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Research Article

Development and Validation of GC - MS Methods for Determination of leaf and root of *Delonix elata* (L.) Gamble

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Abstract

A Gas Chromatography with Mass spectra methods were developed and validated for the quantitative determination of leaves and root (*Delonix elata*) in pharmaceutical dosage form. A total 50 compounds of leaf, 45 compounds of root were identified from the methanolic extracts of *Delonix elata.* The identified compounds of leaf, the highest % Peak area of 7.06 is Quercetin (Retention time 0.00 - 39. 93), rutin % Peak area of 11.78 is rutin (Retention time 0.00 - 39. 93), the identified compounds of flavonoids. A total 45 compounds of root, 45 compounds were identified from the methanolic extracts of *Delonix elata.* The identified compounds of root, the highest % Peak area of 32.54 is β - amyrin (Retention time 0.00 - 39. 94), hesperitin % Peak area of 7.06 is Rutin (Retention time 0.00 - 39. 93), the identified compounds of flavonoids.

Keywords: GCMS, *Delonix elata*, hesperitin, rutin, β - amyrin and quercetin and biologically active compounds

Introduction

Knowledge of the identity and relative amounts of the volatile substances released by plants is of great importance to several fields of basic and applied research in biology, chemistry and may other disciplines. Obtaining this knowledge requires overcoming many analytical challenges posed by these complex mixtures, because they normally present large variations in component amounts, chemical structures and functionalities. Gas chromatography (GC) is recognized as the most suitable technique to find out how many components and in what proportion there are in a complex mixture of volatile compounds. When it is coupled to mass spectrometry (GC-MS), additional information arises about each separated compound molecular mass, elemental composition (when high resolution mass spectrometry is used), functional groups, and in certain cases, molecular geometry and spatial isomerism.

Flavonoids (or bioflavonoid), collectively known as Vitamin P and citrin, are a class of plant secondary metabolites which are ubiquitous in photosynthesizing cells and are commonly found in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. For centuries,

preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases. The function of flavonoids in flowers is to provide colors attractive to plant pollinators (Middleton *et al*., 1993), (Harborne, 1992). In leaves, these compounds are increasingly believed to promote physiological survival of the plant, protecting it from, for example, fungal pathogens and UV- radiation (Harborne, 1999), (Hambone, 1992). In addition, flavonoids are involved in photosensitization, energy transfer, the actions of plant growth hormones and growth regulators, control of respiration and photosynthesis, morphogenesis and sex determination (Middleton *et al*., 1993), (Harborne, 1999). The basic structural feature of flavonoid compounds is the 2-phenyl-benzopyrane or flavane nucleus, which consists of two benzene rings (A and B) linked through a heterocyclic pyrane ring (C). Increasingly, this class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity. The flavonoids inhibit a perplexing number and variety of eukaryotic enzymes and have a tremendously wide range of activities. In the case of enzyme inhibition, this has been postulated to be due to the interaction of enzymes with different parts of the flavonoid molecule, e.g. carbohydrate, phenyl ring, phenol and benzopyrone ring (Havsteen, 1983). The vasodilator property of flavonoids is highly useful for the treatment of heart diseases. Literature search indicated that biflavones and isoflavones are potential blood circulation enhancers in brain. Several reviews showed that flavonoides possess wide spectrum of biological activities in cardio vascular system, which include anti oxidant, anti thrombotic, anti apoptic, anti ischemic, anti arrhythmic, and anti hypertensive activities. Major dietary sources of Flavonoides in the form of flavonols, flavones, isoflavones, flavonones, biflavones are, tea , red wine , apple, tomato, cherry, onion, thyme, parsley, soyabeans, and other legumes, grape fruit, orange, lemon, ginkgo, and neem. The flavonoid groups of poly phenolic compounds have low toxicity in mammals and are widely distributed in plant kingdom. They have been shown to inhibit the growth of various cancer cell lines *invitro* and reduce tumor development. Flavonoides like kaempferol, myricetin, and quercetin are strong inhibitors of xanthine oxidase, and indicated in the treatment of gout, hyperuricemia, and reperfusion injury. The aldoreductase inhibition property of flavonoids found to be useful in diabetes induced retinopathy and cataract.Catechins acts as an antiulcer agent by inhibiting the H+/ K ATPase .Liquritigenin administration in experimental animals showed significant fall in serum cholesterol. The cardio toxicity of doxorubicin can be countered by flavonoides like luteolin. Silymarin is proved as an effective hepatoprotective agent. Tyrosinase inhibitors like Butein (2', 4', 3, 4 - Tetrahydroxychalcone)and other chalcones have become increasingly important in the cosmetic and medicinal products used in the prevention of hyperpigmentaion.

Leaf and root *in vivo* plants of *D. elata* were washed, shade dried and powdered in electric mixer grinder, sieved, labelled and then put in amber coloured containers and stored in referigerator for future use (Harborne, 2005). A higher plant is a solar powered biochemical factory which manufactures both primary and seconday metabolites, the phytopharmaceuticals, from air, water and sun light. Only little information is available about the chemical composition of *D. elata* . Since GC-MS have been sucessfully applied to the analysis of polar compounds in most of the plant materials.GC-MS technique was applied to Leaf and root of *in vivo* plants of *D. elata* in order to identify the highest possible number of compounds.

Materials and Methods

Plant Material

Plant material of *Delonix elata* (L.) Gamble was collected from Narthamalai, Pudukottai, Tiruchirappalli, Tamil Nadu, during the month of December 2008. The plant specimen was identified with Gambles Flora of the Presidency of Madras and the identity is confirmed with the herbarium

specimen deposited in Department of Botany, Periyar EVR College (Autonomous) Tiruchirappalli.

Preparation of the Extract

Plant materials leaf and root was washed with distilled water and shade dried. The dried samples were manually ground to a fine powder. The plant materials was identified and authenticated by Botanical Survey of India (Southern Circle, Coimbatore Tamil Nadu, India). A voucher specimen of both has been deposited for future reference in the Department of Botany Periyar EVR College (Autonomous), Tiruchirappalli - 620 023.

Delonix elata (L.) leaves and root were chopped into small pieces, shade dried. Dried samples were powdered in a Wiley mill. Powdered samples were stored in polythene containers at room temperature. The leaves and root samples were taken for analysis to detect the presence of certain biologically active compound(s). The extract contains polar components of the material and 2µl sample of the solution was employed in HPL and GC-MS for analysis of different compounds.

Identification of certain Biologically active compounds

Delonix elata leaf and root were chemically screened to find out the presence alkaloids (AL), flavonoids (FL), protein (PR), amino acids (AA), carbohydrates (CR), tannins (TA), phenolics (PH), glycosides (GL), saponins (SA), or absence of bio active compounds.

Extraction

50 g of *Delonix elata* (L.) Gamble (Leaves) and (Root) coarse sample using Soxhlet method, extraction 24 hrs and using Methanol (MT) solvent.

Preliminary phytochemical screening

The condensed extracts of different solvent used for preliminary phytochemical screening were carried out using standard procedures to test the presence of bioactive compounds (Amarasingham *et al*., 1964), (Chabra *et al*., 1984) and Harborne (eds). 1984.

Qualitative Analysis of phtyochemicals

The analysis of phytochemicls from the solvent free extract of *Delonix elata* leaves was individually carried out using various qualitative test for alkaloids, flavonoids, protein, amino acid, tannins, phenolics, glycosides, saponins and carbohydrates compounds.

Extraction of phytochemicals

The individual phytochemical was extracted in the appropriate solvent and stored in air –tight containers at 4^{o}C till further use.

Test for alkaloids

The small portion of extract were stored separately with a few drops of dilute hydrochloric acid and filtered. The filterate was tested with various alkaloidal agents, such as Mayer's reagent (White precipitate or turbidity).

Test for flavonoids

Shinoda test

One ml of the extract was treated with magnesium turnings and 1-2 drops of concentrated Hcl. Formation of pink or red colour shows the presence of flavonoids.

Test for protein and amino acids

Ninhydrin test

One ml of the extract, 2 drops of freshly prepared 0.2 per cent ninhydrin reagent was added and heated. The appearance of blue colour indicates the presence of proteins, peptides or amino acids.

Test for tannins and phenolic compounds

One ml of the extract was treated with few ml of the gelatin solution, a white precipitate reveals the presence of tannins and phenolic compounds.

Test for glycosides

Legal test

The extract was dissolved in pyridine and freshly prepared sodium nitoprusside solution was added. The formation of pink to red colour indicates the presence of glycosides.

Test for saponins

To 1 ml of the extract, alcoholic vanillin solution and a few drops of concentrated sulphuric acid were added. A deep violet colour confirms the presence of saponins.

Test for carbohydrates

Benedict's test

Five ml of Benedict's solution was added to the extract and boiled in water bath. The appearance of red yellow or green precipitate indicates the presence of reducing sugars.

Phytochemical screening

The test was performed on methanol extract of *Delonix elata* (L.) Gamble leaves (**Table 1**).

Table 2.1. Phytochemical constitute of the Leaf extracts of *Delonix elata* (L.) Gamble

Extract Name	Chemicla constituent
Methanol	Alkaloids
	Flavonoids
	Protein
	Amino Acid
	Tannins
	Phenolics
	Glycosides
	Saponins
	Carbohydrates

Phytochemical analysis revealed that Methanol extract of *Delonix elata* leaves contains, alkaloids, flavonoids, protein, amino acid, tannins, phenolics, glycosides, saponins and carbohydrates compounds (Table 2. 1).

Maceration

Powdered dried leaves (1g) and root (1 g) were macerated with methanol: water (1:1; v/v, 10 mL) and left at rest (7 days, room temperature). The material was filtered and the crude extract obtained was analyzed directly by GCMS and HPLC-UV. This procedure was repeated in triplicate.

Gas Chromatography (GC)

A common form of mass spectrometry is Gas Chromatography - Mass Spectromertry (GC/MS or GC - MS). In this technique, a gas chromatography is sued to separate compounds. The stream of separated compounds is fed on line into the ion source, a metallic filament emits electrons, which ionize the compounds. The ions can then further fragment, yielding predictable patterns. Itact ions and fragments pass into the mass spectrometer's analyzer and are eventually detected.

The ample were analysed by GC - Mas supplied by FISONS instruments the GC - Mas model is GC 8000 series and MS is MD 800.

GC column dimension : 30 mm × 0.25 mm × 0.5 mm. AB-35 MS fused silica column. Helium gas is engaged as a carrier gas at the rate of 1 ml/min.The GC-MS oven temperature was programmed as follows:

i. 50 °C (hold 2.5 minutes) to 150 °C at the rate of 15 °C/min
ii. ii. increased to 200 °Cat a rate of 3 °C/min
iii. increased to 300 °C at a rate of 8 °C/min

iv. kept for another 8 minutes at 300 °C

GC conditions

Injector temperature 250^{o}C at 6^{o}C/mts and be held at this temperature for 10mts. The ion source temperature is 200^{o}C and the interface temperature is 250^{o}C .

Gas chromatography - mass spectrometry (GC - MS)

The spectras was obtained in the EI mode with 70 eV ionization energy. The compounds were

identified by comparison with the standards, if not available, the mass spectra was matched with the in built library. The methanolic extract of the plant drug powder, viz., leaf, stem bark, flower, seed and root were injected by hypodermic syringe into the inlet port of GC. The full scan MS of the compounds were measured from m/z 80 - 750. MS data were acquired in the negative ionization mode. The results can be expressed in terms of retention time (Rt), which is the time required for elution of sample or RV - the volume of carrier gas required to elute a component from the column. These parameters are nearly always expressed in terms relative to a standard compound (RR_v or RR_T) which may added to the sample extract or which could take the form of the solvent used for dissolving the sample. GC provides both quantitative and qualitative data on plant substances, since measurements of the area under the peaks shown on the GC trace are directly related to the concentrations of the different components in the original mixture.

GC is automatically linked to mass spectrometry and the value of the technique is that it requires only microgram amounts of material, that it may yield a complex fragmentation pattern, which is often characteristic of that particular compound. MS, in essence, consits of degrading trace amounts of an organic compound and recording the fragmentation pattern according to mass. The sample vapor diffuses in to the low-pressure system of the mass spectrometer where it is ionized with sufficient energy to cause fragmentation of chemical bonds. The resulting positively charged ions are accelerated in a magnetic field, which disperses and permits relative abundance measurements of ions of give mass - to - charge ratio. The resulting record of ion abundance versus mass constitutes the mass spectral graph, which thus consits of a series of lines of varying intensity at different mass units. In many cases, some of the parental compound will survive the vaporization process and will be recorded as a molecular ion peak. Very accurate mass measurement (0. 0001 mass units) can then be performed on this and particular ion. The accuracy is such as to indicate the exact molecular formula of the substance, so that conventional lethal analysis no longer necessary.

MS is frequently used in conjunctions with GLC, and the combined operation provides both qualitative and quanitative identification of the many structurally complex components that my be present together in a particular plant extract (Zarba *et al* ., 2005). Some of the many applications of MS data to plant biochemical research are covered in two Treatises (Waller, 1972; Waller and Dermer, 1980). Leaves of *D. elata* were found to contain Quercetin(Fig c) and Rutin (Fig. d) represented chromatogram (Fig. c) represent GC - MS data of leaf extract (Table 2). Phytochemicals found in the root extracts contain β - amyrin (Fig. a) and hespertin (Fig. b) are listed in the (Table 3). Fig represent gas chromatogram, Fig represent GC - Mass spectral data of stem extract.

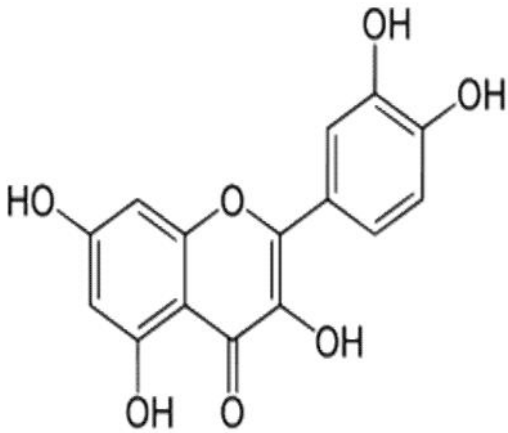
Results and Discussion

Qualitative and Quantitative Analysis

The quantitative determination of the chemical compounds was based on the comparison of peak areas of samples with those in GCMS library. Some of the identified compounds are quercetin, rutin, beta amyrin and hesperitin as described below:

Quercetin

The quercetin was identified in *Delonix elata* L. (leaves) in GCMS analysis. Figure 2.1 Figure 1 shows that the comparison of abundance of quercetin in leaf parts of the plant extracted by the methanol solvents. The leaves sample contain the least quercetin compounds.

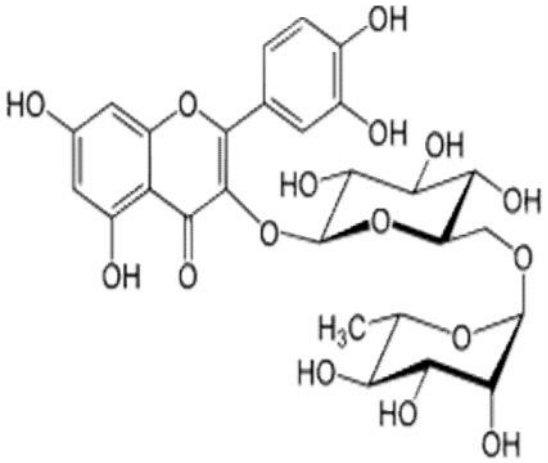


Structure of Quercetin ($C_{15}H_{10}O_7$)

As a result, the best extraction conditions for quercetin compound in *Delonix elata* L. were 24 hours extraction period with methanol as solvent and the most favourable parts of the plant for Soxhlet extraction process was leaves.

Rutin

Besides quercein, rutin was also one of the chemical compounds that can be determined from *Delonix elata* L. leaves in the GCMS analysis which was next to the peak of rutin. The rutin was identified in *Delonix elata* L. (leaves) in GCMS analysis. Figure 2.1 Figure 1 shows that the comparison of abundance of rutin in leaf parts of the plant extracted by the methanol solvents. The leaves sample contain the least rutin compounds.

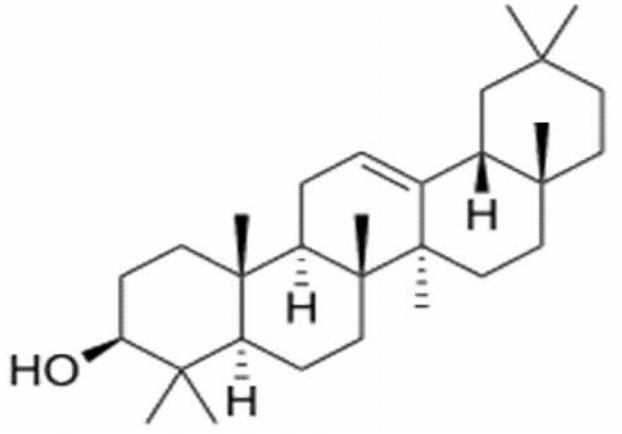


Structure of Rutin ($C_{27}H_{30}O_{16}$)

As a result, the best extraction conditions for rutin compound in *Delonix elata* L. were 24 hours extraction period with methanol as solvent and the most favourable parts of the plant for Soxhlet extraction process was leaves. *Delonix elata* (L.) leaves show the presence of quercetin and rutin. In addition, methanol has been identified as the better extraction solvent. This was because almost all the parts of plant which were extracted with methanol solvent proposed the higher percentage area in the result of gas chromatography as shown in **Figure .**

Beta amyrin

The beta amyrin was identified in *Delonix elata* L. (root) in GCMS analysis. Figure 2. 2 Figure 1 show that the comparison of abundance of beta amyrin in root parts of the plant extracted by the methanol solvents. The root sample contains the least rutin compounds.

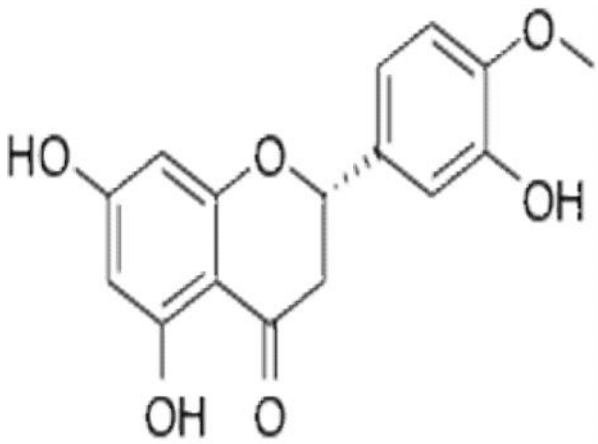


Structure of Beta amyrin ($C_{30}H_{50}O$)

As a result, the best extraction conditions for beta amyrin compound in *Delonix elata* L. were 24 hours extraction period with methanol as solvent and the most favourable parts of the plant for Soxhlet extraction process was leaves.

Hesperitin

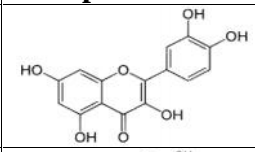
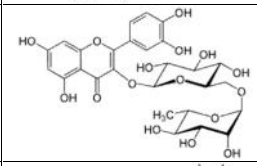
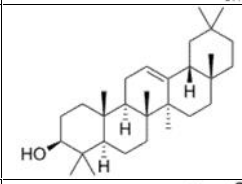
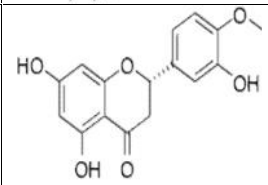
The hesperitin was identified in *Delonix elata* L. (root) in GCMS analysis. Figure 2.2 Figure 1 shows that the comparison of abundance of hesperitin in root parts of the plant extracted by the methanol solvents. The root sample contains the least hesperitin compounds.



Structure of Hesperitin ($C_{16}H_{14}O_6$)

As a result, the best extraction conditions for hesperitin compound in *Delonix elata* L. were 24

Table 2. 3 Chemical components of the methanolic leaf and root extract of the *Delonix elata* (L.) using GC - MS analysis

S.NO.	Plant part	Name of the Compounds	Structure of the Compound	Rt (min)	Molecular weigt (*m/z*)	Molecular formula
1.	Leaf	Quercetin		7.06	302.236	$C_{15}H_{10}O_7$
2.		Rutin		11.78	610.52	$C_{27}H_{30}O_{16}$
1.	Root	Beta amyrin		7.06	426.72	$C_{30}H_{50}O$
2.		Hesperitin		32.54	302.27	$C_{16}H_{14}O_6$

Sample ID: EM-65 Low Mass(m/z): 50 Sample Name: PLANTSAMPLE16
Operator: DSQ High Mass(m/z): 650 Comments:
Run Time(min): 36.93 Instrument Name: DSQ Acquisition Date: 05/20/11 12:46:08 PM

EQUIPMENT : THERMO GC - TRACE ULTRA VER: 5.0,
THERMO MS DSQ II
COLUMN : TR 5 - MS CAPILLARY STANDARD NON - POLAR COLUMN
DIMENSION : 30 Mts, ID : 0.25 mm, FILM : 0.25 µm
CARRIER GAS : He, FLOW : 1 ML/Min
TEMP PROG : 100 - 250 , RATE : 8/Min, HOLDING TIME : 10 Min @250

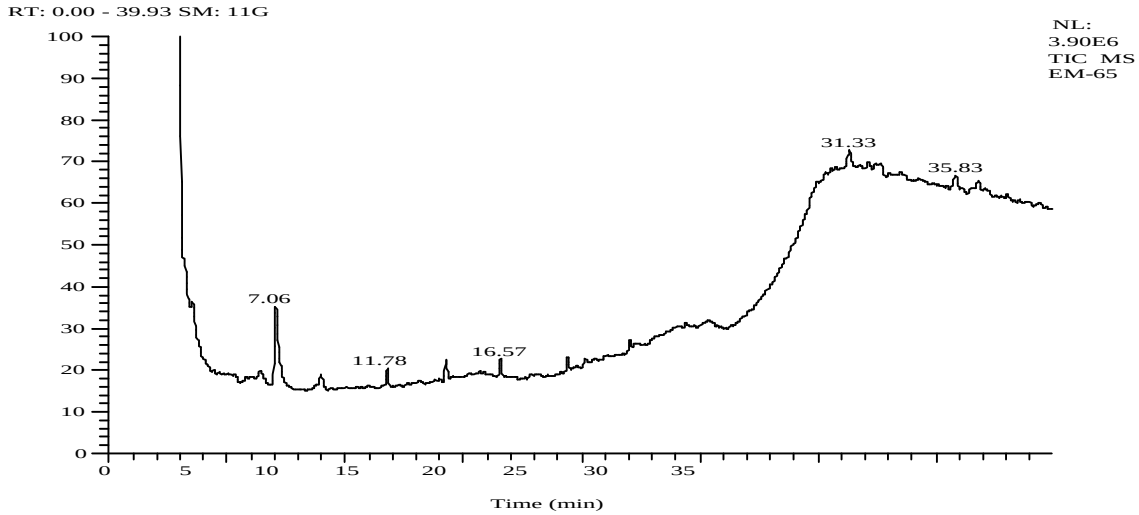


Fig. 3.28 GC-MS chromatogram of *in vivo* leaf of *D. elata*

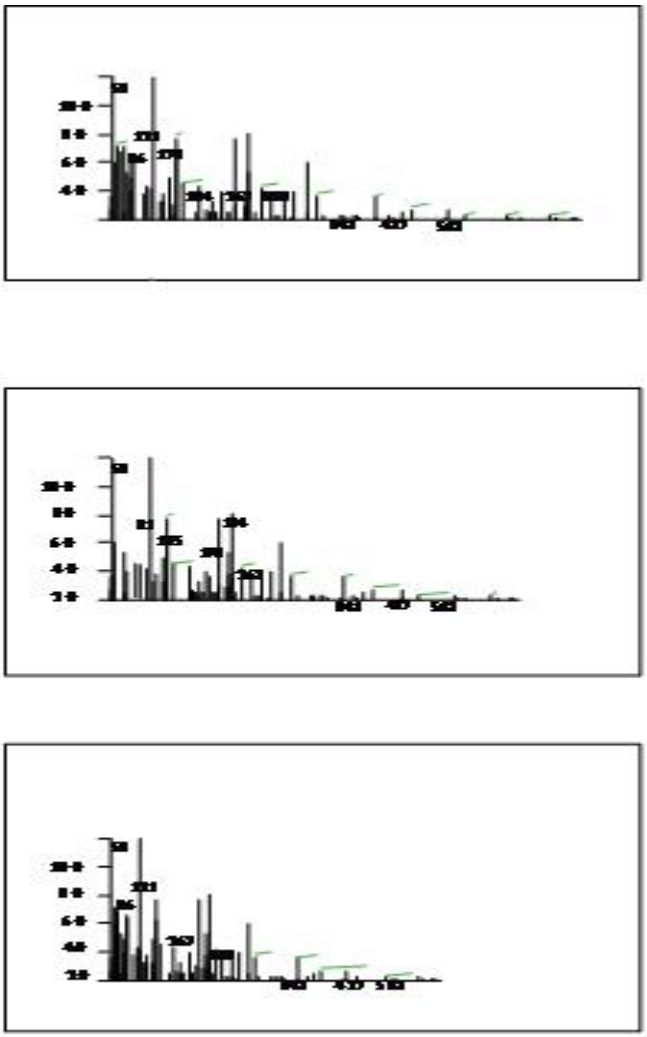


Figure 3.29 GC-MS mass spectrum of *in vivo* leaf of *D. elata*

hours extraction period with methanol as solvent and the most favourable parts of the plant for Soxhlet extraction process was leaves.

GC-MS investigation of Methanolic Extract of *Delonix elata (*Linn) (Fig.2 and 3)

The results of the GC-MS analyses on methanolic extracts of of *Delonix elata* is presented in the leaves (Table 1), (Fig) and root (Table 2). A total 50 compounds of leaf, 45 compounds of root were identified from the methanolic extracts of *Delonix elata*. The identified compounds of leaf, the highest % Peak area of 7.06 is Quercetin (Retention time 0.00 - 39. 93), rutin % Peak area of 11.78 is Rutin(Retention time 0.00 - 39. 93), the identified compounds of flavonoids. The results of the GC-MS analyses on methanolic extracts of of *Delonix elata* is presented in the root (Table 2), (Fig). A total 45 compounds of root, 45 compounds were identified from the methanolic extracts of *Delonix elata*. The identified compounds of root, the highest % Peak area of 32.54 is β - amyrin (Retention time 0.00 - 39. 94), hesperitin % Peak area of 7.06 is Rutin (Retention time 0.00 - 39. 93), the identified compounds of flavonoids.

In the present study 10 compounds were identified by Gas Chromatography - Mass Spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners.

Table 2. Phytoconstituents present in methanolic extract of *Delonix elata* (L.) Gamble leaf sample using GC-MS

S.NO.	Compound Name	Formula	M W
1.	1-[3',4'-Dihydro-1'-(trimethyl silylethynyl) naphtha len-2'-yl]ethanone	$C_{17}H_{20}OSi$	268
2.	(3,4,5-Trimethoxy-7-oxo-1'-allyl-2',6'-dimethoxy)-8.0.4'-Neolignan	$C_{23}H_{28}O_7$	416
3.	N,N-dimethyl-3,4-(methylenedioxy)benzylamine	$C_{10}H_{13}NO_2$	179
4.	Diethyl 1-isopropyl-2,3-dioxobutylphosphonate	$C_{11}H_{21}O_5P$	264
5.	3-(4-Methylphenyl)-3-phenylcyclobutanone	$C_{17}H_{16}O$	236
6.	Dimethyl 2,4,6,6-tetramethylcyclohex-3-ene-1,1-dicarboxylate	$C_{14}H_{22}O_4$	254
7.	3,4-Methylenedioxy-N,N-dimethylbenzylamine	$C_{10}H_{13}NO_2$	179
8.	N-(9-METHYL-9-FLUORENYL)-3.5-DICHLO ROANILINE	$C_{20}H_{15}C_{l2}N$	339
9.	2,5-Dimethoxy-1-(2'-hydroxypropyl)-3,4,6-trimethylbenzene	$C_{14}H_{22}O_3$	238
10.	(2S,3S)-1,2-p-Methoxybenbzylidene-3-tert-butyloxy carbonylamino-1,2-Butanediol	$C_{17}H_{25}NO_5$	323
11.	D-(+)-Raffinose	$C_{18}H_{32}O_{16}$	504
12.	5-Amino-4-cyano-3-(3-methylaminopyopyl)pyrazole	$C_8H_{13}N_5$	179
13.	1-Cyclohexylhexan-3-one	$C_{12}H_{22}O$	182
14.	4-Acetyl-1-amidino-3,5-dimethylpyrazole Amidino hydrazone Dihydrochloride	$C_9H_{16}N_8$	236
15.	4-acetyl-1-amidino-3,5-dimethylpyrazole amidino hydrazole	C_9H16N8	236
16.	15,16-Epoxy-10(9-8 -abeo-labda-13(16),14-diene-7,9-dione	$C_{20}H_{28}O_3$	316
17.	(erythro)-(7R,8S)-[3,4-(Methylenedioxy)-7-acetoxy-1'-allyl-3',5'-dimeth oxy]-8.0.4'-Neolignan	$C_{22}H_{38}O_7$	414
18.	5-[N-(2-(3-Nitropyridin-2-ylamino)phenyl)imino]-4-chloro-5H-1,2,3-dithiazole	$C_{13}H_8ClN_5O_2S_2$	365
19.	9-[3'-(N,N-Dimethylamino)propylamino]-1,4-dimeth oxy acridine	$C_{20}H_{25}N_3O_2$	339
20.	[(t-butyl)dimethylsilyl] [(t-butyl)dimethyl silyl] vanillate	$C_{20}H_{36}O_4Si_2$	396
21.	2-ethylbenzenesulphonamide	$C_8H_{11}NO_2S$	185
22.	(5S)-(+)-3,5-Diphenyl-4,5-dihydroisoxazole	$C_{15}H_{13}NO$	223
23.	1-Hydroxylamino-2-nitro-1-phenylethane	$C_8H_{10}N_2O_3$	182
24.	3-methyl-5-phenylisoxazoline	$C_{10}H_{11}NO$	164
25.	Acetic acid, 2-phenylethyl ester (CAS)	$C_{10}H_12O_2$	164
26.	Styrene	C_8H_8	104
27..	Butanedioic acid, phenyl	$C_{10}H_{10}O_4$	194
28.	m - xylene AND p - xylene	C_8H_{10}	106
29.	(E)-2-Methyl-1,3-diphenyl-2-propen-1-amine	$C_{16}H_{17}N$	223
30.	Bicyclo[4.2.0]octa-2,4-diene	C_8H_{10}	106
31.	trans-2-Acetyl-1-benzyl-3-phenylaziridine	$C_{17}H_{17}NO$	251

(Continued)

S.NO.	Compound Name	Formula	M W
32.	Benzene, ethyl- (CAS)	C_8H_{10}	106
33.	cis-(2R,3S)-2,3-Epoxy-1-phenyl-4-pentene	$C_{11}H_{12}O$	160
34.	1-Methylenespiro[2.4]hept-4-ene	C_8H_{10}	106
35.	tetracyclo[5.1.0(1,6),0(2,7)]octane	C_8H_{10}	106
36.	ethyl 2-acetyl-6-cyclopropylidenehexanoate	$C_{13}H_{20}O_4$	240
37.	6-[(N-Benzylimino-N'-benzylamino) methyl] benzimidazo[1,2-a]benzimidazole	$C_{28}H_{23}N_5$	429
38.	Dimethyl ester of pentylurofuranoic acid isomer	$C_{16}H_{26}O_5$	298
39.	2,3-dicarbomethoxybenzoselenophene	$C_{12}H_{10}O_4$	298
40.	(4-Ethynylphenyl)diphenylmethoxymethane	$C_{22}H_{18}O$	298
41.	(1R*,4S*,7S*)-3,3-Dimethoxy-7-methoxy carbonyl-7-methyl-5-(2,5-diox acyclo pentyl) bicyclo[2.2.2]oct-5-en-2-one	$C_{16}H_{22}O_7$	326
42.	Aziridine, 2-phenyl	C_8H_9N	119
43.	2,2-Dimethoxy-3-oxacyclopentan-1-one	$C_6H_{10}O_4$	146
44.	(E)-3-(1H-Pyrrol-3-yl)prop-2-enenitrile	$C_7H_6N_2$	118
45.	1-Methylthio-7-isopropyl-4-anti-formyl-8,10,12,13-tetraoxa entacyclo [5.5.1.0(2,6). 0(3,11) .0(5,9)]tridecane	$C_{14}H_{18}O_5S$	298
46.	2-(Methylthio)-4-nonenoic isobutyl amide	$C_{14}H_{27}NOS$	257
47.	1-(1,3-Dithian-5-yl)-3-methyl-2-thiourea	$C_6H_{12}N2S_3$	208
48.	4,5-(1,4-Dithianediyl-2,3-dithio)-1,3-dithiol-2-one	$C_7H_6OS_6$	298
49.	Methyl 4-[3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]benzoate	$C_{17}H_{15}FN2O_2$	298
50.	3,6-Dichloromethyldurene	$C_{12}H_{16}Cl_2$	230

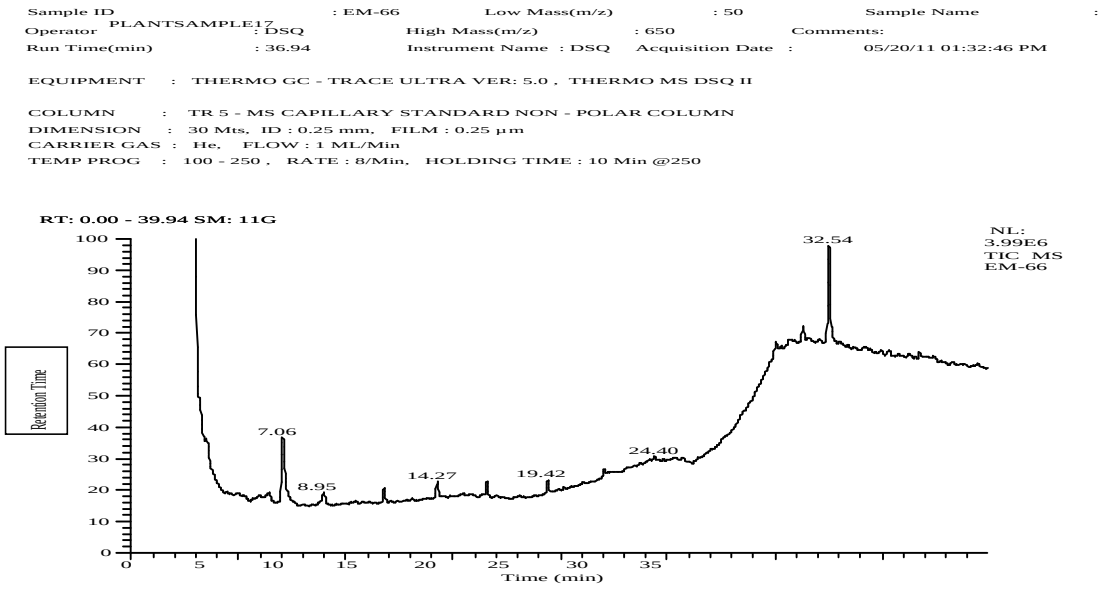


Fig. 3.29 GC-MS chromatogram of *in vivo* root of *D. elata*

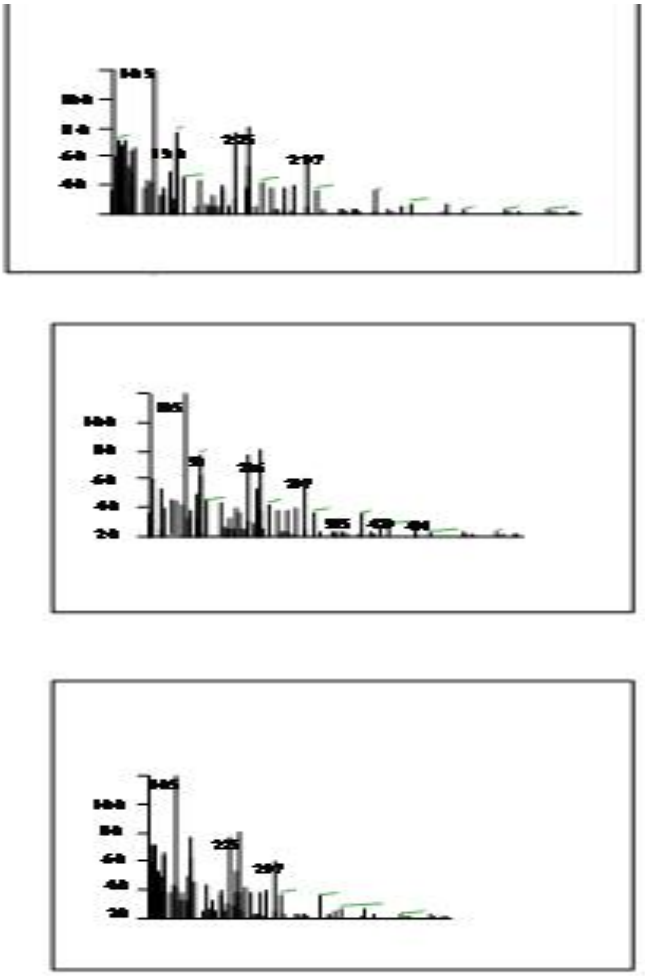


Figure3.30 GC-MS mass spectrum of *in vivo* root of *D.*

However, isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that *Delonix elata* contains various bioactive compounds, so it is recommended as a plant of phytopharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

Conclusions

The *Delonix elata* (Linn) has been subjected to Preliminary evaluation of macroscopic characters, Methanolic extraction by help of Soxhlet apparatus, and screened for phytoconstitents using GC-MS. Finally GC-MS analysed revealed that the methanolic extract of *Delonix elata* were mainly observed to contain Flavonoids and Terpenes eluting at different retention times with varied percentage of peak area. In the present study, 2 compounds (Leaf) and 2 compounds root have been identified from the methanol extract of leaf and root plant of *D. elata* by Gas Chromatography - Mass Spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to pharmacological activity will definitely give fruitful results.

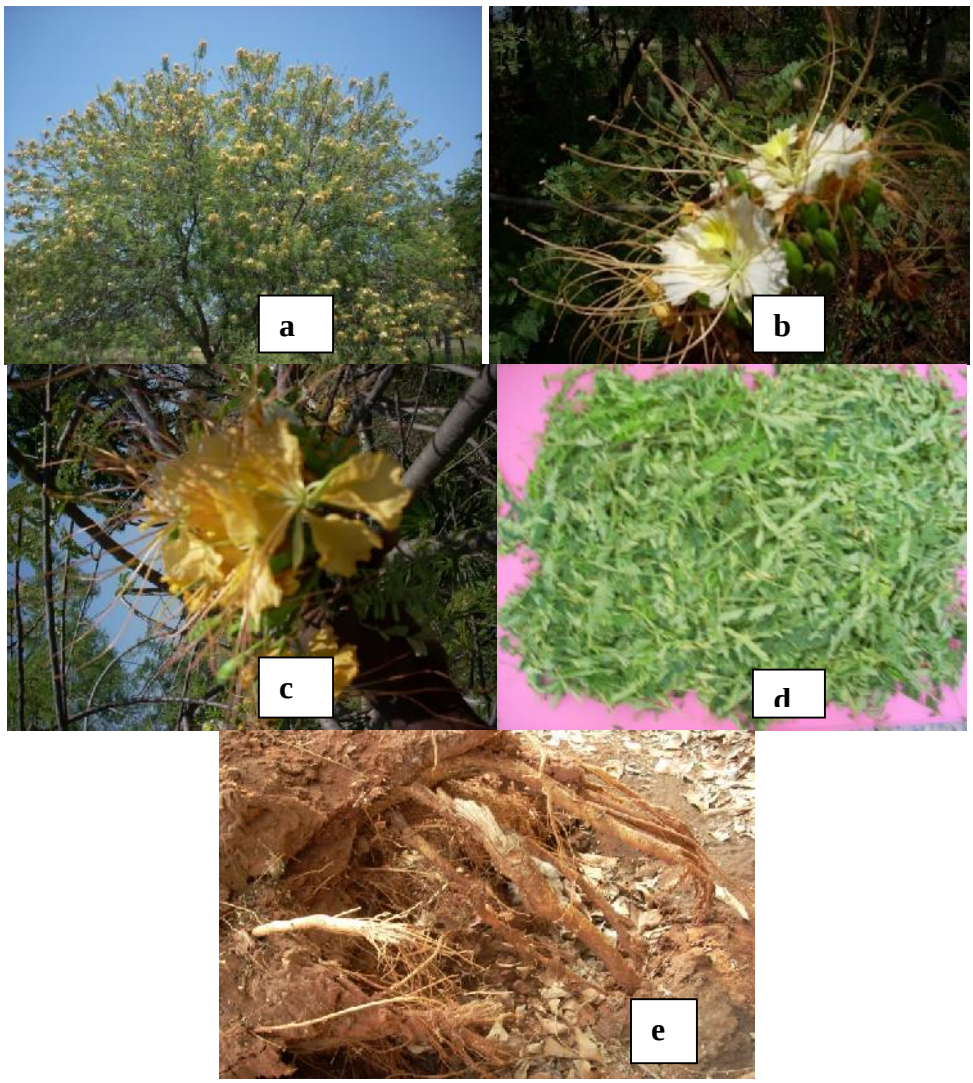
Table 3. Phytoconstituents present in methanolic extract of *Delonix elata* (L.) Gamble root sample using GC-MS

S.NO.	Compound Name	Formula	M W
1.	3-Benzyl-4-hydroxymethyl-2-oxazolidinone	$C_{11}H_{13}NO_3$	207
2.	1-formyl-7-(phenylmethoxy)indane	$C_{17}H_{16}O_2$	252
3.	5-Iodo-2-methyl-4-nitroimidazole	$C_4H_4IN_3O_2$	253
4.	Allylbenzyloxy-t-butylmethylsilane	$C_{15}H_{24}OSi$	248
5.	N-Phenylacetyl-N-methylglycine	$C_{11}H_{13}NO_3$	207
6.	(R)-N-Benzyl-3-hydroxyvaleramide	$C_{12}H_{17}NO_2$	207
7.	2,3-Diphenylpropanenitrile	$C_{15}H_{13}N$	207
8.	1-Benzyloxy-3-methoxymethoxymethyl-3,4-bismethoxy-methoxybutane	$C_{18}H_{30}O_7$	358
9.	Ethyl 4-Benzyloxy-2-[2-methyl-2(E)-butenyl]acetoacetate	$C_{18}H_{24}O_4$	304
10.	2-Ethoxycarbonyloxy-1-phenyl-2-nonene	$C_{18}H_{26}O_3$	290
11.	3,3-Diethoxypropylbenzyloxyamine	$C_{14}H_{23}NO_3$	253
12.	(2s,3s,4s)-5-(benzyloxy)-3,4-bis[(methoxymethyl)oxy]-1-(4-methoxyme thyl)-2-propanol	$C_{23}H_{32}O_7$	420
13.	1-O-Benzyl-2-O-(t-butyldiphenylsilyl)-3,4-O-isopropylidenebutane-1,2,3,4-tetrol	$C_{30}H_{38}O_4Si$	490
14.	(2R,3S)-3-(Benzyloxy)tetrahydropyran-2-carbaldehyde	$C_{13}H_{16}O_3$	220
15.	4-Benzyloxy-1-(p-toluenesulfonyl)-1-butyne 314	$C_{18}H_{18}O_3S$	314
16.	Ethyl 3-Hydroxy-2-(1-benzyloxy-2,2-difluoro-1-vinyl)-2-butenoate 298	$C_{15}H_{16}F2O_4$	298
17.	Benzyl 6,6-diethoxycarbonyl-5-methyl-1,3-cyclohexadien-1-carboxylate	$C_{21}H_{24}O_6$	372
18.	2,3-Diphenylpropionitrile	$C_{15}H_{13}N$	207
19.	4-(2-Phenylethyl)benzonitrile	$C_{15}H_{13}N$	207
20.	2-(tert-Butyldimethylsilyloxy)-4,5-dimethoxybenzyl alcohol	$C_{15}H_{26}O_3Si$	282
21.	4-tert-Butyl-6-(4-chlorophenyl)-2-ethyl-4H-1,4-oxaphosphorin-4-oxide	$C_{16}H_{20}ClO_2P$	310
22.	Ethyl 2-[N-di(ethoxycarbonyl) (methoxy) methyl] amino-4-nitrobenzoate	$C_{16}H_20N_2O_9$	384

(Continued)

S.NO.	Compound Name	Formula	M W
23.	2-[3'-(t-Butyldimethyldimethylsilyloxy)propyl]-1,4-benzoquinone	$C_{15}H_{24}O_3Si$	280
24.	(-)-(1S,2R,3S)-4,4-Dimethyl-1,2-epoxy-1,3-diphenylpentan-3-ol	$C_{19}H_{22}O_2$	282
25.	n-Butyl 2-benzoyloxymethylbenzoate	$C_{19}H_{20}O_4$	312
25.	2,3-cis-2-Acetoxy-3-bromoflavan	$C_{17}H_{15}BrO_3$	346
26.	4-Chloro-4-deuterio-1-phenyl-3-heptanone	$C_13H_{16}DClO$	224
27.	6,6,7,7-tetramethoxy-1,3-dipropylbicyclo[3.2.0]hept-3-en-2-one	$C_{17}H_28O_5$	312
28.	2-Methoxycarbonylbenzo[g]indolizine	$C_{14}H_{11}NO_2$	225
29.	16-Azatricyclo[9.2.2.1(4,8])hexadeca-4,6,8 (16),11,13,14-hexaene,16-oxide	$C_{15}H_{15}NO$	225
30.	2-Cyclopropyl-1-[6-(2-methyl-1,3-dithian-2-yl)cyclohexa-2,4-dien-1-yl]ethanone	$C_{16}H_{22}OS_2$	294
31.	Methyl (1R,3S)-3-Hydroxy-1-phenylpentane-1-sulfonate	$C_{12}H_{18}O_4S$	258
32.	6-[N-(4-Methylphenyl)imino-N'-(4-methylphenyl)aminomethyl] benzim idazo[1,2-a] benzimidazole	$C_{28}H_{23}N_5$	429
33.	Methyl 4-[2'-(3'',6''-Dioxocyclohexa-1'',4''-dienyl)ethenyl]acrylate	$C_{10}H_8O_4$	192
34.	Phenyl 2,6-dimethylbenzoate	$C_{15}H_{14}O_2$	226
35.	4-Phenyl-4-oxo-3-tosylbutanal	$C_{17}H_16O_4S$	316
36.	1,2-Difluoro-3,3-dimethylbut-1-ene	$C_6H_{10}F_2$	120
37.	bis[1,2,3-tri(t-Butyl)-2-cyclopropen-1-yl] ketone	$C_{31}H_{54}O$	442
38.	1H-Indene	C_9H_8	116
39.	1-Naphthalenol, methylcarbamate (CAS)	$C_{12}H_{11}NO_2$	201
40.	,2,3,4,5,6,7-hexafluoro-9,16-methylene-tetracyclo[6.8.0.0(2,7).0(3,6)]hexadeca-4-ene	$C_{17}H_{12}F_6$	330
41.	1H-Indene, 1-chloro-2,3-dihydro	C_9H_9Cl	152
42.	Benzene, 1-ethynyl-4-methyl	C_9H_8	116
43.	N-Succinimidyl 2,3-dimethylbut-2-enedioate - monoester	$C_{10}H_{11}NO_6$	241
44.	(2'-Nitro-2'-propenyl)benzene	$C_9H_9NO_2$	163
45.	3-(Acetoxy)-4-methyl-2-pentanone	$C_8H_{14}O_3$	158

Fig a- e. *Delonix elata* (L.) Gamble. **a.** Habit, **b.**young flower, **c.** mature flower, **d.** leaf and **e.** root



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