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Research Article

STRUCTURAL ELUCIDATION OF CHEMICAL COMPOUNDS OF PHARMACOLOGICAL SIGNIFICANCE FROM *CONVOLVULUS PLURICAULIS* BY GAS CHROMATOGRAPHY: MASS SPECTROSCOPY (GC-MS) ANALYSIS

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A R T I C L E I N F O

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Convolvulus pluricaulis, GC-MS analysis, phytochemical, pharmacology, fatty acids, triterpenoid, alkaloid, cardiac glycoside.

A B S T R A C T

Background: *Convolvulus Pluricaulis* (CP) is a well-known ethnopharmacological herb in Ayurveda and exhibits a comprehensive range of therapeutic potential. **Objective:** The main objective of this research was to study the phytochemical profile of ethanolic extract of CP by Gas Chromatography Liquid Chromatography (GC-MS) analysis and to review the pharmacological activities associated with the compounds identified in this study from the literature. **Materials and Methods:** Ethanolic extract of CP was prepared by soxhlet extraction and the extract was subjected to GC-MS analysis (Agilent 7890AGC System) for chemical characterization of the extract. **Results:** The constituents were analysed by matching mass spectra with MS libraries. Total 19 different compounds were identified for CP extract belonging to different chemical groups like fatty acids, alkaloid, triterpenoid, diterpenoids, tetracycline antibiotic, cardiac glycoside and long chain aldehyde chemical group. The constituents of pharmacological significance include demeclocycline; n-hexadecanoic acid; hexadecanoic acid, ethyl ester; phytol; squalene; digitoxin; ethyl iso-allocholate; 9,12-octadecadienoic acid; 9,12,15-octadecatrienoic acid; Octadecanal 2-bromo; pentadecanoic acid, 14-methyl, methyl ester and 9,12,15- octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester. **Conclusion:** The GC-MS analysis was successful in identifying the pharmacologically important phytochemicals and the eluted compounds could provide the researchers to work with different pharmacological activities related models and studies.

INTRODUCTION

Phytochemicals are plant-derived secondary metabolites that would help in the exploration of various groups of compounds and would assist in the identification of new bioactive compounds.[1-3] In recent times, the research on plants as medicines has been focusing on isolation and characterization of bioactive constituents and secondary metabolites.[4,5] Long chain fatty acids (LCFA) also called as free fatty acids or nonesterified fatty acids, are molecules that act as metabolic intermediates and constituents of membranes and these fatty acids play an important role in metabolic disorders and in chronic diseases where inflammation is involved. Their role as signalling molecules in immune function has been demonstrated. LCFAs are saturated or unsaturated fatty acids containing 13-21 carbons. The role of fatty acids in some diseases such as cancer, inflammation and autoimmune diseases has been well-discussed in several reviews. Palmitic acid is a saturated fatty acid with plasma total lipid levels ranging from 0.3 to 4.1 mmol/L.[6,7] Fatty acids are classified according to their carbon (C) chain-length and the number of double bonds. LCFAs have chain-lengths of C11-20 and FAs longer than C20 (C > 20) are called very long-chain FAs (VLCFAs). Based on double bonds, FAs are classified into saturated FAs (no double bond), monounsaturated FAs (one double bond) and polyunsaturated FAs (two or more double bonds). Polyunsaturated fatty acids are further subdivided into n-3 (or ω3) and n-6 (or ω6) series depending on the position of the terminal double bond (the double bond most distant from the carboxyl group). In the *n*-x series, x indicates the ordinal number of carbon atom with a double bond from the end of the carbon chain.[8,9]

Terpenoids have common biosynthetic origin based on isoprene units [CH_2=C(CH_3)- CH=CH_2]. Carbon skeletons in them are built from the union of two or more of C_5 units. The classification is based on number of units as follows: C_{10}-Monoterpenoids (2 carbon atoms), C_{15}-Sesquiterpenoids (3 carbon atoms), C_{20}-Diterpenoids (4 carbon atoms), C_{30}-

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Triterpenoids (6 carbon atoms) from squalene parent molecule and C_{40}-Tetraterpenoids (8 carbon atoms).[9] Triterpenoids are divided to four groups of compounds: true triterpenes, steroids, saponins and cardiac glycosides or cardenolides. Most cardiac glycosides are toxic and many have pharmacological activity as their name implies on the heart.[9]

Gas Chromatography Mass-Spectrometry (GC-MS), a hyphenated system has become a technological platform for metabolite profiling in plant and the medicinal herbs having numerous bioactive compounds can be identified at less than 1ng by using this technique. This method has proved to be a valuable method for analysis of non-polar components and volatile essential oils, fatty acids, lipids, and alkaloids.[10-14] Thus, in the current research work we have identified the phytoconstituents belonging to long chain fatty acids, alkaloid, terpenoids chemical groups by subjecting the ethanolic extract of CP to GC- MS analysis which may be of therapeutic potential.

MATERIALS AND METHODS

Ethanolic extract of CP: The whole plant was collected, authenticated, and extracted with 70:30 ratio of ethanol and water by using soxhlet extractor. The extract was stored at 8-15°C for further analysis. *GC-MS analysis:* Mobile phase: Ethanol (electron impact (EI)-MS Spectrum was scanned at 70 eV with instrument details as follows:

GC Specification:

Model	Agilent 7890A GC System
Detector Specification	Mass Spectrometer Model: The AccuTOFGCv/JMS-T100GCv Make: JEOL
Column Specifications	
1. Name of column	HP5 Column
2. Length	(30m length*0.25mm internal
3. Dimension	diameter*0.25microfilm thickness)
4. Column material	Column Material is Polysiloxane
Carrier Gas Used	Helium
Carrier Gas Flow Rate	1ml/min
Oven Temperature	280°C
Injection Temperature	200°C
Injection Volume	1 microL
Sample flow rate	1ml/min
Split Ratio	1:10

MS Specification:

Model	Joel, AccuTOF GCV
Isonization Source Used	EI Positive
Mass range	35-800amu
Split Ratio	1:10
Ion Source Temperature	220°C
Solvent Delay	4mins

The compounds were identified by comparing their mass spectra with NIST MS 2.0 Structural library.

RESULTS AND DISCUSSION

In the current research work, compounds [Table 1] belonging to different chemical groups [Table 2] were identified. The mass spectrum of ethanolic extract of *convolvulus pluricaulis* is shown in Figure 1 and the mass spectrum of different compounds of CP extract is shown from Figure 2 to 20. The phytoconstituents that are identified belong to important chemical groups - long chain fatty acids (majorly), terpenoids, alkaloids and tetracycline antibiotic. LCFAs have been implicated in the development autoimmune CNS disease because of their ability to promote proliferation and differentiation of pathogenic Th1 and Th17 cells and enhance the production of IFN-γ, IL-2, IL-6, and IL-17 in experimental autoimmune encephalomyelitis model. 12-h exposure of schwann cells to LCFAs in culture promoted oxidative stress and mitochondrial dysfunction, suggesting a potential toxic mechanism related to alterations in LCFA oxidation in diseases such as diabetic neuropathy.

Table 1 Components found in ethanolic extract of *Convolvulus pluricaulis* by GC- MS analysis

Sr. No.	Components	Retention time	Molecular formula	Molecular weight	Figure Number
1	Demeclocycline	8.10	$C_{21}H_{21}ClN_2O_8$	464	2
2	2-Hexadecanol	8.92	$C_{16}H_{34}O$	242	3
3	Tetracosane, 12-decyl-12- nonyl	12.36	$C_{43}H_{88}$	604	4
4	Hexadecane,1,1-bis(dodecyloxy)	15.47	$C_{40}H_{82}O_2$	594	5
5	3-o-methyl-d-glucose	18.12	$C_7H_{14}O_6$	194	6
6	Octadecanal,2-bromo	19.27	$C_{18}H_{35}BrO$	346	7
7	Pentadecanoic acid, 14- methyl, methyl ester	20.23	$C_{17}H_{34}O_2$	270	8
8	n-Hexadecanoic acid	20.95	$C_{16}H_{32}O_2$	256	9
9	Hexadecanoic acid, ethyl ester	21.21	$C_{18}H_{36}O_2$	284	10
10	9,12,15, octadecatrienoic acid, 2-phenyl-1,3-dioxan- 5yl-ester	22.69	$C_{28}H_{40}O_4$	440	11
11	Phytol	22.86	$C_{20}H_{40}O$	296	12
12	9,12-Octadecadienoic acid (z,z)	23.45	$C_{18}H_{32}O_2$	280	13
13	9,12,15-Octadecatrienoic acid	23.54	$C_{18}H_{30}O_2$	278	14
14	Octadecanoic acid, ethyl ester	23.83	$C_{20}H_{40}O_2$	312	15
15	Digitoxin	26.90	$C_{41}H_{64}O_{13}$	764	16
16	Hexadecanoic acid,2,3-dihydroxy-propylester,(±)	27.50	$C_{19}H_{38}O_4$	330	17
17	Ethyl iso-allocholate	29.53	$C_{26}H_{44}O_5$	436	18
18	Di-n-octyl phthalate	29.76	$C_{24}H_{38}O_4$	390	19
19	Squalene	30.62	$C_{30}H_{50}$	410	20

Table 2 Chemical nature of components found in ethanolic extract of *Convolvulus pluricaulis* through GC-MS analysis

Sr. No.	Components	Chemical nature
1	Demeclocycline	Tetracycline antibiotic
2	2-Hexadecanol (cosmetics, emulsifier, lubricants	Long chain fatty alcohol
3	Tetracosane, 12-decyl-12-nonyl	Alkane hydrocarbon
4	Hexadecane,1,1-bis(dodecyloxy)	Alkane hydrocarbon
5	3-o-methyl-d-glucose (preservative)	D-Aldohexose , methylated sugar
6	Octadecanal,2-bromo	Long chain aldehyde
7	Pentadecanoic acid, 14-methyl, methyl ester	Saturated Long chain Fatty acid methyl ester
8	n-Hexadecanoic acid	Saturated Long chain fatty acid (Palmitic acid)
9	Hexadecanoic acid, ethyl ester	Saturated Long chain fatty acid ethyl ester (Palmitic acid ester)
10	9,12,15, octadecatrienoic acid, 2-phenyl-1,3- dioxan-5yl-ester	Polyunsaturated Long chain fatty acid
11	Phytol	Acyclic diterpenoids
12	9,12-Octadecadienoic acid (z,z)	Polyunsaturated Long chain fatty acid, Linoleic acid
13	9,12,15-Octadecatrienoic acid	Polyunsaturated Long chain fatty acid
14	Octadecanoic acid, ethyl ester (ethyl stearate)	Saturated Long chain fatty acid
15	Digitoxin	Cardiac glycoside
16	Hexadecanoic acid,2,3-dihydroxy-propylester(±)	Saturated Long chain fatty acid
17	Ethyl iso-allocholate	Alkaloid
18	Di-n-octyl phthalate	Phthalate ester and a diester
19	Squalene	Triterpenoid

Therefore, LCFA can be used to improve inflammatory diseases, which also would contribute to control metabolic diseases, thus regulating homeostasis immune and metabolic.[6,7] Triterpenoids are the largest group of phytochemicals and are metabolites of isopentenyl pyrophosphate oligomers. In preclinical animal models, triterpenoids are known to exhibit cytotoxicity against a variety of tumor cells and has a potent anti-inflammatory and anticarcinogenic role specially in breast cancer. *In-vitro* studies has demonstrated the inhibitory effects of various triterpenoids against proliferation, growth and invasion of a large variety of breast cancer cell lines.[15]

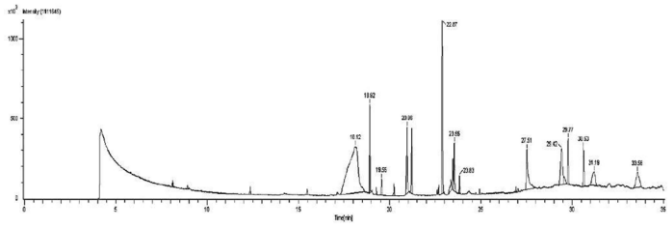

Figure 1 Mass spectrum of ethanolic extract of *Convolvulus pluricaulis*

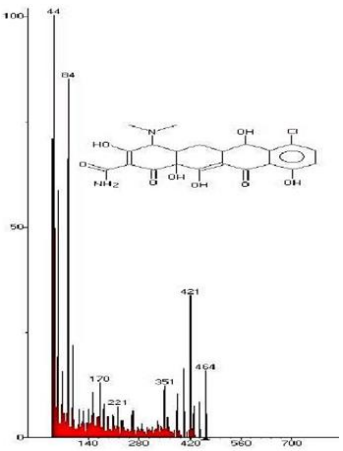

Figure 2 Mass spectrum showing presence of Demeclocycline in *Convolvulus pluricaulis* extract

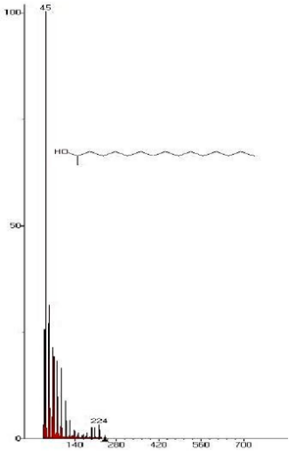

Figure 3 Mass spectrum showing presence of 2-Hexadecanolin *Convolvulus pluricaulis* extract

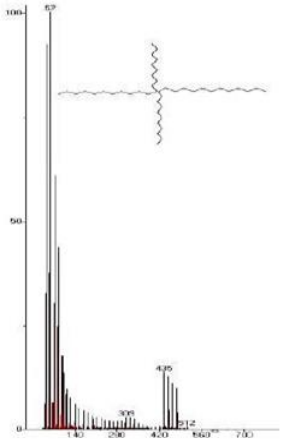

Figure 4 Mass spectrum showing presence of Tetracosane, 12-decyl-12-nonyl in *Convolvulus pluricaulis* extract

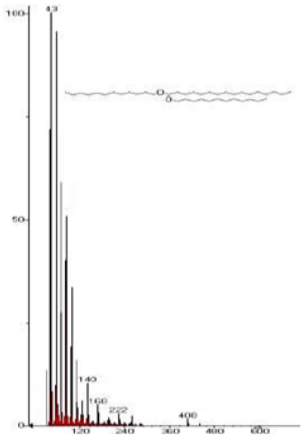

Figure 5 Mass spectrum showing presence of Hexadecane,1,1-bis(dodecyloxy) in *Convolvulus pluricaulis* extract

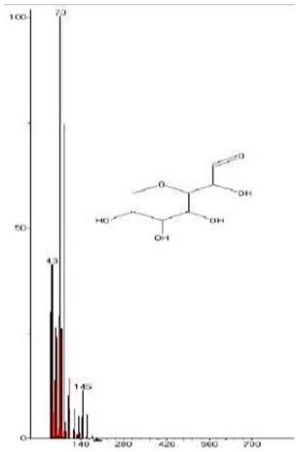

Figure 6 Mass spectrum showing presence of 3-o-methyl-d-glucose in *Convolvulus pluricaulis* extract

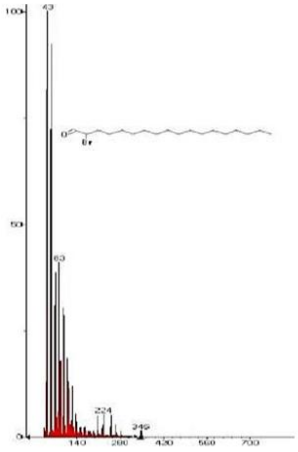

Figure 7 Mass spectrum showing presence of Octadecanal,2-bromo in *Convolvulus pluricaulis*extract

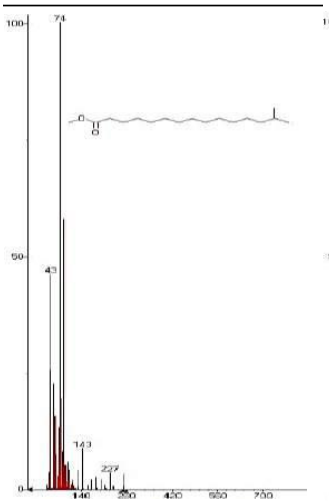

Figure 8 Mass spectrumshowing presence of Pentadecanoic acid, 14-methyl, methyl ester in *Convolvulus pluricaulis*extract

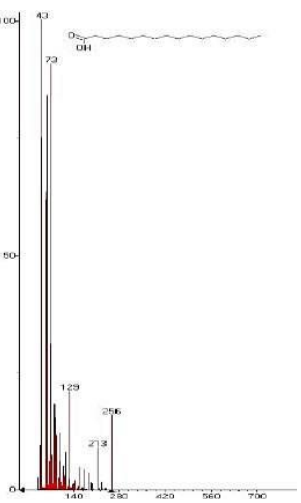

Figure 9 Mass spectrum showing presence of n-Hexadecanoic acid in *Convolvulus pluricaulis* extract

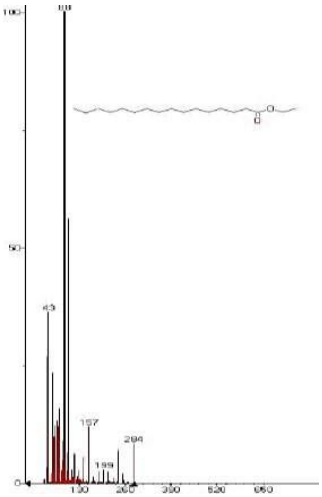

Figure 10 Mass spectrum showing presence of Hexadecanoic acid, ethylester in *Convolvulus pluricaulis* extract

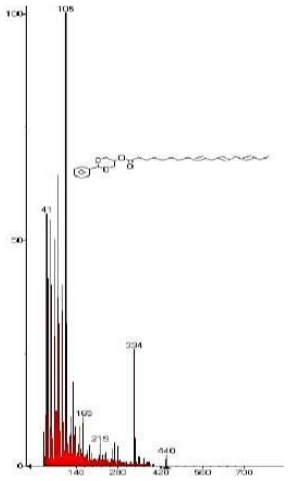

Figure 11 Mass spectrum showing presence of 9,12,15, octadecatrienoic acid, 2-phenyl-1,3-dioxan-5yl-ester in *Convolvulus pluricaulis* extract

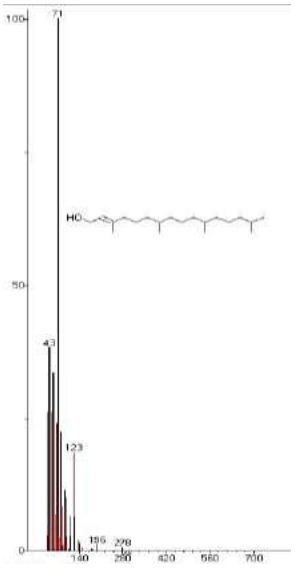

Figure 12 Mass spectrum showing presence of Phytol in *Convolvulus pluricaulis*extract

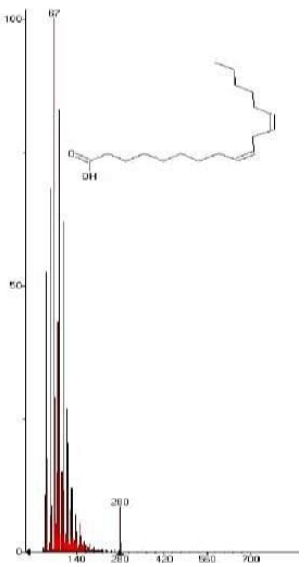

Figure 13 Mass spectrum showing 9,12-Octadecadienoic acid (z,z) in *Convolvulus pluricaulis*extract

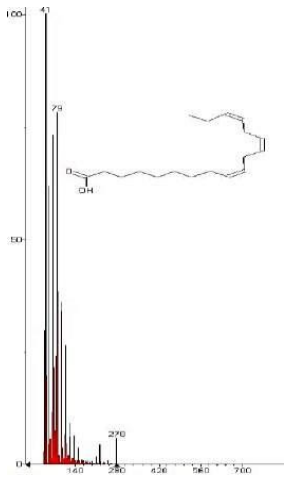

Figure 14 Mass spectrum showing presence of 9,12,15-Octadecatrienoic acid in *Convolvulus pluricaulis* extract

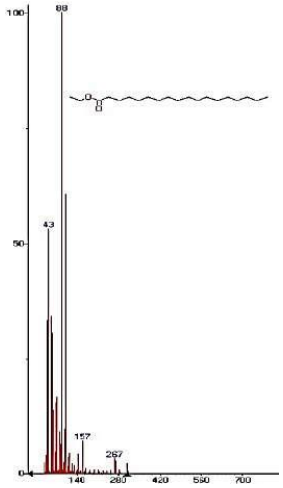

Figure 15 Mass spectrum showing presence of Octadecanoic acid, ethylester in *Convolvulus*

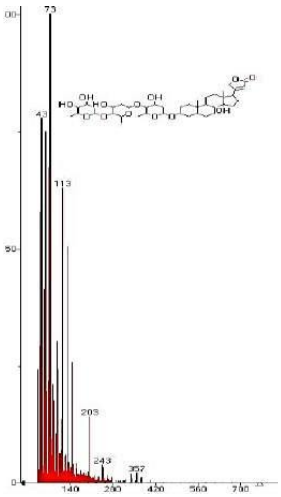

Figure 16 Mass spectrum showing presence of Digitoxin in *Convolvulus pluricaulis* extract

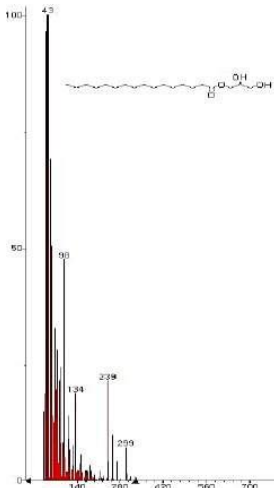

Figure 17 Mass spectrumshowing presence of Hexadecanoic acid,2,3-dihydroxy-propylester,(±) in *Convolvulus pluricaulis* extract

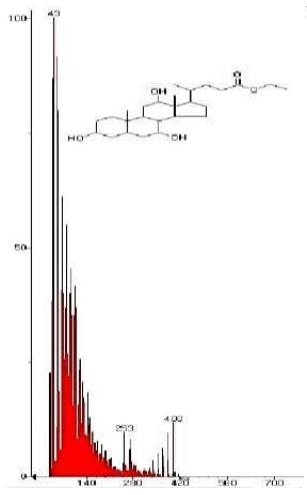

Figure 18 Mass spectrum showing presence of Ethyliso-allocholate in *Convolvulus pluricaulis* extract

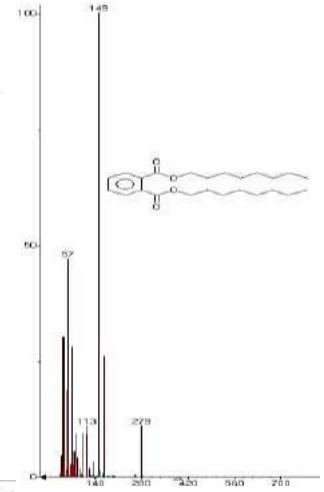

Figure 19 Mass spectrum showing presence of Di-n-octyl phthalate in *Convolvulus pluricaulis* extract

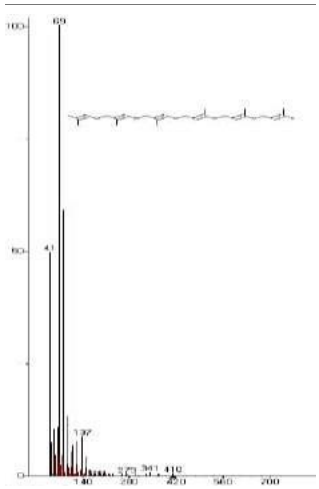

Figure 20 Mass spectrumshowing presence of Squalene in *Convolvulus pluricaulis* extract

Pharmacological significance of compounds identified in the ethanolic extract of CP by GC-MS analysis are discussed below

Demeclocycline is a tetracycline antibiotic and antibiotics not only have the antibacterial activity, but many of them like macrolides, sulphonamides and tetracyclines have immunomodulating effect, affecting functions of lymphocytes, macrophages and costimulatory molecules, macrophage migration and phagocytosis, proinflammatory cytokine secretion.[16] Squalene is a natural triterpene found in CP extract by GC-MS analysis and has use in various disease management. The anti-inflammatory and immunomodulatory effects of squalene have been examined in detail in different studies.

The antibacterial, antioxidant, immunostimulant, chemopreventive, lipoxygenase-inhibitor, pesticide, diuretic activity has been reported for squalene in literature.[17,18] Digitoxin is a cardiac glycoside (CG) found in CP extract by GC-MS analysis and is a natural steroid compound commonly used for various cardiac diseases. Currently CGs are mostly studied as anticancer agents and the anti-tumor effect of CG in breast and prostate cancers has been reported. CGs are strongly connected to immunogenic cell death, a complex mechanism of induction anticancer immune response.[19,20] Phytol an acyclic diterpenoids identified in CP extract has been reported to possess antitumor activity and antioxidant activity. Phytol was able to reduce the production of free radicals, and this activity can be attributed to its structural feature, since phytol is a branched chain unsaturated alcohol and its antioxidant properties may be related to the hydroxyl group (OH) present in its molecule. Phytol, by reacting with a free radical, donates hydrogen atoms with an unpaired electron, converting free radicals into less reactive species.[21-25] Ethyl iso-allocholate has been reported for its anti-inflammatory, anticancer, antimicrobial, antiasthma and diuretic properties.[26,27] Hexadecanoic acid was reported to have activities like flavour, antibacterial, cosmetic and perfumery, hypercholesterolemic and lubricant.[28-30] Octadecanal,2-bromo has been reported to show anti-inflammatory and anti-apoptotic effects. 9,12,15 octadecatrienoic acid has been reported to show anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge antihistaminic,

antiarthritic, anticoronary, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic.[31] Pentadecanoic acid, 14-methyl, methyl ester has been reported to show antioxidant, antifungal and antimicrobial activities. Hexadecanoic acid, ethyl ester has been reported to show antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic and flavor activities. n-hexadecanoic acid has been reported to show antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, haemolytic and 5-Alpha reductase inhibitor activities.[32] Hexadecanoic acid, ethyl ester has been reported to show antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5- alpha reductase inhibitor. 9,12-octadecadienoic acid (Z,Z) has been reported to show anti-inflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge.[18] Octadecanoic acid, ethyl ester has been reported to show antimicrobial activity.[33,34] 9,12,15-Octadecatrienoic acid,2- phenyl-1,3-dioxan-5-yl ester has been reported to show antiviral and anti-obesity properties.[35]

Thus, the results of GC-MS analysis of ethanolic extract of CP demonstrates the presence of secondary metabolites belonging to long chain fatty acids, tetracycline antibiotic, terpenoids and alkaloid chemical groups that possess a potential pharmacological role and the plant can be considered to have therapeutic potential. However, isolation of individual secondary metabolites and screening it for biological activity will be more beneficial.

FUNDING

NIL

CONFLICT OF INTEREST

We declare no conflict of interest.

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