

Evaluation of the Anti-Inflammatory activity and Phytochemical Profiling of *Bougainvillea glabra* Choisy Flower and Bract

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ABSTRACT

Background: *Bougainvillea glabra* is an ornamental plant with reported ethnomedicinal uses. Its genus *Bougainvillea* possesses anti-inflammatory activity and its bract is rich in phenolics. No study has examined the anti-inflammatory activity of the combined flower and bract.

Methods: This study evaluated the anti-inflammatory effect of *B. glabra* flower and bract (both structures parts contribute to the plant's phytochemicals) and profiled their constituents via gas chromatography-mass spectrometry (GC-MS). The air-dried flower and bract were pulverised, macerated in 80% v/v ethanol, and the crude extract was partitioned with n-hexane and dichloromethane (DCM). Each fraction was evaluated for anti-inflammatory activity (anti-denaturation and nitric oxide (NO) scavenging assays), and the active fractions were profiled via GC-MS.

Results: The crude extract yielded 1.300%, from which the n-hexane and DCM fractions were recovered at 32.980 and 17.020%, respectively. Both fractions inhibited protein denaturation comparably to diclofenac sodium at fixed concentration ($p > 0.05$), while diclofenac sodium required statistically lower concentrations to achieve the same effect ($IC_{50} = 0.037 \pm 0.002$ mg/mL; $p = 0.013$). Both fractions exhibited NO scavenging activity comparable to ascorbic acid at fixed percentage inhibition ($p > 0.05$), though ascorbic acid demonstrated significantly greater potency ($IC_{50} = 0.046 \pm 0.001$ mg/ml; $p < 0.0001$) than n-hexane and DCM fractions. GC-MS analysis identified twenty-nine unique compounds across both fractions, with oleic acid, 9,12-octadecadienoic acid, and n-hexadecanoic acid among the dominant constituents.

Conclusion: These findings suggest that the n-hexane and DCM fractions of *B. glabra* flower and bract possess moderate anti-inflammatory activity, which may be associated with the identified compounds.

1.0 Introduction

Inflammation, derived from the Latin 'inflammare' ('to ignite'), is a fundamental response of the vascular system to pathogens, injury, or chemical irritants, aimed at eliminating the injurious stimulus and initiating tissue repair^{1,2}. While acute inflammation is protective, chronic inflammation is a central driver of many pathologies, including rheumatoid arthritis, cardiovascular disease, diabetes, and certain malignancies, and is often sustained

by prolonged antigen exposure, autoimmune reactions, or unresolved acute responses²⁻⁶.

Current pharmacological management relies mainly on non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids⁷⁻⁸, whose long-term use is limited by adverse effects such as gastrointestinal ulceration, renal impairment, and cardiovascular risk. This has renewed interest in ethnopharmacology and plant-derived bioactive compounds that may offer comparable efficacy with a safer

profile⁹.

Bougainvillea glabra Choisy (Nyctaginaceae), commonly known as glory of the garden, is an ornamental climbing shrub widely distributed in tropical and subtropical regions¹⁰. Various parts of the plant are used in traditional medicine to treat respiratory complaints, fever, and digestive disorders¹¹, and its biological activities have been linked to diverse secondary metabolites, including alkaloids, flavonoids (some of which possess antioxidant and membrane-stabilising properties), betacyanins (potent free-radical scavengers), and saponins and tannins (inflammatory modulators)¹².

Prior investigations of *B. glabra*'s anti-inflammatory potential have focused mainly on the leaves. Ogunwande et al.¹³ reported the chemical composition, anti-nociceptive and anti-inflammatory activities of essential oil from *B. glabra* leaves, while Manivannan et al.¹⁴ and Mandal et al.¹⁵ similarly demonstrated significant anti-inflammatory activity (carrageenan-induced paw oedema and cotton pellet granuloma inhibition) in the leaves of the closely related species *Bougainvillea spectabilis*. Markandan et al.¹⁶ determined the total phenolic and flavonoid contents and antioxidant activity of *B. glabra* bracts extracted at varying methanol concentrations, but did not assess the anti-inflammatory effect or extend the investigation to the flower nor profiled the phytochemistry. These studies established that the genus *Bougainvillea* genuinely possesses anti-inflammatory activity and that the bract is a phenolic-rich tissue. However, no study has examined the anti-inflammatory activity of the flower or bract, and none of these studies, whether on the leaf's anti-inflammatory activity or the bract's antioxidant activity, paired the bioactivity data with a detailed phytochemical profile of the tissue tested, so the specific constituents responsible for the reported activities remain unidentified.

The flowers and bracts are of particular interest because, in *Bougainvillea glabra*, the conspicuous coloured structures are largely bracts subtending small true flowers, and both are rich in the pigmented and phenolic metabolites associated with the plant's antioxidant and anti-inflammatory properties. Evaluating the flower and bract together, alongside GC-MS profiling, allows the observed bioactivity to be linked to specific constituents rather than treated in isolation. The *in vitro* models used here (protein anti-denaturation, and NO radical scavenging) were selected because they interrogate distinct but complementary mechanisms of inflammation and provide a practical, reproducible first screen ahead of more resource-intensive *in vivo* work. This study therefore aims to evaluate the anti-inflammatory activity of the n-hexane and

DCM fractions of *B. glabra* flower and bract using these established *in vitro* models, and identify their phytochemical constituents via GC-MS.

2.0 Materials and Methods

2.1 Collection and Identification of the Plant Sample

The fresh flower and bract of *Bougainvillea glabra* were collected at the Institute for Entrepreneurship and Development Studies (IFEDS), Obafemi Awolowo University Ile-Ife, Nigeria, in November, 2024 (dry season): GPS coordinates of the collection site, **7.511067 °N, 4.524998 °E**. The plant sample was identified and authenticated (Voucher: **IFE-18385**) by Mr. Ademoriyo Gabriel at the IFE HERBARIUM, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, where a voucher specimen has been deposited for future reference.

2.1.1 Flower Sample Preparation

The flowers and bracts were air dried for six weeks at laboratory temperature (25 ± 2 °C) and milled into powder using electric machine (Model: Electric feed mill dry grinder, 110V 3000W). The powdered material (70 g) was extracted in 80% (v/v) ethanol (960 mL) inside a glass jar. The mixture was stirred intermittently for 48 h and filtered through cotton wool. Eighty percent (v/v) ethanol was chosen as it is widely reported to give a good balance of polar and non-polar metabolite recovery from plant material, including phenolics and flavonoids relevant to anti-inflammatory activity¹⁷. The process was repeated and the bulked filtrate was concentrated *in vacuo* on a rotary evaporator (at 50 °C) to dryness.

2.1.2 Solvent-Solvent Partitioning of Crude Extract of *B. glabra*

The crude extract (0.94 g) was dissolved in 500 mL of distilled water and partitioned successfully with n-hexane (500 mL x 3) and DCM (500 mL x 3) inside a separatory funnel. The n-hexane and DCM were selected for sequential partitioning to separate non-polar (n-hexane) from semi-polar (DCM) constituents of the crude extract, allowing the anti-inflammatory contribution of each polarity range to be compared. Consistent with reports that phenolic and flavonoid content declines significantly in aqueous fractions relative to fractions of lower polarity¹⁸⁻¹⁹, the aqueous fraction obtained in this study was excluded from further analysis, as it was considered unlikely to contribute meaningfully to the non-polar/semi-polar anti-inflammatory activity under investigation. Consequently, the n-hexane and DCM fractions were collected into

separate containers. The two fractions were allowed to air dry to obtain their respective fractions, labelled as the n-hexane fraction and DCM fraction. The percentage yield of each fraction was calculated as (weight of fraction / weight of crude extract partitioned) $\times$ 100.

2.2 *In vitro* Anti-inflammatory analysis of n-Hexane and DCM Fractions

2.2.1 Inhibition of Albumin Denaturation Assay

The anti-denaturation assay using bovine serum albumin (BSA) as standard protein and diclofenac sodium as control drug was evaluated. The reaction mixture contained 0.5mL (BSA, 0.25 mg/mL) and varying concentrations of the extracts (0-300 μ g/mL) to a volume of 3.0 mL. This was incubated at 37 °C for 20 min before heating at 57 °C for 3 min. The mixture was allowed to cool before addition of 2.5 ml phosphate buffer (0.5 M, pH 6.3). Thereafter, 1.0 mL of the reaction mixture was pipetted into clean test tubes followed by addition of alkaline copper reagent (1.0 mL) and 1.0 mL of Folin-Ciocateu's reagent (10%). The reaction mixture was incubated at 55 °C for 10 min and allowed to cool. Absorbance of the intact protein was measured at 650 nm against the reagent blank²⁰. The assay was carried out in duplicate.

$$\text{Quantity of protein left} = \frac{(\text{Abs of Test} - \text{Abs of Control})}{(\text{Abs of Test})}$$

$$\text{Percentage of Inhibition} = \frac{\text{Quantity of protein left} \times 100}{\text{Total protein}}$$

Where Absorbance of control represents 100% denaturation.

2.2.2 Nitric Oxide Radical Scavenging Assay

The nitric oxide radical scavenging activity of the fractions was measured via Griess' reagent method. The reaction mixture consisted of 0.1 mL of each fraction (0.3125, 0.625, 1.25, 2.5, 5 and 10 mg/mL) and 0.9 mL of sodium nitroprusside (2.5 mM) in phosphate buffer saline. This was incubated under light for 150 min. Then, 0.5 mL of 1% sulphanilamide in 5% phosphoric acid was added and incubated in the dark for another 10 min. Then, 0.5 mL of 0.1% N-1-naphthylethylenediamine dihydrochloride (NED) was added and the absorbance was measured at 546 nm²¹⁻²². The test was conducted in duplicate. The NO scavenging activity was calculated as:

$$\% \text{ Inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100$$

Sample concentration providing 50% inhibition (IC_{50}) was intrapolated from the graph by plotting the percentage inhibition against the extract concentration.

2.3 GC-MS Analyses of n-Hexane and DCM Fractions

The constituents of the n-hexane and DCM fractions were profiled on a Varian 3800/4000 gas chromatograph-mass spectrometer (Agilent Technologies, USA) system equipped with a BP5 capillary column (30 m $\times$ 0.25 mm ID, 0.25 μ m film thickness)²³. The oven temperature was initially held at 70 °C for 3 min. It was then increased to 300 °C at 10°C/min, with a final hold time of 9 min. The injection port and ion source temperatures were maintained at 250 and 230 °C, respectively, while the interface temperature was set to 240 °C. The helium (purity 99.99%) carrier gas was maintained at a constant flow rate of 1.5 mL/min. The sample was introduced in split mode (10:1). The mass spectrometer operated in electron ionisation (EI) mode at 70 eV, scanning a mass range of 40 – 800 m/z. The total run time was 25 min, with an initial solvent delay of 3 min. Also, the mass spectra and retention time of constituents were compared with the online library (National Institute of Standards and Technology NIST08) to identify the sample constituents. Compound identification was confirmed based on a match probability of the fragmentation patterns against the database containing > 62,000 standard compounds.

2.4 Data Analysis

All data were expressed as mean $\pm$ standard error of the mean (SEM; n = 2). Statistical analysis was performed using GraphPad Prism software (version 8.0.2, GraphPad Software, San Diego, California, USA). Differences among groups were analyzed using ordinary one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons post hoc test to determine statistical significance between individual group means. Prior to ANOVA, the Brown-Forsythe test and Bartlett's test were used to assess the homogeneity of variances among groups. A P-value of less than 0.05 was considered statistically significant.

3.0 Results

3.1 Percentage Yields of the Crude Extract and Fractions of *B. glabra* Flower and Bract

The percentage yield of the crude extract was 1.300% while the n-hexane and DCM fractions gave 32.980 and 17.020%, respectively.

3.2 Effect of *B. glabra* Flower and Bract Extract on Protein Denaturation Inhibition

The effect of *Bougainvillea glabra* flower and bract extract on the inhibition of protein denaturation is presented in

Figure 1 while the IC_{50} is presented in **Table 1**. The percentage albumin denaturation inhibitory activity of *B. glabra* flower and bract fractions (n-hexane fraction = $30.660 \pm 4.090\%$, DCM fraction = $23.660 \pm 6.640\%$) did not differ significantly from that of diclofenac sodium standard ($47.840 \pm 9.630\%$), ($F(2, 15) = 3.025$, $p = 0.0788$; $R^2 = 0.2874$). At the concentrations tested, both fractions

possessed comparable protein denaturation inhibitory capacity to the standard anti-inflammatory drug, with the observed differences in mean inhibition not attributable to the treatment (fraction type) rather than random variation. The R^2 value also shows that the treatment accounted for only 28.740% of the overall variability in inhibitory activity.

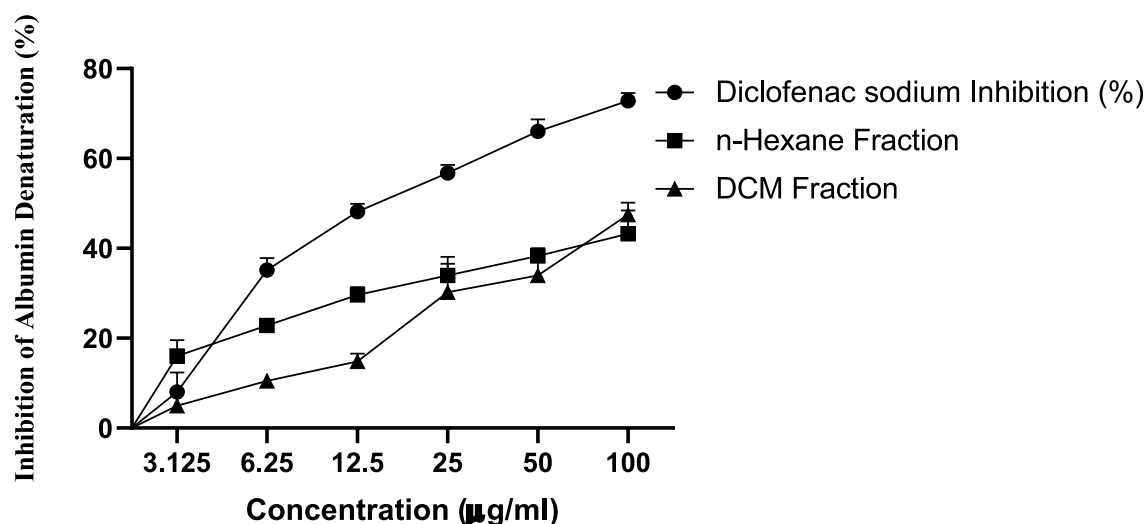


Figure 1. Anti-denaturation activity of *B. glabra* flower and bract fractions and diclofenac sodium at varying concentrations. Values were expressed as Mean $\pm$ SEM ($n=2$).

Conversely, the IC_{50} values for protein denaturation inhibitory activity of *B. glabra* flower and bract fractions (n-hexane fraction = 1.188 ± 0.197 mg/mL, DCM fraction = 0.972 ± 0.065 mg/mL) differed significantly from that of diclofenac sodium (0.037 ± 0.002 mg/mL), ($F(2, 3) = 26.020$, $p = 0.013$; $R^2 = 0.946$). Tukey's post-hoc multiple comparisons showed that diclofenac sodium was significantly lower than both the n-hexane fraction ($p = 0.013$) and the DCM fraction ($p = 0.024$), while the n-hexane and DCM fractions did not differ significantly from each other ($p = 0.495$).

Table 1. IC_{50} values (mg/mL) for protein denaturation inhibitory activity of diclofenac sodium, n-hexane fraction, and DCM fraction of *B. glabra* flower and bract.

Group	IC 50 (mg/ml)	95% CI
Diclofenac sodium	0.037 ± 0.002^a	0.013 - 0.061
n - Hexane fraction	1.188 ± 0.197^b	-1.319 - 3.696
DCM fraction	0.972 ± 0.065^b	0.150 - 1.793

Values are expressed as mean $\pm$ SEM ($n = 2$). Values bearing different superscript letters within the column are significantly different ($p < 0.05$, Tukey's multiple comparisons test); values bearing the same superscript letter are not significantly different ($p = 0.495$).

3.3 Nitric Oxide (NO) Radical Scavenging Activity of *B. glabra* Flower and Bract Extract

The percentage NO radical scavenging activity of *B. glabra* flower and bract is presented in **Figure 2** while the IC_{50} is presented in **Table 2**. The percentage NO radical scavenging activity of the fractions (n-hexane fraction = $33.130 \pm 9.400\%$, DCM fraction = $28.020 \pm 7.580\%$) did not differ significantly from that of ascorbic acid standard ($42.31 \pm 9.79\%$), ($F(2, 15) = 0.651$, $p = 0.536$; $R^2 = 0.079$). At the concentrations tested, both fractions of *B. glabra* possessed comparable NO radical scavenging capacity to that of the standard ascorbic acid, with the observed differences in mean inhibition not attributable to the treatment (fraction type) rather than random variation.

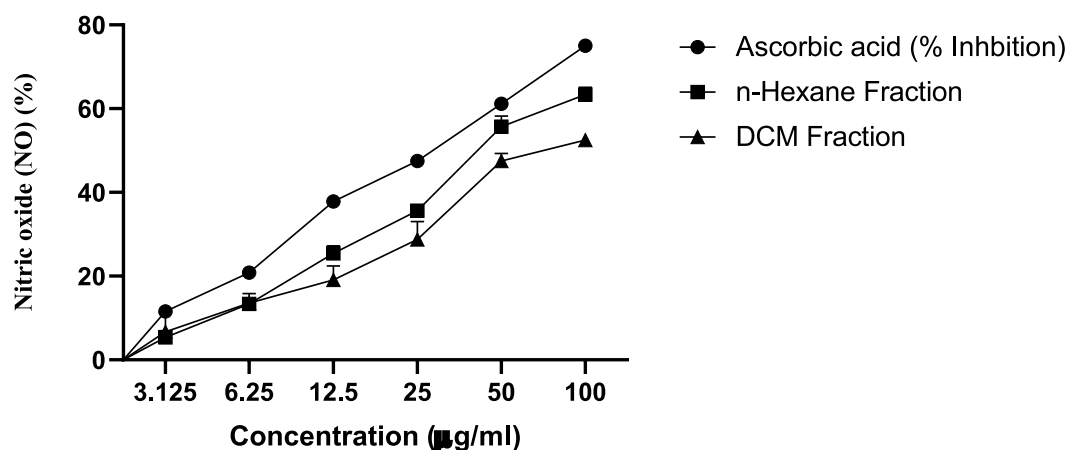


Figure 2. Nitric oxide radical scavenging activity of *B. glabra* flower and bract fractions and ascorbic acid at varying concentrations. Values were expressed as Mean $\pm$ SEM (n=2).

Furthermore, the IC_{50} values for NO radical scavenging activity of *B. glabra* flower and bract fractions (n-hexane fraction = 0.625 ± 0.004 mg/mL, DCM fraction = 0.804 ± 0.006 mg/mL) differed significantly from that of ascorbic acid standard (0.046 ± 0.001 mg/mL), ($F(2, 3) = 9314$, $p < 0.0001$; $R^2 = 0.999$). Tukey's post-hoc multiple comparisons confirmed that all pairwise differences were statistically significant ($p < 0.0001$ in each case), with ascorbic acid exhibiting the lowest IC_{50} and the DCM fraction the highest

Table 2. IC_{50} values (mg/mL) for nitric oxide radical scavenging activity of ascorbic acid, n-hexane fraction, and DCM fraction of *B. glabra* flower and bract

Treatment Group	IC 50 (mg/ml)	95% CI
Ascorbic acid	0.046 ± 0.001^a	0.033 – 0.059
n -Hexane fraction	0.625 ± 0.004^b	0.573 – 0.676
DCM fraction	0.804 ± 0.006^c	0.732 – 0.877

Values are expressed as mean $\pm$ SEM (n = 2). Values bearing different superscript letters within the column are significantly different ($p < 0.0001$, Tukey's multiple comparisons test). Values with (^a) are statistically lower than (^{b,c}) within the column. Also, values with (^b) are statistically lower than (^c).

3.5 Constituents Identified in the n-Hexane and DCM Fractions of *B. glabra*

The chromatogram and identified compounds in the n-hexane fraction are shown in **Figure 3** and **Table 3** whereas those of the DCM fraction are presented in **Figure 4** and **Table 4**. The GC-MS analysis identified eighteen (18) compounds in n-hexane fraction, dominated by seven compounds constituting 63.61% of the total sample weight (**Table 3**). Meanwhile, twelve (12) compounds were identified in DCM fraction dominated by two (2) compounds, constituting 56.58% of the total sample weight (**Table 4**). The major constituents of the n-hexane fraction were octadecanoic acid (14.41%); n-hexadecanoic acid (13.11%), 1,19-eicosadiene (8.63%), oleic acid (7.21%), 5,7,3',4'-tetrahydroxyflavone (7.13%), cholesta-4,6-dien-3-ol, (3 β) (6.62%), and 9,12-octadecadienoic acid (6.50%). Conversely, eugenol (29.5%) and undecane (27.08%) dominated the DCM fraction.

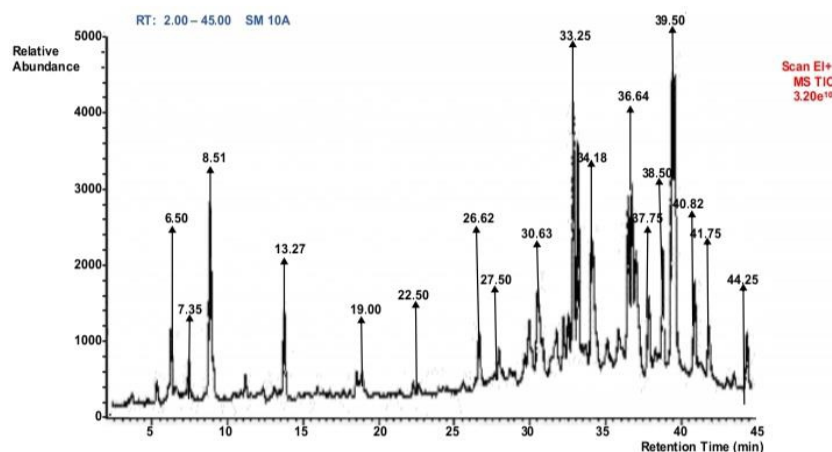


Figure 3: GC-MS chromatogram of *B. glabra* n-hexane fraction showing relative abundance versus retention time.

Table 3: Compounds identified by GC-MS in *B. glabra* n-hexane fraction.

Peak No	Retention Time (min)	Suggested Compound	Compound (wt %)	m/z
1	6.50	1,7,7-trimethyl, (1S-endo) Bicyclo [2.2.1] heptan-2-ol	1.73	108
2	7.35	9-Borabicyclo [3.3.1] nonane	3.28	122
3	8.51	(6R,7aS)-3,6-dimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	5.48	166
4	13.29	3-Azabicyclo [3.2.2] nonane	2.24	125
5	19.00	Terpinen-4-ol	2.19	154
6	22.50	6,10,14-trimethyl-2-Pentadecanone	0.90	268
7	26.62	9-Octadecenoic acid, (E)	2.72	555
8	27.50	Methylhexadecanoate	3.49	270
9	30.63	9,12-Octadecadienoic acid (Z, Z)	6.50 *	280
10	33.25	n-Hexadecanoic acid	13.11 *	256
11	34.18	1,19-Eicosadiene	8.63 *	240
12	36.64	Oleic acid	7.21 *	282
13	37.25	Eicosane	5.49	282
14	38.50	5,7,3',4'-tetrahydroxyflavone	7.13 *	286
15	39.50	Octadecanoic acid	14.41 *	284
16	40.82	Cholesta-4,6-dien-3-ol, (3 β)	6.62 *	384
17	41.75	12-hydroxymethyl ester 9-Octadecanoic acid,	4.75	312
18	44.25	Stigmasterol	4.11	412

* Major constituents present.

Figure 4: GC-MS chromatogram of *B. glabra* DCM fraction showing relative abundance versus retention time.

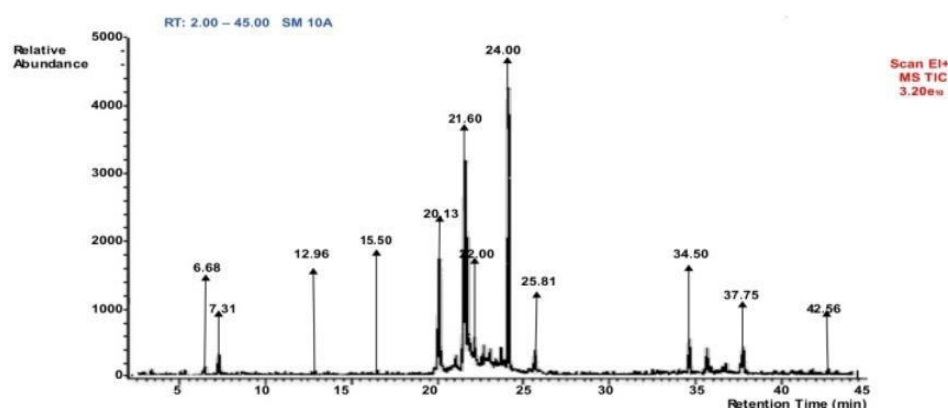


Table 4: Compounds identified by GC-MS in *B. glabra* DCM fraction.

Peak No	Retention Time (min)	Suggested Compound	Comp(wt%)	m/z
1	6.68	2,3- dihydrobenzofuran	3.72	120
2	7.31	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one,	3.51	144
3	12.96	2,4-dimethyl-1-pentanol	2.98	116
4	15.50	1,11 -Dodecadiene	2.65	166
5	20.13	Hexadecane	2.80	226
6	21.60	Undecane	27.08 *	184
7	22.00	Tetradecane	6.91	198
8	24.00	Eugenol	29.50 *	164
9	25.81	n-Hexadecanoic acid	5.15	256
10	34.50	Phytol	6.65	296
11	37.75	(2E,6E,10E)-3,7,11,15 -Tetramethylhexadeca -2,6,10,14-tetraen-1-yl formate	6.21	318
12	42.56	Squalene	2.59	410

* Major components of the mixture.

4.0 Discussion

This study evaluated the anti-inflammatory property of *B. glabra* flower and bract and profiled the constituents that may be associated with its anti-inflammatory activity. The percentage yield of the crude extract of *B. glabra* flower and bract obtained in this present study (1.300%) was markedly lower than the yield range (20-38%) reported by Markandan et al.¹⁶ for *B. glabra* bracts extracted with varying methanol concentrations (50, 70, and 100%). This divergence could be attributed to differences in the extraction solvent employed. Methanol is generally more efficient than ethanol at solubilizing a broader range of

phytoconstituents, including sugars, glycosides, and polar phenolics, compared to ethanol used in the present study²⁴. Also, the study by Markandan et al. explored the bract alone, whereas the present study combined the flower and bract extract, which may have introduced compositional differences that affected the overall extractive yield. Following the partitioning of the crude extract (1.300%), the n-hexane fraction accounted for the highest percentage recovery (32.980%), followed by the DCM fraction (17.020%), together representing 50% of the crude extract mass. This suggests that a substantial proportion of the non-polar and moderately polar constituents of *B. glabra* flower

and bract were preferentially partitioned into n-hexane and DCM solvent systems. The remainder, presumably comprising more polar constituents, would be expected in fractions of higher polarity not examined in the present study.

Given that several classes of plant-derived anti-inflammatory agents (including terpenoid-rich essential oil components) are lipophilic in nature²⁵, the n-hexane fraction may represent a concentrated pool of potentially bioactive nonpolar metabolites in *B. glabra* flowers and bracts. Similarly, dichloromethane has been shown to selectively extract flavonoid compounds with anti-cyclooxygenase-2 and anti-15-lipoxygenase activities²⁶⁻²⁷, suggesting that the DCM fraction of *B. glabra* flowers and bracts may likewise be enriched in intermediate-polarity anti-inflammatory constituents.

Furthermore, protein (albumin) denaturation is a well-established biomarker of inflammation, as denatured proteins expose antigenic determinants associated with hypersensitivity reactions and are implicated in the pathophysiology of conditions such as rheumatoid arthritis²⁸. Consequently, inhibition of protein denaturation is widely used as an *in vitro* screening model for anti-inflammatory activity. In this present study, both the n-hexane and DCM fractions of *B. glabra* flower and bract exhibited appreciable inhibition of protein denaturation, though this activity did not differ significantly from that of diclofenac sodium reference standard, ($F(2, 15) = 3.025$, $p = 0.0788$).

When the concentration-dependent response was evaluated via IC_{50} determination, a significant difference emerged among the groups, $F(2, 3) = 26.020$, $P = 0.013$, with diclofenac sodium requiring markedly lower concentrations than the n-hexane and fractions to achieve 50% inhibition. This apparent divergence between the non-significant ANOVA at fixed percentage inhibition and the significant ANOVA at IC_{50} reflects the greater sensitivity of the IC_{50} parameter, which integrates the full concentration-response relationship rather than comparing means at a single concentration point. It also suggests that while the fractions are capable of inhibiting protein denaturation, they do so with lower potency than the standard drug.

Comparable trends have been reported for other plant species evaluated by this method. For instance, the crude ethanolic extracts of Jamaican plants (*Cajanus cajan*, *Cinnamomum zeylanicum*, *Cordia alba*, *Mangifera indica*, and *Tecoma stans*) significantly inhibited the denaturation of BSA, exhibiting anti-denaturation activity of 62.31, 49.61, 65.47, 72.60, and 61.50% respectively, compared to aspirin at 35.30%²⁸. Also, the methanol and acetone extracts of *Lecaniodiscus cupanioides* and related species similarly

demonstrated significant inhibition of albumin denaturation²⁹. Although, the fractions evaluated in this present study did not statistically outperform diclofenac sodium, their numerically substantial percentage inhibition, particularly that of the n-hexane fraction, supports the presence of bioactive constituents in *B. glabra* flower and bract capable of stabilising protein structure under heat-induced denaturing conditions.

Furthermore, the nitric oxide (NO) radical scavenging activity of the n-hexane and DCM fractions of *B. glabra* flower and bract was not significantly different from that of ascorbic acid reference standard, $F(2, 15) = 0.6507$, $p = 0.5358$. However, when evaluated on the basis of IC_{50} , a significant difference emerged, $F(2, 3) = 9314$, $p < 0.0001$, indicating that although the fractions scavenge NO radicals, they do so with considerably lower potency than ascorbic acid.

This pattern parallels the findings reported for the crude extract and solvent fractions of *Artocarpus altilis* stem bark, where the NO radical scavenging activity was found to be concentrated in moderately polar solvents³⁰. The extracts and fractions displayed good activity, but their activity did not compare with that of ascorbic acid standard, and the difference in IC_{50} values between the extracts/fractions and ascorbic acid was statistically significant. This recurring pattern across independent studies reinforces the broader understanding that while non-polar to moderately polar plant fractions consistently demonstrate genuine NO-scavenging capacity, they typically require substantially higher concentrations than pure reference antioxidants to achieve an equivalent effect. Moreover, the GC-MS analysis identified 29 unique compounds in both n-hexane and DCM fractions of *B. glabra* flower and bract. The n-hexadecanoic acid appears in both n-hexane and DCM fractions and was counted once. Out of these constituents, nine were dominant and some of which have been reported to elicit anti-inflammatory and antioxidant properties which supported our findings.

The 9,12-Octadecadienoic acid was identified as the most potent bioactive compound present across the extracts, consistent with the report of Muzahid *et al.*²³, who similarly detected this compound as the most abundant bioactive constituent in the extracts of *Benincasa hispida* and *Cucurbita moschata* seeds. The 9,12-Octadecadienoic acid has been reported to possess anti-inflammatory and antiarthritic properties³¹. Methylhexadecanoate (methyl palmitate) has been reported to possess anti-inflammatory activity, having been shown to reduce carrageenan-induced paw oedema and diminish prostaglandin E2 (PGE2) levels in inflammatory exudates in experimental rat models³². Oleic acid has been reported to possess potent anti-

inflammatory and antioxidant properties, exerting its anti-inflammatory effects through inhibition of proinflammatory cytokines and modulation of antioxidant enzyme activity³³. The 5,7,3',4'-tetrahydroxyflavone (also known as luteolin) has been reported to possess potent anti-inflammatory and antioxidant properties, with documented efficacy in regulating inflammation-related symptoms and diseases³⁴. Eugenol has also been shown to possess anti-inflammatory and antioxidant activities, modulating the inflammatory response and reducing oxidative stress in joint, lung, and liver tissues.

4.1 Conclusion

This study evaluated the anti-inflammatory effect of the n-hexane and DCM fractions of *Bougainvillea glabra* flower and bract, and characterised their phytochemical composition via GC-MS analysis. Both fractions demonstrated NO radical scavenging activity that was not significantly different from ascorbic acid at the percentage inhibition level, although a significant difference emerged when evaluated on the basis of IC₅₀, indicating that the fractions scavenge NO radicals with lower potency than ascorbic acid standard. Similarly, both fractions exhibited anti-denaturation activity that was statistically comparable to diclofenac sodium at fixed concentration; although, the standard drug required statistically lower concentrations to achieve equivalent inhibition on an IC₅₀ basis. Extraction yield data revealed that the crude extract of *B. glabra* flower and bract had a relatively low overall yield, from which the n-hexane fraction accounted for the greater proportion of the partitioned mass, followed by the DCM fraction. GC-MS profiling of the two fractions identified twenty-nine unique bioactive compounds, several of which, including oleic acid, 9,12-octadecadienoic acid, and methylhexadecanoate, have been independently reported in the literature to possess anti-inflammatory, antioxidant, and related pharmacological properties. These findings provide preliminary evidence that the n-hexane and DCM fractions of *B. glabra* flower and bract possess moderate anti-inflammatory activity, which may be associated with the bioactive fatty acid derivatives and related constituents identified by GC-MS. This study represents, to the best of our knowledge, the first combined bioactivity-phytochemistry profile of the flower and bract of *B. glabra*.

4.2 Study Limitations

The anti-inflammatory activity of the n-hexane and DCM fractions were assessed solely through *in vitro* assays, without corroboration by *in vivo* models. Therefore, the extrapolation of these findings to physiological anti-inflammatory efficacy should be made with caution, and

further *in vivo* studies are recommended to validate the bioactivity observed. Furthermore, the mechanisms underlying the observed effects were not investigated, and no toxicity studies were conducted to establish a safety profile for the flower and bract fractions. Future work should therefore prioritise bioassay-guided isolation and structure elucidation of the active constituents, mechanistic studies, toxicity and pharmacokinetic evaluation, and *in vivo* validation before *B. glabra* flower and bract extracts can be considered for therapeutic development.

Conflict of Interest

There is no conflict of interest.

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