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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2016, Vol. 32, No. (3):
Pg. 1329-1346

In *vitro* Antibacterial, Antifungal and Phytochemical Analysis of Methanolic Extract of Fruit *Cassia fistula*

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http://dx.doi.org/10.13005/ojc/320307

(Received: April 02, 2016; Accepted: May 05, 2016)

ABSTRACT

The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, MS Fragment- ions and Pharmacological actions. GC-MS analysis of *Cassia fistula* revealed the presence of the Oxacyclododecan-2-one, Imidazole ,2-amino-5-[(2-carboxy)vinyl], D-Glucose , 6-O-á-D-galactopyranosyl, 2-Nonanone, Eicosanoic acid , phenylmethyl ester, Phenol , 4-(2-propenyl), Eugenol, Caryophyllene, ß-copaene, Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl), á-acorenol, Spiro[5.5] undec-8-en-1-one, Isoaromadendrene epoxide, Tetraacetyl-d-xylonic nitrile , Benzyl Benzoate, N-Isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide, Phenethylamine , 3-benzyloxy-2-fluoro-ß-hydroxy, 4a-Hydroxy-4-nitroperhydronaphthalen-1-one, Dasycarpidan -1-methanol, acetate (ester), Propanoic acid , 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl), Carda-4,20(22)-dienolide,3-[(6-deoxy-3-O-methyl-á-L-mannopyranosyl, Cis-13-Eicosenoic acid , 16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one, Strychane ,1-acetyl-20á-hydroxy-16-methylene, 2,4,6-Decatrienoic acid , 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydro, Vitamin E and Glycine ,N-[(3á,5ß,12á)-3,12-dihydroxy-24-oxocholan-24-yl]. The FTIR analysis of *Cassia fistula* leaves proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters, Nitro Compounds, Alkanes, Alcohols and Phenol. *Cassia fistula* was highly active against *Aspergillus terreus* (6.99±0.29). Methanolic extract of bioactive compounds of *Cassia fistula* was assayed for *in vitro* antibacterial activity against eleven pathogenic bacteria by using the diffusion method in agar. The zone of inhibition were compared with different standard antibiotics. The diameters of inhibition zones ranged from 1.00±0.05 to 6.02±0.23 mm for all treatments.

Keywords: GC/MS, Bioactive compounds, FT-IR, *Cassia fistula*.

INTRODUCTION

Cassia fistula plant have naturally occurring bioactive compounds and are mostly secondary metabolites which are now a days being used as medicines, dietary supplements and other useful commercial products[1]. Also, has been reported to contain anthraquinone the principal laxative constituent of many plants used as purgative[2]. Plants have been an important source of medicine

with qualities for thousands of years[3,4]. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines[5,6]. Since thousands of years back, plants are used as a major source for medicine as they found to possess a reservoir of bioactive compound[7-9]. *Cassia fistula* contains alkaloids, tannins, flavonoids,terpenes, sugars, and glucosides. Tannins are naturally occurring and water soluble phenolic compounds, which precipitate proteins from aqueous media[10]. *Cassia fistula* shows the presence of glycoside a natural product, which is used to enhance the cardiac contractile force in patient with congestive heart failure[11] glycoside also plays major role in the cancer therapy[12]. They also play major role in controlling topical disease as eczema etc. Many metabolites have found to possess interesting biological activities such as bactericidal, fungicidal, hepatoprotective and muscle relaxant[13]. A fruit is cylindrical pod and seeds many in black, sweet pulp separated by transverse partitions. The long pods which are green, when unripe, turn black on ripening after flowers shed[14]. Pulp is dark brown in colour, sticky, sweet and mucilaginous, odour characteristic, and somewhat disagreeable[15]. Each compartment contains one seed which is flat, oval, reddish brown with a well-marked raphe. The seed contains a whitish endosperm in which the yellowish embryo is embedded[16]. Fruits are used in the treatment of diabetes, antipyretic, abortifacient, demulcent, lessens inflammation and heat of the body; useful in chest complaints, throat troubles, liver complaints, diseases of eye and gripping[17]. The aim of this research was study the phytochemical composition of *Cassia fistula* and to evaluate the isolates for possible in vitro antifungal and antibacterial activities.

MATERIALS AND METHODS

Solvent extraction

The shade dried powdered of *Cassia fistula* was extracted with methanol. The extract was filtered with Whatman's filter paper. Filtrate was concentrated under reduced pressure and preserved at 5Cº in dark air tight bottles[18,19].

Sample preparation for GC-MS analysis

50 µl of sample (*Cassia fistula*) was dissolved in 2ml of methanol and kept in ultrasonic bath for 25 min and centrifuged for 10 min. at 6000 rpm and supernatant was injected in GC-MS for analysis[20,21].

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS of methanol extract was performed using Agilent 7890A. The run time was 30 minutes. ionization of sample components were performed on EI mode (70 eV). The carrier gas was helium at 1.0ml/min flow rate. 0.5 ml of sample was injected in split mode of 20:1.The mass spectrum scan range was set at 29.0 to 500(m/z) [22].

Identification of compounds

Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST). The mass spectrum of phytochemicals was compared with the spectrum of known compounds stored in the NIST library[23].

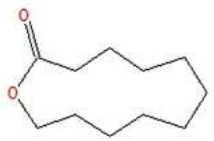
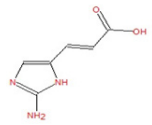
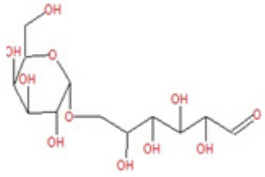
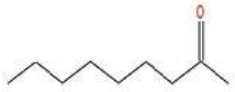
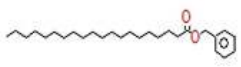
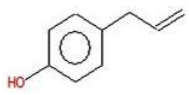
Determination of antibacterial activity of crude bioactive compounds of *Cassia fistula*

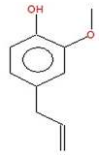
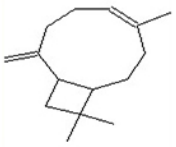
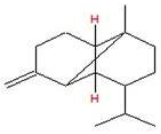
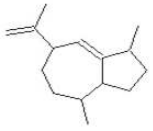
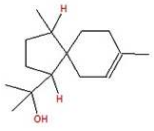
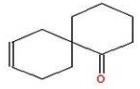
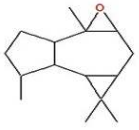
The anti-bacterial activity was evaluated using Mueller-Hinton agar. The bacterial plates were incubated at 37 °C for 24 h. After incubation, the diameter of the inhibition zone was measured to evaluate the antimicrobial activity. Each test was performed twice and the average of the results was calculated. The extraction solvents were used as negative control[24,25]. The test pathogens were swabbed in Muller Hinton agar plates. 60ìl of plant extract was loaded on the bored wells. The wells were bored in 0.5 cm in diameter. The plates were incubated at 37C° for 24 h and examined. After the incubation the diameter of inhibition zones around the discs was measured[26].

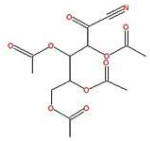
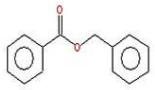
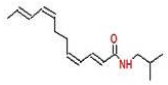
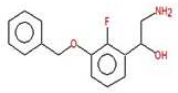
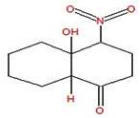
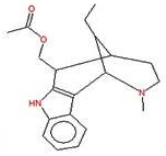
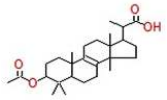
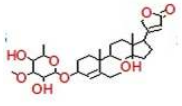
Determination of antifungal activity

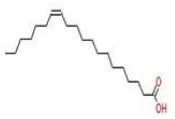
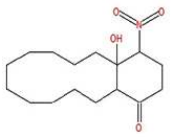
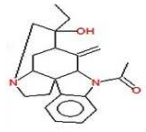
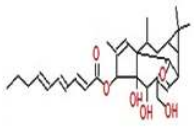
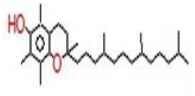
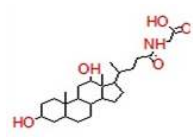
Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 ìl of the samples solutions *Cassia fistula* was delivered into the wells. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation[27,28].

Table 1: Major phytochemical compounds identified in methanolic extract of Cassia fistula

S. No.	Phytochemical compound	RT (min)	Formula	Molecular Weight	Exact Mass	Chemical structure	MS Fragment- ions	Pharmacological actions
1	Oxacyclododecan-2-one	3.367	$C_{11}H_{20}O_2$	184	184.146		55,69,84,98	Biological activities such as antitumor, anti-inflammatory and antiplatelet
2	Imidazole ,2-amino-5-[(2-carboxy)vinyl]-	3.613	$C_6H_7N_3O_2$	153	153.054		55,69,109,135	Antimicrobial and anti-inflammatory activity
3	D-Glucose , 6-O-±-D-galactopyranosyl-	3.751	$C_{12}H_{22}O_{11}$	342	342.116		60,73,85,110, 126,182,212,261	Anti-inflammatory activities
4	2-Nonanone	4.878	$C_9H_{18}O$	142	142.136		58,67,71,85,99, 113,127,142	Anti-bacterial activity
5	Eicosanoic acid , phenylmethyl ester	5.833	$C_{27}H_{46}O_2$	402	402.35		57,71,85,91,97, 108,126,147,167,207	Anti-inflammatory activity
6	Phenol , 4-(2-propenyl)-	7.344	$C_9H_{10}O$	134	134.073		51,65,77,91,107,115,134	Antioxidant, anti microbial and anti inflammatory

7	Eugenol	8.769	$C_{10}H_{12}O_2$	164	164.084		51,55,65,77,91,103,115, 121,131,137,149,164	Anti-insect activity
8	Caryophyllene	9.644	$C_{15}H_{24}$	204	204.188		55,69,79,93,105,120,133, 147,161,175,189,204	Anti-inflammatory activity
9	ß-copaene	10.606	$C_{15}H_{24}$	204	204.188		55,67,79,91,105,119, 133,147,161,189,204	Anti-inflammatory, antibiotic, antioxidant, anticarc inogenic and local anaesthetic
10	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-	10.411	$C_{15}H_{24}$	204	204.188		55,81,91,107,121,133, 161,175,189,204	Anti-Candida
11	±-acorenol	10.331	$C_{15}H_{26}O$	222	222.198		59,81,93,105,119,147, 161,175,189,204,222	Antioxidant and anti-inflammatory activities
12	Spiro[5.5]undec-8-en-1-one	11.183	$C_{11}H_{16}O$	164	164.12		55,67,79,91,120,135,164	Antibacterial agents, anticonvulsant agents and anti- tuberculosis
13	Isoaromadendrene epoxide	12.253	$C_{15}H_{24}O$	220	220.183		55,67,93,107,135,149, 162,220	Biological activities including antimicrobial, antioxidant and anti-inflammatory

14	Tetraacetyl-d-xylonic nitrile	12.797	$C_{14}H_{19}NO_9$	343	343.09		60,73,112,133,197,223, 251,281	Anti-viral effects
15	Benzyl Benzoate	13.346	$C_{14}H_{12}O_2$	212	212.084		51,65,77,91,105,167, 194,212	Anti-inflammatory
16	N-Isobutyl-(2E,4Z,8Z,10E) -dodecatetraenamide	14.228	$C_{16}H_{25}NO$	247	247.194		57,66,81,95,115, 152,167,247	Analgesic, anti-inflammatory
17	Phenethylamine , 3-benzyloxy-2-fluoro- ß-hydroxy-	14.416	$C_{15}H_{16}FNO_2$	261	261.117		65,77,91,105,15,166,243	Unknown
18	4a-Hydroxy-4-nitroperhydronaphthalen -1-one	15.468	$C_{10}H_{15}NO_4$	213	213.1		55,67,98,125,149167,213	Unknown
19	Dasycarpidan -1-methanol, acetate (ester)	15.87		326	326.199		60,69,83,97,111,124,167, 180,222,256,284,326	Unknown
20	Propanoic acid , 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	16.745	$C_{27}H_{42}O_4$	430	430.308		55,69,121,159,187,213, 233,281,337,355,415	Anti-microbial and anti-tumor
21	Carda-4,20(22)-dienolide, 3-[(6-deoxy-3-O-methyl -±-L-mannopyranosyl	18.473	$C_{30}H_{44}O_8$	532	532.304		56,74,91,105,145,179,201, 231,245,275,321,336,354, 368,384,446	Anti-inflammatory activity

22	Cis-13-Eicosenoic acid	18.611	$C_{20}H_{38}O_2$	310	310.287		55,69,83,97,125,138,208, 249,292,310	Anti-inflammatory activity
23	16-Nitrobicyclo[10.4.0] hexadecane-1-ol-13-one	19.08	$C_{16}H_{27}NO_4$	297	297.194		55,69,81,98,126,158, 173,209,267,297	Anti-inflammatory and antiviral agents
24	Strychane ,1-acetyl-20 ±-hydroxy-16-methylene-	19.846		338	338.199		57,70,88,130,166,239, 281,338	New chemical compound
25	2,4,6-Decatrienoic acid , 1a,2,5,5a,6,9,10, 10a-octahydro-5,5a-dihydro	21.929	$C_{30}H_{40}O_6$	496	496.282		55,79,91,122,149,284, 312,330,380,413,465	Anti-Candida
26	Vitamin E	26.484	$C_{29}H_{50}O_2$	430	430.381		57,71,91,121,165,205, 246,275,303,344,372,430	Anti-inflammatory activities and their role in disease prevention and therapy
27	Glycine ,N-[(3±,5ß,12±) -3,12-dihydroxy-24 -oxocholan-24-yl]-	28.893	$C_{26}H_{43}NO_5$	449	449.314		55,67,81,117,147,238,255, 273,314,356,398,413,431, 447	Unknown

Statistical analysis

Results of the study were based on analysis of variance (ANOVA) and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Identification of phytochemical compounds

Plants have been a common source of medicinal property, either in the form of pure active

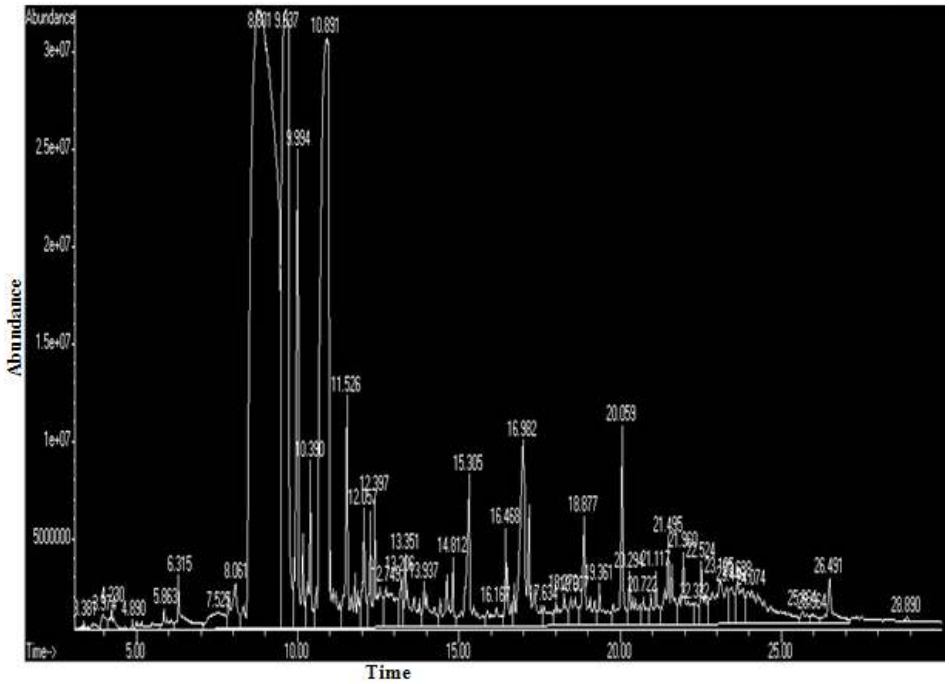


Fig. 1: GC-MS chromatogram of *Cassia fistula*

Table 2: FT-IR peak values of *Cassia fistula*

No.	Peak (Wave number cm^{-1})	Intensity	Bond	Functional group assignment	Group frequency
1	675.09	57.314	C-H	Alkenes	675-995
2	904.61	72.826	C-H	Alkenes	675-995
3	921.97	72.347	C-H	Alkenes	675-995
4	1014.56	51.417	C-F stretch	Aliphatic fluoro compounds	1000-10150
5	1072.42	57.43	C-F stretch	Aliphatic fluoro compounds	1000-10150
6	1242.16	72.837	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
7	1317.38	74.466	NO2	Nitro Compounds	1300-1370
8	1361.74	72.514	NO2	Nitro Compounds	1300-1370
9	1373.32	72.15	-	Unknown	-
10	1595.13	69.134	-	Unknown	-
11	1732.08	83760	-	Unknown	-
12	2752.42	90.502	-	Unknown	-
13	2848.86	77.274	-	Unknown	-
14	2918.3	72.553	C-H	Alkanes	2850-2970
15	3277.06	76.261	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600

compound or as traditional preparations and it are reasonable to use local plants[29]. Identifying on the plant phytochemistry provides a fundamental use of plants as storage of chemical agents in the field of medicine[13]. The phytochemical analyses of plant from *Cassia fistula* was studied extensively. Identifying the importance of secondary metabolites in the field of medicine, the presence of tannins, phlobatanins, saponins, flavonoids, terpenoids, glycosides and steroids was detected. Folklore medicine are widely used in our ancient period[13]. Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic leaves extract of *Cassia fistula*, shown in **Table 1.** The GC-MS chromatogram of the 27 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Cassia fistula* showed the presence of twenty seven major peaks and the components corresponding to the peaks were determined as follows. The first set up peak were determined to be 1,7-Dioxaspiro[5,5] undec-2-ene **Figure 2**. The second peak indicated to be 2,4-Dihydroxy-2,5-dimethyl-39(2H)-furan-3-one **Figure 3**. The next peaks considered to be á-D-Glucopyranoside , O-á-D-glucopyranosyl-(1. fwdarw.3)-ß-D-fruc, d-Mannose, 5,7-Dodecadiyn -1,12-diol, 3-Trifluoroacetoxypentadecane, 3-Trifluoroacetoxypentadecane, Pterin-6-carboxylic acid, Imidazole-4-carboxylic acid ,2-fluoro-1-methoxymethyl-,ethyl ester, D-Carvone, Pyrrolizin-

Table 3: Antimicrobial activity of *Cassia fistula*

Microbe	Plant	Cefotoxime	Streptomycin	Amphotericin B	Fluconazol
Streptococcus pneumonia	4.19±0.15	1.00±0.07	2.00±0.11	-	-
Pseudomonas eurogenosa	5.73±0.20	1.30±0.09	1.07±0.06	-	-
Staphylococcus epidermidis	3.00±0.10	1.31±0.09	1.56±0.09	-	-
Salmonella typhi	4.15±0.14	1.01±0.08	1.22±0.08	-	-
Bacillus subtilis	4.00±0.12	1.49±0.10	1.00±0.04	-	-
Escherichia coli	4.83±0.19	2.03±0.11	2.08±0.10	-	-
Proteus mirabilis	6.02±0.23	1.90±0.09	1.00±0.05	-	-
Streptococcus pyogenes	3.04±0.11	1.16±0.09	1.82±0.05	-	-
Staphylococcus aureus	5.20±0.20	2.00±0.10	2.11±0.12	-	-
Streptococcus faecalis	4.65±0.20	1.58±0.08	1.00±0.05	-	-
Klebsiella pneumonia	5.23±0.23	1.27±0.07	1.95±0.09	-	-
Fungi/ Yeast					
Aspergillus niger	5.89±0.22	-	-	1.94±0.08	2.11±0.19
Aspergillus terreus	6.99±0.29	-	-	4.00±0.16	1.95±0.18
Aspergillus flavus	5.96±0.28	-	-	2.00±0.20	3.00±0.22
Aspergillus fumigatus	6.00±0.29	-	-	2.06±0.09	2.85±0.17
Candida albicans	5.25±0.20	-	-	2.00±0.19	2.00.±0.11
Saccharomyces cerevisiae	3.92±0.18	-	-	1.89±0.09	2.90±0.19
Fusarium sp.	5.01±0.24	-	-	2.99±0.10	3.00±0.21
Microsporum canis	4.00±0.19	-	-	3.31±0.19	1.97±0.10
Streptococcus faecalis	3.80±0.27	-	-	3.73±0.14	2.60±0.20
Mucor sp.	4.00±0.19	-	-	2.00±0.17	1.99±0.18
Penicillium expansum	4.12±0.19	-	-	2.61±0.19	2.60±0.17
Trichoderma viride	5.08±0.23			1.99±0.15	2.30±0.18
Trichoderma horzianum	3.95±0.17			0.94±0.01	2.99±0.19
Trichophyton mentagrophytes	4.00±0.19			2.71±0.14	1.00±0.14

[a] The values (average of triplicate) are diameter of zone of inhibition at 100 mg/mL crude extract, 30 µg/mL of antibiotics (Streptomycin; Rifambin; Kanamycin; Cefotoxime and chloramphenicol).

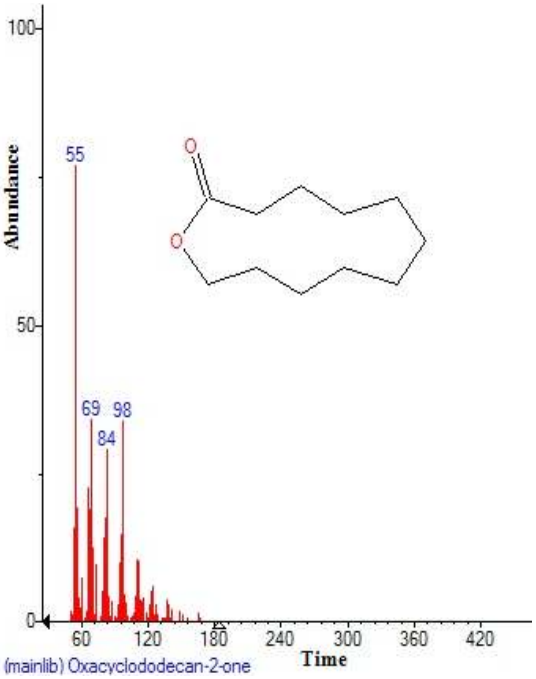


Fig. 2: Structure of Oxacyclododecan-2-one with RT: 3.367 present in *Cassia fistula*

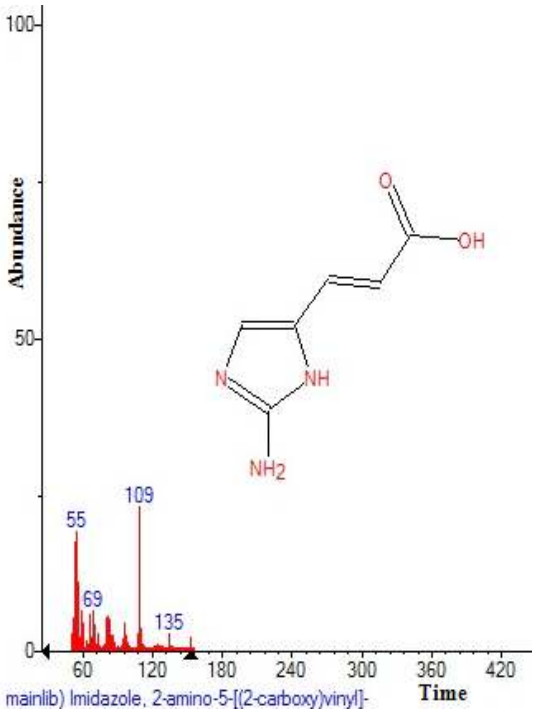


Fig. 3: Structure of Imidazole ,2-amino-5-[(2-carboxy)vinyl] with RT: 3.613 present in *Cassia fistula*

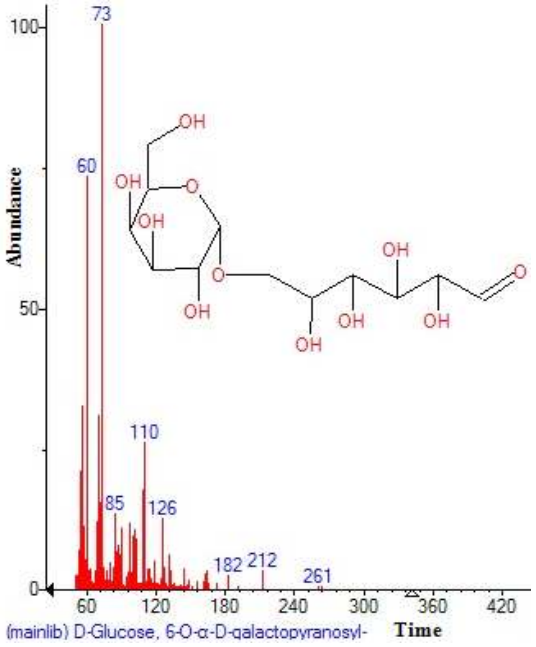


Fig. 4: Structure of D-Glucose , 6-O-α-D-galactopyranosyl with RT: 3.751 present in *Cassia fistula*

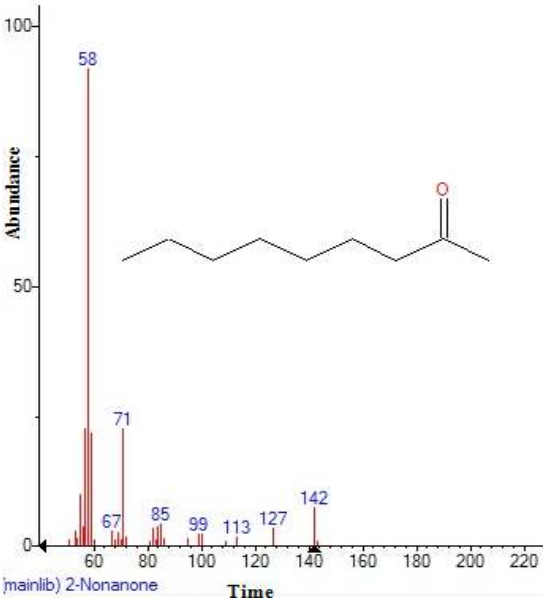


Fig. 5: Structure of 2-Nonanone with RT: 4.878 present in *Cassia fistula*

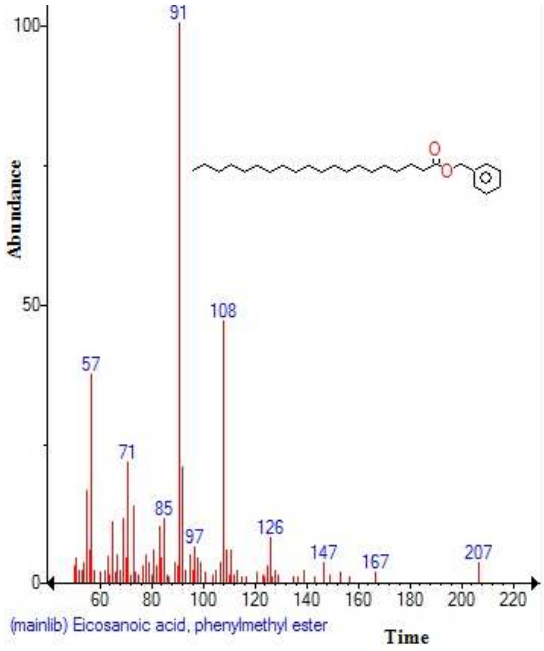


Fig. 6: Structure of Eicosanoic acid , phenylmethyl ester with RT: 5.833 present in *Cassia fistula*

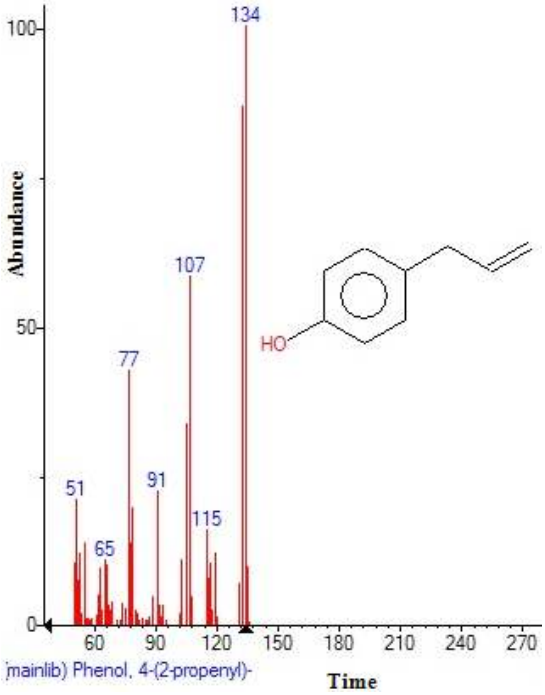


Fig. 7: Structure of Phenol , 4-(2-propenyl) with RT: 7.344 present in *Cassia fistula*

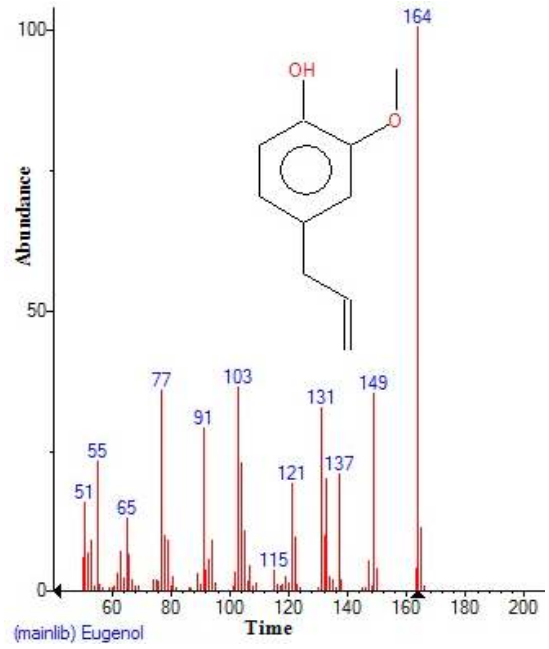


Fig. 8: Structure of Eugenol with RT: 8.769 present in *Cassia fistula*

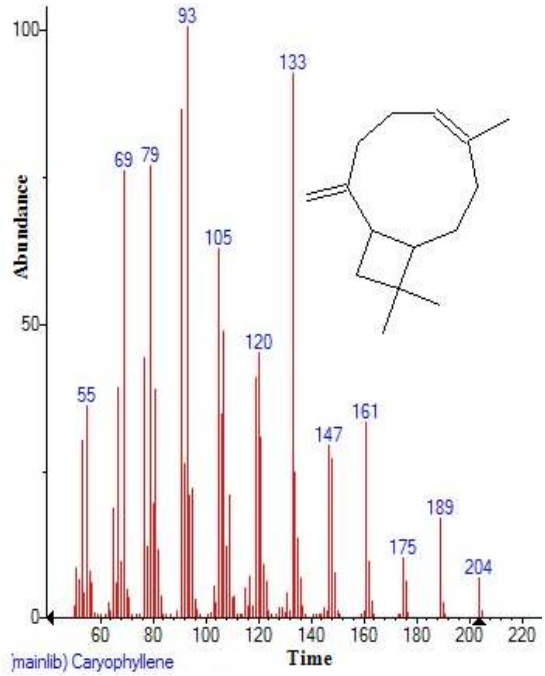


Fig. 9: Structure of Caryophyllene with RT: 9.644 present in *Cassia fistula*

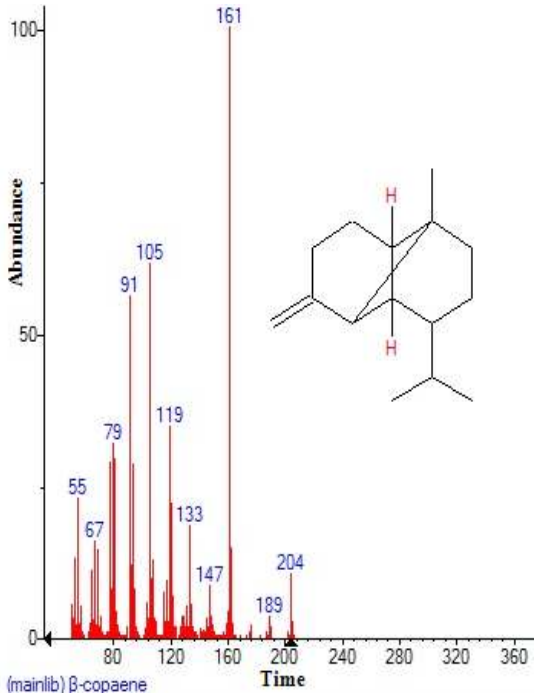


Fig. 10: Structure of ß-copaene with RT: 10.606 present in *Cassia fistula*

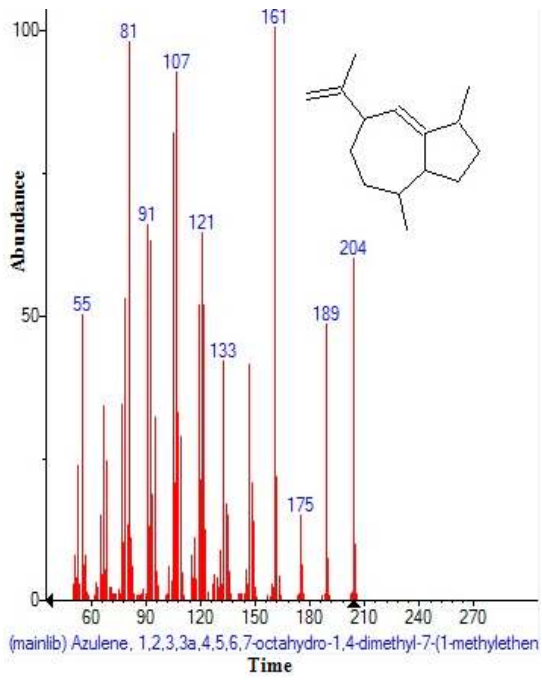


Fig. 11: Structure of Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl) with RT: 10.411 present in *Cassia fistula*

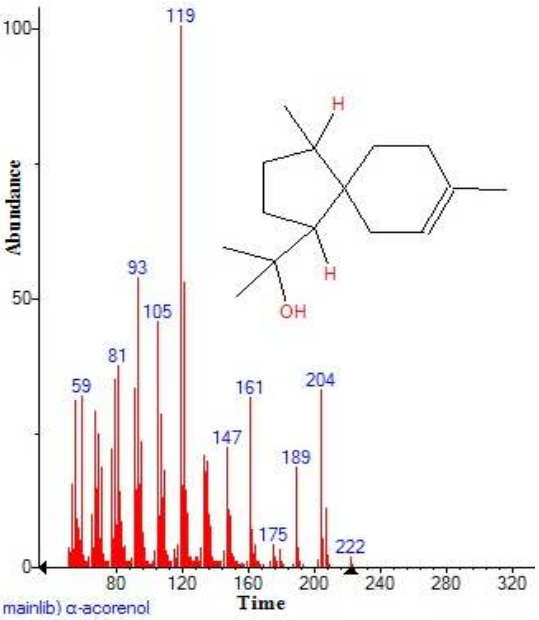


Fig. 12: Structure of á-acorenol with RT: 10.331 present in *Cassia fistula*

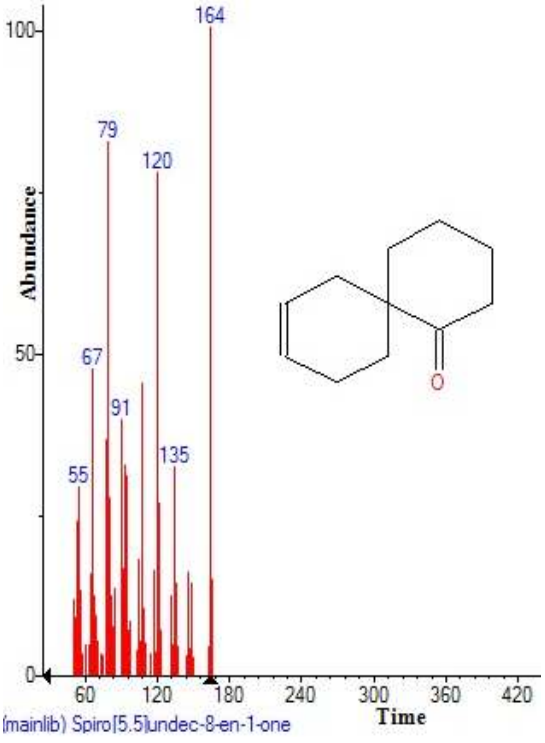


Fig. 13: Structure of Spiro[5.5]undec-8-en-1-one with RT: 11.183 present in *Cassia fistula*

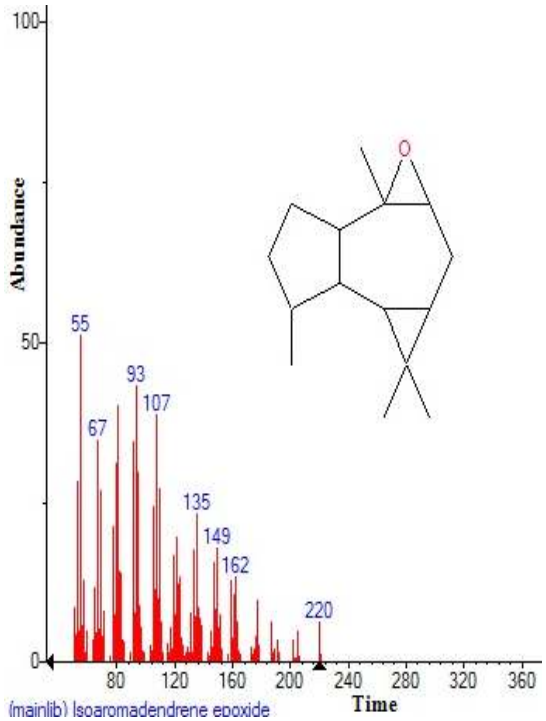


Fig. 14: Structure of Isoaromadendrene epoxide with RT: 12.253 present in *Cassia fistula*

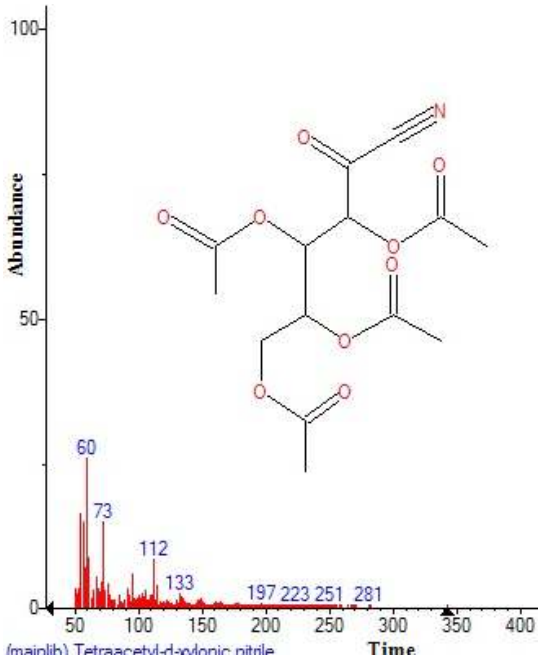


Fig. 15: Structure of Tetraacetyl-d-xylonic nitrile with RT: 12.797 present in *Cassia fistula*

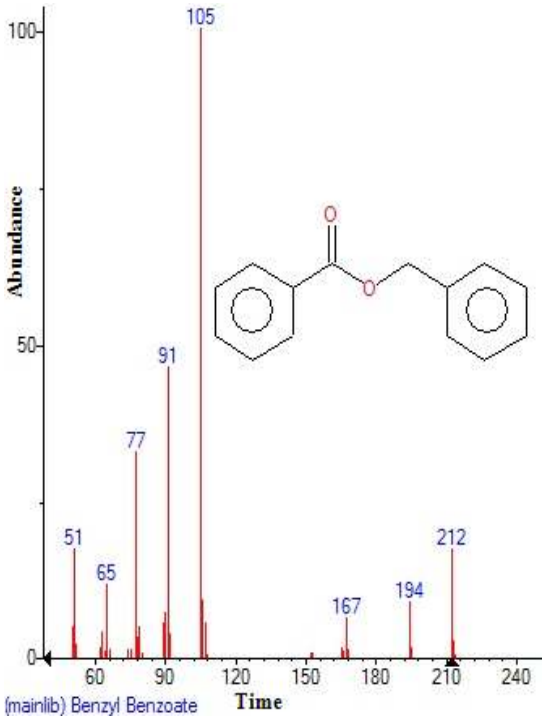


Fig. 16: Structure of Benzyl Benzoate with RT: 13.346 present in *Cassia fistula*

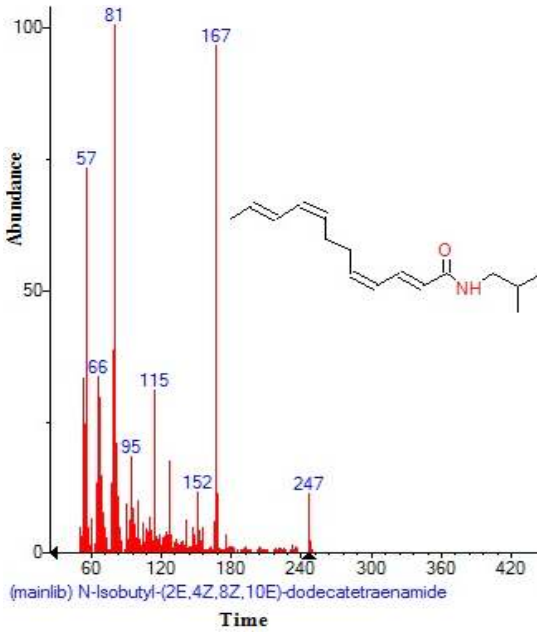


Fig. 17: Structure of N-Isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide with RT: 14.228 present in *Cassia fistula*

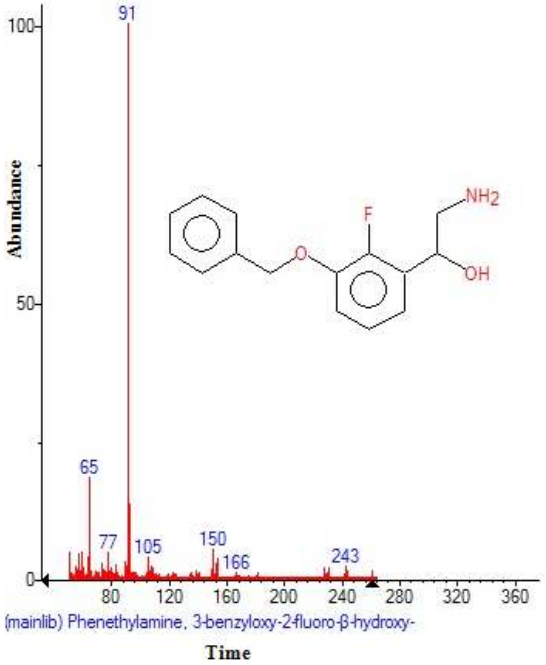


Fig. 18: Structure of Phenethylamine , 3-benzyloxy-2-fluoro-ß-hydroxy with RT: 14.416 present in *Cassia fistula*

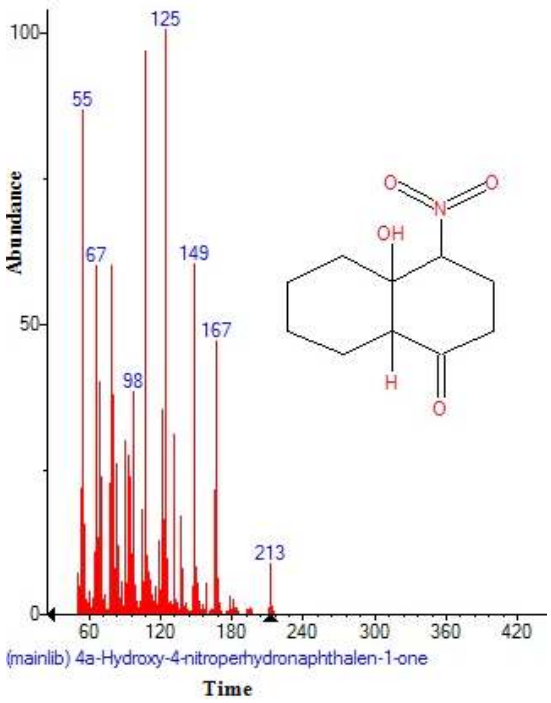


Fig. 19: Structure of 4a-Hydroxy-4-nitroperhydronaphthalen-1-one with RT: 15.468 present in *Cassia fistula*

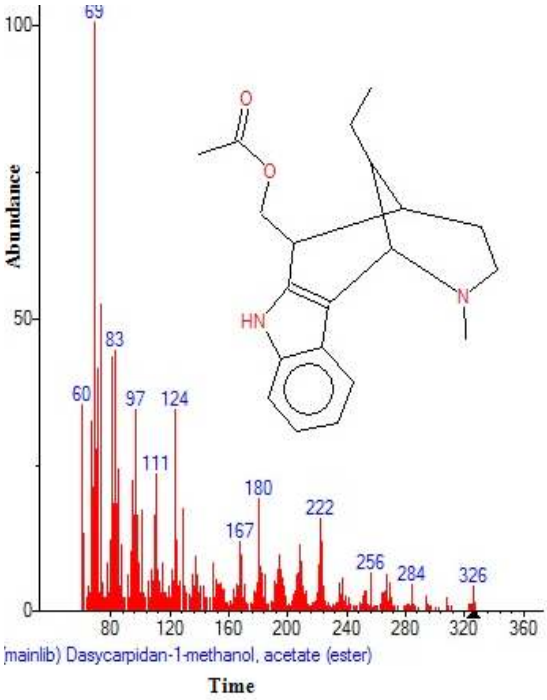


Fig. 20: Structure of Dasycarpidan -1-methanol, acetate (ester) with RT: 15.870 present in *Cassia fistula*

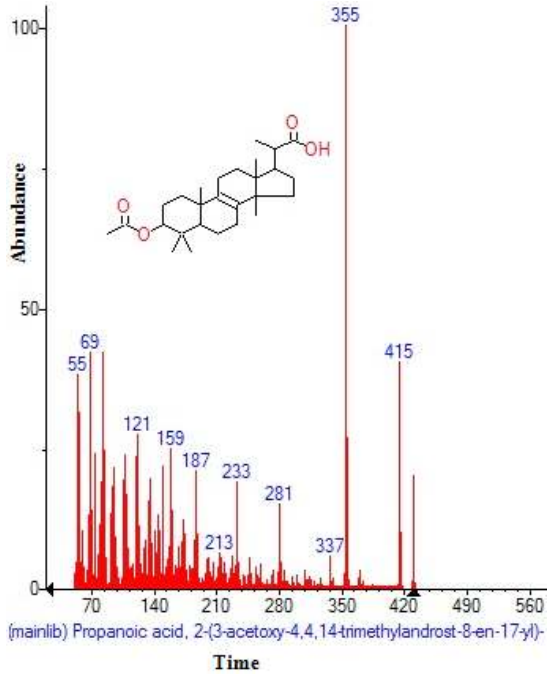


Fig. 21: Structure of Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) with RT: 16.745 present in *Cassia fistula*

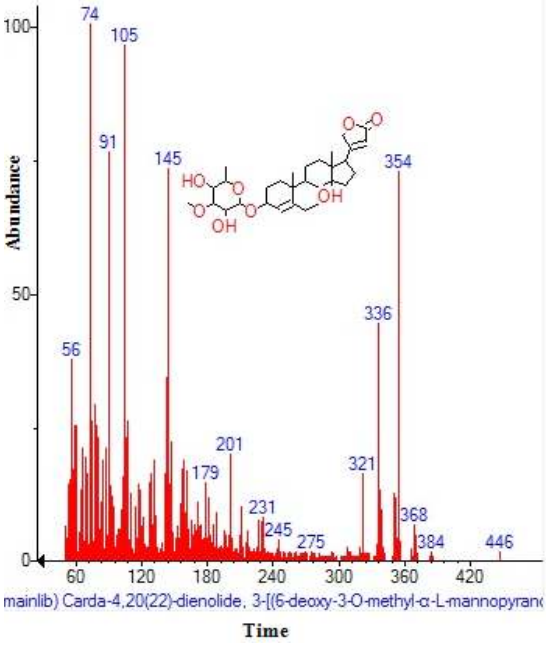


Fig. 22: Structure of Carda-4,20(22)-dienolide,3-[(6-deoxy-3-O-methyl-α-L-mannopyranosyl with RT: 18.473 present in *Cassia fistula*

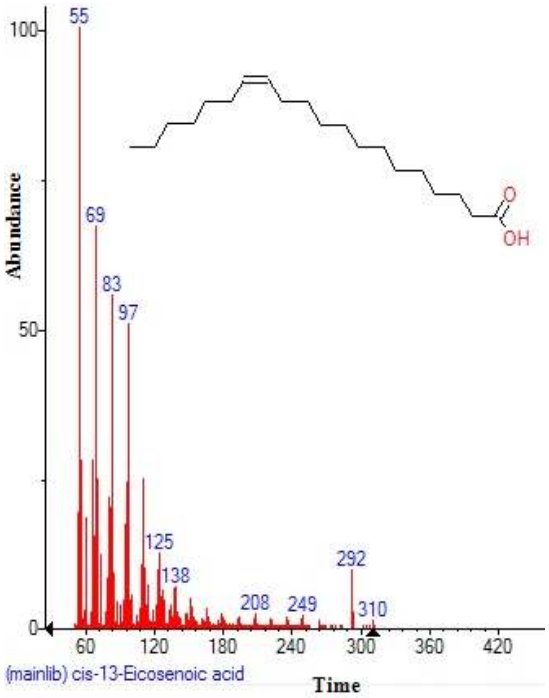


Fig. 23: Structure of Cis-13-Eicosenoic acid with RT: 18.611 present in *Cassia fistula*

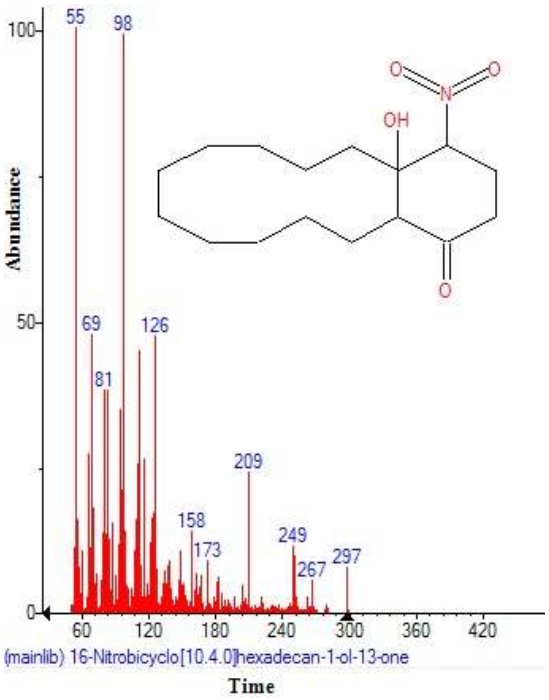


Fig. 24: Structure of 16-Nitrobicyclo[10.4.0] hexadecane-1-ol-13-one with RT: 19.080 present in *Cassia fistula*

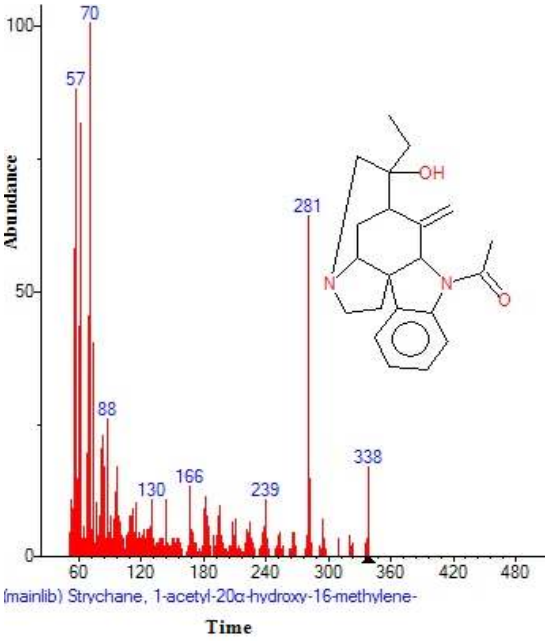


Fig. 25: Structure of Strychane ,1-acetyl-20α-hydroxy-16-methylene with RT: 19.846 present in *Cassia fistula*

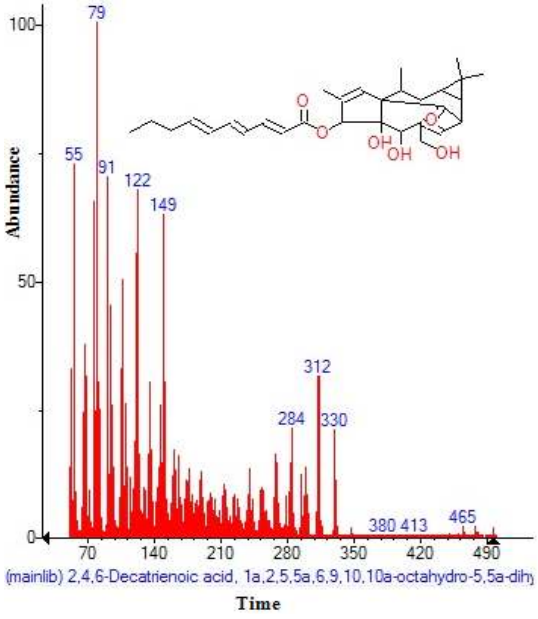


Fig. 26: Structure of 2,4,6-Decatrienoic acid , 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydro with RT: 21.929 present in *Cassia fistula*

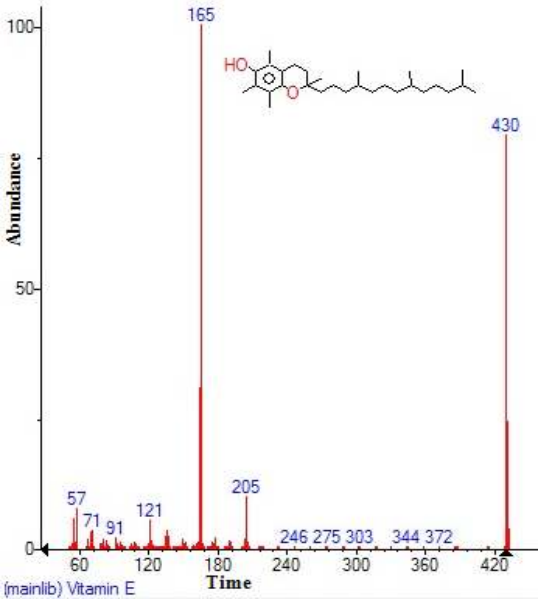


Fig. 27: Structure of Vitamin E with RT: 26.484 present in *Cassia fistula*

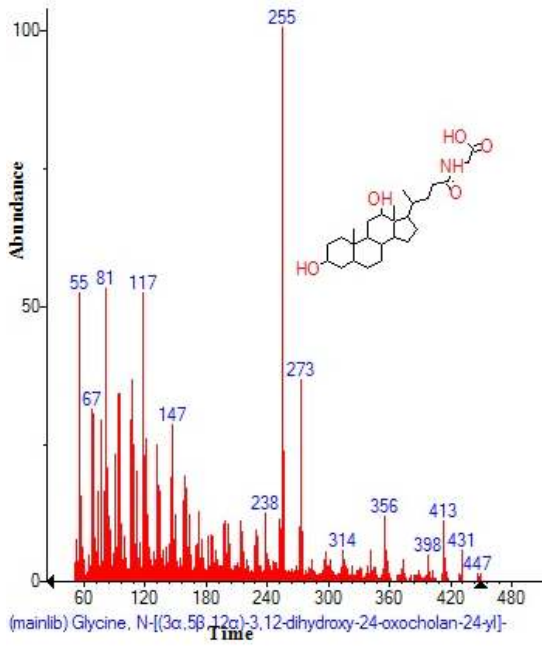


Fig. 28: Structure of Glycine ,N-[(3α,5ß,12α)-3,12-dihydroxy-24-oxocholan-24-yl] with RT: 28.893 present in *Cassia fistula*

1,7-dione-6-carboxylic acid , methyl (ester), D-Glucose ,6-O-á-D-galactopyranosyl, Estragole, Phenol,2-methyl-5-(1-methylethyl), 3-Allyl-6-methoxyphenol, Ppropiolic acid , 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl), 7-epi-trans-sesquisabinene hydrate, Tetraacetyl-d-xylonic nitrile, y-Sitosterol, Ergosta-5,22-dien-3-ol, acetate , (3ß,22E), Curan-17-oic acid ,2,16-didehydro-20-hydroxy-19-oxo,methyl ester, 9,10-Secocholesta -5,7,10(19)-triene-1,3-diol,25-[(trimethylsilyl) oxy], Cis-Vaccenic acid, L-Ascorbic acid , 6-octadecanoate, L-Ascorbic acid , 6-octadecanoate, Deoxyspergualin, Tributyl acetylcitrate, 10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2-carboxylic acid, 18,19-Secoyohimban-19-oic acid , 16,17,20,21-tetradehydro-16, 9-Octadecenamide ,(Z), Olean-12-ene-3,15,16,21,22,28,-hexol,(3ß,15á,16á,21ß,22á), (22S)-21-Acetoxy-6á,11ß-dihydroxy-16á,17ápropylmethylenedioxy, Ethyl iso-allocholate, Olean-12-ene-3,15,16,21,22,28-hexol,(3ß,15á,16á,21ß,22á) and Olean -13(18)-ene (**Figure 3-28**). The FTIR analysis of *Cassia fistula* leaves proved the presence of alkenes, aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters, nitro compounds, hydrogen bonded

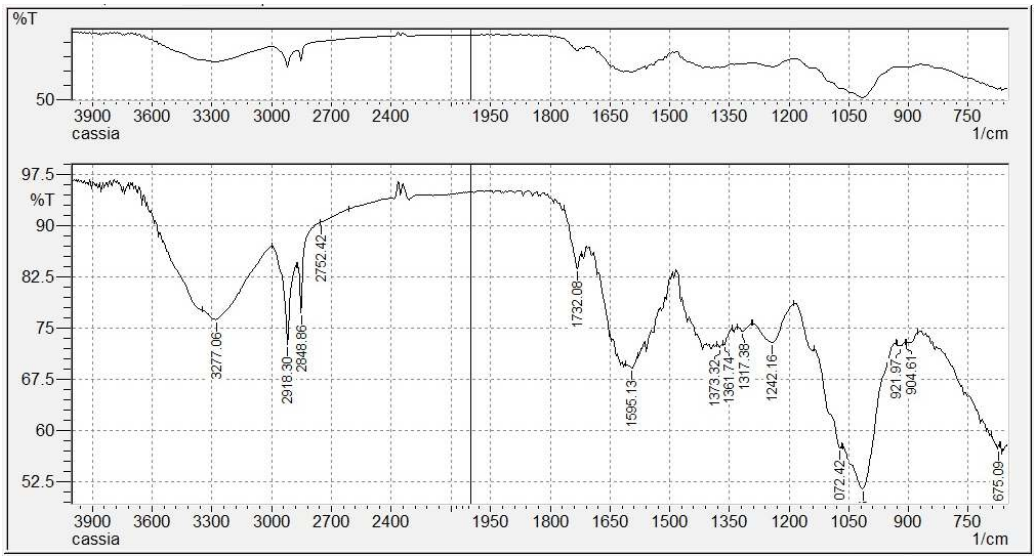


Fig. 29: FT-IR profile of *Cassia fistula*

alcohols and phenols which shows major peaks at 675.09, 904.61, 1014.56, 1072.42, 1242.16, 1317.38, 1361.74, 2918.30 and 3277.06 (**Table 2; Figure 29**). *Cassia fistula* Linn. (Cassia) family Caesalpiniaceae commonly known as Amulthus has been extensively used in Ayurvedic system of medicine for various ailments[30]. *Cassia fistula* is widely used in traditional medicinal system of India has been reported to possess hepatoprotective, anti-inflammatory, antitussive, antifungal and used also check wounds healing and antibacterial[15]. Fruits are used as catharatic and in snake bite. Juice of leaves is used in skin diseases[31-33]. Flowers and pods are used as purgative, febrifugal, biliousness and astringent. The ethanolic 50% extract of pods show antifertility activity in female albino rats. The heated pods are applied to swellings on the neck due to cold. The fruits are reported to be used for asthma. Padma, (2006) [17] tested the methanolic leaf extract of *Cassia fistula* for activity against *Candida albicans* showed highest activity i.e., upto 21 mm which was comparable with the standard antifungal antibiotic, clotrimazole.

Evaluation of antimicrobial activity

In the current study, the anti-microbial activity of the methanolic extract was evaluated by determining the zone of inhibition against five bacteria and fourteen fungi and yeast. Clinical pathogens were selected for antibacterial activity namely, (*Bacillus subtilis*, *Pseudomonas eurogenosa, Streptococcus faecalis*, *Salmonella typhi* and *Staphylococcus aureus*. Maximum zone formation was against *Streptococcus faecalis*. Methanolic extraction of plant showed notable antifungal activities against *Aspergillus niger, Aspergillus terreus, Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Saccharomyces cerevisiae, Fusarium sp., Microsporum canis, Streptococcus faecalis, Mucor sp., Penicillium expansum, Trichoderma viride, Trichoderma horzianum* and *Trichophyton mentagrophytes*. *Cassia fistula* was very highly active against *Aspergillus terreus* (7.09±0.32). Results of antimicrobial activity are presented in Table 3. In comparison to the antibiotics used in this study, the plants extracts were far more active against the test bacterial strains.

CONCLUSION

Medicinal property of plant extract is due to presence of secondary metabolites. Twenty seven phytoconstituents were identified from methanol extract of *Cassia fistula* by gas chromatogram and mass spectrometry (GC-MS) analysis. This plant derived bioactive compounds used as source of antibiotic properties and pharmaceutical industries used for drug formulation. This plant crude extract

showed the phytochemical constituent has great potential for food resource and malnutrition of human health.

ACKNOWLEDGMENTS

Special thanks to Prof. Abdul-Kareem, Babylon University, Faculty of science for women, for his special care.

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