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Phytochemical Analysis, Antioxidant Properties and Vermicidal Activity of Flower Extract of *Delonix regia*

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Abstract: *Delonix regia* belongs to subfamily Caesalpinioideae and family Fabaceae. The purpose of this study was to determine the phytochemicals that are present in the flowers of the *Delonix regia* plant and to assess the antioxidant activity of these flowers. In order to ascertain the antioxidant activity, the DPPH radical scavenging assay was utilized. Carbohydrates, proteins, amino acids, fatty acids, resins, alkaloids, cardiac glycosides, polyphenols, flavonoids, phytosterols, anthocyanins, phlobotannins, saponins, xanthoproteins, terpenoids, and coumarins are all components that were found in the floral extract of *Delonix regia*. Therefore, it is evident that the flower extract of *Delonix regia* possesses a substantial amount of antioxidant activity, which in turn, makes a major contribution to the reduction of the risk of a variety of diseases, such as heart disease, the creation of cancer cells, and physiological abnormalities in cells. The floral extract was subjected to GC-MS analysis, which revealed the presence of catechol, nonanol and 1,2-benzenedicarboxylic acid, diethyl ester as the primary components. The Indian earthworms (*Pheritima posthuma*) were employed to study the vermicidal activity of the floral extract of *Delonix regia*. The plant extract exhibited vermicidal activity which was dose dependent. The results indicate the presence of significant bioactive chemicals in the plant extract, which may be responsible for its pharmacological effects.

Keywords: *Delonix regia*, GC-MS, FT-IR, Vermicidal activity, Antioxidant, Phytochemical

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Introduction

The *Delonix regia* tree, also known as Gulmohar, belongs to the Fabaceae family, specifically the subfamily Caesalpiniaceae. The plant is an ornamental species characterized by its deciduous nature and huge size, featuring leaves that resemble ferns. Ayurvedic and traditional

medicines utilize the stem, bark, leaves, flower, and seed of this plant to address a range of ailments (Saroj *et al*., 2022).

The *Delonix regia*, often known as Gulmohar, possesses diverse therapeutic and traditional characteristics. Different portions of this plant are utilized to address various illnesses. The infusion of leaves is customarily employed in the treatment of gastrointestinal issues and rheumatic aches in the joints. Flowers were employed in the treatment of gynaecological diseases and diarrhoea. Additionally, the leaves have been documented for their antibacterial, anti-inflammatory, and anti-diabetic properties (Chakraborty et al., 2016).

The purpose of this study was to determine the phytochemicals that are present in the flowers of the *Delonix regia* plant and to assess the antioxidant activity of these flowers. The Indian earthworms (*Pheritima posthuma*) were employed to study the vermicidal activity of the floral extract of *Delonix regia*.

Materials and Methods

Collection of Plant Materials:

Delonix regia flowers were freshly collected from Enathur, Kanchipuram, India. The flowers underwent a thorough washing process using regular tap water, followed by sterile distilled water. Subsequently, the flowers were dried in the shade at ambient temperature and transformed into a fine powder by using a grinding machine. A crude extract was made from the powdered flower. The solvents employed were water and ethanol.

Preparation of extracts:

The aqueous and ethanolic floral extract was prepared by soaking 7.5 g of coarsely powdered *Delonix regia* powder in a mixture of 150 ml of ethanol and water for a period of 24 h. Afterwards, the extract was filtered using Whatman filter paper No. 1, resulting in the collection of the filtrate. The resulting filtrate was hermetically sealed in an airtight container and kept in a light-free environment for further examination.

Preliminary phytochemical analysis:

The aqueous and ethanolic floral extracts of *Delonix regia* were subjected to qualitative analysis of phytochemicals using established methodologies, as described by Harborne *et al*. (1998), to determine the components.

Preliminary detection of potential bioactive metabolites by GC-MS analysis:

The Shimadzu QP2010 Plus apparatus, which included an auto sampler and a gas chromatograph that was coupled to a mass spectrometer (GC-MS), was utilized in order to conduct the analysis of the ethanolic floral extract of *Delonix regia*. Gas chromatography-mass spectrometry, sometimes known as GC-MS, is the most efficient technique for determining the phytochemicals or components that can be found in plant extracts. With the assistance of the SRM Nanotech Research Centre, the analysis was carried out. The database that was provided by the National Institute of Standard and Technology (NIST) was utilized in order to carry out the analysis of the mass spectrum in GC-MS.

FTIR Spectroscopic Analysis:

The Fourier transform infrared spectrophotometer, often known as FTIR, is one of the most effective instruments for determining the many kinds of chemical bonds (functional groups) that are present in individual substances. An FTIR analysis was performed using particles that had been dried from an ethanolic extract of *Delonix regia*. For the purpose of preparing a translucent sample disc, 10 mg of the powdered dry extract was carefully encapsulated within 100 mg of KBr pellet. An FTIR Spectroscope (Shimadzu, IR Affinity1, Japan) was used to load the powdered sample of plant specimen. The spectroscope has a scan range of 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1}.

UV-Visible Spectroscopic analysis:

The ethanolic floral extract of *Delonix regia* was

subjected to UV-visible spectrophotometric examination at room temperature. The instrument used for this analysis was a Hitachi UH5300 UV-visible spectrophotometer. An examination of the extract was carried out using visible and ultraviolet light with wavelengths ranging from 190 to 900 nm for the purpose of proximate analysis.

Quantitative analysis of free radical scavenging activity of Delonix regia:

In accordance with the methodology outlined by Babu *et al*. (2015), the antioxidant activities were evaluated by employing DPPH, which is manufactured by Sigma-Aldrich.

Collection of earthworms:

The earthworms (*Pheritima posthuma*) were collected from Orikkai, Kanchipuram, India and subsequently identified.

Vermicidal activity:

The vermicidal action of the *Delonix regia* flower extract has been documented against *Pheritima posthuma* (Indian Earthworm). The study was conducted on mature earthworms due to their morphological and physiological similarity to the intestinal roundworm parasites found in humans. The widespread accessibility of earthworms encourages their substantial utilization for first *in vitro* assessment of vermicidal substances.

The vermicidal activity was conducted using the methodology described by Ghosh *et al*. (2005) on the adult Indian earthworm species *Pheritima posthuma*. Albendazole, the established medication, was mixed with normal saline to achieve concentrations of 50 mg/ml and was then placed onto Petri dishes. The aqueous floral extract of *Delonix regia* was mixed with normal saline to create concentrations of 25, 50, and 100 mg/ml. The negative control consisted solely of normal saline solution containing 0.9% sodium chloride.

The dilutions were poured into the Petri dishes. The study involved a total of five groups of earthworms, with each group consisting of three individuals. Earthworms of approximately identical dimensions (around 8 cm) were introduced into individual petridishes at the ambient temperature. The test solutions and standard medication solutions were newly made prior to commencing the experiment. Three equitably sized groups of earthworms were introduced into 20 ml solutions with three distinct concentrations (25, 50, 100 mg/ml) in petridishes containing the aforementioned extract solutions.

The reference standard employed was Albendazole at a concentration of 50 mg/ml, while the control was saline. The worm's time of paralysis and time of death were determined. The occurrence of paralysis was observed when the worms exhibited no movement whatsoever, except when they were severely shaken. The time of death for the worms was determined by confirming their immobility when subjected to both strong shaking and immersion in warm water at a temperature of 50°C. The duration of paralysis and time till death were measured in minutes.

Results and Discussion

Preliminary Phytochemical screening:

The study showed that ethanolic flower extracts of *Delonix regia* were found to be high in all phytochemical elements that were evaluated. Tables 1–3 provide an overview of the qualitative phytochemical screening of *Delonix regia* that was conducted. Based on the investigation, *Delonix regia* is the plant that has the majority of the phytochemicals. The majority of the tests yielded comparable outcomes for both ethanol and aqueous extracts.

It was found that the water-based extract of *Delonix regia* gave positive results for carbohydrates, proteins, amino acids, fatty acids, resins, alkaloids, cardiac glycosides, flavonoids, phlobotannins, saponins, xanthoproteins, terpenoids, and coumarins. To put this into perspective, the ethanolic extract of Delonix regia produced positive results for all of the following:

Table 1: Phytochemical Screening of Aqueous and Ethanolic Extract of *Delonix regia*

Test	Aqueous Extract	Ethanol Extract
Test For Primary Metabolites		
Test For Carbohydrates		
a, Benedict's test	+	+
b, Molisch's test	+	+
c. Iodine test	--	--
Test For Proteins		
a. Heller's test	+	--
b. Biuret test	+	+
Test For Amino acids		
a. Ninhydrin test	+	+
Test For Fatty acids	+	+
Test For Resins	+	+
Test For Fixed oil and Fats		
a, Spot test	--	+
b, Saponification test		
c. Gums and Mucilage	--	+

+ indicates the presence of Phytochemicals; -- indicates the absence of Phytochemicals

Table 2: Phytochemical Screening of Aqueous, and Ethanolic Extract of *Delonix regia*

Test	Aqueous Extract	Ethanol Extract
Test For Secondary Metabolites		
Test For Anthraquinones	--	--
Test For quinones	--	--
Test For Alkaloids		
a. Mayer's test	--	--
b. Wagner's test	+	+
c. Dragendorff test	+	+
Test For Glycosides		
Borntrager's test	--	+
Test For Cardiac Glycosides		
Keller - Killani test	+	+
Test For Phenolic compounds		
a. Gelatin test	--	--
b. Lead acetate test	--	+
Test For Polyphenols	--	+
Test For Tannins	--	--
Test For Flavanoids	+	+
Test For Phytosterols	--	+
Test For Phlobotannins	+	+
Test for Saponin	+	--
Test For Sterols	--	+

+ indicates the presence of Phytochemicals; -- indicates the absence of Phytochemicals

Table 3: Phytochemical Screening of Aqueous, and Ethanolic Extract of *Delonix regia*

Test	Aqueous Extract	Ethanol Extract
Test For Secondary Metabolites		
Test For Xanthoproteins	**+**	**+**
Test For Chalcones	--	--
Test For Terpenoids (Salkowsky test)	**+**	**+**
Test for Triterpenoids	**+**	**+**
Test For Anthocyanin	--	**+**
Test For Leuco-Anthocyanin	--	--
Test For Coumarins	**+**	**+**
Test For emodins	--	--

+ indicates the presence of Phytochemicals; -- indicates the absence of Phytochemicals

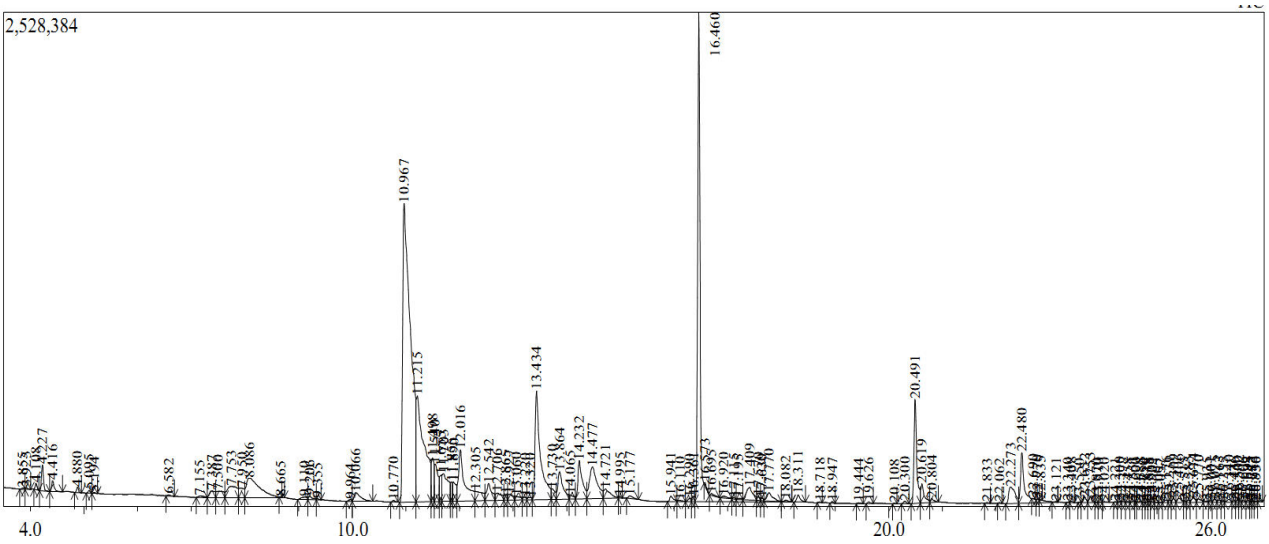


Fig. 1: Preliminary detection of potential bioactive metabolites by GC-MS analysis.

carbohydrates, proteins, amino acids, fatty acids, resins, fixed oils, alkaloids, glycosides, cardiac glycosides, polyphenols, phytosterols, phlobo-tannins, sterols, xanthoproteins, terpenoids, anthocyanins, and coumarins. Leuco-anthocyanins, emodins, quinones, anthrax-quinones, and chalcones were lacking in both extracts.

Saponin, alkaloid, terpenoids, flavonoids, steroids, phenols, cardiac glycosides, quinine coumarins, and tannins are all types of compounds that can be found in the flower extract of the *Delonix alata* plant. Accordingly, it is evident that the floral extract of *Delonix elata* possesses a substantial amount of antioxidant activity, which, in turn, makes a significant contribution to the reduction of the risk of a wide range of diseases, such as cardiovascular disease, the creation of cancer cells, and physiological abnormalities in cells (Teklit *et al*., 2016).

Flower samples were analyzed and found to contain a variety of compounds, including alkaloids, flavonoids, proteins, tannins, carbohydrates, phenols, triterpenes, and steroids

Table 4: Chemical constituents identified in GC-MS analysis of Ethanolic extract of Delonix regia

S. No.	Chemical Name of the Compound	Molecular formula	Mol. Wt	Retention time	Peak Area %
1	1,2-Cyclohexanedione	C6H8O2	112	7.753	1.26%
2	7-Oxabicyclo[2.2.1]hept-5-en-2-one	C6H6O2	110	8.086	2.98%
3	Catechol	C6H6O2	112	10.967	26.42%
4	1,2-Benzenediol,o-(3-chloropropionyl)-	C9H9ClO3	200	11.215	9.32%
5	1,2-Benzenediol, mono (methylcarbamate)	C8H9NO3	167	11.498	1.27%
6	N-Ethylcyclohexane-1,1-Dicarboximide	C10H15NO2	181	11.540	1.46%
7	o-Hydroxyphenyl methylcarbamate	C8H9NO3	167	11.703	1.89%
8	6,7,8,8a-Tetrahydro-2H,5ah-cyclopenta[b][1,4]dioxepine	C8H12O2	140	12.016	3.45%
9	Cycloheptanone	C7H12O	112	12.542	0.99%
10	Nonanal	C9H18O	142	13.434	6.58%
11	6-Hydroxyhexahydro-2h-Cyclopenta [B] Furan-2-One	C7H10O3	42	13.864	1.59%
12	Aziridine, 2,3- Dimethyl-1- (1-Methyl ethyl)-	C7H15N	113	14.232	1.91%
13	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	C4H9NO5	151	14.477	2.76%
14	1,2-Benzenedicarboxylic Acid, Diethyl Ester	C12H14O4	222	16.460	15.16%
15	4-O-Methylmannose	C7H14O6	194	17.409	0.89%
16	9-Octadecenoic acid	C18H34O2	282	20.491	3.19%
17	17-Octadecynoic acid	C18H32O2	280	22.273	1.18%
18	9-Octadecenoic acid	C18H34O2	282	22.480	2.55%

(Khursheed *et al*., 2012).

Identification of Chemical Compounds present in hydroalcoholic extract of Delonix regia by GC-MS:

The chromatogram of the GC-MS spectral analysis of *Delonix regia* reflecting the peaks of the distinct compounds, their elution, and their retention time are shown in Figure 1. GC-MS analysis of the ethanolic flower extract of *Delonix regia* (Table 4) revealed the presence of 18 compounds. Of these, four major chemical compounds are catechol, nonanal, 9-octadecanoic acid, and 17-octadecynoic acid. The compounds with their retention time (RT), molecular formula, molecular weight (MW), and peak area (%) have been presented in Table 4. Table 5 illustrates the pharmacological activity and structure of *Delonix regia.*

It is possible that the existence of a wide variety of secondary metabolites, including alkaloids, flavonoids, cardiac glycosides, phenols, saponins, steroids, and so on, is the cause of the therapeutic capabilities that medicinal plants possess. Therefore, it is necessary to conduct the preliminary screening test in order to identify the

Table 5: GC – MS analysis of *Delonix regia*

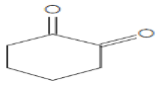
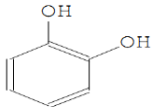
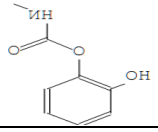
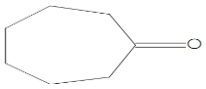
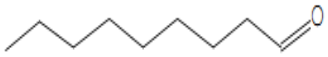
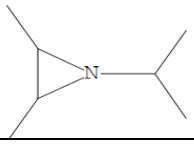
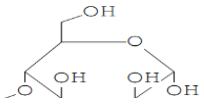
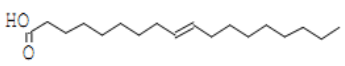
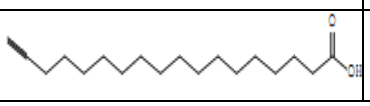
S. No.	Name of the compound	Structure	Pharmacological activity
1	1,2-Cyclo hexane dione		Anticancer activity
2	7-Oxabicyclo [2.2.1] hept-5-en-2-one	--	Nil
3	Catechol		Antibacterial effects Antioxidant Antihyperlipidemic Anticancer effect
4	1,2-Benzenediol, o-(3-chloro propionyl)-	--	Nil
5	1,2-Benzenediol, mono (methyl carbamate)		Hypercholinergic activity
6	N-Ethylcyclohexane-1,1-Dicarboximide	--	analgesic activity, anti-allergic activity, Circulatory activity
7	o-Hydroxy phenyl methyl carbamate	--	hypercholinergic activity
8	6,7,8,8a-Tetrahydro-2H,5ah-cyclopenta [b][1,4] dioxepine	--	**Nil**
9	Cycloheptanone		Antispasmolytic, Anti-inflammatory, Vasodilator agent
10	Nonanal		Antimicrobial , Anti-diarrhoeal, Antifungal activity, Antioxidant activity.
11	6-Hydroxy hexa hydro -2h- Cyclo penta [B] Furan-2-One	--	**Nil**
12	Aziridine, 2,3- Dimethyl-1- (1-Methyl ethyl)-		Antitumor, Antimicrobial, Anticancer activity,
13	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	--	**Nil**
14	1,2-Benzenedicarboxylic Acid, Diethyl Ester	--	**Nil**
15	4-O-Methylmannose		Antibacterial activity
16	9-Octadecenoic acid		Antimicrobial, Antioxidant activity, Anti-inflammatory, Anticancer, Dermatitigenic, Hypocholesterolemic
17	17-Octadecynoic acid		Antifungal and antioxidant activity

Table 6: Analysis of Functional groups in Ethanolic extract of *Delonix regia* by FTIR

Peak No.	Peak Value	Nature of Functional Group
1.	408.05	Alkyl & Aryl Halides-C-I stretch
2	435.91	Alkyl & Aryl Halides-C-Br stretch
3.	879.54	Aromatic Compounds- C-H bend
4.	1045.42	Alkyl & Aryl Halides-C-F stretch
5.	1087.85	Alkyl & Aryl Halides-C-F stretch
6.	1651.07	Amides - C=O stretch
7.	2098.55	Alkynes- C≡C stretch
8.	2885.51	Alkanes - C-H stretch
9.	2970.38	Carboxylic Acids-O-H stretch

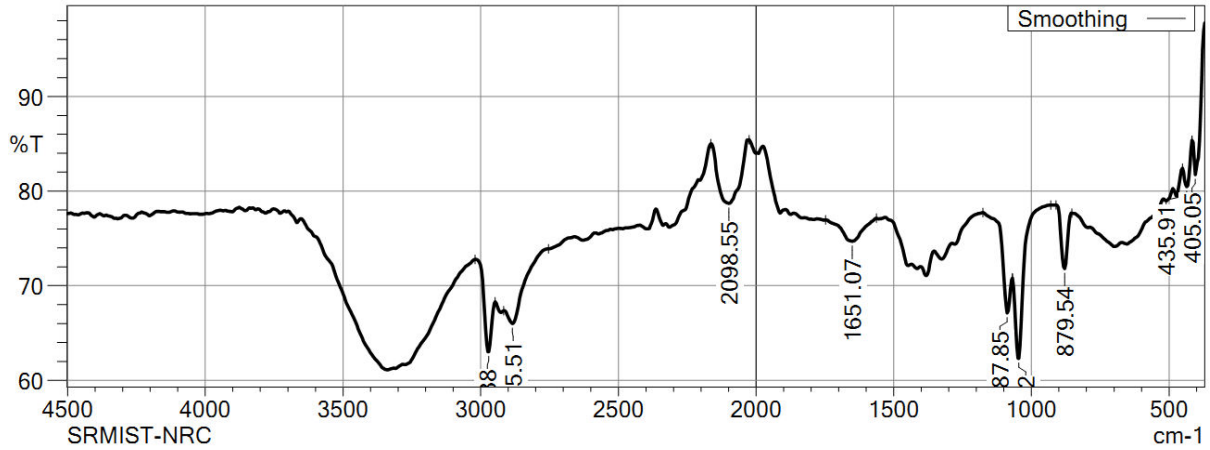


Fig. 2: FT-IR analysis of Ethanolic extract of *Delonix regia.*

bioactive principles, which will ultimately result in the discovery and development of potential pharmaceuticals (Adeneye *et al*., 2006).

Fourier Transform Infrared (FTIR) analysis of a plant extract:

To find the functional groups of the active ingredients in the extract, the FT-IR spectrum was used to look at the peak values in the IR radiation region. By passing the extract through the FT-IR, the functional groups of the components were separated based on their peak ratios. The results of the FT-IR analysis confirmed the presence of various functional groups (Table 6), including C-I, C-Br, C-H, COOH, C=C, C-H stretch, C-F stretch, and C=O **(**Fig. 2). FTIR spectroscopy has been demonstrated to be a reliable and sensitive method for detecting the composition of biomolecules.

In addition to the functions that were initially mentioned, *Delonix regia* possesses a number of therapeutic properties. All these therapeutic applications of this extract is absolutely due to the phytoconstituents like flavonoids, sterols, saponins, glycosides, alkaloids and tannins present in the plant. It has been observed that all of the flavonoids that have been identified are involved in biological activities. Flavonoids are widely distributed throughout the plant world (Mariajancyrani *et al*., 2016)

UV Spectroscopic Analysis:

The ethanolic flower extract of *Delonix regia* was analyzed using a double-beam ultraviolet spectrophotometer (Hitachi UH5300) over a wavelength range of 190 to 900 nm. The purpose

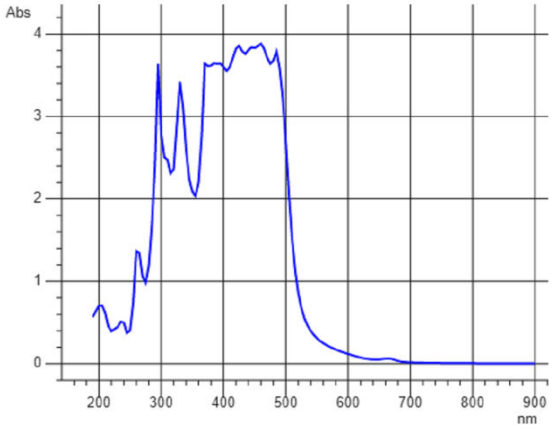


Fig. 3: Ultra Violet-Visible Spectroscopy Analysis of Ethanolic extract of *Delonix regia.*

Table 7: UV-VIS Spectrum Peak values of Ethanolic extract of *Delonix regia*

S. No.	Wavelength nm	Absorbance
1.	205	0.708
2	260	1.367
3.	295	3.638
4.	330	3.418
5.	395	3.641
6.	425	3.857
7.	460	3.883
8.	485	3.786

was to identify the specific peaks in the UV spectrum. The recorded peak values are presented in Table 7. The UV-VIS profile exhibited various peaks between 200 and 500 nm, each with different absorption levels.

The profile displays peaks at specific wavelengths (205, 260, 295, 330, 395, 460, and 485 nm) with corresponding absorption values (0.708, 1.367, 1.638, 3.418, 3.641, 3.857, 3.883, and 0.625). Figure 3 illustrates the absorption spectrum of the extract from the *Delonix regia* plant, which is mostly transparent in the wavelength range of 300-800 nm. The absorption bands related to the *Delonix regia* plant extract is depicted in Table 7.

The presence of unsaturated groups and heteroatoms such as S, N, and O can be clearly identified in the UV-VIS spectra by the appearance of multiple peaks in the 200 to 500 nm region. The UV-VIS spectrum of the *Delonix regia* extract exhibits two peaks at 425 nm and 460 nm, confirming the presence of organic chromophores in the extract. However, the use of UV-visible spectrophotometry in analyzing complex media is limited due to the challenges in assigning absorption peaks to specific constituents in the system. Therefore, UV-VIS findings should be complemented with other analytical techniques such as GC/MS to accurately characterize the extract and identify its constituents.

The ethanolic extract of *Delonix regia* demonstrated a notable dose-dependent suppression of DPPH activity, indicating its electron-donating capability. The ethanolic flower extract of *Delonix regia* had a strong free radical scavenging effect on DPPH that depended on the concentration.

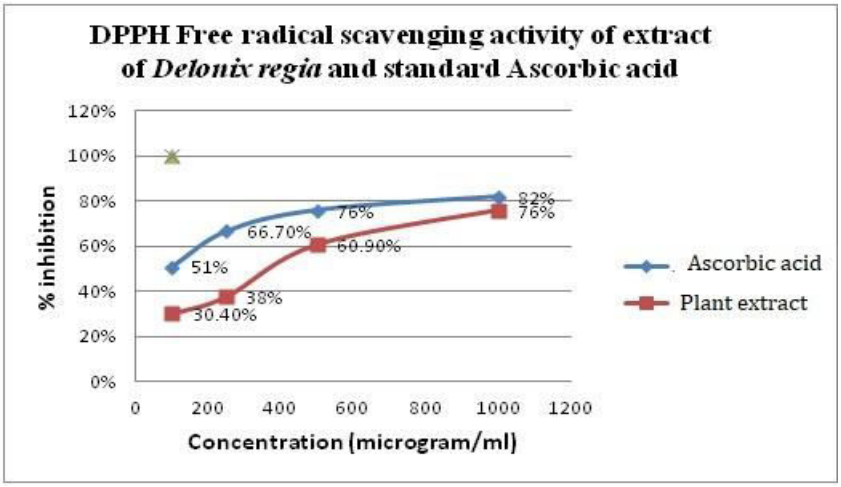


Fig. 4: Assay of antioxidant activity.

Table 8: Vermicidal activity with aqueous floral extract of *Delonix regia* and standard drug

Concentration (mg/ml)	Negative control Saline (0.9%)	Positive control Standard drug Albendazole (50 mg/ml)	Aqueous floral extract of *Delonix regia*		
			(25 mg/ml)	(50 mg/ml)	(100 mg/ml)
Time of paralysis (Mean ± SD) (Minutes)	Nil	20.5 ± 1.04	Nil	17.8 ± 1.16	12.8 ± 1.17
Time of death (Mean ± SD) (Minutes)	Nil	29.3 ± 1.36	Nil	Nil	17.3 ± 1.63

All values represent Mean +SD; n= 3 in each group

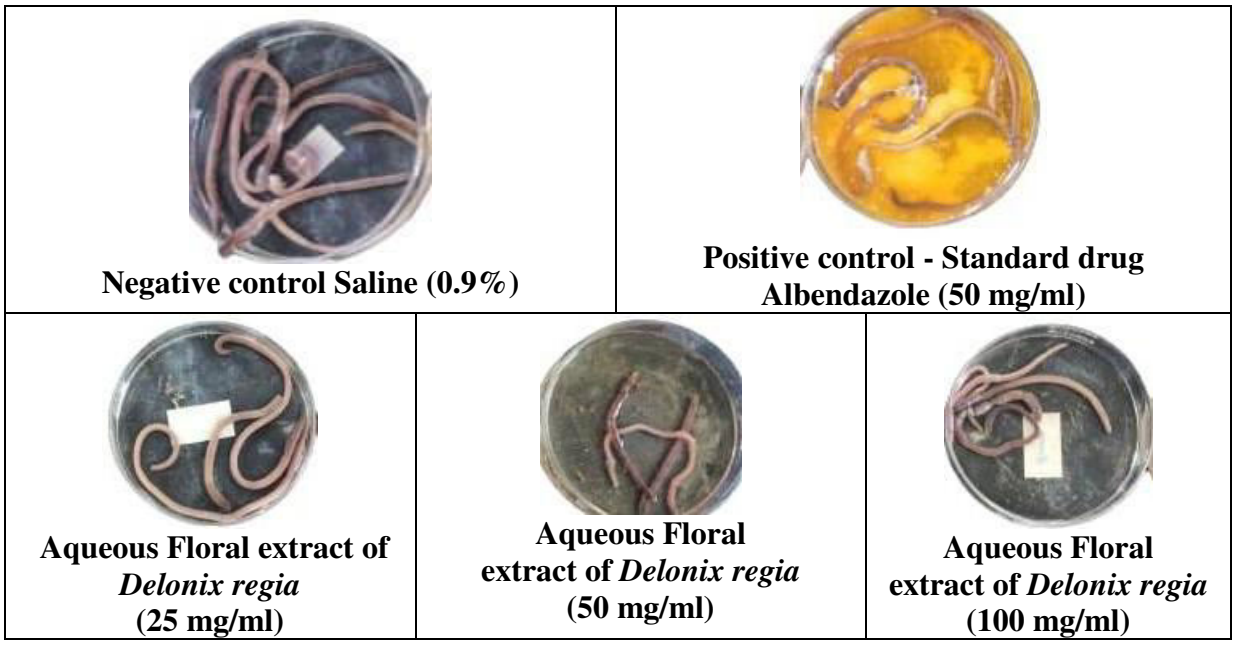


Fig. 5: Anthelmintic activity with aqueous floral extract of *Delonix regia* and standard drug.

An assay was conducted to determine the concentration dependence of these extracts, and the findings are illustrated in Figure 4. The study utilized four different concentrations of ethanolic floral extract derived from *Delonix regia*, ranging from 100 to 1000 µg/ml in ethanol. The corresponding percentages of DPPH scavenging activity were determined to be 30.4%, 38%, 60.9%, and 70.6%, respectively.

In addition to the investigation, varying dosages (100–1000 mg/ml) of standard ascorbic acid were administered. The resulting DPPH scavenging activity percentages were determined to be 51%, 66.7%, 76%, and 82%, respectively.

For the most part, the quantity of DPPH radicals that the ethanolic flower extract of *Delonix regia* was able to scavenge was comparable to the amount of DPPH radicals that ascorbic acid was able to neutralize. The flower extract of *Delonix regia* that was mixed with alcohol showed a high level of free radical scavenging activity. This is because the plant contains phenolic compounds, flavonoids, and other antioxidants.

Phenolic compounds and flavonoids have a negative impact on free radicals and also boost the activity of antioxidative enzymes. The findings indicate that the test plants exhibit high phenolic contents and antioxidant capacity, rendering them a superior source of dietary antioxidants. Using α-tocopherol and butylated hydroxyl toluene as the standard antioxidant, ethanolic crude extracts of *Delonix regia* were evaluated for their antioxidant and free radical scavenging properties. The efficacy of these extracts was evaluated using the ferric thiocyanate (FTC) assay, and the results were compared with the thiobarbituric acid (TBA) method. The diphenyl picryl hydrazyl (DPPH) radicals were utilized in order to assess the free radical scavenging activity. A high amount of antioxidant activity was demonstrated by the extract. The FTC method, which uses α-tocopherol and butylated hydroxy toluene (BHT) as standard antioxidants, demonstrates that the alcoholic extract of this plant had the potential to exhibit beneficial antioxidant properties **(**Ahmed *et al*., 2009).

Vermicidal activity:

The aqueous solution of *Delonix regia* plant extract, at a concentration of 50 mg/ml, induced paralysis in worms within a time period of 20 min. The aqueous extract of the *Delonix regia* plant, at a concentration of 100 mg/ml, induced paralysis in worms within 13 min and resulted in their death within 18 min (Fig. 5). Albendazole, a commonly used medication, induced paralysis within 21 min and resulted in death after 29 min when administered at a dose of 50 mg/ml (Table 8).

Conclusion

Through the use of techniques such as GC/MS, FTIR, and UV-VIS, this study has provided preliminary information that can be used to establish the chemical composition of *Delonix regia.* With the help of gas chromatography and mass spectrometry (GC/MS) analysis, the present investigation was able to determine the identities of eighteen different chemical compounds that were present in the ethanolic floral extract of *Delonix regia*.

Aqueous floral extract of *Delonix regia* used for study showed profound vermicidal activity. In addition, it is imperative to identify and separate the potential active phytoconstituents that are responsible for the vermicidal activity and thoroughly investigate their pharmacological effects in future research. In conclusion, future studies should focus on conducting comprehensive investigations into the phytochemical, clinical, and molecular mechanisms of action. Simultaneously, it is necessary to standardize the plant extracts that exhibit effective vermicidal properties and develop optimal herbal medicines as viable alternatives to or in conjunction with the existing synthetic drugs.

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