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Research Article

COMPARATIVE PHYTOCHEMICAL ANALYSIS OF *CUSCUTA REFLEXA* PARASITE GROWN ON *CASSIA FISTULA* AND *FICUS BENGHLENSIS* BY GC-MS

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ABSTRACT

Objective: The present study was aimed to determine the phytochemicals present in *Cuscuta reflexa* parasite grown on two different hosts. The comparative GC-MS analysis of ethyl acetate extract of *Cuscuta reflexa* grown on *Cassia fistula* and *Ficus benghalensis* was performed.

Method: Powdered sample of *Cuscuta reflexa* from both the host trees was extracted with petroleum ether, ethyl acetate, methanol and water. 50µl of ethyl acetate extract was dissolved in 2ml of methanol and kept in ultrasonic bath for 15mins and centrifuged for 10mins at 6000 rpm and supernatant was analyzed by GC-MS

Result: This analysis revealed that 1, 2, 3-propanetriol 1-acetate, benzofuran 2, 3-dihydro, glycerol, 1, 2-diacetate, 2-methoxy-4-vinylphenol and triacetin are present in *Cuscuta reflexa* grown on both the host trees. *Cuscuta reflexa* grown on *Cassia fistula* showed the presence of 1H-1,2,4-triazol-5-amine-1-ethyl, D-glucitol-4-O-hexyl, 3,4,5-trimethoxy cinnamic acid, 3,6-dimethoxy phenanthrene and 3,5-di tert butyl 4-hydroxyanisol while these compounds are absent in *Cuscuta reflexa* grown on *Ficus benghalensis.* Vanillin, 3-aminopyrrolidine, cetene, sarcosine N-isobutyryl-tetra decyl ester, 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol, 1,5-diphenyl-2H-1,2,4 triazoline-3-thione, 1-octadecene, heptanamide,N-(1-cyclohexylethyl)-2-methyl, scoparone, 3'methyl-2-benzyledine-coumaran-3-one are present only in *Cuscuta reflexa* grown on *Ficus benghalensis* while absent in *Cuscuta reflexa* grown on *Cassia fistula.*

Conclusion*:* This comparative study confirms that phytochemicals present in *Cuscuta reflexa* parasite depends on nature of host.

Keywords: *Cuscuta reflexa* parasite, *Cassia fistula*, *Ficus benghalensis*, GC-MS analysis, Phytochemicals.

INTRODUCTION

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines[1]. Plants are capable of synthesizing low molecular weight organic compounds called as secondary metabolites, usually with unique and complex structure. Many metabolites have found to posses interesting biological activities such as bactericidal, fungicidal, hepatoprotective and muscle relaxant.

The plant *Cuscuta reflexa Roxb* is a perennial parasitic herb, commonly known as akashbela or amarbel. It is an unusual parasitic vine belongs to the Convolvulaceae family. The parasitism of *C. reflexa* is by rapping itself around the host plant after attaching to it. If the host contains food beneficial to *C. reflexa*, it will produce the haustoria inserting themselves into the vascular system of the host [2].

Cuscuta reflexa is the valuable medicinal herb. Stem of this plant is antibacterial and used externally to treat itch and internally in fever [3]. It is useful in treatment of androgen induced alopecia [4]. It also gives anti inflammatory and anti cancer activity [5]. The aqueous and alcoholic extract of *C. reflexa* has diuretic activity [6]. The crude water extract of the *C. reflexa* also shows the anti HIV activity [7]. It is the parasitic plant completely dependent on host plant for food and nutrition. The organic matter is transported from the phloem of the host to the parasite through the haustorium [8]. It is believed that the parasitic herbs extract healthy and potential sap from host plant and if their host plant is medicinal plants then these parasitic herbs show many similar properties to host plants. *Cuscuta* species feeding on commonly used medicinal herbs are given special attention by traditional healers.

In present work different chemical constituents present in ethyl acetate (EA) extract of C. *reflexa* grown on two different host i.e. *Cassia fistula* and *Ficus benghalensis* has been analyzed by GC-MS

MATERIAL AND METHOD

Collection of plant material

Cuscuta stem were collected from the tree of *Cassia fistula* and *Ficus benghalensis* tree near village areas of Gokulpur in Jabalpur district Madhya Pradesh, India in the month of September and November 2010 respectively. Stems were washed thoroughly with water. Immense care was taken to avoid the mixing of host plant with that of targeted *Cuscuta* stem. Stems of *Cuscuta* were cleaned and completely separated from the stems of host plant.

Solvent extraction

Thoroughly washed stems of *Cuscuta reflexa* from both the host trees were shade dried for 15 days and the powdered in the grinder. The shade dried powdered was extracted with petroleum Ether, ethyl acetate, methanol and water in increasing polarity. The extracts were filter with Whatman's filter paper. Filtrates were concentrated under reduced pressure and preserved at 5^{o}C in dark air tight bottles

Sample preparation for GC-MS analysis

50 µl of sample was dissolve in 2ml of methanol and kept in ultrasonic bath for 15 min and centrifuged for 10 min at 6000 rpm and supernatant was injected in GC-MS for analysis.

GC-MS analysis

GC-MS of ethyl acetate extract was performed using Agilent 7890A. Compounds were separated on Agilent 1909S-433: 2065.49541 HP - 5MS. 5% phenyl methyl silox column (30m x 250µm x 0.25µm). Oven temperature was programmed as follows: isothermal temperature of 50^{o} C for 2 min then increased to 150^{o}C at the rate of 5^{o}C /min and held for 1.75 min then increased to 280^{o}C at the rate of 8^{o}C /min and kept constant for 5min. The run time was 45 min. 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).

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.

RESULT

Phytochemicals present in EA extract of *C. reflexa* grown on *C. fistula* and *F. benghalensis* are summarized in Table 1 and Table 2

Table 1: Phytochemicals identified in *C. reflexa* grown on *C. fistula*

S. No.	RT	Name of Compound	MF	M W	Total peak %
1	11.773	1,2,3 Propanetriol, 1- acetate	$C_5H_{10}O_4$	134.13	14.346
2	15.358	Benzofuran, 2,3- dihydro	C_8H_8O	120.14	4.645
3	16.252	Glycerol 1,2-diacetate	$C_7H_{12}O_5$	176.17	11.51
4	17.246	1H-1,2,4-triazol-5-amine 1-ethyl-	$C_4H_8N_4$	112.13	0.478
5	18.045	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	0.498
6	19.028	Triacetin	$C_9H_{14}O_6$	218.21	4.525
7	27.812	D-Glucitol,4-O-hexyl	$C_{12}H_{26}O_6$	266.17	11.038
8	31.542	3,4,5-trimethoxy cinnamic acid	$C_{12}H_{14}O_5$	238.24	0.652
9	32.327	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.48	0.941
10	32.749	3,6-di methoxy phenanthrene	$C_{16}H_{14}O_2$	238.09	1.35
11	32.992	3, 5- di-tert-Butyl-4-hydroxyanisol	$C_{15}H_{24}O_2$	215.35	2.622

RT: Retention time MF: Molecular formula MW: Molecular weight

Table 2: Phytochemicals identified in *C. reflexa* grown on *F. benghalensis*

S. No.	RT	Name of Compound	MF	MW	Total peak %
1	11.769	1,2,3-propanetriol,1-acetate	$C_5H_{10}O_4$	134.13	9.898
2	15.363	Benzofuran,2,3-dihydro	C_8H_8O	120.14	2.987
3	16.252	Glycerol,1,2-diacetate	$C_7H_{12}O_5$	176.17	5.429
4	18.043	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.177	0.781
5	19.033	Triacetin	$C_9H_{14}O_6$	218.21	1.213
6	20.272	Vanillin	$C_8H_8O_3$	152.15	0.599
7	21.944	3-aminopyrrolidine	$C_4H_{10}N_2$	86.14	0.720
8	25.361	Cetene	$C_{16}H_{32}$	224.42	1.395
9	27.168	Sarcosine, N-isobutyryl, tetradecyl ester	$C_{21}H_{41}NO_3$	355.55	1.135
10	28.430	4-((1E)-3-hydroxy-1-propenyl)-2-methoxy phenol	$C_{10}H_{12}O_3$	180.20	2.113
11	29.241	1,5-diphenyl-2H-1,2,4-triazoline-3-thione	$C_{14}H_{11}N_3S$	253.33	1.976
12	29.361	1-octadecene	$C_{18}H_{36}$	252.48	2.219
13	30.649	Heptanamide, N-(1-cyclohexylethyl)-2-methyl	$C_{16}H_{31}NO$	253.42	1.562
14	32.099	Scoparone	$C_{11}H_{10}O_4$	206.2	30.05
15	32.321	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.48	4.612
16	32.994	3'-Methyl-2-benzylidene-coumaran-3-one	$C_{16}H_{12}O_2$	236.26	24.910

RT: Retention time MF: Molecular formula MW: Molecular weight

GC chromatogram of EA extract grown on *C. reflexa* grown on *C. fistula* is given in Fig.ure 1.The chromatogram shows many peaks out of which eleven phytochemicals were characterized and identified on comparison of mass spectra of constituents with NIST library (Fig.ure 3- Fig.ure14).

Fig.ure 2 shows the chromatogram of EA extract of *C. reflexa* from *F. benghalensis* from which sixteen compounds were identified from mass spectrum (Fig.ure14- Fig.ure 28).

Retention time of the components identified in EA extract of *C. reflexa* grown on *C. fistula* were found to be 11.773, 15.358, 16.252, 17.246, 18.045, 19.028, 27.812, 31.542, 32.327, 32.749, 33.992.

Phytochemicals identified from C. reflexa grown on C. fistula were 1,2,3 propanetriol, 1-acetate (14.346%), Benzofuran, 2, 3-dihydro (4.645%), Glycerol, 1,2-diacetate (11.51%), 1H-1,2,4-triazol-5-amine-1-ethyl- (0.478%), 2-methoxy-4-vinylphenol(0.498%), triacetin (4.525%), D-glucitol-4-O-hexyl (11.038%), 3,4,5-trimethoxy cinnamic acid (0.652%), Hexadecanoic acid, ethyl ester (0.41%), 3,6-dimethoxy phenanthrene (1.35%), 3,5 di tert. Butyl - 4-hydroxy anisole (2.622%).

GC-MS analysis of EA extract of C. reflexa grown on F. benghalensis showed the presence of sixteen compounds. Retention times of these phytochemicals were 11.767, 15.636, 16.252, 18.043, 19.033, 20.272, 21.944, 25.361, 27.168, 28.430, 29.241, 29.361, 30.649, 32.099, 32.321 and 32.994.

These compounds were identified as 1,2,3-propanetriol, 1-acetate (9.898%), Benzofuran,1,2-dihydro (2.987%), glycerol,1,2-diacetate (5.429%), 2-methoxy-4-vinylphenol (0.781%), triacetin (1.213%), vanillin (0.599%), 3-aminopyrrolidine (0.720%), cetene (1.395%), sarcosine, N-isobutyryl-tetradecyl ester (1.135%), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (2.113%), 1,5-diphenyl-2H-1,2,4 triazoline-3-thione (1.976%), 1-octadecene (2.219%), Heptanamide, N-(1-cyclohexylethyl)-2-methyl (1.562%), scoparone (30.05%), Hexadecanoic acid, ethyl ester (4.612%), 3'-methyl-2-benzylidene-coumaran-3-one (24.910%).

DISCUSSION

In the present study the GC-MS analysis of ethyl acetate extract of *C. reflexa* grown on *C. fistula* and F. *benghalensis* showed the presence of eleven and sixteen compounds respectively. 1, 2, 3 propanetriol, 1-acetate, benzofuran, 2, 3-dihydro, glycerol 1, 2-diacetate, 2-methoxy-4-vinyl phenol, triacetin and hexadecanoic acid ethyl ester are common in *C. reflexa* from both the host trees.

1H-1, 2, 4-triazol-5-amine, 1-ethyl-, D-glucitol-4-O-hexyl, 3, 4, 5-trimethoxy cinnamic acid, 3,6-dimethoxy phenanthrene and 3,5-di-tert-Butyl-4-hydroxyanisol are present only in *C. reflexa* grown on *C. fistula.*

Vanillin, 3-aminopyrrolidine, cetene, sarcosine, N-isobutyryl-tetra decyl ester, 4-((1E)-3-hydroxy-1-propenyl)-2-methoxy phenol, 1,5-diphenyl-2H-1,2,4-triazoline-3-thione, 1-octadecene, heptanamide, N-(1-cyclohexylethyl)-2-methyl, scoparone, 3'-methyl-2-benzylidine-coumaran-one are present only in *C. reflexa* grown on *F. benghalensis.*

In terms of percentage amount 1, 2, 3 propanetriol 1-acetate, glycerol, 1, 2-diacetate and D-glucitol 4-O-hexyl predominates in C. reflexa grown on C. fistula. Scoparone and 3'-methyl-benzylidene coumaran-3-one predominates in *C. reflexa* grown on *F. benghalensis*.

Glycerol and its derivatives are known to have bacterial inhibiting effect [9]. Scoparone is very potent biological compound and possess many important biological activities like, anti-ulcerogenic [10] hepatoprotective and anti-inflammatory activity [11]. Benzylidene 3-

coumaranones are investigated for antioxidant activity [12]. Benzofurans are known to possess anti-oxidant, anti-inflammatory and antimicrobial effect [13]. Hexadecanoic acid ethyl esters show antioxidant, hypocholesterolemic, and nematicidal activity [14]. 1, 2, 4 triazole derivatives are worked out for their antimicrobial activity [15]. Phenanthrene derivatives are known to be cytotoxic agents [16].

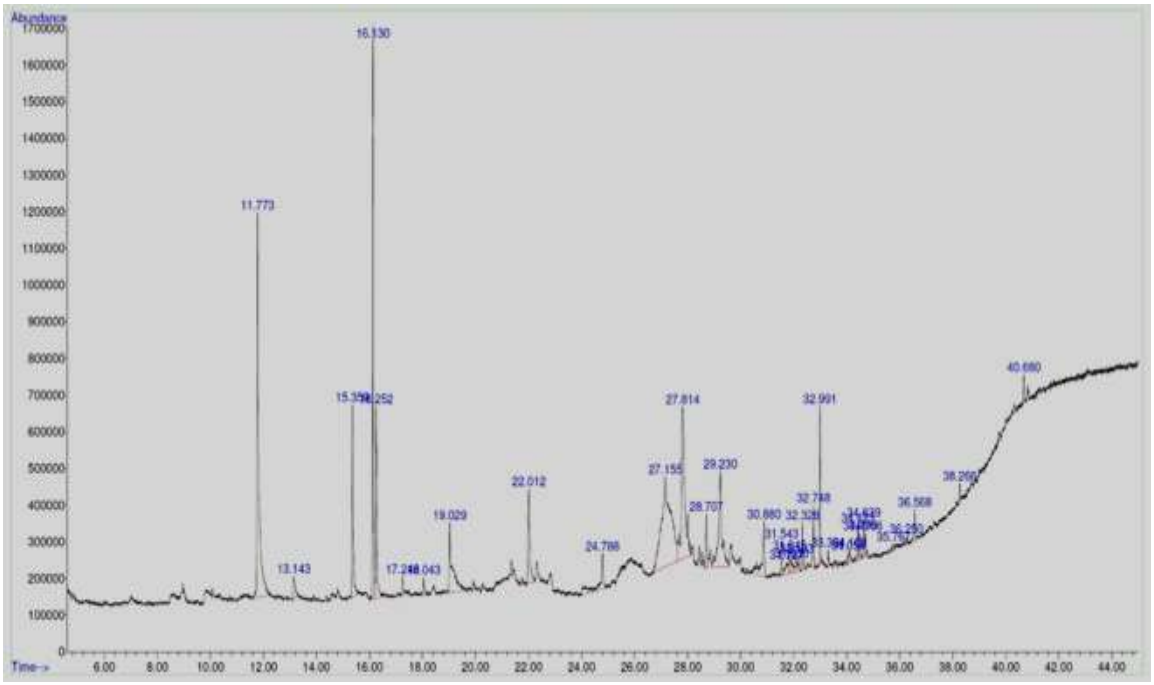

Fig. 1: GC-MS Chromatogram of ethyl acetate extract of *C. reflexa* grown on *C. fistula*

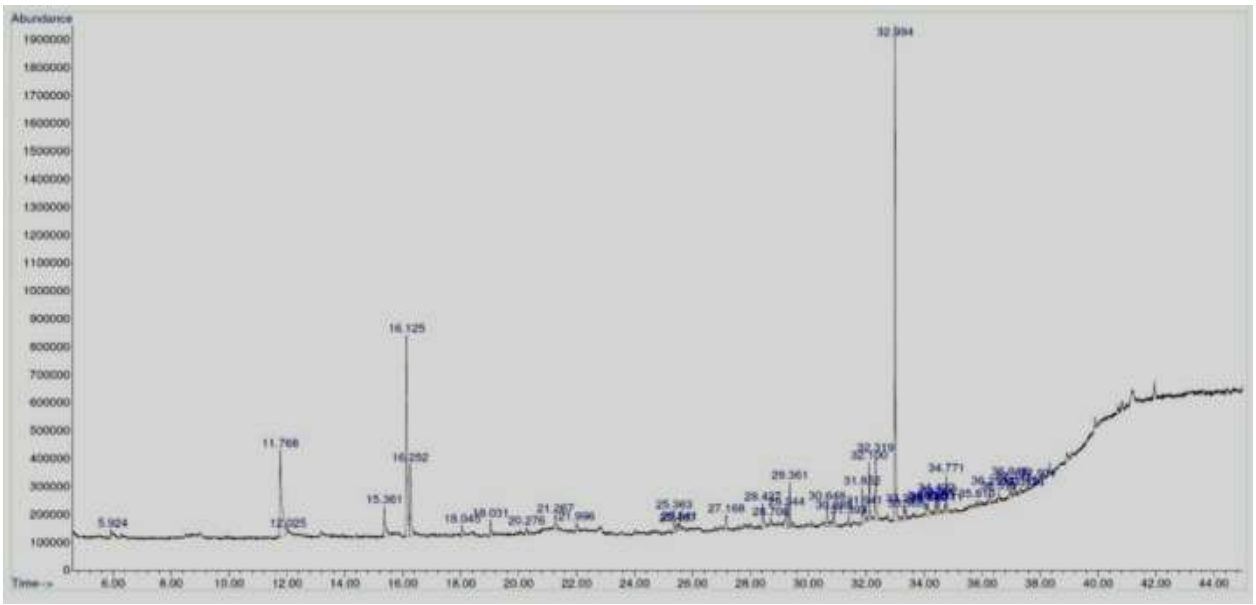

Fig. 2: GC-MS Chromatogram of ethyl acetate of *C. reflexa* grown on *F. benghalensis*

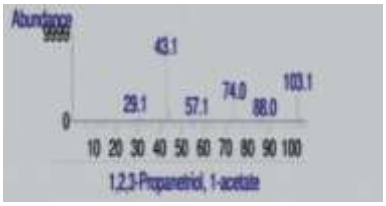

Fig. 3: MS of 1, 2, 3-propanetriol, 1-acetate

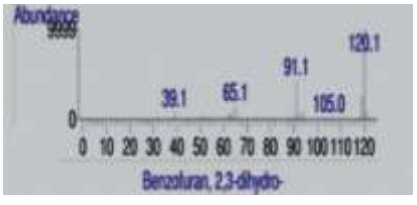

Fig. 4: MS of Benzofuran,2 ,3-dihydro

Fig. 5: MS of Glycerol 1,2diacetate

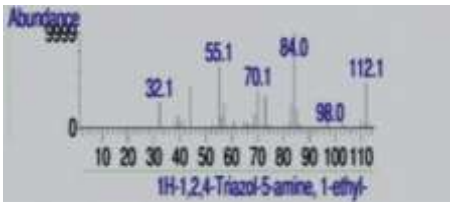

Fig. 6: MS of 1H-1, 2, 4-triazol-5-amine, 1-ethyl

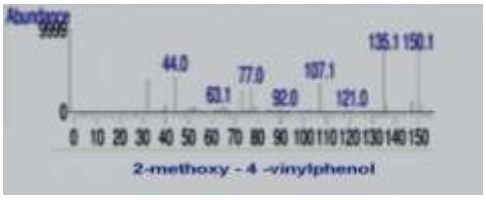

Fig. 7: MS of 2-methoxy-4-vinyl-phenol

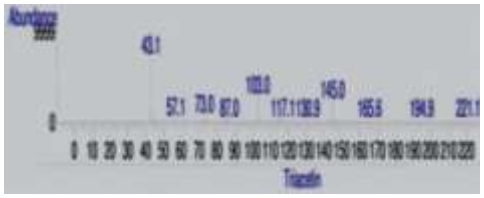

Fig. 8: MS of Triacetin

Fig. 9: MS of D-Glucitol, 4-O-hexyl

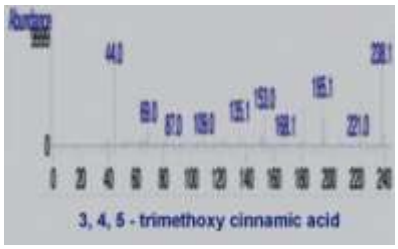

Fig. 10: MS of 3, 4, 5-trimethoxy cinnamic acid

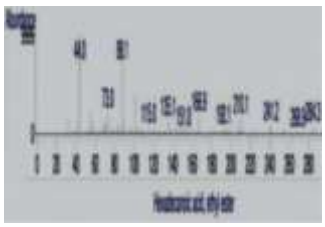

Fig. 11: MS of Hexadecanoic acid, ethyl ester

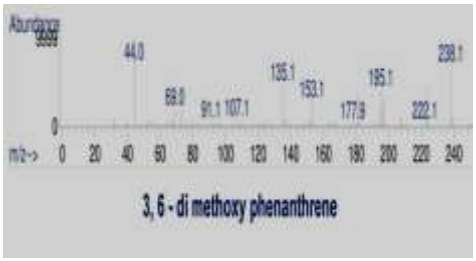

Fig. 12: MS of 3, 6-di methoxy phenanthrene

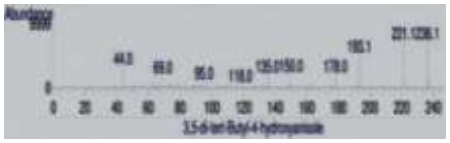

Fig. 13: MS of 3, 5-di- tert-butyl-4-hydroxyanisol

Fig.. 3-13: Mass spectrum of Phytochemicals identified in EA extract of *C. reflexa* grown on *C. fistula*

Fig. 14: MS of 1, 2, 3-propanetriol, 1-acetate

Fig. 15: MS of Benzofuran, 2, 3-dihydro

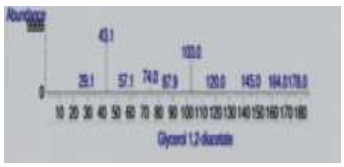

Fig. 16: MS of Glycerol, 1, 2-diacetate

Fig. 17: MS of 2-methoxy-4-vinylphenol

Fig. 18: MS of Vanillin

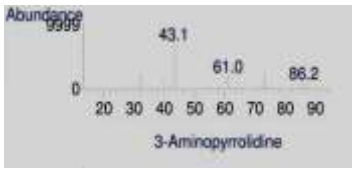

Fig. 19: MS of 3-Aminopyrrolidine

Fig. 20: MS of Cetene

Fig. 21: MS of Sarcosine, N-isobutyryl, tetradecyl ester

Fig. 22: MS of 4-((1E)-3-hydroxy- 1-propenyl)-2-ethoxyphenol

Fig. 23: MS of 1, 5-diphenyl-2H-1, 2, 4-triazoline-3-thione

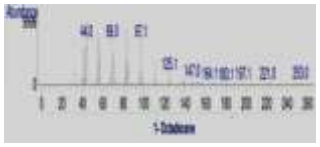

Fig. 24: MS of 1-octadecene

Fig. 25: MS of Heptanamide, N-(1-cyclohexylethyl)-2-methyl

Fig. 26: MS of Scoparone

Fig. 27: MS of Hexadecanoic acid, ethyl ester

Fig. 28: MS of 3'-Methyl-2-benzylidene-coumaran-3-one

Fig. 14-28: Mass spectrum of phytochemicals identified in EA extract of *C. reflexa* grown on *F.benghalensis*

CONCLUSION

It is revealed from this study that *C. reflexa* from both the host trees is rich in secondary metabolites which possess wide range of biological activities. Different compounds are present in *C. reflexa* on two different host thus it is concluded that variation in phytochemicals in *C. reflexa* is host dependent. Further study need to be undertaken to investigate the biological activity and other phytochemicals present in *C. reflexa* grown on *C .fistula* and *F. benghalensis*.

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