



COMPARATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENT, IN-VITRO ANTIOXIDANT AND ANTI- INFLAMMATORY ACTIVITIES OF *Annona muricata* LEAF EXTRACT

Adebayo Masaudat Adeola^{1*}, Asimi Tajudeen², Gbadosi Taofeek Remilekun¹, Suleiman Ismail Ochu¹,
Bamisaye Funmilola Docas¹, Rasheed Olanike Muinat², Ayoola Adekunle Stanley³, Alake Comfort
Olamide¹, Egbemakin Comfort Dolapo¹, Amuda Opeyemi Funmilayo¹, Popoola Bukunmi Damilola¹

¹Department of Biochemical Science, Federal Polytechnic Ede, Osun State.

²Department of Chemical Science and Technology, Federal Polytechnic, Ede Osun State

³Department of science laboratory technology, Federal Polytechnic Ayede, Oyo state.

*Correspondence email: smdeola@gmail.com

ABSTRACT: *Annona muricata* L. (soursop) is a tropical plant recognized for its diverse phytochemical constituents and reported biological activities. This study investigated the phytochemical profile and in-vitro antioxidant and anti-inflammatory-related activities of *Annona muricata* leaves extracted with methanol, ethyl acetate, and n-hexane. Fresh leaves were collected, washed, air-dried, pulverized, and extracted separately with the three solvents. The extracts were evaluated for total flavonoid content, DPPH and hydroxyl radical-scavenging activities, inhibition of lipid peroxidation, protein denaturation, and proteinase inhibition at 50 μ l, 100 μ l, and 150 μ l. HPLC analysis were also performed to identify major phytochemical constituents. Total flavonoid content ranged from 0.57 to 0.63 mg QE/g, with the highest value at 100 μ l. The n-hexane extract showed the highest DPPH scavenging activity of 52.82% at 50 μ l, while ethyl acetate recorded the highest hydroxyl radical-scavenging activity of 86.45% at 150 μ l. Lipid peroxidation inhibition was highest with n-hexane at 26.27% at 50 μ l. Protein-denaturation inhibition was highest with n-hexane at 66.78% at 50 μ l, whereas methanol showed the highest proteinase inhibition of 57.44% at 150 μ l. HPLC revealed diverse phytochemicals, including flavonoids, phenolic compounds, alkaloids, acetogenin-related compounds, and phytosterols. The findings demonstrate measurable antioxidant and anti-inflammatory-related activities, influenced by extraction solvent and concentration, and support further investigation of *Annona muricata* leaves as a source of biologically active phytochemicals.

Keywords: *Annona muricata*, Phytochemical constituents, Antioxidant activity, Anti-inflammatory activity, Leaf extract

1.0 INTRODUCTION

Comparative analysis is an important approach in scientific research used to examine and identify similarities and differences between two or more samples, conditions, or variables. In medicinal plant research, comparative analysis can be used to determine how differences in extraction methods, solvents, or plant materials influence the chemical composition and biological activities of plant extracts. Such comparisons are useful for establishing relationships between the constituents present in an extract and the biological effects it produces. In the present study, comparative analysis is used to examine the phytochemical constituents as well as the *in-vitro* antioxidant and anti-inflammatory activities of *Annona muricata* leaf extracts obtained using different solvents (Hernandez-Fuentes et al., 2024).

Phytochemicals are naturally occurring chemical compounds found in plants, particularly secondary metabolites that contribute to the plant's defense, adaptation and interaction with its environment. Common classes of phytochemicals include flavonoids, phenolic compounds, alkaloids, tannins, saponins, terpenoids, glycosides and steroids. These compounds have attracted considerable scientific interest because of their potential biological activities, including

antioxidant, anti-inflammatory, antimicrobial and other pharmacological effects. The presence and concentration of phytochemical constituents in a plant may vary depending on factors such as plant species, geographical location, stage of maturity, environmental conditions, method of processing and extraction procedure. Therefore, the identification of phytochemical constituents is an important preliminary step in evaluating the potential biological properties of medicinal plants (Adewole et al., 2022; Onuoha et al., 2023).

Annona muricata L., commonly known as soursop, is a tropical plant belonging to the family Annonaceae and is widely distributed in tropical and subtropical regions. Although the plant is widely recognized for its edible fruit, various parts of *Annona muricata*, including the leaves, fruits, seeds, bark and roots, have received scientific attention because of their phytochemical composition and potential biological activities. The leaves have been reported to contain several classes of secondary metabolites, including flavonoids, phenolic compounds, alkaloids, tannins, saponins, terpenoids and annonaceous acetogenins. These constituents may contribute to some of the biological activities associated with the plant (Onuoha et al., 2023; Thant et al., 2024).

A leaf extract refers to a preparation obtained by treating plant leaves with an appropriate solvent or extraction method to remove soluble chemical constituents from the plant material. Extraction is an important stage in phytochemical research because the type of solvent used can influence the quantity and type of compounds extracted. Solvents differ in polarity and therefore have different abilities to dissolve particular classes of phytochemicals. N-hexane, methanol and ethyl acetate are solvents that can produce extracts with different phytochemical profiles. N-hexane is relatively non-polar and may favour the extraction of non-polar constituents, while methanol is more polar and can extract a broader range of polar and moderately polar compounds. Ethyl acetate has intermediate polarity and may extract compounds with properties different from those obtained using n-hexane or methanol (Hernandez-Fuentes et al., 2024; Optimization of Drying and Extraction Techniques, 2024).

Antioxidants are substances capable of reducing or preventing oxidative damage caused by reactive molecules known as free radicals and reactive oxygen species. Free radicals are highly reactive chemical species that contain unpaired electrons and can interact with important cellular components such as lipids, proteins and nucleic acids. When the production of reactive oxygen species exceeds the body's antioxidant defense mechanisms, oxidative stress may occur. Oxidative stress has been associated with cellular damage and has been implicated in several pathological processes. Plants contain naturally occurring antioxidant compounds, particularly phenolic compounds and flavonoids, which can contribute to the ability of plant extracts to scavenge free radicals and reduce oxidative reactions (Adewole et al., 2022; Hernandez-Fuentes et al., 2024).

In-vitro antioxidant activity refers to the ability of a substance or plant extract to demonstrate antioxidant effects under laboratory conditions outside a living organism. Several methods can be used to evaluate the antioxidant activity of plant extracts, including the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and other free-radical-scavenging methods. These assays provide an experimental means of assessing the capacity of plant extracts to interact with or neutralize reactive species. Previous studies have reported antioxidant activity in *Annona muricata* leaf extracts and have associated this activity with the presence of phenolic and flavonoid constituents (Adewole et al., 2022; Hernandez-Fuentes et al., 2024).

Inflammation is a protective biological response that occurs when the body encounters harmful stimuli such as infection, tissue injury or irritation. It forms part of the body's defense and tissue-repair processes. Although inflammation is essential for maintaining normal physiological functions and responding to injury, excessive or prolonged inflammatory responses may contribute to tissue damage and disease development. Anti-inflammatory activity therefore refers to the ability of a substance to reduce, inhibit or regulate processes involved in inflammation. Several naturally occurring plant compounds, including flavonoids, phenolic compounds, terpenoids and other secondary metabolites, have been investigated for their potential anti-inflammatory properties (Polyphenols-Rich Fraction from *Annona muricata* Leaves, 2022).

The anti-inflammatory properties of *Annona muricata* have also received scientific attention because of the presence of bioactive compounds in different parts of the plant. Bioactive fractions obtained from *Annona muricata* leaves have been investigated for their effects on biological processes associated with inflammation and oxidative stress. The presence of phenolic compounds, flavonoids and other secondary metabolites may contribute to the potential anti-

inflammatory activity of the leaf extracts (Polyphenols-Rich Fraction from *Annona muricata* Leaves, 2022; Thant et al., 2024).

The relationship between phytochemical composition and biological activity is therefore important in the study of medicinal plants. The presence and concentration of particular phytochemicals may contribute to the antioxidant and anti-inflammatory properties of plant extracts. Phenolic compounds and flavonoids, for example, have been widely investigated for their antioxidant properties, while several classes of secondary metabolites have also been associated with modulation of inflammatory processes. Differences in the phytochemical profiles of extracts obtained using different solvents may consequently result in differences in their antioxidant and anti-inflammatory activities (Adewole et al., 2022; Hernandez-Fuentes et al., 2024).

Although *Annona muricata* has been investigated for its phytochemical composition and biological properties, differences in extraction solvents and experimental conditions may produce different results. The use of solvents with different polarities, such as n-hexane, methanol and ethyl acetate, may result in extracts with different concentrations and combinations of phytochemical constituents. Comparing these extracts may therefore provide useful information on the influence of solvent selection on the phytochemical profile, antioxidant activity and anti-inflammatory activity of *Annona muricata* leaves (Thant et al., 2024; Optimization of Drying and Extraction Techniques, 2024).

The extraction of solvent is an important factor in phytochemical investigation because solvent differ in polarity and therefore extract different groups of chemical constituents. The use of n-hexane, methanol and ethyl acetate can provide extracts with different phytochemical profiles. Chromatographic techniques such as high-performance liquid chromatography (HPLC) can further assist in the separation and characterization of compounds present in plant extracts (*Annona muricata* Leaves as a Source of Bioactive Compounds, 2022; Hernandez-Fuentes et al., 2024).

Therefore, this study aimed to investigate the phytochemical profile and in-vitro antioxidant and anti-inflammatory properties of *Annona muricata* leaves extracted using n-hexane, methanol and ethyl acetate, with emphasis on their chemical constituents and biological activities.

2.0 MATERIALS AND METHODS

2.1 Collection and Preparation of Plant materials

Fresh leaves of *Annona muricata* L. were collected from the Federal Polytechnic Ede North Campus, Osun State, Nigeria. The collected leaves were carefully examined to remove unwanted materials and damaged portions. The leaves were washed thoroughly with clean water to remove dust, soil particles, and other visible contaminants. After washing, the leaves were spread on clean surfaces and allowed to air-dry at room temperature until completely dry. The dried leaves were pulverized into a fine powder using a clean grinder and passed through a sieve to obtain a relatively uniform particle size. The powdered plant material was collected into clean, dry, properly labelled containers and stored in a cool, dry place until required for extraction and subsequent laboratory analyses. Similar procedures involving washing, drying, pulverization, and storage of plant materials have been reported for phytochemical investigations of *Annona muricata* leaves (Ginting et al., 2024).

2.2 Chemicals and Reagents

Analytical-grade ethyl acetate, n-hexane, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, trichloroacetic acid (TCA), and thiobarbituric acid (TBA), together with other reagents required for the phytochemical, antioxidant, anti-inflammatory, and related analyses, were used in this study. All chemicals and reagents were procured from Olabest Chemical Supplier and services and Labtrade Nig. Company and were used as supplied without further purification.

2.3 Preparation of Extract

Fifty grams (50 g) of powdered *Annona muricata* leaves were weighed separately into appropriate containers and soaked in 500 mL each of methanol, n-hexane, and ethyl acetate. The mixtures were allowed to stand for 48 hours with intermittent agitation to facilitate the extraction of soluble constituents. After extraction, each mixture was filtered through Whatman No. 1 filter paper to remove the plant residues. The resulting filtrates were allowed to evaporate at room temperature until completely dry, yielding powdered extracts. The dried extracts were collected, weighed, labelled according to the respective solvents used, and stored in airtight containers until required for further analysis.

2.4 Invitro Anti-Oxidant Assay

2.4.1 Total Flavonoid Content

The total flavonoid content (TFC) of the *Annona muricata* leaf extracts was determined using the aluminum chloride colorimetric method, with quercetin as the reference standard (Gutiérrez-Montiel et al., 2023). Different concentrations of each extract were prepared, and their absorbance was measured at **415 nm using a visible spectrophotometer**. The TFC was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

2.4.2 DPPH Scavenging Activity

The DPPH radical-scavenging activity of the *Annona muricata* leaf extracts was determined following the method described by Gutiérrez-Montiel et al. (2024). Different concentrations of each extract were prepared and reacted with DPPH solution. The mixtures were incubated in the dark, and the absorbance was measured at 517 nm using a visible spectrophotometer. The percentage of DPPH radical-scavenging activity was calculated using the equation:

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where: A_0 represents the absorbance of the DPPH control and A_1 represents the absorbance of the extract-treated sample.

2.4.3 Hydroxyl Radical Scavenging

The hydroxyl radical-scavenging activity of the *Annona muricata* leaf extracts was determined using the 2-deoxyribose degradation method with slight modifications (Rahman et al., 2022). Different concentrations of each extract were reacted with the hydroxyl radical-generating system and incubated under the specified conditions. Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were subsequently added, and the absorbance was measured at 532 nm using a visible spectrophotometer. The percentage hydroxyl radical-scavenging activity was calculated as:

$$\text{Hydroxyl radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where: A_0 represents the absorbance of the control and A_1 represents the absorbance of the extract-treated sample.

2.4.4 Inhibition of Lipid Peroxidation

The inhibitory effect of the *Annona muricata* leaf extracts on lipid peroxidation was determined using the thiobarbituric acid reactive substances (TBARS) assay, following a previously reported method (Rahman et al., 2022). Different concentrations of the extracts were incubated with the lipid peroxidation reaction mixture, followed by the addition of trichloroacetic acid (TCA) and thiobarbituric acid (TBA). The reaction mixture was heated and subsequently cooled, and the absorbance was measured at 532 nm using a visible spectrophotometer. The percentage inhibition of lipid peroxidation was calculated as:

$$\text{Lipid peroxidation inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where: A_0 represents the absorbance of the control and A_1 represents the absorbance of the extract-treated sample.

2.5 Invitro Anti-inflammatory

2.5.1 Inhibition of Protein Denaturation

The anti-inflammatory activity of the *Annona muricata* leaf extracts was evaluated using the protein denaturation inhibition assay following the method of Matotoka et al. (2023). Different concentrations of each extract were mixed with egg albumin and phosphate-buffered saline, incubated, and subsequently heated to induce protein denaturation. After cooling, the absorbance was measured at 660 nm using a visible spectrophotometer. The percentage inhibition of protein denaturation was calculated as:

$$\text{Protein denaturation inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where: A_0 represents the absorbance of the control and A_1 represents the absorbance of the extract-treated sample.

2.5.2 Inhibition of Proteinase Activity

The proteinase inhibitory activity of the *Annona muricata* leaf extracts was determined using an in-vitro proteinase inhibition method described by Mba *et al.* (2022). Different concentrations of the extracts were incubated with the proteinase enzyme and substrate under the required conditions. Following incubation, the absorbance was measured at 660 nm using a visible spectrophotometer. The percentage inhibition of proteinase activity was calculated using the equation:

$$\text{Proteinase inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where: A_0 represents the absorbance of the control and A_1 represents the absorbance of the extract-treated sample.

3.0 DISCUSSION OF RESULTS AND FINDINGS

3.1 ANTI-OXIDANT ASSAY

3.1.1 Total Flavonoid Content

The total flavonoid content of the *Annona muricata* extract was determined using the aluminum chloride colorimetric method. The result was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g). The obtained value is presented in Table 1.

Table 1: Total Flavonoid Content of *Annona muricata* Extract

Concentration (µl)	Total Flavonoid Content (mg QE/g)
50	0.57±0.02
100	0.63±0.00
150	0.62±0.02

Discussion: The total flavonoid content ranged from 0.57 ± 0.02 to 0.63 ± 0.02 mg QE/g. The lowest value was recorded at 50 µl (0.57 ± 0.02 mg QE/g), while the highest value was observed at 100 µl (0.63 ± 0.00 mg QE/g). At 150 µl, the value slightly decreased to 0.62 ± 0.02 mg QE/g. The result therefore did not show a continuous increase in flavonoid content with increasing concentration. The slight reduction observed at 150 µl may be related to the response characteristics of the colorimetric assay or interactions among extract constituents at higher concentrations. Flavonoids are important secondary metabolites in plants and have been associated with antioxidant properties because of their ability to participate in reactions involving reactive oxygen species. The presence of flavonoids in *Annona muricata* leaves has been reported in previous phytochemical investigations, although their concentration may vary according to extraction solvent, geographical location, processing conditions and analytical method (Nolasco-González *et al.*, 2022; Hernández-Fuentes *et al.*, 2024).

3.1.2 DPPH Scavenging Activity

The antioxidant activity of the *Annona muricata* extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The extracts obtained using the three solvents were analyzed separately, and their antioxidant activities were expressed as percentage DPPH radical inhibition. The results for the methanolic, ethanol, and aqueous extracts are presented in Tables 2.

Table 2: Percentage Inhibition of DPPH Scavenging Activity of *Annona muricata*

Concentration (µl)	Methanolic Extract (%)	Ethylacetate Extract (%)	N-Hexane Extract (%)
50	41.62	35.35	52.82
100	43.45	28.25	52.61
150	44.72	22.39	52.39

The DPPH radical-scavenging activity varied among the three extracts. At 50 µl, the methanol, ethyl acetate and *n*-hexane extracts showed inhibition values of 41.62%, 35.35% and 52.82%, respectively. At 100 µl, the values were 43.45%, 28.25% and 52.61%, while at 150 µl they were 44.72%, 22.39% and 52.39%, respectively. The *n*-hexane extract recorded the highest DPPH inhibition at all three concentrations, although its activity decreased slightly from 52.82% at 50 µl to 52.39% at 150 µl. The methanol extract showed a gradual increase in activity as the concentration increased, whereas the ethyl acetate extract showed a progressive decrease.

The differences observed among the extracts indicate that the solvent used for extraction influenced the DPPH radical-scavenging response. The relatively high activity of the *n*-hexane extract suggests that compounds soluble in the non-polar fraction may have contributed to the observed activity. However, solvent polarity alone may not account for the differences because antioxidant activity depends on the individual compounds present, their concentrations and possible interactions. Similar solvent-dependent differences in antioxidant activity have been reported for *Annona muricata* extracts (Hartati et al., 2024; Ginting et al., 2024).

3.1.3 Hydroxyl Radical Scavenging

The antioxidant activity of the *Annona muricata* extracts was further evaluated using the hydroxyl radical scavenging assay. The methanol, ethyl acetate, and *n*-hexane extracts were analysed separately at different concentrations, and the results were expressed as percentage hydroxyl radical scavenging activity. The results obtained are presented in Tables 3.

Table 3: Percentage Inhibition of Hydroxyl Radical Scavenging Activity of *Annona muricata*

Concentration (µl)	Methanolic Extract (%)	Ethylacetate Extract (%)	N-Hexane Extract (%)
50	81.22	82.62	81.51
100	82.50	85.17	82.33
150	85.06	86.45	84.19

The hydroxyl radical-scavenging activity increased with increasing concentration in all three extracts. The methanol extract increased from 81.22% at 50 µl to 82.50% at 100 µl and 85.06% at 150 µl. Similarly, the ethyl acetate extract increased from 82.62% to 85.17% and 86.45%, while the *n*-hexane extract increased from 81.51% to 82.33% and 84.19% at the respective concentrations.

The ethyl acetate extract recorded the highest hydroxyl radical-scavenging activity at 150 µl (86.45%). The progressive increase observed in all three extracts suggests that increasing extract concentration generally increased the ability of the samples to reduce or interact with hydroxyl radical-related reactions under the conditions of the assay. The stronger activity of the ethyl acetate extract at higher concentration may be associated with the presence of compounds that were efficiently extracted by ethyl acetate and possess electron- or hydrogen-donating properties. Phenolic and flavonoid compounds have been associated with antioxidant activity because of their ability to participate in reactions involving reactive species. The presence of these classes of compounds in *Annona muricata* leaves has been reported in previous studies (Hernández-Fuentes et al., 2024; Ruiz-López et al., 2025).

3.1.4 Inhibition of Lipid Peroxidation

The antioxidant potential of the *Annona muricata* extracts was further assessed by their ability to inhibit lipid peroxidation. The methanol, ethyl acetate, and *n*-hexane extracts were analysed separately at different concentrations, and the results were expressed as percentage inhibition of lipid peroxidation. The results obtained are presented in Table 4.

Table 4: Percentage Inhibition of Lipid Peroxidation of *Annona muricata*

Concentration (µl)	Methanolic Extract (%)	Ethylacetate Extract (%)	N-Hexane Extract (%)
50	8.18	10.16	26.27
100	12.12	18.16	26.00
150	11.51	18.98	17.79

The inhibition of lipid peroxidation varied according to both extraction solvent and concentration. At 50 µl, the methanol, ethyl acetate and *n*-hexane extracts showed inhibition values of 8.18%, 10.16% and 26.27%, respectively. At 100 µl, the corresponding values were 12.12%, 18.16% and 26.00%, while at 150 µl they were 11.51%, 18.98% and 17.79%, respectively.

The *n*-hexane extract recorded the highest inhibition at 50 µl (26.27%) and maintained a similar value at 100 µl (26.00%) before decreasing to 17.79% at 150 µl. The ethyl acetate extract, however, showed a gradual increase from 10.16% to 18.98%, while the methanol extract increased from 8.18% to 12.12% before slightly decreasing to 11.51%.

The relatively high activity of the *n*-hexane extract at the lower concentrations may indicate the contribution of non-polar constituents to the inhibition of lipid oxidation. However, the reduction at 150 μ l indicates that the response was not strictly concentration-dependent. Such variations may result from interactions among extract constituents or assay-specific effects. Antioxidant and oxidative-stress-related activities have previously been reported for *Annona muricata* leaf extracts and fractions (Badmus et al., 2022; Saraiva et al., 2022).

3.2 Anti-inflammatory Assay

3.2.1 Inhibition of Protein Denaturation

The anti-inflammatory activity of the *Annona muricata* extracts was evaluated based on their ability to inhibit protein denaturation. The methanol, ethyl acetate, and *n*-hexane extracts were analysed separately at different concentrations, and the results were expressed as percentage inhibition of protein denaturation. The results obtained are presented in Table 5.

Table 5: Percentage Inhibition of Protein Denaturation of *Annona muricata*

Concentration (μ l)	Methanolic Extract (%)	Ethylacetate Extract (%)	N-Hexane Extract (%)
50	44.76	55.45	66.78
100	42.60	44.90	54.45
150	39.40	39.86	52.72

The results showed that the inhibition of protein denaturation decreased with increasing concentration in all three extracts. At 50 μ l, the methanol, ethyl acetate and *n*-hexane extracts recorded inhibition values of 44.76%, 55.45% and 66.78%, respectively. At 100 μ l, the values decreased to 42.60%, 44.90% and 54.45%, while at 150 μ l they decreased further to 39.40%, 39.86% and 52.72%, respectively.

The *n*-hexane extract showed the highest inhibition at all concentrations, with the highest value of 66.78% recorded at 50 μ l. The reduction in activity with increasing concentration indicates that the response was not directly concentration-dependent under the conditions of this assay. Protein-denaturation inhibition is commonly used as an *in-vitro* indicator of anti-inflammatory-related activity because substances capable of preventing protein denaturation may interfere with processes associated with inflammatory responses. The relatively high response of the *n*-hexane extract may be related to the presence of constituents preferentially extracted into the non-polar fraction. Previous investigations have reported anti-inflammatory-related effects of *Annona muricata* leaf extracts and fractions (Nwaehujor et al., 2020; Saraiva et al., 2022).

3.2.2 Inhibition of Proteinase Activity

The anti-inflammatory activity of the *Annona muricata* extracts was further evaluated based on their ability to inhibit proteinase activity. The methanol, ethyl acetate, and *n*-hexane extracts were analysed separately at different concentrations, and the results were expressed as percentage inhibition of proteinase activity. The results obtained are presented in Table 6.

Table 6: Percentage Inhibition of Proteinase Activity of *Annona muricata*

Concentration (μ l)	Methanolic Extract (%)	Ethylacetate Extract (%)	N-Hexane Extract (%)
50	26.69	12.62	12.09
100	43.42	28.72	18.72
150	57.44	40.06	24.53

Proteinase inhibition increased with increasing concentration in all three extracts. The methanol extract increased from 26.69% at 50 μ l to 43.42% at 100 μ l and 57.44% at 150 μ l. The ethyl acetate extract increased from 12.62% to 28.72% and 40.06%, while the *n*-hexane extract increased from 12.09% to 18.72% and 24.53%, respectively.

The methanol extract demonstrated the highest proteinase inhibition at all concentrations and recorded the highest overall value of 57.44% at 150 μ l. The progressive increase in inhibition suggests that increasing the concentration of the extract increased the number of active constituents available to interfere with proteinase activity under the conditions of the assay. The stronger response of the methanol extract may be related to the ability of methanol to extract a broad range of polar and moderately polar phytochemicals, including phenolic and flavonoid constituents. Proteinase inhibition may provide an indication of anti-inflammatory-related potential because proteinases participate in biological processes associated with inflammatory responses. Previous studies have reported anti-inflammatory

activities associated with polyphenol-rich fractions of *Annona muricata* leaves (Saraiva et al., 2022; Shin and Kim, 2023).

3.3 HPLC Analysis

The methanolic, ethyl acetate and *n*-hexane extracts of *Annona muricata* (Soursop leaf) were subjected to high-performance liquid chromatography (HPLC) analysis following the method described by Engelhardt et al. (2023), with slight modifications. The extracts were dissolved in an appropriate HPLC-grade solvent and thoroughly mixed before filtration through a membrane filter. The filtered samples were transferred into HPLC vials and injected into the chromatographic system. Separation of the compounds was achieved using a reversed-phase C18 column, while detection was performed using a diode-array detector (DAD). The chromatographic peaks were identified by comparing their retention times and spectral characteristics with those of available reference standards. The resulting chromatograms were recorded and analyzed for the identification and quantification of the major phenolic compounds present in the extracts.



Figure 1: HPLC chromatogram illustrating twenty-two bioactive compounds of the ethyl acetate extract of *Annona muricata* Leaves

The HPLC chromatogram of the ethyl acetate extract demonstrated the presence of several compounds. The detected compounds included beta-carotene, annonacin, anonaine, gallic acid, ascorbic acid, beta-sitosterol, daidzein, genistein, asimilobine, quercetin, kaempferol, rutin, apigenin, reticuline, atherospermine, coreximine, annomuricin, vitexin, emodin, swainsonine, casuarine and liriodenine. Among the compounds reported, quercetin showed the highest relative abundance at 57.38%, followed by kaempferol at 12.37% and anonaine at 7.74%. Rutin was detected at 3.10%, while apigenin and atherospermine were detected at 1.65% and 1.75%, respectively. Other compounds occurred at relatively lower abundances.

The relatively high abundance of quercetin is notable because quercetin is a flavonoid that has been widely investigated for antioxidant properties. The presence of other flavonoid-related compounds, including kaempferol, rutin, apigenin, genistein, daidzein and vitexin, may also contribute to the biological properties of the extract. Gallic acid and ascorbic acid were also detected and may contribute to antioxidant-related activity. The detection of annonacin, anonaine, reticuline, coreximine, annomuricin and liriodenine further demonstrates the chemical diversity of *Annona muricata* leaves. Previous chromatographic investigations have similarly reported multiple classes of bioactive compounds in *Annona muricata* leaves (Hernández-Fuentes et al., 2024; Ruiz-López et al., 2025).

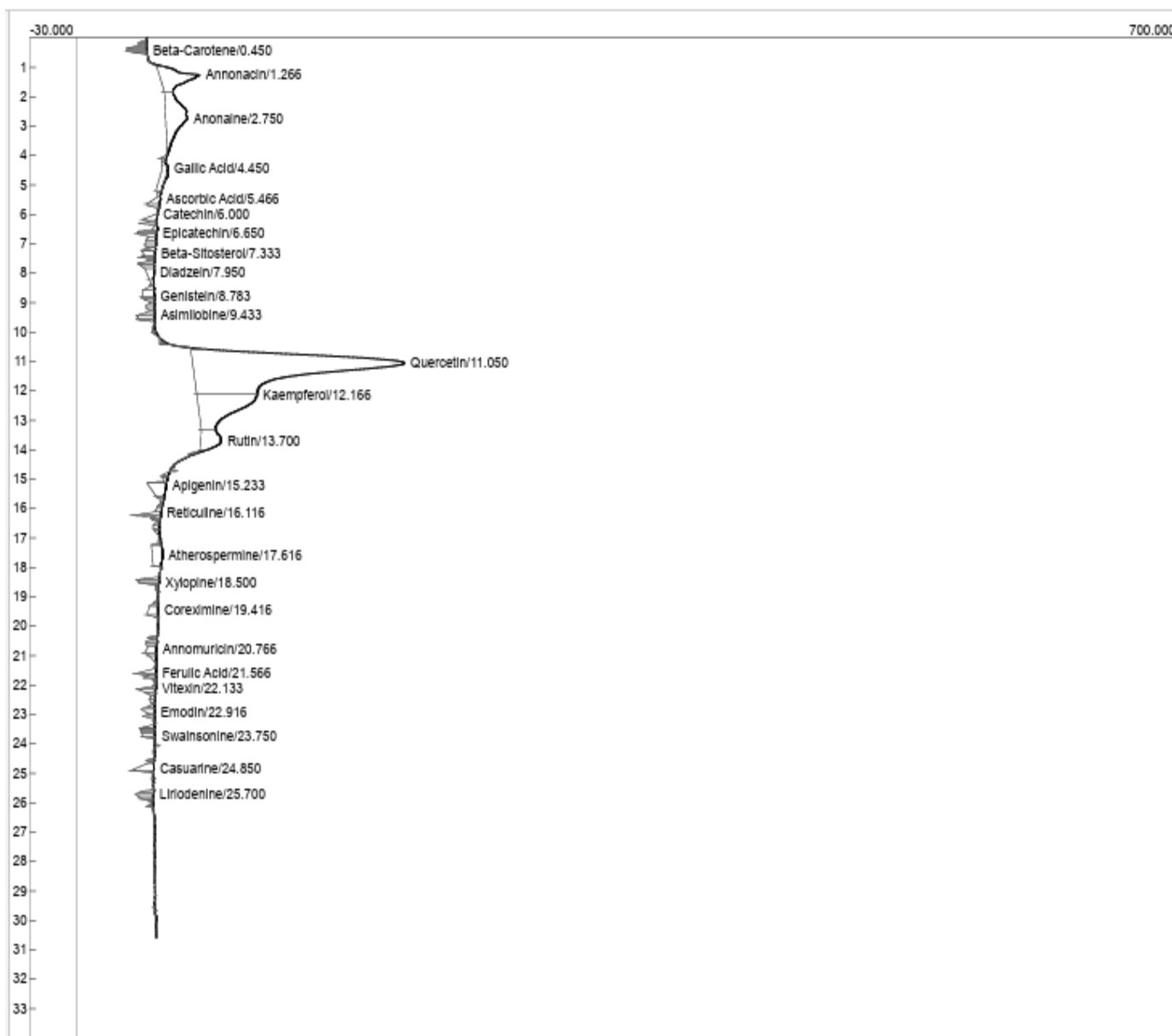


Figure 2: HPLC chromatogram illustrating twenty-five bioactive compounds of the n-hexane extract of *Annona muricata* Leaves.

The HPLC chromatogram of the *n*-hexane extract also demonstrated the presence of several compounds, including beta-carotene, annonacin, anonaine, gallic acid, ascorbic acid, catechin, epicatechin, beta-sitosterol, daidzein, asimilobine, quercetin, kaempferol, rutin, apigenin, reticuline, atherospermine, xylopin, coreximine, annomuricin, ferulic acid, vitexin, emodin, swainsonine, casuarine and liriodenine. Rutin showed the highest reported relative abundance at 13.23%, followed by anonaine at 7.67% and kaempferol at 5.73%. Apigenin was detected at 3.63%, while xylopin and reticuline were detected at 2.03% and 1.95%, respectively. The remaining compounds occurred at lower relative abundances.

The detection of catechin, epicatechin, quercetin, kaempferol, rutin, apigenin, vitexin, gallic acid and ferulic acid demonstrates the presence of several compounds associated with antioxidant-related properties. The detection of annonacin, anonaine, reticuline, xylopin, coreximine, annomuricin and liriodenine also indicates the chemical diversity of the *n*-hexane extract. These findings may help explain some of the antioxidant and anti-inflammatory-

related activities observed in the biological assays. However, the HPLC results demonstrate the presence and relative abundance of detected compounds but do not by themselves establish that a particular compound was solely responsible for a specific biological activity. Similar chemical diversity has been reported in chromatographic investigations of *Annona muricata* leaves (Abdallah et al., 2024).

Overall, the results demonstrate that the phytochemical composition and biological activities of *Annona muricata* leaf extracts varied according to the extraction solvent and assay employed. The *n*-hexane extract showed the highest DPPH radical-scavenging activity and the highest inhibition of lipid peroxidation at 50 µl, as well as the highest protein-denaturation inhibition at all tested concentrations. The ethyl acetate extract showed the highest hydroxyl radical-scavenging activity at 150 µl, while the methanol extract demonstrated the highest proteinase inhibition at 150 µl. These findings indicate that no single extraction solvent produced the highest activity across all assays. The differences may be associated with variation in the chemical composition, solubility and concentration of compounds extracted by the different solvents. Previous studies have similarly reported that extraction conditions can influence the phytochemical composition and biological activities of *Annona muricata* leaves (Nolasco-González et al., 2022; Mutakin et al., 2022; Jadhav and Selvakumarsamy, 2024).

The HPLC findings further support the presence of diverse bioactive constituents in the extracts. Compounds belonging to different chemical classes, including flavonoids, phenolic compounds, alkaloid-related compounds and acetogenin-related constituents, were detected. The differences in the relative abundance of these compounds between extracts may contribute to the differences observed in the antioxidant and anti-inflammatory-related assays. However, because the present biological assays were conducted *in vitro*, the findings should be interpreted as evidence of biological activity under laboratory conditions rather than as proof of therapeutic effectiveness. Further studies involving isolation and structural confirmation of individual compounds, quantitative validation, mechanistic studies and appropriate *in-vivo* investigations would be required to establish the specific contribution of individual constituents to the biological activities of *Annona muricata* leaves.

5.0 CONCLUSION

The findings of this study demonstrate that *Annona muricata* leaves contain diverse phytochemical constituents with measurable in-vitro antioxidant and anti-inflammatory-related activities. The methanol, ethyl acetate, and *n*-hexane extracts showed varying activities across the different assays, indicating that the type of extraction solvent and concentration influenced the biological activities of the extracts.

HPLC analysis further revealed several phytochemical constituents, including quercetin, rutin, kaempferol, apigenin, annonacin, and other phenolic and alkaloid compounds. Overall, the study demonstrates that *Annona muricata* leaves are a potential source of bioactive compounds and provides useful information on their phytochemical composition and in-vitro antioxidant and anti-inflammatory-related activities.

5.1 RECOMMENDATIONS

- Based on the findings of this study, the following recommendations are made:
- Further studies should be carried out to isolate and identify the individual bioactive compounds present in *Annona muricata* leaves and determine their specific contributions to the observed biological activities.
- Quantitative HPLC and other advanced analytical techniques should be employed to confirm and accurately quantify the phytochemical constituents detected in the extracts.
- Further investigations should be conducted using additional antioxidant and anti-inflammatory assays to provide broader evidence of the biological activities of *Annona muricata* leaf extracts.
- In-vivo studies should be conducted to further evaluate the antioxidant and anti-inflammatory-related effects of the extracts and determine their biological relevance beyond the in-vitro findings.
- Further studies should investigate the influence of extraction conditions, including solvent type, concentration, extraction time, and temperature, on the phytochemical composition and biological activities of *Annona muricata* leaf extracts.

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