

Gas Chromatography – Mass Spectroscopy (GC-MS) Analysis of Various Extracts of *Cocculus hirsutus*

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Gas Chromatography - Mass Spectroscopy (GC-MS) analysis was used to study the bioactive compounds present in the petroleum ether, chloroform and methanol extracts of *Cocculus hirsutus*. GC-MS analysis of petroleum ether extracts showed the presence of 5 compounds of which 1 is a nitrogenous compound. Chloroform extract of *Cocculus hirsutus* showed the presence of 15 compounds of which 11 are nitrogenous compounds. In the same way methanol extract of *Cocculus hirsutus* showed the presence of 12 compounds of which 8 are nitrogenous in nature. This GC-MS analysis gives an idea that phytoconstituents present in *Cocculus hirsutus* are rich in nitrogenous substances. Some of the nitrogenous compounds identified from this plant in this study are Aziridine, 2-hexyl-, Azocine octahydro-, Boraneamine-N-ethyl-1,1-dipropyl-, 4-[1,4]Diazepan-1-yl-1,5-dihydro-imidazol-2-one, Pyrazol-3(2H)-one, 4-(5-hydroxymethylfurfurylideneamino)-1,5-dimethyl-2-phenyl-, Cinnamic acid, a-[N-benzoylamino]-3,5-di-*t*-butyl-4-hydroxy- with the peak area of 2.12%, 3.04%, 2.64%, 2.38%, 12.75% and 33.04.

Key words: GC-MS, *Cocculus hirsutus*, Aziridine.

Cocculus hirsutus (L.) Diels (Menispermaceae) is a climbing shrub that grows all over India, especially in dry regions. *Cocculus hirsutus* is widely used in the indigenous system of medicine for curing various diseases. Leaves are used in the treatment of gonorrhea, eczema, neuralgia, ophthalmia and leucorrhoea and the roots are used for the treatment of rheumatism, dyspepsia, skin diseases, impotency and constipation¹. Alcoholic extract of the leaves and stem are also reported to have anticancer and hypotensive activities². The plant is reported to have jantine, cohirsine, hirsudiol, cohirsinine, cohirstinine, haiderine, jamantine, hirsutine, shaheenine³⁻¹¹. GC-MS analysis of petroleum ether, chloroform and methanol extract was carried out in order to investigate various chemical components present in the different extract of *Cocculus hirsutus*.

MATERIALS AND METHODS

Collection and extraction of plant material

The whole plant was collected in October 2011 from Kallakad, Tirunelveli District in South India. The specimen was identified by Prof. V. Chelladurai, Research Officer – Botany, C.C.R.A.S. Govt. of India (Retired). A voucher specimen was prepared in our research lab and maintained with Voucher no. PSGCP/DPC/01 for *Cocculus hirsutus* for further reference. After the collection, the whole plant was washed with water and shade dried. The shade dried plant was used for extraction. The crude drug was successively extracted in Soxhlet apparatus using petroleum ether, chloroform and methanol. The solvents were removed by distillation on a water bath at atmospheric pressure. The final traces of the solvents were removed with the help of rotary evaporator under reduced pressure. Petroleum ether, Chloroform and Methanol extracts of *Cocculus hirsutus* were analyzed in GC-MS.

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Identification of compounds

Petroluem ether extract, Chloroform extract and methanol extract were purified using the respective solvents. These extracts were subjected to the compound identification using GC-MS (model GC-MS - Clarus 500 Perkin Elmer with Turbomass 5.2 software) at a flow rate of 1mL/minute and a split ration of 10:1. The column used is Elite- 5MS (5% Diphenyl/95% Dimethyl poly siloxane), 30 x 0.25mm x 0.25mm df. The initial temperature was 110⁰ C for 2 minutes and then it is increased upto 200⁰ C at the rate of 10⁰ C/min without any hold. Further the temperature is increased upto 280⁰C at the rate of 5⁰ C / min with 9 min hold. The total running time of GC

is 36 minutes. The electron energy given is 70 eV. The detector used is mass detecto turbo mass gold – Perkin Elmer. The peaks of the compounds obtained were compared with the already available compounds in the library NIST Version – Year 2005⁸.

RESULTS AND DISCUSSION

Petroluem ether extract of *Cocculus hirsutuss* pareira showed the presence of 1,2-Benzenedicarboxylic acid, 2-butoxyethyl butyl ether, 1,2-Benzenedicarboxylic acid, disooctyl ether, 2-Propen-1-amine, Pyruvic acid, butyl ester, Propanoic acid, anhydride (Table. 1 and Fig. 1).

Table 1. Compounds identified in the petroleum ether extract of *Cocculus hirsutus*

S. No	Name of the compound Formula	Molecular	MW	RT Area %	Peak
1.	1,2-Benzenedicarboxylic acid, 2-butoxyethyl butyl ether	C ₁₈ H ₂₆ O ₅	322	11.93	31.79
2.	1,2-Benzenedicarboxylic acid, disooctyl ether	C ₂₄ H ₃₈ O ₄	390	20.80	6.25
3.	2-Propen-1-amine	C ₃ H ₇ N	57	26.84	0.54
4.	Pyruvic acid, butyl ester	C ₇ H ₁₂ O ₃	144	27.65	0.82
5.	Propanoic acid, anhydride	C ₆ H ₁₀ O ₃	130	29.64	60.60

MW - Molecular Weight

RT – Retention time

Table 2. Compounds identified in the choloroform extract of *Cocculus hirsutus*

S. No	Name of the compound Formula	Molecular	MW	RT Area %	Peak
1.	1,3-Dimethyl-3-n-propyldiaziridine	C ₆ H ₁₄ N ₂	114	4.97	0.80
2.	Cyclooctanamine	C ₈ H ₁₇ N	127	6.55	0.40
3.	3-Ethyl-1,3-dimethyldiaziridine (Cis)	C ₅ H ₁₂ N ₂	100	7.63	1.00
4.	Phenol,2,5-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206	7.96	0.53
5.	Cyclopentane, butyl-	C ₉ H ₁₈	126	8.89	0.40
6.	Azocine, octahydro-	C ₇ H ₁₅ N	113	10.09	1.00
7.	1H-Pyrrole-2,5-dione, 1-(hydroxymethyl)-	C ₅ H ₅ NO ₃	127	10.22	1.00
8.	Cyclopentane, butyl-	C ₉ H ₁₈	126	11.11	0.40
9.	Ethaneperoxoic acid, 1-cyano-1-[2-[2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl ester	C ₁₉ H ₂₅ NO ₅	347	11.93	4.47
10.	2-Propen-1-amine, N-2-propenyl-	C ₆ H ₁₁ N	97	13.42	0.53
11.	Octane, 1-azido-	C ₈ H ₁₇ N ₃	155	16.03	0.20
12.	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	390	20.88	28.84
13.	3-[3-[1-Aziridiny]propoxy]-2,5-dimethylpyrazine	C ₁₁ H ₁₇ N ₃ O	207	29.03	10.01
14.	Pyrazol-3(2H)-one, 4-(5-hydroxymethylfurfur-2-ylidenamino)-1,5-dimethyl-2-phenyl-	C ₁₇ H ₁₇ N ₃ O ₃	311	29.32	34.65
15.	Cinnamic acid, a-[N-benzoylamino]-3,5-di-t-butyl-4-hydroxy-	C ₂₅ H ₃₁ NO ₄	409	34.97	15.75

MW - Molecular Weight

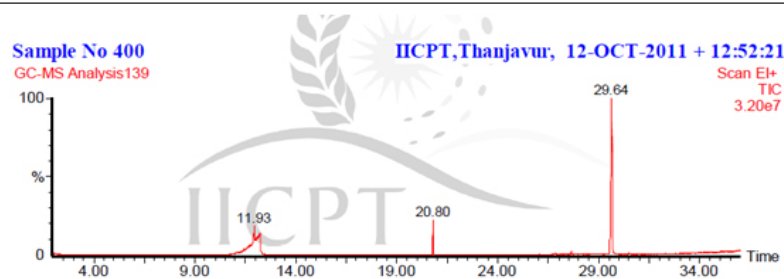
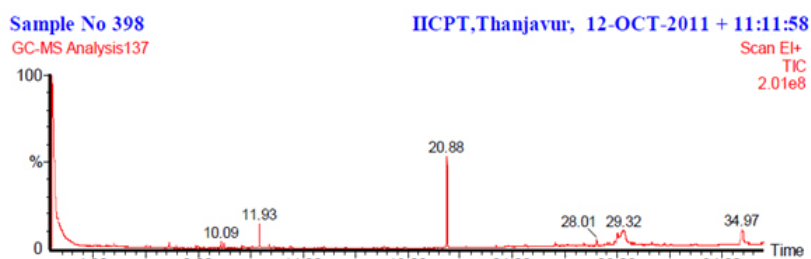
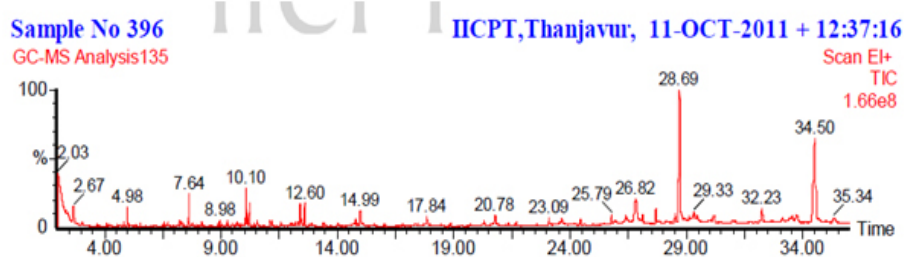
RT – Retention time

Table 3. Compounds identified in the chloroform extract of *Cocculus hirsutus*

S. No	Name of the compound Formula	Molecular	MW	RT Area %	Peak
1.	Aziridine, 2-hexyl-	$C_8H_{17}N$	127	4.98	2.12
2.	Azocine, octahydro-	$C_7H_{15}N$	113	7.64	3.04
3.	Acetic acid, trifluoro-, octyl ester	$C_{10}H_{17}F_3O_2$	226	10.10	3.41
4.	Hexanal, O-methylxime	$C_7H_{15}NO$	129	12.60	1.89
5.	Boraneamine, N-ethyl-1,1-dipropyl-	$C_8H_{20}BN$	141	14.99	2.64
6.	4-[1,4]Diazepan-1-yl-1,5-dihydro-imidazol-2-one	$C_8H_{14}N_4O$	182	17.84	2.38
7.	Acetic acid, trifluoro-, octyl ester	$C_{10}H_{17}F_3O_2$	226	20.78	2.49
8.	Pyrazol-3(2H)-one, 4-(5-hydroxymethylfurfur—ylidenamino)-1,5-dimethyl-2-phenyl-	$C_{17}H_{17}N_3O_3$	311	26.82	12.75
9.	1-Heneicosyl formate	$C_{22}H_{44}O_2$	340	28.69	29.43
10.	N,N-Bis[azirid-1-ylmethyl]cyclopropylamine	$C_9H_{17}N_3$	167	32.23	4.70
11.	Cinnamic acid, a-[N-benzoylamino]-3,5-di-t-butyl-4-hydroxy-	$C_{25}H_{31}NO_4$	409	34.50	33.04
12.	Pregn-4-ene-3,20-dione, (9a,10a)-	$C_{21}H_{30}O_2$	314	35.34	2.12

MW - Molecular Weight

RT – Retention time

**Fig.1.** Petroleum ether extract of *Cocculus hirsutus***Fig.2.** Chloroform extract of *Cocculus hirsutus***Fig.3.** Methanol extract of *Cocculus hirsutus*

Chloroform extract of *Cocculus hirsutus* showed the presence of 1,3-Dimethyl-3-n-propyldiaziridine, Cyclooctanamine, 3-Ethyl-1,3-dimethyldiaziridine (Cis), Phenol, 2,5-bis(1,1-dimethylethyl)-, Cyclopentane, butyl-, Azocine, octahydro-, 1H-Pyrrole-2,5-dione, 1-(hydroxymethyl)-, Cyclopentane, butyl-, Ethaneperoxoic acid, 1-cyano-1-[2-[2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl ester, 2-Propen-1-amine, N-2-propenyl-, Octane, 1-azido-, 1,2-Benzenedicarboxylic acid, diisooctyl ester, 3-[3-[1-Aziridinyl]propoxy]-2,5-dimethylpyrazine, Pyrazol-3(2H)-one, 4-(5-hydroxymethylfurfur-2-ylidenamino)-1,5-dimethyl-2-phenyl-, Cinnamic acid, a-[N-benzoylamino]-3,5-di-t-butyl-4-hydroxy- (Table. 2 and Fig.2)

Methanol extract *Cocculus hirsutus* showed the presence of Aziridine, 2-hexyl-, Azocine, octahydro-, Acetic acid, trifluoro-, octyl ester, Hexanal, O-methyloxime, Boraneamine, N-ethyl-1,1-dipropyl-, 4-[1,4]Diazepan-1-yl-1,5-dihydro-imidazol-2-one, Acetic acid, trifluoro-, octyl ester, Pyrazol-3(2H)-one, 4-(5-hydroxymethylfurfur—yliden amino)-1,5-dimethyl-2-phenyl-, 1-Heneicosyl formate, N,N-Bis [azirid-1-ylmethyl]cyclopropylamine, Cinnamic acid, a-[N-benzoylamino]-3,5-di-t-butyl-4-hydroxy-, Pregn-4-ene-3,20-dione, (9a,10a)- (Table. 3 and Fig 3).

CONCLUSION

This study helps to identify various components present in petroleum ether, chloroform and methanol extract of *Cocculus hirsutus* by GC-MS.

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