

Gas Chromatography – Mass Spectrum Analysis of Volatile Components of Methanolic Leaves Extract of *Cordia Myxa*

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ABSTRACT

Medicinal plants have been used to treat human diseases for thousands of years because they have vast and diverse assortment of organic compounds that can produce a definite physiological action on the human body. The objectives of this study were analysis of the secondary metabolite products of methanolic leaf extract of *Cordia myxa*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC-MS analysis of *Cordia myxa* revealed the existence of the 2-[3-Cyclohexylaminopropylamino] ethylthiophosphate, Spiro[2.4]heptan-4-one, D-Glucose, 6-O- α -Dgalactopyranosyl, ϵ -N-Formyl-L-lysine, Dodecanoic acid, 3-hydroxy, Paromomycin, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 10-Methyl-E-11-tridecen-1-ol propionate, 1,3-Dioxolane, 4-[[2-methoxy-4-octadecenyl]oxy]methyl]-2,2, 5-Hydroxymethylfurfural, 6-Acetyl- β -d-mannose, E-9-Methyl-8-tridecen-2-ol, acetate, 4-Hexenal, 6-hydroxy-4-methyl-, dimethyl acetal, acetate, (Z, α -D-Glucopyranoside, O- α -D-glucopyranosyl-(1.fwdarw.3)- β , 2-Cyclohexylpiperidine, Dodecanoic acid, 3-hydroxy, Cyclopentadecanone, 2-hydroxy, Ethyl iso-allocholate, 3-O-Methyl-d-glucose, 13-Heptadecyn-1-ol, Trans-13-Octadecenoic acid, Dasycarpidan-1-methanol, acetate (ester, 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9-(acetyloxy)-3, α -Tocopheryl acetate.

Keywords: *Cordia myxa*, Chemical analysis, GC-MS, Products, Secondary metabolite.

INTRODUCTION

Plants are rich source of secondary metabolites with various biological activities^{1,2}. The chemical compounds present in the plant should be analyzed not only for discovery of drugs but also for identifying new phytocompounds for the synthesis of complex substance and to discover the actual remedies (*Cordia myxa* fruit locally known as "Bumber" is one of the largest genera in the family Boragiaceae, as about 300 species have been identified worldwide mostly in warmer region³⁻⁵. *C. myxa* is a sweet fruit because it contains the maximum amount of sucrose, glucose, fructose and high total dietary fiber, which plays one important role in decreasing risk of many diseases, *C. myxa* fruits are a rich source of proteins, carbohydrates, ash, fat and essential minerals⁶⁻¹¹. Medical plants are very important in our daily life as these are used for the treatment of many diseases. *C. myxa* contained various compounds which are responsible for protection against pathogenesis. Recently, there are trends for using plants in therapy (back to the nature) as a result of side effects and complications of chemotherapies. *C. myxa* is used in popular folk medicine as abortive in tropical region of the world Analgesic and as inflammatory agents in both acute and chronic phase¹². Chromatography is a separation technique in which a mobile phase carrying the mixture is moved through a selective absorbent stationary phase. It is a technique for quality control and standardization of phyto components. Gas chromatography involves the principle

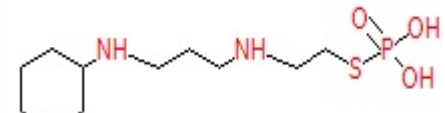
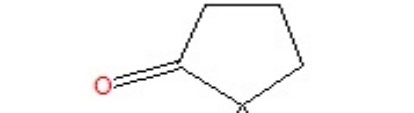
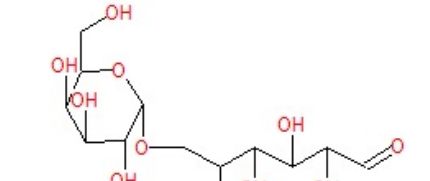
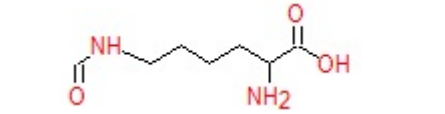
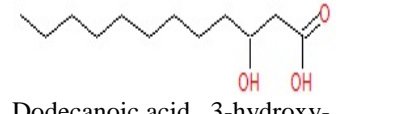
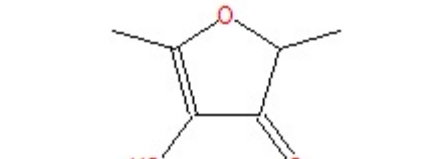
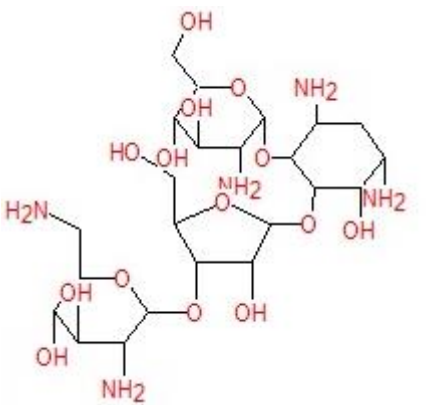
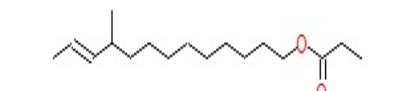
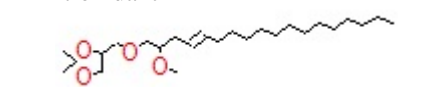
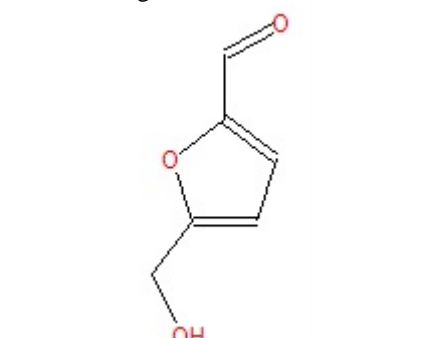
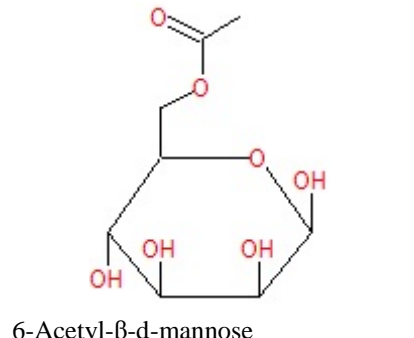
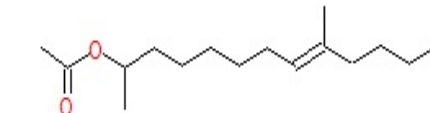
of adsorption and partition, is an important tool for separation of volatile compounds. Medicinal plants are directly analyzed for their existing compounds by GC-MS technique. It is mainly used for the determination of thermochemical constants, for purification of compounds and for qualitative and quantitative analysis of mixtures. Few reports are available on the pharmacological properties of the plant. The fruit of *C. myxa* is popularly used for the treatment of chest, urinary tract infections, diseases of the lung and spleen analgesic, anti-inflammatory cytotoxic, antiviral, antiulcer, anticancer, antihelminthic and anti-HIV-1¹³⁻¹⁶. Mucilage extract of *C. myxa* was shown to be active against promastigotes of *Leishmania major* and *L. infantum*¹⁷. The present study has been undertaken to investigate the bioactive compounds present in methanolic leaves extract of *Cordia myxa*.

MATERIALS AND METHODS

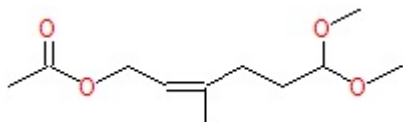
Collection of Plant material, extraction and isolation of Cordia myxa

Cordia myxa leaves were collected from Hilla city, middle of Iraq. *Cordia myxa* was washed with sterile distilled water and shade dried. The leaves were powdered and stored in an air tight container at room temperature. Methanolic leaves extract of *Cordia myxa* powdered were soaked in 500 mL methanol for 14 hours in a rotatory shaker. The filtrates were used for further phytochemical analysis¹⁸⁻²⁰.

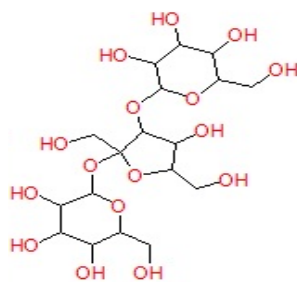
Table 1: Bioactive chemical compounds identified in methanolic extract of *Cordia myxa*.

 <p>2-[3-Cyclohexylaminopropylamino]ethylthiophosphate RT=3.321 Mw=296.13235 Pharmacological activity: anticancer activity</p>	 <p>Spiro[2.4]heptan-4-one RT=3.436 Mw=110.073165 Pharmacological activity: Anti-Bacteria</p>	 <p>D-Glucose,6-O-α-Dgalactopyranosyl- RT=3.545 Mw=342.11621 Pharmacological activity: Anti-carcinogenic</p>
 <p>ε-N-Formyl-L-lysine RT=3.859 Mw=174.100442 Pharmacological activity: Unknown</p>	 <p>Dodecanoic acid, 3-hydroxy- RT=4.014 Mw=216.1725445 Pharmacological activity: anti-HIV activity</p>	 <p>2,5-Dimethyl-4-hydroxy-3(2H)-furanone RT=4.563 Mw=128.047344 Pharmacological activity: Antioxidant</p>
 <p>Paromomycin RT=4.243 Mw=615.296303 Pharmacological activity: anti-HIV-1 agent</p>	 <p>10-Methyl-E-11-tridecen-1-ol propionate RT=4.975 Mw=268.24023 Pharmacological activity: anti-inflammatory, anticancer and diuretic properties</p>	 <p>1,3-Dioxolane, 4-[[2-methoxy-4-octadecenyl]oxy]methyl]-2,2 RT=5.210 Mw=412.35526 Pharmacological activity: antimicrobial and anti-inflammatory activity</p>
 <p>5-Hydroxymethylfurfural RT=6.823 Mw=126.031694 Pharmacological activity:</p>	 <p>6-Acetyl-β-d-mannose RT=7.395 Mw=222.073953 Pharmacological activity: Unknown</p>	 <p>E-9-Methyl-8-tridecen-2-ol, acetate RT=7.807 Mw=254.22458 Pharmacological activity: anti-malarial, anti-allergy</p>

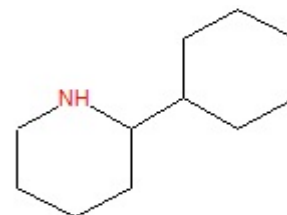
antioxidant



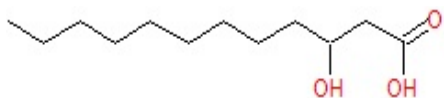
4-Hexenal , 6-hydroxy-4-methyl- ,
dimethyl acetal , acetate ,(Z)
RT=7.836
Mw=216.136159
Pharmacological activity:
Anti-bacterial, Antifungal Activity



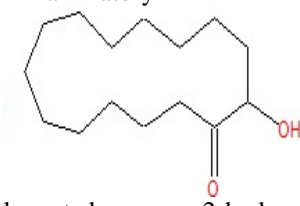
α -D-Glucopyranoside , O- α -D-
glucopyranosyl-(1.fwdarw.3)- β
RT=8.139
Mw=504.169035
Pharmacological activity:
anti-inflammatory



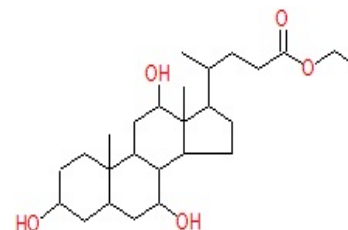
2-Cyclohexylpiperidine
RT=8.820
Mw=167.167399
Pharmacological activity:
anti-inflammatory agent



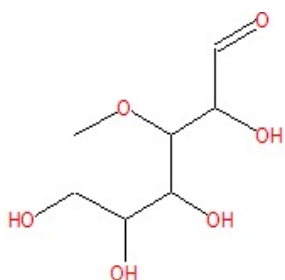
Dodecanoic acid , 3-hydroxy-
RT=9.181
Mw=216.1725445
Pharmacological activity:
anti-HIV activity



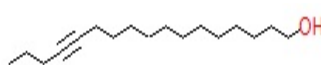
Cyclopentadecanone ,2-hydroxy-
RT=9.701
Mw=240.20893
Pharmacological activity:
anti-inflammatory and *Anti* cancer



Ethyl iso- allocholate
RT=13.564
Mw=436.318874
Pharmacological activity:
Antioxidant



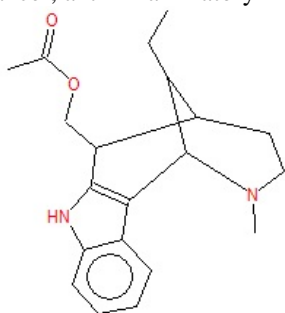
3-O-Methyl-d-glucose
RT=15.486
Mw=194.079039
Pharmacological activity:
anti-cancer, anti inflammatory



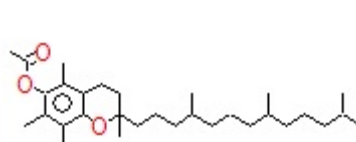
13-Heptadecyn-1-ol
RT=16.556
Mw=252.245316
Pharmacological activity:
anti- inflammatory *activity*



Trans-13-Octadecenoic acid
RT=16.608
Mw=282.25588
Pharmacological activity:
anti-inflammatory activity



Dasycarpidan-1-methanol , acetate
(ester)
RT=18.176
Mw=326.199429
Pharmacological activity:
antimicrobial, antioxidant and anti-
inflammatory



α -Tocopheryl acetate
RT=25.740
Mw=472.391645
Pharmacological activity:
Anti-Inflammatory and Analgesic
activity

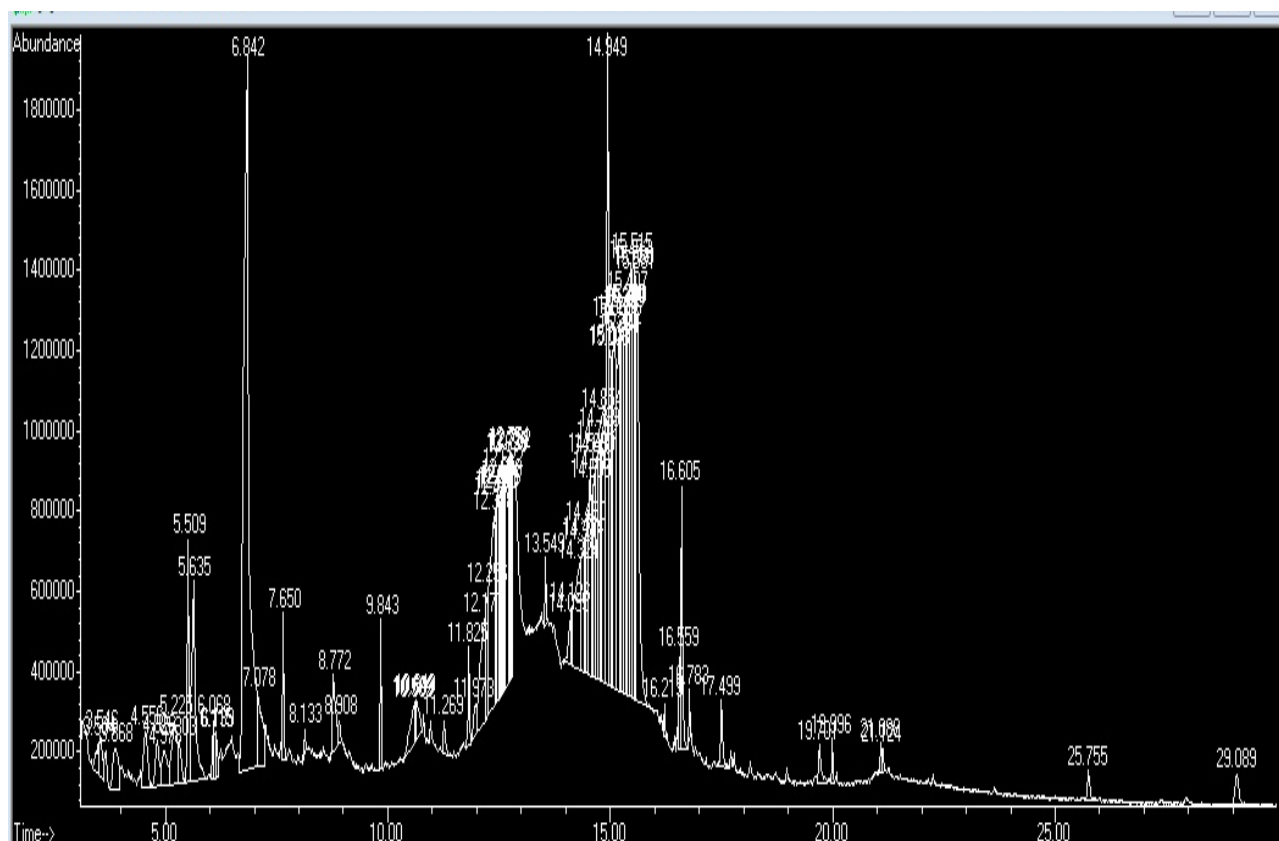


Figure 1: GC-MS chromatogram of methanolic extract of *Cordia myxa*.

Gas chromatography – mass spectrum analysis

The Gas chromatography – mass spectrum analysis of methanolic extract of *Cordia myxa* was made in a (Agilent 789 A) instrument under computer control at 70 eV. About 1 μ L of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes. The time from when the injection was made (Initial time) to when elution occurred referred to as the Retention time (RT). While the instrument was run, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector²⁰⁻³¹. The M/Z (mass / charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using gas chromatography and mass spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries³²⁻⁴⁰.

RESULTS AND DISCUSSION

Characterization of phytochemical compounds

GC-MS is a powerful technique used for many applications which has very high sensitivity and

specificity. GC-MS analysis of compounds was carried out in methanolic extract of *Cordia myxa*, shown in Table 1. The GC-MS chromatogram of the 23 peaks of the compounds detected was shown in Figure 1. The set up peak were determined to be 2-[3-Cyclohexylaminopropylamino]ethylthiophosphate, Spiro[2.4]heptan-4-one, D-Glucose, 6-O- α -Dgalactopyranosyl, ϵ -N-Formyl-L-lysine, Dodecanoic acid, 3-hydroxy, Paromomycin, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 10-Methyl-E-11-tridecen-1-ol propionate, 1,3-Dioxolane, 4-[(2-methoxy-4-octadecenyl)oxy]methyl]-2,2, 5-Hydroxymethylfurfural, 6-Acetyl- β -D-mannose, E-9-Methyl-8-tridecen-2-ol, acetate, 4-Hexenal, 6-hydroxy-4-methyl-, dimethyl acetal, (Z, α -D-Glucopyranoside, O- α -D-glucopyranosyl-(1.fwdarw.3)- β , 2-Cyclohexylpiperidine, Dodecanoic acid, 3-hydroxy, Cyclopentadecanone, 2-hydroxy, Ethyl iso-allocholate, 3-O-Methyl-d-glucose, 13-Heptadecyn-1-ol, Trans-13-Octadecenoic acid, Dasycarpidan-1-methanol, acetate (ester, 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9-(acetyloxy)-3, α -Tocopheryl acetate. Phytochemicals present in herbal medicinal plants, spices, vegetables and fruits have a protective role against many diseases⁴¹⁻⁴⁸. They also inhibit oxidation process through a variety of mechanism and they act as radical scavengers. Quantitative assay for the presence of plant phytochemical analysis of *C. myxa* indicated the presence of restively high levels of glycosides, flavonoids, sterols, saponins, trepenoids, alkaloids, phenolic acids, gums and mucilage. The fruit of *C. myxa* is used for treatment of chest, urinary infection, disease of the lung and spleen and against liver

fibrosis when measured level of hepatic enzymes ALT, ALP, AST¹². Antiradical activity was measured, it contain 40/100g of scorbic acid and antioxidant of *C. myxa* which may be due phenols, scorbic acid and lycopene¹³. Most important of such compounds are alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds^{49,50}. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity¹. A number of such compounds have been isolated from plants which could be used for the development of new drugs to inhibit the growth of bacterial and fungal pathogens and to quench ROS with possibly novel mechanisms of action and low toxicity to the host cell⁵¹⁻⁵⁸.

CONCLUSION

Twenty three bioactive chemical constituents have been identified from methanolic extract of the *Cordia myxa* by gas chromatography-mass spectrometry technique. Secondary metabolite products of *Cordia myxa* forms a primary platform for further pharmacological investigation for the development of new potential anti-insect compounds.

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