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Phytochemical Analysis of *Cynanchum callialatum* through GCMS and LCMS

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Abstract

Medicinal plants are still important source for drug discovery. Herbal medicines have gained importance in recent years because of their efficacy and cost effectiveness. The objective of the present study is to investigate the phytochemical present in the *Cynanchum callialatum*. The phytochemical analysis was done by preliminary phytochemical test for secondary metabolites, GCMS for volatile constituents and LCMS for nonvolatile constituents.

The phytochemical test confirms the presents of alkaloids, flavonoids, terpenoids, tanins etc. The GCMS analysis shows the presents of 52 compounds in which some have medicinal value. The LCMS analysis shows the presents of compounds in which most of them have the medicinal properties. The present study on *Cynanchum callialatum* reveals the presence of various phytochemical constituents like Betulinic acid, Lupeol, Germacrone and Longiverbenone. *Cynanchum callialatum* may be a potential source for anticancer, antiHIV, antiinflammatory, antimicrobial drug discovery.

Keywords: *Cynanchum callialatum*; Phytochemical analysis; GC-MS; LC/MS

Introduction

Plant still remains a major source for drug discovery in development of synthetic molecules. The use of traditional plant extract in the treatment of various diseases has been flourished. In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. The World Health Organization estimated that about 80% of the world population relays on herbal medicines.

Herbal medicines have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are invariable single plant extracts or mixtures of extracts from different plants, which have been carefully standardized for their safety and efficacy. Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma and infectious diseases [1]. Nowadays medicinal plants receive more attention to researchers because of their safety and curative property which is due to the complex mixtures secondary metabolites.

Cynanchum is a genus of about 300 species including some swallowwort's, belongs to the milkweed family Asclepiadaceae. Most species are non-succulent, climbers or twiners. These plants are perennial herbs or sub shrubs, often growing from rhizomes. The leaves are usually oppositely arranged and sometimes are borne on petioles. The inflorescences and flowers come in a variety of shapes. These plants bear follicles, which are pod like dry fruits. These species are found worldwide in the tropics and subtropics. Several species also grow in temperate regions. *Cynanchum* varieties are prescribed in chinese medicine to treat fever, cough, pneumonia and asthma [2]. *Cynanchum callialatum* twiner to 4 m, latex milky, flowers white, widely distributed in India. *Cynanchum callialatum* has been used to treat wounds, headaches, infections and other skin related problems by tribes in Tamil Nadu, India.

Based on the literature review there is no scientific reports on phytochemical constituents of *Cynanchum callialatum*. The present study has made an attempt to identify the chemical constituents from the areal parts of *Cynanchum callialatum* through GCMS and LCMS.

Materials and Methods

Plant material

The plant *Cynanchum callialatum* was collected from Pollachi, Coimbatore District, Tamil Nadu, India and It has been identified and authenticated by Dr. Udyan P.S., Professor, Sreekrishna College, Guruvayur, Thrissur, Kerala, India.

The areal parts of the *Cynanchum callialatum* were collected during March-April month and washed with water. Then the plant material was shade dried for 10 days. The dried plant materials have been powdered using mechanical grinder to get uniform coarse particles. The powdered plant material was stored in polythene air tight containers at room temperature for further use.

Preparation of the crude plant extract

The shade dried coarse powdered bark of *Cynanchum callialatum* (250 g) was packed in the soxhlet extraction apparatus and extracted with 2 L of 95% ethanol at a temperature of 40-50°C for 72 hr. The extract was filtered and the filtered extract was then concentrated to dryness in a rotary evaporator under reduced pressure at temperature of 40°C. The resultant green color residue was stored in a desiccator for use in subsequent experiments and considered as the crude ethanol extract. The yield of the ethanolic extract was 14% w/w.

Phytochemical analysis

The preliminary phytochemical screening test was carried out in

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ethanolic extract of *Cynanchum callialatum* to find out the nature of chemical compounds as per the standard procedures [3-6] and the phytoconstituents were identified through GCMS and LCMS (Tables 1- 3).

GCMS specifications

Make: PerkinElmer Clarus 500

Software: Turbomass ver 5.2.0

Column Type: Capillary Column Elite-5MS (5%Phenyl 95% dimethylpolysiloxane)

Column length: 30 m

Column id: 250 µm

GC conditions

Oven Program: 50°C@6°C/min to 220°C (2 min)@6°C/min to 270°C (10min)

Injector temperature: 280°C

Carrier gas: Helium @ flow rate 1 ml/min

Split ratio: 1:20

MS conditions

Mass Range: 40-600 amu

Type of Ionization: Electron Ionization (EI)

Electron energy: 70 ev

Transfer line and source temperature: 200°C, 180°C

Library: NIST 2005

Sample injected: 1.0 microlitre

LCMS specifications

S.No	Test done for	Name of the test	Quantity present
1	Phenol	Lead acetate test	+
2	Flavonoids	Shinoda's test	+
3	Alkaloids	Dragondroff's test Wagners test Mayer's test Hager's test	+
4	Saponins	Foam test	-
5	Glycosides	Borntragers test	+
6	Proteins	Biuret test	-
7	Amino acids	Ninhydrin test	-
8	Carbohydrates	Anthrone test	+
9	Tannins	Ferric chloride test	+
10	Gums and Mucilage	Ruthenium red test	-
11	Flavones	NaoH test	+
12	Sterols	Liberman's test	+
13	Terpenoids	Tin and thionyl chloride test	+
14	Reducing sugars	Molishch's test	+
15	Terpenes	Plate derivatisation	+
16	Aromaticity	Organoleptic tests	+
17	Essential oil	Filter paper test	-

+ Presence - Absence

Table 1: Preliminary Phytochemical carried out in ethanolic extract of *Cynanchum callialatum.*

S.No.	Peak Name	Retention time	% Peak Area	
1	Name: Hexanal Formula: $C_6H_{12}O$ MW: 100	4.32	0.2696	
2	Name: Octanal Formula: $C_8H_{16}O$ MW: 128	8.85	0.0067	
3	Name: Hexanoic acid Formula: $C_6H_{12}O_2$ MW: 116	8.97	0.0914	
4	Name: 1,3-Dioxane, 2-heptyl- Formula: $C_{11}H_{22}O_2$ MW: 186	9.48	0.8036	
5	Name: Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- Formula: $C_{10}H_{18}O$ MW: 154	13.41	0.0347	
6	Name: Octanoic Acid Formula: $C_8H_{16}O_2$ MW: 144	13.65	0.6199	
7	Name: Myrcenylacetate Formula: $C_{12}H_{20}O_2$ MW: 196	13.90	0.0958	
8	Name: L-Glucose, 6-deoxy-3-O-methyl- Formula: $C_7H_{14}O_5$ MW: 178	14.31	0.7977	
9	Name: á-d-Allopyranoside, methyl 6-deoxy-2-O-methyl- Formula: $C_8H_{16}O_5$ MW: 192	15.19	2.1420	
10	Name: 2H-Pyran-2-one, tetrahydro-6-propyl- Formula: $C_8H_{14}O_2$ MW: 142	16.13	0.0819	
11	Name: Thymol Formula: $C_{10}H_{14}O$ MW: 150	16.50	0.5089	
12	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW: 150	16.88	0.3273	
13	Name: 3-Cyclohexene-1-methanol, à,à,4-trimethyl-, acetate Formula: $C_{12}H_{20}O_2$ MW: 196	17.27	0.5481	
14	Name: Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]- Formula: $C_{15}H_{24}$ MW: 204	18.25	0.0448	
15	Name: à-Zingiberene Formula: $C_{15}H_{24}$ MW: 204	18.41	0.0600	
16	Name: Phenol, 4-(1,1-dimethylpropyl)- Formula: $C_{11}H_{16}O$ MW: 164	18.59	0.0377	
17	Name: (+)-2-Carene, 4-à-isopropenyl- Formula: $C_{13}H_{20}$ MW: 176	19.03	0.0704	
18	Name: 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)- Formula: $C_{15}H_{24}$ MW: 204	19.45	0.1268	
19	Name: Phenol, 2-methoxy-4-(1-propenyl)-, (E)- Formula: $C_{10}H_{12}O_2$ MW: 164	19.91	0.0776	

20	Name: Curcumene Formula: $C_{15}H_{22}$ MW: 202	20.20	8.9064	
21	Name: à-Farnesene Formula: $C_{15}H_{24}$ MW: 204	20.56	0.2632	
22	Name: Di-epi-à-cedrene Formula: $C_{15}H_{24}$ MW: 204	20.76	6.6327	
23	Name: Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]- Formula: $C_{15}H_{24}$ MW: 204	21.08	0.5788	
24	Name: Nerolidol 2 Formula: $C_{15}H_{26}O$ MW: 222	21.83	0.1858	
25	Name: Dodecanoic acid Formula: $C_{12}H_{24}O_2$ MW: 200	22.13	0.0141	
26	Name: Benzenepropanoic acid, á,á-dimethyl-, methyl ester Formula: $C_{12}H_{16}O_2$ MW: 192	22.33	0.4125	
27	Name: Elemenone Formula: $C_{15}H_{22}O$ MW: 218	22.77	1.1606	
28	Name: Asarone Formula: $C_{12}H_{16}O_3$ MW: 208	22.96	0.3469	
29	Name: 6-(p-Tolyl)-2-methyl-2-heptenol Formula: $C_{15}H_{22}O$ MW: 218	23.92	3.2528	
30	Name: Bergamotol, Z-à-trans- Formula: $C_{15}H_{24}O$ MW: 220	24.40	0.1695	
31	Name: Germacrone Formula: $C_{15}H_{22}O$ MW: 218	24.74	1.3890	
32	Name: Longiverbenone Formula: $C_{15}H_{22}O$ MW: 218	25.12	0.3171	
33	Name: Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)- Formula: $C_{15}H_{22}O$ MW: 218	25.80	13.7413	
34	Name:Tetradecanoic acid, ethyl ester Formula: $C_{16}H_{32}O_2$ MW: 256	26.13	1971073	0.2242
35	Name: 2-n-Propyladamantane Formula: $C_{13}H_{22}$ MW: 178	26.35	4544831	0.5170
36	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	27.03	27310200	3.1067
37	Name: 2-Pentadecanone, 6,10,14-trimethyl- Formula: $C_{18}H_{36}O$ MW: 268	27.26	12747370	1.4501
38	Name: Naphthalene, decahydro-1,1-dimethyl- Formula: $C_{12}H_{22}$ MW: 166	28.77	9831310	1.1184
39	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	30.37	214922064	24.4485
40	Name: Hexadecanoic acid, ethyl ester Formula: $C_{18}H_{36}O_2$ MW: 284	30.45	7800140	0.8873
41	Name: Uridine, 2'-deoxy-3-methyl-3',5'-di-O-methyl- Formula: $C_{12}H_{18}N_2O_5$ MW: 270	31.28	4476319	0.5092
42	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	32.75	6885792	0.7833
43	Name: (E)-9-Octadecenoic acid ethyl ester Formula: $C_{20}H_{38}O_2$ MW: 310	33.64	64711056	7.3612
44	Name: Octadecanoic acid Formula: $C_{18}H_{36}O_2$ MW: 284	33.95	37751912	4.2945
45	Name: Hexadecanoic acid, ethyl ester Formula: $C_{18}H_{36}O_2$ MW: 284	34.07	30412262	3.4595
46	Name: 3-Methoxytyrosine Formula: $C_{10}H_{13}NO_4$ MW: 211	34.99	5369919	0.6109
47	Name: 1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl- Formula: $C_{15}H_{26}$ MW: 206	36.04	3164643	0.3600
48	Name: 2-(3,4-Methylenedioxyphenyl) cyclohexanone Formula: $C_{13}H_{14}O_3$ MW: 218	36.71	25409886	2.8905
49	Name: Ethyl 13-docosenoate(ethyl erucate) Formula: $C_{24}H_{46}O_2$ MW: 366	40.30	7670625	0.8726
50	Name: 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one Formula: $C_{30}H_{48}O$ MW: 424	40.84	11640140	1.3241
51	Name: Benzaldehyde, 4-methoxy-3-(3,7,11-trimethyldodeca-2,6,10-trienyl- (E,E)- Formula: $C_{23}H_{32}O_2$ MW: 340	45.03	12875374	1.4646
52	Name: Lupeol Formula: $C_{30}H_{50}O$ MW: 426	46.06	1775649	0.2020

Table 2: Shows the Peak name, peak area during GCMS analysis of ethanolic extract of *Cynanchum calilalatum*.

LC column: ReversePhaseC-18PUMP:SPD10AVP

Mobile Phase: water: Methanol (50:50)

Ionization Mode: Electronic Spray Ionization

Mode: Both Positive and negative

Injection Volume: 10 microlitre

Flow Rate: 2 ml/min

Column Temperature: 250°c

S.No	Compound name	Molecular mass
1	Betulinic acid	456.71
2	Benzoic acid	122.12
3	Palmitic acid	256.43
4	Sinapic acid	242.21
5	Pseudolaric acid A- glucopyranoside	550.60
6	Isoeugenol	164.20
7	Succinic acid	118.09
8	Conduritol	146.14
9	Daocosterol	576.85
10	Lupeol acetate	468.77
11	Taraxasterol	426.73
12	Amino Benzoic acid	137.14
13	Coumarin	146.15
14	Umbelliferone	162.15
15	Syringic acid	198.18
16	Diferulic acid	386.36
17	Beta amyrin	426.73
18	Palmitoyl acetate	660.85
19	Vanillic acid	168.15
20	Glucuronic acid	193.21
21	Abscisic acid	264.33
22	Erucic acid	338.58

Table 3: LCMS Analysis Library Search Results.

Column: PhenomenexRP18

Column Dimension: 25 cmx2.5 mm

LC Detection: 254 nm

M/Z Range: 50-1000

Software: classvp integrated.

Library: Metwin2.0

Results and Discussion

Cynanchum callialatum twiner to 4 m, latex milky, flowers white widely distributed in India. *Cynanchum callialatum* has been used to treat wounds, headaches, infections and other skin related problems by tribes in Tamil Nadu, India. As per our knowledge the chemicals constituents of *Cynanchum callialatum* was not yet scientifically reported. Moreover, identification of chemical constituents in the crude drugs is the basic goal to prove its pharmacological effects behind the folklore uses and ultimate discovery of novel therapeutics. In the present study phytochemical investigation on *Cynanchum callialatum* ethanolic extract has been done by preliminary phytochemical screening, GCMS and LCMS analysis (Figures 1-3). Our study reveals the presence of various natural bioactive compounds shown in table 1 and 2 and these chemical compounds also been found in other species of *Cynanchum* [7].

Expected pharmacological properties of *Cynanchum callialatum*

The photochemical investigations on *Cynanchum callialatum* ethanolic extract have revealed the presence of several natural compounds (Tables 1 and 2) and most of them have various biological activities.

Betulinic acid: Betulinic acid, is a naturally occurring pentacyclicIupane- type triterpenoid which exhibits a variety of biological and medicinal properties such as inhibition of human immunodeficiency virus (HIV), anti-bacterial, anti-malarial, antiinflammatory, anthelmintic, antinociceptive, anti-HSV-1, anti-HSV-1 , and anti- cancer activities [8].

Lupeol: Lupeol, a phytosterol and triterpene, is widely found in edible fruits, and vegetables. In various in vitro and preclinical animal studies suggest that lupeol has a potential to act as an anti-inflammatory, anti-microbial, anti-protozoal, anti-proliferative, anti-invasive, anti-angiogenic and cholesterol lowering agent. Employing various *in vitro* and *in vivo* models, lupeol has also been tested for its therapeutic efficiency against conditions including wound healing, diabetes, cardiovascular disease, kidney disease, and arthritis. Lupeol has been found to be pharmacologically effective in treating various diseases under preclinical settings (in animal models) irrespective of varying routes of administration viz; topical, oral, intra-peritoneal and intravenous. It is note worthy that lupeol has been reported to selectively target diseased and unhealthy human cells, while sparing normal and healthy cells. Lupeol modulates the expression or activity of several molecules such as cytokines IL-2, IL4, IL5, ILβ, proteases,

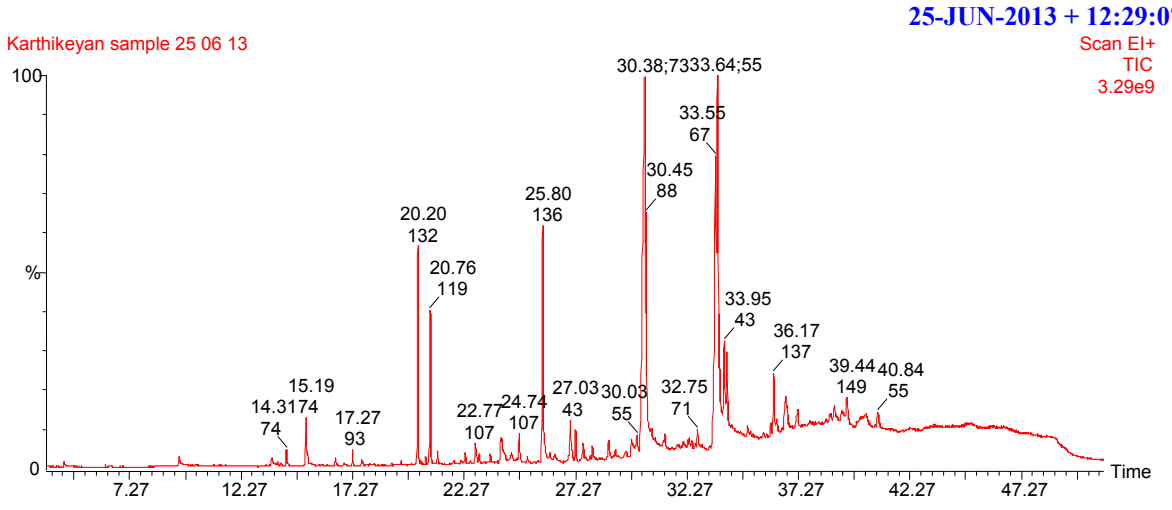

Figure 1: GCMS of *Cynanchum callialatum*.

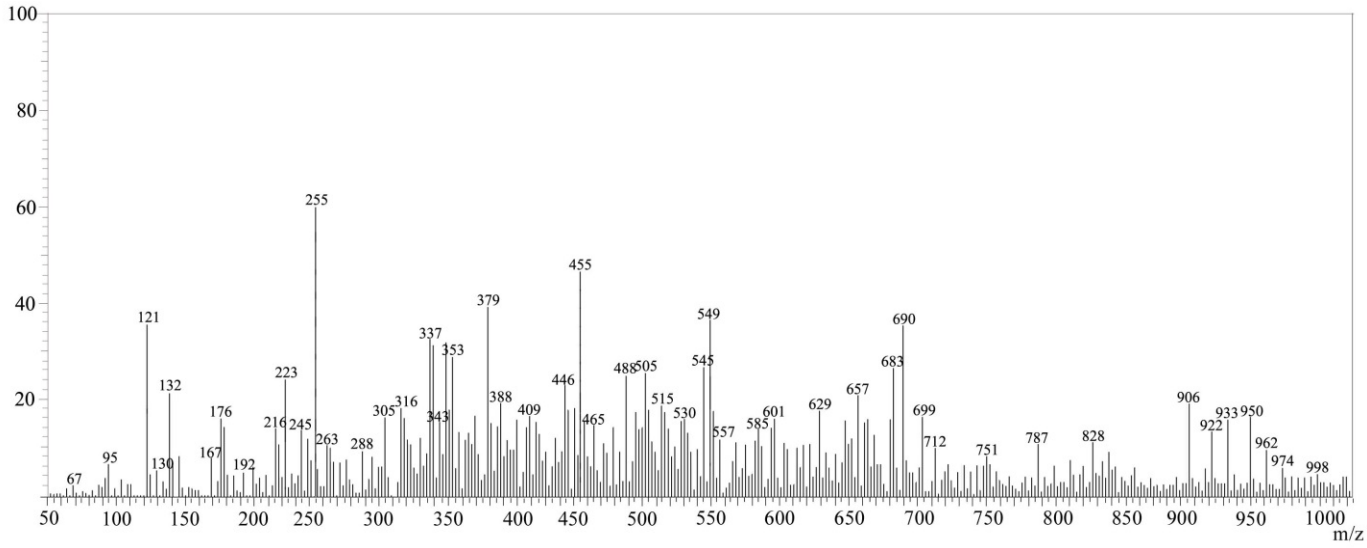

Figure 2: LCMS negative peaks of *Cynanchum callialatum*.

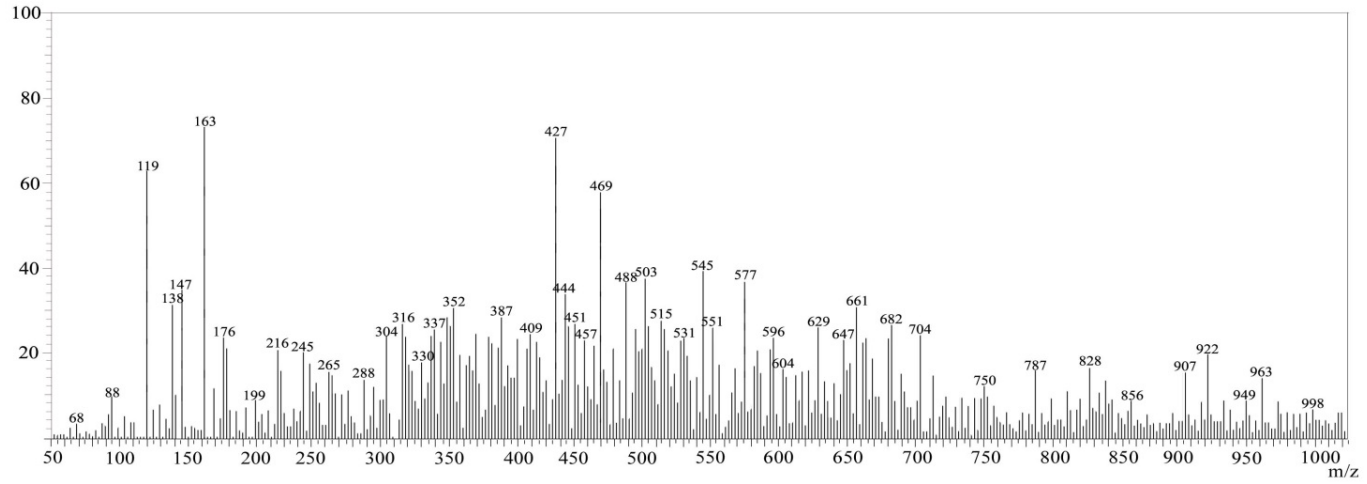

Figure 3: LCMS positive peaks of *Cynanchum callialatum*.

α-glucosidase, cFLIP, Bcl-2 and NFκB [9].

Germacrone: Germacrone possessed antiviral activity against the H1N1 and H3N2 influenza A viruses and the influenza B virus in a dose-dependent manner. The viral protein expression, RNA synthesis and the production of infectious progeny viruses were decreased both in MDCK and A549 cells treated with germacrone. In a time-of-addition study, germacrone was found to exhibit an inhibitory effect on both the attachment/entry step and the early stages of the viral replication cycle. Germacrone also exhibited an effective protection of mice from lethal infection and reduced the virus titres in the lung [10]. The germacrone possessed anti-proliferative effect of on the human hepatoma cell lines. Treatment of human hepatoma cell lines HepG2 and Bel7402 with germacrone resulted in cell cycle arrest and apoptosis in a dose-dependent manner as measured by MTT assay, flow cytometric and fluorescent microscopy analysis, while much lower effect on normal human liver cell L02 was observed. Germacrone might be a new potent chemo preventive drug candidate for liver cancer via regulating the expression of proteins related to G2/M cell cycle and apoptosis, and p53 and oxidative damage may play important roles in the inhibition of human hepatoma cells growth [11].

Longiverbenone: Is a sesquiterpene isolated which possess antibacterial and cytotoxic activity. The cytotoxic activity (LC50) of the compound longiverbenone new born brine shrimp (Artemiasalina) is presented in (Table 3). The LC50 of the compound against the brine shrimp was found to be 14.38 µg/ml. The cytotoxic bioassay result of longiverbenone may lead to the exploration of its potential and practical application as a novel less toxic and antimicrobial compound from this plant. Similar cytotoxic activities of plant constituents have been reported previously [12].

Sinapic acid (SA): shows cerebral protective and cognition-improving medicine.SA has anti-oxidative and anti-inflammatory activities, and may be an efficacious treatment for Alzheimer's disease [13].

Daucosterol: The treatment with DS-given mice with anti-mouse IFNγ, the protection was also abolished. These show that DS protects mice against disseminated candidiasis by the $CD4^+$ Th1 immune response [14]. Daucosterol improved blood circulation by inhibiting ether platelet aggregation and/or blood coagulation [15].

Taraxasterol: Have been shown experimentally to inhibit colon and breast cancer development. They act at various stages of tumor development, including inhibition of tumorigenesis, inhibition of tumor promotion, and induction of cell differentiation. Effectively inhibit invasion of tumor cells and metastasis [16]. Taraxasterol dramatically decreased the total inflammatory cell and reduced the production of Th2 cytokine IL-4, IL-5, IL-13 in BALF and OVA-specific IgE in sera, and suppressed AHR in a dose-dependent manner. Histological studies evidenced that the taraxasterol substantially suppressed OVA-induced inflammatory cells infiltration into lung tissues and goblet cell hyperplasia in airways [17].

Conclusion

The present study on phytochemical investigation of *Cynanchum callialatum* reveals the presence of various phytochemical constituents which support its use in folk medicine. This study also helps us to carry out researches based on the bioactive compounds, and to confirm scientifically the anticancer, antiretroviral, antimalarial, anti-inflammatory activities of *Cynanchum callialatum*. Our study suggests that *Cynanchum callialatum* may be a potential source for anticancer, antiHI, anti-inflammatory, antimicrobial drug discovery.

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