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Fortification of curd using *Delonix regia* flower petal extract and estimation of its phytochemical, antibacterial & antioxidant activity

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Abstract
Delonix regia commonly known as gulmohar is a traditional medicinally used plant in Africa and Asia. The water extract of flowers is used as a healthy beverage. In this study dried floral petals of *Delonix regia* were examined for their phytochemicals including total flavanoid, tannin and phenol content, antioxidant efficacy, fat and monomeric anthocyanin content followed by GC-MS analysis for the detection of secondary metabolites and finally antibacterial activity of the extracts prepared with water, methanol and acetone respectively.
The results revealed a good antioxidant activity (DPPH radical scavenging assay) with highest for water and satisfactory results for antibacterial activity tested against *Shigella, S.epidermidis,* and *E.coli.* The phytochemical analysis showing the presence of phenols, flavanoids and tannins also show these compounds to be present in higher amounts which account for the antioxidant activity as well. The crude alkaloid content was low and fat content was almost negligible <1%. The total monomeric anthocyanin content was tested using the pH differential method. Further the GC-MS analysis provides some data of the secondary metabolites present in the petals, the major ones being- Squalene, Vitamin E, Campesterol, beta. -Sitosterol, Octadecanoic acid, gamma. -Sitosterol.
These properties enable the use of this incredible flower in food fortification. The water extract of the petals of *Delonix regia* was added to the curd subjected to sensory evaluation.

Keywords: *Delonix regia*, phytochemical, antibacterial, anthocyanin, GC-MS, fortification.

1. Introduction
Natural drugs and excipients are gaining a lot of attention now days. Depletion of health day by day is making people run for antioxidants from natural sources and healthy alternatives. Nutraceuticals are the emerging market today and gaining a lot of attention due to their daily healthy keeping effects. Another alternative is the fortification of food. Food fortification is the addition of necessary substances such as vitamins, minerals, etc. to a food product which is either deficient or does not contain these essentials; whereby nutrients are extracted from a natural source or synthesized artificially. *Delonix regia* is one such natural species rich in antioxidants and carotenoids. A flowering plant grown as an ornamental tree and given the name, flamboyant or flame tree, Gulmohar, Peacock and Royal Poinciana [1]. The *D. regia* can be commonly found in India, Mexico, Australia, Caribbean, Northern Mariana Islands, United Arab Emirates and South Florida [2, 3].
It has been extensively studied and found useful as an indicator in acid base titrations [4]. Shows biological activities such as antioxidant [5], antiarthritic [6], antidiarrhoeal [7], hepatoprotective, cytotoxic [1], gastroprotective [8] (studied in Wister rats) antimicrobial [9, 10], anthelmintic, anticancer, antirheumatic, antimalarial, anti-diabetic activity [11] and for controlling insect pest [12]. Various parts of the tree are studied to be used as a natural dye for dyeing silk, for the isolation of gum from seeds [13] and as a tablet binder [14].
It is full of variety of pigments such as anthocyanins, flavonol glycosides [15], carotenoids [16], flavonol and phenolic acid [17], tannins, alkaloids, saponins, steroids, β-sitosterol [18].
Some of the recent studies have revealed the presence of valuable compounds such as astaxanthin, having anti-obesity, anti-oxidant and anti-diabetic properties. Anthocyanins such as- Peonidin-3-O-glucoside and Petunidin-3-O-acetylglucoside were newly identified. The presence of cyanidin-3-O rutinoside in a very high content, a nutraceutically important anthocyanin makes the species more valuable [19].
For the applications and potential use in food products, the toxicological aspects have been studied using murine cell line models [20].

Addressing to the previous studies, Jungalwala and Cama (1962) found 29 carotenoids present in different floral petals of *D. regia* out of which β-carotene being the major one followed by considerable amounts of zeaxanthin, phytofluene, lutein, γ-carotene, rubixanthin, phytoene, lycopene isomers and several epoxy carotenoids. The presence of such valuable substances enables the use of petals as pro-vitamin-A and as a source of natural color; supplement in cosmetics, feed and future foods. The red and white colors of the petals show the presence of another important class of pigments i.e. anthocyanins that are chemically derived from phenol-propanoids. Polyphenols and dietary anthocyanins provide health improving and constructive roles in the human body, such as reducing the risk of cardiovascular diseases as per studied in red wine which is a rich source of anthocyanins [21].

Presence of good amount of antioxidants enhances the use of any natural ingredient in different products. *D. regia* came out to be an important source of antioxidants that reduce the formation of ROS (Reactive Oxygen Species) in the body [16].

2. Materials and methods

2.1 Preparation of extracts

30 gm dried petals were extracted in 300ml methanol using soxhlet extraction. This procedure was followed for 5 consecutive cycles. The solvent was evaporated and extract was obtained. The leftover leaves were then macerated in acetone followed by placing in an incubator shaker for 24 hrs. This mixture of acetone and leaves was filtered and the solvent was evaporated to obtain the acetone extract.

The water extraction was done on the similar basis- maceration of leaves in water, placing in an incubator shaker at 25 °C followed by filtration. Heating this solution to the boiling point of water and obtaining the extract.

2.2 Antioxidant activity

2.2.1 DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay)

The radical scavenging activity of flower extracts was determined on the basis of the radical scavenging effect on the DPPH free radical. This method was given by Blois [22]. Different concentrations of the methanolic and water extracts dissolved in 10ml methanol were prepared. 1ml of the prepared extracts was added to 1 ml 0.3 mM DPPH (1.1829 mg in 10ml methanol) and 1ml methanol and a blank was prepared by just adding DDPH and methanol 1ml each. All the solutions were kept in dark for 10 mins. OD was measured at absorbance of 517 nm. %inhibition was calculated by the following formulae:

$$\%inhb = [(B-A)/B] \times 100$$

Where
B= Absorbance of blank solution
A= Absorbance of sample

2.3 Phytochemical analysis

2.3.1 Total flavanoid analysis using UV-Vis calorimetric method

Total flavanoid content in the methanol and water extracts was determined by the UV-Visible spectrophotometry [23]. Two different concentrations were prepared by dissolving 0.5 and 1mg extracts in 1ml DMSO. Out of this 250µl extract was added to 4.5ml distilled water and 0.3ml 5% $NaNO_2$ and kept for 5 mins. Added 0.3ml 10%$AlCl_3$ and incubate for 6 mins. Add 2 ml 1M NaOH to it and make up the final volume to 10ml with distilled water. Vortex all the test tubes and take OD at 510 nm. A 5-points calibration curve was carried out with Catechin as standard.

2.3.2 Total Phenolic estimation by FC method

2.3.2.1 FC (Folin-Ciocalteu)

Total phenols were estimated by the FC reagent method [24]. The sample extracts were tested in triplicates i.e. 1, 2 & 5mg in 1ml DMSO for methanol and water extracts. Out of these stocks 100µl of the sample was taken and added to 250µl FC reagent with a further addition of 1.15ml distilled water. Vortex the test tubes and added 1.5ml 20% sodium carbonate, incubation for 2 hrs add 2ml distilled water followed by measuring the OD at 765 nm. Standard curve generated using Gallic acid standard.

2.3.3 Total Tannins estimation

Estimation of tannin content of the flower was done by the method of Van Buren (1981) [25]. To 1 gm sample 100ml water was added and allowed to shake for 1 hr in an incubator shaker.

The solution was filtered and 5ml filtrate was taken in a test tube. To this 1ml of 0.1M $FeCl_3$, 1ml, 0.1N HCl and 1ml 0.008M KCN were added. OD was measured at 615 nm within 10 mins. A standard curve was generated using tannic acid as standard.

2.3.4 Total alkaloid content

Alkaloids were determined by gravimetric method of Harborne (1973) [26]. 5gm sample was added to 10% ammonium hydroxide stirred and allowed to stand for 4hrs and filtered. The filtrate was evaporated to $1/4^{th}$ of the original volume on a hot plate. To this conc. NH_4OH was added drop wise in order to precipitate the alkaloids. The precipitates were filtered using a pre weighed filter paper and washed with 10% ammonium hydroxide solution. The precipitates were dried with the filter paper in an oven for 30 mins at 60 °C and reweighed. The amount of alkaloids present was calculated in % alkaloid in the sample by the formulae:

$$\%alk = \frac{W_2 - W_1 \times 100}{W}$$

Where: W_1- weight of the tissue paper
W_2- weight of the tissue with alkaloids
W- Weight of sample

2.4 Total monomeric anthocyanin content

Total anthocyanins content in the flowers was determined by a pH differential method given by Lee (2005) [27].

2.4.1 Principle

The principle behind this experiment is based on a simple rule that monomeric anthocyanins change their color with the

change in pH reversibly. Two different forms exist at pH 1.0 and pH 4.5, the colored oxonium form and the colourless hemiketal form respectively. The polymeric forms of anthocyanins absorb at both pH 1 and 4.5 because they are defiant to color change hence are not measured. The concentration of pigments is in direct relationship to their absorbance difference at 520 nm.

2.4.2 Reagents

Two buffers are to be prepared, one having pH 1.0 (potassium chloride, 0.025M) and other having pH 4.5 (sodium acetate, 0.4M).

For the first buffer, 1L reagent is made by adding 1.86 gm KCl to 930ml distilled water. The pH is measured and adjusted to 1.0 (±0. 05) with HCl (6.3ml) and rest volume made by adding distilled water again.

The second is a pH 4.5 buffer which includes adding 54.43 gm sodium acetate and 960ml distilled water. Measure the pH, and adjust with HCl (20ml) to equalize the pH to 4.5 (±0.05) and making the volume to 1L with distilled water.

2.4.3 Procedure

1 gm dried flowers were powdered and extracted in acidified methanol (1% HCl). The flask was placed in an incubator shaker at 100 rpm/25 °C for 6hrs. Centrifuged at 7000 rpm/10 mins, supernatant decanted and pallet re-extracted thrice. Supernatants combined. Solution diluted with pH buffer 1.0 and 4.5. 1ml supernatant added to 20 ml each of the buffers in separate test tubes. OD measured at 520 nm and 700 nm respectively against distilled water blank. The anthocyanin pigment concentration was expressed as cyanidin-3-glucoside equivalents.

The above values for HCl and distilled water are calculated and may vary accordingly with the experiment.

2.4.4 Calculation

Calculate anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, as follows [27]:

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) =

$$\frac{A \times MW \times D \times 10^3}{\varepsilon \times 1}$$

Where A = (*A*520nm – *A*700nm)pH 1.0 – (*A*520nm – *A*700nm)pH 4.5; DF = dilution factor established in D; l = path length in cm; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); ε (molar extinction coefficient) = 26900 in L mol^{-1} cm^{-1}, for cyd-3-glu; and 10^3 = factor for conversion from g to mg.

Note: Dilution factor is determined by adding pH 1.0 buffer to the sample to be tested till the absorbance sets within the range of 0.2 and 1.0 AU at 520 nm.

2.5 Total Fat content

The crude fat content was estimated by AOAC method Ref. 2003.06 [28]. 2 gm dried petals added to 20ml petroleum ether and kept for 5 hrs at 150-200 rpm/45 °C in an incubator shaker. The solution was filtered and stored. 20ml petroleum ether was again added to previous days left over. The procedure was repeated.

Filtrates combined and kept at 70 °C in an oven. Total fats were calculated by the formulae:

$$\%fat = \frac{W_2\text{-}W_1 \times 100}{W}$$

Where: W_1- Initial weight of beaker
W_2- Final weight of beaker
W- Weight of sample

2.6 Antibacterial activity of flower extract

In order to determine the antibacterial activity of all three flower extracts, the agar well diffusion assay was performed. Antibacterial activity was tested against *Shigella flexneri* (gram -ve), *Staphylococcus epidermidis* (gram +ve) and *Escherichia coli* (gram -ve). The test bacteria were grown in sterile Nutrient broth tubes respectively. The broth cultures of bacteria were then aseptically transferred to the agar plates by pour plate method. Wells of 6 mm diameter were created in the inoculated plates using sterile cork borer. Different concentrations of the extracts were prepared by dissolving in DMSO (500 mg and 100 mg DMSO) and were filled in labelled wells. The plates were incubated at 37 °C for 24 hours and the zone of inhibition was measured.

Methanol and DMSO were separately plated and used as a control for the experiment.

2.7 Determination of other secondary metabolites

The secondary metabolites in the sample were identified by the GC-MS method. For this 1µl of sample extract added to the respective solvent was analyzed. As the name suggests Gas Chromatography- Mass Spectrometry, so the carrier gas here is helium. An Agilent 6890 GC with 5975B mass spectrometric detector (MSD) was used in the scan mode (m/z 35-1050) for all samples. Screening of volatiles and semi volatiles were performed using the automatic RTL screener software in combination with the Agilent NIST'05 library [28]. The detected compounds have been identified by the NIST'05 mass spectrum library.

2.8 Product preparation

A widely consumed food, i.e. curd is selected for the addition of *D. regia* and its valuable properties.

The fortification of curd was carried out in 2 ways:

1. Dried leaves in a concentration of 0.5% were added to milk and subjected to boiling and settling to make curd by adding inoculum.
2. 1 mg/gm water extract was added to the curd.

For the first method the petals were weighed and added to milk while heating, so that the necessary components inherit the milk from petals. The milk was filtered, a little cooled and inoculated with a curd. The sample was left overnight in a warm place. In the second preparation curd was made by the regular household method and added to its water extract. Next day to both these samples color and flavorus of rose were added and subjected to refrigeration at 4 °C. Both the curd preparations along with a control of sample curd were evaluated for sensory aspects on a scale of 0-9.

3. Results & Discussions

3.1 Antioxidant activity

The extracts have shown the increasing value of DPPH radicals scavenging with an increase in the dose. The radical scavenging effect of flower extracts is in the order WATER > METHANOL > ACETONE (subsequent extraction after methanol extract). The DPPH scavenging assay was carried out first to check the antioxidant property of the different extracts. DPPH happens to be a stable free radical with an unpaired electron. It appears as a dark violet colored liquid and shows maximum absorption band at 517 nm. This experiment has a simple principle that if an antioxidant is present in the test sample, then the unpaired electron of DPPH free radical becomes paired by gaining an electron from them and the color changes from violet to colourless or pale yellow. The resulting decolourization is stoichiometric with respect to the number of electrons taken up [29]. According to a data for acetone, methanol and water extracts shows more than 80% DPPH scavenging property [30]. But this study reveals that at 100 ppm all the three extracts give more than 90% DPPH scavenging activity. The water extract showed highest antioxidant activity IC_{50} value. The high antioxidant activity of water extract may be attributed to the presence of water-soluble antioxidants like phenolic acids. Table 1 shows the results for DPPH scavenging activity of methanol, water and acetone extracts respectively.

Table 1: DPPH scavenging activity of *Delonix regia* floral extracts.

Conc. (mg)	Acetone extract (after methanol extract)		Methanol extract		Water extract	
	Absorbance	DPPH scavenging activity (%)	Absorbance	DPPH scavenging activity (%)	Absorbance	DPPH scavenging activity (%)
0.5	0.071	76.56	0.060	80.19	0.055	81.84
1.0	0.027	91.08	0.023	92.40	0.021	93.74

3.2 Phytochemical analysis

Phytochemicals including phenols and flavanoids are gaining a lot of attention now days. Especially the food industry is looking forward to these compounds due to their potential to be used in functional foods as they are reported to provide health benefits. Total phenol content was estimated by the FC reagent method, which includes the transfer of electrons from phenols to the FC reagent thereby reducing it and giving color if present in the sample. The results are expressed in the form of GAE (Gallic acid equivalent) which is taken as standard for the experiment. Total flavanoid content estimated by the $AlCl_3$ method is based on the principle that if flavanoids are present in the sample, then $AlCl_3$ binds with their C_3 or C_4 keto group giving a pink colored complex which is determined in the visible range of spectrophotometer. The results are expressed as RE (Rutin equivalent). Tannins are another class of polyphenols which are found mainly in plants. They are known to hinder with the protein digestibility in body by forming complexes with the proteins and not letting them to be digested [31]. Tannic acid is taken as standard and results are expressed as TAE (tannic acid equivalent). The presence of alkaloids in the food samples represents their therapeutic significance. A diet containing alkaloids may prove helpful for healing wounds, ulcers, heamorrhoids and burns [32]. The results for all the four phytochemicals are represented in table 2, 3 and 4.

Table 2: Total flavanoid content of *Delonix regia* flower

Conc. (mg)	Methanol Extract		Water Extract	
	Absorbance	RE (Rutin equivalent) (mg RE/gm extract)	Absorbance	RE (Rutin equivalent) (mg RE/gm extract)
2	0.027	1.33	0.32	2.68
5	0.034	7.20	0.45	10.30

Table 3: Total phenol content in *Delonix regia* flower.

Conc. (in mg)	Methanol extract		Water Extract	
	Absorbance	GAE (Gallic acid equivalent) (mg GAE/gm extract)	Absorbance	GAE (Gallic acid equivalent) (mg GAE/gm extract)
2	0.266	64.78	0.327	81.72
5	0.873	91.18	0.967	101.29

Table 4: Alkaloid and tannin content in *D. regia* floral petals.

S.no	Total alkaloid content (percentage)	Total tannin content (mg TAE/gm sample)
1	2.6%.	1.8

3.3 Total fat content of the flower

Method given by AOAC was followed [28]. The amount of fats calculated was 0.48%.

3.4 pH differential method

The pH differential method is mainly applied to calculate the monomeric or pure form of anthocyanins in any test sample. At pH 1.0 mostly this pure form exists while the polymeric form does not exist at all. Hence another 4.5 pH buffer is prepared to determine the presence of polymeric or degraded forms.

The Total anthocyanin content in 1 gm floral petals is 5.009 mg/L.

3.5 Antibacterial test

Traditional antimicrobial therapy with antibiotics suffers from the major drawback of rapid development of resistance to existing antimicrobial agents. Thus, it is necessary to develop new antimicrobial agents against drug resistant strains that continuously undergo genetic change [33]. Recently WHO reported that >75% of world population, in particular developing and underdeveloped countries, rely chiefly on traditional medicines that are based on plants and their products [34]. On screening plant extracts for antimicrobial activity, it has been shown that higher plants represent a promising source of new antimicrobial agents [33]. In the present study, flower extracts of *Delonix regia* showed antibacterial activity. The zone of inhibition i.e. where there is no growth of the microbial species shows the efficacy of the extract to inhibit their growth. Control plates were also inoculated for all the three species- *Shigella flexneri, E. coli and S. epidermidis* for DMSO and methanol to prove that the zone of inhibition was due to the extracts only and not because of the solvents used. Methanol and DMSO were found to have no effect on all the three species used in the test. The acetone extract also had no effect (because it was a re-extract after the extraction of petals in methanol) and hence is not included in the results. The water and methanol extracts were most effective against *Shigella.* Table 5 shows the zone of inhibition and the concentrations of the extracts used in the tests.

Table 5: Antibacterial test

Extract used	Concentration (mg)	Zone of Inhibition (mm)		
		Shigella flexneri	*S. epidermidis*	*E.coli*
Control (DMSO & Methanol)	-	NA	NA	NA
Methanol extract	500	22	12	15
	100	16	9	9
Water extract	500	20	10	8
	100	14	7	8

3.6 GC-MS analysis

The GC-MS analysis shows the presence of a wide variety of compounds in higher concentrations that are important. The methanol fraction comprises of compounds such as 4H-Pyran-4-one, 2, 3-dihydro-3, 5-di hydroxy-6-methyl which is a strong antioxidant in glucose-histidine maillard reaction products [35], 2-Methoxy-4-vinylphenol is a flavouring agent and responsible for the aroma of buckwheat, benzoic acid which is a preservative, fats such as palmitic acid, margaric acid, linoleic acid, 7,10-Octadecadienoic acid methyl ester, 3E,12Z)-1,3,12-Nonadecatriene-5,14-diol also found in drumsticks and jasmine flower, stigmasterol is one amongst the plant sitosterols may be useful in prevention of certain cancers and Vitamin E which itself is a good antioxidant agent. Studies have also indicated that a diet high in phytosterols including β-sitosterol, campesterol, ergosterol may inhibit the absorption of cholesterol and lower serum cholesterol levels by competing for intestinal absorption [36]. The water extract comprises of compounds such as Acetic acid, 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl, Benzenecarboxylic acid, n-Hexadecanoic acid, 9,17-Octadecadienal, (Z), 9-Octadecenoic acid, (E), Octadecanoic acid similar to that of methanol fraction.

However the acetone fraction has some compounds such as Naphthalene which is used as a fumigant and Azulene an isomer of naphthalene. 1-Hexadecanol is flavour ingredient, pentadecene, heptadecene, octadecene, nonadecene, eicosene, oleic acid which is an omega 9 fatty acid and sitosterol in higher amounts.

Table 6: List of compounds in Methanol extracted fraction.

S.no.	Compound detected	CAS#	% Area	R.T.
1	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl	028564-83-2	3.79	9.794
2	2-Methoxy-4-vinylphenol	07786-61-0	0.65	12.586
3	Benzoic acid, 3-hydroxy	000099-06-9	3.88	15.312
4	Benzoic acid, 4-hydroxy	000099-96-7	3.88	15.312
5	n-Hexadecanoic acid	000057-10-3	5.39	20.235
6	Heptadecanoic acid	000506-12-7	0.27	21.144
7	7,10-Octadecadienoic acid, methyl ester	56554-24-6	0.57	21.502
8	9,12-Octadecadienoic acid (Z,Z)	000060-33-3	7.70	21.895
9	E,Z-1,3,12-Nonadecatriene	1000131-11-3	2.54	26.415
10	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	000111-02-4	0.70	27.581
11	Squalene	007683-64-9	0.70	27.581
12	Vitamin E	000059-02-9	2.03	31.585
13	Campesterol	000474-62-4	0.83	33.368
14	Ergost-5-en-3-ol, (3.beta.)	004651-51-8	0.83	33.368
15	Stigmasterol	000083-48-7	1.26	34.030
16	Stigmasterol, 22,23-dihydro-.gamma.-Sitosterol	000083-47-6	1.28	35.297
17	.beta.-Sitosterol	00083-46-5	1.28	35.297

Table 7: List of compounds in water extracted fraction.

S.no.	Compounds detected	CAS#	%Area	R.T.
1	Acetic acid	000064-19-7	4.80	3.603
2	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl	028564-83-2	5.08	9.883
3	Benzenecarboxylic acid	000065-85-0	0.53	10.254
4	n-Hexadecanoic acid	000057-10-3	2.02	20.201
5	9,17-Octadecadienal, (Z)	056554-35-9	2.18	21.884
6	9-Octadecenoic acid, (E)	000112-79-8	2.18	21.884
7	Octadecanoic acid	000057-11-4	0.98	22.063

Table 8: List of compounds in the acetone extracted fraction.

S.no.	Compound detected	CAS#	%Area	R.H.
1	Naphthalene	000091-20-3	0.26	10.500
2	Azulene	000275-51-4	0.26	10.500
3	1-Tetradecene	001120-36-1	0.84	13.618
4	1-Hexadecanol	036653-82-4	0.84	13.618
5	Cyclotetradecane	000295-17-0	0.84	13.618
6	Cyclohexane, octyl	001795-15-9	0.50	14.403
7	Pentadecane	000629-62-9	0.34	15.042
8	1-Heptadecene	006765-39-5	4.08	16.198
9	5-Octadecene, (E)	007206-21-5	4.08	16.198
10	1-Nonadecene	018435-45-5	4.08	16.198
11	8-Pentadecanone	000818-23-5	2.85	17.173
12	1-Nonadecene	018435-45-5	6.50	18.463
13	3-Eicosene, (E)	074685-33-9	6.50	18.463
14	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	017851-53-5	11.83	20.280
15	Dibutyl phthalate	000084-74-2	11.83	20.280
16	1-Nonadecene	018435-45-5	5.59	20.504
17	10-Nonadecanone	000504-57-4	1.81	21.289
18	Octadec-9-enoic acid	1000190-13-7	4.82	21.884
19	9-Octadecenoic acid, (E)	000112-79-8	4.82	21.884
20	Bicyclo[10.8.0]eicosane, (E)	1000155-85-0	4.82	21.884
21	1-Docosene	001599-67-3	4.09	22.344
22	Docosane	000629-97-0	2.76	23.342
23	Cyclotetracosane	000297-03-0	2.50	24.048
24	Trichloroacetic acid, hexadecyl ester	074339-54-1	2.50	24.048
25	Trifluoroacetic acid, n-octadecyl ester	079392-43-1	2.50	24.048
26	17-Pentatriacontene	006971-40-0	0.61	24.284
27	9-Hexacosene	071502-22-2	2.07	25.630
28	Ethanol, 2-(octadecyloxy)	002136-72-3	6.95	26.415
29	Octadecane	000593-45-3	4.01	27.233
30	Nonacosane	000630-03-5	13.57	28.243
31	Tetracosane	000646-31-1	13.57	28.243
32	Vitamin E	000059-02-9	3.98	31.596
33	Gamma.-Sitosterol	000083-47-6	1.78	35.297

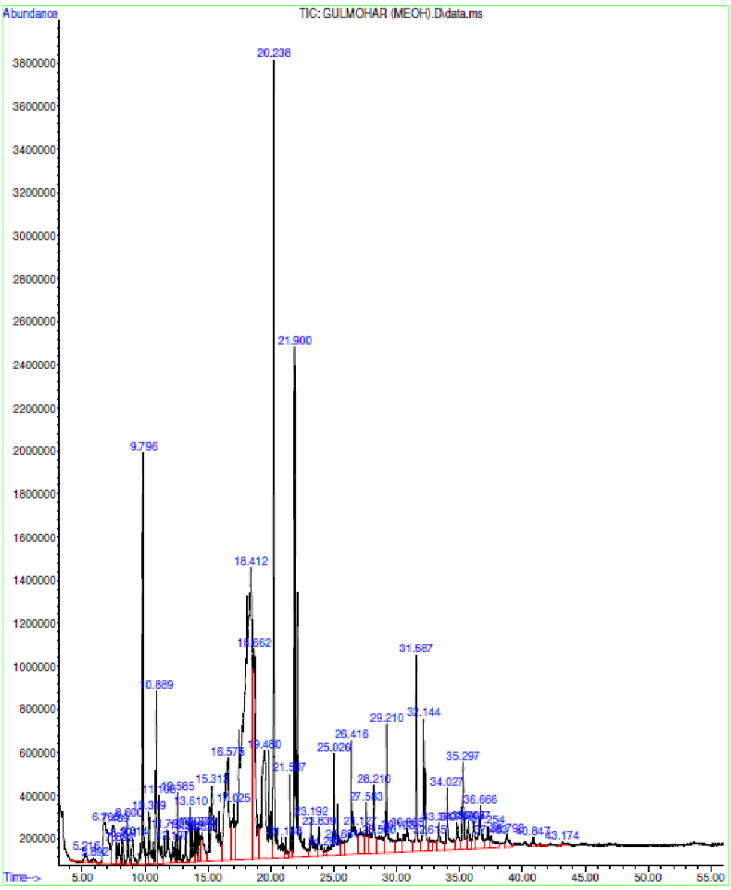

Fig 1: GC-MS Chromatogram for methanolic extract of *Delonix regia*.

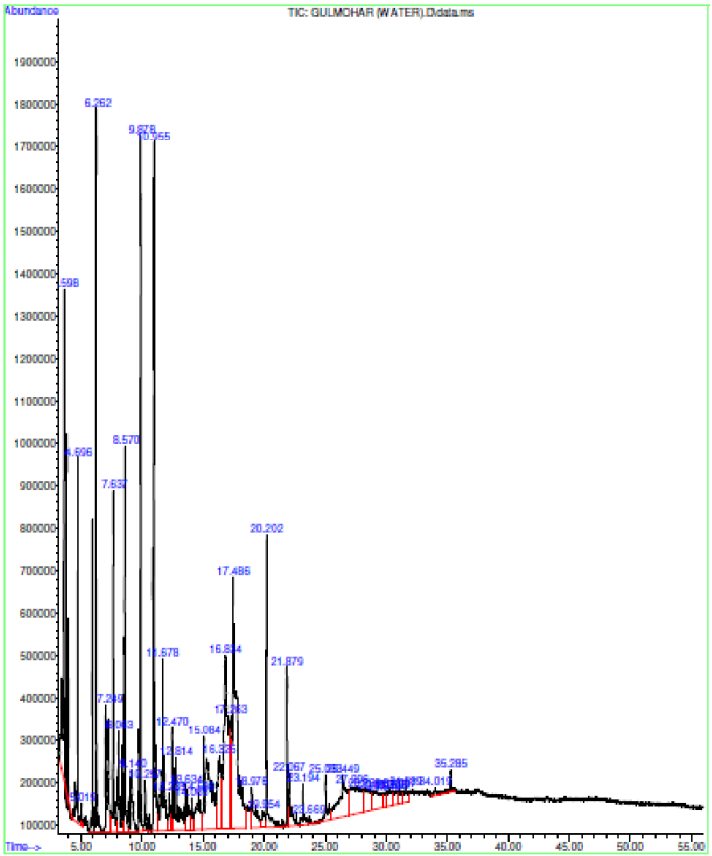

Fig 2: GC-MS chromatogram for water extract of *Delonix regia.*

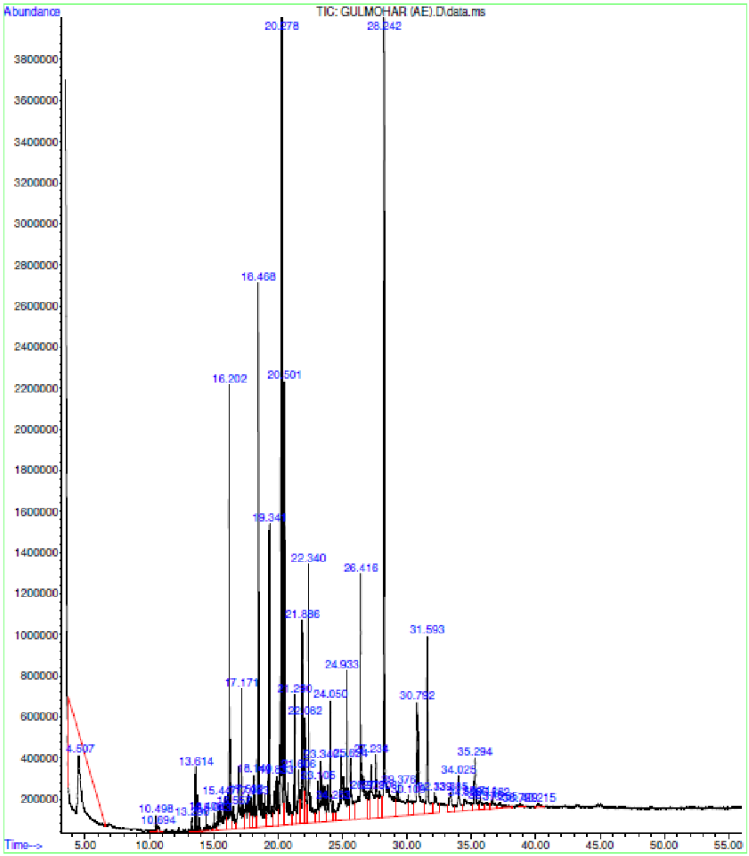

Fig 3: GC-MS chromatogram for acetone extract of *Delonix regia.*

3.9 Sensory evaluation of the product

The product sensory evaluation was measured on the basis of color, odor, taste, appearance and texture. Flavor of rose was added because the extract and flower had no taste or color it. For the formulation 1 i.e. with the petals boiled in milk the curd was not settled and not giving a good texture. Curdling was also not proper and complete this may be due to the metabolites present in the petals which inhibit the growth of bacteria. For the second formulation the water extract was added from outside after curd was formed and gave a good color, taste, texture, odor and flavor of roses. The formulation 2 was acceptable.

4. Conclusion

Delonix regia is an easily available raw material. The flowers appear in abundance during the months of April-July which are wasted across the streets of the country. The petals of this flower are a golden pot filled with a variety of pigments such as anthocyanins, carotenoids and other compounds such as phenols, flavanoids. They are a good source of antioxidants and should be used in a beneficial way such that the wastage is also reduced. Due to the presence of high amount of antioxidants, it can find applications in the pharmaceutical as well as the food industry. However, scaling up of the principal procedure is a much need. And further the analysis is required for its potential use in the food industry and human health after consumption.

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