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Research Article

Some Phaemacological activitie Of Delonix Regia Flower Extracts

Evaluation Of Antioxidant, Antidiabetic, Anti-Inflammatory And Anticancer Activities Of *Delonix Regia* Flower Extracts

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Abstract: Non-communicable diseases like diabetes and cancer are the major cause of death worldwide. Various drugs are used for the treatment of these diseases. However, they cause lots of side effects. There is a need for alternate drugs with fewer side effects. Medicinal plants serve as a good source for alternate form of treatment. Therefore, in this study, ethanolic and aqueous extracts of *D. regia* flowers were evaluated for their antioxidant, antidiabetic, anti-inflammatory and cytotoxic activity to justify its use as a medicinal plant. Total phenol and flavonoid content of the extracts were measured. GC-MS analysis of the extracts were done to investigate the presence of various bioactive compounds. Antioxidant activity was assessed by radical scavenging and reduction assays. Antidiabetic activity was assessed by the ability of extracts to inhibit enzyme alpha amylase. Anti-inflammatory activity was evaluated by membrane stabilization activity. Anticancer activity against MCF-7 and A549 cell lines were measured by the MTT assay.The ethanolic extract contained more phenols (282.940.80 mgGAE/g) and flavonoids (140.912.27 mgQE/g). GC-MS analysis showed the presence of compounds belonging to fatty acids, alkanes, phenols and organic alcohols. The aqueous extract showed strong superoxide radical scavenging activity with a low IC_{50} of 39.35±0.74 µg/mL. The ethanolic extract showed higher ferric reducing power with an IC_{50} of 59.65±0.28µg/mL. Ethanolic extract was more potent in inhibiting alpha amylase with a low IC_{50} value of 47.14±0.6 µg/mL. Ethanolic extract also showed maximum inhibition of 88.86±0.1% against heat induced lysis of cell membrane. Both extracts affected the proliferation of MCF-7 and A549 cell lines at 160 µg/mL. The results of the present study support the use of *D. regia* flower as a potential source of bioactive phytochemicals and can be used as a plant-based antioxidant, antidiabetic, anti-inflammatory and anticancer agent.

Keywords: *Delonix regia;* GC-MS analysis; Antidiabetic Activity; Anti-inflammatory activity, Anticancer Activity

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1. INTRODUCTION

The mortality index has gradually shifted from infectious diseases towards non-communicable diseases (NCDs) in the last century. [1] Spread of western lifestyle has made NCDs a global problem. Main NCDs like cancer, diabetes, and cardiovascular diseases are among the top 10 causes of death worldwide. [2] Type 2 diabetes is a major NCD contributing to illness and deaths worldwide. Over the past three decades, there has been a steady increase in the prevalence of diabetes. In recent times, low- and middle-income countries the rate of prevalence is much faster compared to high-income countries. Diabetes, if not properly treated or managed, can lead to a variety of short- and long-term complications such as blindness, kidney failure, lower limb amputation, and so on. etc. Diabetes can also cause other NCDs like cancer and cardiovascular disorders. [3] A NCD strongly associated with diabetes is cancer. Both diseases share common risk factors and have been a major reason for illness worldwide. [4] The prevalence of cancer is increasing at an alarming rate. Studies show that every fourth person is has a lifetime risk of cancer. As of 2018, a total number of 18 million new cases have been diagnosed. Among different types of cancers, the most frequent are lung (2.09 million cases), breast (2.09 million cases), and prostate (1.28 million cases) cancers. [5] Various forms of treatments have been used to prevent and manage these diseases. However, drugs used for treatment and management have side effects such as weight gain, plasma volume expansion, mild anemia, myalgia, and liver dysfunction.[6] This has necessitated the development of novel therapeutic and preventative strategies. [7] The search for more effective alternative drugs with reduced side effects in the treatment of T2DM, cancers, and conditions correlated to it continues. [8] On the other hand, medicinal plants have been shown to act as an alternative source for the treatment and management of NCDs. [9] *Delonix regia* is one such plant with medicinal properties. *Delonix regia* also known as "Flame tree" or "Gulmohar" is a tropical flowering plant belonging to the family of Fabaceae, is grown in Madagascar, India, and Northern Australia. Most of the bioactive components of the tree are present in flowers, leaves, and bark. [10] *D. regia* has been used for both medicinal and economical purposes. It has a wide array of therapeutic abilities. Apart from acting as an antioxidant agent, it also possesses antidiabetic, anti-inflammatory, antidiarrheal, hepatoprotective, anthelmintic, and wound healing activity.[11] The flower of the plant has been used to treat constipation, inflammation, rheumatoid arthritis, diabetes, pneumonia, and malaria. [12] D. regia flowers have medicinal value, as they contain phytochemicals such as sterols, triterpenes, and flavonoids. .[13]Studies have reported the presence of components such as anthocyanins, carotenoids, phenolic acids like quercetin, and flavonols from the kaempferol and isorhamnetin compound families.[14,15] In previous studies, the medicinal attributes of the flower have been studied. However, very little work has been done on its antidiabetic, anti-inflammatory, and anticancer properties. The objective of the present study was to evaluate the potential use of *D. regia* flowers for the treatment and management of diabetes and cancer. Therefore, the antioxidant, antidiabetic, and anti-inflammatory activities of ethanolic and aqueous extracts of the flowers were studied.The anticancer activity of the extracts against breast (MCF-7) and lung cancer (A549) cell lines was also evaluated. The GC-MS analysis of extracts was done to identify the bioactive compounds.

2. MATERIALS AND METHODS

2.1 Plant collection and crude extract preparation

Delonix regia flowers were collected from Chennai, India. Identification and authentication of *Delonix regia* was done by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu and the voucher number is PARC/2021/4533. Flowers were cleaned, shade dried and powdered using an electric blender. For extraction, a maceration method was used. In 250 mL of 95% ethanol, 500 g of plant powder was soaked and was left to macerate for 72 hours. After 72 hours, the supernatant was filtered through Whatman's filter No.41 Filtrate was then condensed on a hot plate at 40° C. Extracts were then stored in a freezer at -20° C until further analysis.

2.2 Determination of total phenol and flavonoid content

The Folin-Ciocalteu method was used to determine the total phenolic content.[15] In two mL of 95% ethanol, five mg of plant extract was dissolved. Then, to extract, five mL of water and one mL of Folin-Ciocalteu reagent was added and was left for three minutes. Then, to the reaction mixture, one mL of 20% sodium carbonate (Na_2CO_3) was added and incubated at room temperature (28-30° C) for one hour. Phenols in extracts react with phosphomolybdic acid in Folin-Ciocalteu reagent to form a blue coloured complex.[16] Using a UV-vis spectrophotometer, absorbance was measured at 765 nm against ethanol blank. Different concentrations of gallic acid were used to calibrate the standard curve. The total phenolic content was expressed as mg of gallic acid equivalents (GAE)/ g of extract. The total flavonoid content was determined using the aluminum chloride colorimetric method. [17] To one mg of the extracts, three mL of 95% ethanol, 0.5 mL of aluminium chloride ($AlCl_3$), 0.5mL of 1 M potassium acetate (CH_3COOK), and 5.6 mL of distilled water was added. Then, the reaction mixture was left to incubate at room temperature (28- 30° C) for 30 minutes. $AlCl_3$ reacts with the ketone or hydroxyl groups of flavonoids present in extracts to form acid stable complexes.[18] Using a UV-vis spectrophotometer, the absorbance of the reaction mixture was measured at 510 nm against distilled water. Different concentrations of quercetin were used to calibrate the standard curve. The total flavonoid content was expressed as mg of quercetin equivalents (QE)/ g of extract.

2.3 GC-MS analysis

Ethanolic and aqueous extracts of *Delonix regia* flowers were analysed using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 500). Using an electron ionization system with an ionization energy of 70 Ev, compounds were detected. At a flow rate of 1 ml/min, helium was used as the gas carrier. The mass transfer line and injector temperature was set at 220 ° C and 300 ° C respectively. During the first 10 minutes, the oven temperature was set from 50 to 150 ◦ C at 3 ◦ C/min and was raised to 300◦ C at 10 ◦ C/min. In split mode (ratio- 1:120) the diluted extracts (1/100, v/v) were injected into the injector.[19] To analyse the mass spectrum, GC-MS National Institute Standard and Technique (NIST) library was used. The component's name, molecular weight, and biological activity were determined.

2.4 In vitro antioxidant activity of *D. Regia* flower

To determine the antioxidant activity of the extracts 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), Superoxide anion radical, Ferric (Fe^{3}+) reducing power (FRAP), and Phosphomolybdenum reducing assays were used. All assessments were performed in triplicates.

2.5 DPPH radical scavenging assay

DPPH radical scavenging activity of the extracts was assessed by the modified methodology of Baba & Malik. [21] The purple chromatogram of the DPPH radical gets reduced to yellow hydrazine by the plant phytochemicals. [22] To one mL of various concentrations (20-120 μ g/mL) of extracts, one mL of 0.1 mM DPPH solution in methanol was added and left to incubate at room temperature (28- 30° C) for 30 minutes. Using a UV-vis spectrophotometer, the decrease in absorbance was measured at 517 nm. The percentage of inhibition of the DPPH radical was calculated using the following formula:

DPPH radical scavenging effect = [(OD control − OD sample) /OD control] × 100%

2.6 Superoxide anion radical scavenging assay

Superoxide anion radical scavenging activity was determined according to Gulcin et al.[23] To various concentrations of extracts (20-120 μ g/mL), 1.33 × 10− 5 M riboflavin, 4.46 × 15 μ M EDTA, and 8.15 × 10− 8 M nitro blue tetrazolium (NBT) were added. The reaction mixture was illuminated at 25 ° C for 40 minutes. Superoxide radicals generated by non-enzymatic ethylenediaminetetraacetic acid nicotinamide adenine dinucleotide (EDTA/ NADH) system reduce NBT to purple formazan.[24] The change in absorbance was measured at 590 nm using a UV-vis spectrophotometer. The percentage of inhibition of superoxide radical was calculated using the following formula:

Superoxide radical scavenging effect = [(OD control − OD sample) /OD control] × 100%

2.7 FRAP assay

The reducing power of extracts was determined by the FRAP assay using the method of Benzie & Strain[25] with modifications. One mL of plant extracts at various concentrations (20-120 g/mL) was mixed with one mL of phosphate buffer (0.2 M, pH 6.6) and one mL of 1% (w/v) potassium ferricyanide [K3Fe (CN)]. Then the mixture was left to incubate at 50° C for 20 minutes. After incubation, one mL of 10% (w/v) trichloroacetic acid and one mL 0.1% (w/v) ferric chloride (FeCl3) were added. Antioxidants present in extracts reduce ferric ion into ferric 2,4,6-tripyridyl-s-triazine (TPTZ). [26] Absorbance was measured using a UV-vis spectrophotometer at 7 00 nm. The ferric reducing potential was calculated by the following equation:

FRAP = [(OD sample − OD control) /OD sample] × 100%

2.8 Total antioxidant capacity

The total antioxidant capacity (TAC) of extracts was determined by the phosphomolybdenum method. [27] Three mL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate were added to 0.3 mL of various concentrations (20-120 g/mL) of plant extracts. Then the reaction mixture was incubated for 90 minutes at 95 °C. Absorbance was read at 695 nm using a UV-vis spectrophotometer. This assay shows the reduction of Mo (VI) to Mo(V) a green phosphate complex, by the phytochemicals present in plant extracts at an acidic pH. [28] Total antioxidant capacity was calculated using the formula:

TAC= [(OD sample − OD control) /OD sample] × 100%

2.9 In vitro antidiabetic activity of *D. Regia* flower *by* inhibition of alpha amylase

The ability of extracts to inhibit enzymes involved in carbohydrate metabolism was analysed by alpha amylase inhibition assay. The inhibition of alpha-amylase activity was assessed by the method of Ou et al.,[29] with modifications. To different concentrations of *D. Regia* flower extracts, 0.1 mL of 1% alpha amylase was added. After 10 minutes, 0.1mL 1% potato starch was added to the reaction mixture and was incubated at 37° C for 60 minutes. After the incubation period, enzyme activity was terminated by adding 0.1 M sodium hydroxide (NaOH). Then the mixture was centri fuged at 2000 RPM for 15 minutes. Using a UV- vis spectro photo meter, absor bance was measured at 565 nm. Inhibitory activity was calculated using the formula:

Inhibitory activity (%) = [(OD control − OD sample) /OD control] × 100%

2.10 In vitro Anti-inflammatory activity of *D. Regia* flower by Membrane stabilization activity

Anti-inflammatory activity of *D. Regia* flower extracts was assessed by membrane stabilization activity as given by Okoli et al., [30] with slight modifications.

2.11 Preparation of blood sample

Two millilitres of blood were drawn from a healthy volunteer who was not on Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) . Then the blood was centrifuged at 3000 rpm for 10 minutes and washed three times with an equal volume of normal saline. The volume of blood was measured and reconstituted as a 10% v/v suspension with normal saline.

2.12 Heat induced hemolysis

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (20 -120 µg/mL) and 1 ml of 10% RBC suspension, and saline was used as a control. The reaction mixture was incubated in a water bath at 56 °C for 30 minutes. At the end of the incubation, the tubes were cooled under running tap water and were centrifuged at 2500 rpm for 5 minutes. The absorbance of the supernatants was taken at 560 nm. The percentage inhibition of hemolysis was calculated as follows:

Percentage inhibition = [(OD control -OD sample) / OD control] × 100

2.13 *In vitro* Anticancer Activity of *D. regia* flowers

The anticancer activity of the extracts was examined using 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) assay on MCF-7 and A549 cell lines.[31] Cells were seeded at 1×10^5 cells per well in 96-well plates and allowed to settle for 12 hours. Various concentrations of plant extracts were added to the cells and incubated for 24 hours. Then the culture supernatant was aspirated out and was replaced with 100 mL of MTT solution and incubated for three hours at 37 ° C. Following incubation, MTT gets reduced by live cells to form formazan crystals. [32] Then, formazan crystals were dissolved in 100 mL of Dimethyl Sulfoxide (DMSO) and the absorbance was measured at 570 nm using a multi plate reader. Percentage cell inhibition was calculated by the following equations:

Cell viability (%) = 100 - [100× (Ac - At) ÷ AC]
Cell inhibitory (%) =1-cell viability (%).

Where At is the absorbance value of extracts and Ac is the absorbance value of the control.

3. STATISTICAL ANALYSIS

All the tests were performed in triplicates, and the results were expressed as mean ± SD. One-way analysis of variance (ANOVA) was performed to compare the difference between ethanolic and aqueous extracts of *D. regia* flowers for phytochemical content, antioxidant and anticancer activity. The means were separated using Tukey ' s pairwise analysis. The p-values at *P<0.05* were considered statistically significant. All statistical analysis was performed using the SPSS 16.0 software package.

4. RESULTS

4.1 Total phenol and flavonoid content

Total phenol and flavonoid content of *D. regia* flower extracts are presented in Table 1. In the ethanolic and aqueous extract, the total phenol content was significantly higher than the total flavonoid content (*p<0.05*).

4.2 GC-MS Analysis

The bioactive principles with their molecular formulae, retention time (RT), molecular mass, and peak area are represented in Table 2 and Table 3. A total of seven compounds were identified in both ethanolic and aqueous extracts. Compounds belonging to various chemical groups like fatty acid, phenols, alkanes, and flavones were present in both extracts. Fatty acids with therapeutic potential like n-Hexadecanoic acid and Pentadecanoic acid, 3-oxo-, methyl ester in ethanolic extract and Hexadecanoic acid, methyl ester in aqueous extract were identified.

Table 1: Bioactive components present in the different extracts of *D. regia* flowers

Bioactive compounds	Extraction solvent	
	ET	AQ
Total Phenols (mg GAE/g)	282.94±0.80[a]	149.58±1.05[a*]
Total Flavonoids (mg QE/ g)	140.91±2.27[b*]	50.3±1.10[b*]

Mean ±Standard deviation of three independent estimations
[a-b] Values followed by the different superscripts are significantly different within the column
[*]Values are significantly different within the row, p<0.05
ET - Ethanolic extract; AQ - Aqueous extract

Table 2: Bioactive compounds identified in ethanolic extracts of *D. regia* flowers

S.NO	RT (Min)	Compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)
1	17.05	4H-1-1-Benzopyran-2-one,3 hydroxy-2-phenyl	$C_{15}H_{12}O_4$	256.25	14.3
2	17.75	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256.62	13.14
3	18.83	Quinoxaline,2-isopropyl-3phenyl,4 oxide	$C_{17}H_{16}N_2O$	264.32	40.29
4	19.63	Pentadecanoic acid, 3-oxo-, methyl ester	$C_{16}H_{30}O_3$	270.41	7.55
5	20.28	Ethanone, 1-[4-methoxy-3-(4-methylphenoxy)phenyl]-	$C_{16}H_{16}O_3$	256.3	4.68
6	22.42	Phytol	$C_{20}H_{40}O$	296.5	6.79
7	20.57	Eicosane	$C_{20}H_{42}$	282.5	11.17

Table 3: Bioactive compounds identified in aqueous extracts of *D. regia* flowers

S.NO	RT (Min)	Compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)
1	13.07	Ethanone-1,1'(1-4 phenylene) bisdioxime	$C_{10}H_{12}N_2O_{22}$	192.21	16.16
2	13.77	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O_3$	206.32	8.83
3	15.02	7-methoxy-2,2,4,8 tetramethyl tricyclo [5,3,1,0 (4,11)] undecane	$C_{16}H_{28}O$	236.39	13.14
4	15.25	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	29.31
5	16.98	Nonadecane-2,4-dione	$C_{19}H_{36}O_2$	296.5	18.31
6	17.82	Isopropyl stearate	$C_{21}H_{42}O_2$	326.6	8.83
7	19.62	4,4'-dimethoxy-1-1'-dioxo(1,1',2,2')-tetrahydro-2-2' binaphthyl idene	$C_{22}H_{16}O_4$	344.4	5.38

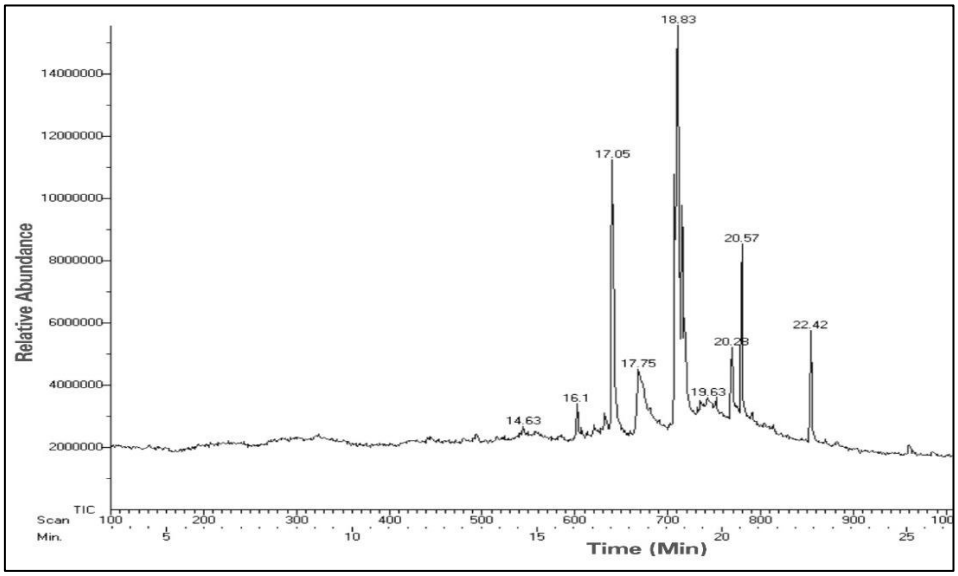


Fig 1: GC-MS Chromatogram of ethanolic extracts of *D. regia* flowers

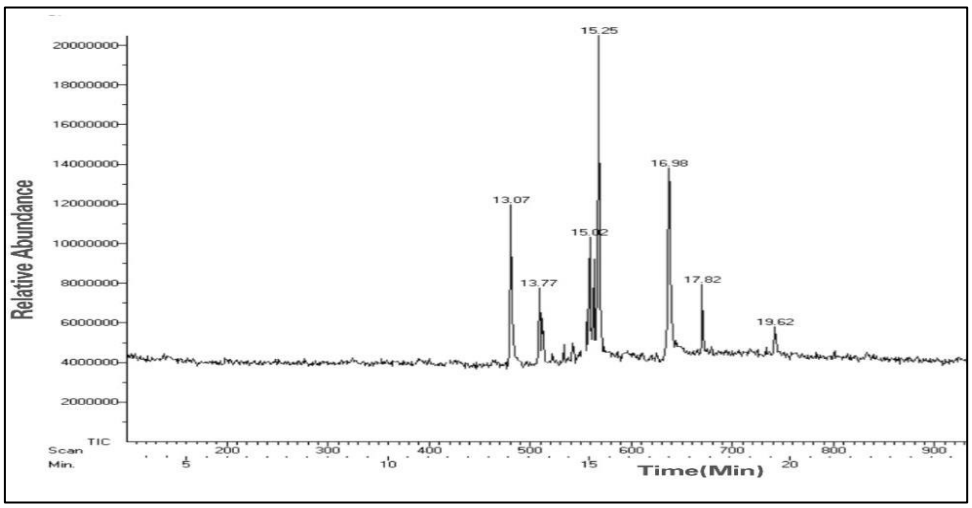


Fig 2: GC-MS Chromatogram of aqueous extracts of *D. regia* flowers

4.3 Antioxidant activity

4.4 DPPH radical scavenging activity

Table 4 shows the DPPH radical scavenging activities of ethanolic and aqueous extracts of D. *regia* flowers. As the concentration of *D. regia* extracts increased, the scavenging ability of the extracts also increased. The scavenging ability of ethanolic extract was significantly higher than aqueous extract ($p<0.05$).

4.5 Superoxide anion radical scavenging activity

The superoxide radical scavenging activity of *D. regia* flower extracts is presented in Table 5. The ability to scavenge superoxide radicals increased with an increase in the concentration of extracts. At a concentration of 120 µg/mL, both ethanolic and aqueous extracts showed maximum scavenging of 67.12 ± 0.91%and 61.64 ± 0.91% respectively. Aqueous extract showed high superoxide radical scavenging activity with a low IC_{50} of 39.35± 0.73µg/mL($p<0.05$) (Table 8).

4.6 FRAP activity

The reducing potential of the extracts increased in a dose dependent manner (Table 6). Ethanolic extract showed a significantly higher reducing potential compared to aqueous extract with an IC_{50} of 59.65±0.28 µg/mL($p<0.05$) (Table 8).

4.7 Total antioxidant capacity

Total antioxidant capacity was assessed by phosphomolybdenum reduction activity as shown in Table 7. Among both extracts, ethanolic extract (90.4± 0.07 %) showed the highest reducing ability when compared to aqueous extract (81.02± 0.12 %) at a concentration of 120 µg/mL.

Table 4 : DPPH radical scavenging activity of different extracts of *D. regia* flowers

Concentration (µg/mL)	% DPPH Scavenging	
	ET	AQ
20	8.27± 0.72[a]	3.1 ± 0.63[a *]
40	19.81 ± 0.85[b]	8.35± 0.70[b*]
60	35.87 ± 0.59[c]	15.59 ±0.59[c, *]
80	39.15 ± 0.88[d]	32.13 ± 0.59[d, *]
100	45.84 ± 0.29[e]	39.13 ± 0.62[e]
120	69.17 ± 0.45[f]	55.81 ± 0.48[f, *]

Mean ±Standard deviation of three independent estimations
[a-f] Values followed by the different superscripts are significantly different within the column
[*]Values are significantly different within the row, p<0.05

Table 5: Superoxide Radical Scavenging activity of different extracts of *D. regia* flowers

Concentration (µg/mL)	% Superoxide Radical Scavenging	
	ET	AQ
20	5.8 ± 1.11[a]	16.43 ± 0.915[a]
40	25.11 ± 0.46[b]	35.02 ± 0.97[b]
60	34.55 ± 1.60[c]	50.83 ± 0.94[c]
80	43.37 ± 1.37[d]	56.31 ±0.94[d]
100	62.25 ± 1.15[e]	59.63 ± 0.55[e]
120	67.12 ± 0.91[f]	61.64 ± 0.91[e]

Mean ±Standard deviation of three independent estimations
[a-f] Values followed by the different superscripts are significantly different within the column
[*]Values are significantly different within the row, p<0.05

Table 6: Ferric reducing power of different extracts of *D. regia* flowers

Concentration (µg/mL)	FRAP	
	ET	AQ
20	17.62 ± 0.54[a]	9.0 ± 0.67[a]
40	35.86 ± 0.33[b]	36.81 ± 0.32[b]
60	50.29 ± 0.23[c]	39.01 ± 0.24[c]
80	53.26 ± 0.22[d]	59.46 ± 0.10[d]
100	62.57 ± 0.33[e]	60.55 ± 0.13[e]
120	87.88 ± 0.52[f]	70.89 ± 0.097[f]

Mean ±Standard deviation of three independent estimations
[a-f] Values followed by the different superscripts are significantly different within the column
[*]Values are significantly different within the row, p<0.05

Table 7: Total antioxidant capacity of different extracts of *D. regia* flowers		
Concentration (µg/mL)	**TAC**	
	ET	**AQ**
20	43.42± 1.35[a]	31.89± 1.61[a]
40	64.29 ±0.87[b]	61.09± 0.53[b]
60	83.89± 2.08[c]	75.71± 0.20[c]
80	87.81± 0.06[d]	76.67± 0.27[c]
100	90.07± 0.05[d]	78.27± 0.16[d]
120	90.4± 0.07[d]	81.02± 0.12[e*]

Mean ±Standard deviation of three independent estimations
[a-f] Values followed by the different superscripts are significantly different within the column
[*]Values are significantly different within the row, p<0.05

Table 8 shows the half maximal inhibitory concentration of the extracts against free radicals and cancer cells. Lower IC_{50} value indicates higher potency.

Table 8: Antioxidant and anticancer activity of different extracts of *D. regia* flowers expressed as IC_{50} values		
Parameters	**ET**	**AQ**
Antioxidant activities		
DPPH	109.08±0.69	107.52±0.93
Superoxide radical scavenging	92.28±2.91	39.35±0.74*
FRAP	59.65±0.28	67.27±0.12*
TAC	31.11±0.42	32.74±0.28
Antidiabetic activity		
Alpha amylase inhibition	47.14±0.6	93.41±0.53*
Anti-inflammatory activity		
Membrane stabilization	20.54±0.03	88.4±0.19*
Anticancer activities		
A549 cell lines	49.83±0.77	76.59±1.11*
MCF7 cell lines	48.78±2.49	80.83±0.65*

[*]Values are significantly different within the row, p<0.05
ET - Ethanolic extract; AQ - Aqueous extract

4.8 In vitro Antidiabetic activity of *D. Regia* flower *by* inhibition of alpha amylase

Table 9 shows the inhibitory activity of D. regia extracts against the alpha amylase enzyme. Both extracts showed good inhibition of alpha amylase, with the aqueous extract having a significantly higher inhibition of 71.31±0.84%at 300µg/mL.

Table 9: Alpha amylase inhibition activity of different extracts of *D. regia* flowers		
Concentration (µg/mL)	**Inhibition (%)**	
	ET	**AQ**
50	47.14±0.6[a]	45.41±0.75[a]
100	54.4±0.47[b]	53.53±0.38[b]
150	56.5±0.56[b]	56.81±0.38[b]
200	58.05±0.91[b,c]	59.72±0.28[b,c]
250	62.63±0.67[c,d]	64.18±0.47[c,d]
300	66.66±0.47[d,e,*]	71.31±0.84[e]

Mean ±Standard deviation of three independent estimations
[a-f] Values followed by the different superscripts are significantly different within the column
[*]Values are significantly different within the row, p<0.05

4.9 In vitro Anti-inflammatory activity of *D. Regia* flower by Membrane stabilization activity

Results of the anti-inflammatory ability of *D. regia* extracts are represented in table 10. Ethanolic extract was significantly potent in protecting human erythrocytes against heat induced lysis with a low IC_{50} value of 20.54±0.03 µg/mL compared to aqueous extract.

Table 10 : Membrane stabilization activity of different extracts of *D. regia* flowers

Concentration (µg/mL)	Inhibition (%)	
	ET	AQ
20	48.68±0.1[a,*]	4.9±0.08[a]
40	61.2±0.16[b,*]	20.21±0.13[b]
60	66.14±0.13[c,*]	40.47±0.1[c]
80	69.92±1.03[c,d,*]	45.25±0.1[c]
100	85.38±0.1[e,*]	68.2±0.1[d]
120	88.86±0.1[e]	78.95±0.12[e]

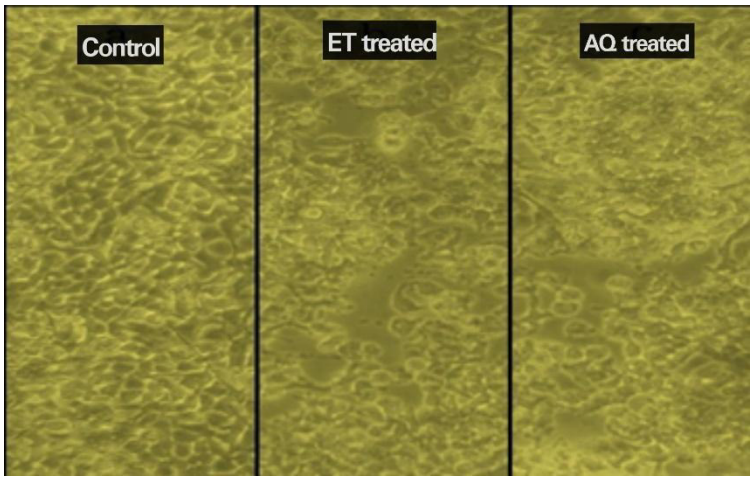
Mean ±Standard deviation of three independent estimations[a-f] Values followed by the different superscripts are significantly different within the column[*]Values are significantly different within the row, p<0.05 **Anticancer activity**

The effect of different concentrations (5-160µg/mL), of *D. Regia* extracts on A549 and MCF-7 cell lines were studied (Table 11) (Figure 3&4). Both extracts significantly inhibited the viability of cancer cells in a dose dependent manner. Ethanolic extract showed higher inhibition of cancer cells compared to aqueous extract with low IC_{50} values of 49.83±0.77µg/mL on A549 cells and 48.78±2.49 µg/mL on MCF-7 cells (*p<0.05*) (Table 8).

Table 11: Anticancer activity of different extracts of *D. regia* flowers

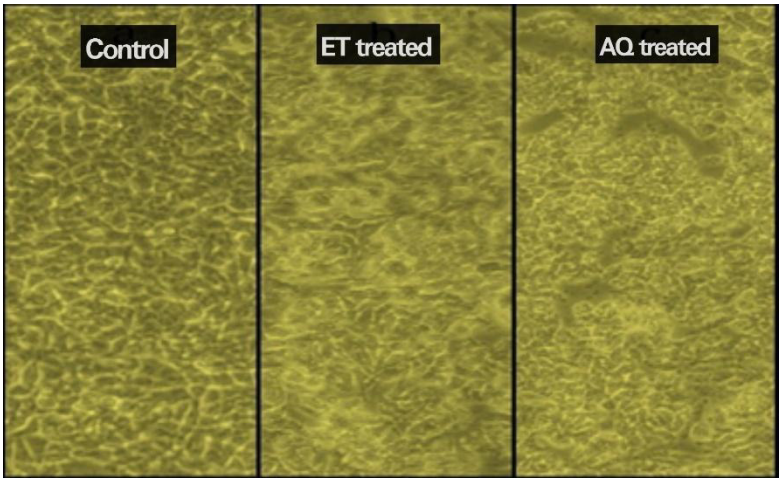
Concentration (µg/mL)	A549 cells		MCF-7 cells	
	ET	AQ	ET	AQ
5	7.24±0.78[a]	2.01±0.78[a,*]	7.92±0.37[a]	1.98±0.55[a,*]
10	15.9±0.49[b]	3.05±0.78[a,*]	15.44±0.44[b]	3.17±0.44[a,*]
20	25.24±0.43[c]	10.53±0.29[b,*]	29.19±0.40[c]	5.05±0.17[b,*]
40	40.14±0.62[d]	21.43±0.70[c,*]	43.31±0.21[d]	18.54±0.29[c,*]
80	62.62±0.60[e]	52.24±0.77[d,*]	63.25±0.64[e]	49.5±0.40[d,*]
160	76.66±0.35[f]	59.97±0.57[e,*]	70.77±0.53[f]	57.79±0.76[e,*]

Mean ±Standard deviation of three independent estimations[a-f] Values followed by the different superscripts are significantly different within the column[*]Values are significantly different within the row, p<0.05



ET - Ethanolic extract; AQ - Aqueous extract

Fig 3: Anticancer activity of different extracts of *D. regia* flowers against A549 cells



ET - Ethanolic extract; AQ - Aqueous extract

Fig 4: Anticancer activity of different extracts of *D. regia* flowers against MCF-7 cells

5. DISCUSSION

The use of natural plant products has gained attention due to the presence of phytochemicals that act as antioxidant agents in preventing oxidative stress and inflammation related diseases such as cancer. [33] Evidence based on research shows that dietary polyphenols prevent and manage metabolic diseases like diabetes. [34] Therefore, in the present study, the antioxidant, anti-inflammatory, antidiabetic, and anticancer activities of ethanolic and aqueous extracts of *D. regia* flowers were evaluated to assess their potential use as natural plant based agents for the treatment of diseases like diabetes and cancer. In countries like India and Africa, *D. regia* flowers are used to prepare homemade water-extracts. It is traditionally known to have medicinal properties such as antimicrobial and antifungal activities. [35] Previous studies also have reported various therapeutic activities of *D. regia* flowers. Flowers' anti-arthritic activity was evaluated in a study by Chitra et al. [36] in Freund's incomplete adjuvant induced arthritis model in rats . The ethanolic extracts showed dose dependant anti-arthritic activity. Studies by Shiramane *et al.* [37, 38] showed the gastroprotective and antidiarrheal activity of ethanolic extracts of *D. regia* flowers in rat models. Results of the study by Husain *et al.* [39] showed the wound healing properties of ethanol and aqueous extracts of D. regia flowers using incision, excision, and dead space wound models. Apart from these, the flower extracts have demonstrated antimicrobial, [40] anthelmintic, [41] and diuretic [42] activities. Results of this study show that both extracts have good levels of phenols and flavonoids. The presence of phenols was significantly higher than flavonoids in both extracts. A similar finding was reported by Sriwatcharakul.[43] where the total phenols and flavonoids present in ethanolic extract were 200.00±11.48 mg GAE/ g and 162.50±13.54 mg QE/g. In another study by Chabra and Gupta [44] the total phenols and flavonoids present in the aqueous extract were 101.29 mg GAE/ g and 10.30 mg RE/g. This variation in levels of phytochemicals among extracts may be due to the nature of the solvent used. [45]To identify compounds of importance like branched chain hydrocarbons, alcohol acids and esters, the Gas Chromatography Mass Spectrometry technique was used. [46] In this study, the ethanolic extract of *D. regia* showed the presence of compounds like n-Hexadecanoic acid, and phytol. Hexadecanoic acid, or palmitic acid, has been reported to have anticancer,[47] antibacterial, [48] and antiviral abilities. [49,50] Previous research suggests that phytol has anticancer,[51] antitubercular, [52] anticonvulsant, [53] antioxidant[54] and antimicrobial[55] potential. Aqueous extract of *D. regia* flowers showed the presence of compounds like phenols, alkanes and fatty acids. Isopropyl stearate was detected and it has been reported to have antibacterial ability. [56] Hexadecanoic acid methyl ester was discovered to have antifungal[57] and antitumor[58] activity. Phenol, 2,4-bis(1,1-dimethylethyl)- was detected and it is reported to have antibacterial [59]and antioxidant[60] ability. In a study by Chabra and Gupta [44] aqueous extract of *D. regia* flowers also showed the presence of similar compounds. Consumption of foods rich in antioxidants can reduce the risk of metabolic disorders or slow down the progression of the disease by decreasing oxidative stress induced DNA damage. [61] Therefore, in the present study, the antioxidant ability of *D. regia* flowers was studied. Results revealed that *D. regia* flower extracts showed good antioxidant activity. Both the extracts were able to scavenge and reduce free radicals effectively in a dose dependent manner. Ethanolic extract showed high reduction potential. The ability of the extracts to reduce free radicals is an indicator of the antioxidant potential of the phytochemicals present in plants.[62] Ethanolic extract was also able to effectively scavenge DPPH and superoxide radicals. Whereas, the aqueous extract showed good free radical scavenging activity with a low IC_{50} value of 39.35± 0.74µg/mL to scavenge superoxide radicals, which play an important role in molecular damage by oxidative stress.[63] During carbohydrate metabolism, the enzyme alpha-amylase breaks down starches and oligosaccharides into glucose. Inhibition of this enzyme, therefore, can reduce the absorption of glucose in the blood and can be considered as a way to manage diabetes. [64]Synthetic drugs like acarbose and voglibose are used as enzyme inhibitors. However, they have been known to cause side effects like flatulence and digestive and liver function disorders. [65]Therefore, the ability of *D. regia* flower extracts to inhibit alpha amylase was assessed as studies show that polyphenolic compounds present in plants can inhibit the enzyme. [66] Results reveal that both extracts have alpha amylase inhibition potential. Among both extracts, the ethanolic extract is more potent as it showed a low IC50 value of 47.14±0.6 µg/mL. Studies have shown that the use of NSAIDs increases the risk of heart failure, elevated blood pressure, and thrombotic events. However, due to the lack of safer alternatives, they

are still extensively used for managing inflammation.[67] Therefore, the need for safe and natural anti-inflammatory agents has increased. In the present study, the anti-inflammatory activity of *D. regia* flowers was assessed by the membrane stabilizing potential against heat induced haemolysis. One way in which NSAIDs reduce inflammation is by protecting the lysosomal membrane from destruction. The Human red blood cell (HRBC) membrane resembles the membrane of a lysosome. Therefore, the protective ability of extracts on HRBC can be implied as its membrane stabilizing ability of lysosomal membrane as well.[68] In heat-induced conditions, both extracts were found to inhibit lysis of the erythrocyte membrane, with ethanolic extract showing the maximum inhibition of 88.86±0.1%. The ability of ethanolic extracts to inhibit lyses can be attributed to the presence of significantly higher levels of phenols and flavonoids. Previous studies show that phenols and flavonoids can protect and stabilize the cell membrane.[69,70] In both developed and developing countries, the death rate from lung and breast cancer is high. [71]Treatment options like chemotherapy, immunotherapy, radiation, hormone therapy, and targeted therapy are available. However, deaths due to cancer and recurrence remain high and these treatments have been known to cause side effects. [72] Several plant-based compounds have been successfully used as anticancer agents. However, there remains a need to evaluate their effectiveness and safety. [73,74] Traditionally, for the treatment of cancer, *Delonix regia* is used. [75] Therefore, the cytotoxic effect of ethanolic and aqueous extracts of *D. regia* flowers was evaluated using cell lines. The cytotoxicity of extracts against lung cancer was assessed with A549, a human non-small cell lung carcinoma cell line. The ethanolic extract significantly reduced the cell viability of A549 cells with 76.66±0.35% cytotoxicity at 160µg/mL when compared to aqueous extract. The effectiveness of extracts against breast cancer was evaluated using the MCF-7 cell line, a commonly used breast cancer cell line. [76] Ethanolic extract had a significantly higher effect on the cell viability of MCF-7 cells compared to aqueous extract, with a maximum inhibition of 76.66±0.35% at 160µg/mL. In the present study, the percentage of cytotoxicity recorded was high compared to the results of the study by Sriwatcharakul[43] where the maximum cytotoxicity was 70.78 ± 8.36 % at 1000 µg/mL against MCF-7 cells.

6. CONCLUSION

D. regia flower extracts showed good antioxidant activity, which can be attributed to the presence of phenols and flavonoids. Extracts also exhibited antidiabetic activity by inhibiting the enzymes involved in the digestion of carbohydrates. Therefore, it can be inferred that extracts can reduce postprandial hyperglycemia. Extracts showed anti-inflammatory activity by acting as the first line of defence in protecting, the cell membrane from lysis during inflammation. Extracts were also able to inhibit the proliferation of breast and lung cancer cells. Results also revealed that the ethanolic extract had higher antioxidant, antidiabetic, anti-inflammatory, and anticancer activity compared to the aqueous extract. Therefore, *D. regia* flowers can be considered to develop new, safe and affordable plant-based antidiabetic, anti-inflammatory, anticancer, and antioxidant agents.

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8. AUTHOR CONTRIBUTION STATEMENT

Miss Benoite. T and Dr. Nora Vigasini K Conceptualized and designed the work process. Miss Benoite. T collected, analysed, interpreted the data and drafted the manuscript. Dr. Nora Vigasini K contributed in editing and revising the manuscript. All the authors read and approved the final version of the manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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