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A CHEMICAL STUDY COMPARED BY USING GC-MS ANALYSIS OF THE ACTIVE INGREDIENTS FROM THE ETHANOLIC EXTRACTS OF LEAVES AND FLOWERS OF *CANNA INDICA* L. PLANT

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Abstract

The current study aimed at diagnosing and identifying the secondary metabolites (active compounds) derived from the ethanolic extracts leaves and flowers of the specie *Canna indica* in Iraq**.** Using the gas separation Gas Chromatography-Mass Spectrometry (GC-MS) for the ethanol extract of leaves and flowers of a plant and dependence on retention and molecular weight compound are known. The results of the present study showed the abundance of the chemical content quantity and quality, in addition to the variation in the types and time of detention of the phytochemical compounds, recorded a 52 chemical compound including eight chemical compounds in the leaves extract and 44 chemical compounds in the flowers extract; Phenolic such as; Benzenemethanol, 3-fluoro, 4-Fluorobenzyl alcohol, Trpenes; 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl). The extracts were combined with a Docosane, and the chemical extracts of the leaves were found to have many effects, including Phytol. The flower extract was Chemical compound distinguished by the abundance and variety of active compounds as Carbohydrates (sucrose); Xylitol and the recurrence of chemical compounds;12- Bis (2-nitrophenoxy) dodecane In the same alcoholic extract with different concentration and retention time. The present results indicate that the ethyl extracts of Leaves and Flowers *C. indica* contains many of the biologically effective secondary metabolites, special medical properties, which can be used to treat various Diseases.

Key words : *Canna indica* L., GC-MS, Phenolic, secondary metabolites, Terpenes.

Introduction

Finding a therapeutic power from plants is an ancient idea dating back to prehistoric times as plants are rich sources of secondary metabolites, also important because of a several biological activities with difference of structural arrangement and properties (Ashal *et al.,* 2017). The chemical variations shown by plant taxonomic hierarchies are similar to those shown in morphological, anatomical, and other traits used to differentiate between these taxa, these variations are also important taxonomic sources, (Stace,1985) pointed out that the chemical evidence has been used since the start of human naming and classification of plants depending on their medical specialists and their economic uses, So, the chemical studies and their relationship to plant classification is important in the distinction between the plants through the smell and taste or both, and this was followed by the herbaceous with the plant.

Canna indica L. (Linnaeus) also known as Indian Shoot is a specie of the *Canna* genus, belonging to the Cannaceae family, table 1 illustrates Botanical Classification as mentioned (Shishir *et al.,* 2008; Al-Snafi, 2015).

States that the Kana family has one species, *Canna*, of which there are 25 species in the world (Guest,1966), three of them in Iraq, including *C. flaccida* and *C. generalis*, mentioned more than 60 species in the world and Iraq, of which one specie is *C. indica* which is currently under study (Al-Musawi,1987). The Canna genus is local tropical and sub-tropical regions of Southern United State, to Philippines, and India, usually live in tropical and subtropical rain forests, premontane, montane, and gallery forests, add to near streams, in uncultivated public

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Table 1: Botanical Classification(Shishir *et al*,2008; Al-Snafi, 2015.

Kingdom	Plantae
Subkingdom	Tracheobiont
Super division	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Zingiberidae
Order	Zingiberales
Family	Cannaceae
Genus	*Canna*
Species	*indica*

lands or on roadsides (Mishra *et al.,* 2013).The description of the plant has been described accurately by(Darsinl *et al.,* 2015) In detail, there is no need to mention them in this research. *C. indica* or Synonyms names as *Canna coccinea* Mill and *Canna edulis* KerGawl, In addition it famous for several names in Arabic: MUZ FAHAL and CANNA HANDI, in English: CANNA INDIAN SHOT, with other names have been mentioned by (Al-Snafi, 2015). considered plant Indian Shot adornment plant, planting for beautiful Flowering and bright green Leaves as such can by benefit from black seeds in industry jewelry like necklace, especially in India as walla as extraction red dye and fibers used in manufacture sling and fabrics (Chakravarty,1976). *C. indica* Roots, Leaves and Flowers are used for medicinal purpose this parts plants are loaded source of phytochemicals like as Flavonoids, and others phenolic compounds, Terpenes and Esters, this compounds received considerable attention because of their physiological function contain, several studies have mentioned various biological activities like; anti-inflammatory, diarrheal, antioxidant (Al-Snafi,2015). Numerous studies have reported the biological effectiveness of several chemical compounds and metastases: Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester is a monoacylglyceride or Palmitic acid derivative, which is added to commercial food products in small quantities.

Materials and Methods

1. Samples Collection

Samples were collect from period 20 September 2018 to 30 November 2019, from AL-Diwaniyah city, which is one of the cities of southern Iraq and the Middle Euphrates region pass by it a branch of the Euphrates River, known as Shat al-Diwaniyah, passed about 180 km from Baghdad.

2. Extraction

The process of extraction was done depending on (Ladd,1978) botanical extracts of plant samples Plant samples were collected, washed from the soil and dried at room temperature and left for a period of one month to dry completely, then grinded by an electric grinder taking 25 gram of dry powder and then extracting the substance from the Saxholate extract with 250 ml of ethyl alcohol at a concentration of 99% for 24 hours, after which the alcohol extract was using a water bath to make extract concentration and placed in sealed, sealed bottles in the refrigerator until use.

Sample analysis in GC-MS device

- The active compounds were diagnosed and evaluated by (Model Agilent 5977A gas chromatograph mass spectrometry)
- System manufactured by the GC Clarus 500 Perkin Elmer system, which includes the AutoSampler [AOC-20i + s] for compounds and the bound gas chromatography By mass spectrometry.
- According to the following conditions: General column of the Elite-1 fused silica capillary column with dimensions (0.25 × 30 Hp -5ms).
- Helium gas (99.999%) was used as a conveyor gas at a continuous flow rate of 1 ml. Min -1 under pressure 6.8psi.
- Size of injected liquid extracts is 2 ìl and works by split (10:1) each one separated.
- Oven temperature is programmed automatically at 40 ° C (the temperature is equal to three minutes) with an increase of (80-250) 50°c / min (hold 10 min), then (250 -310)10°c /min (hold 22min)
- The mass spectra were taken on a 70 EV basis with a time interval of 0.5 seconds.
- The pressure inside the device is 49.5kPa and the rate of 1 ml.Min^{-1}.
- The total time to start and end the device working for each sample is 28 minutes.
- The relative amount of each component was calculated by comparing its average surface area to the total area based on Turbo Mass Ver 5.2.0 for mass spectra and chromatograms supplied with the device, all this information is automatically programmed on the machine.

4. Determination of raw chemical compounds

The components were determined according to GC-MS mass spectrometry and the database of the National Institute of Standards and Technology (NIST) Determination of raw chemical compounds. The output spectrum of the unknown component was compared with

a range of known components stored in the NIST library to confirm the name, structure of the components molecular and weight, of the test materials. This test carried out at of the Lab GC-MS/ Environment and Water Service/ Ministry of Science and Technology. Depending on the highest percentage and importance of compounds were diagnosed in the current study.

Results and Discussion

The studies of the active principles in the Ethanol of leaves and Flowers extract of *C. indica* by GC-MS analysis showed the presence of 52 Chemical compounds. The active principles with their retention time (RT) and percent relative composition are presented in (Fig. 1 and 2). It should be noted that this is the first study of its kind in Iraq for this plan. The study showed important changes in terms of their containment of phenolic compounds, terpenes, alkanes, Fatty acids and another compound. (Table 2), (Fig. 3 and Fig. 4). These forms can be used as classifiers to support and promote other studies of phenotypic, diagnostic, cellular and other types. These forms can be used as classifiers to support and promote other studies of phenotypic, diagnostic, cellular and other types.

Table 2: Types of Chemicals found in species *C. indica* and their location in the plant part.

No.	Type of chemical compounds	Plant part	
		Leaves	Flower
1	Phenolic	+	+
2	Terpenes	+	+
3	Alkaloids	-	+
4	Carbohydrates(sucrose(	-	+
5	Fatty acids	+	+
6	Esters		+
7	Carboxylic acids	-	+
8	Alkene		+

\+ Presence of the compound - Absence of the compound

The current study which was conducted by GC-MS analysis was recorded 52 Chemical compound (Fig.2 and Fig.1), which are identification based on the, molecular weight, retention time, peak areas and molecular formula. These compounds vary in their concentration Table 3, which has; Phytol, the superior purity was recorded in the leaves, as it was concentrated was repeated twice 10.90 % and 22.99% at the time of appearance 15.863 and 18.200, mint respectively. Followed by the compound Hexadecanoic acid, ethyl ester in 22.55 % concentration with a time 17.016 mint which it fatty acid compound, as for the alkanes recorded the compound Docosane highest concentration 6.61% in time 18.830 mint. While less

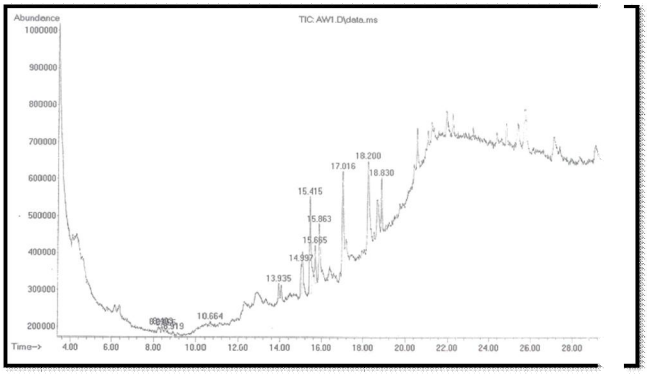


Fig. 1: GC-MS chromatogram of ethanol extract o *C. indica* parts of Leaves.

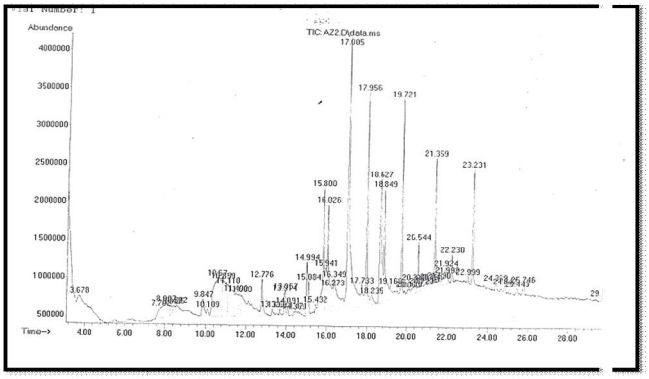


Fig. 2: GC-MS chromatogram of ethanol extract of *C. indica* parts of Flowers.

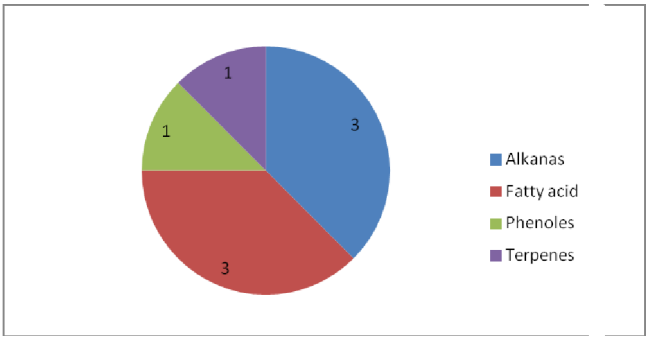


Fig. 3: Type of Chemical compound in Leaves extract of *C.indica* plant.

concentration was 0.95% for Adenosine, 2—methyl (Phenolic – Glycosides) was the first compound it appeared in time 10.664 mint, in Leaves extract.

This compound acts as an emulsifier to mix fat and water with these products and protect them from damage.(Cheesbrough, 2000). Also is Antioxidant, Hypocholesterolemic, Pesticide, Flavor, Hemolytic 5-Alpha-reductase inhibitor Antiandrogenic (Kavitha *et al.,* 2014) Phytol, diterpene, Hypocholesterolemic, Antiarthritic, Hepatoprotective, Hypocholesterolemic, Antihistaminic, Anticoronary, Insectifuge and Antieczemic (Soso *et al.,* 2019) 7-Methy1—Z—tetradecane-1—ol

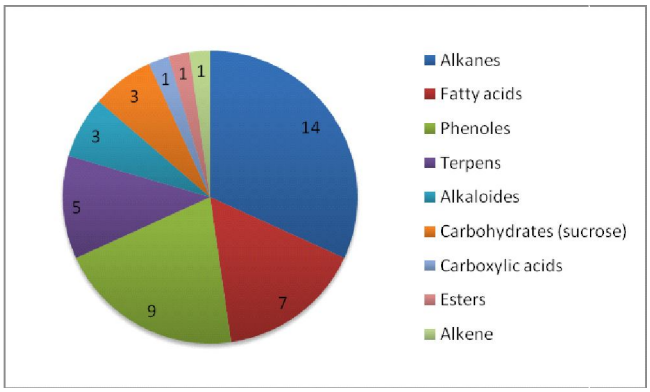


Fig. 4: Type of Chemical compound in Flowers extract of *C. indica* plant.

acetate; Anti-cancer, anti-inflammatory and hepatoprotective (Hameed *et al.,* 2015) H.exadecanoic acid, ethyl ester; Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavour, Hemolytic, Alpha-reductase inhibitor (Sudha *et al.,* 2013).

As for the chemical compounds that were recorded in the extract of Flowers about (44) compounded chemically Fig. 4, varied between Phenolic, Terpens Alkaloids, Carbohydrates (sucrose (Fatty acid and others Table 2).

The current study recorded three carbohydrate (sucrose) like; Xylitol and Ribitol but in small proportions ranged from 0.46% to 0.86% in at different times, also is found nine Phenolic Chemical compound, the highest concentration of the compound 5-Hydroxymethyl furfural 7.93% appeared in min 10.676, This compound has been repeated four times in good proportions table 4, the lowest concentration of 0.09% from the compound Benzenemethanol, 3—fluoro.

As for Terpenes compound this study recorded five Chemical compound; highest concentration reached 1.07% from the 1, 3-Cyclohexadiene, 1-methyl-4-(1-methylethyl) - compound which it appeared in 9.848 min. the lowest concentration was 0.14% from compound the 22—Ergosten-3-ol, acetate, (3.beta, 5.alpha., 22E)-, The compound Cyclohexane is repeated three times but in small proportions ranged from (0.21% - 0.83%) with at different times. The current study also recorded one carboxylic acid compound, Acetic acid, 4, 4a, 6b, 8a, 11, 11, 12b, appeared at 20.935 minute and with concentration 0.44%.

Either for Alkaloids compounds It was the highest concentration 1.51% of the compound Methylbutyl)—5—phenyl yridine, appeared at 15.942 min. 1,12—Bis(2—nitrophenoxy) dodecane, have been repeated twice with the lowest concentration 0.27% and 0.34% respectively. Also results found one Esters compound; Nonadecyl trifluoro acetate in 1.05% concentration.

The study also recorded the presence of 14 Alkanes compounds; 9—Nonadecene recorded the highest concentration 5.71% appeared at 15.798 min), the lowest

Table 3: Analysis GC-MS of an extract of Leaves of *C. indicza.*

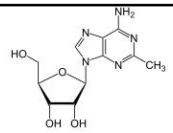
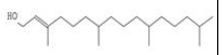
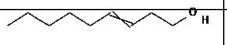
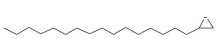
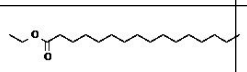
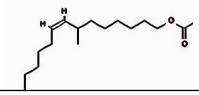
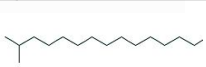
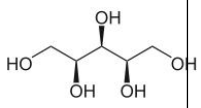
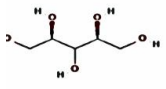
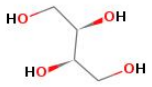
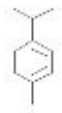
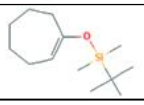
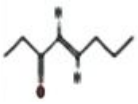
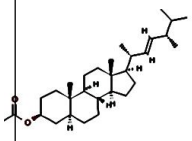
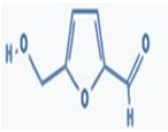
N. Prak	CAS.N0	Retention time (min)	Peak Area%	Molecular Formula	Structure	Compound Name	No.	Nature of compounds
5	016526-56-0	10.664	0.95	$C_{11}H_{15}N_5O_4$		Adenosine, 2—methyl	1	Phenolic - Glycosides
10	1000375—01-4	15.863	10.90	$C_{20}H_{40}O$		Phytol	2	Terpenes
12		18.200	22.99					
8	010340—23—5	15.415	19.16	$C_9H_{18}O$		3-Nonen-1-ol, (Z)-	3	Faty acids
9	007390—81—0	15.665	6.23	$C_{21}H_{44}O_3$		Oxirane, hexadecyl	4	
13	000628-97—7	17.016	22.55	$C_{18}H_{36}O_2$		Hexadecanoic acid, ethyl ester	5	
6	1000130—99—6	13.935	2.15	$C_{17}H_{32}O_2$		7-Methyl—Z—tetradecen-1—ol acetate	6	Alkanes
7	001560—93—6	14.997	4.32	$C_{16}H_{34}$		Pentadecane, 2-methyl-	7	
14	000629-97—0	18.830	6.61	$C_{22}H_{46}$		Docosane	8	

Table 4: Analysis GC-MS of an extract of Flowers *C.indica.*

Pr. Prak	CAS.N0	Retention time (min)	Peak Area%	Molecular Formula	Structure	Compound Name	No.	Nature of compounds
2	000087—99—0	7.762	0.86	$C_5H_{12}O_5$		Xylitol	1	Carbohydrates sucrose)
8	000488-81—3	10.106	0.29	$C_5H_{12}O_5$		Ribitol	2	
4	002319-57—5	8.316	0.46	$C_4H_{10}O_4$		1,2,3,4-Butanetetrol, [S-(R*,R*)]—	3	
7	000099—86-5	9.848	1.07	$C_{10}H_{16}$		1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	4	Terpenes
24	1000189—30—7	15.434	0.34	$C_{13}H_{26}$		OSiButyl—cycloheptene	5	
15	01 4 129 — 4 8 — 7	13.324	0.25	$C_8H_{14}O$		4-Octen—3-one	6	
38	056009—20—2	20.063	0.28	C_6H_{12}		Cyclohexane	7	
40		20.351	0.83					
46		21.247	0.21					
39	055515—02—1	20.139	0.14	C_{30}		$H_{50}O_2$22—Ergosten-3-ol, acetate, (3.beta,5.alpha.,22E)-	8	
9	000067-47—0	10.676	7.93	$C_6H_6O_3$		5-Hydroxymethyl furfural	6	Phenolic
10		10.858	3.85					
11		11.108	4.41					
12		11 488	1.77					

concentration 0.27% from Nonacosane compound, as for Docosane compound the compound has been repeated twice with different concentration and impression time.

The results of the present study showed the presence of seven fatty acid compounds like Hexadecanoic acid, ethyl ester compound obtained the highest concentration who reached 16.43% between fatty acids and others Chemical compound and it subscribe between both extracts.

Finally, a single Alkene compound, Pentadec-7-ene, 7-bromomethyl, was recorded but repeated four times in succession with concentrations; 0.25%, 0.22%, 0.29% and 1.37% appeared at 20.723, 21.155, 21.588 and 21. 922 min respectively

Previous studies have shown that secondary metabolites have a biological significance: 9,17-Octadecadienoic acid (Z,Z): Hypocholesterolemic,, Antiarthritic, Insectifuge-Anti-inflammatory, Cancer preventive, Anticoronary, Hepatoprotective, Nematicide Antihistaminic, Insectifuge, Antiandrogenic and reductase inhibitor (Al-Mayyahi, 2017). Xylitol; is a naturally occurring pentose sugar alcoholusedassweetener, Sweetening power of xylitol is equivalent to sucrose; however, other polyols possess sweetness than low sucrose. It has one-their caloric content than conventional sugar and thus has potentialtore place sucrose in less caloric products (Rehmana *et al.,* 2015). The current study, together with a study of (Ashal *et al.,*2017) found

that *C.indica* contains Terpens and alkanes. And agreed with study (Lamaeswari and Ananthi, 2012) that phytochemical analysis of *C. indica* flower showed that they contained various phytochemicals including alkaloids, carbohydrates, flavonoids, terpenoids, glycosides, steroids, tannins, phlobatinins and saponins.

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