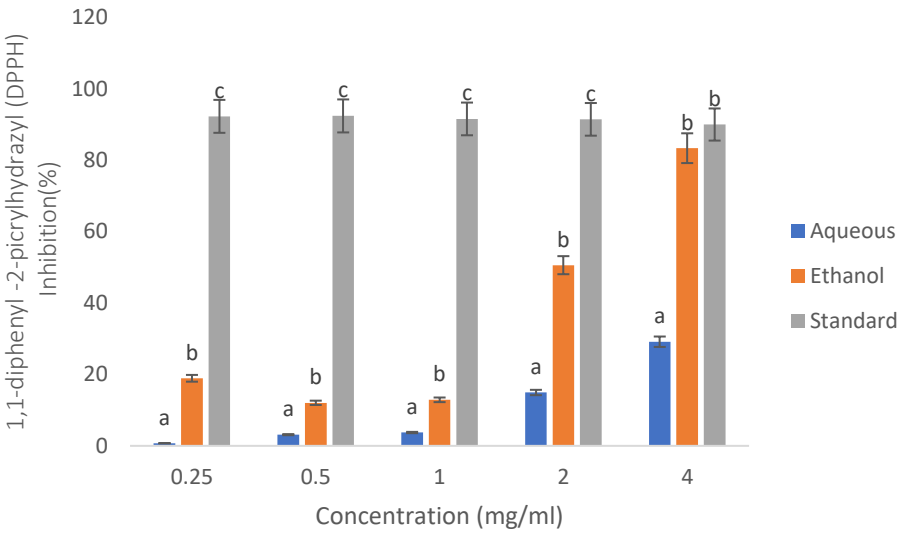

Figure 2: 2,2-diphenyl-1-picrylhydrazyl scavenging activities of aqueous and ethanolic leaf extracts of *C. pubescens* Benth

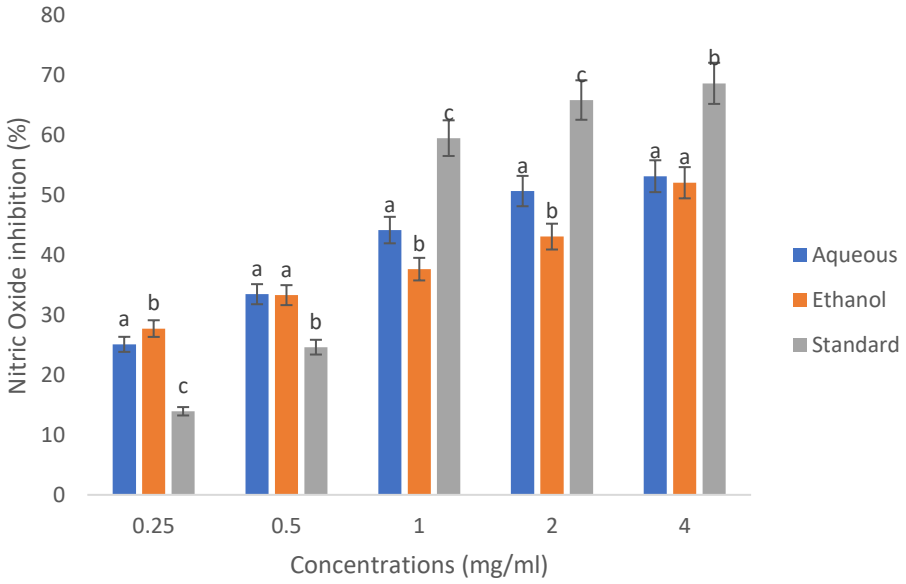

Figure 3: Nitric oxide scavenging activities of aqueous and ethanolic leaf extracts of *C. pubescens* Benth

Ferric reducing antioxidant power in leaf extracts of *C. pubescens* Benth

The outcome of the *in-vitro* ferric reducing antioxidant power in aqueous and ethanolic leaf extracts of *C. pubescens* Benth is shown in Figure 4. The ethanolic extract is seen to demonstrate a higher ferric reducing power than the aqueous extract

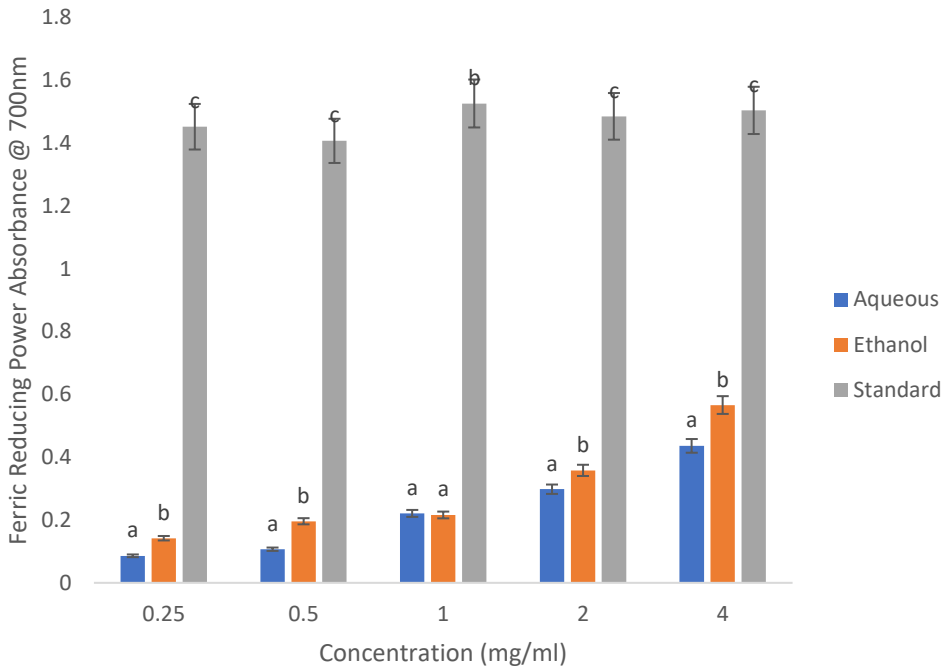

Figure 4: Ferric reducing antioxidant power of aqueous and ethanolic leaf extracts of *C. pubescens* Benth

Total antioxidant capacity in leaf extracts of *C. pubescens* Benth

The observation of the *in-vitro* total antioxidant capacity in aqueous and ethanolic leaf extracts of *C. pubescens* Benth is shown in Figure 5. Similar to previous assays, the ethanolic extract likely shows a higher total antioxidant capacity compared to the aqueous extract.

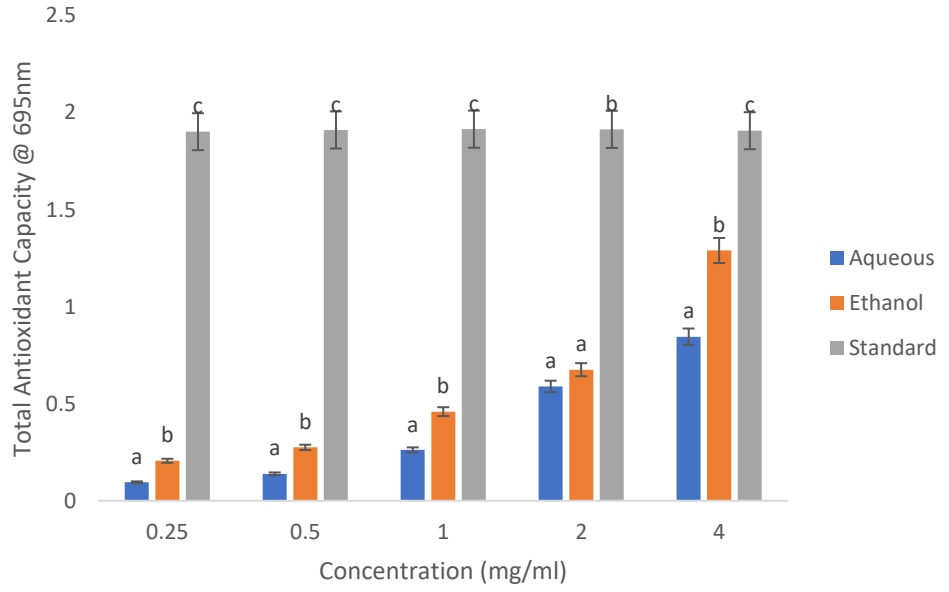

Figure 5: Total antioxidant capacity of aqueous and ethanolic leaf extracts of *C. pubescens* Benth

***In-vitro* enzyme inhibition assay: pancreatic lipase inhibition assay of leaf extracts of *C. pubescens* Benth**

The results of the *in-vitro* enzyme inhibition assay of aqueous and ethanolic leaf extracts of *C. pubescens* Benth is shown in Figure 6. It infers that both extracts inhibit pancreatic lipase. However, the ethanolic extract exhibit a stronger inhibitory effect.

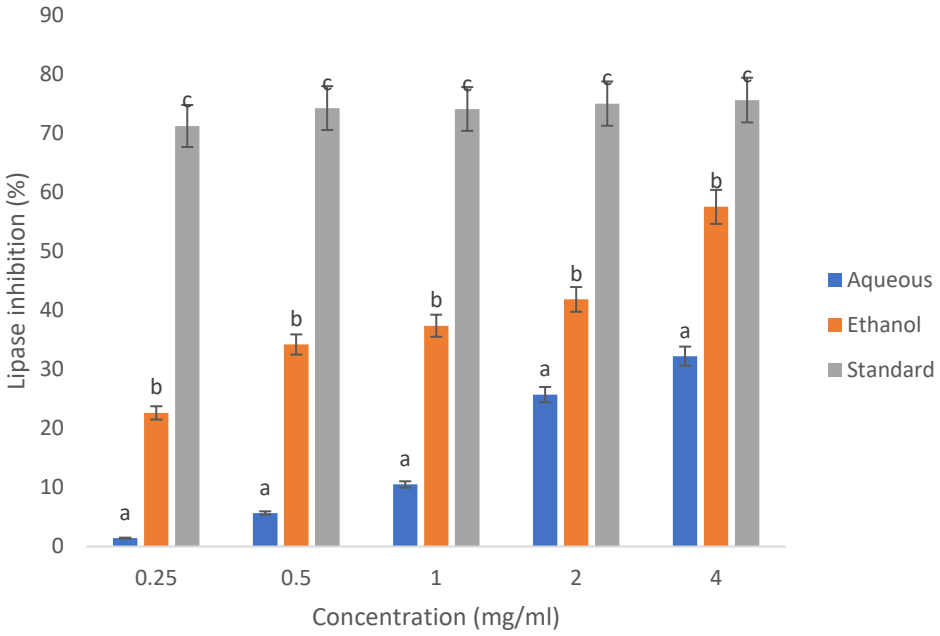

Figure 6: Pancreatic lipase inhibition assay of aqueous and ethanolic leaf extracts of *C. pubescens* Benth

DISCUSSION

Obesity and overweight have become a substantial worldwide health issue, influencing approximately a third of the world's population and generating severe health dangers (WHO, 2024). Oxidative stress, generated by an imbalance between reactive oxygen species (ROS) and the body's defensive systems, is heightened in obesity owing to increased fat storage and chronic inflammation. This stress damages cells and changes insulin signaling, resulting to insulin resistance and increases the risk of type 2 diabetes. It also promotes inflammation, endothelial dysfunction, and lipid peroxidation, leading to cardiovascular diseases and non-alcoholic fatty liver disease (NAFLD). Thus, oxidative stress plays a crucial part in the challenges related with obesity (Marseglia *et al.*, 2014; Manna and Jain, 2015).

This research examined the bioactive components, antioxidant activity, and anti-lipase potential of aqueous and ethanolic extracts of *C. pubescens* Benth.

The aqueous extract displayed a higher percentage yield (15.79%) than ethanolic extract (8.03%) but qualitative analysis, given in Table 1, showed the presence of several bioactive components in both aqueous and ethanolic extracts of *C. pubescens* Benth. Key findings include the presence of tannins, phlobatannin, flavonoids, cardiac glycosides, alkaloids, phenol, proteins, thiols, saponins, carbohydrates, and terpenes in the aqueous extract, and tannins, phlobatannin, flavonoids, alkaloids, phenol, steroids, proteins, and carbohydrates in the ethanolic extract (Table 1). The quantitative phytochemical analysis, depicted in Figure 1, reveal that distinct solvents, extract varied profiles of bioactive compounds, which can be related to the variable polarity of the solvents utilized (Khan *et al.*, 2022; Altemimi *et al.*, 2017). Studies have suggested that phytochemicals such as flavonoids, tannins, and phenols have high antioxidant capabilities (Anigboro *et al.*, 2019, Mariraj *et al.*, 2020)). These chemicals scavenge free radicals and chelate metal ions, lowering oxidative stress and associated

damage (Lobo *et al.*, 2010). The choice of solvent varies on the target phytochemicals, since each solvent may selectively extract distinct kinds of bioactive compounds, affecting the overall therapeutic potential and effectiveness of the plant extract (Altemimi *et al.*, 2017).

In-vitro tests such as DPPH and Nitric oxide scavenging activity, ferric reducing power, and total antioxidant capacity are useful for assessing the antioxidant potential of natural compounds. The DPPH test comprises the use of a persistent free radical that, when reduced by an antioxidant, changes color from deep violet to colorless or light yellow, enabling the antioxidant capacity to be measured by measuring absorbance at 517 nm. This hue shift directly indicates the compound's capacity to neutralize free radicals and prevent oxidative damage. Similarly, the nitric oxide scavenging test assesses a plant extract's potential to neutralize nitric oxide radicals, which are associated to oxidative stress. In this experiment, a decrease in nitric oxide levels, assessed by the Griess reagent, reveals a stronger antioxidant capacity and offers potentially therapeutic advantages against illnesses connected to oxidative stress. Ferric reducing power assesses a compound's electron-donating ability by its capacity to reduce Fe3+ to Fe2+, while total antioxidant capacity provides an overall evaluation of a substance's effectiveness in counteracting oxidative stress, which is vital for preventing chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. Together, these studies reveal essential insights into the therapeutic potential and health-promoting characteristics of natural extracts.

The findings from all assays (DPPH, nitric oxide scavenging, FRAP, and TAC) consistently demonstrate that the ethanol extract displays better antioxidant properties compared to the water extract. This accords with the results of Nguyen *et al.* (2020), which revealed that the ethanol extract of *Clerodendrum Cyrtophyllum Turcz* had both anti-inflammatory and antioxidant properties, this shows that ethanol is a better solvent for extracting bioactive compounds in plants. However, despite the ethanol extract's excellent antioxidant activity, the reference molecule, ascorbic acid, nonetheless displayed much greater antioxidant capacity across all studies. This underlines the usefulness of the standard component while underlining the promise of the ethanol extract as a natural antioxidant source, albeit somewhat less efficient than ascorbic acid.

Pancreatic lipase is the primary enzyme responsible for converting triglycerides into monoglycerides and fatty acids (Pirahanchi and Sharma, 2023). The pancreatic lipase suppression experiment (Figure 6) demonstrates that both (aqueous and ethanolic) extracts lower pancreatic lipase activity, a critical enzyme in fat digestion. However, the ethanol extract displays a more effective inhibitory effect, suggesting its promise for treating obesity by reducing fat absorption by blocking the enzyme responsible for fat digestion (Anigboro *et al.*, 2021; Anigboro *et al.*, 2024).

The conclusions of this study are consistent with earlier research that stresses the antioxidant and anti-lipase obesity properties of several plant's extracts. For instance, Kumar *et al.* (2020) and Anigboro *et al.* (2021) underlined the utility of plant-based antioxidants in lowering oxidative damage and regulating obesity, while Aziz *et al.* (2019) revealed the efficiency of many plant extracts in suppressing pancreatic lipase. Although a study has shown the presence of antioxidants in the leaf extract of *C. pubescens* Benth (Murugan *et al.*, 2020), yet, none has revealed its anti-obesity potential, it has remained a traditional claim with no scientific authentication. Hence, this study revealed the anti-obesity potential by highlighting is antioxidant and pancreatic lipase activity.

CONCLUSION

This experiment reveals the substantial antioxidant and anti-obesity potential of *C. pubescens* Benth extracts, notably the ethanol extract, which demonstrated better efficacy in both antioxidant tests and pancreatic lipase inhibition. The incorporation of bioactive substances, such as flavonoids, tannins, and phenols, adds to the extract's capacity to alleviate oxidative stress, a crucial aspect in obesity-related issues. Notably, the ethanol extract consistently outperformed other extracts, distinguishing it as the most effective choice for both antioxidant and anti-obesity action. These results coincide with past studies, further confirming the therapeutic potential of plant-based extracts in reducing obesity and its related metabolic illnesses via antioxidant activity and inhibition of fat digesting enzymes. However, more studies should be done to re-affirm this *in-vivo.*

Conflict of interest

The authors have no conflict of interest to declare.

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