

Full Length Research Paper

***In vitro* anti-inflammatory and antioxidant activities of *Delonix regia* (Bojer Ex Hook.) Raf. leaf and stem**

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Inflammation and oxidative stress are closely connected and play crucial roles in the development of diseases. Several synthetic drugs have been employed to treat these conditions, but not without side effects. *Delonix regia* has been used traditionally to treat inflammation and other health problems. Hence, this study validated the *in vitro* anti-inflammatory and antioxidant potential of leaf and stem extracts and fractions of *D. regia*. The dried and pulverized leaves and stems were extracted with methanol at room temperature, and the extracts were screened for secondary metabolites. The anti-inflammatory activity was determined using the membrane stabilising assay, and antioxidant activity was determined using DPPH, ferric ion reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) assays. FT-IR was used to identify the functional groups in the crude extracts. GC-MS was used to analyse the compounds in the stem fraction. Phytochemical screening of the crude extracts revealed the presence of saponins, tannins, flavonoids, cardiac glycosides, terpenoids, alkaloids, and phenols. The leaf extract showed a greater membrane stabilizing activity (47.77%) compared to the stem extract (40.46%) and Indomethacin, the standard drug (41.46%). The DPPH, TAC content, and reducing power ability were concentration dependent. The stem extract presented a high antioxidant capacity. Functional groups such as alcohols, amines, alkanes, aromatic, nitro compounds, and alkyl and aryl halides were identified and common to both extracts. The leaf and stem extracts possess significant anti-inflammatory and antioxidant properties. Further studies on the toxicological profile, isolation, and characterization of bioactive compounds are recommended.

Key words: *Delonix regia*, Leaf and stem extracts, Anti-inflammatory and antioxidant activities.

INTRODUCTION

Inflammation is a natural physiological response that activates the immune system to protect the body against microbial infections and other threats. It often serves as a defense mechanism against diseases, including cancers (Chen et al., 2017; Back et al., 2019). Chronic

inflammation has been shown to lead to many pathogenic diseases due to the over-secretion of inflammatory mediators. It alters normal physiological processes by activating several signaling pathways, particularly nuclear factor kappa B (NF- κ B) transcription factor. NF- κ B is

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responsible for inducing many inflammatory genes, thus amplifying the inflammatory response, as well as maintaining it (Liu et al., 2017; Parker et al., 2019). Chronic inflammation in this case, can have dangerous effects in the human body, as it usually accumulates in a silent way and is a real risk for the health and lifespan. Diabetes, heart diseases, cancers, digestive disorders, autoimmune ailments, and disorders related to the progressive loss of nerve cells are only a few of the diseases that are largely caused by chronic inflammation (Braz-Silva et al., 2019). Therefore, reducing inflammation has emerged as a promising treatment approach for illnesses linked to inflammation. Due to the significant undesirable effects associated with many conventional anti-inflammatory medications, like non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal drugs currently on the market (Saeed et al., 2023), there is a growing need for new anti-inflammatory drugs. This demand drives ongoing research focused on developing safer and efficient anti-inflammatory molecules that can circumvent the negative side effects of existing medications. Thus, it is evident that a substantial number of plant-derived substances are integral to the therapeutic arsenal of modern medicine.

Delonix regia (Bojer ex Hook.) Raf. has gained attention because of its use in traditional medicine and its interesting phytochemical properties (Havaladar et al., 2024). *D.regia* is a flowering plant in the pea family, *Fabaceae*, which is found in many regions of the tropics and subtropics (National Parks Board, 2025). Valued for its striking flowers, it has long been used to treat ailments, including bronchitis, anemia and liver disorders (Havaladar et al., 2024). Through selected assays, the study seeks to evaluate their efficacy in inhibiting oxidative stress and inflammatory mediators. This research may contribute to the growing body of evidence supporting the use of medicinal plants as alternative or complementary treatments for inflammation- and oxidative stress-related disorders, ultimately enhancing our understanding of plant-based pharmacology and aiding in the discovery of safer therapeutic agents.

MATERIALS AND METHODS

Plant collection and preparation

Mature leaves and stems of *D. regia* were collected on 18th May, 2024, along Amina way, University of Ibadan, Ibadan, Oyo State, Nigeria. The plant was identified and authenticated at Forest Herbarium, Ibadan (FHI) with voucher number FHI-114056 and deposited at the Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI). The plant samples were air-dried and pulverized by grinding, using a mechanical grinder.

Plant extraction

Pulverized leaves and stem (1 kg each) of *D. regia* were extracted with methanol at room temperature (25°C). One kilogram each of

the plant samples were transferred into different glass containers and 10 L of pure methanol was added, stirred occasionally and the extraction process was allowed to take place for 72 h. The solvent (now containing the extract) was collected using muslin bag and was further filtered using Whatman filter paper 1mm. This process was repeated on the macerate with another 10 L of pure methanol. After the combined filtrates were concentrated using a rotary evaporator (Heidolph Laboratory, Germany) set at 40°C, the concentrate was further dried using a vacuum oven set at 40°C and 700 mmHg of pressure. According to Sheneni et al. (2018), the percentage yield of the plant samples was calculated using the formula:

$$\text{Percentage yield} = \frac{\text{weight of dried extract}}{\text{weight of dried plant sample}} \times 100$$

Fractionation of crude extracts

Fifteen grams (15 g) of the concentrated crude methanol extract of *D. regia* stem was poured into a 500 mL clean beaker, 50 mL of methanol was added to it, to make a solution and 50 g of silica gel was mixed with it, for pre-adsorption. The pre-adsorbed extract (dried) was transferred into a VLC (vacuum liquid chromatography) setup that had been already packed with 100 g of silica gel. *n*-Hexane, ethyl acetate and methanol were respectively added after a clear solution of the preceding solvent was gotten. The respective fractions were concentrated with rotary evaporator using a temperature of 40°C and a vacuum oven at 40°C with a pressure of 700 mmHg.

Phytochemical analyses of crude extracts and determination of *in vitro* anti-inflammatory activity

Qualitative and quantitative phytochemical screening for secondary metabolites was determined using standard procedures (Muhongo et al., 2021). The membrane stabilising assay was used to determine the anti-inflammatory activity of *D. regia* leaves and stem (Ajayi et al., 2014).

Preparation of rat erythrocyte suspension

200 g healthy Male Wistar rat was anesthetized with an intraperitoneal injection of a mixture of 50 mg/kg Ketamine and 10 mg/kg Xylazine, which were prepared as a cocktail. Blood was collected from the rat by cardiac puncture. The blood was centrifuged twice at 4000 rpm for 7 min followed by removal of the supernatant. The packed cells were washed with isosaline (0.85%, pH 7.2), which was then used to prepare a suspension (10% V/V). Alsever solution was prepared by dissolving D-glucose (2.05 g), sodium citrate (0.8 g), citric acid (0.055 g), and sodium chloride (0.42 g) in distilled water and made up to 100 mL. It was then mixed in a plain tube with an equal volume of fresh whole blood (3 mL) collected from the rat (Ajayi et al., 2014).

Preparation of sample extract

The sample extract (1 mg) was made soluble in distilled water using Tween-80 (5%) and serially diluted into five concentrations (1000, 500, 250, 125, and 62.5 µg/mL) with distilled water (Ajayi et al., 2014).

Rat erythrocyte membrane stabilizing assay

The hypotonic membrane stabilizing activity of extracts determined

on rat erythrocytes using the method of Ajayi et al. (2014). Various concentrations (1000, 500, 250, 125, and 62.5 µg/mL) of the extracts were prepared by serial dilution with distilled water. The assay mixture was prepared by adding 400 µL of hypotonic solution in a plain tube, followed by 200 µL of the extracts, 200 µL of RRBC (Rat Red Blood Cell) and 1.2 mL of Isosaline solution after 10 mins. The mixture lacking drug was used as a blank control, while drug control was prepared by omitting the erythrocyte suspension. Indomethacin (1 mg/mL) at varying concentrations (1000, 500, 250, 125, and 62.5 µg/mL) was used as a reference standard. Each sample was prepared in triplicate. The reaction mixtures were incubated at 56°C for 30 min in a water bath, then cooled down at room temperature and placed in a freezer followed by centrifugation at 3000 rpm for 5 min. The supernatant was aliquoted in 96 wells plate and absorbance was read at 540 nm using a Microplate reader. The release of haemoglobin determined erythrocyte lysis in hypotonic solutions. All experiments were performed within 24 h of blood collection. The percentage membrane stability was estimated using the expression:

$$\% \text{Membrane stability} = \frac{C - T}{C} \times 100$$

where C is the absorbance of the control and T is the absorbance of the test sample (Ajayi et al., 2014). Absorbance of Test Sample = (Average absorbance of the sample – Absorbance of blank control). An inhibition graph against various doses was used to calculate the IC₅₀. A sample with a low value was considered to have a high membrane stabilization effect.

Determination of antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

As stated by Coker et al. (2024), free radical scavenging was used to assess the test samples' antioxidant capacity. To do this, the optical density of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was tracked. Methanol was used to prepare the various extracts and fractions in five different concentrations, ranging from 50 to 250 µg/mL. After transferring 1 mL of each concentration into a test tube, 1 mL of DPPH solution (0.3 mM) was added. The absorbance at 517 nm was measured 30 min later after incubation in obscurity for 15 min. The percentage of the DPPH radical scavenging was calculated using the equation as given:

$$\% \text{ inhibition of DPPH radical} = \frac{[A_{br} - A_{ar}]}{A_{br}} \times 100$$

where A_{br} is the absorbance before reaction (blank) and A_{ar} is the absorbance after reaction (Coker et al., 2024).

Ferric ion reducing antioxidant power assay (FRAP)

The reducing power of ferric ions in the plant extracts were measured using the method of Rohan and Anup (2014). The mixtures were incubated at 50°C for 30 min after the crude extracts and fractions in varying concentrations (20 to 100 µg/mL) were combined with 2.5 mL of 20 mM phosphate buffer and 2.5 mL of 1%, w/v potassium ferricyanide. The mixtures were then left for 10 min after being mixed with 2.5 mL of 10% w/v trichloroacetic acid and 0.5 mL of 1% w/v ferric chloride. At 700 nm, the absorbance was finally measured. The positive reference standard that was employed was ascorbic acid. Every assay was performed three times and averaged. The values gotten from the FRAP assay were

obtained by comparing the absorbance change in the test reaction mixtures at 700 nm with those containing ferrous ions in a known concentration of the standard, Ascorbic acid. The values were expressed as µg ascorbic acid equivalence (AAE) using the standard curve equation: $y = 0.1903x - 0.3048$

Total antioxidant capacity determination (phosphomolybdenum assay)

After adding 1 mL each of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate to 20 mL of distilled water, the volume was increased to 50 mL using the same solvent. 3 ml of distilled water and 1 ml of Molybdate reagent solution were placed in separate test tubes, and samples in varying concentrations (200 to 1000 µg/mL) were added. For 90 min, the test tubes were incubated at 95°C. After incubation, the absorbance of the reaction mixture was measured at 695 nm and normalized to room temperature for 20-30 min. For every extract, the mean values of three separate samples were determined. The positive reference standard employed was ascorbic acid. The values were expressed as µg ascorbic acid equivalence (AAE) using the standard curve equation (Srividhya et al., 2019):

$$y = 0.515x - 0.5006$$

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) analysis was performed on all the crude extracts in order to investigate the presence of the functional groups of compounds. This analysis was conducted using a Perkin Elmer Spectrophotometer system at 16 scan speeds in the mid-IR range of 400–4000 cm⁻¹.

Gas chromatography mass spectrometer

The analyses of the compounds in the *n*-hexane fraction of *D. regia* stem were run on a Varian 3800/4000 gas chromatography mass spectrometer (GC-MS Agilent Technologies, USA). The mass spectral data obtained from GC-MS were examined using Mnova 11.0.1, with data interpretation referencing the National Institute of Standards and Technology (NIST) database. The software assessed the name, molecular weight, and peak area percentage of identified compounds as seen in the chromatogram. The identification and structural confirmation of the constituents in the test samples were achieved.

RESULTS

Extraction from the plant

The percentage yields of the methanolic extraction of *D. regia* leaf and stem were 10.12 and 2.64% respectively. The percentage yield of the stem fractions *n*-hexane, ethyl acetate and methanol were 0.96, 9.25 and 89.73%, respectively.

Phytochemical analyses of extracts

Qualitative analysis

From the crude extracts obtained from the leaves and

Table 1. Qualitative phytochemical constituents of *D. regia* leaf and stem.

Phytochemicals	DL	DS	<i>n</i> -Hexane fraction (DS)	Ethyl acetate fraction (DS)	Methanol fraction (DS)
Saponins	+	+	-	+	+
Tannins	+	+	-	+	+
Flavonoids	+	+	-	+	+
Cardiac glycosides	+	+	+	+	+
Anthraquinones	-	-	-	-	-
Terpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Phenol	+	+	-	+	+

DL (*D. regia* leaf), DS (*D. regia* stem), + (Present), - (Absent).

Table 2. Quantitative estimation of the methanol extracts of *D. regia* leaf and stem.

Plant sample	Alkaloids (%w/v)	Saponins (%w/v)	Terpenoids (%w/w)	Flavonoids (mg/g QE)	Tannins (µg/g GAE)	Phenol (mg/g GAE)
DL	0.90±0.003	0.60±0.001	0.45±0.002	0.78±0.110	1.39±0.005	1.09±0.001
DS	1.70±0.001	0.40±0.003	1.70±0.001	4.46±0.100	1.21±0.001	0.77±0.004

DL (*D. regia* leaf), DS (*D. regia* stem).

stems, the qualitative phytochemical screening as shown in Table 1 confirmed the presence of phytochemicals such as saponins, tannins, flavonoids, cardiac glycosides, terpenoids, steroids, alkaloids and phenol while anthraquinones were absent.

Quantitative analysis

The total alkaloids, saponins, terpenoids, flavonoids, tannins and phenol contents of the crude leaf (DL) and stem (DS) extracts were shown in Table 2.

In vitro anti-inflammatory activity (Membrane stabilising assay)

The leaf and stem extracts had membrane stabilizing activities of 47.7 and 40.46% respectively at the highest working concentration of 100 µg/mL. At the same concentration, Indomethacin, being the standard/positive control had 41.46% as its %membrane stabilizing activity (Table 3). The membrane stabilizing activity of the ethyl acetate (DSEtOAc) and methanol fractions of Delonix stem (DSM) also showed a concentration-dependent activity, with the % membrane stabilizing activity being 41.91 and 40.33% respectively, at the highest working concentration of 100 µg/mL. Table 4 shows the half-maximal inhibitory concentration (IC₅₀) of the leaf extract (DL); stem extract (DS), ethyl acetate stem fraction

(DSEtOAc), methanolic stem fraction (DSM), as well as that of the standard drug, Indomethacin.

In vitro antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

From the DPPH radical scavenging activity results (Figures 1 and 2), the leaf and stem extracts exhibited lower scavenging abilities, as compared to the standard drug (Ascorbic acid). Ascorbic acid, at the highest concentration of 250 µg/mL, had a scavenging percentage of 72.24%. The scavenging percentage of extracts, DL and DS increased as their concentrations increased and at the highest concentration (250 µg/mL), the leaf extract had a scavenging percentage of 57.95% while the stem extract had a scavenging percentage of 58.28%. The ethyl acetate fraction of DS had a higher radical scavenging ability, with a scavenging percentage of 41.94%, in comparison to the *n*-hexane and methanol fractions.

Ferric ion reducing antioxidant power assay

The crude extracts of *D. regia* leaf and stem exhibited good reducing power with the leaf having a Ferric ion reducing antioxidant power assay (FRAP) content of 3.86 µg/g ascorbic acid equivalence (AAE) and the stem,

Table 3. Membrane stabilising assay for extracts and fractions.

Concentration ($\mu\text{g/mL}$)	DL	DS	DSEtOAc	DSM	Indo
	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM
100	47.77 $\pm$ 0.89	40.46 $\pm$ 0.96	41.91 $\pm$ 1.22	40.33 $\pm$ 0.97	41.46 $\pm$ 0.66
50	40.33 $\pm$ 1.31	39.88 $\pm$ 1.16	39.48 $\pm$ 1.06	39.87 $\pm$ 1.57	39.58 $\pm$ 0.66
25	37.12 $\pm$ 0.73	39.55 $\pm$ 1.37	37.92 $\pm$ 1.18	39.32 $\pm$ 1.79	40.12 $\pm$ 1.47
12.5	36.85 $\pm$ 0.77	39.72 $\pm$ 0.82	37.70 $\pm$ 0.57	37.62 $\pm$ 0.72	39.13 $\pm$ 1.22
6.25	30.49 $\pm$ 1.13	39.27 $\pm$ 1.03	35.66 $\pm$ 0.03	36.37 $\pm$ 0.49	38.83 $\pm$ 1.41

DL (*D. regia* leaf), DS (*D. regia* stem),) DSEtOAc (*D. regia* stem ethyl acetate fraction), DSM (*D. regia* stem methanol fraction) Indo (Indomethacin).

Table 4. Half-maximal inhibitory concentration (IC_{50}) ($\mu\text{g/mL}$) of extracts and fractions.

Extracts and fractions	IC_{50} ($\mu\text{g/mL}$)
	Mean $\pm$ SEM
DL	30.38 $\pm$ 0.33
DS	37.43 $\pm$ 3.28
DSEtOAc	30.93 $\pm$ 0.59
DSM	17.37 $\pm$ 2.15
Indo	42.69 $\pm$ 1.68

DL (*D. regia* leaf), DS (*D. regia* stem), DSEtOAc (*D. regia* stem ethyl acetate fraction), DSM (*D. regia* stem methanol fraction) Indo (Indomethacin).

3.53 $\mu\text{g/g}$ AAE at the least concentration of 20 $\mu\text{g/mL}$. Also, the methanol fraction of DS had a higher activity as compared to the *n*-hexane and ethyl acetate fractions (Figures 3 and 4).

Total antioxidant capacity determination

Total antioxidant capacity (TAC) was one of the parameters used to test the antioxidant properties of the extracts and fractions. Relative to the standard (ascorbic acid), *D. regia* extract also exhibited total antioxidant capacity, with its leaf and stem having a TAC content of 1.13 $\mu\text{g/g}$ and 1.07 $\mu\text{g/g}$ AAE, respectively at the least concentration of 200 $\mu\text{g/mL}$. The ethyl acetate fraction of *D. regia* showed a higher activity in comparison to the *n*-hexane and methanol fractions (Figures 5 and 6).

Fourier transform infrared spectroscopy

The functional groups of the active components in the plant extracts were identified using the FT-IR spectrum. FT-IR analysis result of crude leaf extract (DL) confirmed the presence of functional groups like O-H stretch (Alcohols), N-H stretch (Amines), C-H stretch (Alkanes), C=C stretch (Aromatic compounds), NO_2 stretch (Nitro compounds), C-F stretch (Alkyl and Aryl Halides) and C-I stretch (Alkyl and Aryl Halides) which showed 9 major

peaks at 3359.14, 2922.35, 2851.83, 1610.75, 1517.10, 1444.89, 1376.10, 1033.91 and 582.00 cm^{-1} , respectively (Figure 7 and Table 5). The FT-IR spectrum of crude stem extract, DS (Figure 8 and Table 6) also confirmed the presence of functional groups like O-H stretch (Alcohols), N-H stretch (Amines), C-H stretch (Alkanes), C=C stretch (Aromatic compounds), NO_2 stretch (Nitro compounds), C-F stretch (Alkyl and Aryl Halides) and C-I stretch (Alkyl and Aryl Halides) which showed 13 major peaks at 3351.18, 2922.25, 2851.50, 1606.96, 1518.40, 1447.33, 1376.35, 1243.85, 1119.20, 1032.97, 499.66, 420.10 and 410.95 cm^{-1} , respectively (Figures 9)

DISCUSSION

Qualitative analysis showed the rich presence of alkaloids, flavonoids, saponins, tannins, terpenoids and steroids, while anthraquinones were absent. Confirmation of the phytochemicals present in the leaves and stems of *D. regia* goes in line with the previous study carried out by Bhokare et al. (2018) and Sheikh et al. (2024). Such secondary metabolites are regarded as important combatants against oxidative stress and inflammatory reactions (Li et al., 2023; Smith et al., 2024). Flavonoids are strong antioxidants to scavenge free radicals and bolster cellular antioxidant defenses, especially through activating Nrf2 signaling pathways (Jones and Ahmed,



Figure 1. 2, 2-Diphenyl-1-picrylhydrazyl scavenging activity of leaves and stem extracts.

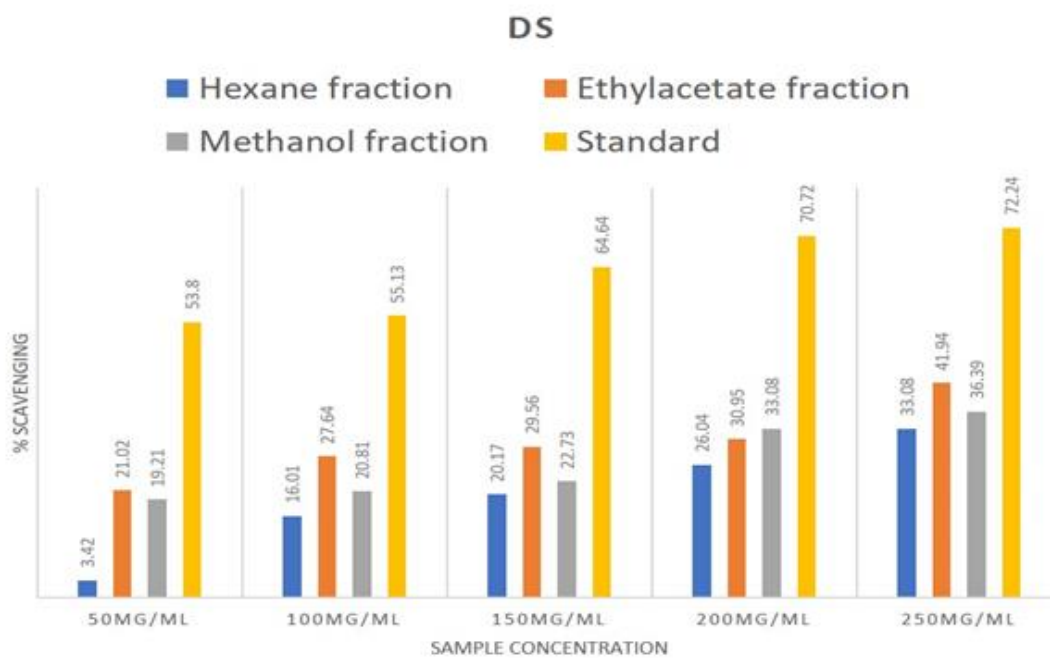


Figure 2. 2, 2-Diphenyl-1-picrylhydrazyl scavenging activity of *D. regia* stem fractions.

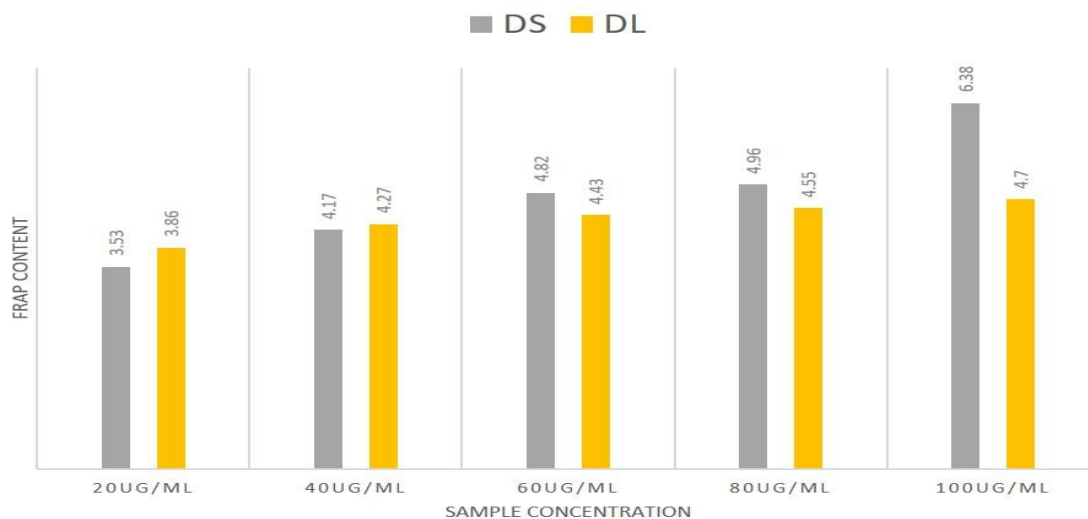


Figure 3. Ferric ion reducing antioxidant power determination of leaves and stem extracts.

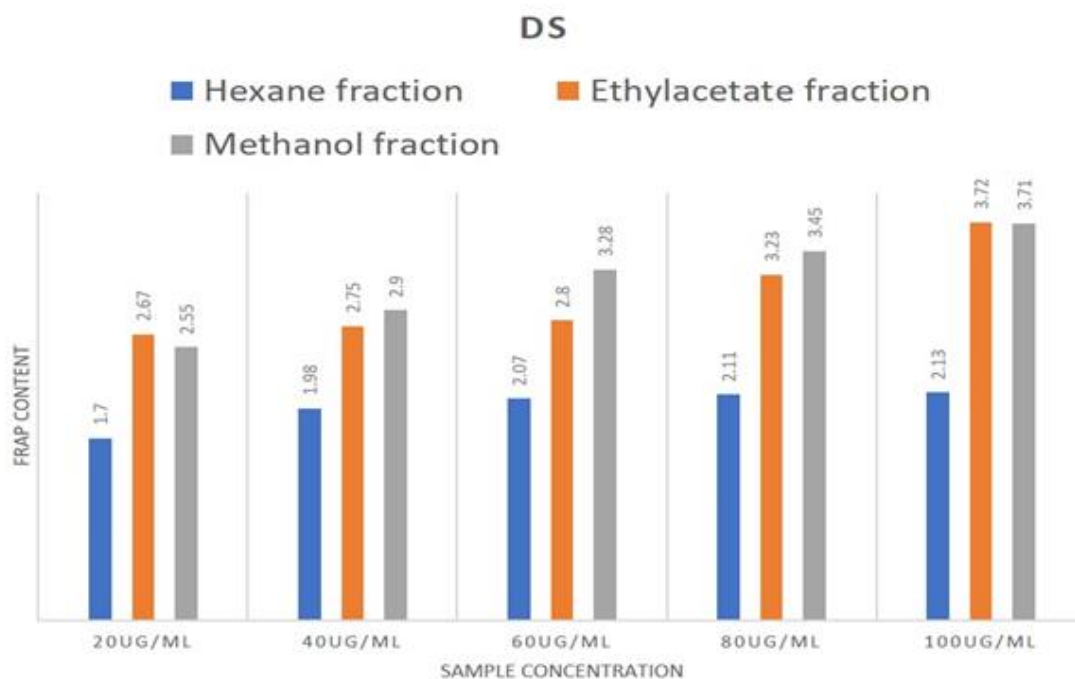


Figure 4. Ferric ion reducing antioxidant power determination of *D. regia* stem fractions.

2023). In contrast, alkaloids exhibit anti-inflammatory actions due to their ability to suppress pro-inflammatory cytokines; such as, TNF- α and interleukin-6 (Garcia et al., 2022). According to Singh et al. (2023) and Zhao et al. (2023), tannins prevent damage caused by oxidative

stress, contributing to their anti-inflammatory activity, and were present in both extracts, with the leaf extract exhibiting the concentration, 1.39 $\mu\text{g/g}$ GAE. Terpenoids, which are found in the amount of 1.70% w/v in stem extract, have been shown to downregulate COX-2 and

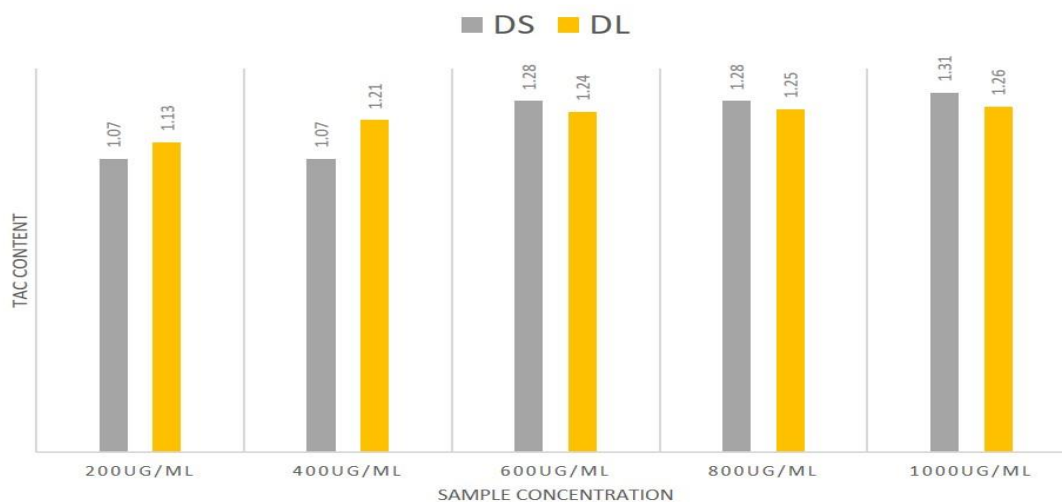


Figure 5. Total antioxidant capacity determination of leaves and stem extracts.

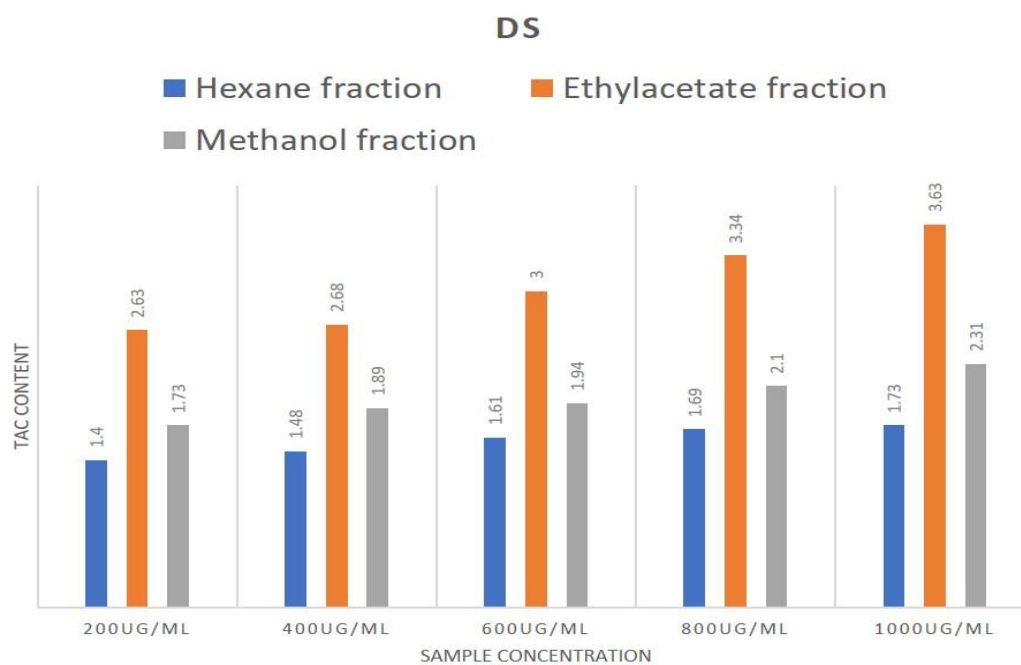


Figure 6. Total antioxidant capacity determination of *D. regia* stem fractions.

LOX enzymes, as well as inhibit inflammation and lipid peroxidation (Olajide et al., 2022). This is corroborated by the phenolic content in DL (1.09 µg/g GAE), since phenolic compounds are known to chelate metal ions and neutralize reactive oxygen species. Also, alkaloids, phenols, flavonoids, saponins, tannins, coumarins,

sterols, terpenoids and essential oils have been reported by Yilma et al. (2023) to exert anti-inflammatory and antioxidant activities alongside other pharmacological activities. The outcomes of qualitative and quantitative phytochemical screening correlate well with the identification of secondary metabolites found to

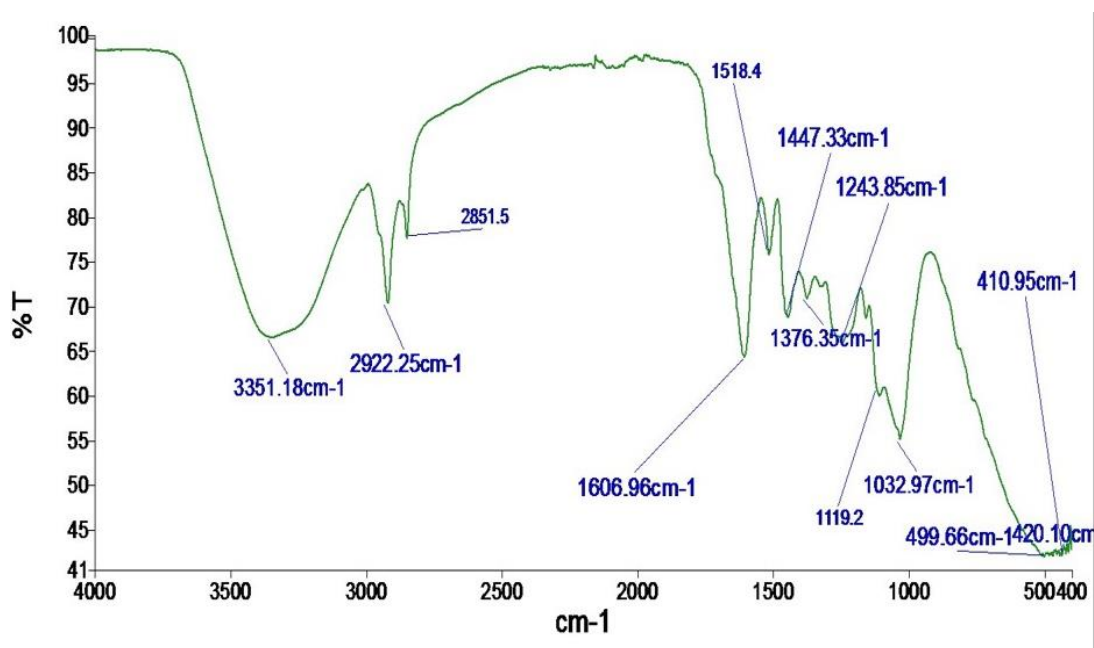


Figure 7. Fourier Transform Infrared Spectrum of *D. regia* leaf crude extract.

Table 5. Compounds detected in Fourier Transform Infrared Spectrum of *D. regia* crude leaf and stem extracts.

Compounds detected	Leaf extract	Stem extract
	Peaks (cm ⁻¹)	Peaks (cm ⁻¹)
O-H stretch (Alcohols), N-H stretch (Amines)	3359.14	3351.18
C-H stretch (Alkanes)	2922.35, 2851.83	2922.25, 2851.50
C=C stretch (Aromatic compounds, alkenes conj.)	1610.75	1606.96
C=C stretch (Aromatic compounds), NO ₂ stretch (Nitro compounds)	1517.10	1518.40
C=C stretch (Aromatic compounds)	1444.89	1447.33
NO ₂ stretch (Nitro compounds)	1376.10	1376.35
C-F stretch (Alkyl and Aryl Halides)	1033.91	1243.85, 1119.20, 1032.97
C-I stretch (Alkyl and Aryl Halides)	582.89	499.66, 420.10, 410.95

play significant roles in the antioxidant and anti-inflammatory mechanisms of action of *D. regia*. This provides scientific background on its use in traditional medicine to treat oxidative stress-linked diseases and inflammatory diseases.

Inflammation is induced by injuries to the tissue(s), which happen when enzymes are released into the cytosol by lysosomal membrane, thereby leading to destruction of membranes. Anti-inflammatory drugs thereby function by inhibiting the release of the lysosomal enzymes, hence, stabilizing the lysosomal and erythrocyte membranes from inflammation (Tanwy and Gupta, 2022). The membrane stabilization assay is a method of screening anti-inflammatory compounds based on their capacity to stop the hemolysis of red blood cells

that is heat-induced. The inhibition of the destruction of membranes would prevent fluids and serum proteins from leaking into the tissues when permeability increases, which is often caused by inflammatory mediators (Ajayi et al., 2014). Phytoconstituents including flavonoids, saponins and tannins identified in the extracts are known to exhibit membrane-stabilizing and anti-inflammatory effects, thus curtailing oxidative lesions and inflammatory mediator release (Yilma et al., 2023). In line with the results of membrane stabilization, the IC₅₀ values are backing up the effectiveness of these plant extracts in membrane stabilization, as shown in Table 4 where DL was the most potent based on the IC₅₀ value 30.38 µg/mL. These extracts also have potential as anti-inflammatory effects due to the fact that the bioactive compounds

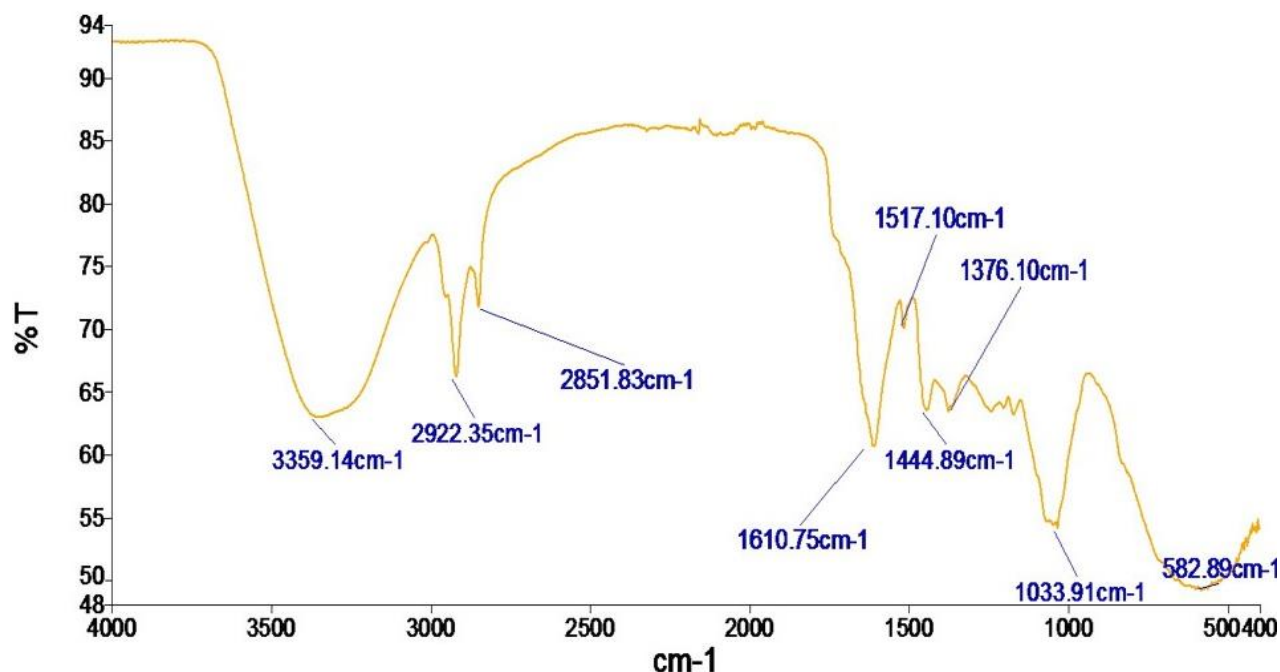


Figure 8. Fourier Transform Infrared Spectrum of *D. regia* stem crude extract.

within them act at lower doses (Yilma et al., 2023). The membrane stabilizing assay results show that *D. regia* leaf and stem have shown their potential for development as natural source of anti-inflammatory agents. The bioactive components maintain membrane integrity, thus minimizing cell lysis and preventing inflammatory damages. These results are consistent with their traditional application in the treatment of inflammation-related diseases.

Antioxidants help in inhibiting oxidation (a reaction that produces radicals) by mopping up free radicals or interrupting their propagation. In large quantity, free radicals can damage cells, but antioxidants can stop or lessen this damage. Free radicals are produced by the body as a response to pressure from the environment or other factors, which may increase the chances of getting sick and getting inflamed. Antioxidants can be gotten from natural or synthetic sources, but mostly in plant-based foods. Phyto-nutrients (plant-based nutrients) contain natural antioxidants (Helberg and Pratt, 2021). Based on the antioxidant assays, the radical-scavenging activity of DL and DS were found to be significant. The DPPH radical scavenging assay showed a dose-dependent scavenging activity of the crude extracts with DS extract achieving up to 58.28% inhibition at 250 µg/mL (comparable to the standard ascorbic acid~72.24%). Several reports have investigated the DPPH radical scavenging activity of *D. regia* plant (Ifelolu et al., 2020) and they are in conformation with the result of our

assay. The antioxidant potential of phenolic compounds is widely known to minimize oxidative stress, suppress lipid peroxidation, and alter all antioxidant enzymes functional (Rudrapal et al., 2022). Phenolic compounds have been proven to eradicate free radicals and militate against their interaction with DNA whilst also increasing the activities of the enzymes responsible for antioxidation and detoxification.

Ferric ion reducing antioxidant power shows the ability of a plant extract to produce reducing agents whose atoms help in reacting with or donating electrons to free radicals thereby turning them to stable metabolites, eradicating radical chain reactions. Bioactive components of plant extracts with ferric ion reducing antioxidant power donate hydrogen and are linked with antioxidant activity (Chaudhary et al., 2023). Results from the FRAP (Ferric Ion Reducing Antioxidant Power) assay confirmed that all extracts showed significant reducing power. The FRAP content of DL extract was 3.86 µg/g ascorbic acid equivalent (AAE) at 20 µg/mL while the highest reducing capacity (6.38 µg/g AAE at 100 µg/mL) was found for DS extract. This result is supported by Ifelolu et al. (2020), who reported the reducing power capacity of *D. regia* leaf. This adds support to previous reports that phenolic and flavonoid compounds can transfer electrons and neutralize oxidative species, strengthening cellular defense mechanisms (Singh et al., 2023; Zhao et al., 2023). The results of the TAC assay showed that both extracts were potent in terms of antioxidant capacity.

Table 6. Compounds detected in the Mass Spectrum for n-hexane fraction of DS (*Delonix regia* stem).

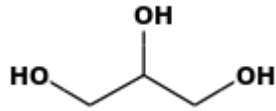
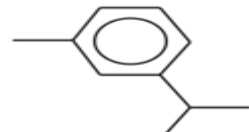
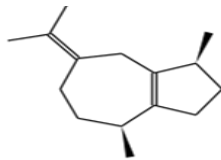
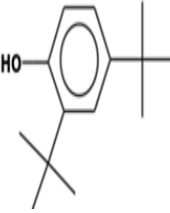
Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp. %wt	m/z	Structures
1	4.00	1,2,3-Propanetriol	C ₃ H ₈ O ₃	92	13.41	5.18	43, 61, 92	
2	4.42	Benzene, 1-methyl-3-(1-methylethyl)-	C ₁₀ H ₁₄	134	8.46	3.84	91, 119, 134	
3	5.25	β-Guaiene	C ₁₅ H ₂₄	204	9.91	3.54	41, 105, 204	
4	6.50	2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206	12.25	8.62	41, 57, 206	
5	9.00	Methyl tetradecanoate	C ₁₅ H ₃₀ O ₂	242	8.16	9.67	43, 74, 242	
6	13.00	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	9.62	13.97	43, 73, 256	
7	14.25	Methyl stearate	C ₁₉ H ₃₈ O ₂	298	0.58	3.64	43, 74, 298	

Table 6. Cont'd.

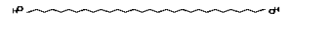
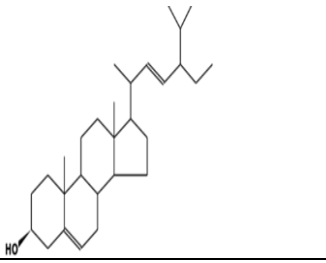
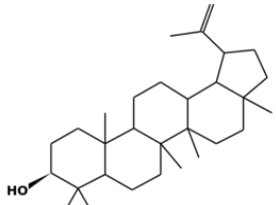
8	15.00	7,10,13-Hexadecatrienoic acid	$C_{16}H_{26}O_2$	250	10.79	12.73	43, 74, 250	
9	17.50	Eicosane	$C_{20}H_{42}$	282	1.46	3.97	43, 57, 282	
10	20.83	Phytol	$C_{20}H_{40}O$	296	4.08	5.52	43, 71, 296	
11	25.21	n-Tetracosanol-1	$C_{24}H_{50}O$	354	2.04	3.61	43, 57, 354	
12	26.50	1-Heptacosanol	$C_{27}H_{56}O$	396	1.17	3.70	43, 57, 396	
13	27.25	1,30-Triacontanediol	<u>$C_{30}H_{62}O_2$</u>	454	11.95	13.31	55, 82, 454	
14	31.98	Squalene	$C_{30}H_{50}$	410	4.96	5.90	41, 69, 410	
15	34.00	Stigmasterol	$C_{29}H_{48}O$	412	0.73	1.53	43, 55, 412	

Table 6. Cont'd.

16	37.00	Lupeol	C ₃₀ H ₅₀ O	426	0.44	1.27	43, 95	
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Collectively, these results authenticate the potent antioxidant activities of *D. regia* extracts. The antioxidants have also been traditionally used to treat disorders related to oxidative stress, at least hypothetically, their ability to scavenge free radicals, donate electrons, and/or inhibit oxidative stress-related damages validate their traditional usage. The functional groups of the active components in the plant extracts were identified using the FT-IR spectrum. The identification was achieved based on the values of the peak in the infrared radiation region. As stated by Al-Kelani and Buthelezi (2024), FT-IR has been proven to be a sensitive and reliable method for determining the composition of biomolecules. The wide bands of absorption near 3350 cm⁻¹ mean hydroxyl (O-H) and amine (N-H) groups, further suggesting the presence of polyphenols and flavonoids with antioxidant and anti-inflammatory activities (Smith et al., 2024; Brown et al., 2023). These extracts contain compounds that scavenge free radicals and modulate inflammatory signaling cascades, thereby decreasing oxidative damages (Jones and Ahmed, 2023).

The 2920 and 2850 cm⁻¹ peaks indicative of alkyl (C-H) stretching vibrations, characteristic of terpenoid components, serve as COX and LOX enzyme inhibitors, thus reducing inflammation.

Moreover, the bands in the region of 1600-1450 cm⁻¹ are due to the C=C stretching vibrations, which signifying the flavonoids contents that showed to inhibit NF-κB, which is one of the key regulators of inflammation (Garcia et al., 2022). The peak observed at 1515 cm⁻¹ is associated with nitro compounds (NO₂), indicative of nitric oxide scavenging activity responsible for protecting against damage related to oxidative stress (Jomova et al., 2023). The carbonyl (C=O) stretching around 1700 cm⁻¹ is characteristic of aldehydes and ketones which amplify membrane stabilization and antioxidative protection (Singh et al., 2023; Zhao et al., 2023). The detection of halides (C-F and C-I) at 1250-1030 cm⁻¹ and below 600 cm⁻¹, respectively, suggests the ability of bioactive compounds linked to cell-mediated activity and antioxidative mechanisms (Olajide et al., 2022).

Collectively, the FT-IR data present compelling evidence of the therapeutic potential of *D. regia* leaf and stem, thus, validating their folkloric use for controlling inflammation and oxidative stress. These results give them a scientific basis of their use in phytomedicine. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the *n*-hexane fractions of *D. regia* stem extract (DS) showed that DS contains a variety of bioactive

compounds that contribute to its anti-inflammatory and antioxidant activity. Phytol, squalene and stigmasterol are among those identified as promising leads in this research area (Smith et al., 2024).

Phytol has antioxidant effects and induces cellular defense via modulation of Nrf2 signaling and decreasing oxidative stress (Jones and Ahmed, 2023). Squalene (39.00 min retention time) is associated with anti-inflammatory and skin-protective effects through ROS-scavenging and downregulation of pro-inflammatory cytokines, such as TNF-α and interleukins (Garcia et al., 2022). The peak area in Table 6 shows that stigmasterol is present in large amount, thus suggesting that they could play a role in the modulation of inflammatory pathways. These plant sterols inhibit cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), the critical enzymes within the inflammatory response that subsequently alleviates inflammation-associated tissue injuries (Kim et al., 2024; Chandra et al., 2023). The 2,4-Di-tert-butylphenol and hexadecanoic acids observed in this study have shown notable antioxidant activity by scavenging free radicals and inhibiting peroxidation of lipid membranes (Olajide et al., 2022). It is a significant point regarding whether the extracts have the

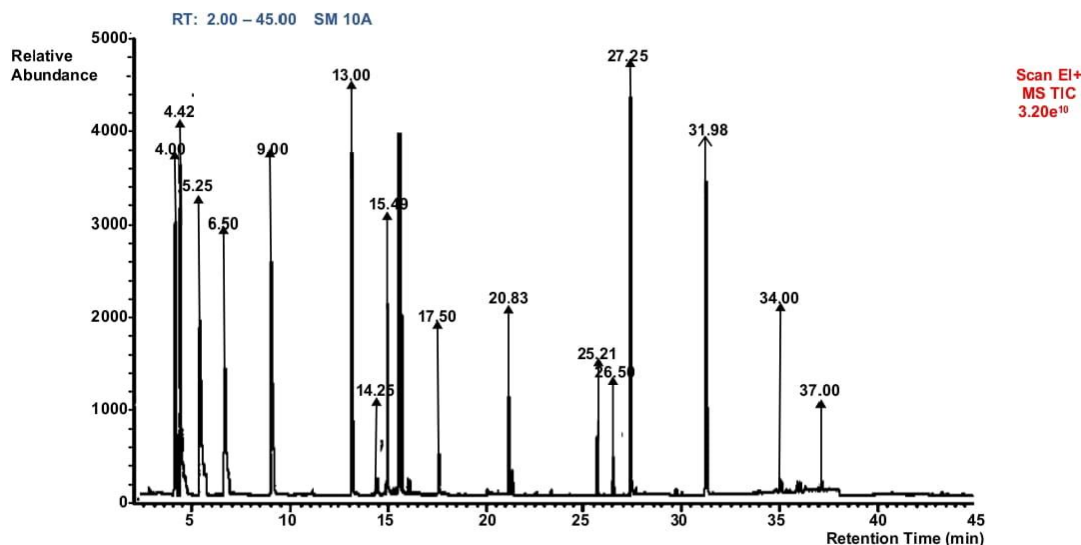


Figure 9. GC-MS Spectrum for *n*-hexane fraction of *D. regia* stem.

potential to modulate inflammatory and oxidative stress pathways also noted by GC-MS profile along with their activity presents the bioactive molecules which possess the anti-inflammatory and antioxidant activity. The results confirm the traditional use of *D. regia* in the management of oxidative stress – associated disorders, and inflammatory diseases.

Conclusion

The methanolic crude extracts of *D. regia* leaf and stem have been observed to possess appreciable anti-inflammatory and antioxidant properties which could be attributed to the wide range of phytochemicals present in them. Based on the findings of this study, it is recommended that *D. regia* can be further explored for toxicological and other therapeutic effects. Further studies can be directed towards the isolation, identification and characterization (using advanced techniques like HPLC, NMR, GC-MS spectroscopy) of pure bioactive compounds present in the plant. The extracts can be formulated as external preparations such as gels, creams, lotions, ointments, etc., for administration as treatment or management of inflammation and oxidative stress-related diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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