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Research Article

# DETERMINATION OF BIOACTIVE COMPONENTS OF THE LEAVES OF *COCCULUS HIRSUTUS* (L.) DIELS USING GC-MS ANALYSIS

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**ABSTRACT**

Objectives: The aim of the present study was to analyzed the methanolic extract of the *Cocculus hirsutus* leaf by GC-MS technique to study the major and minor phytoconstituents.

Methods: In the present study, preliminary phytochemical analysis and Gas Chromatography–Mass Spectrum (GC–MS) was carried out on the methanol extract of leaf of *Cocculus hirsutus* for identification of phytocompounds in the plant while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library.

Results: The preliminary phytochemical analysis of methanolic extract of *C. hirsutus* leaves showed the presence of bioactive components like carbohydrates, steroids, alkaloids, glycosides, flavonoids, tannins and saponins. 32 bioactive compounds from methanolic extract of the above said plant were identified. The GC-MS results showed the presence of a number of bioactive phytocompounds like Quinic acid, 2,3,4,5-Tetrahydroxypentanal, Vitamin E, Linolenic acid, stearic acid, Phthalic acid, beta-sitosterol, campesterol, lupeol, betulin and squalene, all of which possessed a wide range of proven therapeutic uses. The highest peak area (%) of 36.29 was obtained by Quinic acid (Retention time 17.866) and the lowest peak area (%) of 0.08 was obtained by 5,5-Dibutylnonane (Retention time 29.396).

Conclusion: Results confirmed the presence of therapeutically potent compounds in the leaf extract. This study will also helps to predict the formula and structure of biomolecules which can be used as drugs and further investigation may lead to the development of drug formulation.

**Keywords:** Phytochemical, Methanolic extract, GC-MS, *Cocculus hirsutus*.

## INTRODUCTION

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties[1]. Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides [2,3]. Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant because these may serve as talented sources of book antibiotic prototypes [4,5]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [6].

Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action. A special feature of higher plants is their capacity to produce a large number of secondary metabolites [7]. Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases[8,9]. Knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agent as well as new sources of economic materials like oil and gums. The most important bioactive constituents of the plants are alkaloids, tannins, flavonoids and phenolic compounds. In India large number of plant species had been screened for their pharmacological properties but still a vast wealth of endangered species are unexplored. Medicinal plants are at interest to the field of biotechnology, as most of the drug industries depend in part on plants for the production of pharmaceutical compounds[10]. Several bioactive compounds have been detected by several workers using GC-MS[11-13].

*Cocculus hirsutus* (L.) Diels is a widely distributed climber mainly found in tropical and subtropical climates. Traditionally the plant was patronised for its unique property of healing all types of cuts, wounds and boils in very less time and less pain. It is also used in treatment of gonorrhoea, spermatorrhoea, urinary troubles, diarrhoea and hyperglycemia etc.[14]. A decoction of the leaves is taken in eczema, dysentery and urinary problem. Leaves and stem are used for treating eye diseases. Roots and leaves are given for Sarsaparilla, as diuretic and in gout [15].Literature survey revealed that the leaves of the plant have been evaluated for anti hyperglycemic[16]; antibacterial[17]; diuretic and laxative[18] effects. In the present study methanolic extract of the plant leaf was analyzed by GC-MS technique to study the major and minor phytoconstituents of the plant.

## MATERIALS AND METHODS

### Collection of plant material

The leaves of *Cocculus hirsutus* were collected from the Kulish Smriti Van, Jaipur, India. They were identified and authenticated by the herbarium of Department of Botany, University of Rajasthan, Jaipur.

### Preparation of powder and extract

Leaves were shade dried, powdered and extracted with methanol for 6-8 hours using soxhlet apparatus. The extract was then filtered through muslin, evaporated under reduced pressure and vacuum dried to get the viscous residue. The methanolic extracts of the plant was used for preliminary phytochemical and GC-MS analysis. 1 µl of the methanolic leaves extract of *C. hirsutus* was employed for GC-MS analysis.

### GC-MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1µM df, composed of 100% Dimethyl poly diloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 250 $^0$C; ion-source temperature 280 $^0$C. The oven temperature was programmed from 110$^0$C (isothermal for 2 min), with an increase of 10$^0$C/min, to 200$^0$C, then 5$^0$C/min to 280$^0$C, ending with a 9 min isothermal at 280$^0$C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 45 minutes.

**Identification of Components**

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials was ascertained.

**RESULTS AND DISCUSSION**

**Phytochemical characterization**

Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history it has been used as a popular folk medicine. Several studies have proved that the phytocomponents present in a medicinal plant are widely responsible for the therapeutic potential of the plant. Phytochemical investigation of methanolic extract of leaves showed the presence of carbohydrates, steroids, alkaloids, glycosides, flavonoids, tannins and saponins. Results are summerised in Table 1.

**Table 1: Preliminary phytochemical analysis of methanolic extract of *Cocculus hirsutus* leaves**

| S. No. | Phyto Constituents | Methanol Extract |
|---|---|---|
| 1 | Carbohydrates | Present |
| 2 | Steroids | Present |
| 3 | Alkaloids | Present |
| 4 | Triterpenoids | Nil |
| 5 | Glycosides | Present |
| 6 | Tannins | Present |
| 7 | Proteins and Amino acids | Nil |
| 8 | Saponins | Present |
| 9 | Flavonoids | Present |

**GC-MS: Phytocomponents in methanolic extract of *Cocculus hirsutus* by GC-MS report** GC-MS chromatogram of the methanolic leaf extract of *C. hirsutus* (Figure 1) showed 32 peaks indicating the presence of thirty two compounds. The chemical compounds identified in the methanolic extract of the leaves of *C. hirsutus* are presented in Table 2. The active principles with their retention time (RT), molecular formula, area %, compound name, RI are presented in Table 2. The total ion chromatograph (TIC) showing the peak identities of the compounds identified have been given in Figure1.

**Table 2: Compounds identified from methanolic extract of *Cocculus hirsutus* (L.) Diels using GC-MS analysis**

| Peak | R.Time | Area% | Molecular Formula | Compound Name | RI |
|---|---|---|---|---|---|
| 1 | 8.277 | 2.00 | $C_6H_8O_4$ | 3,5- Dihydroxy-6- methyl-2, 3- dihydro-4H-pyron-4-one | 1269 |
| 2 | 8.913 | 0.18 | $C_{13}H_{28}$ | Tridecane | 1313 |
| 3 | 12.816 | 0.75 | $C_{14}H_{30}$ | Tetradecane | 1413 |
| 4 | 16.740 | 0.55 | $C_{15}H_{32}$ | Pentadecane | 1512 |
| 5 | 17.866 | 36.29 | $C_7H_{12}O_6$ | Quinic acid | 1852 |
| 6 | 21.416 | 28.07 | $C_5H_{10}O_5$ | 2,3,4,5-Tetrahydroxypentanal or d- ribose | 1436 |
| 7 | 21.983 | 0.44 | $C_{17}H_{34}O_2$ | Palmitic acid | 1878 |
| 8 | 22.336 | 0.38 | $C_{18}H_{28}O_3$ | Benzene propanoic acid | 2134 |
| 9 | 22.492 | 0.78 | $C_{16}H_{32}O_2$ | n-Hexadecanoic acid | 1968 |
| 10 | 22.603 | 0.62 | $C_{16}H_{22}O_4$ | Phthalic acid | 1908 |
| 11 | 22.767 | 0.29 | $C_{20}H_{40}O_2$ | Stearic acid, ethyl ester | 2177 |
| 12 | 24.118 | 1.08 | $C_{19}H_{32}O_2$ | Linolenic acid, methyl ester | 2101 |
| 13 | 24.249 | 1.04 | $C_{20}H_{40}O$ | 3,7,11,15- Tetramethyl-2-hexadecen-1-ol | 2045 |
| 14 | 24.656 | 0.18 | $C_9H_{20}O_3$ | 1-Butoxy ethoxy-2-propanol | 1230 |
| 15 | 24.748 | 0.91 | $C_{20}H_{38}O_2$ | Oleic acid, ethyl ester | 2185 |
| 16 | 25.907 | 0.13 | $C_7H_{13}NO_2$ | Dimethylaminoethylacrylate | 924 |
| 17 | 26.307 | 0.31 | $C_{19}H_{38}O_3$ | Methyl 4-hydroxyoctadecanoate | 2239 |
| 18 | 27.531 | 0.25 | $C_{10}H_{21}N$ | Propylhexedrine | 1213 |
| 19 | 27.768 | 0.43 | $C_{25}H_{48}O_3$ | Tetracosanoic acid, 3 oxo-methyl ester | 2810 |
| 20 | 28.267 | 1.83 | $C_{24}H_{38}O_4$ | Diisooctyl phthalate | 2704 |
| 21 | 29.396 | 0.08 | $C_{17}H_{36}$ | 5,5- Di butylnonane | 1627 |
| 22 | 30.760 | 2.31 | $C_{30}H_{50}$ | Squalene | 2914 |
| 23 | 31.455 | 0.29 | $C_{19}H_{40}O$ | 10-Nonadecanol | 2072 |
| 24 | 33.751 | 0.19 | $C_{28}H_{48}O_2$ | Gamma-Tocopherol | 3036 |
| 25 | 34.017 | 3.28 | $C_{20}H_{35}F_5O_2$ | Pentafluoropropionic acid | 1872 |
| 26 | 35.011 | 10.27 | $C_{29}H_{50}O_2$ | Vitamin-E | 3149 |
| 27 | 37.362 | 0.35 | $C_{28}H_{48}O$ | Campesterol | 2632 |
| 28 | 37.962 | 0.75 | $C_{16}H_{26}O_3$ | Dodecenyl succinic anhydride | 2159 |
| 29 | 39.247 | 1.85 | $C_{29}H_{50}O$ | Beta - sitosterol | 2731 |
| 30 | 40.459 | 0.70 | $C_{30}H_{50}O$ | Alpha-Amyrin | 2873 |
| 31 | 40.959 | 0.34 | $C_{30}H_{50}O$ | Lupeol | 2848 |
| 32 | 41.564 | 3.06 | $C_{30}H_{50}O_2$ | Betulin | 3090 |

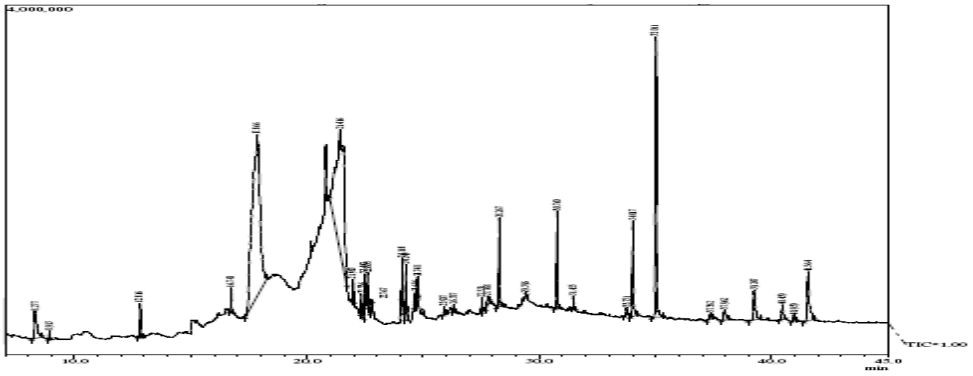


**Fig. 1: Shows GC-MS Chromatogram of methanolic leaf extract of *Cocculus hirsutus***

GC MS analysis revealed the presence of palmitic acid, oleic acid, phthalic acid, stearic acid, Linolenic acid etc. Quinic acid is present in maximum amount (36.29%), followed by 2,3,4,5-Tetrahydroxypentanal (28.07%) in the methanolic extract of leaves of *C. hirsutus*. Steroids like beta-sitosterol, campesterol, squalene were also detected. Vitamin E was also present in considerable amount i.e. 10.27%. GC-MS analysis revealed that the minimum presence of 5,5- Dibutylnonane, Dimethy aminoethylacrylate, Tridecane, 1-Butoxy ethoxy-2-propanol and Gamma tocopherol. The GC-MS analyses revealed that the methanolic extract is mainly composed of esters, phenolics and steroids. These phytochemicals are responsible for various pharmacological actions like antimicrobial, anti-oxidant, and anti-inflammation activities. By interpreting these compounds, it is found that *C. hirsutus* possess various therapeutic applications. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in *C. hirsutus*. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This study is only a preliminary study of the occurrence of certain properties of *C. hirsutus* leaf extract an in-depth study will provide a good concrete base for all the biochemical and phytochemical functions mentioned above. New scientific strategies for the evaluation of natural products with specific biological activities require the implementation of large screening process. The aim of the present study is to provide more information about the essential phytoconstituents of *Cocculus hirsutus*, the results from the present investigation are very encouraging and indicates that this plant should be studied more extensively to explore its potential to use as plant medicinal nutritive.

## CONCLUSION

Phytochemical investigation and GC-MS analysis of methanolic extract of leaves showed the presence of carbohydrates, steroids, alkaloids, glycosides, flavonoids, tannins and saponins. The presence of various bioactive compounds confirms the application of *Cocculus hirsutus* for various ailments by traditional practitioners. This report is the first of its kind to analyze the chemical constituents of methanolic extract of leaves of *Cocculus hirsutus.* However, isolation of individual phytochemical constituents may proceed to find a novel drug.

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