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RESEARCH ARTICLE

CHEMICAL COMPOSITION OF *CORDIA MYXA* FRUIT: PHYTOCHEMICAL SCREENING AND IDENTIFICATION OF SOME BIOACTIVE COMPOUNDS.

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Abstract

Cordia myxa is a fruit grown around the globe for its nutritional and medicinal benefits. The identity and relative concentration of some compounds in the fruit extract was reported and preliminary phytochemical screening was performed. Ethanol extract was obtained by heated maceration using ethanol. The extract was screened for the presence of carbohydrates, proteins, glycosides, alkaloids, flavonoids, phenolic compounds, saponins, and tannins. Gas Chromatography-Mass Spectroscopy was performed to identify the volatile compounds with their relative concentrations. The fruit was found to contain all tested compounds except saponins; and notable compounds identified with GC-MS include phytol, linoleic acid, olealdehyde, stigmastanol, γ -sitosterol, several esters and phosphate ester-derivatives, and a fat-soluble form of ascorbic acid. The study concludes that the fruit contain many bioactive compounds –with hyperlipidemic, hyperglycemic, cytotoxic, anti-inflammatory, and thyroid-inhibiting properties– but quantitative analysis is yet to be performed. More studies are needed to fully understand the chemical composition of the fruit –not just the volatile potion.

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Introduction:-

Plants have been used as medicines with qualities all over the world and for thousands of years. The study of the chemical composition and biological activities of these plants have received renewed scientific interest in the past few years [1].

Cordia myxa is a species of flowering plant in the borage family. The broad-leaved deciduous tree of *Cordia myxa* grows in many parts of the world, including tropical Africa, tropical Asia, the Americas, and the region stretching from eastern Mediterranean to eastern India [2-3].

The light pale fruit begins appearing in July-August, and tends to darken as ripening proceeds. When fully ripe, the translucent pulp, being full of mucilage, is quite sweet in taste [2-3].

The fruit is widely used in traditional medicine especially in the Middle east, India, and China. Due to certain demulcent properties, the fruit has been widely used to treat respiratory infections, coughing, and sore throat; also

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used as a diuretic. The pulp is used to mature abscesses, calm rheumatic pain, treat ringworm, and as an anthelmintic [2] [5-7].

Scientific literature indicates that *Cordia myxa* has certain analgesic, anti-inflammatory, immunomodulatory, antimicrobial, antiparasitic, insecticidal, cardiovascular, respiratory, gastrointestinal and protective effects [4] [8-10].

Prior studies have confirmed the presence of the nontoxic pyrrolizidine alkaloid macrophylline but no quantitative data is available. Moreover, *Cordia myxa* samples collected from the region have been found to contain trace amounts of 6 Polycyclic Aromatic Hydrocarbons (PAHs) with a total concentration 411.6 ng/g [12,13].

Cordia myxa fruit is regarded as a good source for fibers, proteins and carbohydrates, and some studies suggest promoting the fruit as a carbohydrate and protein supplement for cereal-based diets in poor rural communities [4].

The aim of the study was to perform preliminary phytochemical screening and identify some volatile compounds found in the fruit extract via Gas Chromatography-Mass Spectroscopy. Ethanol was chosen for its ability to extract the maximum plant constituents (flavonoids, alkaloids, tannins, glycosides, steroids, phenols, cardiac glycosides, saponins, carbohydrates, and proteins) compared with other solvents [14-16].

Materials and Methods:-

Fruit Sample:-

Cordia myxa was cultivated during August in Basra, Iraq. The fruit was identified by morphological comparison with traits described by AP database provided by Conservatory and Botanical Garden/ Geneva and South African National Biodiversity Institute.

Extraction Method:-

The extraction method was designed to extract maximum amount of bioactive compounds without altering thermosensitive and volatile compounds. The whole fruit was shade dried and pulverized to a powder using a Philips HL7720 mechanical grinder. The powder was immersed with ethanol absolute of 99.8% concentration and the extract heated to 70 °C with stirring for 1 hour. Vacuum-filtration was performed drying over anhydrous sodium sulfate to remove traces of water. All materials were obtained from Sigma Aldrich.

GC-MS Analysis:-

A diluted volume of 8.00 µl was injected in the system using AOC-20i/s auto-injector with split injection mode; injector temp. was 250 °C. The test was run using a Shimadzu GCMS-QP2010 Ultra equipped with polar standard capillary column of 59 m length. Helium gas was used as the carrier gas with a total flow of 34.0 mL/min (column flow of 1.00 mL/min). The oven temp. was programmed from 40 °C (5 min. hold time) to 180 °C (1 min hold time) and to 300 °C (5 min. hold time). The total running time was 37.0 min. For the mass spectroscopic test, ion-source temp. was 200 °C; solvent cut time was 3.00 min.; detector gain was 0.70 kV. Compounds were identified by their mass spectra and retention indices using the NIST Mass Spectral Library and the Retention Index Database. GCMS Insight Software Package was used to process the data.

Phytochemical Qualitative Analysis:-

Phytochemical screening for the following compounds was performed using standard methods [17]:

1. **Carbohydrates:** Extract was treated with Benedict's reagent and heated gently. Formation of orange red precipitate indicated a positive result.
2. **Proteins:** The extracts were treated with a few drops of concentrated nitric acid; yellow coloration of the sample indicates a positive result.
3. **Glycosides:** Extract was dissolved in bromine water; the formation of yellow precipitate which indicates a positive result.
4. **Saponins:** Extract was mixed with water and shaken; formation of froth that's stable for 15 minutes indicates a positive result.
5. **Alkaloids:** Extract was treated with few drops Wagner's reagent; precipitation indicates a positive result. Wagner's reagent was prepared by dissolving 2g of iodine and 6g of KI in 100ml of water.
6. **Flavonoids:** A few drops of Ferric chloride solution were added to the extract; the formation of dark red color indicates positive results.

7. **Phenolic compounds and tannins:** Sodium hydroxide solution was added to the extract; the formation of yellow to red precipitate indicates a positive result.

Results:-

A total of 20 volatile compounds was identified in the extract using GC-MS analysis; their identities, retention indices (compared to a series of n-alkanes), and relative concentrations (percentage of area under the chromatogram) are shown in Table 1. In addition, the chromatogram is shown in Figure 1.

The highest identified concentration belongs to 1-(+)-Ascorbic acid 2,6-dihexadecanoate followed by - Octadecenal, (Z) and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester; with 9.94%, 9.54%, and 8.89% respective concentrations. Phytol and Stigmasterol had lower concentrations of 6.38% and 5.86%, respectively. Concentration of other compounds ranged between 2-5%.

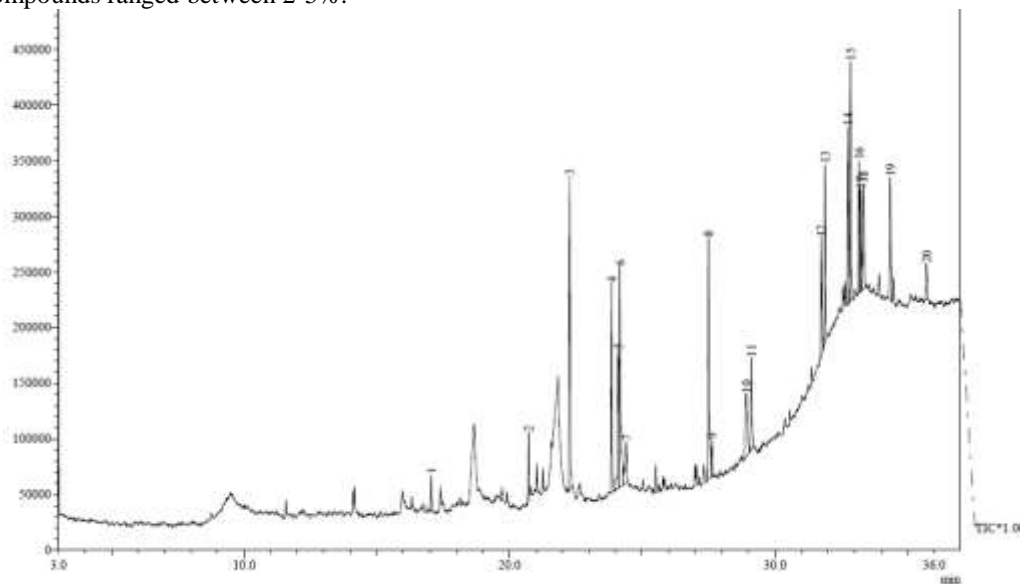


Figure 1:-GC-MS chromatogram for the ethanol extract of *Cordia myxa* fruit.

Table 1:-Description of identity and concentration of compounds identified with GC-MS.

Name	RI*	RC** (%)
Phosphoric acid, diethyl octyl ester	-	0.99
9-Octadecenal, (Z)-	2007	9.54
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2045	1.87
Phytol	2045	6.38
Oleoyl chloride	2131	5.16
Octadecanoic acid	2167	2.72
9,12-Octadecadienoic acid (Z,Z)-	2183	5.29
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2498	8.89
Octadecanoic acid, 2,3-dihydroxypropyl ester	2681	3.48
1,2-Benzenedicarboxylic acid, diisooctyl ester	2704	1.17
Cholestan-3-one, 4,4-dimethyl-, (5 α)-	2704	8.25
Stigmastanol	2720	4.86
γ -Sitosterol	2731	3.46
Stigmasterol	2739	5.86
1-Heptacosanol	2948	4.16
1-Triacontanol	3246	5.30
Dihydro-iso-solanidine 3,5-dinitrobenzoate	4279	1.92
α -Tocopherol- β -D-mannoside	4489	5.43
1-(+)-Ascorbic acid 2,6-dihexadecanoate	4765	9.94

Total		100.00
* Retention Index (series of n-alkanes).		
** Relative Concentration (area %)		

The preliminary phytochemical screening indicated the presence of carbohydrates, proteins, glycosides, alkaloids, flavonoids, phenolic compounds, and tannins. Saponins test produced a negative result.

Discussion:-

This study discusses the biological effects of some identified volatile compounds according to previous scientific literature, which might provide a preliminary reference for further studies regarding the importance of the fruit. The identified biologically-active ingredients of the fruit had a relative concentration falling between 3-8%, except the fat-soluble form of ascorbic acid which accounted for around 10% of the identified portion.

A summary for the bioactivity of some identified compounds and their relative concentrations is provided in Table 2. Other active and inactive compounds were present in the extract; notably, stearic acid and several types of esters.

Table 2:- Bioactivity of some identified compounds and their relative concentrations

Compound	Bioactivity	RC (%)
Stigmasterol	(i) Prevents certain types of cancers including ovarian, prostate, breast, and colon cancers. (ii) Posses certain antioxidant, hypoglycemic, hyperlipedimic, and thyroid inhibiting properties. [18,19]	5.86
Phytol	(i) Posses certain cytotoxic effects on human HeLa cells and antiproliferative effects on different types of human cells. (ii) An active anti-inflammatory agent. (iii) Linked to regulating blood glucose and can possibly restore the metabolic functions of type two diabetic patients. [20,21]	6.38
Linoleic acid	(i) Pro-inflammatory and anti-inflammatory effects. (ii) Helps in weight control. (iii) Inhibits blood clotting inhibitor (iv) Aids in the treatment of a broad-spectrum of diseases. [22-33]	5.28
Phosphate ester-deriv.	Enhance drug penetration through skin (emollient properties).	0.99
Ascorbic acid	(i) Must be ingested in human diet for survival. (ii) Linked to reduction of incidence of cancer, regulating blood pressure, and enhancing immunity. (iii) Essential for drug metabolism, urinary excretion, and tissue regeneration. (iv) Needed for enzymes stimulation, collagen biosynthesis, hormonal activation, antioxidant activity, detoxification of histamine, amongst others. [34]	9.94
Olealdehyde	FAAH inhibition effects [35]	5.16
Stigmastanol	Hypolipidemic agent (lowers the levels of certain lipids in the blood and inhibit the absorption of cholesterol from the diet) [36,37].	4.86
γ -Sitosterol	Hypolipidemic agent (lowers the levels of certain lipids in the blood and inhibit the absorption of cholesterol from the diet) [38].	3.46

The phytochemical screening indicated the presence of many phytochemicals: carbohydrates, proteins, glycosides, alkaloids, flavonoids, phenolic compounds, and tannins. These results are consistent with previous scientific literature except that saponins were not detected [4].

The chemical composition of *Cordia myxa* fruit is not yet fully understood. More identification techniques are necessary to confirm the chemical composition of the fruit, notably HPLC (injecting with standards) and LC-NMR.

In conclusion, *Cordia myxa* fruit contains many bioactive compounds and can be part of a healthy diet. We suggest carrying out more studies to assess the bioactivity of the fruit, as well as more phytochemical studies.

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Conflicts of Interest:-

The authors declare no conflict of interest.

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