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Contents

RESEARCH ARTICLES

**Phytochemical Analysis, Antioxidant and Anti-ulcer Potential of
Gossypium barbadense L. Leaf Extract (GBLE): In Vitro and In
Silico Approaches** 41

*Hannah Ireoluwa Alabi, Olu Israel Oyewole and Julianah Bunmi
Adetunji*

**Antidepressant Effects of White Ginger (*Mondia whitei*) and
Turmeric (*Curcuma longa*) Composite Tea in Wistar Rat Model of
Chronic Unpredictable Mild Stress** 62

*Gbolagade-Akanbi Abiola Monsurat, Oyewole Olu Isreal, Salawu Sule
Ola Adedeji Victor Adedapo and Gbolagade Omolola Hubeidah*

**Human–AI Collaboration in Digital Marketing: Evidence from an
Emerging Economy, Somalia** 82

Ibrahim Farah Hilowle

RESEARCH ARTICLE



Phytochemical Analysis, Antioxidant and Anti-ulcer Potential of *Gossypium barbadense* L. Leaf Extract (GBLE): *In Vitro* and *In Silico* Approaches

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Abstract: *Gossypium barbadense* L. has traditionally been used to manage various ailments. This study evaluated the phytochemical composition, antioxidant potential, and anti-ulcer potential of *G. barbadense* leaf extract (GBLE) using *in vitro* and *in silico* approaches. Qualitative and quantitative phytochemical analyses were performed, while antioxidant activity was assessed using H₂O₂, NO, DPPH, and ABTS assays. GC-MS was used to identify bioactive compounds, followed by molecular docking with PyRx/AutoDock against selected ulcer-related targets (IPSO, NF-κB, and KEAP-1). Phytochemical screening revealed xanthoproteins, tannins, triterpenes, glycosides, saponins, and flavonoids. GBLE showed notable antioxidant activity, while GC-MS identified several bioactive compounds, including methyl stearate, dodecanoic acid methyl ester, tetra-cosane, α-copaene, heneicosane, methyl tetradecanoate, heptadecane, hexadecanoic acid methyl ester, humulene, methyl 9-octadecenoate, β-caryophyllene, and eicosane. Molecular docking revealed favourable binding affinities ranging from −7.0 to −3.4 kcal/mol, suggesting stable ligand–protein interactions. Overall, the findings support the antioxidant and anti-ulcer potentials of *G. barbadense* leaves and suggest that their bioactive constituents may contribute to these effects.

Keywords: Scavenging assays, binding affinities, antioxidants and Ulcer pathogenesis

1. INTRODUCTION

The need to substitute certain medications owing to their adverse effects underscores the universal role of medicinal plants in health maintenance, disease prevention and treatment. Since antiquity, plant therapy has been widely used to manage diseases, including acute and recurrent gastrointestinal disorders such as inflammatory bowel disease and ulcers (Kuna *et al.*, 2019). This reliance is due to the healing potential of medicinal plants, which contain diverse secondary metabolites, including terpenoids, saponins, flavonoids, glycosides, tannins, and alkaloids, which are widely distributed across various plant parts as defensive agents against pathogens. Many of these compounds have shown significant therapeutic benefits in humans, thus attributing their multiple pharmacological actions (Kim *et al.*, 2018). Despite these promising benefits, their applications remain limited due to insufficient scientific evidence, limited pleiotropy, potential toxicity, and standardisation issues. Targeting specificity and efficacy, ensuring safety, and minimising the use of animals in *in vivo* studies are essential steps in optimising the use of medicinal plants. These objectives can be achieved through phytochemical screening and *in silico* analyses to identify and quantify bioactive compounds and to predict their mechanisms of action, toxicity, and efficacy prior to *in vivo* investigations.

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Gastric ulcer, a widespread gastrointestinal disorder, is among the conditions traditionally managed with medicinal plants. It is a type of peptic ulcer characterised by open, painful lesions in the gastric mucosa that often lead to bleeding and increased susceptibility to infection, making it a significant global health concern. Factors contributing to gastric ulcer include the use of Non-Steroidal Anti-inflammatory Drugs (NSAIDs), alcohol consumption, stress, *Helicobacter pylori* infection, and excessive intake of irritant foods such as spicy meals, carbonated beverages, and coffee (Shahzad *et al.*, 2024). Its pathophysiology involves an imbalance between mucosal defence mechanisms and aggressive factors (e.g. gastric acid and pepsin). In addition, gastric inflammation, oxidative stress and impaired antioxidant defences contribute to disease progression. The dysregulation of KEAP-1 and NF- κ B signalling pathways, followed by gastric mucosal injury, further exacerbates ulcer pathogenesis, suggesting that modulation of these pathways may provide therapeutic benefit.

Gossypium barbadense (cotton plant) is widely used in Ayurvedic medicine for conditions such as wound healing, haemorrhoids, haemorrhage control, bronchitis, fungal infections and rheumatism (Ade-Ademilua and Okpoma, 2018). It is a perennial shrub indigenous to South America and is widely cultivated in Senegal and Nigeria. Various parts, such as seeds, leaves, bark, and roots, are used in traditional medicine for their therapeutic potential. The species has been reported to exhibit anticancer, anti-ulcer, antihypertensive, anti-inflammatory, antimalarial, antioxidant, and antimicrobial activities (Morales-Aranibar *et al.*, 2023; Balkrishnan *et al.*, 2023; Olanrewaju *et al.*, 2025). Among all of its traditional applications, wound healing is most prominent, possibly due to gossypol and related compounds identified in the leaf essential oil (Sabiou *et al.*, 2017). However, comprehensive scientific validation of its pharmacological effects, particularly regarding anti-ulcer activity and wound healing, remains limited. This calls for further investigation to elucidate its phytochemical constituents and *in vitro* antioxidant properties as preliminary steps in drug discovery before advanced preclinical studies.

Accordingly, this study was designed to qualitatively and quantitatively analyse the leaf extract of *Gossypium barbadense* by evaluating its *in vitro* antioxidant activity and validating its anti-ulcer potential through *in silico* analysis.

1.1. Hypothesis and Objective

We hypothesise that GBLE will have antioxidant and anti-ulcer potential against oxidative stress-related diseases. The study, therefore, aimed to qualify and quantify *Gossypium barbadense* L. Leaf Extract and also evaluate its antioxidant and anti-ulcer potential using *in vitro* and *in silico* Approaches.

2. MATERIALS AND METHODS

2.1. Materials

Absolute ethanol, 2,2'-Azinobis (3-Ethylbenzothiazoline-6-sulphonic (ABTS), Folin-Ciocalteu reagent, aluminium chloride, distilled water, iron (III) Chloride (FeCl_3), Griess reagent, methanol, potassium persulphate, DPPH (2,2'-diphenyl-1-picrylhydrazyl) reagent, sodium carbonate, hydrochloric acid, ascorbic acid, tannic acid, chloroform, sodium hydroxide, and sodium dodecyl sulphate were product of Sigma-Aldrich (St. Louis, USA), while all other laboratory reagent used were of analytical grade.

2.2. Plant Collection and Authentication

Gossypium barbadense leaves were sourced from Ikirun town, Osun State, Nigeria. A Botanist identified these leaves at the Department of Plant Biology, Osun State University, Osogbo, Nigeria, and a voucher specimen (Voucher No. 2026020001) was deposited in the herbarium.

2.3. Preparation of GBLE

At room temperature, the plant's fresh leaves were air-dried, pulverised using an electric blender to obtain a fine powder, and then stored in an airtight container to preserve their integrity until further use. 600 g of powdered *G. barbadense* leaves was macerated in 3.6 litres of absolute ethanol (1:6 w/v) for 14 days

to extract bioactive phytochemicals. The resulting mixture was filtered, and the filtrate was concentrated at 40°C using a rotary evaporator. The resulting residue was further dried in a water bath to obtain a concentrated crude extract, which was refrigerated for later use.

2.4. Qualitative and Quantitative Phytochemical Analysis of the GBLE

In accordance with the method of Odebiyi and Sofowora, (1978), the presence of the following phyto-bioactive compounds, flavonoids, saponins, glycosides, tannins, triterpenes and xanthoprotein were qualitatively assessed, while their quantitative analysis was conducted following their respective methods; Glycoside content was determined by a colorimetric reaction between the deoxysugar portion of cardiac glycosides and specific reagents in an acidic medium which produced a coloured complex that was spectrophotometrically measured at 495 nm following the method of Harborne (1998); the formation of blue-coloured phosphomolybdic-phosphotungstic acid complexes on reaction with Folin-Ciocalteu reagent was used for tannins determination, which was measured spectrophotometrically (Swain, 1979); Aluminium chloride colorimetric method was employed for determination of total flavonoid content, which resulted in formation of stable acid complexes on reaction with either the hydroxyl groups of carbon-3 (C-3) of flavonols or flavone and its carbon-4 (C-4) keto group as reported by Kaufman *et al.* (1999); Saponins content was quantified spectrophotometrically using the method of Brunner (1984) which involved the formation of blood red coloured products formed by the reaction of the oxidised triterpene of saponin with iron (III) Chloride; Triterpene was determined using Liebermann-Burchard reaction which involved of a reagent mixture containing acetic anhydride and concentrated sulfuric acid with triterpene to produce a coloured complex whose absorbance is proportional to the triterpene's concentration in GBLE (Harborne, 1998) and Xanthoprotein contents measurement is based on the nitration of aromatic amino acids present in proteins by concentrated nitric acid, forming yellow nitro derivatives whose intensity was measured spectrophotometrically (Wilson & Walker, 2000).

2.5. In Vitro Antioxidant Assay of the *G. barbadense* Leaf Extract (GBLE)

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was determined spectrophotometrically by measuring the absorbance of the yellow-coloured products formed upon reducing the DPPH radical with the antioxidants present in the GBLE at 517 nm, which aligned with the prior method of Brand-Williams. (1995). Briefly, varying concentrations of the extract (50-500 µg/ml) and the standard (ascorbic acid) were prepared in distilled water, mixed with a methanolic DPPH solution (0.5 ml, 0.1 M), shaken, and incubated in the dark at room temperature for 30 minutes. Upon spectrometric measurement, the results were expressed as percentages of DPPH scavenging Effect.

The 2,2'-Azinobis (3-Ethylbenzothiazoline-6-Sulphonic acid) (ABTS) scavenging activity of GBLE was determined by measuring the level at which compounds in the extract neutralise the ABTS⁺ radical, resulting in a spectrophotometric decrease in colour intensity at 734 nm (Re *et al.*, 1999). For this, varying concentrations of 0.01-5.0 mg/ml of GBLE and 0.01- 5.0 mg/ml of ascorbic acid (standard) were prepared and mixed with 2.0 ml of diluted ABTS radical cation solution. The result was expressed as the percentage of ABTS scavenging effect.

In accordance with the protocol of Bhaskar & Balakrishnan (2009), the Nitric Oxide (NO) scavenging activity was measured by oxidation of nitric oxide produced from sodium nitroprusside by GBLE.

Briefly, the GBLE solution at varying concentrations (1-20 µg/ml) in distilled water was mixed with 1 ml of 10 mM sodium nitroprusside and phosphate buffer (pH 7.4), incubated, and treated with 1 ml of Griess reagent. The mixture was kept standing for 5-10 minutes, and the absorbance of the coloured product formed was measured at 540 nm.

Hydrogen peroxide radical scavenging activity was determined spectrophotometrically at 230 nm by measuring GBLE's ability to donate electrons to Hydrogen peroxide, thereby decomposing it into water (H₂O) and Oxygen (O₂), thereby reducing the concentration in the reaction mixture (Gülçin *et al.*, 2002).

2.6. GC-MS analysis of GBLE

GBLE was characterised using a 7890B gas chromatograph coupled to a 5977C inert mass spectrometer with an electron impact source (Agilent Technologies). GBLE sample (2.5 g) was extracted by adding 20ml of ethanol and sonicating in an ultrasonic bath. It was cleaned up with a mixture of graphite, carbon black, Magnesium sulphate, and a primary-secondary amine to remove the matrix pigment. The mass spectrometer was operated at 70 eV in electron-impact ionisation mode with an ion source temperature set at 230°C, a quadrupole temperature of 150°C, and a transfer line temperature of 280°C. Possible compounds were scanned within a mass-to-charge ratio (m/z) range of 50 to 550 amu at a scan speed of 2.62 s/scan rate, and the identification of compounds was carried out by comparing the obtained mass spectral data with standard reference spectra available in the NIST 14 Mass Spectral Library.

2.7. In Silico Studies

The three-dimensional (3D) structures of the selected ligands identified from *Gossypium barbadense* leaf extract (GBLE) were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in Structure Data File (SDF) format. The ligand structures were converted to Protein Data Bank (PDB) format using Open Babel and subjected to energy minimisation to optimise their geometries and reduce structural strain. The minimised ligands were subsequently converted to PDBQT format using AutoDock Tools for molecular docking.

The three-dimensional structures of the selected target proteins were obtained from the Protein Data Bank (PDB). Protein preparation was performed using Discovery Studio Visualizer by removing co-crystallised ligands, water molecules, and other non-essential heteroatoms. Polar hydrogen atoms were added, and Gasteiger partial charges were assigned using AutoDock Tools. The binding sites of the target proteins were defined based on the positions of co-crystallised ligands and/or previously reported active-site residues. The docking grid boxes were centred on the corresponding binding sites and adjusted to adequately encompass the active-site regions.

Molecular docking simulations were performed using AutoDock Vina through the PyRx platform. Each ligand was individually docked into the predefined binding pocket of its respective target protein. Unless otherwise specified, the docking parameters were maintained at their default settings. The predicted binding affinities were expressed in kcal/mol, and the top-ranked ligand conformations based on docking scores were selected for subsequent interaction analysis. The docking results were interpreted as predicted ligand–protein binding poses and affinities rather than as experimental evidence of binding.

Post-docking analysis was performed using Discovery Studio Visualizer to examine the predicted protein–ligand complexes and characterise the principal non-covalent interactions. These included hydrogen bonds, hydrophobic interactions, π – π interactions, and other relevant contacts between the ligands and amino acid residues within the binding sites.

In silico pharmacokinetic and drug-likeness properties of the selected ligands were evaluated using the SwissADME web server. The assessment included Lipinski's rule of five, gastrointestinal absorption, bio-availability, lipophilicity, and aqueous solubility. These computational predictions were used to complement the molecular docking analysis and provide an initial assessment of the drug-likeness and ADME characteristics of the selected GBLE-derived compounds.

2.8. Statistical Analysis

Data were analysed using a one-way analysis of variance (ANOVA), followed by a post hoc Bonferroni multiple comparisons test to assess differences between mean values. The results were expressed as mean $\pm$ standard deviation (SD). All analyses were carried out using GraphPad Software Prism.

3. RESULTS

3.1. Extraction Yield of GBLE

The percentage yield of GBLE upon extraction was calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{Mass of Extract Obtained}}{\text{Mass of plant material used}} \times 100 \quad \dots\dots\dots (1)$$

Given: Initial plant material weight of 600 g,

Mass of extract obtained = 31.68 g,

Mass of plant material used = 600 g

$$\begin{aligned} \text{Yield (\%)} &= \frac{31.68}{600} \times 100 \\ &= 0.0528 \times 100 \\ &= 5.28\% \end{aligned}$$

The crude extract yield was about 5.28 % of the sample (600 g).

The phytoactive compounds identified and quantified in GBLE, along with their respective compositions, are highlighted in Table 1 below. The findings indicated the presence of bioactive compounds, including xanthoproteins, glycosides, tannins, saponins, and flavonoids, in the plant. Xanthoprotein has the highest value (73.90 ± 42.60 g/100g), followed by saponins (64.05 ± 36.92 g/100g), glycosides (41.57 ± 23.96 g/100g), flavonoids (4.94 ± 2.85 g/100g), and Triterpenes (3.45 ± 1.99 g/100g), while tannin has the lowest value (1.97 ± 1.14 g/100g).

Table 1. Quantitative phytochemical composition of GBLE.

Phytobioactive Compound	Crude Calculated (g/100 g)
Flavonoids	4.94 ± 2.85
Triterpenes	3.45 ± 1.99
Glycosides	41.57 ± 23.96
Tannin	1.97 ± 1.14
Saponin	64.05 ± 36.92
Xanthoprotein	73.90 ± 42.60

Figure 1 compared the DPPH radical scavenging inhibition of GBLE to a standard antioxidant compound (Ascorbic acid), n=3. At the chosen concentrations, GBLE scavenged DPPH radicals more effectively than ascorbic acid. At concentrations of 1, 2, 5, 10, and 20 µg/ml, the percentage scavenging inhibition of GBLE was 62.5, 75.6, 83.80, 85.60, and 91.30%, while that of the standard was 8.13, 68.13, 77.50, 81.25, and 85.00%, respectively. The DPPH's half concentration inhibition (IC₅₀) is 2.36, while that of Ascorbic acid is 31.50.

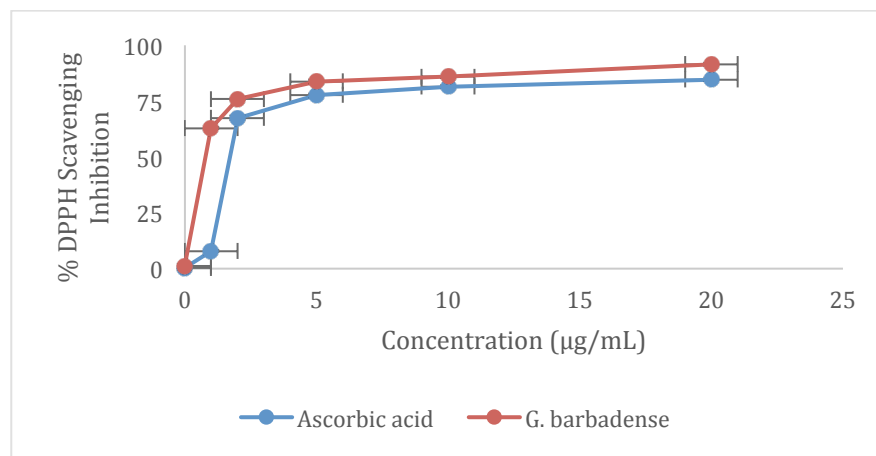


Figure 1: DPPH Radical Scavenging Inhibition of GBLE and Ascorbic acid

Figure 2 presents the nitric oxide radical scavenging inhibition of GBLE and ascorbic acid, $n = 3$, at varying concentrations ranging from 1–20 $\mu\text{g/mL}$. At all tested concentrations, GBLE exhibited greater nitric oxide radical scavenging activity, expressed as percentage inhibition, than ascorbic acid. At concentrations of 1, 2, 5, 10, and 20 $\mu\text{g/mL}$, the percentage inhibition of nitric oxide radicals by GBLE was 32.4, 49.00, 64.80, 74.50, and 80.70 %, respectively. In comparison, the corresponding inhibition values for ascorbic acid were 11.03%, 42.76%, 61.38%, 71.03%, and 78.62%, respectively. Furthermore, the IC_{50} value for GBLE was determined to be 15.55 $\mu\text{g/mL}$, whereas that of ascorbic acid was 62.16 $\mu\text{g/mL}$.

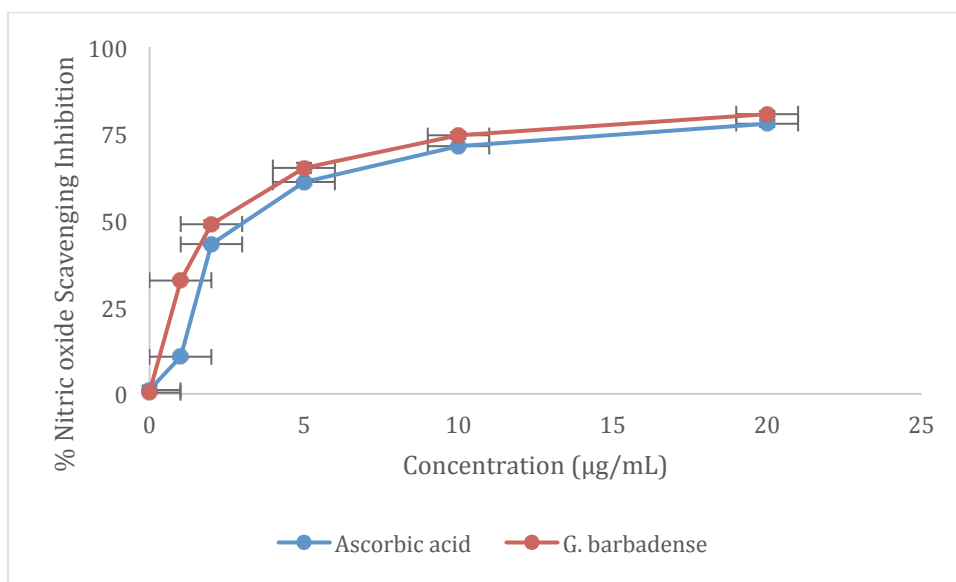


Figure 2: Nitric Oxide Radical Scavenging Inhibition of GBLE and Ascorbic Acid

Figure 3 illustrates the hydrogen peroxide scavenging inhibition of GBLE compared with ascorbic acid, $n = 3$, at varying concentrations. Similar to the nitric oxide radical-scavenging assay, the hydrogen peroxide-scavenging inhibition of GBLE, expressed as percentage inhibition, was higher than that of ascorbic acid across all tested concentrations. At concentrations of 1, 2, 5, 10, and 20 $\mu\text{g/mL}$, the percentage inhibition of hydrogen peroxide by GBLE was 14.1, 38.30, 74.00, 77.20, and 81.10%, respectively. In comparison, the corresponding inhibition values for ascorbic acid were 10.68%, 36.89%, 64.56%, 71.85%, and 77.18%, respectively. However, the estimated IC_{50} value for GBLE (60.63 $\mu\text{g/mL}$) was greater than that of ascorbic acid (28.2 $\mu\text{g/mL}$), indicating that ascorbic acid exhibited stronger scavenging potency despite the higher percentage inhibition observed for GBLE at the tested concentrations.

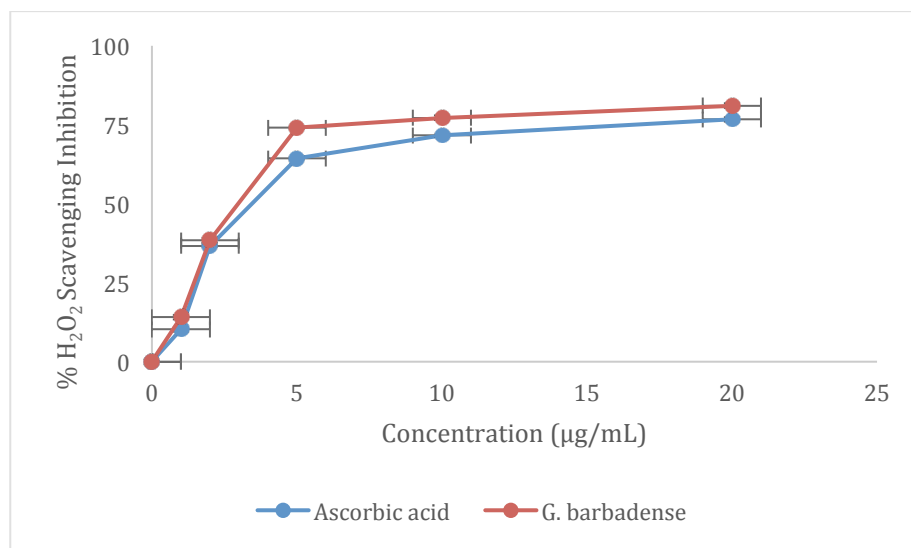


Figure 3: H_2O_2 Scavenging Inhibition of GBLE and Ascorbic acid

Figure 4 presents the 2, 2'-Azinobis (3-Ethylbenzothiazoline-6-Sulphonic acid (ABTS) radical scavenging inhibition of GBLE compared with ascorbic acid, $n=3$, at varying concentrations. The results show that GBLE exhibited markedly greater ABTS radical-scavenging inhibition than ascorbic acid across all concentrations tested. At concentrations of 1, 2, 5, 10, and 20 $\mu\text{g/mL}$, the percentage inhibition of the ABTS radical by GBLE was 56.30, 68.90, 78.80, 85.40, and 88.10 %, respectively. In contrast, the corresponding inhibition values for the standard antioxidant, ascorbic acid, were 8.61%, 37.09%, 59.70%, 67.55%, and 81.46%, respectively. However, the estimated IC_{50} value for GBLE was 3.43 $\mu\text{g/mL}$, while that of ascorbic acid was 83.05 $\mu\text{g/mL}$. Thus, GBLE showed stronger antioxidant activity than the standard.

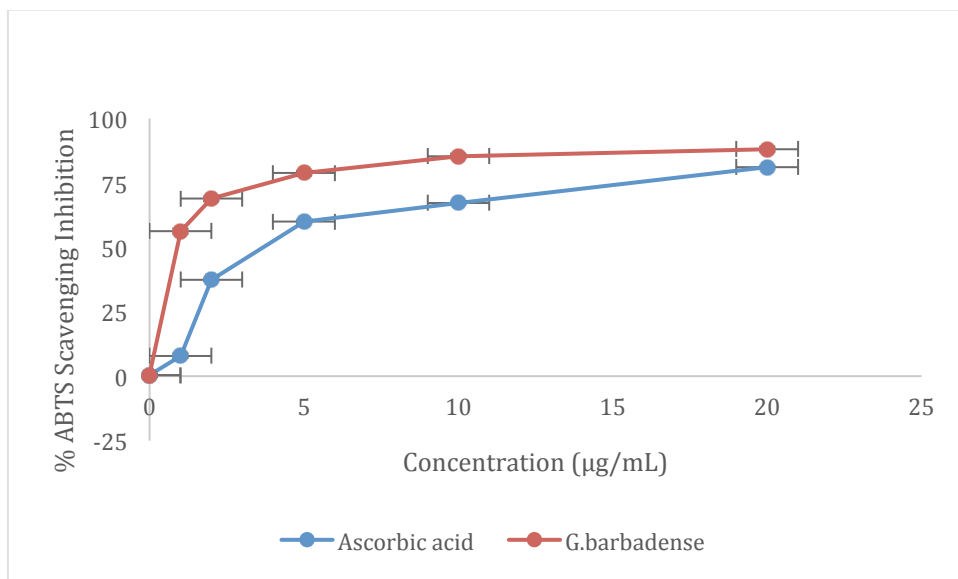


Figure 4: ABTS Radical Scavenging Inhibition of GBLE and Ascorbic acid

3.2. Determination of IC_{50}

The IC_{50} was defined as the concentration of the test sample required to inhibit 50% of the measured biological activity. IC_{50} values were expressed in the corresponding concentration units and used to compare the inhibitory activities of the tested samples. The half-maximal inhibitory concentration (IC_{50}) was determined using GraphPad Prism 9.0 (GraphPad Software, USA). Concentration-response data were plotted as percentage inhibition versus the corresponding test concentrations and fitted by nonlinear regression using a variable-slope four-parameter logistic model.

3.3. GC-MS Results of GBLE

Table 2 presents a list of selected therapeutic phytobioactive compounds identified in GBLE through qualitative characterisation and Gas Chromatography-Mass Spectrometry analysis, with each compound's molecular formula, molecular weight, area, retention time (RT), and chemical class provided.

Table 2. GC-MS of GBLE

Peak No	Compound	Area %	Retention Time (RT), min	Molecular weight (amu)	Molecular Formula	Chemical Class
1	Eicosane, 10-methyl	1.20	4.605	296.344	$\text{C}_{21}\text{H}_{44}$	Branched Alkane
2	Tetracosane, 3-Ethyl	2.53	4.773	366.423	$\text{C}_{26}\text{H}_{54}$	
3	Heptadecane, 9-octyl	0.91	6.396	352.407	$\text{C}_{25}\text{H}_{52}$	
4	Heneicosane	1.19	6.490	296.344	$\text{C}_{21}\text{H}_{44}$	
5	Alpha-copaene	2.67	5.569	204.188	$\text{C}_{15}\text{H}_{24}$	Sesquiterpene

Peak No	Compound	Area %	Retention Time (RT), min	Molecular weight (amu)	Molecular Formula	Chemical Class
6	Caryophyllene	14.85	5.946	204.188	C ₁₅ H ₂₄	
7	Humulene	4.43	6.239	204.188	C ₁₅ H ₂₄	
8	Dodecanoic acid, methyl ester	3.30	6.877	214.193	C ₁₂ H ₂₄ O ₂	Fatty acid
9	Methyl tetradecanoate	2.90	8.594	242.2256	C ₁₅ H ₃₀ O ₂	
10	Hexadecanoic acid, methyl ester	8.51	10.154	270.256	C ₁₇ H ₃₄ O ₂	
11	9-Octadecenoic acid, methyl ester (E)-	5.20	11.358	296.272	C ₁₉ H ₃₆ O ₂	
12	Methyl stearate	1.29	11.568	298.287	C ₁₉ H ₃₈ O ₂	

GC-MS analysis revealed 12 compounds from GBLE that are of therapeutic benefit, as in Table 2. These compounds are Methyl tetradecanoate (2.90%), Eicosane, 10-methyl (1.20%), Tetracosane, 3-Ethyl (2.53%), Alpha-copaene (2.67%), Hexadecanoic acid, methyl ester (8.51%), β-Caryophyllene (14.85%), Heptadecane, 9-octyl (0.91%), Heneicosane (1.19%), Dodecanoic acid, methyl ester (3.30%), Methyl stearate (1.29%), 9-Octadecenoic acid-methyl ester (E) (5.20%), humulene (4.43%).

Figure 5 presents the GC-MS Chromatogram obtained from the analysis, showing the various peaks with their retention times and abundances. However, among these bioactive compounds, two compounds with the prominent peaks, named β-Caryophyllene (14.85%) and Hexadecanoic acid- methyl ester (8.51%) have been previously documented to exhibit antibacterial properties (Constani *et al.*, 2019), antioxidant and analgesic properties (Machado *et al.*, 2020), gastroprotective activity (Sharma *et al.*, 2023) and anti-inflammatory properties (Francomano *et al.*, 2019; Rodríguez-Mejía *et al.*, 2022; Dinku *et al.*, 2022),

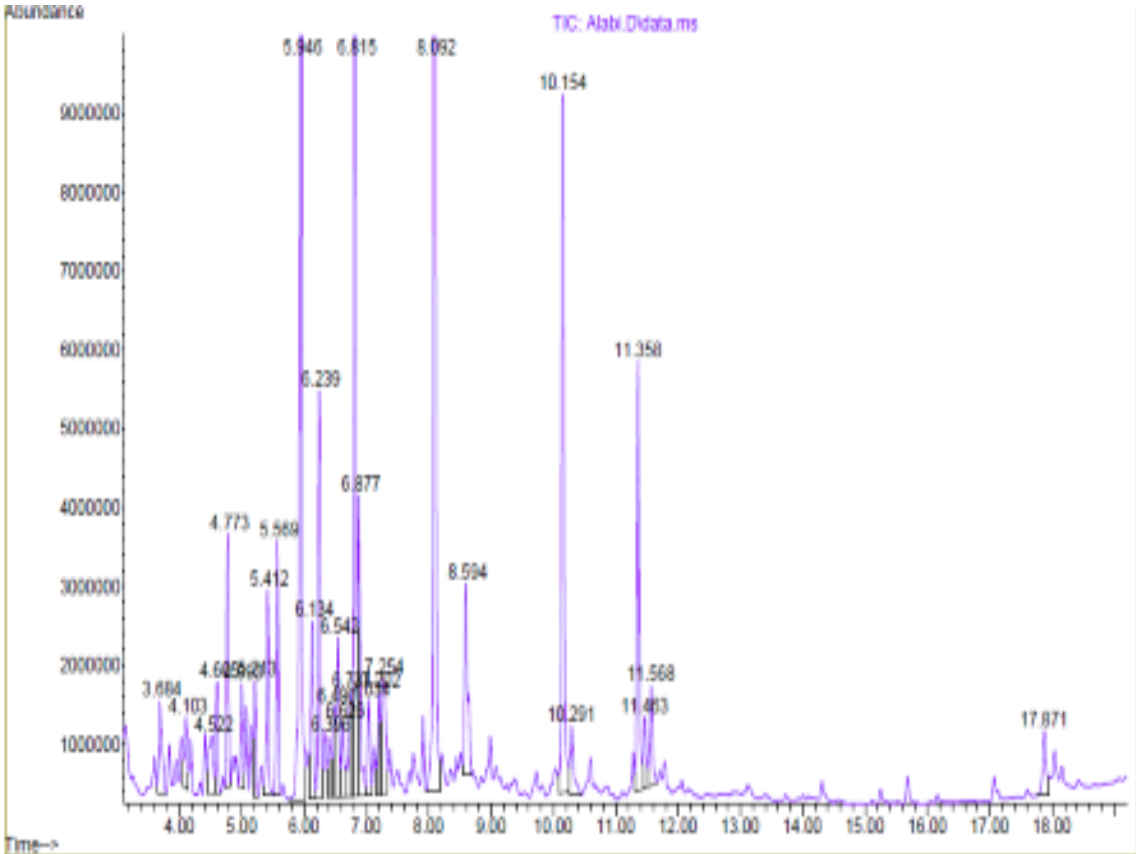


Figure 5: GC-MS Chromatogram and Identified Bioactive Compounds in GBLE

3.4. *In Silico* Results and Molecular Docking

Table 3. GBLE ligands with 1NFK.

Ligand	Binding Affinity (kcal/mol)
1VX	-7.5
Caryophyllene	-5.7
Alpha-copaene	-5.7
Pepstatin	-5.5
Humulene	-5.4
Octadecane, 5-methyl-	-4.6
9-Octadecenoic acid, methyl ester, (E)-	-4.2
Dodecanoic acid, methyl ester	-4.0
Eicosane, 10-methyl-	-3.9
Hexadecanoic acid, methyl ester	-3.9
Heptadecane, 9-octyl-	-3.8
Heneicosane	-3.5
Methyl stearate	-3.5
Tetracosane, 3-ethyl-	-3.4

Table 4. GBLE with IPSO.

Ligand	Binding affinity (kcal/mol)
Pepstatin	-8.4
1VX	-7.3
Alpha-copaene	-5.9
Heptadecane, 9-octyl-	-5.6
Methyl stearate	-5.6
Methyl tetradecanoate	-5.5
Hexadecanoic acid, methyl ester	-5.4
Eicosane, 10-methyl-	-5.4
Humulene	-5.2
Caryophyllene	-5.1
Tetracosane, 3-ethyl-	-5.1
Dodecanoic acid, methyl ester	-5.0
Heneicosane	-4.2
9-Octadecenoic acid, methyl ester, (E)-	-4.0

Table 5. GBLE with 4L7D.

Ligand	Binding affinity (kcal/mol)
1VX	-10.6
Pepstatin	-8.2
Caryophyllene	-7.0

Ligand	Binding affinity (kcal/mol)
Humulene	-6.9
Alpha-copaene	-6.6
Heptadecane, 9-octyl-	-5.4
9-Octadecenoic acid, methyl ester (E)-	-5.2
Heneicosane	-5.2
Tetracosane, 3-ethyl-	-5.1
Methyl stearate	-5.0
Hexadecanoic acid, methyl ester	-4.9
Eicosane, 10-methyl-	-4.9
Methyl tetradecanoate	-4.9
Dodecanoic acid, methyl ester	-4.8

Table 6. Physicochemical properties of GBLE ligands with Pepstasin and IVX

Parameters	Compounds							
	Alpha-copaene	Caryophyllene	Humulene	9-Octadecenoic acid, methyl ester (E)-	Methyl stearate	Tetracosane, 3-ethyl-	Pepstasin	IVX
Molecular Formula	C ₁₅ H ₂₄	C ₁₅ H ₂₄	C ₁₅ H ₂₄	C ₁₉ H ₃₆ O ₂	C ₁₉ H ₃₈ O ₂	C ₂₆ H ₅₄	C ₃₄ H ₆₃ N ₅ O ₉	C ₂₇ H ₃₀ N ₂ O ₄
Molecular weight (g/mol)	204.35	204.35	204.35	296.49	298.50	366.74	685.54	446.54
Number of heavy atoms	15	15	15	21	21	26	48	33
Number of aromatic Atoms	0	0	0	0		0	0	12
Topological polar surface area (TPSA)	0	0	0	26.3 Å	26.3 Å	0	223.26 Å	77.92 Å
Fraction Csp3	0.87	0.73	0.60	0.84	0.95	1	0.82	0.44
Number of rotatable bonds	1	0	0	16	17	22	27	5
Number of H-bond acceptors	0	0	0	2	2	0	9	4
Number of H-bond donors	0	0	0	0	0	0	8	1
Molecular refractivity	67.14	68.78	70.42	94.26	94.73	127.1	184.66	133.34

Table 7. Lipophilicity and water solubility of GBLE ligands with Pepstasin and IVX

Compounds	Lipophilicity	Water Solubility					
	Consensus log P _{o/w}	Log S (ESOL)	Solubility Class	Log S (all)	Solubility Class	Log S (sili- cos-it)	Solubility Class
Alpha-copaene	3.62	-3.86	Soluble	-4.19	Moderately soluble	-3.07	Soluble
Caryophyllene	4.24	-3.87	Soluble	-4.1	Moderately soluble	-3.77	Soluble
Humulene	4.26	-3.97	Soluble	-4.27	Moderately Soluble	-3.52	Soluble
9-Octadecenoic acid, methyl ester (E)-	5.95	-5.32	Moderately solu- ble	-7.83	poorly Soluble	-6.09	Poorly solu- ble
Methyl stearate	6.24	-5.83	Moderately solu- ble	-8.77	Poorly soluble	-6.81	Poorly solu- ble
Tetracosane, 3-ethyl-	10.05	-9.49	Poorly soluble	-14.09	Insoluble	-9.49	Poorly solu- ble
Pepstatin	2.37	-4.19	Moderately solu- ble	-7.34	Poorly soluble	-4.55	Moderately soluble
IVX	3.26	-4.72	Moderately solu- ble	-4.77	Moderately soluble	-5.83	Moderately soluble

Table 8. Pharmacokinetics of GBLE ligands with Pepstasin and IVX

PARAMETERS	COMPOUNDS							
	α -copaene	Caryophyllene	Humulene	9-Octadecenoic acid, methyl ester (E)-	Methyl stearate	Tetracosane, 3-ethyl-	Pepstasin	IVX
Blood-brain barrier permeation	Yes	No	No	No	No	No	No	Yes
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.11	0.56
Gastrointestinal absorption	Low	Low	Low	High	High	Low	Low	High
P-glycoprotein substrate	No	No	No	No	No	Yes	No	Yes
Cytochrome P450 1A2 inhibitor	No	No	No	Yes	Yes	No	No	No
Cytochrome P450 2C19 inhibitor	Yes	Yes	No	No	No	No	No	No
Cytochrome P450 2C9 inhibitor	No	Yes	Yes	No	No	No	No	Yes
Cytochrome P450 3A4 inhibitor	No	No	No	No	No	No	No	Yes
Cytochrome P450 2D6 inhibitor	No	No	No	No	No	No	No	Yes

Table 9. Drug-likeness and medicinal chemistry

COMPOUNDS	Drug-likeness rules					Medicinal Chemistry			
	Lipinki #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	PAINS #alerts	Brenk #alerts	Lead likeness #violations	Synthetic accessibility
Alpha-copaene	1	0	0	0	1	0	1	2	4.62
Caryophyllene	1	0	0	0	1	0	1	2	4.51
Humulene	1	0	0	0	1	0	1	2	3.66
9-Octadecenoic acid, methyl ester (E)-	1	1	1	1	2	0	1	2	3.16
Methyl stearate	1	1	1	1	2	0	0	2	2.76
Tetracosane, 3-ethyl-	1	2	1	1	3	0	0	3	3.44
Pepstatin	3	3	2	1	4	0	0	2	6.65
IVX	0	1	0	0	0	0	0	1	4.11

Table 10. Summary of the docking scores of the six shortlisted ligands with Pepstasin and IVX across the three targets

Ligand	IPSO (kcal/mol)	INFK(kcal/mol)	4LFD(kcal/mol)	Observation
Pepstatin (standard)	-8.4	-5.5	-8.2	Best on IPSO, strong on 1NFK, and very strong on 4LFD
IVX (standard)	-7.3	-7.5	-10.6	Best on 1NFK, Best on 4LFD, and very strong on IPSO
Alpha-copaene	-5.9	-5.7	-6.6	strong on 1NFK, strong on IPSO, and good on 4LFD
Methyl stearate	-5.6	-3.5	-5.0	Weak across all proteins
Humulene	-5.2	-5.4	-6.9	Weak across all proteins
Caryophyllene	-5.1	-5.7	-7.0	Weak on IPSO, strong on 1NFK and 4LFD
Tetracosane, 3-ethyl-	-5.1	-3.4	-5.1	Weak across all proteins
9-Octadecenoic acid, methyl ester (E)-	-4.0	-4.2	-5.2	Weak across all proteins

4. DISCUSSION

The phytochemical analysis conducted on *G. barbadense* leaf extract, as represented in Table 1, revealed the presence of flavonoids, triterpenes, xanthoprotein, saponins, tannins and glycosides, which have been reported to exhibit antioxidant, anti-inflammatory, anti-viral properties, antitumor, anti-ulcer, gastroprotective, anti-convulsant, anti-diabetic, antibacterial, anthelmintic and anti-fertility activities (Vishwakarma *et al.*, 2022; Morales-Aranibar *et al.*, 2023; Alzandi *et al.*, 2025; Adib *et al.*, 2024).

Glycosides are compounds consisting of a glycosyl group and an aglycone (Johnson *et al.*, 2021), characterised by a sugar moiety linked to a non-sugar aglycone. They can exhibit diverse biological functions that are exploited for therapeutic benefit. Flavonoids are secondary metabolites which comprise a

Table 11. Summary of the key parameters used for the final selection of the ligands

Parameters	Alpha-copaene	Caryophyllene	Humulene	9-Octadecenoic acid, methyl ester (E)-	Methyl stearate	Tetracosane, 3-ethyl-	Pepstatin	IVX
Molecular weight (g/mol)	204.35	204.35	204.35	296.49	298.50	366.74	685.54	446.54
Topological polar surface area (TPSA)	0	0	0	26.3Å	26.3 Å	0	223.26 Å	77.92 Å
Gastrointestinal absorption	Low	Low	Low	High	High	Low	Low	High
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.11	0.56
Muegge Violations	1	1	1	2	2	3	4	0
Lipinski violations	1	1	1	1	1	1	3	0
Drug likeness	Good	Good	Good	Moderate	Moderate	Poor	Poor	Very Good

1PSO with 1TVX

1PSO with 9-Octadecenoic acid, methyl ester, (E)-

1PSO with Heptadecane, 9-octyl-

1PSO with Methyl stearate

1PSO with Pepstatin

1PSO with 1TVX

Figure 6: IPSO with GBLE Ligands and IVX and Pepstatin

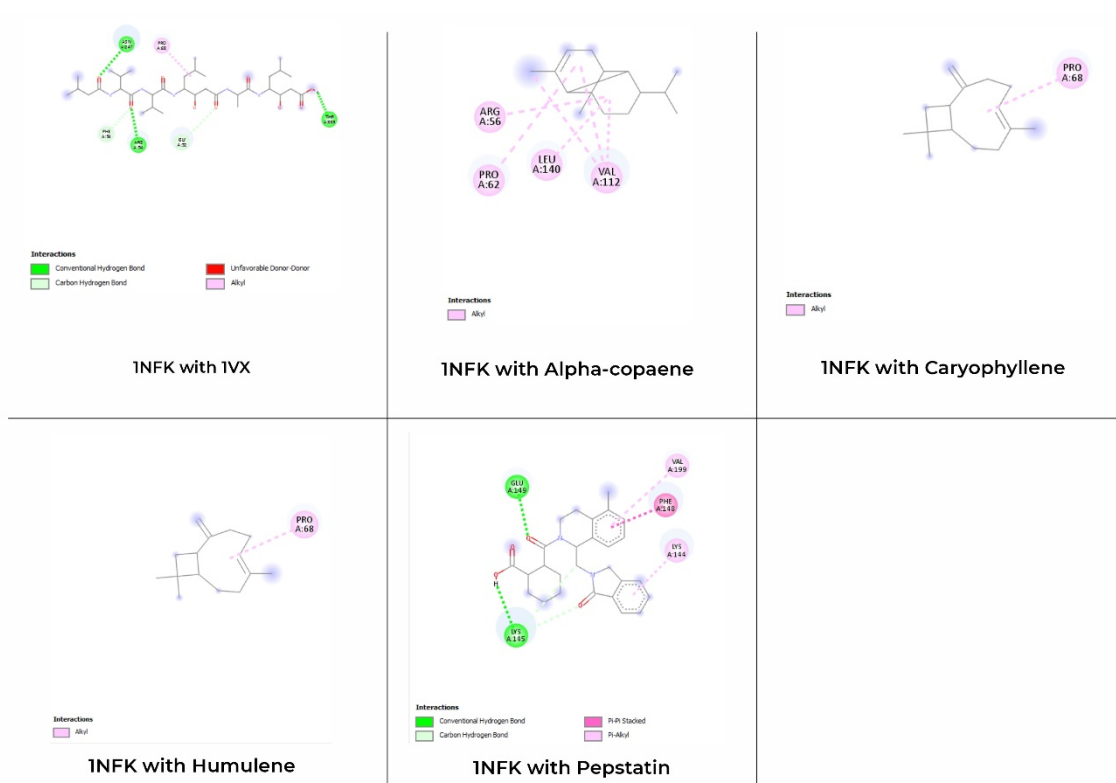
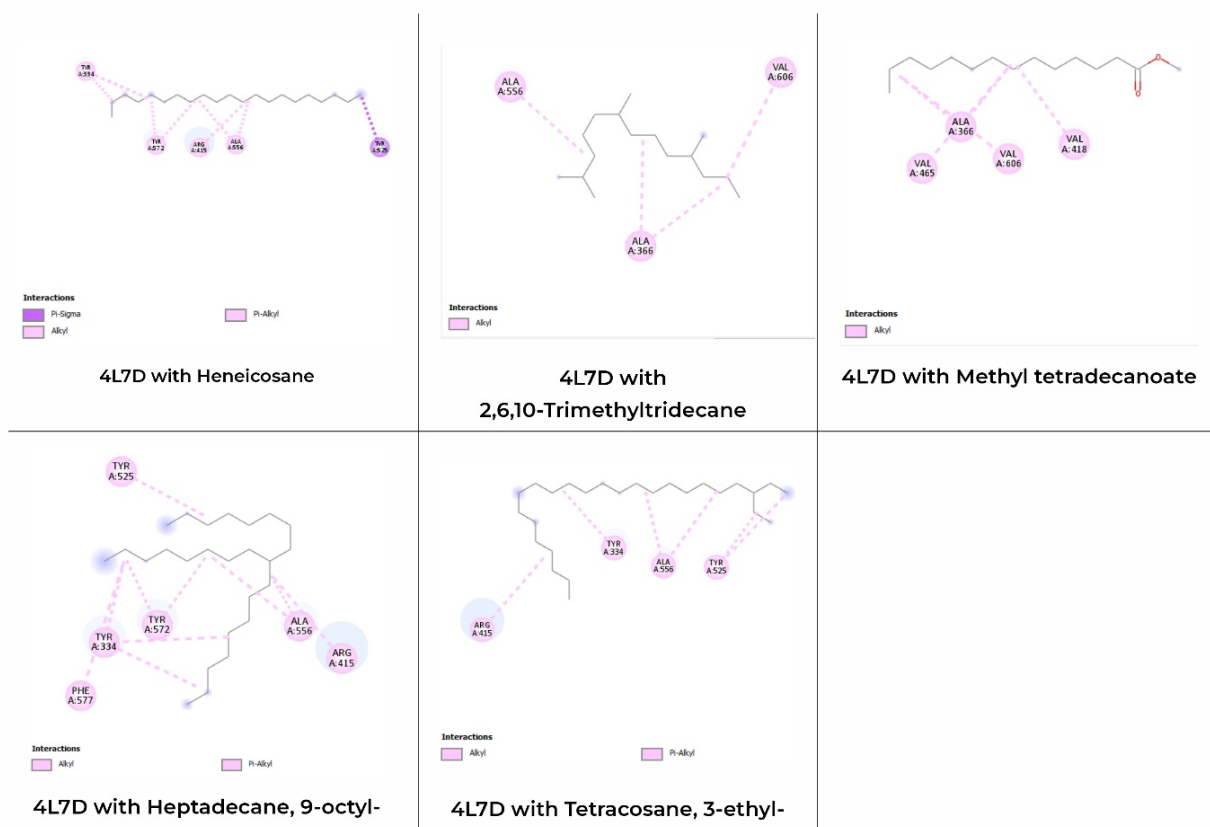
**Figure 7:** 1NFK with GBLE Ligands and IVX and Pepstatin**Figure 8:** 4L7D with GBLE Ligands and IVX and Pepstatin

Table 12. Choice of Target Proteins

SN	Protein Targets	Pathway	Mechanistic Role in Gastrotoxicity	Top Ligands
1.	1NFK	Gastric inflammation	Reduces immune –mediated gastric mucosal injury and pain	Alpha-copaene and Caryophyllene strong on 1NFK, strong on 1PSO, and good on 4LFD
2.	IPSO	Gastric proteolytic injury	Reduces direct chemical damage	
3.	4L7D	Oxidative stress	Increases antioxidant defense and healing	

The attachment of multiple phenolic hydroxyl groups to a conjugated aromatic ring system in flavonoids enables hydrogen atom or electron donation to neutralise reactive oxygen species and free radicals, thereby conferring strong antioxidant activity and contributing to other pharmacological effects, such as cardioprotective, antimicrobial, anti-ulcer, and anti-inflammatory properties. In addition, flavonoids can chelate transition metals such as iron and copper, thereby reducing oxidative stress and enhancing their therapeutic significance in medicinal plants (Ferraz *et al.*, 2020). However, the aglycone and glycone moieties of glycosides are primarily responsible for their therapeutic activity. While the sugar component improves solubility, stability, and bioavailability, the aglycone typically mediates the primary pharmacological Effect. Glycosides undergo enzymatic hydrolysis *in vivo*, releasing the active aglycone, which exerts diverse therapeutic effects depending on its chemical structure (Tang *et al.*, 2025; Xie *et al.*, 2022). Numerous plant-derived flavonoids and glycosides exhibit anti-ulcer and gastroprotective properties, including quercetin and rutin from *Allium cepa* and *Fagopyrum esculentum*, as well as geniposide from *Gardenia jasminoides* and salidroside from *Rhodiola rosea* (Liu *et al.*, 2022; Feng *et al.*, 2022).

Saponins are plant-derived glycosides with triterpenoid or steroidal aglycones, whereas tannins are high-molecular-weight polyphenols with strong protein- and metal-binding capacities. Both are secondary metabolites involved in plant defence and exhibit diverse pharmacological activities, including hypolipidaemic, antioxidant, hypoglycaemic, antimicrobial, antihypertensive, antiasthmatic, cardioprotective, and anti-inflammatory effects (Shi *et al.*, 2022; Sharma *et al.*, 2023; Cosme *et al.*, 2025). These bioactivities are largely attributed to their structural properties and redox-modulating capabilities. Tannins' pharmacological activity is primarily linked to their polyphenolic structure, which enables free-radical scavenging, protection against oxidative damage, and protein precipitation, thereby forming a protective layer on the gastric mucosa (Cosme *et al.*, 2025). The amphiphilic structure of saponins, consisting of a lipophilic aglycone and hydrophilic sugar chains (Juang *et al.*, 2020), enables anti-inflammatory, membrane-stabilising and cytoprotective effects. These findings support the extract as a plausible therapeutic candidate for managing ulcers and gastrointestinal disorders.

Triterpenes, which form the aglycone backbone of many saponins, may further enhance the anti-ulcer activity of this extract by modulating inflammatory pathways and reinforcing mucosal defence systems (Baltina *et al.*, 2022; Shen *et al.*, 2025). Triterpenes are C₃₀ isoprenoid compounds produced via the mevalonate pathway and exhibit pharmacological activity due to their pentacyclic structures and functional groups such as carboxyl and hydroxyl moieties. These compounds inhibit the synthesis of inflammatory mediators and reactive oxygen species by modulating key cellular signalling pathways, including NF-κB and oxidative stress pathways (Khan *et al.*, 2023; Darshani *et al.*, 2022).

Evidence suggests that triterpenoid saponins significantly inhibit gastric lesions and improve mucosal integrity in experimental models (Baltina *et al.*, 2022). The presence of xanthoproteins (aromatic amino acid-containing compounds) in GBLE may also contribute to its overall protection, antioxidant capacity, and support for tissue repair. Xanthoproteins are derived from aromatic amino acids such as tyrosine, tryptophan, and phenylalanine, whose aromatic rings undergo nitration and donate electrons to neutralise reactive oxygen species, thereby conferring cytoprotective and antioxidant properties.

Earlier reports have identified triterpenes such as oleanolic acid from *Olea europaea*, lupeol from *Mangifera indica*, betulinic acid from *Betula pendula*, and astragaloside IV from *Astragalus membranaceus* (Darshani *et al.*, 2022; Khan *et al.*, 2023; Li *et al.*, 2025), as well as xanthoproteins such as pro-

tein-derived peptides from *Glycine max* and aromatic amino acid-rich proteins from *Oryza sativa* (Chen *et al.*, 2019), which exhibit anti-ulcer and gastroprotective effects by suppressing inflammatory mediators and oxidative stress associated with gastric injury, while enhancing prostaglandin synthesis and gastric mucus secretion.

Collectively, flavonoids and glycosides in medicinal plants may synergistically contribute to gastro-protective effects through antioxidant and anti-inflammatory activities, enhanced mucus production, and the formation of a protective mucosal barrier. This multimodal action is consistent with the established mechanisms of action of plant-derived anti-ulcer agents. It supports GBLE's potential as a promising agent for managing gastric ulcers and other gastrointestinal disorders.

To further assess the antioxidant activity of GBLE and guide dose selection and optimisation *in vivo* studies, *in vitro* antioxidant assays were conducted using hydrogen peroxide (H_2O_2), DPPH, Nitric oxide (NO) radical scavenging and ABTS⁺ inhibition methods. The results of DPPH scavenging activity of GBLE versus the Ascorbic acid (Figure 1) and ABTS radical scavenging activity versus the Ascorbic acid (Figure 4) demonstrate that GBLE exhibits greater potency in scavenging DPPH and ABTS radicals than ascorbic acid, as the percentage radical scavenging at the selected concentrations was significantly higher than that of the ascorbic acid. This may be due to the electron- or hydrogen-donating capacity of bioactive constituents present in GBLE, such as tannins, flavonoids, triterpenes and saponins as identified in the qualitative and quantitative phytochemical screening (Table 1). This redox potential enables the GBLE to neutralise free radicals by stabilising their unpaired electrons. In addition, the half-maximal inhibitory concentration (IC_{50}) values further confirm the superior free radical-scavenging capacity of GBLE compared to ascorbic acid, with IC_{50} values of 3.43 (ABTS) and 2.36 (DPPH), relative to 83.05 and 31.50 for the reference standards, respectively. These findings indicate that GBLE exhibits higher efficacy and stronger antioxidant activity, requiring lower concentrations to achieve comparable effects, thereby suggesting its potential as a promising candidate for further drug discovery and development.

Consistent with the ABTS and DPPH results, the nitric oxide (NO) radical-scavenging and hydrogen peroxide (H_2O_2) inhibition activities of GBLE (Figures 2 and 3) were greater than those of the reference standard, ascorbic acid, at all tested concentrations. This indicates that GBLE is more potent at scavenging NO radicals, which are key mediators of inflammation and oxidative stress (Babatunde *et al.*, 2025), and at mitigating the cytotoxic effects of hydrogen peroxide than ascorbic acid. These findings suggest that GBLE possesses enhanced antioxidant and anti-inflammatory properties relative to ascorbic acid, which may be effective in attenuating peroxide-induced and nitrosative cellular damage. Overall, the pharmacological efficacy of GBLE demonstrated in these *in vitro* assays suggests its potential to promote wound healing, suppress oxidative stress and reduce inflammation in the gastrointestinal tract and other organs.

The results obtained agreed with the report by Hussen & Endalew (2023) on the *in vitro* antioxidant activity of GBLE, showing that it exhibits Nitric oxide, DPPH, ABTS radical-scavenging, and H_2O_2 -inhibitory activities.

Mounting evidence from *in vivo* studies has also shown that β -Caryophyllene inhibits the production of interleukin-17 (IL-17), nitric oxide, TNF- α , hydrogen peroxide, and interferon gamma (IFN- γ), thereby reducing the extent of macrophage infiltration in gastric injury (Francomano *et al.*, 2019). In addition, the lipid-lowering, anti-inflammatory, antioxidant, and antibacterial potential of Hexadecanoic acid methyl ester has been documented in another *in vivo* study (Elwekeel *et al.*, 2023). This evidence affirmed the therapeutic use of the plant extract for the management of malabsorption, gastrointestinal, and stomach disorders. Based on the phytoactive compounds identified by GC-MS analysis, this study further affirmed the antioxidant, free radical-scavenging activity, and potential of GBLE to suppress inflammatory responses. The finding also suggests that the extract may be further exploited for anti-ulcer activity via an *in vivo* study.

Table 12 shows the rationale for selecting the target proteins NF- κ B (p65 subunit) (INFK), PEPSIN A (IPSO), and KEAP-1 (4LFD) as the most relevant and mechanistically informative targets. The abbreviations (INFK, IPSO and 4LFD) represent the PDB IDs of the proteins.

NF- κ B was selected as a primary docking target for gastric ulcer because it is a central regulatory node in gastric inflammation (Saha *et al.*, 2022). Pepsin A was selected due to its direct involvement in gastric mucosal damage (Heda *et al.*, 2023), while Keap1 was selected as the representative oxidative stress-related target due to its regulatory role in the Nrf2 antioxidant signalling pathway (Verza *et al.*, 2025). In summary, INFK, IPSO and 4LFD were selected as molecular docking targets because they directly correspond to the key pathological processes modulated *in vivo*, namely inflammation, gastric proteolytic injury, and oxidative stress. This strategic selection ensures a focused, mechanistically consistent, and molecular-docking analysis that complements the experimental findings.

By combining docking results with physicochemical parameters, lipophilicity, water solubility, pharmacokinetics, and druglikeness/medicinal chemistry traits as seen in Tables 6-11, ligand selection was improved. Molecular docking of the 12 initially selected GBLE ligands against the three target proteins produced binding affinities that range from -7.0 to -3.4 kcal/mol, while Pepstatin and IVX standards exhibited comparatively stronger affinities (-10.6 to -5.5 kcal/mol), out of which 6 ligands with the best molecular docking scores were selected for further studies. The six ligands were selected for their high docking scores, which indicate predicted binding affinities for the target proteins. Through several interaction types, including Pi-Sigma bonds, conventional hydrogen bonds, and Pi-Alkyl bonds, the compounds interact with specific regions of the protein. Ligand selection was improved by combining docking results with physicochemical properties, pharmacokinetics, lipophilicity, water solubility, and drug-likeness/medicinal chemistry characteristics.

Variables such as molecular weight, topological polar surface area (TPSA), and lipophilicity (LogP) are predictive of oral absorption and membrane permeability; this multifactorial approach is well established in virtual screening pipelines (Çevik *et al.*, 2025).

Predictions of water solubility were deemed significant because even high-affinity ligands can have poor solubility, which can limit systemic exposure. In addition, predicted gastrointestinal absorption, bioavailability scores, and P-glycoprotein substrate status are examples of pharmacokinetic metrics that provide insight into the potential for systemic distribution (Murakami *et al.*, 2021).

Predictions regarding water solubility were considered significant, as poor solubility can limit systemic exposure even for high-affinity ligands (Basharat *et al.*, 2025). Pharmacokinetic parameters, including predicted gastrointestinal absorption, bioavailability scores, and P-glycoprotein substrate status, provide insight into the likelihood of effective systemic delivery (Nguyen *et al.*, 2021). *In silico* CYP450 inhibition profiling was employed to predict metabolic stability and potential drug-drug interactions (Dmitriev *et al.*, 2021). The cytochrome P450 enzyme system is crucial in drug metabolism and elimination in biological systems.

Compounds with undesired structural liabilities or low development potential were eliminated using medicinal chemistry filters (PAINS, Brenk alerts) and drug-likeness rules (Muegge, Lipinski, Ghose, Egan, Veber) (Mahmud *et al.*, 2022). Hence, from this combined evaluation, Caryophyllene and α -copaene emerged as the top candidates, each exhibiting favourable ADMET properties and consistently high binding affinities across all targets. Previous studies have reported some biological activities of both compounds, including antioxidant and analgesic properties (Machado *et al.*, 2020), gastroprotective activity (Sharma *et al.*, 2023), and anti-inflammatory properties (Francomano *et al.*, 2019; Rodríguez-Mejía *et al.*, 2022; Dinku *et al.*, 2022). The present study revealed that neither compound inhibits the majority of Cytochrome P450 enzymes, indicating significant potential for bioavailability and transformation upon oral administration without causing adverse responses, as supported by (Saravanakumar *et al.*, 2019).

From the *in silico* results, Caryophyllene and α -copaene are likely to exhibit better gastro-protective effects than the conventional standards pepstatin and IVX (standards) in gastric ulcer healing due to their reduced toxicity, strong anti-inflammatory properties, improved mucosal regeneration and protection, increased safety, multimodal activity and antioxidant properties (Bolat *et al.*, 2025). This positions them as promising candidates for anti-ulcer therapeutics.

CONCLUSION

The present study evaluated the antioxidant activity and possible gastroprotective potential of *Gossypium barbadense* leaf extract using an *in silico* approach. *In vitro* antioxidant assays demonstrated appreciable free radical-scavenging activity, indicating the potential of the extract to mitigate oxidative stress. GC–MS profiling identified several bioactive compounds, while molecular docking predicted favourable interactions between selected compounds and target proteins, suggesting their possible contribution to the observed bioactivities of the extract.

Docking Validation

The docking protocol was evaluated retrospectively using the pepsin–pepstatin complex (PDB ID: 1PSO) as the reference structure. Redocking of the crystallographic pepstatin using PyRx/AutoDock Vina yielded a binding affinity of -8.4 kcal/mol, consistent with the initial docking result. Comparable redocking validation could not be performed for the NF- κ B and Keap-1 targets because suitable co-crystallised reference ligands were not available in the structures used. Accordingly, the docking results for these targets are considered predictive and should be interpreted with appropriate caution.

AUTHORS' CONTRIBUTIONS

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None

CONFLICT OF INTEREST

The author confirms that the content of this article has no conflicts of interest.

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RESEARCH ARTICLE



Antidepressant Effects of White Ginger (*Mondia whitei*) and Turmeric (*Curcuma longa*) Composite Tea in Wistar Rat Model of Chronic Unpredictable Mild Stress

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Abstract: Monoaminergic dysfunction, cellular oxidative damage, hyperkinesis of hypothalamic–pituitary–adrenal (HPA) axis as well as neuroinflammation are relevant to chronic unpredictable mild stress model (CUMS)-induced depression. This study evaluates the possible depression-reducing effects, mechanistic pathways, sedative interaction and sensory acceptability of *Mondia whitei*–*Curcuma longa* composite tea in Chronic Unpredictable Mild Stress (CUMS)-exposed rats. Male Wistar rats were placed under chronic unpredictable mild stress conditions for a period of 3 weeks, followed by oral administration of functional tea blends (100:0, 75:25, 50:50, 25:75, 0:100; w/w) for 21 days. Forced swim, Tail suspension, and open field tests were used as behavioral analysis. Sedative modulation was assessed by measuring the diazepam-induced sleeping time. The neurochemicals (dopamine, monoamine oxidase, acetylcholinesterase), oxidative stress (GSH, GST, GPx, SOD, CAT), corticosterone and inflammatory cytokines (TNF- α , IL-6, IL-4 and IL-10) were assessed. CUMS Group showed increased immobility time, MAO and AChE activities, corticosterone and pro-inflammatory cytokines ($p < 0.05$), and decreasing dopamine & antioxidant enzyme activities. *Mondia whitei*–*Curcuma longa* treatment significantly inhibited depressive-like behaviors and corrected biochemical changes, with the 50:50 mixture showing the greatest antidepressant-like effect. Composite formulations also prolong the duration of diazepam-induced sleep and exhibit suitable sensory acceptability. The findings provide preliminary preclinical evidence supporting further investigation of the composite tea in experimental models of stress-related depressive-like behaviors.

Keywords: Depression, chronic stress, turmeric, white ginger; oxidative stress.

1. INTRODUCTION

Depression is a complex neurological condition characterized by constant sadness, anhedonia, cognitive impairment, and altered neurotransmission. According to a world health report, about 450 million individuals worldwide suffer from a mental or behavioral disease. Around the world, depression affects around 300 million people of all ages, and it frequently results in disability and suicide (Leis *et al.*, 2019). By 2020, depression is predicted to be the next principal source of disease burden worldwide, behind heart disease.

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Despite neuropsychiatric advances, the precise pathogenesis remains elusive (Milic *et al.*, 2025). Recent study implicates monoaminergic dysfunction (Hamon *et al.*, 2013), HPA axis impairment (Correia *et al.*, 2024), reduced brain-derived neurotrophic factor (BDNF) (Thornton, 2023), altered neuroplasticity (Fuchs *et al.*, 2004), increased pro-inflammation (Maeng & Hong, 2019), oxidative stress (Bhatt *et al.*, 2020), and gut microbiome imbalance (Peirce & Alviñ, 2019) in neuronal dysfunction and behavioral abnormalities associated with depression. While antidepressants have been clinically available for several decades, their efficacy remains suboptimal (Blackburn, 2019). Only about 33% of patients respond to the first antidepressant prescribed, which has significant side effects such as sexual dysfunction, agitation, or insomnia, among other side effects. Because HPA-axis dysregulation, oxidative stress, and neurological inflammation contribute to the pathogenesis of depression, alternative therapy targeting this specific pathway are required (Ruberto *et al.*, 2020; Elias *et al.*, 2022).

There is mounting evidence that food choices and dietary patterns may influence the prevention and treatment of depression (Ortega *et al.*, 2022). Consequently, foods with strong antidepressant effects are being investigated as potential supplements or alternatives to antidepressants. *Mondia whitei* (Hook.f.) Skeels belong to the subfamily Periplocoideae of the Apocynaceae (formerly the family Asclepiadaceae). An endemic species found in Africa, *M. whitei* has been beneficial to the people since ancient times. It has several significant applications, including nutritional, cultural, and medical. It is used in traditional medicines to treat diverse illnesses, according to Aremu *et al.*, (2011) ethanolic extract of *Mondia whitei* displayed substantial anti-inflammatory, antiepileptic, anticonvulsant and antidepressant-like effects both *in vivo* and *in vitro*, and moderate inhibitory effect on neuronal uptake of serotonin (Aremu *et al.*, 2011).

Similarly, *Curcuma longa* L, also called turmeric, is a perennial herb belonging to the Zingiberaceae (ginger) family and is widely grown throughout Asia, India, China, and other tropical nations. Curcumin's therapeutic qualities have attracted a lot of research interest (Lopresti, 2022). Due to its potent antioxidant and anti-inflammatory qualities, curcumin which is the major active ingredient in *Curcuma longa* has been demonstrated to enhance a number of medical conditions, such as cancer, nervous system disorders, metabolic syndrome, oral diseases, and others. Curcumin's neuroprotective properties are demonstrated by experimental studies via a variety of routes. By regulating JNK/NF- κ B/Akt signaling, Khan *et al.* demonstrated that dietary curcumin protects Lipopolysaccharide-induced neuronal inflammation and cognitive decline in rats (Khan *et al.*, 2019). In both Alzheimer's disease and depression models, treatment with curcumin supplementation has been demonstrated to restore levels of brain-derived neurotrophic factor (BDNF), regulate monoaminergic neurotransmission, and mitigate various pathological processes such as oxidative stress and neuroinflammation (Alam *et al.*, 2024). Furthermore, curcumin's strong antioxidant qualities regulate stress reactions, reducing anxiety-like behaviors while maintaining the benefits of acute stress on cognition (Haider *et al.*, 2015). This dual modulation implies that when taken as a dietary supplement, curcumin may maximize physiological stress responses.

Although both *Mondia whitei* and *Curcuma longa* individually possess neuroprotective, antioxidant, anti-inflammatory, and antidepressant-like activities, no previous study has explored the combined formulation as functional tea. Therefore, the current research explored the combined effect of this combination in a Chronic Unpredictable Mild Stress (CUMS)-induced depression rat model through modulation of oxidative stress, neuroinflammation and neurotransmitter disturbances.

2. METHODOLOGY

2.1. Sample Collection and Preparation

White ginger and turmeric rhizome were purchased at the Ibadan, Oyo State, Nigeria, local market. The Department of Plant Science and Biotechnology at the Federal University of Technology in Akure, Ondo State (FUTA), Nigeria, verified the authenticity of the plant samples (voucher number FUTA/0467 for *Curcuma longa* and FUTA/0468 for *Mondia whitei*). After washing and thinly slicing the plants, each sample was dried at room temperature until a constant weight was obtained and ground into a fine powder using a laboratory grinder. The resulting powder was weighed and blended or formulated in the weight to weight ratio (w/w): 100% *Mondia whitei*, 75% *Mondia whitei*:25% *Curcuma longa*, 50% *Mondia*

whitei:50% *Curcuma longa*, 25% *Mondia whitei*:75% *Curcuma longa*, and 100% *Curcuma longa*. and packaged in a tea bag (containing one gram of the mixture of samples). For tea preparation, one tea bag containing 1 g of the composite tea was infused in 20 mL of freshly boiled distilled water for 10 min and allowed to cool to room temperature. The resulting infusion had a concentration of 50 mg/mL. Fresh tea was prepared daily and administered orally by gavage at a dose of 50 mg/kg body weight once daily for 21 consecutive days. The volume administered to each animal was calculated according to its body weight to achieve the required dose. The dose was selected based on the human-equivalent dose calculated using the body surface area conversion factor of 6.2, as described by Reagan-Shaw *et al.* (2008).

2.2. Handling of Experimental Animals and Design

Adult male Wistar rats (180–220g), were obtained from the Animal Breeding Unit, Federal University of Technology Akure, Nigeria. The rats were kept in standard cages in a regulated environment (12-hour light/dark cycle, $22 \pm 2^\circ\text{C}$ temperature, $55 \pm 10\%$ humidity), were given normal rat feed and had free access to water *ad libitum* and were allowed to acclimatize for 7days prior to experimentation. All procedures complied with the ARRIVE guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. CUMS Procedure

Depressive-like phenotypes were induced in the rats ($n = 6$, each group) by chronic unpredictable mild stress daily for 21 days (Dai *et al.*,2010). Stressors were administered daily with variable inter-stressor intervals ranging from 3 to 8 hours. To prevent habituation and stressor adaptation, two distinct stressors were randomly selected and paired each day, ensuring that no individual stressor was repeated on 2–3 consecutive days. The animals utilized as controls were those that were allowed to roam freely in their cages without any stressors. The rats received treatment for 21 days. The stressor used are shown in the Table 1 below.

Table 1. Chronic Unpredictable Mild Stress (CUMS) protocol and stressors applied during the experimental period.

Stressors	Duration	Days
Cold water immersion	1 minutes	1, 4, 7, 10, 13, 16, 19
Deprivation of Food and Water	12 hours intervals	2, 4, 8, 10, 14, 16, 19
Cage Tilting	12 hours intervals	3, 5, 9, 12, 15, 18, 21
Tail pinch	1 minutes	1, 6, 8, 11, 15, 18, 21
Cage Shaking	10 minutes	2, 5, 7, 11, 13, 17, 20
Physical restraint	1 hour	3, 6, 9, 12, 14, 17, 20

2.3.1. Experimental Design

After acclimatization, the animals were randomly allocated to groups by an independent colleague using body-weight-stratified block randomization to minimize baseline weight differences. Daily treatments and CUMS stressors were administered by the primary investigator. A total of 48 rats were randomly assigned to eight groups, with six rats in each group, as shown below:

Group I: (Normal Control): Received 1 mL of distilled water.

Group II: (Stressed group): Received 1 mL of distilled water.

Group III: Stressed +Standard Drug (10 mg/kg fluoxetine)

Group IV: Stressed + *Mondia whitei* tea (100: 0)

Group V: Stressed + *Mondia whitei* and *Curcuma longa* tea (ratio 75:25)

Group VI: Stressed + *Mondia whitei* and *Curcuma longa* tea (ratio 50:50)

Group VII: Stressed + *Mondia whitei* and *Curcuma longa* tea (ratio 25:75)

Group VIII: Stressed + *Curcuma longa* tea ratio (0:100)

At the end of the treatment all of the animals were sacrificed by cervical dislocation after being subjected to anesthetics and their brains removed rapidly washed in ice cold 1.15% (v/v) potassium chloride solution, weighed after being blotted with filter paper and homogenized using a Teflon homogenizer in 10% (w/v) phosphate-buffered saline PBS (pH 7.4), the resulting homogenate was centrifuged at 10,000 x g for 25 minutes at 4°C, and the supernatant was utilized for biochemical studies. Before the brain was removed, blood was obtained via cardiac puncture into the tube for further biochemical analysis. The research involving the use of animal (Wistar rats) were conducted according to the approved ethical standard and guidelines approved by Osun State Health Research Ethics Committee (OSHREC), Ministry of Health, Osogbo, Osun State, Nigeria (Approval No.: OSHREC/PRS/2024/842/01/244; Date of approval: 31 January 2024) (Figure 1).

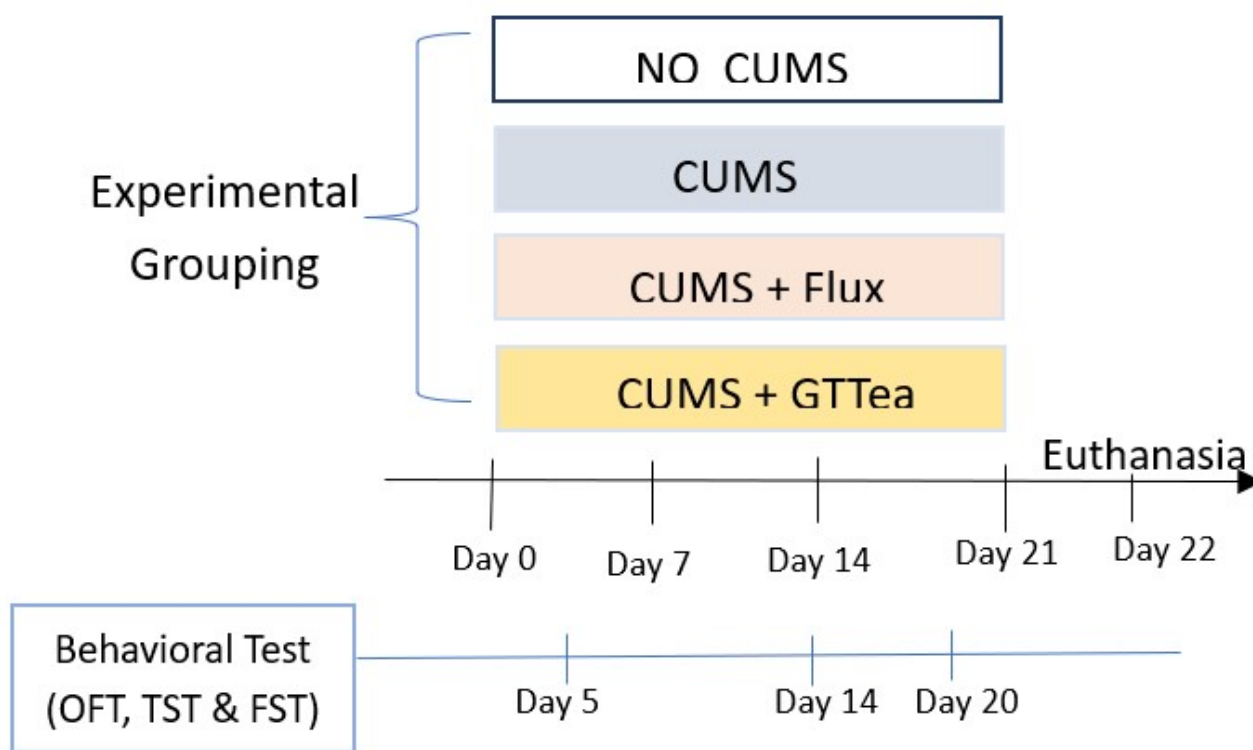


Figure 1: Experimental procedure for CUMS induction, treatments and behavioral tests.

Abbreviations: CUMS: chronic unpredictable mold stress; GTTea: White Ginger-turmeric tea; OFT-Open field test; TST: Tail suspension test; FST: Forced swim test; Flux: Fluoxetine.

2.4. Preliminary Evidence of an Effect on Diazepam-induced Sleeping Time

Two sets of three animals each were used for each sample in the diazepam-induced sleeping time test, with a third group serving as a control. While the other group received the proper tea, the control group was administered diazepam. The tea was given orally at the appropriate dosages (50mg/ml/kg). Both groups received an intraperitoneal dose of diazepam (1.5 mg/kg body weight) 30 min after receiving corresponding extract. They were monitored for the loss of their righting reflex, or normal posture, which was thought to be an indication that sleep had begun. The test samples' hypnotic activity was measured using the duration between the loss and the subsequent regaining of the righting reflex (Akingbesote & Salawu, 2023).

2.4.1. Experimental Design

Group	Drug/Tea	Purpose
NC	Diazepam	Control
SC	SC + Diazepam	-ve control
SC + Fluoxetine	SC + Diazepam + Fluoxetine	+ve control
100G	Diazepam + SC + 100T	Test sample
75G:25T	Diazepam + SC + 75G:25T	Test sample
50G:50T	Diazepam + 50G:50T	Test sample
25G:75T	Diazepam + 25G:75T	Test sample
100T	Diazepam + 100T	Test sample

2.5. Measurement of Antistress, Anxiolytic and Antidepressant Effect of the Tea

This study used the Open Field Test (OFT) to evaluate anxiety and the Forced Swim Test (FST) and Tail Suspension Test (TST) to study depression. These models have been used extensively in evaluating anxiety reducing and depression alleviating properties of the tea.

2.5.1. Behavioural Tests

Behavioral testing was conducted on Day 5, 14, 20 early in the morning. To eliminate test-induced stress carryover and fatigue, assays were performed in sequence from least to most inherently stressful (OFT, TST and FST)

2.5.2. Forced Swim Test

Rat behavior was noted and examined. OFT for neurobehavioral assessments. For this evaluation, a modified version of Porsolt methodology (Porsolt *et al.*, 1997) was used.

2.5.3. Tail Suspension Test

For this TST evaluation, a modified Steru (Steru *et al.*, 1985) approach was used.

2.5.4. Open-Field Test

Abdelsalam and Safar's modified method was used for this test (Abdelsalam & Safar, 2015).

2.5.5. Sensory Evaluation of the Functional Tea

Thirty judges, both male and female, from the Biochemistry Department at FUTA, Ondo State, comprised the semi-trained panel. The panelists were given tea cups and hot water to infuse the medicinal tea samples that were wrapped in their own tea bags. The panelists were instructed to use a 9-point Hedonic scale, with nine representing extreme like and 1 representing extreme dislike, to assess several samples for appearance, taste, aroma, aftertaste, and overall acceptability after learning the descriptive terms of the sensory scales. Drinkable water was offered for mouthwash in between each evaluation, and coded samples were presented at random (Umeh *et al.*, 2025).

2.6. Biochemical Analysis

According to Perry *et al.* (2000) (Perry *et al.*, 2000), the substrate acetylthiocholine iodide was used to measure AChE activity in brain tissue. Using adrenaline (0.06 mg/ml) as a substrate, the activity of superoxide dismutase (SOD) of brain tissue was measured using the method outlined by Alia *et al.* (2003) (Alia *et al.* 2003). According to Sinha (1972) (Sinha 1972), catalase activity was also examined in the experimental rats' brains. A Coomassie blue-based method was used to determine the protein content (Bradford, 1976). While glutathione peroxidase (GSH-Px) activity in brain tissue was measured using the procedure

outlined by Paglia and Valentine (1967) (Paglia & Valentez, 1967), glutathione-S-transferase (GST) activity and reduced glutathione (GSH) levels in brain tissue were measured using the technique employed by Akinyemi et al. (2014) (Akinyemi *et al.* 2014). The EIA kit (Cayman Chemical Company, US) was used to estimate the serum corticosterone level. A previously described approach was used to measure the tea's impact on MAO (EC 1.4.3.4) activity in rats' brains (Turski *et al.*, 1972).

2.7. Inflammatory Cytokine

Serum Tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), interleukin-4 (IL-4), and interleukin-10 (IL-10) levels were evaluated by means of ELISA with Quantikine Immunoassay kits (R&D Systems, Minneapolis, MN), following the manufacturer's protocol and the method described by Paim et al. (2011) (Paim *et al.*, 2011).

2.8. Statistical Analysis

Data were analyzed using the GraphPad Prism 8.02 (GraphPad Software Inc., CA, USA). The data are shown as the mean $\pm$ standard error of the mean (mean $\pm$ SEM). Analysis for significance level was done by calculating the Mean values using one-way ANOVA. The significance was accepted at $p < .05$ after Tukey's post hoc test. The estimated number of animals required is based on a power analysis program from InStat. The experiment required 6 animals by power analysis based on the following: variance of up to 25% (standard deviation), significance at a minimum difference between means of 50%, 95% power, and 5% type-I error rate

3. RESULTS

Table 2 displays the 30 panelists' sensory evaluations of the various tea blends. Some samples showed no notable difference ($p > 0.05$) in appearance, whereas others showed a significant difference ($p < 0.05$). The control sample had the highest sensory appearance, whereas sample 100T (100% turmeric) had the lowest, with the appearance result ranging from 6.32 to 8.96 with 75G:25T showing the highest appearance when compared to the other *Mondia whitei*–*Curcuma longa* composite tea samples. Significant differences in taste were noted. Panelists gave samples 75G:25T (75% white ginger:25% turmeric) the lowest taste rating (6.33), while the COM received the highest grade (8.96). Although the panelists found all of the samples to have acceptable tastes, there were significant differences ($p < 0.05$) in each sample. The panelists' perceptions of the tea based on Aroma also showed significant variations ($p < 0.05$). Aroma received a mean score between 5.59 and 8.33. The results indicated that sample 100G (100% white ginger) had the lowest aroma score, whereas the control sample (commercial tea) had the greatest aroma score. It was found that the aroma improved with the amount of ginger. The respondents preferred the control sample's scent above the others. There was a significant difference ($p < 0.05$) in aftertaste between the composite tea and the commercial tea. With the commercial tea showing the highest after taste followed by 75G:25T and 50G:50T (50% white ginger: 50% turmeric), with 25G:75T (25% white ginger:75% turmeric) and 100T (100% turmeric) showing the least after taste. All of the teas were found to be generally acceptable, with no discernible differences, according to the overall acceptability. With the greatest overall acceptability scores—the others ranged from 7.75 to 6.97—the commercial (COM) tea samples were, nevertheless, the most favored. The preference for these samples may have resulted from the higher ginger content, which was deemed acceptable by the majority of panelists. Additionally, in terms of general acceptability, 100T was the least favored product.

Preliminary evidence of the effect of the composite tea on diazepam-induced sleeping time was assessed. Administration of diazepam produced marked differences in sleep parameters across the experimental groups (Table 3). The stress-control group showed a longer latency (10.55 min) to loss of the righting reflex and a shorter sleep duration (30.83 min) than the normal control group, which showed a latency of 6.20 min and a sleep duration of 70.03 min. Treatment with Fluoxetine and the composite tea restored more balanced sleep patterns, increasing duration while maintaining moderate latency. Interestingly, there was an increase in sleep duration and decrease in sleep latency with increased turmeric.

The forced swim test in Figure 2a demonstrated the behavioral impact of ginger and turmeric tea; there was a significant difference in immobility time ($p < 0.0001$) among the stressed, NC group, and the treated groups. However, the SC groups showed highest immobility time indicating depression based on the length of time spent immobile in the water. Interestingly, these were reduced or reversed in groups treated with fluoxetine and the composite tea. However, the composite 50G:50T tea exhibits the shortest immobility time, indicating reverse in depression or behavioral despair upon addition of turmeric and white ginger tea treatment.

Additionally, the tail suspension test was displayed in Figure 2b. The results indicate a substantial change ($p < 0.0001$) in the stressed group when compared to the normal control group signifying depression, this was reversed with the administration of fluoxetine and composite tea. 50G:50T tea composite exhibited the shortest immobility period when compared to other treated groups.

Rats' depression-like activity during the OFT was evaluated by counting the number of entrances into the middle zone (Figure 2c) and the amount of time they spent there (Figure 2d). The number of entries in the center zone and the amount of time spent there indicated a higher level of despair in the second control group (stress only). For the treated groups, there was a considerable increase in both the quantity of entries and the amount of time spent ($p < 0.0001$) in the central zone.

Table 2. Sensory Analysis of the Different Tea Blend

Sample	Appearance	Taste	Aroma	After taste	Overall Acceptability
100G	6.44 ^c ± 1.25	5.85 ^d ± 1.32	7.20 ^b ± 1.21	7.00 ^a ± 1.60	7.75 ^b ± 1.25
75G:25T	7.46 ^b ± 1.63	6.33 ^c ± 2.10	7.00 ^b ± 1.88	7.20 ^a ± 1.88	7.84 ^b ± 1.43
50G:50T	7.25 ^b ± 1.84	6.43 ^c ± 1.63	6.67 ^c ± 1.19	7.10 ^a ± 1.98	7.55 ^b ± 1.71
25G:75T	7.15 ^b ± 1.46	7.59 ^b ± 1.76	6.35 ^c ± 0.59	6.88 ^b ± 1.7	7.01 ^b ± 1.71
100T	6.32 ^c ± 1.16	7.69 ^b ± 1.80	5.59 ^d ± 0.89	6.30 ^b ± 0.94	6.97 ^c ± 1.25
Com	8.96 ^a ± 1.24	8.63 ^a ± 1.25	8.33 ^a ± 1.03	7.90 ^a ± 0.99	8.45 ^a ± 1.54

Note: Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ significantly ($p < 0.05$). Means sharing the same superscript letter are not significantly different ($p \geq 0.05$).

Table 3. Sedative effect of the composite tea

GROUP	DOSE OF DIAZEPAM (mg/kg)	SLEEP LATENCY (MIN)	SLEEP DURATION (MIN)
NC	1.5	6.20 ^b ± 0.33	70.03 ^c ± 0.21
SC	1.5	10.55 ^a ± 0.25	30.83 ^c ± 0.95
SC + Fluoxetine	1.5	7.71 ^b ± 1.08	62.27 ^d ± 0.71
100G	1.5	7.92 ^b ± 1.01	71.73 ^c ± 0.34
75G:25T	1.5	6.83 ^c ± 1.11	77.33 ^c ± 1.04
50G:50T	1.5	6.06 ^d ± 0.47	83.54 ^b ± 1.34
25G:75T	1.5	5.74 ^c ± 0.88	88.47 ^b ± 0.12
100T	1.5	5.31 ^f ± 0.74	93.24 ^a ± 0.16

Note: Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ significantly ($p < 0.05$). Means sharing the same superscript letter are not significantly different ($p \geq 0.05$).

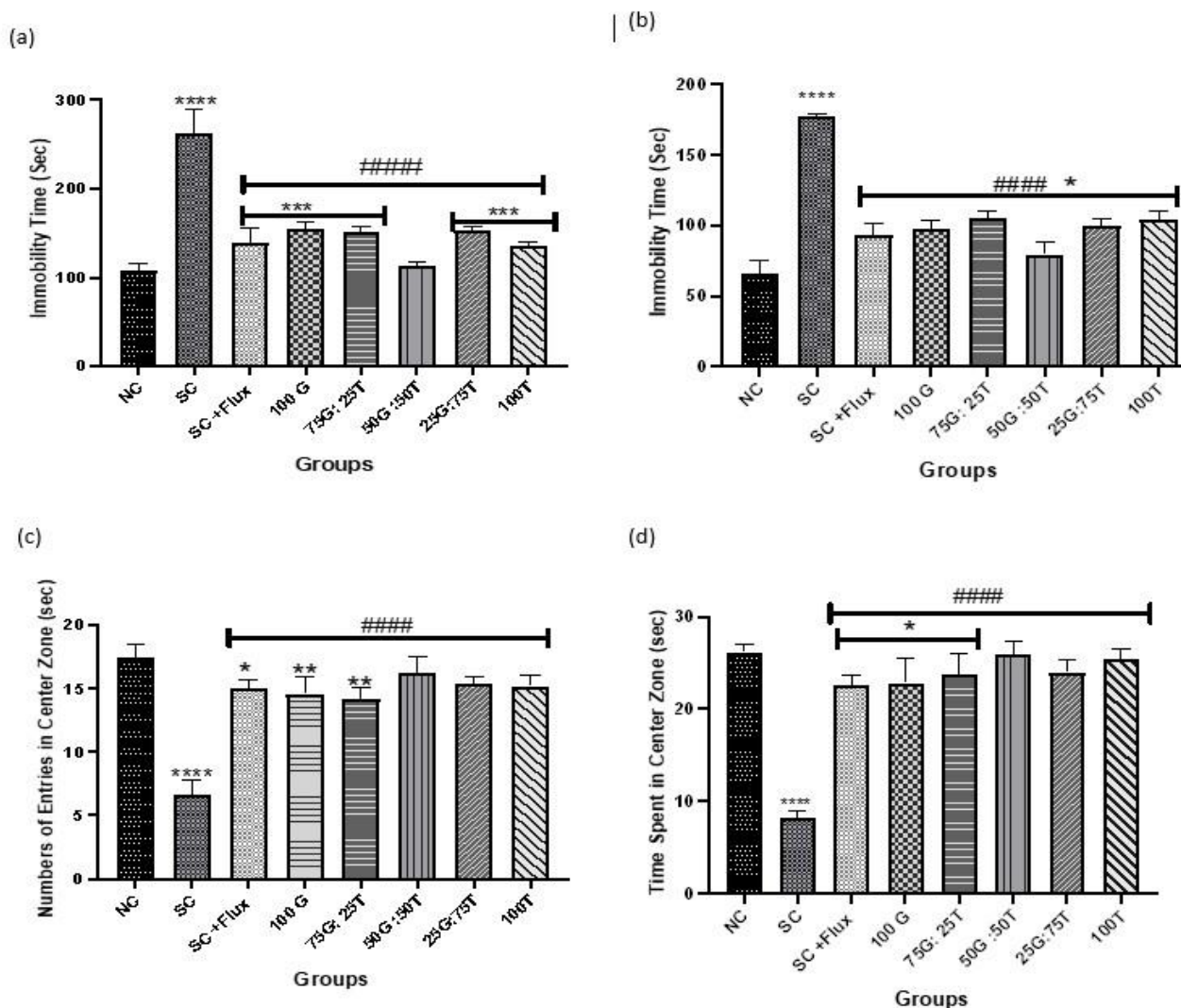


Figure 2: Effects of CUMS, fluoxetine and white ginger-turmeric tea administration on behavioral patterns. (a)FST (b)TST (c)OFT (No. of times) (d) OFT (No. of entries). Bars represent mean $\pm$ SEM (n = 6). Values are statistically different at * $p < .05$; ** $p < .01$; *** $p < .001$; **** $p < .0001$ Vs NC, # $p < .05$; ## $p < .01$; ### $p < .001$; #### $p < .0001$ Vs. SC.

Abbreviations: NC: normal control; Sc: stressed; SC + FLUX: stress + fluoxetine; G: white ginger; T: turmeric

Figure 3a displays the effect of fluoxetine and the composite tea treatment on dopamine levels of the rats. The results show that Dopamine levels significantly differed among experimental groups ($p < 0.0001$), a notable distinction between the stressed rats and the normal control was noted, indicating a condition of illness or depression. However, upon treatment with fluoxetine.

Furthermore, a significant difference was noted between the NC, the stressed group and treated groups ($p < 0.0001$). The activity of MAO was increased in the SC group compared to NC indicating a depressed state (Figure 3b). Low MAO activity was observed in the treated group compared to SC untreated group. 50G:50T, on the other hand, had the least activity of MAO.

The AChE was accurately depicted in Figure 3c. High levels of AChE activity, a sign of depression, were seen in the SC group. Comparing the SC group to NC rats revealed a substantial difference in AChE activity ($p < 0.0001$). Upon treatment with composite tea and fluoxetine, the activity was reduced. When all the treated groups were compared, 50G:50T showed the least amount of activity; there was no discernible difference between the NC group and the treated rat.

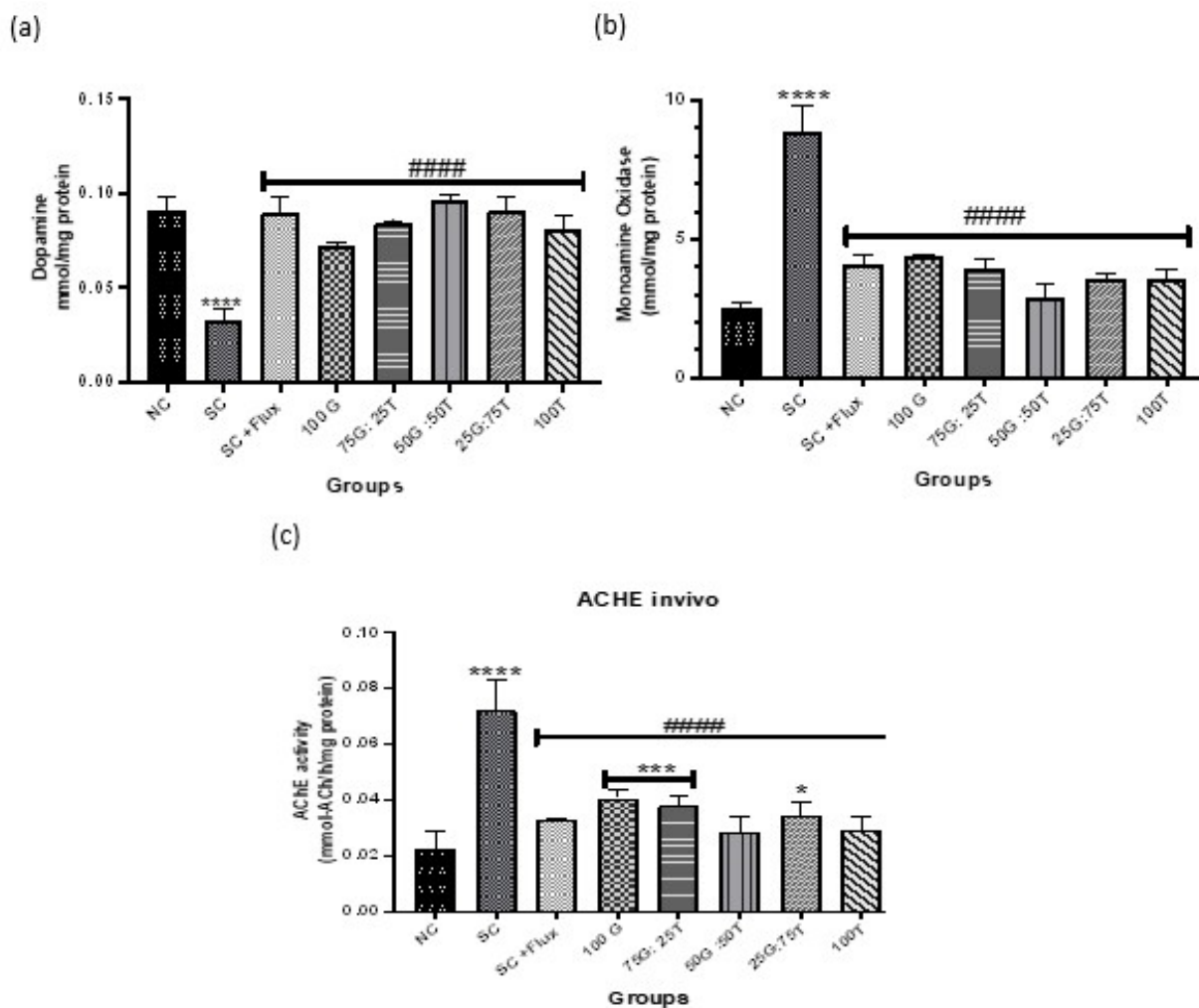


Figure 3: Effects of CUMS, fluoxetine and white ginger-turmeric tea administration on Dopamine, Monoamine Oxidase and AChE. (a) Dopamine (b) Monoamine Oxidase (c) AChE. Bars represent mean $\pm$ SEM (n = 6). Values are statistically different at * $p < .05$; ** $p < .01$; *** $p < .001$; **** $p < .0001$ Vs NC, # $p < .05$; ## $p < .01$; ### $p < .001$; #### $p < .0001$ Vs SC.

Abbreviations: NC: normal control; Sc: stressed; SC + FLUX: stress + fluoxetine; G: white ginger; T: turmeric

Figures 4a (GSH), 4b (GST), and 4c (GPX) display the antioxidant parameters in the brains of the rats. While the untreated group exhibited a substantial decrease ($p < 0.0001$) in the activity of reduced glutathione, white ginger and turmeric composite tea demonstrated an increase in antioxidant capacity of stress-induced depression in rats when compared to the rats that were not treated, indicating an improvement in GSH (Figure 4a). The SC group's glutathione-S-transferase activity was shown to be significantly decreased ($p < 0.0001$) (Figure 4b). This was reversed, by administering a white ginger and turmeric tea blend. Additionally, 50G:50T showed the highest levels of GSH and GST, while the other treatment groups' levels were similar to the normal control level.

Glutathione peroxidase activity significantly differed among treatment groups ($p < 0.0001$). Untreated depressed rats (SC) had a much-reduced level of GPx, as shown in Figure 4c. However, after receiving the tea, the activity of GPx increased to a level that was both greater and equivalent to the normal control. The greatest GPx activity was shown by 50G:50T and 75G:25T revealing the antioxidant potential of the tea mix.

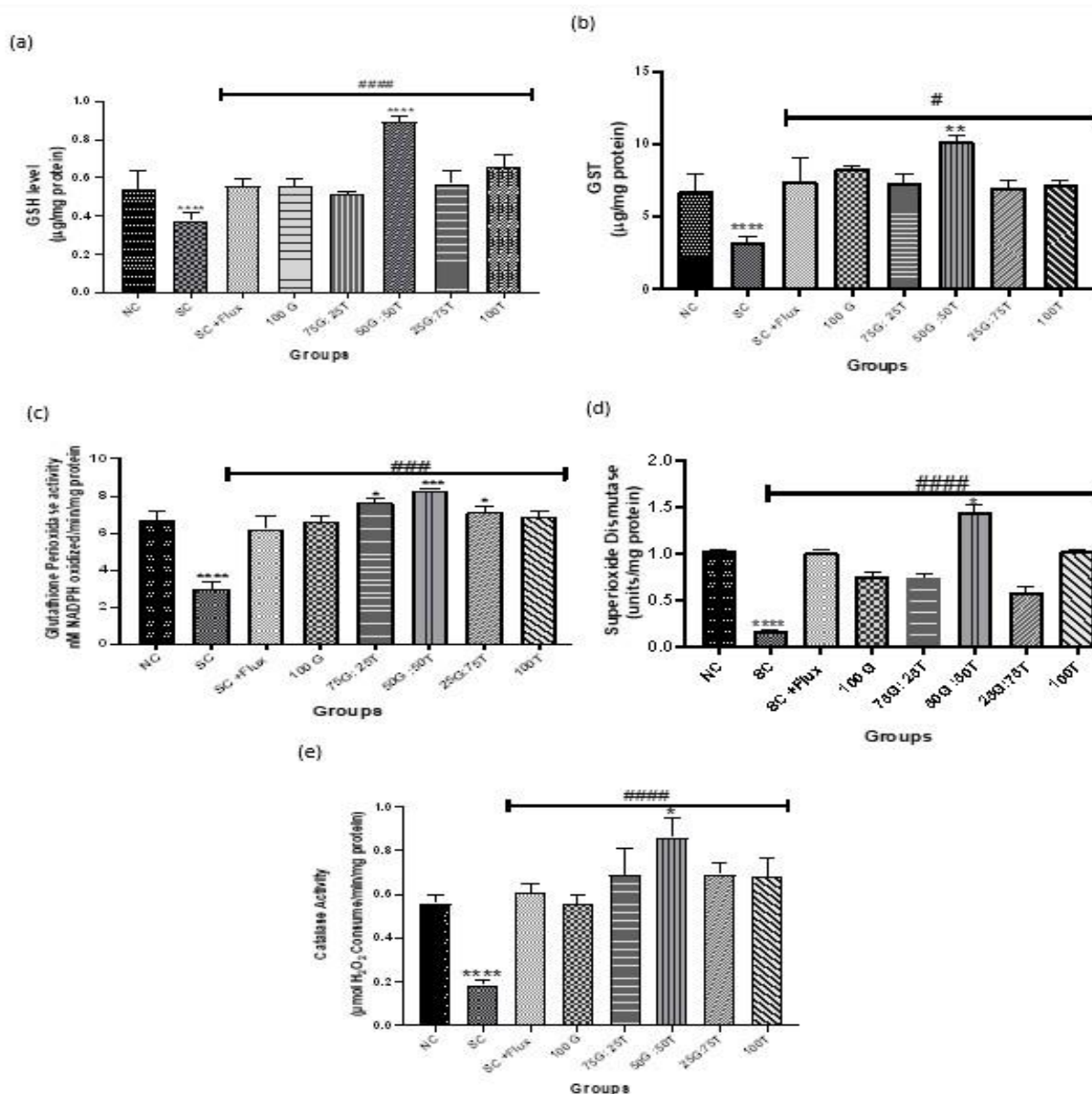


Figure 4: Effects of CUMS, fluoxetine and white ginger-turmeric tea administration on antioxidant status. (a): GSH, (b): GST, (c): GPx, (d): SOD (e): CAT. Bars represent mean $\pm$ SEM (n = 6). Values are statistically different at * $p < .05$; ** $p < .01$; *** $p < .001$; **** $p < .0001$ Vs NC, # $p < .05$; ## $p < .01$; ### $p < .001$; #### $p < .0001$ Vs. SC.

Abbreviations NC: normal control; Sc: stressed; SC + FLUX: stress + fluoxetine; G: ginger; T: turmeric

When the SC group of SOD and CAT activity were compared to the NC group, significant differences were observed ($p < 0.0001$) as seen in Figures 4d and 4e, respectively. Nevertheless, the enzyme activities were increased by treatments using the fluoxetine and the tea blend. Amazingly, among the rats that received treatment, the group that received 50G:50T exhibited the highest levels of SOD and CAT activity in both normal and depressed rats.

The rats' corticosterone levels are shown in Figure 5. The findings demonstrated that, in comparison to the NC group, stress significantly raises ($p < 0.001$) corticosterone in the SC group. Stress-induced corticosterone increase is attenuated ($p < 0.0001$) even near the normal control level in the SC + FLUX group treated with fluoxetine and the tea blend. When compared to the SC + FLUX group and normal control, the 50G:50T and 25G:75T tea formulations exhibited the lowest level showing that they have the highest effect on corticosterone.

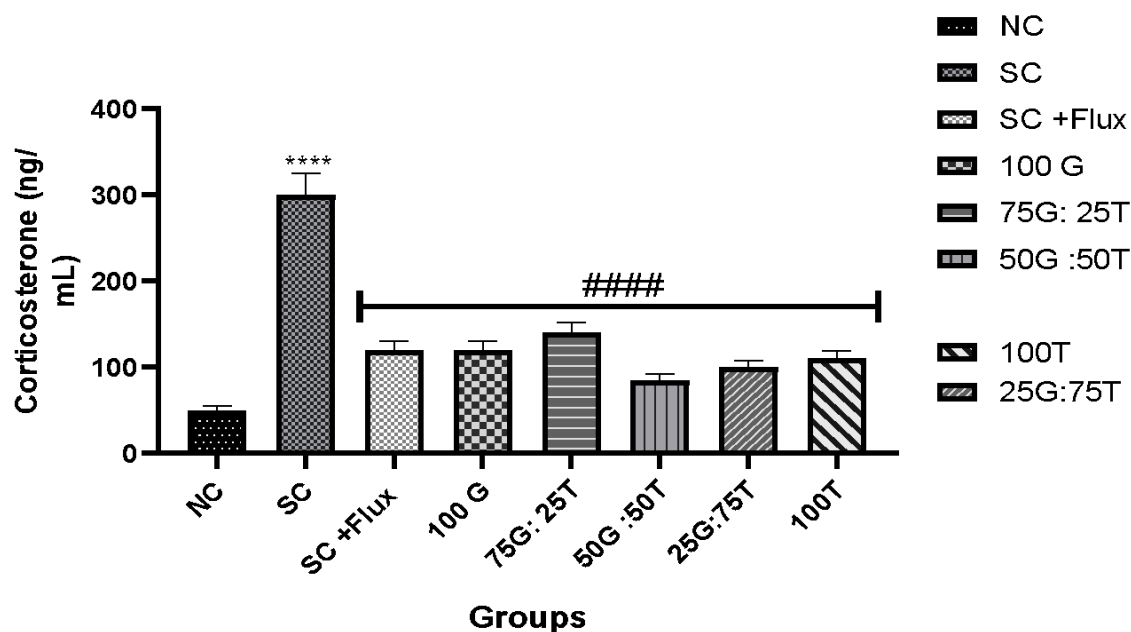


Figure 5: Effects of CUMS, fluoxetine and white ginger-turmeric tea administration on serum corticosterone. Bars represent mean $\pm$ SEM (n = 6). Values are statistically different at * $p < .05$ Vs NC, # $p < .05$ Vs. SC. Values are statistically different at * $p < .05$; ** $p < .01$; *** $p < .001$; **** $p < .0001$ Vs NC, # $p < .05$; ## $p < .01$; ### $p < .001$; #### $p < .0001$ Vs. SC.

Abbreviations NC: normal control; Sc: stressed; SC + FLUX: stress + fluoxetine; G: white ginger; T: turmeric

The effects of different tea compositions on pro-inflammatory cytokines (TNF- α and IL-6) were shown in Figures 6a and 6b significantly differed ($p < 0.0001$). Stress-induced untreated rats showed a considerable rise in these cytokines. When the rats were given tea samples, a reduction in these cytokines was observed. The 50G:50T group had the lowest levels of pro-inflammatory cytokines among the treatment groups. Similarly, the tea blend's anti-inflammatory (IL-4 and IL-10) effect was evaluated as seen in Figure 6c and 6d, in which a significant difference ($p < 0.0001$) in IL-4 and IL-10 was observed. There was a significant decrease in the stressed untreated group (SC) but a significant rise after treatment with the composite tea and the commercial drug.

4. DISCUSSION

Chronic low mood, disinterest, and reduced activity are hallmarks of depression, a prevalent mental illness that can result into suicidal thoughts and actions. Because the CUMS model can mimic the pathophysiological changes observed in human depression, it is therefore often used in depression studies (Zheng *et al.*, 2010). Dietary strategies have been encouraged in health care for the treatment and management of depression. Appropriate application or incorporation of functional food can help reduce the risk of developing depression or mitigating the severity (Ortega *et al.*, 2022). However, functional food showed no or low advised side effects when compared to the conventional drugs and these foods are generally accepted making it a good approach. Previous research findings revealed that both *Mondia whitei* and *Curcuma longa* are rich in diverse phytochemicals. Both plants are rich sources of flavonoids, phenolics, alkaloids, terpenoids, saponins and other secondary metabolites (Ikuomola *et al.*, 2024). Flavonoids and polyphenols have been reported to have antidepressant-like effects by modulating monoaminergic neurotransmitters, reduction of oxidative stress, and neuroinflammation (Figueiredo *et al.*, 2025). Studies reveal that Curcuma regulates serotonin, dopamine and norepinephrine neurotransmission while suppressing inflammatory pathways in depression (Ramaholimihaso *et al.*, 2020). Alkaloids in *Mondia* have been reported to have modulated monoaminergic systems (Edah *et al.*, 2020). In addition, the antioxidant and anti-inflammatory properties of these phytochemicals help in protecting the neurons, reducing stress-induced behavioral changes caused by oxidative stress and improving synaptic transmission (Tkaczenko *et*

al., 2025). Therefore, the combined effect of the phytochemicals present in both *Mondia whitei* and *Curcuma longa* contributes to the antidepressant-like effect observed in this study.

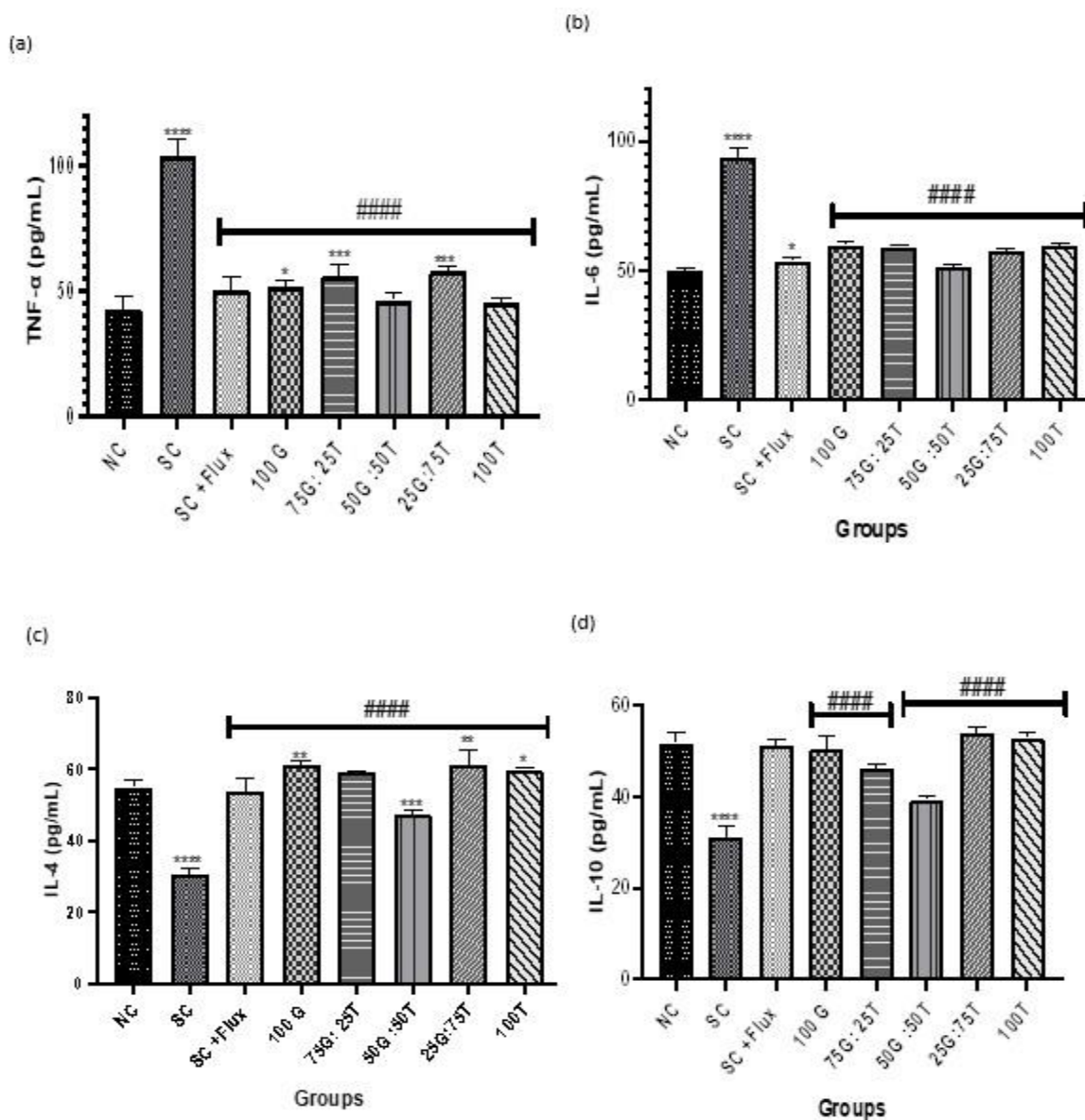


Figure 6: Effects of CUMS fluoxetine and white ginger-turmeric tea administration on inflammatory markers. (a): TNF- α , (b):IL-6, (c): IL-4, (d): IL-10. Bars represent mean $\pm$ SEM (n = 6). Values are statistically different at * $p < .05$; ** $p < .01$; *** $p < .001$; **** $p < .0001$ Vs NC, # $p < .05$; ## $p < .01$; ### $p < .001$; #### $p < .0001$ Vs. SC.

Abbreviations: NC: normal control; Sc: stressed; SC + FLUX: stress + fluoxetine; G: white ginger; T: turmeric

As seen in Figure 2 composite tea treatment showed antidepressant-like effect on the CUMS rats in the OFT, FST and TST models. Treatment with the fluoxetine and the composite tea showed increase in both the number of center zone admissions and the duration of time spent in the center zone. This improvement suggests a reduction in stress-induced depressive-like behavior and a return to regular exploratory activity in OFT. Two verified models for evaluating potential antidepressant drugs and depressive-like in rodents are the TST and FST. The immobility time depicts the anti-depressant-like activity. In this research a decrease in immobility time was observed in both FST and TST as compared to the untreated group showing the anti-depressant-like effect of the tea blend.

The sensory profile of the formulations reveals that the amounts of turmeric (T) and white ginger (G) significantly affected consumer perception as compared to the commercial sample. Moderate blending of white ginger and turmeric (50G:50T) appeared to enhance overall sensory balance, suggesting that their flavor and bioactive ingredients complement one another. In food manufacturing, appearance is crucial since it greatly influences whether or not food products are accepted by consumers (Meijer *et al.*, 2021). Given that visual attributes significantly influence consumer acceptability and expectations, 100T (100% turmeric) had the lowest appearances which may be due to the more intense yellow coloration of turmeric. However, color blending likely improved appearance with 75G:25T showing the highest score (Lawless & Heymann, 2010). In sensory evaluation, taste is also seen to be quite significant. One crucial characteristic of a food product is taste, which is the flavor sensation that is experienced in the mouth and throat when coming into contact with a material. In this study, 75G:25T had the lowest taste rating which may be due to the higher percentage of white ginger present. However, the unique warm, slightly spicy flavor of white ginger balanced the sharpness of white ginger, resulting in improved taste as more turmeric was added. Given that flavor is a major factor in food selection (Drewnowski, 1999), this probably helped to make foods more palatable at moderate substitution levels. However, higher turmeric concentrations reduced the aroma's acceptability, perhaps as a result of the dominant volatile chemicals masking the white ginger's crisper notes (Chen *et al.*, 2020). In organoleptic estimation, overall acceptability is a significant metric since it affects panelists' acceptance and selection of the various samples and is essential to decision-making. Because higher concentrations of turmeric contain more polyphenolic chemicals, which can contribute astringency or bitterness, they may be associated with poorer aftertaste ratings (Singh *et al.*, 2025). Intermediate white ginger-turmeric blends often performed better in terms of sensory equilibrium than single-component formulations (Al-Khazaleh, 2021). This is in line with the concepts of product development optimization.

Insomnia or sleep disorder are closely linked with depression, although the underlying biological mechanism is not fully understood. Persistent lack of sleep or insomnia has been independently a risk factor for the development and progression of depression and is associated with poorer clinical outcome and reduced sensitivity to antidepressant medications. Studies have shown that both sleep latency and duration can be used to predict the severity of depression. Evidence also suggests that treatment of insomnia alongside antidepressant drugs can have an immense effect on insomnia and depression. The present research demonstrated that white ginger–turmeric tea improves therapeutic effectiveness by extending sleep duration while maintaining latency within a physiologically acceptable range. This effect may be associated with the bioactive constituent presence in white ginger and turmeric, particularly gingerol and curcuminoids which have been reported to influence GABAergic and serotonergic pathways implicated in sleep regulation, depression, and stress-related disorders (Sochacka & Lachowicz, 2025; Mohammadzadeh *et al.*, 2024). The study reveals that ginger-turmeric tea blend can alter the sleep-inducing effects of diazepam in a manner similar to that of antidepressants. The best outcomes were obtained with moderate turmeric-rich blends (specifically, 25G:75T) which prolonged sleep duration while maintaining regulated onset. However, the specific mechanisms underlying these effects were not directly investigated in the present study. Therefore, the potential of these formulations as complementary interventions for stress-related depressive conditions warrants further investigation.

The cholinergic system is made up of acetylcholine which is a neurotransmitter released from cholinergic neurons, as well as cholinesterase enzymes that control their function (Kokaz *et al.*, 2020). The cholinergic scheme in the central nervous system (CNS) is essential for memory functions and depressive-like behaviors in experimental animal models (Pannu, 2025). Acetylcholine facilitates nerve impulse transmission between neurons by acting within the synaptic cleft. Following synaptic transmission, the enzyme acetylcholinesterase (AChE) rapidly hydrolyzes acetylcholine into choline and acetate. Choline can be recycled back into the presynaptic neuron for acetylcholine resynthesis since this enzymatic breakdown stops the neurotransmitter signal. Memory problems and emotional disorders including depression and depressive-like behaviors can arise from any imbalance in the activity of these neurotransmitters, which can lead to poor or nonexistent signal transmission from the brain to peripheral organs (Pannu, 2025; Halász *et al.*, 1975). Therefore, substances that inhibit the acetylcholine esterases can help alleviate depres-

sive-like effects through modulation of cholinergic neurotransmission. AChE activity was considerably higher in the untreated rats in this study, which suggests that it may be one of the main causes of depression-like effect observed in stressed untreated rats. However, AChE activity was shown to have decreased following treatment with the composite tea under study, as illustrated in Figure 3. This result is aligned with other published research showing that plant extracts and phytochemicals, particularly phenolic compounds, are strong AChE inhibitors and that ingesting them may help treat neurological conditions (Akomolafe *et al.*, 2019; Oboh *et al.*, 2022).

A class of enzymes known as monoamine oxidases (MAO) uses oxygen to cleave the amine group from monoamines during the process of oxidative deamination. In addition to inactivating monoamine neurotransmitters, MAOs show a vital role in the breakdown of monoamines consumed through diet (Hou *et al.*, 2026, Xiao *et al.*, 2024). Since MAOs are essential for the inactivation of neurotransmitters, a variety of neurological and psychiatric conditions are believed to be influenced by MAO dysfunction, or excessive or insufficient MAO activity including Schizophrenia (Ding & Bao, 2026; Xu & Yang, 2022), depression (Kim *et al.*, 2022), attention deficit disorder (Nikolaus *et al.*, 2022), substance misuse (Edinoff *et al.*, 2022), and migraines (García-Azorín *et al.*, 2020). One of the main pharmacological types recommended to treat depression is monoamine oxidase inhibitors. According to some studies, MAO inhibitors (MAOIs) are more effective than other classes of antidepressants in treating specific phenotypes of depression. When treatment-resistant depression does not improve with tricyclic antidepressants, MAOIs may be useful (Shaldam *et al.*, 2026; Hoffman *et al.*, 2023). Dopamine, a type of Monoamine neurotransmitter that controls mood, motivation, reward, and emotional reactions is dopamine. The mesolimbic pathway, often known as the reward pathway, is one of the main dopaminergic circuits linked to depression. The nucleus accumbens, a part of the brain closely linked to pleasure and reward processing, is connected to the ventral tegmental area (VTA) by this pathway (Ranaldi, 2023). When this route malfunctions, it can lead to anhedonia, a primary sign of depression marked by a diminished capacity for pleasure, which is commonly seen in distressed albino rats. In this study, The SC-untreated rats showed a markedly elevated MAO activity and reduced dopamine level (Figure 3) which may indicate alterations in monoaminergic neurotransmission associated with depressive-like and anhedonia-related behaviors.

Interestingly, a significant reduction in MAO activity and an increase in dopamine was observed upon treatment with white ginger and turmeric tea formulations resulting in improved mood and activity with 50G:50T tea blend showing highest ameliorative effect. The potential inhibitory effect of this tea blend may be associated to the phytochemicals present and it supports previous findings that phytochemicals such as flavonoids and polyphenols have powerful neuroprotective effects.

It has been established that behavioral depression in male Wistar rats is caused by antioxidant depletion, a reduction in the amount of glutathione peroxidase (GSH-Px), and a rise in lipid peroxidation and nitric oxide (NO) in the brain's cortex and medulla (Atagün & Canbek, 2022). Superoxide dismutase (SOD) levels and depression severity were discovered to be significantly correlated, indicating that antioxidant enzymes may be state markers of depression (Mucci, 2024).

An enzyme called superoxide dismutase catalyzes the dismutation (or partitioning) of the superoxide O_2^- into H_2O_2 and regular molecular oxygen (O_2) alternatively. Superoxide, a byproduct of oxygen metabolism, can harm cells in multiple ways if it is not properly regulated. (Fujii *et al.*, 2022). Glutathione-S-transferase helps to conjugate harmful electrophilic chemicals with GSH, which improves cellular detoxification, while catalase and glutathione peroxidase further detoxify hydrogen peroxide. Stressed untreated rats in this study had low levels of GST, GSH, GPx, SOD, and catalase (Figure 4), indicating an altered antioxidant status that may be associated with oxidative stress in the stressed animals. Upon administration of the tea blend, the activities of these antioxidant enzymes were restored, suggesting that the tea may have the potential to attenuate oxidative stress. The seen antioxidant effect correlates with previous findings that phytochemicals found in common herbs, spices, fruits, vegetables, and medicinal plants like flavonoids and phenolics can prevent oxidative stress or damage as observed in the *Mondia whitei*–*Curcuma longa* composite tea (Hossain *et al.*, 2025).

Corticosterone, the main glucocorticoid hormone in rodents, is a crucial marker of stress and hypothalamic-pituitary-adrenal (HPA) axis activity (Knezevic *et al.*, 2023), the secretion is strictly controlled to help the body cope with stress by releasing stored energy and modifying neurological and immunological functions (Sitorus & Silitonga, 2025). However, in animal models, the development of depressive-like behaviors has been closely associated with prolonged increase of corticosterone levels brought on by excessive or chronic activation of the HPA axis (Nandam *et al.*, 2020). Neurogenesis, synaptic plasticity, and neurotransmitter balance are negatively impacted by prolonged high corticosterone exposure in brain regions linked to mood regulation, particularly the prefrontal cortex and hippocampus. In depression experimental models, stress-induced corticosterone hypersecretion is commonly utilized as an indicator of HPA axis dysfunction. Elevated corticosterone levels have been linked to depressive symptoms such as anhedonia, anxiety, behavioral despair, and cognitive impairment (Wu *et al.*, 2025). Therefore, it is proposed that interventions capable of normalizing corticosterone levels may have antidepressant and stress-relieving properties. Figure 5 shows that CUMS exposure significantly increased serum corticosterone levels in the stress control (SC) group compared with the normal control (NC) group (**** $p < 0.001$), indicating an altered stress response in the CUMS-exposed animals. This finding is consistent with previous research demonstrating that prolonged stress can increase corticosterone release and is associated with behavioral and physiological changes resembling depression. The observed reduction in corticosterone levels following tea treatment suggests that the formulations may help attenuate the stress response and may be associated with modulation of HPA-axis activity. Plant-derived compounds such as flavonoids, polyphenols, and saponins have been investigated for their potential to modulate corticosterone responses (Balogun *et al.*, 2025). This reduction in corticosterone may contribute to the behavioral improvements observed in the treated rats, potentially through effects on stress-related neuronal and neurotransmitter processes. Overall, these findings suggest that the antidepressant-like effects of the tea formulations may be partly associated with their phytochemical constituents and their potential to attenuate stress-related increases in circulating corticosterone. However, because other components of HPA-axis activity were not directly assessed, the involvement of HPA-axis modulation remains a proposed mechanism rather than a mechanism directly demonstrated in the present study.

In this study, TNF- α and IL-6, which are pro-inflammatory cytokines, were detected in the rats' blood. Results indicated that untreated rats (stressed group) had significantly greater levels of these cytokines than the control group ($p < 0.001$) (Figure 6). Fluoxetine and tea treatment significantly ($p < 0.001$) decreased TNF- α levels in the serum compared with the stress group. Nevertheless, the 50G:50T treatment exhibited the lowest TNF- α level, suggesting a greater decrease in inflammatory response among the tea formulations. Increased interleukin (IL)-6 has been recognized as a potential marker associated with depression (Yamasaki *et al.*, 2020). Reduced IL-10 and IL-4 expression may influence HPA-axis activity and glucocorticoid responsiveness observed in depressive conditions, and IL-10 has been reported to suppress adrenocorticotrophic hormone-induced steroid production, which may contribute to the regulation of hypothalamic-pituitary-adrenal (HPA) axis homeostasis (Drela, 2025). Furthermore, previous studies have reported concurrent increases in IL-6 and decreases in IL-10/IL-4 in experimental models associated with depressive-like behaviors. Thus, altered levels of these cytokines may be associated with the severity of depressive-like symptoms seen in the stressed rats.

Additionally, compared with controls, the stressed rats in the present study showed significantly greater TNF- α levels, and IL-6 and significantly higher IL-4/IL-10 ratios (Figure 6). When compared with the stressed group, the tea blend treatment groups had much lower IL-6 and TNF- α levels, while their IL-10/IL-4 levels were higher. In contrast, the fluoxetine group showed noticeably greater levels of IL-10/IL-4 and much lower IL-6 and TNF- α . These findings suggest that the antidepressant-like effects observed following white ginger–turmeric tea and fluoxetine treatment may be associated with modulation of the inflammatory response, characterized by reduced pro-inflammatory cytokines such as IL-6 and TNF- α . However, the present study did not directly establish a causal relationship between cytokine modulation and the observed behavioral effects; therefore, inflammatory pathways may represent one of the mechanisms contributing to the effects of the tea formulations.

CONCLUSION

The present research revealed that *Mondia whitei*–*Curcuma longa* composite tea displays significant antidepressant-like effects in CUMS-induced rats. The observed effect mechanism may be through modulation of monoaminergic neurotransmission, attenuation of oxidative stress, suppression of neuroinflammation and changes in corticosterone levels. Among the tested formulations 50:50 blend demonstrated the most pronounced effect *in vivo*. The findings provide preliminary evidence that the composite tea may modulate behavioral and biochemical alterations associated with CUMS in rats. Further studies, including mechanistic, dose-response, safety, and clinical investigations, are required before any conclusions regarding human therapeutic efficacy can be drawn

AUTHORS' CONTRIBUTION

The authors confirm their contributions to the paper as follows: study conception and design, methodology: Gbolagade-Akanbi A.M, Oyewole O.I., Salawu S.O. data collection: Gbolagade-Akanbi A.M, Adedeji V. A, Gbolagade O.H; analysis and interpretation of results, draft manuscript preparation, writing – review and editing:, Gbolagade-Akanbi A.M, Oyewole O.I., Salawu S.O, Adedeji V. A , Gbolagade O.H. All authors reviewed the results and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The author confirms that this article's content has no conflict of interest.

DATA AVAILABILITY STATEMENT

Data can be obtained upon request

ACKNOWLEDGEMENTS

Declared none.

STATEMENT OF ETHICS

This Study protocol was reviewed and approved by the Osun State health Research Ethic committee (OSHREC), Ministry of Health, Osogbo, Osun state, Nigeria, approval number Approval No.: OSHREC/PRS/2024/842/01/244; Date of approval: 31 January 2024.

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RESEARCH ARTICLE



Human–AI Collaboration in Digital Marketing: Evidence from an Emerging Economy, Somalia

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Abstract: Artificial intelligence (AI) is reshaping digital marketing through automation, customer analytics, personalization, and data-driven decision-making. However, empirical evidence on human–AI collaboration in emerging and fragile economies remains limited. This study examined the adoption of human–AI collaboration in digital marketing among businesses in Somalia, identified key barriers to AI adoption, and assessed the association between human–AI collaboration and customer engagement, marketing efficiency, and business growth. The study used a positivist research approach and a quantitative cross-sectional survey design. Data were collected from 86 business owners, marketing managers, digital marketing specialists, and ICT/technology officers using purposive sampling. Data were analyzed using descriptive statistics, Spearman's rank correlation, and multiple linear regression. Respondents reported frequent use of AI-assisted content creation, customer analytics, marketing automation, social media management, and AI-supported decision-making. The principal barriers were limited technical skills, limited infrastructure, implementation costs, data privacy concerns, and organizational resistance to change. Spearman's correlations indicated positive associations between human–AI collaboration and customer engagement ($p = .684$), marketing efficiency ($p = .725$), and business growth ($p = .658$), all reported at $p < .001$. Regression models explained 50.7% of the variance in customer engagement, 54.9% in marketing efficiency, and 46.4% in business growth. Human–AI collaboration was positively associated with each outcome after controlling for organizational size and industry sector. Because the study is cross-sectional and based on a purposive sample, the findings should be interpreted as associations rather than causal effects. The results highlight the importance of combining AI capabilities with human judgment, creativity, contextual knowledge, and responsible data practices in Somalia's digital marketing environment.

Keywords: Artificial intelligence; Human–AI collaboration; Digital marketing; Customer engagement; Marketing efficiency; Business growth; Somalia

1. INTRODUCTION

Digital technologies have transformed marketing by making it more data-driven, automated, and personalized. Artificial intelligence (AI) supports customer segmentation, predictive analytics, conversational technologies, content creation, recommendation systems, and campaign optimization. Research in marketing suggests that AI can augment human decision-making by handling repetitive tasks and processing large volumes of information, while human marketers contribute contextual judgment, creativity, ethical reasoning, and strategic direction (Davenport et al., 2020; Huang & Rust, 2021; Petrescu & Krishen, 2023).

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Digital marketing is particularly relevant in emerging economies because increased internet access, smartphone use, social media adoption, and digital financial services create new channels for customer interaction and business development. Somalia has experienced growth in mobile connectivity and digital financial services, creating opportunities for businesses to engage customers through digital channels (World Bank, 2023). These developments provide a context in which AI-enabled marketing tools may be useful, but adoption may also be constrained by infrastructure, skills, cost, privacy, and governance challenges.

Human–AI collaboration refers in this study to the use of AI capabilities together with human expertise in marketing activities. The collaboration perspective is important because AI and human intelligence have different strengths. AI can support large-scale data processing, automation, and analytical tasks, whereas human actors remain important for contextual interpretation, creativity, relationship management, and ethical judgment (Huang & Rust, 2022; Petrescu & Krishen, 2023).

Evidence from developing and emerging markets indicates that AI adoption is uneven and shaped by organizational capabilities, infrastructure, employee skills, financial resources, and concerns about data privacy and responsible use. These issues may be especially relevant in fragile digital ecosystems, where technological development can move faster than institutional capacity and regulatory arrangements.

Despite growing international interest in AI-enabled marketing, empirical evidence focused specifically on human–AI collaboration in Somalia remains limited. This study therefore investigates AI-supported marketing practices among businesses operating in Somalia, identifies barriers to adoption, and examines the statistical associations between human–AI collaboration and three organizational outcomes: customer engagement, marketing efficiency, and business growth.

2. RESEARCH OBJECTIVES

To examine the level of adoption of human–AI collaboration in digital marketing activities among businesses in Somalia.

To identify the key challenges affecting the adoption of AI technologies in digital marketing among businesses in Somalia.

To assess the association between human–AI collaboration and customer engagement, marketing efficiency, and business growth in Somalia.

3. METHODS

3.1. Research Approach and Design

The study adopted a positivist research approach and a quantitative cross-sectional survey design. The approach was selected because the study sought to measure reported AI adoption practices, perceived barriers, and organizational outcomes using structured quantitative data. The cross-sectional design captured respondents' perceptions and reported practices at a single point in time. Accordingly, the study examines statistical associations rather than causal effects.

3.2. Study Population

The target population comprised business owners, marketing managers, digital marketing specialists, and ICT/technology personnel involved in digital marketing activities within businesses operating in Somalia. Participants were considered appropriate because their roles provide direct knowledge of digital marketing practices and the use of AI-related tools. The study focused on businesses using digital channels such as social media, websites, e-commerce platforms, and mobile applications.

3.3. Sample and Sampling Technique

A total of 86 respondents were selected using purposive sampling. Participants were selected because of their involvement in digital marketing and familiarity with AI-related technologies. The sample comprised business owners, marketing managers, digital marketing specialists, and ICT/technology officers.

3.4. Data Collection Instrument

Data were collected using a structured questionnaire consisting primarily of closed-ended items measured on a five-point Likert scale: 1 = Strongly Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, and 5 = Strongly Agree. The questionnaire contained four sections:

Section A: demographic and organizational information, including gender, age, organizational role, relevant work experience, type of business, organizational size, and industry sector.

Section B: human–AI collaboration adoption, covering AI-assisted content creation, customer analytics, marketing automation, social media management, and AI-supported decision-making.

Section C: barriers to AI adoption, including technical skills, implementation costs, data privacy, technological infrastructure, and organizational resistance to change.

Section D: perceived organizational outcomes associated with human–AI collaboration, focusing on customer engagement, marketing efficiency, and business growth.

3.5. Measurement of Constructs

The study used multi-item constructs to measure human–AI collaboration and the three organizational outcomes. Items were informed by the literature on AI in marketing and human–AI collaboration. The measurement framework was aligned with the variables subsequently analyzed in the results.

Table 1. Measurement of Study Constructs.

Construct	No. of items	Measurement focus	Key sources
Human–AI Collaboration	5	AI support for content creation, analytics, automation, social media management, and decision support.	Huang & Rust (2021, 2022); Petrescu & Krishen (2023)
Customer Engagement	4	Responsiveness, personalization, and continuity of customer interaction.	Huang & Rust (2021, 2022); Petrescu & Krishen (2023)
Marketing Efficiency	4	Time savings, productivity, automation, and campaign optimization.	Davenport et al. (2020); Huang & Rust (2021, 2022)
Business Growth	4	Customer acquisition, market reach, competitiveness, and value creation.	Huang & Rust (2021); Paschen et al. (2022)

3.6. Data Collection Procedure

Questionnaires were distributed electronically through email, WhatsApp, and web-based survey platforms. Participants were informed of the purpose of the study, the voluntary nature of participation, and the confidentiality of their responses. Completed questionnaires were collected electronically and prepared for statistical analysis.

3.7. Data Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS). Descriptive statistics included frequencies, percentages, means, and standard deviations. Spearman's rank-order correlation was used to examine bivariate associations because the questionnaire responses were measured using Likert-type scales. Multiple linear regression was used to examine the predictive association between human–AI collaboration and each outcome while controlling for organizational size and industry sector. The statistical results are reported using coefficients, R^2 , adjusted R^2 , F-statistics, t-values, p-values, and confidence intervals where available.

3.8. Validity and Reliability

Content validity was addressed by developing questionnaire items from the literature on AI and digital marketing. Internal consistency reliability was assessed using Cronbach's alpha. The reported coefficients were above the commonly used 0.70 threshold.

Construct	Items	Cronbach's α
Human–AI Collaboration	5	0.82
Customer Engagement	4	0.79
Marketing Efficiency	4	0.84
Business Growth	4	0.81

3.9. Ethical Considerations and Common-Method Procedures

Participation was voluntary, informed consent was obtained, confidentiality and anonymity were respected, and data were used for academic purposes. No identifying participant information was reported. Procedural measures included anonymous responses, voluntary participation, and neutral wording.

The manuscript also reports a Harman single-factor test, with the first factor accounting for 38% of the total variance. This is below the 50% heuristic used in the original manuscript. However, Harman's single-factor test alone does not establish that common-method bias is absent; the result is therefore treated as an indicator rather than definitive evidence that common-method bias is not present.

4. RESULTS

4.1. Respondent Characteristics and Demographic/Organizational Profile

Table 2. Demographic and Organizational Characteristics of Respondents (N = 86)

Variable	Category / Measure	Frequency (n)	Percentage (%)
Professional role	Business owners	20	23.26
	Marketing managers	20	23.26
	Digital marketing specialists	30	34.88
	ICT/technology officers	16	18.60
	Total	86	100.00
Gender	Male	53	61.63
	Female	33	38.37
Variable	Category / Measure	Frequency (n)	Percentage (%)
	Total	86	100.00
	18–25 years	11	12.79
	26–35 years	34	39.53
Age	36–45 years	27	31.40
	46–55 years	10	11.63
	56 years and above	4	4.65
	Total	86	100.00

The sample of 86 respondents consisted mainly of digital marketing specialists (34.88%), followed by business owners and marketing managers (23.26% each), with ICT/technology officers forming the smallest group (18.60%). Thus, 81.40% were business, marketing, or digital-marketing personnel, providing both strategic and technical perspectives relevant to human–AI collaboration. Males constituted the majority (61.63%) compared with females (38.37%), indicating a moderate gender imbalance but still a mixed sample. Most respondents were in the 26–35 (39.53%) and 36–45 (31.40%) age brackets, together accounting for 70.93% of the sample. Only 4.65% were 56 years or older. This young-to-mid-career pro-

file is favourable for a study on AI-enabled marketing, as such respondents tend to be more familiar with digital and AI tools.

4.2. Adoption of Human–AI Collaboration

Respondents reported relatively high use of AI-enabled activities in digital marketing. The highest reported mean was for AI-assisted content creation ($M = 4.12$, $SD = 0.78$), followed by customer analytics ($M = 4.08$, $SD = 0.81$), social media management ($M = 4.01$, $SD = 0.76$), marketing automation ($M = 3.95$, $SD = 0.84$), and AI-supported decision-making ($M = 3.88$, $SD = 0.87$).

Table 3. AI Adoption in Digital Marketing

AI-supported activity	Mean	SD
AI-assisted content creation	4.12	0.78
Customer analytics	4.08	0.81
Marketing automation	3.95	0.84
Social media management	4.01	0.76
AI-supported decision-making	3.88	0.87

4.3. Challenges Affecting AI Adoption

The reported barriers were primarily related to technical skills, infrastructure, implementation costs, data privacy, and organizational resistance to change. Lack of technical skills had the highest mean ($M = 4.28$, $SD = 0.73$), followed by limited infrastructure ($M = 4.20$, $SD = 0.76$), high implementation costs ($M = 4.15$, $SD = 0.79$), and data privacy concerns ($M = 4.02$, $SD = 0.82$). Organizational resistance to change had the lowest mean among the listed barriers ($M = 3.72$, $SD = 1.34$), although it remained above the neutral midpoint.

Table 5. Challenges Affecting AI Adoption

Challenge	Mean	SD
Lack of technical skills	4.28	0.73
Limited technological infrastructure	4.20	0.76
High implementation costs	4.15	0.79
Data privacy concerns	4.02	0.82
Organizational resistance to change	3.72	1.34

4.4. Perceived Organizational Outcomes

Respondents reported positive perceptions of the three organizational outcomes. Marketing efficiency had the highest mean ($M = 4.25$, $SD = 0.68$), followed by customer engagement ($M = 4.18$, $SD = 0.71$) and business growth ($M = 4.10$, $SD = 0.75$).

Table 6. Perceived Organizational Outcomes of Human–AI Collaboration

Outcome	Mean	SD
Customer engagement	4.18	0.71
Marketing efficiency	4.25	0.68
Business growth	4.10	0.75

4.5. Spearman Rank Correlation Analysis

Spearman's rho was used to examine the bivariate associations between human–AI collaboration and the three outcome variables. All reported correlations were positive and statistically significant at $p < .001$.

Table 7. Spearman Rank Correlation Matrix (N = 86)

Variable	1	2	3	4
1. Human–AI Collaboration	1.000			
2. Customer Engagement	.684***	1.000		
3. Marketing Efficiency	.725***	.652***	.000	
4. Business Growth	.658***	.701***	.683***	1.000

4.6. Multiple Linear Regression Analysis

Three multiple linear regression models were reported, with human–AI collaboration as the focal independent variable and organizational size and industry sector as control variables. The reported models explained 50.7% of the variance in customer engagement, 54.9% in marketing efficiency, and 46.4% in business growth. The corresponding adjusted R^2 values were 48.9%, 53.2%, and 44.4%, respectively.

Table 8. Multiple Linear Regression Model Summary (N = 86)

Dependent variable	R	R ²	Adjusted R ²	Std. error	F	p
Customer engagement	.712	.507	.489	.629	28.11	< .001
Marketing efficiency	.741	.549	.532	.650	33.27	< .001
Business growth	.681	.464	.444	.753	23.66	< .001

The focal independent variable was human–AI collaboration; organizational size and industry sector were included as control variables.

Model 1 explained 50.7% of the variance in customer engagement. Model 2 explained 54.9% of the variance in marketing efficiency. Model 3 explained 46% of the variance in business growth.

*** $p < .001$.

4.7. Regression Coefficients

Table 9. Regression Coefficients for the Three Outcome Models

Outcome	Predictor	B	Beta	t	p	95% CI for B
Customer engagement	Constant	1.12	—	2.45	.017	[0.21, 2.03]
	Human–AI Collaboration	.562	.588	6.82	< .001	[.398, .726]
	Organizational size (log)	.087	.102	1.23	.222	[-.053, .227]
	Industry sector	-.124	-.089	-1.02	.311	[-.366, .118]
Marketing efficiency	Constant	.98	—	2.01	.048	[.01, 1.95]
	Human–AI Collaboration	.643	.623	7.54	< .001	[.473, .813]
	Organizational size (log)	.112	.124	1.56	.123	[-.031, .255]
	Industry sector	-.089	-.060	-0.72	.474	[-.336, .158]
Business growth	Constant	1.45	—	2.56	.013	[.32, 2.58]
	Human–AI Collaboration	.521	.573	6.35	< .001	[.358, .684]
	Organizational size (log)	.143	.149	1.69	.095	[-.025, .311]
	Industry sector	-.211	-.135	-1.52	.132	[-.488, .066]

Abbreviations: B = unstandardized coefficient; Beta = standardized coefficient; CI = confidence interval.

Across the three models, human–AI collaboration was positively associated with each outcome after controlling for organizational size and industry sector. The standardized coefficients were $\beta = .588$ for customer engagement, $\beta = .623$ for marketing efficiency, and $\beta = .573$ for business growth, all with $p < .001$. The control variables were not statistically significant in the reported models. These results indicate statistical association and predictive contribution; they should not be interpreted as evidence of causal impact because the study is cross-sectional.

5. DISCUSSION

5.1. Interpretation of Findings

The results indicate that respondents in Somali businesses reported substantial use of AI-supported activities in digital marketing, particularly content creation, customer analytics, social media management, marketing automation, and decision support. This pattern is consistent with the broader marketing literature, which describes AI as a tool for automation, analysis, personalization, and campaign support (Davenport et al., 2020; Huang & Rust, 2021).

The findings also support the human–AI collaboration perspective. AI can perform high-volume analytical and repetitive tasks, while human marketers provide contextual interpretation, creativity, ethical judgment, and strategic direction. This complementarity is central to collaborative AI frameworks in marketing (Huang & Rust, 2022; Petrescu & Krishen, 2023).

The reported positive association between human–AI collaboration and customer engagement is consistent with the role of AI in personalization, conversational interaction, and customer analytics. In practice, AI-supported responsiveness and analysis may help firms tailor digital interactions, although the present study does not establish a causal mechanism.

The positive association with marketing efficiency is also consistent with the use of AI for automation, data processing, targeting, and campaign support. The relatively high mean for marketing efficiency and the regression result suggest that respondents perceived a relationship between collaborative AI use and more efficient marketing operations.

The association with business growth should likewise be interpreted cautiously. AI-supported segmentation, targeting, customer interaction, and automation may contribute to business performance, but growth is influenced by many factors beyond marketing technology. Because the study used cross-sectional self-reported data, the results show an empirical relationship rather than a demonstrated causal effect.

The barriers identified by respondents—skills, infrastructure, implementation cost, privacy, and organizational resistance—are particularly relevant to emerging and fragile digital environments. These constraints suggest that AI adoption is not only a question of access to software; it also depends on digital capabilities, organizational readiness, reliable infrastructure, and responsible data practices.

5.2. Theoretical and Practical Implications

- The study adds empirical evidence from Somalia to the growing literature on human–AI collaboration in marketing, a literature that has largely developed in more digitally mature settings.
- The findings suggest that human–AI collaboration should be considered as an augmentation approach rather than an assumption that AI automatically replaces marketing professionals.
- For businesses, investment in staff AI literacy, data practices, and digital infrastructure should accompany investment in AI tools.
- For policymakers and higher-education institutions, skills development and responsible AI practices are relevant to strengthening the digital business ecosystem.

5.3. Limitations

- The sample consisted of 86 purposively selected respondents, so the findings should not be generalized to all businesses in Somalia without further evidence.
- The cross-sectional design does not establish temporal order or causality. The terms 'effect' and 'impact' have therefore been replaced throughout this revised manuscript with 'association' where appropriate.
- The data were self-reported and collected through a common questionnaire, which may introduce common-method and response biases. The reported Harman single-factor result should not be treated as a definitive test of the absence of common-method bias.
- The analysis controls for organizational size and industry sector, but the submitted manuscript does not provide detailed sampling distributions for these variables. Their coding and descriptive statistics should be retained in the final dataset and reported if required by the journal.

CONCLUSION

This study examined human–AI collaboration in digital marketing among businesses in Somalia. Respondents reported frequent use of AI for content creation, customer analytics, marketing automation, social media management, and decision support. The principal barriers were technical skills, infrastructure, implementation costs, data privacy, and organizational resistance to change. Human–AI collaboration was positively associated with customer engagement, marketing efficiency, and business growth in the reported correlation and regression analyses. These findings support the relevance of combining AI capabilities with human judgment and contextual expertise in emerging digital markets. However, the cross-sectional design and purposive sample mean that the results should be interpreted as associations rather than causal effects.

7. RECOMMENDATIONS

- Invest in practical AI and digital marketing skills for business owners, marketing professionals, and ICT personnel.
- Improve access to reliable and affordable digital infrastructure and AI-enabled tools, particularly for small and medium-sized businesses.
- Use AI to augment human judgment, creativity, and strategic decision-making rather than treating automation as a substitute for human oversight.
- Develop internal policies covering data privacy, transparency, human oversight, and responsible AI use in marketing.
- Strengthen collaboration among government, universities, technology providers, and businesses to support AI skills development and responsible digital innovation.

SUGGESTIONS FOR FUTURE RESEARCH

- Use larger probability-based samples to improve representativeness and generalizability.
- Conduct comparative studies across Somalia and other African emerging markets.
- Examine organizational culture, employee AI readiness, trust in AI, customer perceptions, and responsible-AI practices as additional explanatory variables.
- Where feasible, combine survey data with objective marketing indicators such as conversion rates, customer retention, campaign costs, or engagement metrics.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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APPENDIX A. COMPLETE QUESTIONNAIRE AND SCORING PROCEDURE

Instructions to respondents. This questionnaire asks about the use of artificial intelligence (AI) in digital marketing within your organization. Please answer based on your actual experience. There are no right or wrong answers. Your responses are confidential and will be used for academic research.

Section A. Demographic and Organizational Information

Variable	Values
	Business owners
	Marketing managers
Professional role	
Digital marketing specialists	
ICT/technology officers	
Total	
	Male
Gender	
Female	
Total	
	18–25 years
	26–35 years
Age	
36–45 years	
46–55 years	
56 years and above	

Section B. AI Adoption in Digital Marketing

- Response scale: 1 = Never, 2 = Rarely, 3 = Sometimes, 4 = Frequently, 5 = Very frequently.
- Our organization uses AI-assisted tools to create or generate marketing content.
- Our organization uses AI tools for customer or market analytics.
- Our organization uses AI tools to automate marketing activities or campaigns.
- Our organization uses AI tools to manage or optimize social-media marketing.
- Our organization uses AI-supported tools to assist marketing decisions.

Scoring: AI Adoption Score = mean of Items B1–B5. Higher scores indicate more frequent reported use of AI-enabled marketing applications.

Section C. Human–AI Collaboration

Response scale: 1 = Strongly Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, 5 = Strongly Agree.

- AI tools complement rather than replace human expertise in our marketing activities.
- Marketing employees use AI-generated information together with their own professional judgment.
- Marketing employees review and interpret AI-generated outputs before important marketing decisions are made.

- Our organization combines AI capabilities with human creativity and contextual knowledge when developing marketing activities.
- Human staff retain responsibility and oversight when AI tools are used for marketing decisions.

Scoring: Human–AI Collaboration Score = mean of Items C1–C5. Higher scores indicate stronger integration of AI capabilities with human expertise. No items are reverse-coded.

Section D. Perceived Benefits of Human–AI Collaboration

Response scale: 1 = Strongly Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, 5 = Strongly Agree.

D1. Customer Engagement

- AI-supported marketing helps our organization respond to customers more quickly.
- AI-supported marketing enables more personalized customer interactions.
- AI-supported marketing helps us maintain more continuous customer engagement.
- AI-supported marketing improves the relevance of our communications to customers.

D2. Marketing Efficiency

- AI-supported marketing reduces the time required to perform routine marketing tasks.
- AI-supported marketing improves the productivity of our marketing team.
- AI-supported marketing improves campaign planning, execution, or optimization.
- AI-supported marketing enables faster analysis of marketing data.

D3. Business Growth

- AI-supported marketing helps our organization reach new or broader customer segments.
- AI-supported marketing helps improve customer acquisition.
- AI-supported marketing strengthens our competitive position.
- AI-supported marketing contributes to overall business growth.

Scoring: Customer Engagement Score = mean of D1 items 1–4; Marketing Efficiency Score = mean of D2 items 1–4; Business Growth Score = mean of D3 items 1–4. The three benefit constructs are kept separate in analysis.

Section E. Barriers to AI Adoption

Response scale: 1 = Strongly Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, 5 = Strongly Agree.

- Lack of technical and AI-related skills is a barrier to wider AI adoption in our organization.
- The cost of implementing AI tools is a barrier to wider adoption.
- Data privacy and security concerns are barriers to wider adoption.
- Limited digital and technological infrastructure is a barrier to wider adoption.
- Resistance to organizational change is a barrier to wider adoption.

Scoring: Barrier Score = mean of Items E1–E5. Higher scores indicate greater perceived barriers to AI adoption.