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Research Article

GC - MS ANALYSIS OF LEAF AND STEM BARK OF *CLEIDION NITIDUM* (MUELL. – ARG.) THW. EX KURZ. (EUPHORBIACEAE)

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ABSTRACT

Objective: GC-MS (Gas chromatography and mass spectroscopy) analysis of ethanol extracts of leaf and stem bark of *Cleidion nitidum* was carried out to detect the bioactive components.

Methods: The chemical compositions of the ethanolic extract of leaves and bark of *Cleidion nitidum* were investigated using Perkin – Elmer Gas Chromatography – Mass Spectra while mass spectra of the compounds found in the extract was matched with the National Institute of Standards of Technology (NIST) library.

Results: Gas chromatography mass spectra (GC-MS) analysis revealed the presence of 16 compounds from leaves and 5 compounds from bark of the *Cledion nitidum* were identified. In GC-MS analysis , some of the phytocomponents screened were squalene, 3, 7, 11, 15-Tetramethyl-2, hexadecen-1-ol , Phytol and Vitamin E in leaves whereas, Thiophene-3-carbonitrite tetrahydro-4-oxo-, Dimethyl-2,6,10-dodecatrien-1-ol , D-Mannotetradecane-1,2,3,4,5-pentaol and Octanal 7-methoxy-3-7-dimethyl (5.13%) major compounds in the bark extract.

Conclusion: These results indicate the ethanol extract of leaf and stem bark of *Cleidion nitidum* possesses potent anti-tumor, anticancer, cancer preventive, anti-inflammatory, antioxidant, antibacterial and pesticide effects so that it can be recommended as a plant of phytopharmaceutical importance.

Keywords: *Cleidion nitidum*, GC-MS, Squalene, Phytol, vitamin-E and various applications

INTRODUCTION

Higher plants are sources of bioactive compounds to play a crucial role in the maintenance of human health since time immemorial. Reports available on green plants represent a reservoir of effective chemotherapeutants; these are non-phytotoxic, more systemic and easily biodegradable[1-4].

Cleidion nitidum (Muell. – Arg.) Thw. ex Kurz. belongs to Euphorbiaceae family. This genus comprises about 33 species, which are pantropical in Asia and the South West Pacific[5]. The taxa are represented as 3 species with 4 varieties in India [6], of which *C. nitidum* is distributed in Andaman and Nicobar Islands of Indian subcontinent. Recently it has been reported in Eastern Ghats of Peninsular India [7] and in Southern Western Ghats of Tamilnadu [8].

Two species of this genus *Cleidion viz C. javanicum* BC and *C. speciflorum* Merr. are used as medicine traditionally in Thailand and Philiphines. Several parts of the above two plant species have been employed as analgesic, antipyretic and diaphoretic[9]. Decoction of its leaves is reputed to cause abortion; where as a decoction of the bark is used for treatment of stomachic. Its seeds are used for treatment of constipation[10]. GC-MS analysis of phytochemical compounds present in the various medicinal plants was made by various workers[11-14]. Isolation and structure determination of two flavone glycosides, a phenylpropanoid glycoside, D–glucopyranoside, a linear diterpene, trans–phytol and a lanostane triterpene from leaves of *C. spiciflorum* was already reported[15]. The chemical composition and the biological activities of the essential oil from the leaves of *C. javanicam* were studied [16]. However, perusal of literature reveals that GC-MS analysis of *C. nitidum* is totally lacking and hence the present investigation was undertaken.

MATERIALS AND METHEODS

Plant material

The leaf and stem bark of *C. nitidum* were collected from Thadagamalai Reserve Forests, 8o 18. 960′N – 77o 29.845′ E, alt. ca. 750m (msl) Kanyakumari wildlife sanctuary, Kanyakumari District, Tamilnadu. The plant materials were taxonomically identified and authenticated by the Sri Ganesan Herbarium (SGH), Madura College Herbarium, Madurai (Ac. No. 1583). Voucher specimen (No. BPN & SSS 2674) was deposited in the STHC herbarium, Dept of Botany, South Travancore Hindu College, Nagercoil, Kanyakumari District, Tamilnadu, India.

Preparation of extract

The leaf and stem bark were shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppered flask and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

Gas Chromatography –Mass Spectroscopy Analysis

GC-MS analysis of the extract was performed using a Perkin – Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite-1, fused silica capillary column (30 mm x 0.25 mm 1DX 1 μMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization

system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/ min and an injection volume of 2 µl was employed (Split ratio of 10:1); injector temperature 250°C; Ion – source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/ min, to 200°C, then 5°C / min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area, to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass.

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. For the identification of biological potential of the analyzed compounds were checked the Dr. Dukes library and enumerated the biological properties of the known compounds[17].

RESULTS AND DISCUSSIONS

The components present in the ethanol extracts of leaves and barks of *C. nitidum* were identified by GC-MS analysis (Figures 1 and 2).

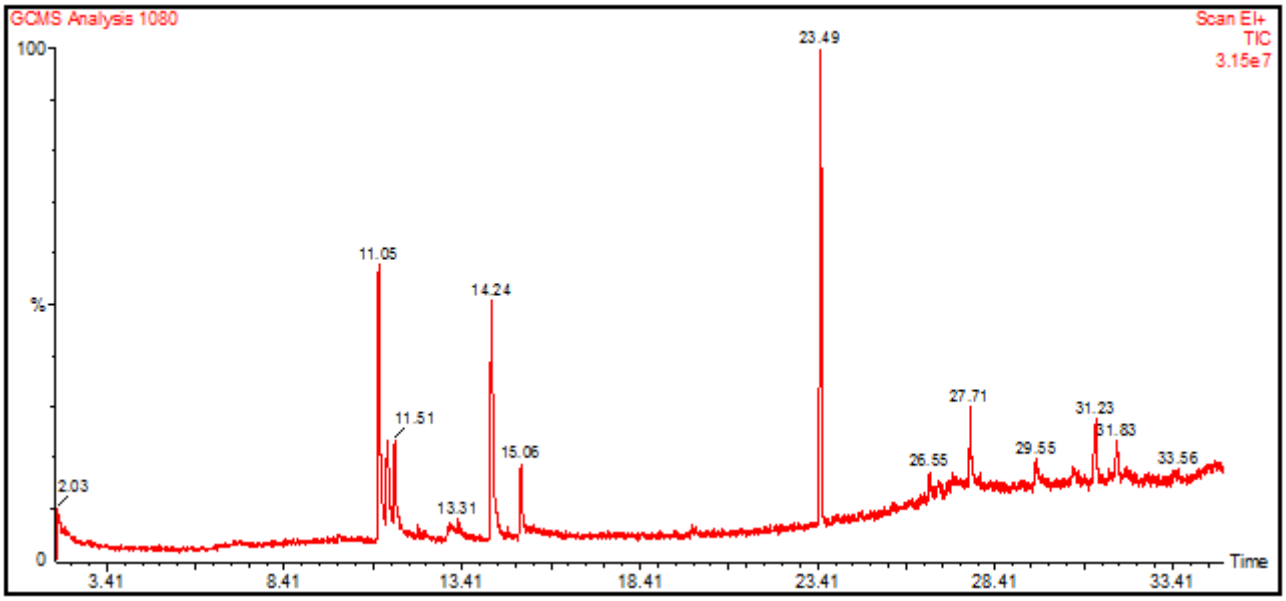


Fig. 1: GC-MS chromatogram of ethanolic extract of leaves of *C. nitidum*

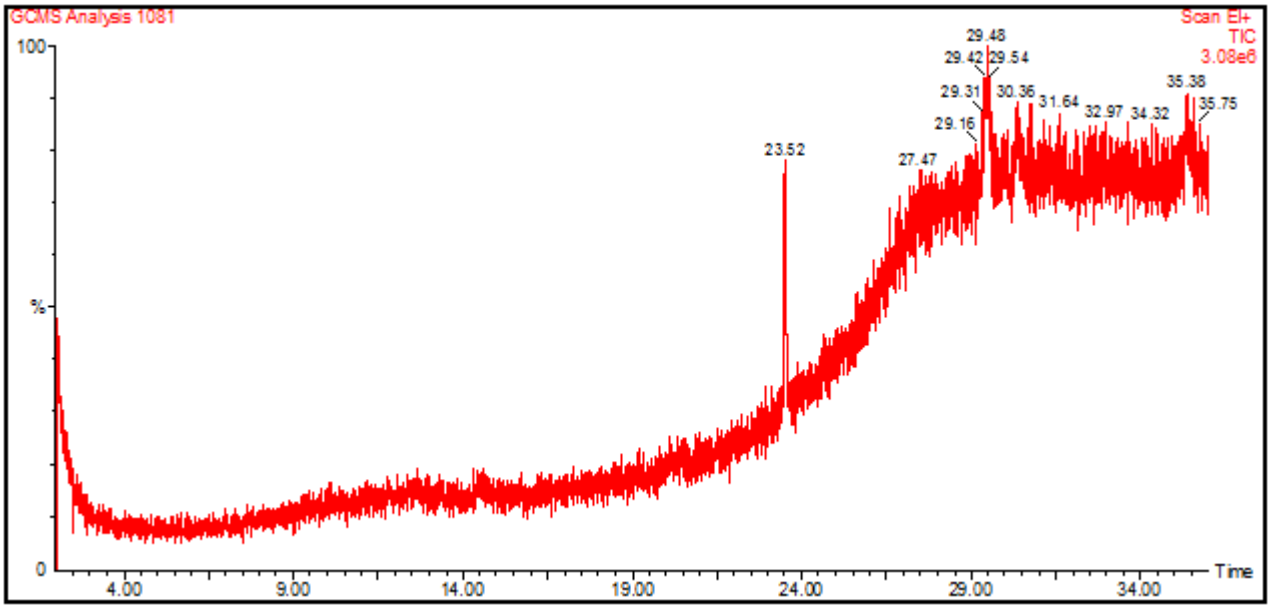
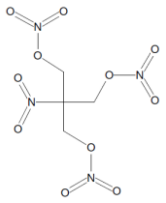
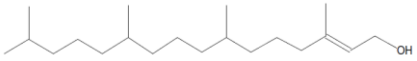
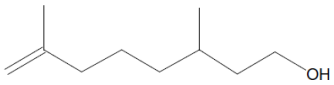
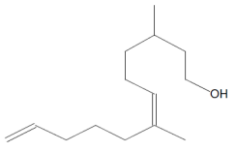
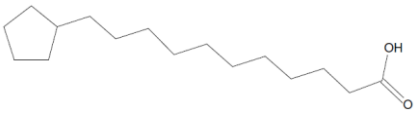
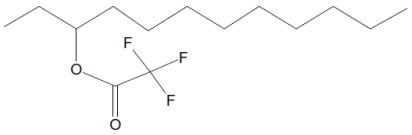
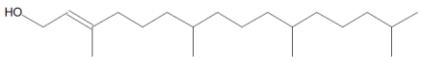
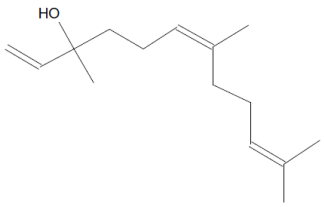
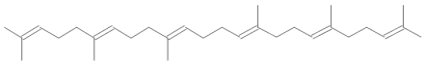
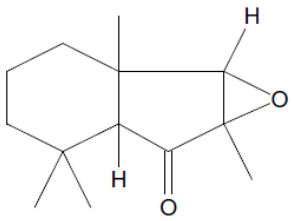


Fig. 2: GC-MS chromatogram of ethanolic extract of bark of *C. nitidum*

The active principles with their retention time (RT), Molecular formula, Molecular weight (MW) and concentration (%), compound nature and biological activity of the compounds in the ethanol extracts of leaves and bark of *Cleidion nitidum* are presented in Tables 1 and 2 respectively.

Table 1: Phytocomponents identified in the ethanolic leaf extract of *C. nitidum* by GC – MS

No.	RT	Name	Mol. Formula	MW	Peak Area %	Structure
1	2.03	1,3-Propanediol, 2-nitro-2-[(nitrooxy)methyl]-, dinitrate (ester)	$C_4H_6N_4O_{11}$	286	6.42	
2	11.05	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	17.52	
3	11.31	7-Octen-1-ol, 3,7-dimethyl-, (S)-[.alpha.-Citronellol]	$C_{10}H_{20}O$	156	4.86	
4	11.51	Dodeca-1,6-dien-12-ol, 6,10-dimethyl-	$C_{14}H_{26}O$	210	5.50	
5	13.07	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254	0.78	
6	13.31	2-Trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	282	0.69	
7	14.24	Phytol	$C_{20}H_{40}O$	296	15.61	
8	15.06	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- [D-nerolidol]	$C_{15}H_{26}O$	222	5.51	
9	23.49	Squalene	$C_{30}H_{50}$	410	17.68	
10	26.55	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	208	2.65	

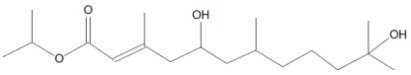
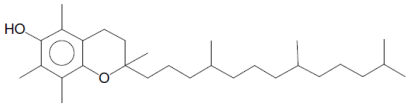
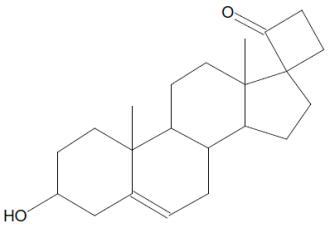
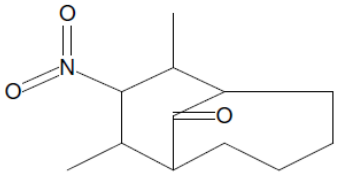
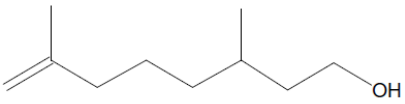
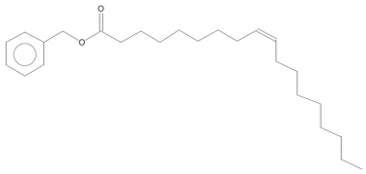
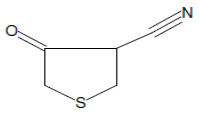
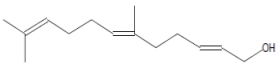
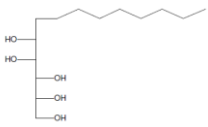
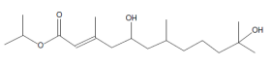
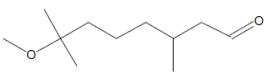
11	27.21	Isopropyl 5,11-dihydroxy-3,7,11-trimethyl-2-dodecenoate	$C_{18}H_{34}O_4$	314	2.18	
12	27.71	Vitamin E	$C_{29}H_{50}O_2$	430	7.88	
13	29.55	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	$C_{22}H_{32}O_2$	328	2.84	
14	31.23	7,9-Dimethyl-8-nitrobicyclo[4.3.1]decan-10-one	$C_{12}H_{19}NO_3$	225	5.17	
15	31.83	5à-Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	350	3.22	
16	34.56	9-Octadecenoic acid (Z)-, phenyl methyl ester	$C_{25}H_{40}O_2$	372	1.47	

Table 2: Phytocomponents identified in the ethanolic extract of the stem bark of *C. nitidum* by GC - MS

No.	RT	Name	Mol. Formula	MW	Peak Area %	Structure
1	2.04	Thiophene-3-carbonitrile, tetrahydro-4-oxo-	C_5H_5NOS	127	51.12	
2	23.52	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	$C_{14}H_{24}O$	208	21.43	
3	29.48	D-Mannotetradecane-1,2,3,4,5-pentaol	$C_{14}H_{30}O_5$	278	20.31	
4	30.36	Isopropyl 5,11-dihydroxy-3,7,11-trimethyl-2-dodecenoate	$C_{18}H_{34}O_4$	314	2.01	
5	35.38	Octanal, 7-methoxy-3,7-dimethyl-	$C_{11}H_{22}O_2$	186	5.13	

Sixteen compounds were identified in ethanolic extracts of leaves of *C. nitidum*.The results showed the presence of 1,3-Propanediol, 2-nitro-2-[(nitrooxy)methyl]-, dinitrate (ester) 6.42(%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol1(17.52%),7-Octen-1-ol, 3,7-dimethyl-, (S)-(4.86%), Dodeca-1,6-dien-12-ol, 6,10-dimethyl-(5.50%), Cyclopentaneundecanoic acid (0.78%), 2-Trifluoroacetoxydodecane (0.69%), Phytol (15.61%), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- (5.51%), Squalene (17.68%), 1b, 5, 5, 6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one (2.65%), Isopropyl 5,11-dihydroxy-3,7,11-trimethyl-2-dodecenoate (2.18%), Vitamin E (7.88%), Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)- (2.84%), 7,9-Dimethyl-8-nitrobicyclo[4.3.1]decan-10-one (5.17%), 5à-Androstan-16-one, cyclic ethylene mercaptole (3.22%) and 9-Octadecenoic acid (Z)-, phenylmethyl ester (1.47%) are the major compounds available in the leaf of the C. nitidum (Table-1). The major phyto components and its biological activities obtained through the GC-MS study of leaves of C.nitidum was provided in table 3.

Table 3: Activity of phytocomponents identified in the ethanolic extracts of leaf of *C. nitidum*

No.	Name of the compound	Nature of Compound	**Activity
1	1,3-Propanediol, 2-nitro-2-[(nitrooxy)methyl]-, dinitrate (ester)	Nitrogen compound	Antimicrobial
2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial Anti-inflammatory Fragrance compound
3	7-Octen-1-ol, 3,7-dimethyl-, (S)-[.alpha.-Citronellol]	Alcoholic compound	Antimicrobial Anti-inflammatory Insecticide
4	Cyclopentaneundecanoic acid	Fatty acid compound	Antimicrobial
5	2-Trifluoroacetoxydodecane	Fluro compound	Antimicrobial
6	Phytol	Diterpene	Antimicrobial Anticancer Anti-inflammatory Diuretic Fragrance compound Antimicrobial Anti-inflammatory
7	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	Sesquiterpene alcohol	Insecticide
8	[D-nerolidol]	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide
9	Vitamin E	Vitamin compound	Antiageing, Analgesic, Antidiabatic Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, .Antibronchitic, Anticoronary
10	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	Steroid	Antiarthritic Anticancer Hepatoprotective Antimicrobial Antiasthma Diuretic
11	7,9-Dimethyl-8-nitrobicyclo[4.3.1]decan-10-one	Nitrogen compound	Antimicrobial Antiarthritic Anticancer Hepatoprotective Antimicrobial Antiasthma
12	5à-Androstan-16-one, cyclic ethylene mercaptole	Steroid	Diuretic Anti-inflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic
13.	9-Octadecenoic acid (Z)-, phenyl methyl ester	Oleic acid ester	Insectifuge, Flavor

****Activity Source: Dr Duke's Phytochemical and Ethnobotanical databases**

Five compounds were identified from the ethanolic extract of stem bark of *C. nitidum.* The results revealed Thiophene – 3 – carbonitrile, tetrahydro – 4- oxo – (51.12%), 6, 11 – Dimethyl – 2, 6, 10 – dodecatrien – 1 – ol (21.43%), D – Mannotetradecane – 1, 2, 3, 4, 5 – pentaol (20.31%), Isopropyl 5, 11 – dihydroxy – 3, 7, 11 – trimethyl – 2 – dodecenoate (2.01%) and Octanal, 7 – methoxy – 3, 7 – dimethyl – (5.13%) were found as the major components in the ethanolic extracts of bark of *C. nitidum* (Table-2). Major phyto compounds and its biological activities obtained through GC-MS study of bark of *C. nitidum* has been tabulated (Table 4)

Table 4: Activity of phyto components identified in the ethanolic extracts of bark of *C. nitidum*

No.	Name of the compound	Nature of Compound	**Activity
1.	Thiophene-3-carbonitrile, tetrahydro-4-oxo-	Sulfur compound	Antimicrobial
2.	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	Alcoholic compound	Antimicrobial
3.	D-Mannotetradecane-1,2,3,4,5-pentaol	Alcoholic compound	Antimicrobial
4.	Octanal, 7-methoxy-3,7-dimethyl-	Aldehyde compound	Antimicrobial Anti-inflammatory

****Activity Source: Dr Duke's Phytochemical and Ethnobotanical databases**

The GC-MS analysis of *C. nitidum* leaves revealed the presence of 16 compounds. The identified compounds possess many biological properties. Among the identified phytochemicals, Squalene has the property of antioxidant activity [18,19] and chemopreventive activity against colon carcinogensis [20,21]. Squalene has been reported in *Canthium parviflorum* and its anticancer activity was mentioned [22].

Phytol is one among the 16 compounds from the leaves of *C. nitidum*. Presence of Phytol in the leaves of *Kirganelia reticulata* aerial parts, which was also found to be effective in different stages of arthritis [19]. It was found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as Phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases [23]. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamin E and K1[24]. It is used along with simple sugar on corn syrup as a hardener in candies [25].

In the present study 16 and 5 phyto-components have been identified from ethanol extract of leaves and stem bark of *C. nitidum* respectively by Gas Chromatography and Mass Spectrometry analysis. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants and this type of study will be helpful for further detailed study. However, isolation of individual phytochemical constituent and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, *C. nitidum* contains various bioactive compounds. So it is recommended as plant of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

CONCLUSIONS

The result of the present investigation reveals that the ethanol extract of leaves and stem bark of *C. nitidum* possessed significant anti-inflammatory, anticancer, antioxidant, antitumor, immunostimulant and antimicrobial properties. The GC-MS analysis of the ethanol extract of *C. nitidum* reveals the presence of phytoconstituents belonging to the type acids, esters, alcohols, ethers, etc. Thus, the medicinal plant *C. nitidum* is found to possess significant phytoconstituents such as Squalene, Phytol, Vitamin E and Octanol, 7 – methoxy – 3, 7 – dimethyl etc. The presence of such a variety of phytochemicals may be attributed to the medicinal characteristics of this plant *C. nitidum*.

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