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Isolation, Characterization and Antimicrobial Activity of Quercetin-3-O-(2”-α-Methyl-p-Coumaryl)-Rutinoside from *Delonix elata* Flowers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the isolated flavonoid compound, characterization and antimicrobial activity of Quercetin-3-O-(2”-α-methyl-p-Coumaryl)-rutinoside from *Delonix elata* flowers.
Place and Duration of Study: The research work was carried out at Research laboratory, Department of chemistry, Periyar E.V.R College, Trichy-23, between May 2016 to January 2018.
Methodology: Extraction and fractionation was carried out from the solvents of ethanol, benzene, petroleum ether, diethyl ether and ethyl acetate. The structure of the isolated compound (Quercetin-3-O-(2”-α-methyl-p-Coumaryl)-rutinoside) was elucidated through their physical and chemical methods. The isolated compound was characterized by using various spectral data such

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as UV, ^{1}H NMR, ^{13}C NMR, MS. Four bacterial strains *Salmonella typhi, Escherichia coli, Enterococcus faecalis, Bacillus cereus* and two fungal strains *Curvularia lunata and Candida albicans* were tested by using disc diffusion method.

Conclusion: The present study was concluded that the dry sample of ethyl acetate fraction of *Delonix elata* flowers was Quercetin-3-O-(2"-α-methyl-p-Coumaryl)-rutinoside and it possesses effective antimicrobial activity against bacteria and fungi.

Keywords: Antimicrobial activity; Delonix elata; ^{1}H & ^{13}C NMR; MS; 7-O-methyl quercetin-3-O-(6"-acetyl)-β-d-glucoside.

1. INTRODUCTION

Medicinal plants are the richest source for bioactive herbal metabolites [1]. These are best remedial for most of the diseases and less toxic, less side effects than the synthetic drugs [2]. The ayurvedic medicines represents flavonoids and other organic compounds of the plants have been used in herbal medicines for the treatment of many human diseases [3]. Many researchers have already exposed the presence of phytochemicals namely alkaloids, flavonoids, steroids, phenols, glycosides and saponins in different plant extracts [4,5]. The medicinal plant materials has been used in traditional medicines in India, China and other countries against infectious diseases [6]. Many plants are available in rural areas and cheaper than the synthetic medications [7]. The tribal communities of many countries are still using medicinal plants to treat disease [8].

Delonix elata is a tree species belongs to the family Fabaceae (Leguminosae) and its sub family is Caesalpinioideae. It is widely distributed in India, Cango, Egypt etc [9]. *Delonix elata* is commonly known as vadha mudakki in Tamil. It bears flowers normally in the region of December and August-March in India [10]. The leaves and barks of the plant are widely used by Siddha and Ayurveda practitioners for treating several conditions [11]. The plant materials are used to treat anti – inflammatory [12,13], anti-microbial [14], antioxidant activities [15]. The medical usefulness of the tree is acknowledged by people living in the villages who take a decoction of the leaves and barks to get relief from rheumatic problems like pain and stiffness of the joints, especially affecting the knees [16]. The phytochemical luteolin (2-(3,4-dihydroxyphenyl)-5,7- dihydroxy-4*H*-chromen-4-one) present in the ethyl acetate fraction of methanol extract of leaf of *Delonix elata* [17]. Thus this study set out to isolate the new compound Quercetin-3-O-(2"-α-methyl-p-Coumaryl)-rutinoside and investigate its antibacterial and anti fungal activities of ethyl acetate fraction of ethanolic extract of flowers of *Delonix elata*.

2. MATERIALS AND METHODS

2.1 Collection of Flowers

The fresh flowers of *Delonix elata* (3 kg) were collected from Periyar E.V.R.College (Autonomous) campus, Tiruchirappalli, Tamil Nadu, India, during the month of May and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. AR004 dated: 30/03/2015). St. Joseph's College Campus, Tiruchirappalli, Tamil Nadu, India.

2.2 Extraction and Fractionation

The collected flowers of *Delonix elata* were socked in 90% ethanol for 7 days and extracted (5x500 ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80ºC) (4x250 ml), Peroxide free diethyl ether (4x250 ml) and ethyl acetate (8x250 ml). Ethyl acetate fraction alone was taken for further study.

2.3 Ethyl Acetate Fraction (Quercetin-3-O-(2"-α-methyl-p-Coumaryl)-rutinoside)

The ethyl acetate fraction was concentrated in vacuo. The yellow colour solid obtained from EtOAc fraction was taken up in acetone and left in an ice chest for two hours. A yellow solid separated was purified by preparative paper chromatography and recrystallised from methanol (m.p. 188-189ºC). It gave yellow colour with NaOH and NH_3. It gave green colour with alc Fe^{3+} and dark pink colour with Mg-HCl. It responded to Gibb's test, Wilson's boric acid test and Molisch's test [18,5]. But did not respond to Horhammer-Hansel test [19]. It had R_f values as depicted in Table 1.

It had λ_{max} MeOH 257, 280, 292sh, 359; + NaOMe 262, 327, 410; + $AlCl_3$ 273, 303sh, 433; + $AlCl_3$ – HCl 268, 303, 363, 401; + NaOAc 269, 302 sh, 312, 376; and + NaOAc - H_3BO_3 264, 302, 379 nm.

2.4 Hydrolysis of the Glycoside (G2)

2.4.1 Acid hydrolysis

To a solution of the glycoside (G2) (50 mg) in hot aqueous methanol (5 ml, 50%), an equal volume of H_2SO_4 (7%) was added and the mixture was refluxed at 100ºC for 2 hours. The aqueous hydrolysate was extracted with Et_2O. The residue obtained from Et_2O was studied further [20,21].

2.5 Identification of the Aglycone (flavonol - Quercetin)

The yellow solid that separated from the Et_2O layer was recrystallizad. The yellow solid obtained had the m.p. 318-319ºC. This solid was soluble in organic solvents and sparingly soluble in hot water. It gave olive green colour with Mg-HCl and golden yellow colour with NaOH and NH_3. It answered to Gibb's test, Wilson's boric acid test and Horhammer-Hansel test [19], but did not respond to Molisch's test [18]. It had R_f values as depicted in Table 1.

It had λ_{max} MeOH 257, 271, 302sh, 373; + NaOMe 248, 327sh, 419; + $AlCl_3$ 272, 303sh, 333, 462; + $AlCl_3$ – HCl 265, 301sh, 360, 430; + NaOAc 275, 328, 391 and + NaOAc - H_3BO_3 258, 303sh, 393 nm.

2.6 Identification of the Sugars (Glucose and Rhamnose)

After the removal of the aglycone, the aqueous filtrate was neutralized with $BaCO_3$. This was concentrated and examined by paper chromatography. This gave R_f values corresponding to those of glucose and rhamnose. Presence of bioside was also confirmed by its running properties. The identity of the sugars was confirmed by comparison with authentic samples of glucose and rhamnose [20].

2.7 Partial Hydrolysis of the Glycoside G2

50 mg of the glycoside (G2) was subjected to partial hydrolysis by treatment with 10% formic acid in cyclohexanol. The resulting solution was found to possess a glycoside G2' and rhamnose. Presence of rhamnose was confirmed by its R_f values and thus it was proved that the terminal sugar was rhamnose. The glycoside obtained (G2') was again subjected to acid hydrolysis by following the procedure mentioned already, which gave a aglycone and a sugar. When subjected to PC, the products were identified as quercetin and glucose. R_f values of sugars obtained were presented in Table 2.

2.8 Antimicrobial Procedure

2.8.1 Screening of antibacterial activity

Four bacterial strains were used throughout the investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

2.8.2 Preparation of inoculums

Stock cultures were maintained at 4ºC on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton Broth (MHB) that were incubated without agitation for 24 hrs at 37ºC. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to $2.0x10^6$ colony forming units (CFU/ml).

2.8.3 Antibacterial susceptibility test

The disc diffusion method was used to screen the antibacterial activity. *In-vitro* antibacterial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The compound of concentration 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37ºC for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Standard antibiotic *Chloramphenicol* of concentration 1 mg/ml was used as positive control [22,13].

Table 1. R_f X 100 values of glycoside and aglycone from the flowers of *Delonix elata* (Whatman No.1, Ascending, 30±2ºC)

Compound	Developing solvents								
	a	b	c	d	e	f	g	h	i
Glycoside (G2)	32	43	54	61	66	53	50	75	54
Aglycone (A2) (Complete hydrolysis)	-	01	04	17	40	86	45	48	72
Aglycone (A2') (Partial hydrolysis)	06	04	22	32	59	58	54	63	64

** Solvent Key; a = H_2O; e = 60 % aq. HOAc;b = 5% aq. HOAc; f = n. BuOH : HOAc : H_2O = 4:1:5 (Upper phase); c = 15% aq. HOAc; g = Phenol saturated with water; d = 30 % aq. HOAc; h = HOAc : Conc. HCl : H_2O = 30:3:10; i = t BuOH : HOAc : H_2O = 3:1:1*

Table 2. R_f x 100 values of the sugars from the glycoside (G2) from the flowers of *Delonix elata* (Whatman No.1, Ascending, 30±2ºC)

Compound	Developing solvents				
	e	f	g	h	j
Sugar from G2	-	17	39	37	23
Glucose (authentic)	-	17	38	37	24
Sugar from G2 (complete hydrolysis)	74	30	60	91	78
Rhamnose (authentic)	75	30	60	92	79
Sugar from G2 (Partial hydrolysis)	74	29	59	92	79

j = n BuOH : Benzene : Pyridine: H_2O = 5:1:3:3; Spray reagent : Aniline hydrogen phthalate

2.9 Screening of Antifungal Activity

2.9.1 Culture media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt Ltd, Bombay, India.

2.9.2 Inoculum

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 105 CFU/ml.

2.9.3 Determination of antifungal activity

The agar well diffusion method (Perez) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with sample solution and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37ºC for 72 hrs. The diameters of zone of inhibition observed were measured.

3. RESULTS AND DISCUSSION

The flowers of *Delonix elata* have been found to contain (Quercetin-3-O-(2"-α-methyl-p-coumaryl)-rutinoside).

The UV spectrum of the glycoside showed two major absorption peaks at 359 nm (band I) and at 257 nm (band II). This indicates the presence of flavonoid skeleton. A bathochromic shift of 14 nm was noticed in the aglycone (band I) as compared to the glycoside in MeOH spectrum. This confirms the site of glycosylation at C-3 [20], which is also supported by the fact that the glycoside was not responded to Horhammer – Hansel test, but the aglycone did. An additional peak at 327 nm was appeared both in the glycoside and in the aglycone in the NaOMe spectra indicate the presence of free 7-OH in both [23]. Bathochromic shifts of 42 nm and 57 nm were noticed in the glycoside and the aglycone respectively in $AlCl_3$-HCl spectra as compared with their respective MeOH spectra, which indicate the presence of free –OH at C-5 in the glycoside and also in the aglycone [24]. This is also supported by the fact that both glycoside and aglycone were responding to Wilson's boric acid test.

Bathochromic shift of 51 nm and 46 nm seen in the NaOMe spectrum of (band I) the glycoside and aglycone respectively, indicated the presence of 4'-OH in both.

Presence of O-dihydroxy group in B-ring is evident from the bathochromic shift of 20 nm seen in the glycoside and 20 nm seen in the aglycone (band I), in the NaOAc-H_3BO_3 spectra. This catechol type of substitution in B ring is also evidenced by the additional bathochromic shift

seen in $AlCl_3$ spectrum (band I) in comparison with the respective $AlCl_3$-HCl spectrum in glycoside and also in aglycone [25].

In the ^{1}H-NMR spectrum (DMSO-d_6, TMS) (Fig 1) of the glycoside, The 5-OH proton appears at δ 12.57 ppm. The proton at C-6 and C-8 appears as doublet at δ 6.21 ppm and at δ 6.42 ppm due to metacoupling by C-8 and C-6 respectively. The C-5' proton due to orthocoupling with C-6' appears at δ 6.9 ppm as a doublet. C-2' proton appears as a doublet at δ 7.9 ppm due to metacoupling with C-6 proton. C-6' proton appears at 7.5 ppm (dd) due to ortho and meta coupling. Anomeric protons of glucose and rhamnose appear at δ 5.08 ppm and at δ 4.54 ppm respectively. $-CH_3$ protons of rhamnose resonates at δ 1.1 ppm. The remaining sugar protons appear in the range of δ 3.0 ppm to δ 3.5 ppm. $-CH_3$ protons of α-methyl-p-coumaryl group resonate at δ 1.53 ppm. C-2'", C-6'" protons and the proton at β carbon atom of the above group appear at δ 7.37 ppm. C-3'" proton and C-5'" proton appear at δ 6.8 ppm [20,25].

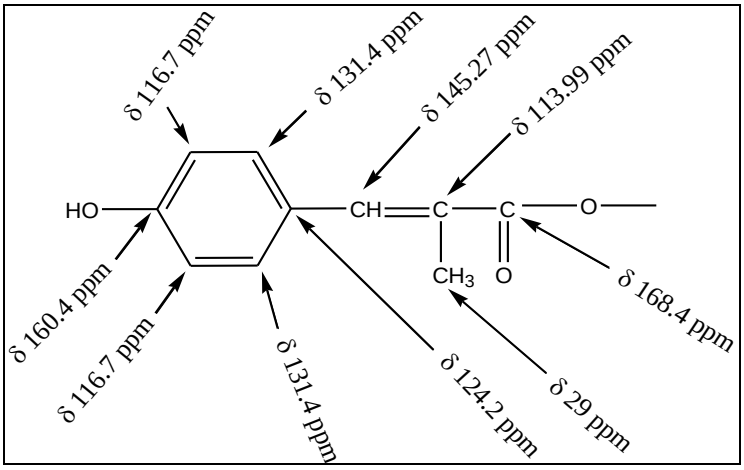
Supporting evidence is given by ^{13}C-NMR data (DMSO-d_6, TMS) (Fig 2). The signal positions and their complete assignments to different carbons are given in Tables 1, 2, 3.

Due to glycosylation, the C-3 carbon show an upfield shift and two Orthocarbons C-2 and C-4 show downfield shift. Presence of α-methyl p-coumaryl group in C-2"' carbon is evidenced by the downfield shift found in C-2"' carbon and upfield shift found in the ortho carbons C-1"' and C-3"'. Glycosylation at C-6"' is evidenced by the downfield shift found at that carbon. Methyl carbon of rhamnose appears at δ 18.1 ppm. Rhamnosyl-(1-6)-glucoside linkage is evidenced by the downfield shift found at C-6 and upfield shift found at C-1"' [20,25].

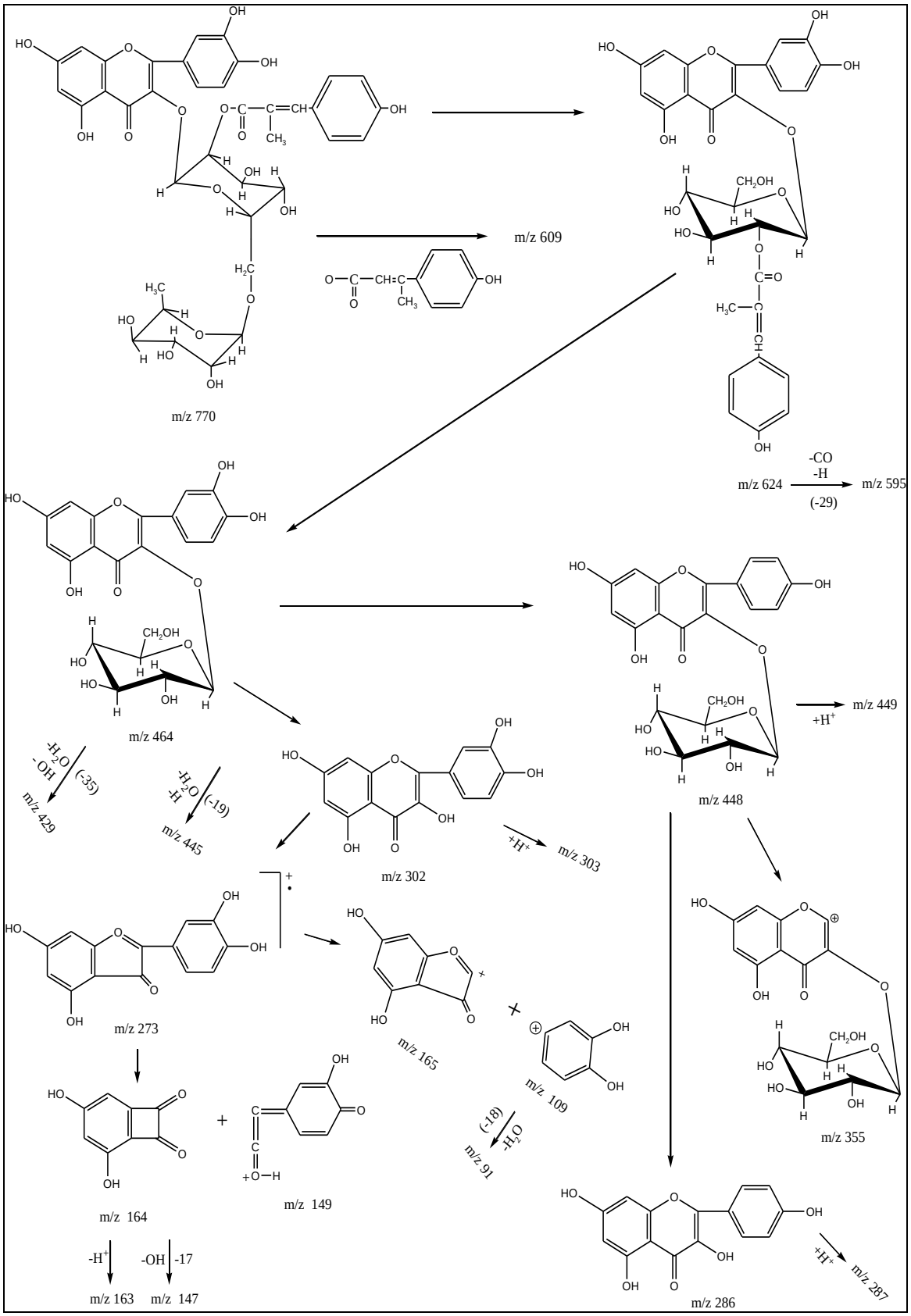
The structure of the compound is further evidenced by the mass spectrum of the glycoside. The fragmentation pattern (Fig. 3) following RDA and other common fragmentation pattern is also in favour of the structure of the compound. Presence of Quercetin glycoside is evidenced by the peaks found at m/z 445, m/z 429, m/z 303, m/z 149 and m/z 91. Presence of α-methyl p-coumaryl group is evidenced by the peaks found at m/z 624 and at m/z 595 [20,25].

Based on the above evidences the glycoside has been characterized as Quercetin-3-O-(2"-α-methyl-p-coumaryl)-rutinoside).

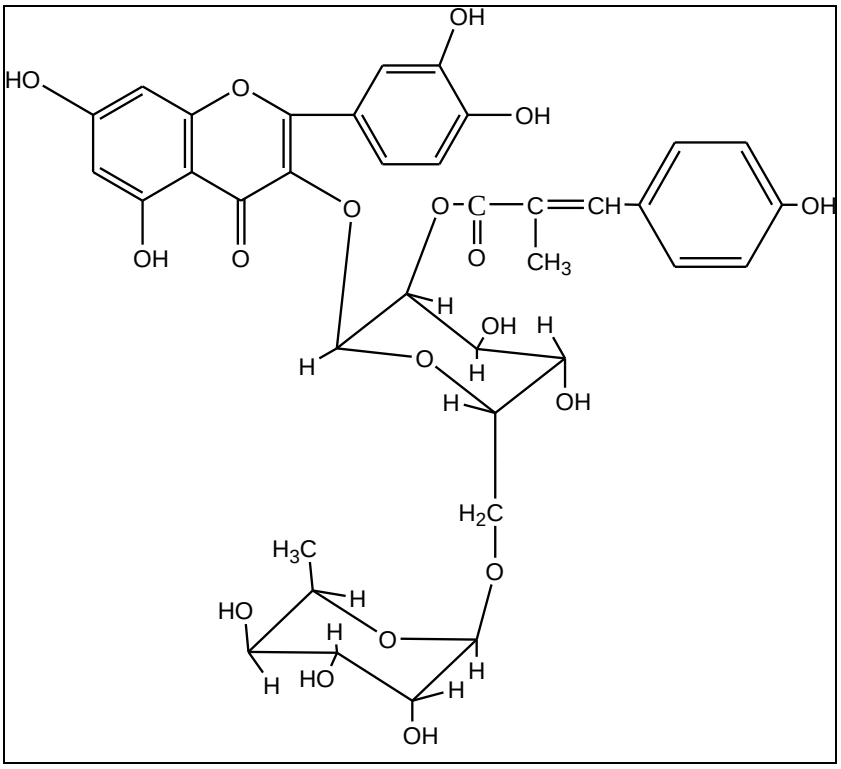
The new compound Quercetin-3-O-(2"-α-methyl-p-coumaryl)-rutinoside isolated from *Delonix elata* flowers were screened for antimicrobial activity and compared with standard drug. It is evident from the data presented in Table 1 that the compound isolated from the ethyl acetate fraction of *Delonix elata* flowers possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 0 mm, 7 mm , 9 mm and 10 mm, for 30 mg/ml as 16 mm, 17 mm, 17 mm and 20 mm, for 40 mg/ml showed as 21 mm, 24 mm, 23 mm and 22 mm, and for 50 mg/ml as 27 mm, 27 mm , 28 mm and 30 mm, for the test sample against *Salmonella typhi, Escherichia coli*, *Enterococcus faecalis* and *Bacillus cereus* respectively when compared with standard drug chloramphenicol showing 22 mm, 20 mm , 21 mm and 19 mm zone of inhibition respectively.



Scheme 1. Mass fragmentation pattern of glycoside



Scheme 2. Characterization of glycosid



Scheme 3. Quercetin-3-O-(2”-α-methyl-p-coumaryl)-rutinoside

Table 3. Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Delonix elata*

S. no.	Organisms	Zone of inhibition(mm)				
		Standard (Chloramphenicol)	Sample concentration (mg/ml)			
			20	30	40	50
1	*Salmonella typhi*	20	10	15	21	26
2	*Escherichia coli*	17	13	17	19	23
3	*Enterococcus faecalis*	15	11	12	22	26
4	*Bacillus cereus*	19	11	13	20	23

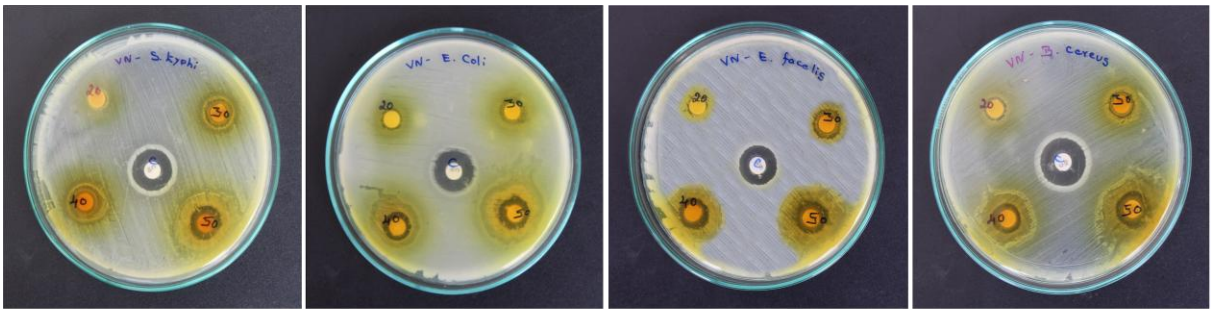


Fig. 1. Images showing the antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Delonix elata*

It is evident from the data presented in Table 2 that the test sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as

11 mm and 12 mm, for 30 mg/ml as 16 mm and 18 mm, for 40 mg/ml as 21 mm and 21 mm and for 50 mg/ml as 29 mm and 28 mm for the test solution against *Curvularia lunata*, and *Candida albicans* respectively when compared with standard drug Fluconazole showing 20 mm and 18mm zone of inhibition respectively.

Table 4. Antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Delonix elata*

S. no.	Organisms	Zone of inhibition(mm)				
		Standard (Fluconazole)	Sample concentration (mg/ml)			
			20	30	40	50
1	*Curvularia lunata*	17	12	15	18	22
2	*Candida albicans*	16	13	15	18	25

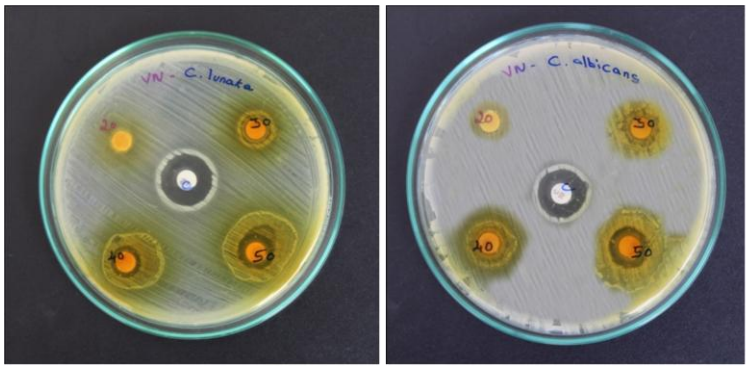
Table 5. ^{13}C-NMR spectral data and their assignments for the glycoside G2 from the flowers of *Delonix elata*

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Quercetin from literature (δ ppm)	146.9	135.5	175.8	160.7	98.2	163.9	93.3	156.2	103.1
Glycoside G2(δ ppm)	156.9	133.7	177.9	161.6	99.1	164.5	92.7	156.9	102.5
Rutin	156.6	135.5	177.8	161.3	99.0	164.1	93.9	156.8	104.2

Compound	C-1’	C-2’	C-3’	C-4’	C-5’	C-6’
Quercetin from literature (δ ppm)	122.1	115.3	145.0	147.6	115.6	120.0
Glycoside G2 (δ ppm)	122.0	115.5	145.2	146.9	115.8	121.3
Rutin	121.4	115.4	144.8	148.5	116.5	121.5

Compound	C-1’’	C-2’’	C-3’’	C-4’’	C-5’’	C-6’’
Glucose from literature (δ ppm)	100.1	76.4	73.9	69.9	77.4	61
Glycoside G2(δ ppm)	99.1	78.0	72.8	69.3	77.3	67.3
Rutin	101.4	74.3	76.6	70.3	76.1	67.3

Compound	C-1’’’	C-2’’’	C-3’’’	C-4’’’	C-5’’’	C-6’’’
Rhamnose from literature (δ ppm)	101.9	70.4	70.6	71.5	70.1	17.3
Glycoside G2(δ ppm)	101.2	70.6	70.6	72.2	70.3	18.1
Rutin	100.9	70.6	70.6	72.1	68.5	18



Curvularia lunata *Candida albicans*

Fig. 2. Images showing the antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Delonix elata*

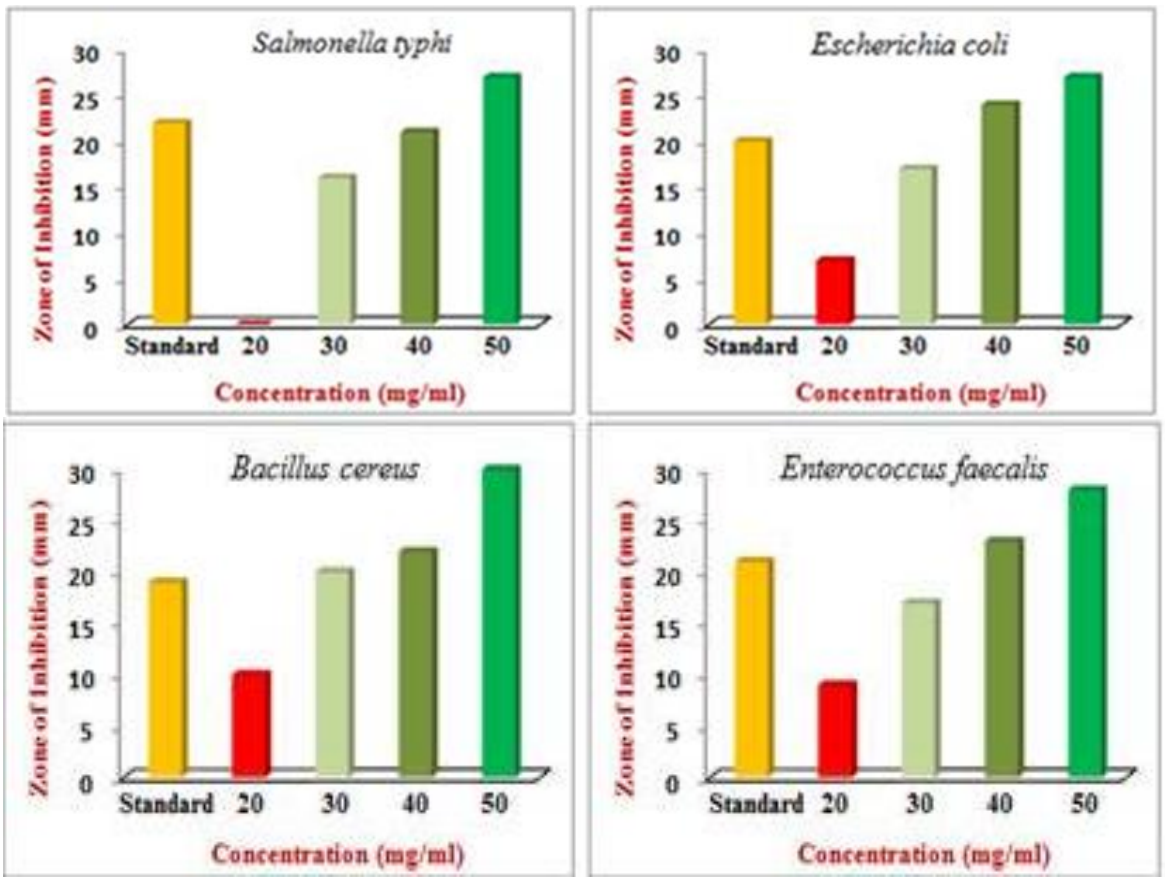


Fig. 3. Graphical representation of anti bacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Delonix elata* (Standard: Chloramphenicol, concentration 1 mg/ml)

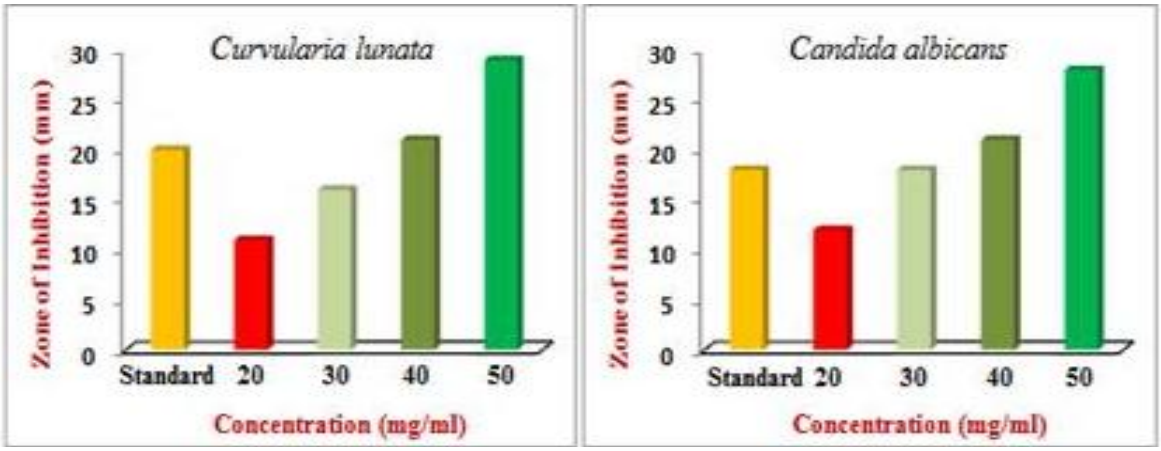


Fig. 4. Graphical representation of anti fungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Delonix elata* (Standard: Fluconazole, concentration 1 mg/ml)

4. CONCLUSION

Delonix elata possess wide range of biological and pharmacological properties. In the present research work, the phytopharmaceutical important compound Quercetin-3-O-(2”-α-methyl-p-coumaryl)-rutinoside was isolated from ethyl acetate extract of *Delonix elata* flowers. The compound was identified by the UV, ^{1}H NMR, ^{13}C NMR and MS spectroscopy. However, the

isolated compound can be used for further investigations to reveal its other unexplored pharmacological properties and believed to be safe for human life.

The antibacterial and antifungal results revealed that the bioactive compound Quercetin-3-O-(2"-α-methyl-p-coumaryl)-rutinoside isolated from the ethyl acetate fraction of flowers from *Delonix elata* is effective antimicrobial agent and it can be a source of high pharmacological importance and potential source of new drugs.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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