Research Article

Vedpal Singh*, Namrita Lall, Ashish Wadhwani and Sangai P. Dhanabal

GC-MS analysis of *curculigo orchiodes and medicinal* herbs with cytotoxic, hepatoprotective attributes of ethanolic extract from Indian origin

https://doi.org/10.1515/jcim-2020-0545
Received December 31, 2020; accepted February 27, 2022; published online March 23, 2022

Abstract

Objectives: Liver illnesses are a major public health issue all over the world. Medicinal plants constituents a viable alternative for the development of phytopharmaceuticals with hepatoprotective activity in order to solve some of these health-related problems. The present study is focused on the phytochemical and biological investigation on Indian traditional medicinal plant extracts, for their cytotoxic and hepatoprotective activity. The isolated compounds showed the presence of phenolic constituents which lead to cytotoxicity and hepatoprotective activity of medicinal plant. Cancer causes about 13% of all human deaths in 2007 (7.6 million) (American Cancer Society and WHO December 2006–07). The American Cancer Society estimates that 12,990 new cases of cervical cancer will be diagnosed in the United States year 2016. Cancer-related deaths are expected to increase, with an estimated 11.4 million deaths in 2030.
Methods: The ethanolic extracts of *Centella asiatica*, *Myristica fragrans*, *Trichosanthes palmata*, *Woodfordia fruticosa*, *Curculigo orchioides* were evaluated against HEP-G2 cell lines for hepatoprotective activity and *Curculigo orchioides* was further promoted for the isolation of secondary metabolites based on inhibitory concentration.
Results: The ethanolic extracts of *C. asiatica*, *M. fragrans*, *T. palmata*, *W. fruticosa*, *Curculigo orchioides* shown significant cytotoxic activity (IC_{50}≤100 μg/mL). The plant extracts also shown significant hepatoprotective activity in a dose dependent manner when tested against HEP-G2 cell lines and cytotoxicity studies against HeLa and HEP-G2 cells.
Conclusions: The extract of *Curculigo orchiodes* rhizome showed significant cytotoxicity results. Hence the *Curculigo orchiodes rhizome* was selected for further phytochemical studies to isolate active compounds and their Characterization by GCMS.

Keywords: GCMS; HeLa cell lines; HEP-G2 cell lines; medicinal plants; MTT assay; silymarin.

***Corresponding author: Dr. Vedpal Singh**, Asst. Professor, Department of Pharmacognosy, College of Pharmacy, JSS Academy of Technical Education, C-1/A, Sector 62, Noida, UP, India, Phone: +919927411605, E-mail: vsphd@jssuni.edu.in. https://orcid.org/0000-0003-3523-1469
Namrita Lall, Department of Plant and Soil Sciences, University of Pretoria, Pretoria, South Africa, E-mail: namrita.lall@up.ac.za
Ashish Wadhwani, Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Ooty, Tamilnadu, India; and School of Pharmacy, Faculty of Health Science, JSS Academy of Higher Education and Research, Mauritius campus. Droopnath Ramphul St, Vacoas-Phoenix, Mauritius, E-mail: dradwadhwani@jssuni.edu.in. https://orcid.org/0000-0001-8102-9486
Sangai P. Dhanabal, Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Tamilnadu, India, E-mail: spdhanabal@jssuni.edu.in

Introduction

Natural products and structural counterparts have long played an important role in pharmacotherapy, particularly in the treatment of cancer and infectious disorders. Natural products have long been important in therapeutic development, particularly for cancer and infectious disorders. Natural products are also of tremendous interest to drug discovery programmers because to their wide variety in nature [1]. Furthermore, natural compounds with a wide range of biological activity usually have a complex and diversified structure, posing significant difficulties in organic synthesis [2]. Since the beginning of time, medicinal plants and their compounds have been utilized as medications to cure a variety of maladies. Consumers feel that herbal goods are natural and safe; hence their use of herbal treatments is fast expanding over the world [3]. The nutraceutical industry in India is currently valued at USD 2.2 billion, with the southern region leading the way, followed by the eastern region, which includes the four major states of Andhra Pradesh, Karnataka, Tamilnadu and West Bengal [4, 5]. As a result, a thorough examination of their gene, cytotoxic, and overall toxicity is

required. Many redox activities produce reactive oxygen species (ROS), which cause oxidative damage to biological components such as carbohydrates, lipids, proteins, and DNA [6]. Antioxidant chemicals have been proposed as a way to prevent ageing by scavenging free radicals and delaying or preventing the oxidation of biological components. Plants that contain phenolic compounds such phenolic acids, phenolic diterpenes, flavonoids, tannins, and coumarins are recognised to be natural antioxidant sources [7]. Numerous investigations have indicated that natural antioxidants have a wide range of pharmacological properties, including neuroprotective, anticancer, and anti-inflammatory properties [8]. Despite the effectiveness of the natural-products strategy to drug discovery, scientific knowledge on these natural sources is available in South Africa [9]. As a result, the National Research Foundation (NRF) of South Africa and the Department of Science and Technology (DST) collaborated on a research project to screen medicinal plants from both India and South Africa for prospective anticancer and antitubercular drug leads. Cancer is a type of disease in which a group of cells display uncontrolled growth, invasion and sometimes leading to metastasis. Cancer affects people at all ages with the risk for most types increasing with age. Cancer causes about 13% of all human deaths in 2007 (7.6 million) (WHO February 2006, American Cancer Society December 2007). Cancer-related deaths are expected to increase, with an estimated 11.4 million deaths in 2030. The American Cancer Society estimates that 12,990 new cases of cervical cancer will be diagnosed in the United States year 2016. In the United States, cervical cancer is one of the top causes of death among women [10]. The World Health Organization (WHO) estimates that 2.4 million people death each year as a result of liver disease, with cirrhosis accounting for about 800,000 of those fatalities. On the other hand, epidemiological studies undertaken by the National Institute of Statistics and Geography (INEGI in Spanish) show that over 600,000 deaths occurred in Mexico in 2013. Diabetes mellitus was the leading cause (14.25%), followed by ischemic heart disease (12.63%), cerebrovascular disease (5.29%), and liver disease (5.29%). Despite contemporary medicine's advancements and the development of new hepatoprotective medications [11]. Given the aforementioned, the current study attempted to evaluate *in vitro* anti-cancer activity on cervical cell lines as well as hepatoprotective efficacy using rat hepatocytes.

Materials and methods

Chemicals, reagents and instruments

HeLa cell lines were procured from National Centre for Cell sciences (NCCS), Pune, India. CCl_4 was purchased from Avi chem. Industries, Khopat, Mumbai, India. Silymarin, MTT Dyes, HEPES Buffer were purchased from Sigma Aldrich, USA. DMEM, MEM-PR, isopropanol was purchased from HI Media, Mumbai Maharashtra, India. Phenobarbitol sodium was purchased from Medopharm Pvt. Ltd, Chennai, India. Rota vapour (BUCHI R 210) was purchased from Flawil, Switzerland. Micro plates were purchased from Tarsons, Mumbai, Maharashtra, India. Vaccum desiccators from Tarsol, Chennai, India. GC-MS, Shimadzu Analytical (India) Pvt. Ltd. Mumbai, India, was used.

Procurement of plant material

The plants were collected by qualified plant collectors from various parts of India and authenticated by Dr. S. Rajan, field taxonomist, Survey

Table 1: Specimen voucher reference number of the selected plants, showing the data of % yield (w/w) of selected plants and *in vitro* cytotoxicity screening of selected plants.

Sl. no	Name of plant	Family	Parts used	Voucher specimen no.	Total weight, g	% yield w/w	IC_{50}, µg/mL
1.	*Abrus precatorius*	Fabaceae	Seeds	JSSPG-01	24.75	2.47	288
2.	*Bacopa monnieri*	Scrophulariaceae	Aerial	JSSPG-277	58.06	5.80	179
3.	*Cedrus deodara*	Pinaceae	Aerial parts	JSSPG-278	5.48	1.09	250
4.	*Celastrus paniculatus*	Celastraceae	Seeds	JSSPG-279	32.84	3.24	330
5.	*Centella asiatica*	Apiaceae	Aerial	JSSPG-176	35.87	3.58	32
6.	*Curculigo orchioides*	Amaryllidaceae	Rhizomes	JSSPG-184	30.16	3.01	76
7.	*Ficus benghalensis*	Moraceae	Bark	JSSPG-66	17.08	8.54	270
8.	*Foeniculum vulgare*	Umbelliferae	Fruits	JSSPG-67	13.50	6.75	185
9.	*Glycyrrhiza glabra*	Fabaceace	Rhizomes	JSSPG-279	40.67	12.32	390
10.	*Holarrhena antidysenterica*	Apocynaceae	Bark	JSSPG-71	35.18	7.81	320
11.	*Myristica fragrans*	Myristicaceae	Fruits	JSSPG-90	54.80	5.48	110
12.	*Pterocarpus marsupium*	Pteridaceae	Wood	JSSPG-101	29.11	7.27	250
13.	*Trichosanthus palmata*	Cucurbitaceace	Aerial	JSSPG-280	25.12	5.02	76
14.	*Valeriana wallichii.*	Valerianaceae	Rhizomes	JSSPG-281	45.38	9.07	220
15.	*Woodfordia fruticosa*	Lythraceae	Aerial	JSSPG-282	85.29	8.52	87
16.	*Symplocos cochinchinensis*	Symplocaceae	Bark	JSSPG-283	31.56	6.48	186

of Medicinal Plants and Collection Unit, Department of Ayush, Emerald, Tamilnadu, India. The specimen voucher reference number (Table 1) was deposited at JSS College of Pharmacy, Department of Pharmacognosy and Phytopharmacy, Taminadau for future reference, Ooty.

Extraction

Plant materials were harvested fresh and dried in the shade before being crushed into a fine powder. The powdered plant material (1 kg) was subjected to triple kinetically hydro-alcoholic maceration with 90% v/v ethanol, and the filtrates were distilled and concentrated under vacuum using a Rotary evaporator (Buchi R 210). Vacuum desiccators were used to dry the concentrated extracts, and the % yield of extracts was measured on a dried basis (Table 1) [12].

Isolation

The crude hydro-alcoholic extract (13 g) was partitioned by using separating funnel (liquid-liquid) with petroleum ether, chloroform, ethyl acetate and *n*-butanol and water. The filtrates were evaporated in a rotary evaporator with reduced pressure (Buchi Rotavap (R-210)) [13].

Compound C1: The compound, C1, was isolated from petroleum ether extract by solvent precipitation method, The isolated fraction was further purified by preparative TLC over silica gel GF254 using ethyl toluene and diclhormethane (1:1). It was crystallized from methanol as white powder (R_f 0.89). The compound C1 was idetified as (1Propanone, 1(2-furanyl)).

Compound C2: The Compound C2 was isolated from chloroform fraction evaporated 2 gms of residue soluble in toluene, the clear fraction was subjected to column chromatography (2 Mm with, 30 cm length with silica gel (100–200 Mesh) column using (Chloroform: Methanol- 7:3), (R_f 0.90, toluene: DCM- 1:1)). The compound C2 was identified as (9, 12-Octadecadienoic acid (z, z) -, methyl ester).

Compound F1: The ethyl acetate fraction was evaporated under reduced pressure. The shiny soft residue was obtained which was subjected to column chromatography (2 Mm with, 30 cm length with silica gel (100–200 Mesh) column using (Chloroform: EA: Methanol-6:3:1)). A greenish yellow residue was obtained. This was dissolved in ethyl acetate and white powder was obtained after recrystallization. White recrystallize compound obtained (F1) was subjected to GCMS and was identified as (3-Methly-2-furoic acid).

Compound F2: The n-butanol fraction was subjected to silica gel column using n-hexane-ethyl acetae-methanol (2:3:5) as eluents in increasing order of polarity. Similar fractions were pooled together based on TLC profile and evaporated, a reddish brown crystal (3 mg) was obtained which was subjected to TLC using (Hexane: Methanol (50:50) R_f (0.64). A white colour substance was obtained and was identified as (F2) Phthalic acid, di (6methylhept2yl) ester].

Compound S1: The aqueous fraction was evaporated. The obtained 5 gms of residue was dissolved, filtered and concentrated and fractionated with 20 mL butanol. The butanol layer was evaporated and 2 g of the residue was subjected to column chromatography (2 Mm with, 30 cm length) with amberlite IR 120. Two fractions were obtained during this process. Both the fractions were pooled together and were evaporated. A brown colour compound (S1) was obtained. (0.59 g) TLC showed purity of single spot with n-butanol: glacial acetic acid: water (3:4:3) compound (R_f 0.79) [14].

Phytochemical screening

Based on standard protocol, the produced extracts and fractions were subjected to a preliminary qualitative phytochemical screening [15].

In vitro cytotoxic activity

The study was carried out according to standard protocol and cytotoxicity was expressed as the concentration of extracts inhibiting cell growth by 50% (IC_{50}). Cytotoxicity was expressed as the concentration of extracts inhibiting cell growth by 50% (IC_{50}) and the study was carried out as reported earlier based on standard protocol [16].

In vitro hepatoprotective studies on rat hepatocytes

Hepatoprotective effect of the plant extract in freshly isolated rat hepatocytes isolation and culture of hepatocytes: Pentobarbital sodium (35 mg/kg bw) was used for anesthesia. Initially heparin was injected into the femoral vein (1,000 IU) followed by perfusion with calcium free HEPES buffer 20 min (37 °C), which contained 1% bovine serum albumin fraction V at a flow rate of 30 mL/min. The liver swells during this time, slowly changing its color from dark red to grayish white. The swollen liver was then perfused with a TPVG solution (50 mL) followed by perfusion with calcium-free HEPES buffer, which contained additional collagenase solution (0.075%) and calcium chloride (4 mM) at a flow rate of 15 mL/min for 20 min. After the perfusion, the lobes were removed and transferred into a sterile Petri dish containing calcium-free HEPES buffer and dispersed gently. It was transferred into a sterile conical flask and the crude cell suspension was stirred with the help of a magnetic stirrer for 5 min to release hepatocytes into the solution. The cell suspension was filtered through a nylon mesh (250 µ) and the preparation was centrifuged at 1,000 rpm for 15 min. The supernatant was aspirated and the loosely packed pellet of cells was gently re-suspended in the calcium-free HEPES buffer. This washing procedure was repeated three times. Cell viability was determined by the Trypan blue dye exclusion method. The isolated hepatocytes were cultured in Ham's F12 medium, supplemented with 10% newborn calf serum, antibiotics, 10^{-6} M dexamethasone, and 10^{-8} bovine insulin, and the cell suspension was incubated at 37 °C for 30 min in a humidified incubator under 5% CO_2.

Carbon tetrachloride-induced *in vitro* hepatocytes injury: In brief, carbon tetrachloride (CCl_4)-induced hepatocytes injury was carried out. After an incubation of 24 h, the hepatocytes were exposed to the fresh medium containing CCl_4 (1%) along with/without various concentrations of the extracts or the medium alone (as normal). Standard drug Silymarin with 250 µg/mL concentration was used for the study. After 60 min of CCl_4 challenge, concentrations of aspartate amino transferase (AST), Alkaline phosphatase (ALP), alanine amino transferase (ALT), total proteins (TP) in the medium were measured as an indication of hepatocytes necrosis using Ecoline diagnostic kits [17–19].

Technical specifications of GCMS instruments

GCMS Analysis was performed using shimadzu GCMS-QP 2010 equipped Thermo Trace 1300GC coupled with Thermo TSQ 800 Triple Quadrupole MS. For GC – THERMO TRACE 1300 GC For MS – THERMO TSQ 8000 Software used: XCalibur 2.2SP1 with Foundation 2.0SP1 Column: TG 5MS (30 m × 0.25 mm, 0.25 µm) Injector: S/SL (Split/Split less) Injection volume: 1.0 µL Injector temp: 250 °C MS transfer line temp: 280 °C, Ion source temp: 230 °C Mass Range: 50–600 Scan time: 0.5 s, Desired scans per peak: 6 Minimum baseline peak width: 3 s, Carrier Flow: 1 mL/min, Oven Program: Initial Temp: 60 °C; Hold time: 2.0 min, Final Temp: 280 °C; Hold Time: 10.0 min Temperature Rate: 10 °C/min, Detector: MS TSQ 8000 Library used: NIST 2.0, Carrier gas used was helium.

Characterization of isolated compounds

The ethanol extract of *Curculigo orchiodes* was fractionated using a bioassay-guided method, resulting in the identification of five components. **C1** (1Propanone, 1(2-furanyl) (RT 20.87, Molecular formula- $C_7H_8O_2$ CAS# 3194-15-8). **C2** (9, 12-Octadecadienoic acid (z, z) -, methyl ester (RT 19.31, molecular formula $C_{19}H_{34}O_2$ CAS# 112-63-0. **F1** (3-Methly-2-furoic acid) (RT 12.76, molecular formula $C_6H_6O_3$ CAS# 4412-96-8). **F2** (Phthalic acid, di (6methylhept2yl) ester) – and (RT 23.14, Molecular formula $C_{24}H_{38}O_4$ CAS# NA) and **S1**. (2-Hexanol, 2-methyl- (RT 4.28, Molecular formula $C_7H_{16}O$, CAS# 625-23-O). The isolated compound were characterized using GCMS finger printing as earlier described by Casuga et al. [20].

Statistical analysis

All data was reported as mean ± SD. values and was analysed using ANOVA and Dunnett's multiple comparisons test, with $p<0.05$ considered significant in the case of ALT and AST.

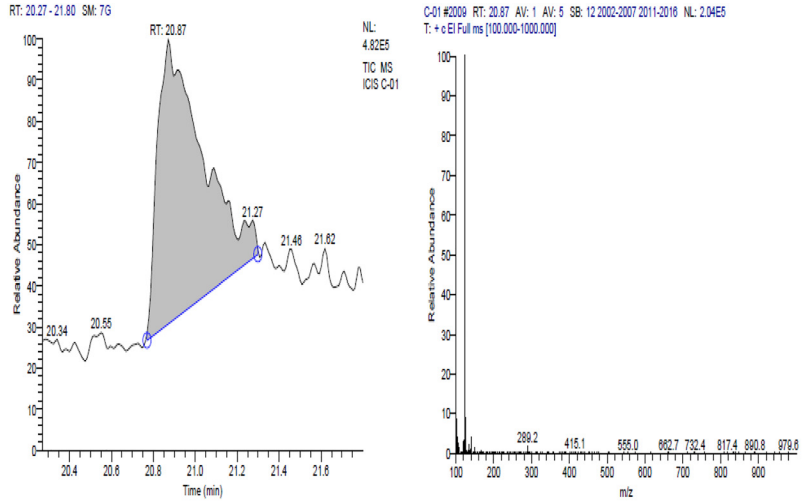
Results and discussion

When ethanolic extracts of *Centella asiatica*, *Myristica fragrans*, *Trichosanthes palmata*, *Woodfordia fruticosa*,

Table 2: Hepatoprotective activity of selected plant extract.

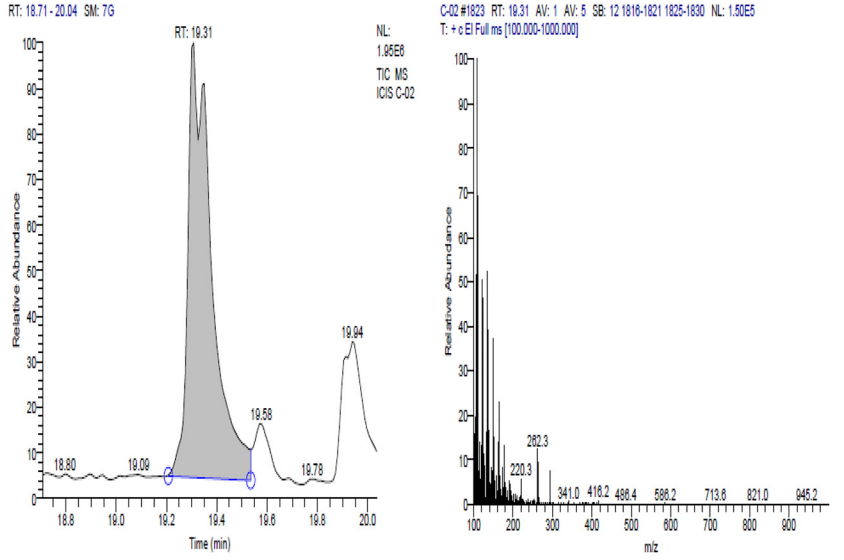
Sl. no	Plant name	Estimated parameter	Concentration, µg/mL		
			250 µg/mL	125 µg/mL	62.5 µg/mL
01	*Centella asiatica* (Apiaceae)	ALT	25.06 ± 4.34	35.53 ± 8.10	40.99 ± 9.98
		AST	30.98 ± 4.25	33.46 ± 7.65	37.07 ± 3.76
		TP	4.64 ± 7.18	5.78 ± 12.22	6.81 ± 16.12
		ALP	155.68 ± 4.74	170.17 ± 14.49	190.12 ± 6.51
02	*Myristica fragrans (Myristicaceae)*	ALT	30.64 ± 9.06	32.04 ± 2.74	41.57 ± 6.14
		AST	27.96 ± 11.71	31.08 ± 13.58	35.03 ± 15.02
		TP	5.70 ± 3.45	6.72 ± 6.54	7.47 ± 7.25
		ALP	166.05 ± 26.00	172.88 ± 17.75	190.41 ± 13.24
03	*Trichosanthes palmata (Cucurbitaceae)*	ALT	31.87 ± 7.27	32.48 ± 17.93	40.65 ± 14.06
		AST	31.79 ± 6.18	33.72 ± 9.83	37.68 ± 4.87
		TP	5.87 ± 1.22	5.73 ± 3.12	6.90 ± 2.86
		ALP	235.19 ± 99	181.68 ± 13.73	204.50 ± 12.00
04	*Woodfordia fruticosa (Lythraceae)*	ALT	32.04 ± 4.73	39.96 ± 6.79	49.45 ± 8.32
		AST	28.67 ± 3.19	30.10 ± 7.39	37.40 ± 8.31
		TP	5.52 ± 4.84	5.81 ± 2.56	6.52 ± 3.84
		ALP	186.46 ± 21.27	205.39 ± 36.99	225.02 ± 32.49
05	*Curculigo orchioides (Amaryllidaceae)*	ALT	29.42 ± 2.84	30.65 ± 6.06	41.61 ± 11.09
		AST	26.60 ± 22.99	35.76 ± 16.81	39.14 ± 2.98
		TP	5.87 ± 2.27	6.81 ± 4.67	8.71 ± 1.84
		ALP	181.99 ± 14.97	209.83 ± 9.74	228.25 ± 11.01
06	Control	ALT	32.65 ± 2.01	–	–
		AST	29.52 ± 7.20	–	–
		TP	5.19 ± 1.49	–	–
		ALP	190.52 ± 3.48	–	–
07	Silymarin	ALT	65.27 ± 12.75	–	–
		AST	54.06 ± 7.15	–	–
		TP	6.80 ± 5.07	–	–
		ALP	247.22 ± 15.50	–	–
08	1% CCl_4	ALT	80.22 ± 3.10	–	–
		AST	140.51 ± 6.48	–	–
		TP	11.71 ± 11.51	–	–
		ALP	350.47 ± 8.52	–	–

Values represent the mean ± SD of three determinations.



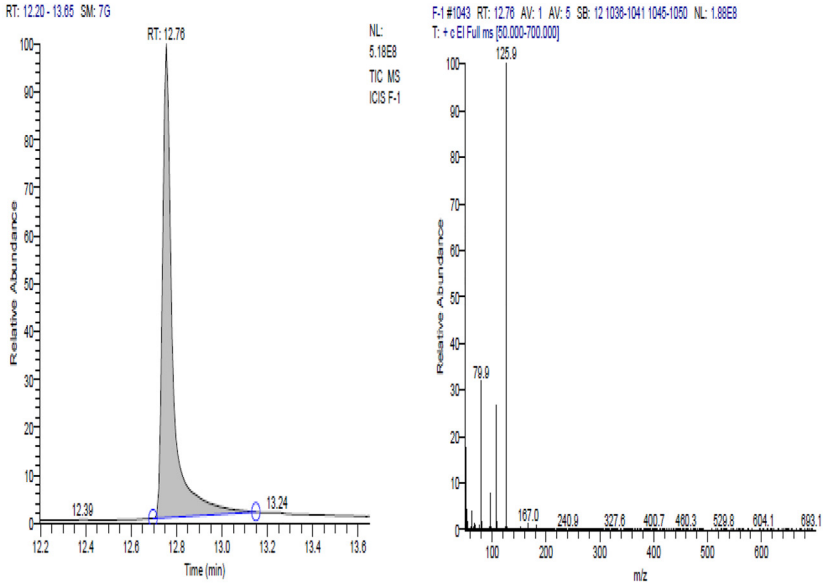
Compound Name	RT	Molecular Formula	Cas #
Atropine	20.87	C17H23NO3	51-55-8
1-Propanone, 1-(2-furanyl)-	20.87	$C_7H_8O_2$	3194-15-8
Propyl-2-iden-5-amino-1,2,4-triazole	20.87	C5H8N4	NA

Figure 1: GC-MS interpretation of compound C1.



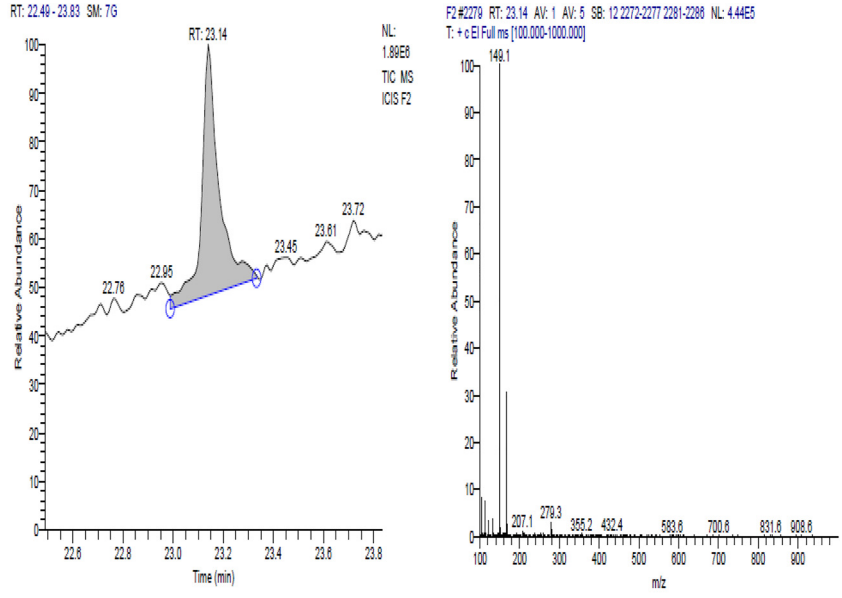
Compound Name	RT	Molecular Formula	Cas #
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	19.31	$C_{19}H_{34}O_2$	112-63-0
Methyl 9-cis,11-trans-octadecadienoate	19.31	C19H34O2	NA
8,11-Octadecadienoic acid, methyl ester	19.31	C19H34O2	56599-58-7

Figure 2: GC-MS interpretation of compound C2.



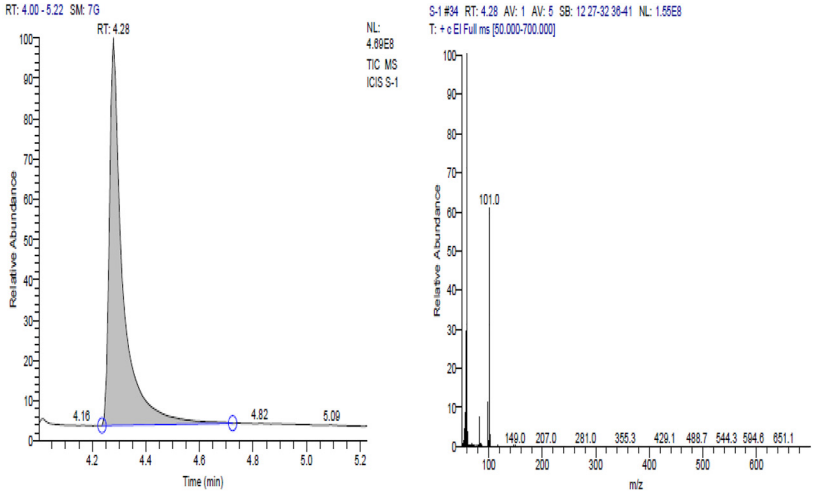
Compound Name	RT	Molecular Formula	Cas #
1,2,3-Benzenetriol	12.76	C6H6O3	87-66-1
1,2,4-Benzenetriol	12.76	C6H6O3	533-73-3
3-Methyl-2-furoic acid	12.76	$C_6H_6O_3$	4412-96-8

Figure 3: GC-MS interpretation of compound F1.



Compound Name	RT	Molecular Formula	Cas #
Phthalic acid, di(2-propylpentyl) ester	23.14	C24H38O4	NA
Phthalic acid, di(6-methylhept-2-yl) ester	23.14	$C_{24}H_{38}O$	NA
Phthalic acid, di(oct-3-yl) ester	23.14	C24H38O4	NA

Figure 4: GC-MS interpretation of compound F2.



Compound Name	RT	Molecular Formula	Cas #
2-Pentanone, 4-hydroxy-4-methyl-	4.28	C6H12O2	123-42-2
2-Hexanol, 2-methyl-	4.28	$C_{17}H_{16}O$	625-23-0
2-Pentanol, 2,3-dimethyl-	4.28	C7H16O	4911-70-0

Figure 5: GC-MS interpretation of compound S1.

and *Curculigo orchioides* were compared to other extracts, the ethanolic extracts of *C. asiatica*, *M. fragrans*, *T. palmata*, *W. fruticosa*, and *Curculigo orchioides* showed significant activity (<100 µg/mL). The extract's phenolic components may be responsible for the action (Table 2). Hepatocytes exposed to CCl4 had significantly higher levels of AST, ALT, and ALP, as well as significantly lower levels of total protein and cholesterol, as compared to rat hepatocytes treated with CCl4 + silymarin.

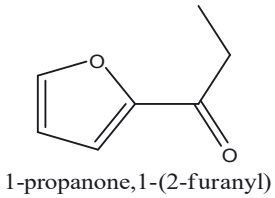
When these cells were treated with all of the plant extracts, the abnormal biochemical parameters were significantly restored. When CCl4-intoxicated hepatocytes were treated with conventional silymarin, similar outcomes were achieved. The hepatoprotective effect of ethanolic extracts of *C. asiatica*, *M. fragrans*, *T. palmata*, *Woodifordia fructicosa*, *Curculigo orchiodes* was observed at the low concentration of 125 µg/mL when compared to the standard silymarin. The reduction in AST, ALT, and ALP levels in newly isolated hepatocytes treated with the aforesaid extracts at 250 g/mL was considerable, and it was more than that produced by conventional silymarin at 250 µg/mL. In newly separated hepatocytes treated with all extracts at 250 g/mL, the rise in total protein and total cholesterol was considerable and greater than that produced by conventional silymarin at 250 µg/mL (Table 2).

GC-MS finger print of isolated fractions

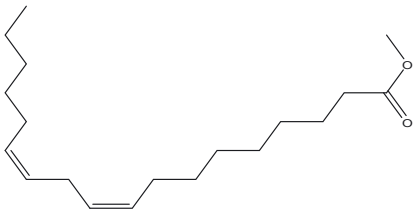
Based on the cytotoxic screening on HeLa cell lines and *in-vitro* hepato-protective studies, secondary metabolites were identified from the ethanol extract of rhizomes of *Curculigo orchiodes* (Hypoxidaceae), Five compounds were identified from different fractions namely C**1**-(1Propanone,1 (2-furanyl). **C2** (9, 12-Octadecadienoic acid (z, z)-, methyl ester. **F1** (3-Methly-2-furoic acid). **F2** (Phthalic acid, di (6methylhept2yl) ester]-and **S1** (2-Hexanol, 2-methyl-). The isolated compounds were analyzed and identified through GCMS spectral studies. The total data were given in (Figures 1–5) and the structures of isolated compounds were given in (Figure 6).

Conclusions

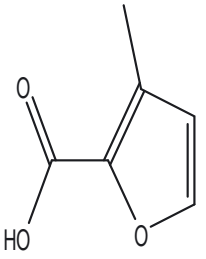
In conclusion, the ethanolic extracts of *C. asiatica*, *M. fragrans*, *T. palmata*, *W. fruticosa*, and *Curculigo orchioides* showed significant (<100 µg/mL) cytotoxic activity when compared to other plant extracts. Among the five plant extracts tested, the *C. asiatica* (Apiaceae) showed the highest toxicity at the lowest concentration of 32 µg/mL. The activity may be due to the phenolics



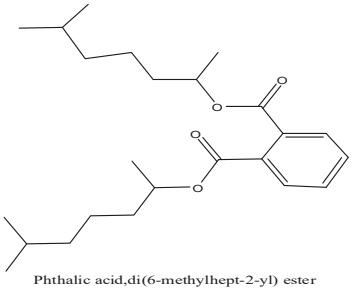
Componud C1



Compound C2



Compound F1



Compound F2

Compouind S1

Figure 6: Structure of isolated compounds from *curculigo orchiodes*.

present in the extract (Eltoumy et al. 2014). A significant increase in the level of AST, ALT, ALP and significant reduction in level of total protein were observed in hepatocytes exposed to CCl_4 when compared to CCl_4 + silymarin treated rat hepatocytes. The decrease in the level of AST, ALT and ALP in freshly isolated hepatocytes treated with the extracts at 125 µg/mL was found to be more significant when compared to standard silymarin at 250 µg/mL. The increase in the level of total protein and total cholesterol in freshly isolated hepatocytes treated with all extracts at 125 µg/mL were significant and more compared to standard silymarin at (250 µg/mL). First time total five new compounds were isolated from *Curculigo orchioides* **C1** (1Propanone, 1(2-furanyl) **C2** (9, 12-Octadecadienoic acid (z, z) -, methyl ester **F1** (3-Methly-2-furoic acid) **F2** (Phthalic acid, di (6methylhept2yl) ester) and **S1.** (2-Hexanol, 2-methyl).

Acknowledgment: The authors are grateful to the Department of Science and Technology of Govt. of India, Medical Council Research South Africa, University of Pretoria, and JSS College of Pharmacy, Ooty, JSS Academy of Higher Education & Research, Mysuru, India for their assistance in conducting this study and also thankful to department of Pharmacognosy, College of Pharmacy, JSS Academy of Technical Education, Noida, UP, India.

Research funding: The financial assistance was provided by the Department of Science and Technology, Govt. of India, New Delhi, India, (DST/INT/SOUTHAFRICA/P/05/2014-06/2014).

Author contributions: Vedpal and Ashish Wadhwani participated to the experimental work and text writing, Namrita Lall contributed to the interpretation section, and SP Dhanabal assessed the paper. All authors have read and approved the final manuscript for submission.

Competing interests: The authors reported no conflicts of interest

Ethical approval: The authors declare that experimentation were performed on animals for this study as per the ethical norms and IAEC guidelines.

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