

Original Article

DETERMINATION OF BIOACTIVE CONSTITUENTS OF LEAVES OF *CORCHORUS AESTUANS* (L.)
BY GC - MS ANALYSIS

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ABSTRACT

Objective: To investigate the bioactive constituents of ethanol extract of *Corchorus aestuans* (L.) using GC-MS technique.

Methods: The ethanol extract of leaves were subjected to GC- MS analysis using GC Clarus 500 (Perkin Elmer) with Elite- 5MS (5%Diphenyl / 95% Dimethyl poly siloxane, 30 mm x 0.25 mm x 0.25 μ m df). The 2 μ l extract sample injected and the components were separated using Helium (1 mL/min) as the carrier gas. Bioactive constituents were detected by the Turbo mass gold detector (Perkin Elmer) with the aid of the Turbomass 5.2 software.

Results: The analysis revealed the presence of fourteen different bioactive constituents namely 3, 7,11,15-tetramethyl-2-hexadecen-1-ol (5.6%), Trans-2-undecen-1-ol (1.26%), E-7-Tetradecenol (1.97%), n-Hexadecanoic acid (25.82%), Phytol (22.34%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (20.23%), Docosanoic acid, ethyl ester (1.99%), 1-Eicosanol (2.11 %), 9,9-dimethoxybicyclo[3.3.1] nona-2,4-dione (0.60%), Heptadecanoic acid, heptadecyl ester (0.95%), Pentadecanoic acid, 2,6,10,14-tetramethyl-methylester (0.91%), 3-Hexadecyloxy carbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion (0.90%), Squalene (8.03%), Vitamin E (7.24%).

Conclusion: The presence of various bioactive compounds confirms the application of *Corchorus aestuans* in various disorders. However, isolation of individual phytochemical constituents may proceed to find a lead for herbal therapy.

Keywords: *Corchorus aestuans*, Ethanol extract, GC-MS analysis, Bioactive constituents.

INTRODUCTION

Nature has been a source of medicinal plants for thousands of years. Medicinal plants are the chemical gold mines existing in the ecosystems and play a dominant role in the development of novel drugs for the prevention and treatment of disease in men and animals [1]. World Health Organization (WHO) supports the use of medicinal plants, provided it is proven to be efficacious, safe, less toxic, available and reliable natural resource [2]. Phytochemical constituents are responsible for medicinal activity of plants species [3]. The chemical features of these constituents differ considerably among different species. A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phyto compounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies [4]. Hence, for the discovery of lead compounds for use as therapeutic drugs, the active principals in medicinal plants needs to be identified [5]. Extraction is the main step for the recovery and isolation of bioactive phytochemical from plant materials, before component analysis [6]. *Corchorus aestuans* (L.) (Syn. *Corchorus acutangulus* Lam), family Tiliaceae is an annual herb occurring throughout the warmer parts of India as weed in the microclimatic areas of river banks where sandy soil is available and also in shade conditions undisturbed areas [7]. They are reported to exhibit various pharmacological activities such as stomachic, anti-inflammatory and pneumonia [8]. But there have been no reports on the bioactive constituents from ethanol extract of leaves of *Corchorus aestuans* Linn. Hence, the present study is to determine the bioactive constituents present in the Ethanol extract of *Corchorus aestuans* leaves by Gas chromatography and Mass spectroscopy (GC-MS) technique.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh leaves of the selected plant *Corchorus aestuans* were collected from Thirunelveli district, Tamil Nadu, India. The plant materials were taxonomically identified and authenticated by Dr. V. Chelladurai,

Research officer - Botany (scientist C), Central council for research in Ayurveda and Siddha, Govt. of India; Thirunelveli. The plant was thoroughly washed in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The leaves of the plant alone were segregated and dried under shade, pulverized by a mechanical grinder into fine powder. The powdered materials were stored in air tight polythene bags till use.

Preparation of extracts

The powdered plant materials were extracted with ethanol (99.9%). The extraction was done by hot continuous percolation method in Soxhlet apparatus for 24 hrs [9]. The extract was concentrated by using a rotary evaporator till dry powder was obtained. The final residue thus obtained was then subjected to GC-MS analysis [10].

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The ethanol extract of *Corchorus aestuans* leaves was analyzed through GC-MS for the identification of different compounds. The GC-MS analysis was carried out by using Clarus 500 (Perkin - Elmer) Gas chromatograph equipped and coupled to a mass detector Turbo mass gold (Perkin - Elmer) spectrometer with an Elite - 5MS (5% Diphenyl / 95% Dimethyl poly siloxane, 30m x 0.25 mm x 0.25 μ m df) of capillary column. The oven was set to an initial temperature 110 $^{\circ}$ C for 2 min, further increased up to 200 $^{\circ}$ C at the rate of 10 $^{\circ}$ C/min. Finally temperature was raised up to 280 $^{\circ}$ C, at the rate of 5 $^{\circ}$ C /min for 9 min. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min. An aliquot of 2 μ l of sample was injected into the column with the injector temperature at 250 $^{\circ}$ C (Split ratio of 10:1). The electron ionization system with ionizing energy of 70 eV was used. Mass spectral scan range was set at 45-450 (m/z). Total GC running time was 36 minutes.

Identification of compounds

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technique (NIST Version-Year 2005) having more patterns. The relative percentage amount of each component was calculated by comparing its average

peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST data library (version 2005). The name, molecular weight, molecular formula and structure of the components of the test material were determined.

RESULTS AND DISCUSSION

GC-MS chromatogram of the ethanol extract of leaves of *Corchorus aestuans* (Fig. 1) clearly showed fourteen peaks indicating the presence of fourteen phytochemical compounds.

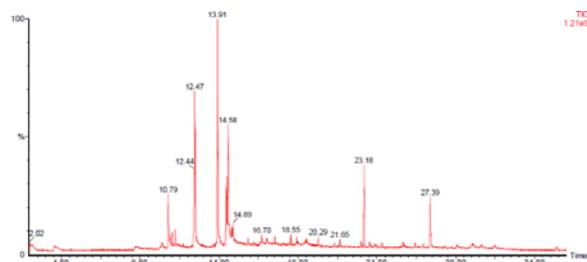


Fig. 1: The GC - MS Chromatogram of ethanol extracts of leaves of *Corchorus aestuans*

The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and peak area in percentage (%) were tabulated in Table 1. The components corresponding to the peaks were determined as follows 3,7,11,15-tetramethyl-2-hexadecen-1-ol (5.67%), Trans-2-undecen-1-ol (1.26%), E-7-Tetradecenol (1.97%), n-hexadecanoic acid (25.82 %), Phytol (22.34 %), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (20.23 %), Docosanoic acid, ethyl ester (1.99 %), 1-Eicosanol (2.11 %), 9,9-dimethoxybicyclo[3.3.1]nona-2,4-dione (0.60 %), Heptadecanoic acid, heptadecyl ester (0.95 %), Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester (0.91 %), 3-hexadecyloxy carbonyl-5-(2-hydroxyethyl)-4- methylimidazolium ion (0.90 %), Squalene (8.03 %), Vitamin E (7.24%).

The spectrum sketch out of GC-MS confirmed the presence of fourteen components with the retention time 10.79, 11.04, 11.22, 12.47, 13.91, 14.58, 14.89, 16.70, 17.55, 18.55, 20.29, 21.65, 23.18, 27.39 min respectively which is shown in Figure 1. The phytochemical compounds recognized through GC-MS analysis showed many biological activities are listed in Table 2. The biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA.

Table 1: Phytochemical compounds identified in ethanol extract of leaves of *Corchorus aestuans*

| S. No. | RT | Name of the compound | Molecular Formula | Molecular Weight | Peak Area % |
|--------|-------|---|---|------------------|-------------|
| 1. | 10.79 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C ₂₀ H ₄₀ O | 296 | 5.67 |
| 2. | 11.04 | trans-2-Undecen-1-ol | C ₁₁ H ₂₂ O | 170 | 1.26 |
| 3. | 11.22 | E-7-Tetradecenol | C ₁₄ H ₂₈ O | 212 | 1.97 |
| 4. | 12.47 | n-Hexadecanoic acid | C ₁₆ H ₃₂ O ₂ | 256 | 25.82 |
| 5. | 13.91 | Phytol | C ₂₀ H ₄₀ O | 296 | 22.34 |
| 6. | 14.58 | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | C ₁₉ H ₃₂ O ₂ | 292 | 20.23 |
| 7. | 14.89 | Docosanoic acid, ethyl ester | C ₂₄ H ₄₈ O ₂ | 368 | 1.99 |
| 8. | 16.70 | 1-Eicosanol | C ₂₀ H ₄₂ O | 298 | 2.11 |
| 9. | 17.55 | 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione | C ₁₁ H ₁₆ O ₄ | 212 | 0.60 |
| 10. | 18.55 | Heptadecanoic acid, heptadecyl ester | C ₃₄ H ₆₈ O ₂ | 508 | 0.95 |
| 11. | 20.29 | Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester | C ₂₀ H ₄₀ O ₂ | 312 | 0.91 |
| 12. | 21.65 | 3-Hexadecyloxy carbonyl-5-(2-hydroxyethyl)-4- methylimidazolium ion | C ₂₄ H ₄₅ N ₂ O ₃ | 409 | 0.90 |
| 13. | 23.18 | Squalene | C ₃₀ H ₅₀ | 410 | 8.03 |
| 14. | 27.39 | Vitamin E | C ₂₉ H ₅₀ O ₂ | 430 | 7.24 |

Table 2: Biological activities of phytochemical compounds identified in ethanol extract of leaves of *Corchorus aestuans*

| S. No. | Name of the compound | Compound Nature | **Biological Activity |
|--------|---|---------------------------|--|
| 1. | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | Terpene Alcohol | Anti microbial, Anti-inflammatory activity |
| 2. | trans-2-Undecen-1-ol | Alcohol | No activity reported |
| 3. | E-7-Tetradecenol | Alcohol | Anti microbial activity |
| 4. | n-Hexadecanoic acid | Palmitic acid | Antioxidant, Hypocholesterolemic |
| 5. | Phytol | Diterpene | Nematicide, Pesticide, Lubricant, Antiandrogenic Hypocholesterolemic, Antimicrobial, Anticancer, Cancer preventive, Diuretic Anti inflammatory |
| 6. | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | Fatty acid ester compound | Anti inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge |
| 7. | Docosanoic acid, ethyl ester | Behenic acid ethyl ester | No activity reported |
| 8. | 1-Eicosanol | Alcohol | Anti microbial activity |
| 9. | 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione | Ketone | No activity reported |
| 10. | Heptadecanoic acid, heptadecyl ester | Fatty acid | No activity reported |
| 11. | Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester | Ester compound | No activity reported |
| 12. | 3-Hexadecyloxy carbonyl-5-(2-hydroxyethyl)-4- methylimidazolium ion | Carbonyl | No activity reported |
| 13. | Squalene | Triterpene | Antibacterial, Antioxidant, Pesticide, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor |
| 14. | Vitamin E | Vitamin | Antiageing, Analgesic, Antidiabetic, Anti inflammatory, Antioxidant, Antileukemic, |

**Source: Dr. Duke's Phytochemical and Ethnobotanical Databases

The individual fragmentation patterns of necessary components were illustrated in Figures A-J.

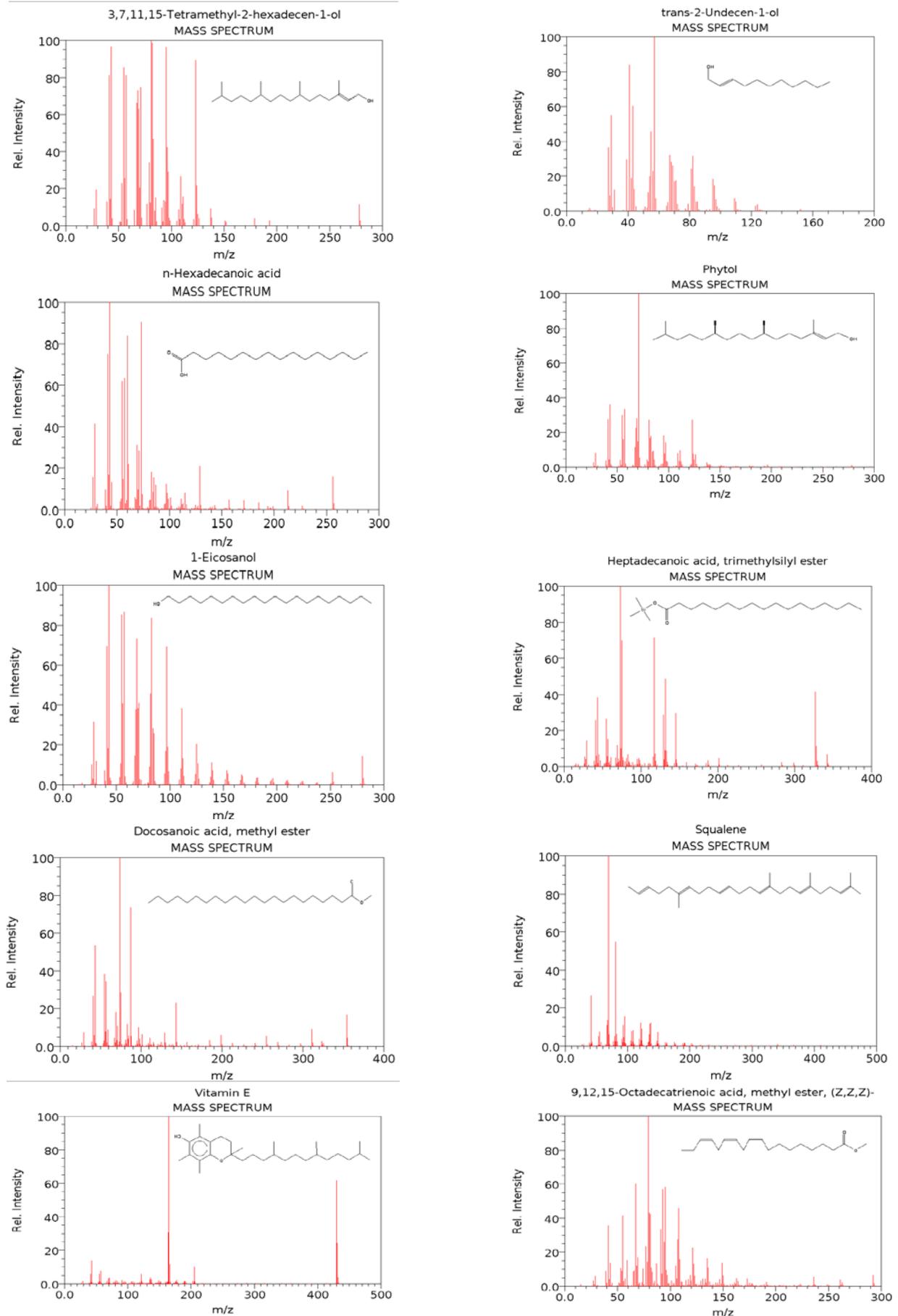


Fig. A-J): The individual fragmentation pattern of the important compounds

CONCLUSION

In the present study, fourteen phytochemical constituents have been identified from the ethanol extract of leaves of *Corchorus aestuans* by Gas Chromatogram - Mass Spectrometry (GC - MS) analysis. The presence of these phytochemical constituents justifies the use of this plant for various ailments by traditional practitioners. Isolation of individual photochemical constituents and subjecting it to biological activities are being undertaken.

CONFLICT OF INTERESTS

Declared None

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