Bioactive Chemical Compound Characterization in Dried Leaves and Flower Powder Extract of *Canna indica* L. using GC MS

Baranidharan R[1]*, D. Keisar Lourdusamy[1], K. Chandrakumar[2], K. Rajamani[1], S. Karthikeyan[3]

Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore

*Corresponding author E-mail id: arunbarani72@gmail.com

Short title: Metabolites present in *Canna indica* L. by GC MS

[1] Department of Floriculture and Landscape Architecture, HC & RI, Coimbatore

[2]Department of Renewable Energy Engineering, AEC&RI, TNAU, Coimbatore

[3]Center for Post-Harvest Technology, TNAU, Coimbatore

Abstract

Canna indica contains numerous natural bioactive compounds which having complex intriguing chemical structures. Plants that release of active compounds have been isolated, purified and employed in a wide range of application. The present study aimed at analyzing and identifying the bioactive compounds (Secondary metabolites) derived from the methanolic extracts of *Canna indica* leaves and flowers using the GC-MS (Gas Chromatography Mass Spectrometry) were analyzed dependence on retention and molecular weight compound are known. And its natural properties being available in pure form, nontoxic with a wide spectrum of biological function was found this application in the formation of various medicinal properties. The variation in the types and time of detention of the phytochemical compounds, recorded an 80 chemical compound including fifty chemical compounds in the leaves extract and 40 chemical compounds in the flowers extract. The current results indicate that the methanolic extracts of leaves and flowers of *Canna indica* L. contains many of the biologically effective secondary metabolites, especially medicinal properties, which can be used to treat many diseases.

Keywords: Leaves, Flowers, *Canna indica* L., GC-MS, Secondary metabolites.

1. Introduction

Indian Shot, botanically known as *Canna indica* L., is a species of the single genus *Canna* and a member of the Cannaceae family (Shishir et al., 2008; Al-Snafi, 2015). The parts of plants that are used for medicine are the leaves, flowers, roots and rhizomes. These parts are a rich source of phytochemicals like flavonoids, phenolic compounds, terpenes, and esters. These compounds had drawn attention because of the physiological functions; several studies have mentioned they enclose biological activities like anti-inflammatory, diarrhoeal, antimicrobial,

antioxidant etc. (Al-Snafi,2015). Several research have documented the biological efficacy of various chemical substances and metastases. Little amounts of the monoacylglyceride or palmitic acid derivative, hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester are added to commercial food products.

2. Materials and methods

2.1 Sample collection and screening:

The fresh plant materials (Leaves and flowers) were collected from the research field trial (Field No. 10 C) located at 11.01'E latitude and 76.9'W longitude at a mean altitude of 458.44±19 m above the mean sea level in the Botanical garden, Tamil Nadu Agricultural University, Coimbatore. The leaves and flowers were washed thoroughly under tap water to clean and allowed to shade dry for 2 weeks until get ≤ 5 % of moisture content. Further the dried material was using electronic blender to pulverized and sieved to obtain a fine powder. The powdered leaves and flowers were stored with air free container until further use for extraction.

2.2 Extraction involved GC MS analysis

A powdered samples about 10 g were weighed and extracted with 300 ml mixture of GC MS grade Methanol (99%) using Soxlet apparatus. After 6 hrs the resulting methanolic extract was filtered and concentrated using rotary evaporator at 55 °C at 150 rpm. The concentrated extract was dissolved in 2 ml of GC MS grade methanol and filtered with PVDF hydrophilic membrane (0.45 mm pore size, Himedia) and it was stored in refrigerator condition till further use.

2.3 Sample analysis in GC-MS:

To analyze methanolic extract of leaves and flowers of *canna indica* L. by GC MS technique, facility was Centre of Excellence, Centre for Plant Molecular Biology and Biotechnology (CPMB&B), Tamil Nadu Agricultural University, Tamil Nadu. The active compounds were diagnosed and evaluated by (Model GCMS TQ series 8040 NX). System manufactured by GC2030 O21755800561 AE system, which includes the auto sampler (AOC-20i+s) for compounds and the bound gas chromatography by mass spectrometry. General column of the SH-Rxi-5 Sil MS capillary column with dimensions (30 m x 0.25 mm ID x 0.25µm df). Helium gas (99.99%) was used as a conveyor gas at a continuous flow rate of 1 ml per minute under pressure 6.8 psi. size of injected liquid extract is 1.00 ml. the injector temperature was set at 200˚ C during GC run and maximum temperature set at 350˚C. Oven temperature is programmed automatically maximum at 350˚C. the extract 1 µl was injected into the instrument and the temperature was 60˚C for 1 minutes followed by 300˚C at the rate where it was held for six minutes. The mass detector conditions were transfer line temperature at 200˚C; ion source temperature 200˚C and ionization mode the mass spectra were taken on a 69 eV basis with sampling period of 1.00 seconds and time interval auto off 180 seconds. The primary pressure 500-900 kPa and inlet pressure inside the device is 100 kPa and the flow rate of 1 ml per minute. The total time to start and end the device working for each sample is 30 minutes. The relative amount of each component was calculated by comparing its average surface are to the total area based on mass version. 1.20 for mass spectra and chromatogram supplied with the device, all this information is automatically programmed on the machine. This current study was diagnosed depending upon the highest percentage and important chemical compounds which is present in

the both leaves and flower. The spectrum of the components was compared with the GC MS NIST library.

3. Results and discussion:

The active principles in the methanolic extract of leaves and flowers of *C. indica* in tall red type by GC-MS analysis showed the presence of 40 Chemical compounds, which are identification based on the molecular weight, retention time, peak areas and molecular formula. The active principles with their retention time (RT) and percent relative composition are presented in (Fig. 1 and 2). These forms can be used as classifiers to support and promote other studies of phenotypic, diagnostic, cellular and other types. In flowers of *Canna indica* (table 1), the compounds present are Sucrose was observed in maximum area of 10.379% at the retention time of 10.927 mins. It is followed by Dihydroxyacetone in 9.137 % at 3.103 mins. The other compounds are cis-13-Octadecenoic acid, Propanal, 2,3-dihydroxy-, (S)-4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl with maximum area. The least concentration was Dodecyl cis-9, 10-epoxyoctadecanoate with area 0.491 at 25.652 mins.

Compounds vary in their concentration in leaves of *Canna indica* (table 2). Dodecanoic acid, 3-hydroxy- the superior purity was recorded in the leaves, as it was concentrated with 12.50% at the time of appearance 14.890 mins respectively. Followed by the compound 2-(Isobutoxymethyl) oxirane, in 10.30 % concentration with a time 14.384 mins. While less concentration was Methyl erucate in 0.02% at the time of 43.312 mins. Hexadecanoic acid is a fatty acid. It is found in all extracts examined. It possess some biological activity such as antioxidants, hypocholestrolemic, and pesticide (Sudha *et al*., 2013; Ahmed *et al*., 2019). Phytol show anti-inflammatory activity (Soso *et al*., 2019). Phytol is a diterpene. It is found in leaves of

Canna indica. The enzyme kinetics study proved that n-hexadecanoic acid inhibits phospholipase A (2) in a competitive manner.

Octadecadienoic acid was found higher retention time and high area of 5.560%. It is Hypocholesterolemic, Antiarthritic, Anti-inflammatory, Cancer preventive, Anticoronary, Hepatoprotective, Nematicide, Antiandrogenic and reductase inhibitor (Al-Mayyahi, 2017).

The result in the present study showed that Butyl 2-acetoxyacetate was found with higher retention time (13.812) and high area of 10.25% in leaves of *Canna indica*. Butyl Acetate is a colorless and flammable liquid. It is found in many types of fruit, where along with other chemicals, it imparts characteristic flavors and has a sweet smell of banana or apple. It is used as a synthetic fruit flavoring in foods such as candy, ice cream, cheeses, and baked goods. Butyl acetate is often used as a high-boiling solvent of moderate polarity. It is also used as a solvent in nail polish along with ethyl acetate. It is used as a solvent for paints, lacquers and thinners. It is used in the manufacture of artificial leather, photographic films, plastics and safety glass. It is also a synthetic flavoring agent for foods and pharmaceuticals. At room temperature it is a flammable, carcinogenic, mutagenic, irritating, and anesthetic gas.

Dodecanoic acid also known as Lauric acid, belongs to the class of organic compounds known as medium-chain fatty acids. These are fatty acids with an aliphatic tail that contains between 4 and 12 carbon atoms. Lauric acid is a weakly acidic compound. They are caproic acid (hexanoic acid), caprylic acid (octanoic acid), capric acid (decanoic acid), and lauric acid (dodecanoic acid), respectively. Most of them can be found in palm and coconut oil, whole milk, and butter, where they are stored as medium chain triglycerides (MCTs) (Boateng *et al*., 2016; Mohamad *et al*., 2018). Lauric acid is used for treating viral infections including influenza (the flu); swine flu; the common cold.

The current study agreed to Asha *et al*., 2017; Lamaeswari and Ananthi, 2012 whose results were similar with identification various chemical compounds including alkaloid, flavanoid, terpenes, etc. through GCMS.

4. Conclusion:

From this study, it showed that the compounds present in both leaf and flower extracts are helpful as antioxidants and fatty acids which will be useful for treating antidiarrheal, antidiabetic, antiinflamatory, antinociceptive, anthelmintic, anticancer and cytotoxicity etc., Further study can be used in this particular crop like HPLC which shows the highly present compounds and also since the crop contains high amount of sucrose in flower it can be used as food additives.

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Table 1. Bioactive chemical compound identified in methanolic extract of *Canna indica* L. flowers

S.No.	Compound name	RT	Area	Area %
1.	Sucrose	10.927	13,109,932.0	10.379
2.	Dihydroxyacetone	3.103	11,541,317.0	9.137
3.	cis-13-Octadecenoic acid	24.777	7,022,769.5	5.560
4.	Propanal, 2,3-dihydroxy-(S)-	6.560	3,830,502.8	3.032
5.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	6.695	2,797,711.5	2.215

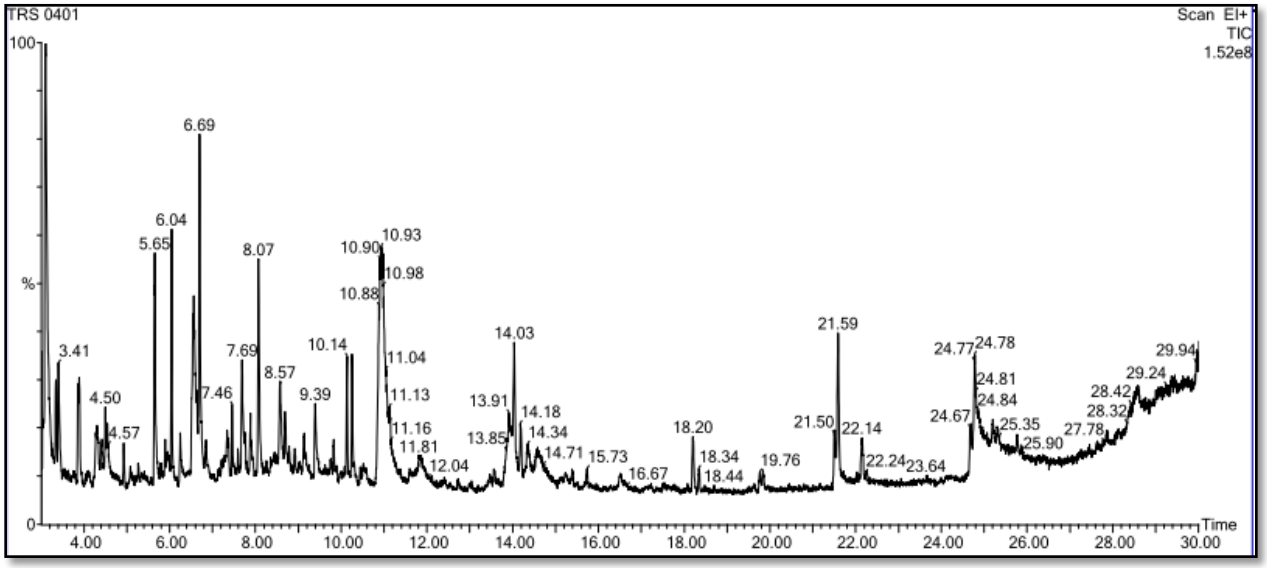

Figure 1. GC MS Chromatogram analysis of *Canna indica* L. Flowers

Table 2. Bioactive chemical compound identified in methanolic extract of *Canna indica* L. leaves

S.No.	Compound Name	RT	Area	Area %
1.	Dodecanoic acid, 3-hydroxy-	14.890	7335113	12.50
2.	2-(Isobutoxymethyl) oxirane	14.384	6042962	10.30
3.	n-Hexadecanoic acid	15.840	4414654	7.53
4.	3-Deoxy-d-mannoic lactone	14.808	3881927	6.62
5.	1,2,3-Propanetriol, 1-acetate	13.679	3275510	5.58

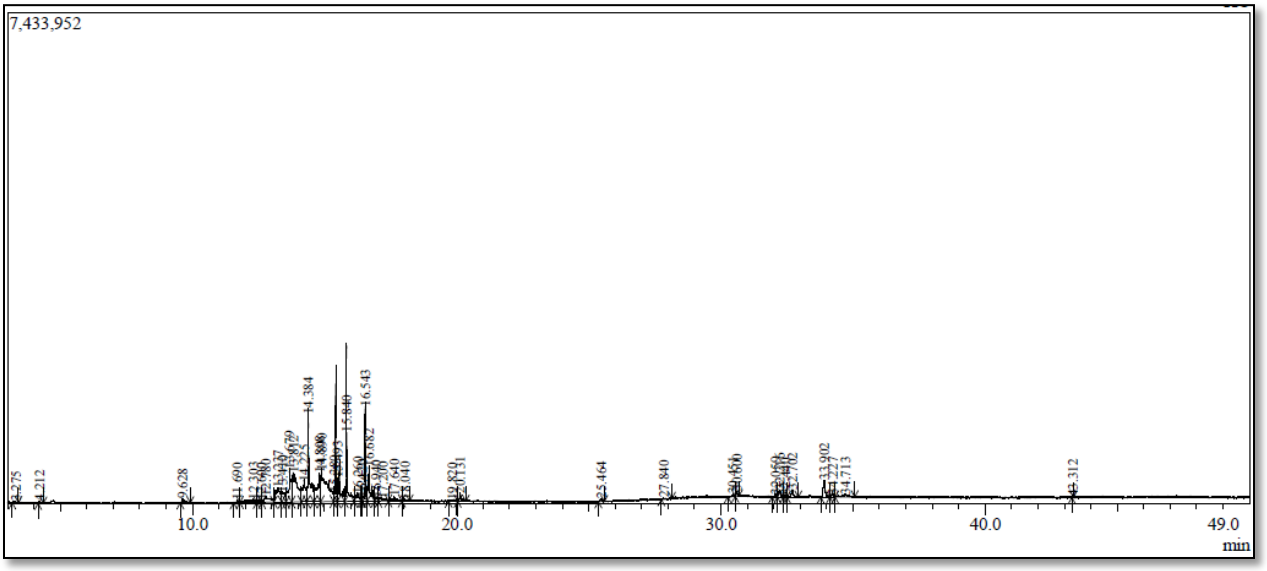

Figure 2. GC MS Chromatogram analysis of *Canna indica* L. Leaves