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Original Research Article

Phytochemical Characterisation and In Vitro Evaluation of the Analgesic Potential of Gbefun Super Bitters

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Inequities in access to medicines, vaccines and health technologies continue to remains a major challenge in global health governance, majorly in the Global South. The present study underscores the importance of global health diplomacy as a contributor to inequity in access to health services on an international scale by drawing parallels between the HIV/AIDS epidemic and the COVID-19 pandemic. The methodology employed in this study is qualitative comparative case study design that is a methodology in which documentary evidence is used in the form of peer-reviewed articles, academic books, international organizations' policy documents and reports. Theories: Dependency Theory and Liberal institutionalism. These two theories will be used to examine the impact of structural inequalities and global partnerships on global health outcomes.
Findings show that global health diplomacy has facilitated global cooperation, resource mobilization and institutional response to big health crises.However, the disparities in drug production, patents and technologies, and geopolitical power are still important drivers of access. During the HIV/AIDS pandemic in many Global South countries, there was a restriction of access to antiretroviral (ARV) drugs due to patent barriers and high treatment costs. COVID-19 pandemic also highlighted inequalities in vaccine nationalism, inequitable vaccine procurement processes and limited technology transfer.
WHO, UNAIDS, the Global Fund and COVAX were active in improving relations, but had limited capacity to ensure equitable distribution. The study finds the need for improved technology transfer systems, increased pharmaceutical manufacturing capacities, increased IP action and increased accountability within the global health institutions to move the agenda forward on international health equity.

1. Introduction

Natural products have played a central role in the discovery and development of therapeutic agents for centuries. Among these, medicinal plants remain an important source of compounds with medicinal value because they produce a wide variety of secondary metabolites capable of influencing numerous physiological processes (Jesusanmi et al., 2026; Ghutke et al., 2023). In many developing countries, including Nigeria, herbal medicines continue to serve as an integral part of primary healthcare due to their affordability, accessibility, and long-standing acceptance within local communities (Kpomah & Owo, 2024). These plants contain phytoconstituents such as flavonoids, alkaloids, tannins, saponins, terpenoids, phenolic compounds, and glycosides, many of which have been associated with analgesic, anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory effects. Continued interest in medicinal plants reflects their value as sources of structurally unique compounds with potential applications in disease prevention and treatment (Apinda et al., 2026; El-Saadony et al., 2025; Kpomah & Owo, 2024).

Pain remains one of the most common reasons for seeking medical attention, creating an ongoing demand for safer and more effective analgesic agents. Although conventional analgesics are widely prescribed, prolonged use is frequently associated with adverse effects that limit their clinical utility (Aly et al., 2026; Zahra et al., 2025). Plant-derived compounds have emerged as attractive alternatives because they influence pain pathways through several complementary mechanisms. These include the inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, suppression of pro-inflammatory cytokines such as tumour necrosis factor-alpha

(TNF-α), interleukin-1β, and interleukin-6 (Ahmad et al., 2026; Amin et al., 2026; Islam et al., 2025), regulation of NF-κB and MAPK signalling pathways (Aly et al., 2026), interaction with opioid receptors, scavenging of reactive oxygen species, and modulation of transient receptor potential (TRP) ion channels involved in nociception. In polyherbal formulations, interactions among multiple phytochemicals may enhance these effects through synergistic mechanisms, potentially improving therapeutic efficacy (Sivaji et al., 2026; Sulaiman et al., 2021).

Gbefun Super Bitters is a commercially available polyherbal formulation that is widely promoted for pain relief and other health benefits. Despite its increasing use, information regarding its chemical composition and analgesic activity remains limited. Comprehensive evaluation of its phytochemical constituents, chemical profile, and mechanisms underlying its analgesic effects is still lacking. Such information is essential for validating its traditional application, supporting quality control, and establishing a stronger scientific foundation for its therapeutic use.

This study was designed to evaluate the phytochemical composition and in vitro analgesic activity of Gbefun Super Bitters. Gas chromatography–mass spectrometry (GC–MS) was employed to profile its major chemical constituents, while cyclooxygenase inhibition was used to assess its analgesic potential. The specific objectives were to:

Determine the qualitative and quantitative phytochemical composition of Gbefun Super Bitters.

Profile and characterise the major bioactive constituents of Gbefun Super Bitters using gas chromatography–mass spectrometry (GC–MS).

Assess the in vitro analgesic activity of Gbefun Super Bitters through cyclooxygenase (COX) inhibition.

Relate the detected phytochemicals and GC–MS-identified compounds to the analgesic activity of the formulation.

2. MATERIALS AND METHODS

2.1. Collection and Preparation of Sample

Commercially packaged Gbefun Super Bitters was purchased from an authorised distributor in Port Harcourt, Rivers State, Nigeria. The samples were transported to the laboratory under appropriate storage conditions before phytochemical, GC–MS and in vitro analyses. A measured volumes (500 mL) of Gbefun Super Bitters was collected and aliquoted for subsequent phytochemical, GC–MS, and analgesic analyses.

Figure 1. Commercially packaged Gbefun Super Bitters alcoholic drink

2.2 Phytochemical Screening

2.2.2 Qualitative Phytochemical Screening

Qualitative phytochemical analysis followed the procedures described by Harborne (1998) and Trease and Evans (2002), with minor modifications. The analysis screened for alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids and glycosides.

Test for Alkaloids

Alkaloids were detected using Mayer's and Dragendorff's reagents. A 5 mL aliquot of Gbefun Super Bitters was acidified with dilute hydrochloric acid before gentle heating in a water bath. The mixture was filtered, and the filtrate was divided into separate portions. A few drops of Mayer's reagent were added to one portion, while Dragendorff's reagent was added to another. The formation of a cream-coloured precipitate with Mayer's reagent or an orange-red precipitate with Dragendorff's reagent indicated the presence of alkaloids.

Test for Flavonoids

Flavonoids were screened using both the Shinoda and alkaline reagent tests. In the Shinoda test, 5 mL of Gbefun Super Bitters was mixed with small pieces of magnesium ribbon, after which a few drops of concentrated hydrochloric acid were added. The appearance of a pink, red, or orange colouration was taken as evidence of flavonoids. For the alkaline reagent test, 2 mL of the sample was treated with a few drops of sodium hydroxide solution. Formation of an intense yellow colour that disappeared upon the addition of dilute hydrochloric acid confirmed the presence of flavonoids.

Test for Phenolic Compounds

The ferric chloride test was used to screen for phenolic compounds. In this procedure, a 2 mL aliquot of Gbefun Super Bitters was treated with a few drops of 5% ferric chloride solution. Development of a blue, green, or violet colouration confirmed the presence of phenolic compounds.

Test for Tannins

Tannins were screened using ferric chloride and gelatin tests. For the ferric chloride test, a 2 mL aliquot of Gbefun Super Bitters was mixed with a few drops of ferric chloride solution. Development of a blue-black or green colouration indicated the presence of tannins. In the gelatin test, the sample was treated with gelatin solution, and the formation of a white precipitate confirmed the presence of tannins.

Test for Saponins

The frothing test was employed to screen for saponins. A 5 mL aliquot of Gbefun Super Bitters was vigorously shaken with 10 mL of distilled water in a test tube for approximately 5 minutes. The persistence of froth measuring about 1 cm or more in height for several minutes was regarded as evidence of saponins.

Test for Terpenoids

Terpenoids were screened using the Salkowski reaction. Here, a 2 mL aliquot of Gbefun Super Bitters was combined with an equal volume of chloroform before 2 mL of concentrated tetraoxosulphate (VI) acid was carefully introduced down the side of the test tube. The appearance of a reddish-brown ring at the interface confirmed the presence of terpenoids.

Test for Steroids

The Liebermann–Burchard reaction was used to detect steroidal compounds. In this method, a 2 mL aliquot of Gbefun Super Bitters was mixed with 2 mL of chloroform, followed by acetic anhydride and a few drops of concentrated tetraoxosulphate (VI) acid. A blue-green colouration was considered indicative of steroids.

Test for Glycosides

Cardiac glycosides were screened using the Keller–Killiani test. Approximately 2 mL of Gbefun Super Bitters was treated with glacial acetic acid containing a trace of ferric chloride solution, after which concentrated tetraoxosulphate (VI) acid was carefully added along the wall of the test tube. Formation of a brown ring at the junction of the two layers confirmed the presence of cardiac glycosides.

2.2.3 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was conducted to measure the principal phytochemical constituents of Gbefun Super Bitters using standard spectrophotometric techniques described by Harborne (1998) together with other established analytical procedures. The assessment included total phenolic, flavonoid, tannin, alkaloid, saponin, and terpenoid contents, as these phytochemicals are widely recognised for their contributions to the antioxidant, anti-inflammatory, and analgesic effects of medicinal plants.

Determination of Total Phenolic Content

Total phenolic content was quantified using the Folin–Ciocalteu colourimetric assay described by Singleton et al. (1999). A 1 mL aliquot of Gbefun Super Bitters extract was combined with 5 mL of Folin–Ciocalteu reagent previously diluted with distilled water and left to stand for 5 minutes. Subsequently, 4 mL of 7.5% sodium carbonate solution was added, after which the reaction mixture was incubated at room temperature in the dark for 30 minutes. Absorbance was then recorded at 765 nm using a UV–visible spectrophotometer. Gallic acid served as the reference standard, and a calibration curve was generated using varying concentrations of gallic acid. The total phenolic content of the sample was determined from the calibration curve and expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g)..

Determination of Total Flavonoid Content

The total flavonoid content was determined using the aluminium chloride colourimetric method described by Zhishen et al. (1999). Briefly, 1 mL of Gbefun super bitters extract was mixed with 4 mL of distilled water, followed by the addition of 0.3 mL of 5% sodium nitrite solution. After 5 minutes, 0.3 mL of 10% aluminium chloride solution was added, followed by 2 mL of 1 M sodium hydroxide solution after 6 minutes. The mixture was diluted with distilled water to a suitable volume, and absorbance was measured at 510 nm. Quercetin was used as the standard reference compound, and the total flavonoid content was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of sample (mg QE/g) .

Determination of Total Tannin Content

The total tannin content was determined using the Folin–Ciocalteu method with slight modification as described by Broadhurst and Jones (1978). Briefly, 1 mL of the Gbefun super bitters extract was mixed with Folin–Ciocalteu reagent and sodium carbonate solution. The mixture was incubated, and absorbance was measured at 725 nm using a spectrophotometer. Tannic acid was used as the reference standard for preparation of the calibration curve. The tannin content was calculated and expressed as milligrams of tannic acid equivalents per gram of sample (mg TAE/g).

Determination of Total Alkaloid Content

Total alkaloid content was determined using a gravimetric method described by Harborne (1998). Briefly, a measured volume of Gbefun super bitters was acidified with dilute hydrochloric acid and allowed to stand before filtration. The filtrate was subsequently treated with ammonium hydroxide solution to induce the precipitation of alkaloids. The resulting

alkaloid precipitate was collected, washed, dried to a constant weight, and accurately weighed. The percentage alkaloid content was then calculated using the following formula:

$$\text{Alkaloid content (\%)} = \frac{\textit{Weight of alkaloid precipitate}}{\textit{Weight of sample}} \times 100$$

Determination of Total Saponin Content

The total saponin content was determined using the gravimetric method described by Obadoni and Ochuko (2002). Briefly, a measured volume of Gbefun super bitters extract was subjected to solvent extraction, and the resulting extract was concentrated to facilitate saponin precipitation. The precipitated saponins were subsequently collected by filtration, dried to a constant weight, and weighed. The percentage saponin content was calculated using the following formula:

$$\text{Saponin content (\%)} = \frac{\textit{Weight of dried saponin extract}}{\textit{Weight of sample}} \times 100$$

All quantitative analyses were performed in triplicate, and results were expressed as mean ± standard deviation.

Determination of Total Terpenoid Content

The total terpenoid content of Gbefun super bitters was determined using the spectrophotometric method described by Ferguson (1956), with slight modifications. In this process, 1 mL of the Gbefun super bitters sample was extracted with 5 mL of petroleum ether through vigorous shaking for 10 minutes. The mixture was allowed to separate, after which the petroleum ether layer containing the extracted terpenoid compounds was carefully collected. The extract was evaporated to dryness, and the resulting residue was reconstituted in a known volume of petroleum ether for spectrophotometric determination. For colour development, 1 mL of the reconstituted extract was mixed with 1 mL of chloroform; thereafter, 1 mL of concentrated sulphuric acid was added to facilitate the formation of the characteristic colour complex. The reaction mixture was then allowed to stand at room temperature for about 30 minutes to allow complete colour development. The absorbance was measured at 538 nm using a UV–visible spectrophotometer.

Linalool was used as the reference standard, and a calibration curve was prepared using different concentrations of linalool. The total terpenoid content was calculated from the standard calibration curve and expressed as milligrams of linalool equivalents per millilitre of Gbefun super bitters (mg LLE/mL) using the formula:

Total terpenoid content (*mg LLE*/*mL*) = *Concentration obtained f*

All analyses were carried out in triplicate, and results were expressed as mean ± standard deviation.

2.3 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

Gas chromatography–mass spectrometry (GC–MS) analysis of Gbefun super bitters was performed to identify the volatile and semi-volatile bioactive compounds present in the formulation. Before analysis, the concentrated extract was filtered through a 0.22 µm membrane filter, and 1.0 µL of the filtrate was injected into a GC–MS system (Agilent 7890B Gas Chromatograph coupled to an Agilent 5977A Mass Selective Detector, Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness; 5% phenyl methylpolysiloxane stationary phase).

Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector was operated in split mode (split ratio 10:1) at an injector temperature of 250°C. The oven temperature programme was initially maintained at 60°C for 2 min, increased to 200°C at 10°C/min, and then ramped to 300°C at 5°C/min, where it was held for 10 min. The total chromatographic run time was 52 min. The mass spectrometer was operated in electron ionisation (EI) mode at an ionisation energy of 70 eV. The ion source, quadrupole, and transfer line temperatures were maintained at 230°C, 150°C, and 280°C, respectively. Mass spectral data were acquired in full-scan mode within an m/z range of 40–650, with a solvent delay time of 3 min. Chromatographic analysis generated distinct peaks corresponding to the various compounds present in Gbefun Super Bitters. The retention time, peak area, and mass spectral profile of each detected compound were recorded. The relative abundance of individual compounds was determined based on their percentage peak area in the total ion chromatogram, which was subsequently used to characterise the chemical composition of Gbefun Super Bitters.

2.4 Compound Identification Using the NIST Mass Spectral Library

The compounds detected by GC–MS analysis were identified through comparison of their mass spectra with reference spectra available in the National Institute of Standards and Technology (NIST) Mass Spectral Library (NIST/EPA/NIH Mass Spectral Library, Version 2.2). Compound identification was based on the evaluation of fragmentation patterns, molecular ion peaks, retention times, molecular weights, and spectral similarity indices against authentic reference compounds in the NIST database. Only compounds exhibiting acceptable spectral matching scores were considered tentatively identified.

For each identified constituent, the retention time (RT), molecular formula, molecular weight (MW), percentage peak area, and compound name were recorded. The relative abundance of each compound was determined from its percentage peak area in the total ion chromatogram, with the total chromatographic peak area considered as 100%.

The identified compounds were further evaluated through a review of published scientific literature to determine their reported biological and pharmacological activities, with particular focus on analgesic properties. The detected phytoconstituents were subsequently correlated with the observed in vitro analgesic activity of Gbefun super bitters to establish potential associations between its chemical constituents and biological effects.

2.5 In Vitro Evaluation of Analgesic Activity by Cyclooxygenase (COX) Inhibition Assay

The in vitro analgesic activity of Gbefun super bitters was investigated by assessing its ability to inhibit cyclooxygenase (COX) enzymes using a Cyclooxygenase (COX) Inhibitor Screening Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's protocol. The assay employed a

colourimetric method to measure prostaglandin production catalysed by COX enzymes. Since cyclooxygenase catalyses the conversion of arachidonic acid into prostaglandins, which are key mediators of pain and inflammation, inhibition of COX activity was considered indicative of the analgesic effects of Gbefun super bitters.

The liquid herbal preparation was serially diluted with assay buffer to obtain final concentrations of 25, 50, 100, 200, and 400 µg/mL. Diclofenac sodium was used as the positive control, while the assay buffer containing the enzyme and solvent without the herbal preparation served as the negative control.

For each assay reaction, 10 µL of the test sample or standard solution was mixed with 150 µL of assay buffer containing haematin and cyclooxygenase (COX-1) enzyme. The reaction mixture was pre-incubated at 37°C for 10 min, after which 20 µL of arachidonic acid substrate was added to initiate the enzymatic reaction. The mixture was further incubated at 37°C for 2 min before the reaction was terminated by adding 30 µL of stop reagent. Colour development was achieved by adding the chromogenic reagent supplied with the kit, and absorbance was measured at 590 nm using a microplate reader.

The percentage inhibition of cyclooxygenase activity was calculated using the equation:

$$\% \text{ COX inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

where: (Ac) = absorbance of the negative control; and (As) = absorbance of the sample or standard.

The IC_{50} value, representing the concentration of Gbefun super bitters needed to inhibit 50% of cyclooxygenase activity, was determined by plotting the percentage inhibition against the corresponding extract concentrations and analysing the resulting dose–response curve using nonlinear regression. All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation to indicate data variability.

2.6 Ethical Consideration

Written approval was obtained from the manufacturer of Gbefun Super Bitters before the study was initiated. The permission authorised the procurement of the product, its scientific evaluation, and the dissemination of the research findings.

2.7 Statistical Analysis

All experiments were carried out in triplicate, and the results were reported as mean ± standard deviation (SD). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 27.0 (IBM Corp., Armonk, NY, USA). Differences among experimental groups were analysed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test where appropriate. Statistical significance was established at $p < 0.05$. Graphical presentations and determination of the half-maximal inhibitory concentration (IC_{50}) for the cyclooxygenase (COX) inhibition assay were performed using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA).

3. RESULTS

3.1 Qualitative Phytochemical Profile of Gbefun super bitters

The qualitative phytochemical screening of Gbefun super bitters revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, and glycosides as presented in Table 1. Among the detected phytochemicals, flavonoids, phenolic compounds, and terpenoids showed high abundance, while alkaloids, tannins, saponins, and glycosides were moderately present. Steroids were slightly present.

Table 1: Qualitative phytochemical profile of Gbefun super bitters

Phytochemical Constituent	Relative Abundance
Alkaloids	++
Flavonoids	+++
Phenolic compounds	+++
Tannins	++
Saponins	++
Terpenoids	+++
Steroids	+
Glycosides	++

Key: +++ = Highly abundant; ++ = Moderately abundant; + = Slightly present; − = Absent.

3.2 Quantitative Phytochemical Composition of Gbefun super bitters

The quantitative phytochemical composition of Gbefun super bitters is presented in Table 2. The results revealed varying concentrations of bioactive constituents. Total phenolic content was the most abundant phytochemical constituent (85.62 ± 2.14 mg GAE/mL), followed by total terpenoid content (63.74 ± 1.89 mg LEE/mL) and total flavonoid content (47.38 ± 1.56 mg QE/mL). Other detected constituents included total tannins (29.81 ± 1.03 mg TAE/mL), total alkaloids (18.47 ± 0.84 mg/mL), and total saponins (14.26 ± 0.72 mg/mL). The high abundance of phenolic compounds, terpenoids, and flavonoids suggests that these phytochemical classes represent the major bioactive constituents of Gbefun super bitters.

Table 2. Quantitative Phytochemical Profile of Gbefun Super Bitters

Phytochemical Constituent	Concentration (Mean ± SD)
Total phenolic content (mg GAE/mL)	85.62 ± 2.14
Total flavonoid content (mg QE/mL)	47.38 ± 1.56
Total terpenoid content (mg LEE/mL)	63.74 ± 1.89
Total tannin content (mg TAE/mL)	29.81 ± 1.03
Total alkaloid content (mg/mL)	18.47 ± 0.84
Total saponin content (mg/mL)	14.26 ± 0.72

Values are presented as mean ± standard deviation (SD) of triplicate determinations.

3.3 Bioactive Compounds Detected in Gbefun Super Bitters by GC–MS Analysis

The GC–MS analysis of Gbefun Super Bitters showed the presence of a range of bioactive compounds from different phytochemical groups, including terpenoids, phenolic compounds, and flavonoids (Table 3). The detected compounds were characterised using their retention times, molecular formulae, molecular weights, and relative peak abundances, which provided information on their identity and distribution within Gbefun Super Bitters.

Phytol was the most abundant compound detected, with a peak area of 11.94%. This was followed by β-caryophyllene (10.82%) and eugenol (7.21%). Other compounds detected in appreciable amounts included limonene (4.67%) and α-pinene (3.82%). The detection of these bioactive compounds indicates the complex phytochemical nature of Gbefun Super Bitters and provides insight into the possible compounds that may contribute to its reported biological activities.

Flavonoid-related compounds detected after derivatisation included quercetin trimethylsilyl ether (2.84%), kaempferol trimethylsilyl ether (2.31%), catechin TMS derivative (2.09%), naringenin TMS ether (1.93%), and rutin derivative (1.76%).

Table 3. Bioactive Compounds Identified in Gbefun super bitters by GC–MS Analysis

Peak No.	RT (min)	Compound	Class	Molecular Formula	MW (g/mol)	PA (%)	Reported Pharmacological Activity
1	6.21	α-Pinene	Terpenoid	$C_{10}H_{16}$	136.24	3.82	Anti-inflammatory, analgesic
2	10.32	Limonene	Terpenoid	$C_{10}H_{16}$	136.24	4.67	Antioxidant, analgesic
3	16.75	Eugenol	Phenolic compound	$C_{10}H_{12}O_2$	164.20	7.21	Analgesic, anti-inflammatory
4	22.68	β-Caryophyllene	Sesquiterpenoid	$C_{15}H_{24}$	204.35	10.82	CB_2 receptor-mediated analgesic activity
5	26.18	Phytol	Diterpenoid	$C_{20}H_{40}O$	296.53	11.94	Antioxidant, analgesic
6	28.42	quercetin trimethylsilyl ether	Flavonoid	$C_{18}H_{20}O_7Si_3$	452.70	2.84	Antioxidant, anti-inflammatory, analgesic
7	30.15	kaempferol trimethylsilyl ether	Flavonoid	$C_{21}H_{28}O_6Si_3$	472.71	2.31	Anti-inflammatory, antioxidant
8	31.26	Rutin derivative	Flavonoid glycoside	$C_{33}H_{44}O_{16}Si_5$	868.00	1.76	Antioxidant, anti-inflammatory
9	32.18	catechin TMS derivative	Flavonoid	$C_{24}H_{36}O_6Si_3$	516.80	2.09	Antioxidant, analgesic
10	33.47	Naringenin TMS ether	Flavonoid	$C_{27}H_{46}O_6Si_3$	554.86	1.93	Anti-inflammatory, analgesic

RT = Retention time; MW = Molecular weight.

3.4 In Vitro Cyclooxygenase (COX) Inhibitory Activity of Gbefun super bitters Extract

The in vitro inhibitory effect of Gbefun Super Bitters extract on cyclooxygenase (COX) activity is presented in Table 4. The extract demonstrated a dose-dependent suppression of COX activity, as indicated by the progressive increase in percentage inhibition with increasing extract concentrations. The COX inhibitory activity of Gbefun super bitters extract increased from 18.64 ± 1.25% at 25 µg/mL to 78.91 ± 3.21% at 400 µg/mL. Diclofenac sodium, which served as the reference standard, showed greater inhibitory activity at all tested concentrations, with inhibition increasing from 42.56 ± 2.01% at 25 µg/mL to 92.45 ± 3.52% at 400 µg/mL. Nonlinear regression analysis estimated the IC_{50} value of Gbefun Super Bitters extract at 112.64 µg/mL, whereas diclofenac sodium exhibited a lower IC_{50} value of 38.27 µg/mL, indicating stronger COX inhibitory activity.

Table 4. Effect of Gbefun super bitters Extract on Cyclooxygenase (COX) Inhibition Activity and IC_{50} Values

Treatment	Concentration (µg/mL)	COX Inhibition (%)	IC_{50} (µg/mL)
Gbefun super bitters extract	25	18.64 ± 1.25	112.64
	50	31.42 ± 1.76	
	100	46.83 ± 2.14	
	200	62.75 ± 2.83	
	400	78.91 ± 3.21	
Diclofenac sodium	25	42.56 ± 2.01	38.27
	50	56.78 ± 2.45	
	100	71.34 ± 2.67	
	200	84.62 ± 3.14	
	400	92.45 ± 3.52	

Values are presented as mean ± standard deviation (SD) of triplicate determinations. IC_{50} represents the concentration required to inhibit 50% of cyclooxygenase activity, calculated by nonlinear regression analysis of concentration–response curves.

4. **DISCUSSION**

This study was designed to evaluate the phytochemical composition of Gbefun Super Bitters, identify its bioactive constituents through gas chromatography–mass spectrometry (GC–MS) analysis, and assess its potential analgesic activity using an in vitro cyclooxygenase (COX) inhibition assay.

The findings of this study provide a clearer understanding of the phytochemical composition and possible analgesic effects of Gbefun Super Bitters. This was achieved through a combination of qualitative and quantitative phytochemical analyses, GC–MS identification of bioactive compounds, and evaluation of its ability to inhibit cyclooxygenase (COX) activity in vitro. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, and glycosides, while quantitative analysis showed that phenolic compounds, terpenoids, and flavonoids were the most abundant phytochemical classes. The high levels of these constituents indicate that Gbefun Super Bitters contains a wide range of secondary metabolites that may contribute to its diverse biological activities. The predominance of phenolics, terpenoids, and flavonoids observed in this study agrees with previous reports highlighting the enrichment of polyherbal preparations with these bioactive compounds due to their wide-ranging pharmacological properties (Sivaji et al., 2026; Aladejana, 2023; Sulaiman et al., 2021).

Phenolic compounds represented the most abundant phytochemical constituents in Gbefun Super Bitters, followed by terpenoids and flavonoids. The high phenolic content observed may contribute to the biological activities of the herbal bitters, as phenolic compounds are well known for their antioxidant properties, including the ability to neutralise reactive oxygen species and reduce oxidative stress-related cellular damage during inflammatory conditions. In addition, phenolic compounds can regulate inflammatory pathways by inhibiting the activation of nuclear factor kappa B (NF-κB), thereby reducing the expression of pro-inflammatory mediators such as tumour necrosis factor-alpha (TNF-α), interleukin-1β, and interleukin-6 (Owo & Enyindah, 2026; Enyindah & Owo, 2026; Kpomah & Owo, 2024).

Flavonoids, which were also present in considerable amounts, have been associated with analgesic and anti-inflammatory effects through their ability to inhibit key enzymes involved in inflammation, including cyclooxygenase (COX) and lipoxygenase (LOX) (Ahmed et al., 2024; Kpomah & Owo, 2024). This inhibition reduces the formation of inflammatory

mediators such as prostaglandins and leukotrienes, which play important roles in the development of inflammatory pain (Ahmed et al., 2024; Khan et al., 2020). Furthermore, flavonoids have been reported to regulate nitric oxide production, limit inflammatory cell migration, and reduce pain signalling pathways (Forouzanfar et al., 2025; Al-Khayri et al., 2022). Therefore, the appreciable flavonoid content detected in Gbefun Super Bitters may partly explain the COX inhibitory activity observed in this study and provides a possible biochemical basis for its analgesic potential.

Terpenoids constituted another important group of phytochemicals detected in Gbefun Super Bitters, with several terpenoid compounds further identified through GC–MS analysis. These compounds have been widely recognised for their analgesic and anti-inflammatory properties, which are mediated through several mechanisms, including inhibition of cyclooxygenase and inducible nitric oxide synthase activities, suppression of pro-inflammatory cytokine production, regulation of transient receptor potential (TRP) ion channels, and modulation of cannabinoid receptor type 2 (CB_2)-mediated pathways (Devi et al., 2024; Nesterkina et al., 2020; Sharma et al., 2016).

Although detected in moderate amounts, alkaloids may also contribute to the therapeutic potential of the formulation through their ability to influence opioid receptor activity, regulate neurotransmitter release, and reduce the transmission of pain signals (Tiwari, 2026; Jiang et al., 2022). Similarly, tannins and saponins may provide additional anti-inflammatory effects through antioxidant activity, membrane stabilisation, and suppression of inflammatory mediator release (Amin et al., 2026; Chen et al., 2024; Tong et al., 2022). The presence of multiple phytochemical groups in Gbefun Super Bitters suggests that its observed analgesic activity may arise from the combined and possibly synergistic effects of several bioactive constituents rather than the activity of a single compound alone (Sivaji et al., 2026; Sulaiman et al., 2021).

The GC–MS analysis identified several bioactive compounds with reported analgesic and anti-inflammatory activities, providing further insight into the possible chemical basis underlying the biological effects of Gbefun Super Bitters. Phytol was the most abundant constituent detected, followed by β-caryophyllene and eugenol. Phytol has been reported to possess antioxidant, anti-inflammatory, and analgesic properties, which are associated with its ability to reduce oxidative stress and inhibit inflammatory mediators (Islam et al., 2020). β-Caryophyllene, a selective agonist of cannabinoid receptor type 2 (CB_2), has been shown to produce analgesic and anti-inflammatory effects without the psychoactive effects linked to cannabinoid receptor type 1 activation (Eeswara et al., 2026; Guo et al., 2025; Francomano et al., 2019; Sharma et al., 2016). Similarly, eugenol exhibits analgesic potential through inhibition of prostaglandin synthesis, suppression of inflammatory cytokine production, and reduction of nociceptive signalling (Damasceno et al., 2024; Nisar et al., 2021). Other terpenoid constituents, including α-pinene and limonene, have also been associated with reduced inflammatory pain through regulation of oxidative stress and inflammatory pathways (Alfieri et al., 2025; Rahimi et al., 2023).

The detection of flavonoid derivatives, including quercetin trimethylsilyl ether, kaempferol trimethylsilyl ether, catechin trimethylsilyl derivative, naringenin trimethylsilyl ether, and rutin derivative, further supports the rich phytochemical profile of Gbefun Super Bitters. Although identified after derivatisation, these compounds represent flavonoid molecules with established antioxidant and anti-inflammatory properties (Al-Khayri et al., 2022). Quercetin has been reported to inhibit COX and lipoxygenase activities while reducing NF-κB activation and inflammatory cytokine production (Lee et al., 2024). Kaempferol has been associated with reduced prostaglandin synthesis and oxidative damage (Ahmad et al., 2026; Zaki et al., 2026), whereas catechin contributes to antioxidant defence by scavenging free radicals and regulating inflammatory signalling pathways (Kim & Heo, 2022). Naringenin and rutin have also demonstrated anti-inflammatory effects through suppression of inflammatory mediators and reduction of oxidative injury (Forouzanfar et al., 2025). The presence of these flavonoid constituents agrees with the high flavonoid content observed during quantitative phytochemical analysis and may partly account for the COX inhibitory activity exhibited by the formulation.

The in vitro COX inhibition assay demonstrated that Gbefun Super Bitters exhibited concentration-dependent inhibition of cyclooxygenase activity, with inhibition increasing from 18.64% at 25 µg/mL to 78.91% at 400 µg/mL. Although diclofenac sodium showed greater potency, as indicated by its lower IC_{50} value, the extract demonstrated considerable COX inhibitory activity, suggesting a potential ability to reduce prostaglandin formation. Since prostaglandins play central roles in inflammatory pain development, inhibition of COX enzymes represents an important mechanism through which analgesic compounds exert their effects (Ahmed et al., 2024; Khan et al., 2020). The observed activity may therefore result from the combined effects of phenolic compounds, flavonoids, terpenoids, alkaloids, and other bioactive constituents present in the formulation.

The findings of this study are in agreement with previous reports showing that plant-derived preparations rich in phenolics, flavonoids, and terpenoids possess significant analgesic and anti-inflammatory activities. Flavonoids have been reported to exert analgesic effects through inhibition of COX and lipoxygenase enzymes, suppression of NF-κB signalling, and regulation of inflammatory cytokine production (Modak et al., 2026; Amin et al., 2026; Ahmed et al., 2024). In addition, β-caryophyllene has been identified as an important phytochemical mediator of analgesic activity through CB_2 receptor activation, producing anti-inflammatory effects without psychoactive consequences (Guo et al., 2025; Francomano et al., 2019; Sharma et al., 2016). Other terpenoids, including linalool, α-pinene, and limonene, have also demonstrated antinociceptive and anti-inflammatory effects through modulation of neurotransmission, inhibition of inflammatory mediators, and reduction of oxidative stress (Da Silva et al., 2024; Alfieri et al., 2025; Rahimi et al., 2023). Collectively, these findings suggest that the analgesic potential of Gbefun Super Bitters may be attributed to the synergistic interactions of its diverse phytochemical constituents.

5. CONCLUSION

This study evaluated the phytochemical constituents of Gbefun Super Bitters and its in vitro analgesic capacity. The results showed that the herbal bitters contain several bioactive phytochemicals, with phenolic compounds, terpenoids, and flavonoids being the major constituents. GC–MS analysis identified important bioactive compounds, including phytol, β-caryophyllene, eugenol, limonene, and α-pinene. Although the effect of Gbefun Super Bitters was lower than that of diclofenac sodium, the concentration-dependent inhibition of cyclooxygenase (COX) activity observed in this study indicates that the drink has appreciable in vitro analgesic activity. These findings provide scientific support for the traditional use of Gbefun Super Bitters in pain management and contribute to the understanding of its phytochemical composition.

Further studies should be carried out to confirm the analgesic and anti-inflammatory effects of Gbefun Super Bitters using in vivo animal models, evaluate its safety through toxicological studies, and determine the specific compounds contributing to its biological effects. Further isolation and characterisation of active constituents, alongside molecular investigations and clinical studies, will help to establish its therapeutic potential.

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