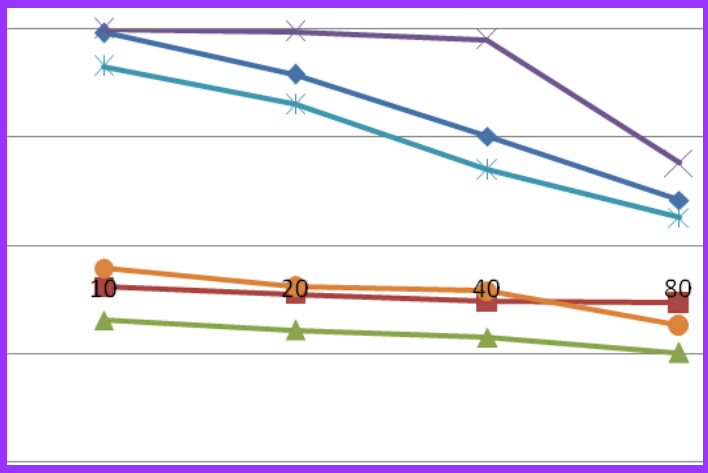

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In vitro anti-cancer potential and GC-MS analysis of *Drimia nagarjunae*, an endangered medicinal plant

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Abstract

The aim of the present study was to investigate the anti-cancer activity of *Drimia nagarjunae* (Liliaceae) extracts against Colo205 Human Colon Cancer cell lines by SRB assay for the first time. The bulbs and leaves of the plant were sequentially extracted using solvents with increasing polarities (hexane> chloroform>ethyl acetate>methanol>water). Ethyl acetate and chloroform bulb extracts showed potent anti-cancer activity compared to standard, adriamycin. Both the extracts exhibited total growth inhibition of cell at 20.1 and 32.1 µg/mL whereas adriamycin shown 33.1 µg/mL and 50% lethal concentration was found to be 61.5 and >80 µg/mL respectively. The active extracts were subjected to GC-MS analysis for identification of phyto-compounds and it showed seven and ten major compounds respectively. Therefore, the present study demonstrated that *D. nagarjunae* can be a promising candidate as an anti-cancer agent.

Introduction

Colon cancer is referred to as large intestine cancer, commonly in people over age 50. It is caused due to high fat diet and diet low in vegetables fruits and fibres (Winawer et al., 1995). Several chemotherapeutic, cytotoxic and immunomodulation agents are available in Western medicine to treat cancer (Newman et al., 2003). Besides being enormously expensive, these drugs are associated with serious side effects. Hence, at present, natural products have been contemplated to be of exceptional value in the development of effective anti-cancer drugs, with minimum host cell toxicity and cost effective (Samuel et al., 2014).

The genus *Drimia* is composed of about 100 species and belongs to liliaceae family. It is widely distributed around equatorial regions and in India (Stedje, 1987). These plants are having various medicinal virtues and possess potent anti-cancer activity. The genus has impor-tant applications as cardiotonics, anticarcinomics and expectorant. These species contain large number of cardiac glycosides (scillaren A, proscillaridin A and bufadienolides) which are used in the treatment of cardiac dysfunction and antitumor activities (Kameshwari et al., 2014). Other bioactive constituents found in the genus are alkaloids, phenolic compounds, quercetin and kaempferol derivatives which show various pharmacological activities including antioxidant and anti-inflammatory (Kameshwari, 2013). Among *Drimia* species, *D. indica* and *D. maritima* have reported to exhibit anti-cancer activity (Ganesh et al., 2012; Bozcuket al., 2011). *Drimia nagarjunae,* an endangered native India medicinal plant, is a member of liliaceae family (Sunil, 2011). No pharmacological investigation in the perspec-tive of anti-cancer activity has yet been reported on *D. nagarjunae*. Therefore, in the present study we aimed to investigate the anti-cancer activity of *D. nagarjunae* on Colo205 HCC cell line.

Materials and Methods

Sample preparation and extraction

The plant was collected from Bhata village, Sri Potti Sriramulu Nellore district, Andhra Pradesh, India and was authenticated by National Ayurveda Dietetics Research Institute, Bangalore with authentication number SMPU/NADRI/ BNG/2013-14/83. The bulbs (B) and leaves (L) were cleaned, dried and powdered. The powdered materials were sequentially extracted using solvents with increasing polarities [hexane (HEX) >chloroform (CHL)>ethyl acetate (EAE)>methanol (MET)>water (WAT)] at 1:10 (w/v) concentrations by using Soxhlet apparatus. The extracts were filtered through Whatman No. 1 filter paper. The filtrate was concentrated by rotary evaporator and used for further studies.

SRB assay

The anti-cancer potential of plant was evaluated by SRB assay according to standard procedure (Skehan et al., 1990). The anti-cancer activities of extracts were studied at Advanced Center for Treat-ment, Research and Education in Cancer (ACTREC), Mumbai where the cell lines were maintained in ideal laboratory conditions. The Colo205 HCC cell lines were grown in 96 well microtiter plates for 24 hours prior to addition of experimental drugs at standard conditions. To measurement of the cell population for cell line at the time of drug addition after 24 hours, the wells were fixed *in situ* with TCA. To each well 10 µL of the extracts was added with final drug concentrations of 10, 20, 40 and 80 µg/mL. The known anti-cancer drug, adriamycin was used as standard drug.

End point measurement

The cold TCA was added to terminate the assay after 48 hours incubation of plates at standard condition. 50 µL of cold 30% (w/v) TCA (final concentration, 10% TCA) was added to cells for *in situ* fixation and incubated for 60 min at 4°C. The supernatant was discarded and the plates were washed, dried. After dry, to each well sulforhodamine B (SRB) solution (50 µL) at 0.4% (w/v) in 1% acetic acid was added. The plates were incubated for 20 min at room temperature. The residual dye was removed by washing five times with 1% acetic acid after recover of unbound dye and plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an ELISA plate reader at a wave length of 540 nm with 690 nm reference wave-length. The total growth inhibition of cells (TGI), growth inhibition of 50% (GI_{50}) and 50% lethal concentration (LC_{50}) were calculated.

GI_{50} value of = 20 µg/mL is considered to demonstrate activity.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The EAE-B and CHL-B extracts were subjected for GC-MS analysis. The thermo GC –Trace ultra Ver: 5.0, thermo MS DSQ II and equipped with column DB 5 - MS capillary standard non – polar with a dimension of 35 Mts x 0.25 mm x 0.25 µm was used for analysis. Helium gas was used as the carrier gas at constant flow rate 1 mL/min and an injection volume of 1 µL. The oven injector temperature 70°C and raised to 260°C at 6°C/min. Total GC running time was 37.5 min.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with relative retention time and mass spectra of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results

The inhibitory effects of extracts from *D. nagarjunae* on the viability of Colo205 cells were determined by SRB assay. LC_{50}, TGI and GI50 of bulb and leaf extracts of *D. nagarjunae* were assessed (Table I). Percentage growth control of bulb and leaves extracts were shown in Figure 1. The anti-cancer activity of extracts was found in the order of EAE-B>CHL-B>EAE-L>WAT-B>MET-L>HEX-B>CHL-L>MET-B>HEX-L>WAT-L. In all the tested extracts, EAE-B, CHL-B and EAE-L extracts have exhibited prominent and dose dependent anti-cancer activity when compared with standard drug, ADR. EAE-B exhibited more anti-cancer activity than CHL-B and EAE-L extract. The EAE-B extracts showed lower LC50 of 61.5 µg/mL while the ADR has 76.0 µg/mL. Total growth inhibition of CHL-B, EAE-B and EAE-L has 32.1, 20.1 and 43.6 µg/mL respectively when

Table I

LC_{50}, TGI and GI_{50} of *Drimia nagarjunae* extracts (µg/mL)

Extracts	HEX-B	CHL-B	EAE-B	MET-B	WAT-B	HEX-L	CHL-L	EAE-L	MET-L	WAT-L	ADR
LC50	>80	>80	61.5	>80	>80	>80	>80	>80	>80	>80	76.0
TGI	>80	32.1	20.1	>80	>80	>80	>80	43.6	>80	>80	33.1
GI50	49.7	<10	<10	77.5	39.4	>80	64.2	<10	21.9	>80	<10

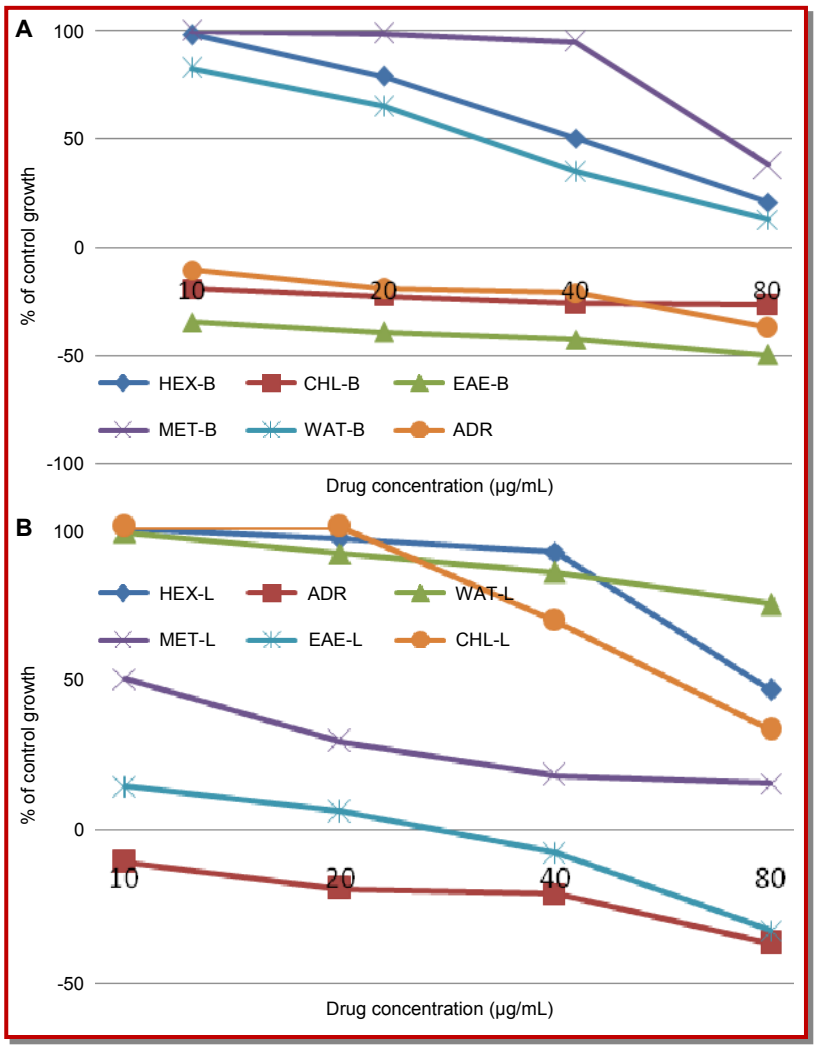

Figure 1: Effect of bulb (A) or leaf (B) extracts of *Drimia nagarjunae* on the percentage control growth of Colo205

Table II

Chemical profile identified by GC-MS analysis of EAE-B of *Drimia nagarjunae*

SL. No.	RT	Compounds	Molecular formula	MW	Area %
1	3.03	Acetic acid	$C_4H_8O_2$	88	2.4
2	6.93	(D,L)-malic acid	$C_4H_6O_5$	134	3.5
3	22.74	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.0
4	28.77	Ethyl[4-t-Butyl-2,6-bis(1-methoxy-1-methylethyl)phenyl] phosphinate	$C_{20}H_{35}O_4P$	370	7.9
5	30.11	{[Thorium-(pentamethylcyclopentadienyl)]-tris [(trimethylsilylamino)-1',2'-ethylideneamino]}	$C_{25}H_{54}N_4S\ i_3Th$	726	3.1
6	32.72	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	10.2
7	36.65	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{42}O_4$	358	4.7

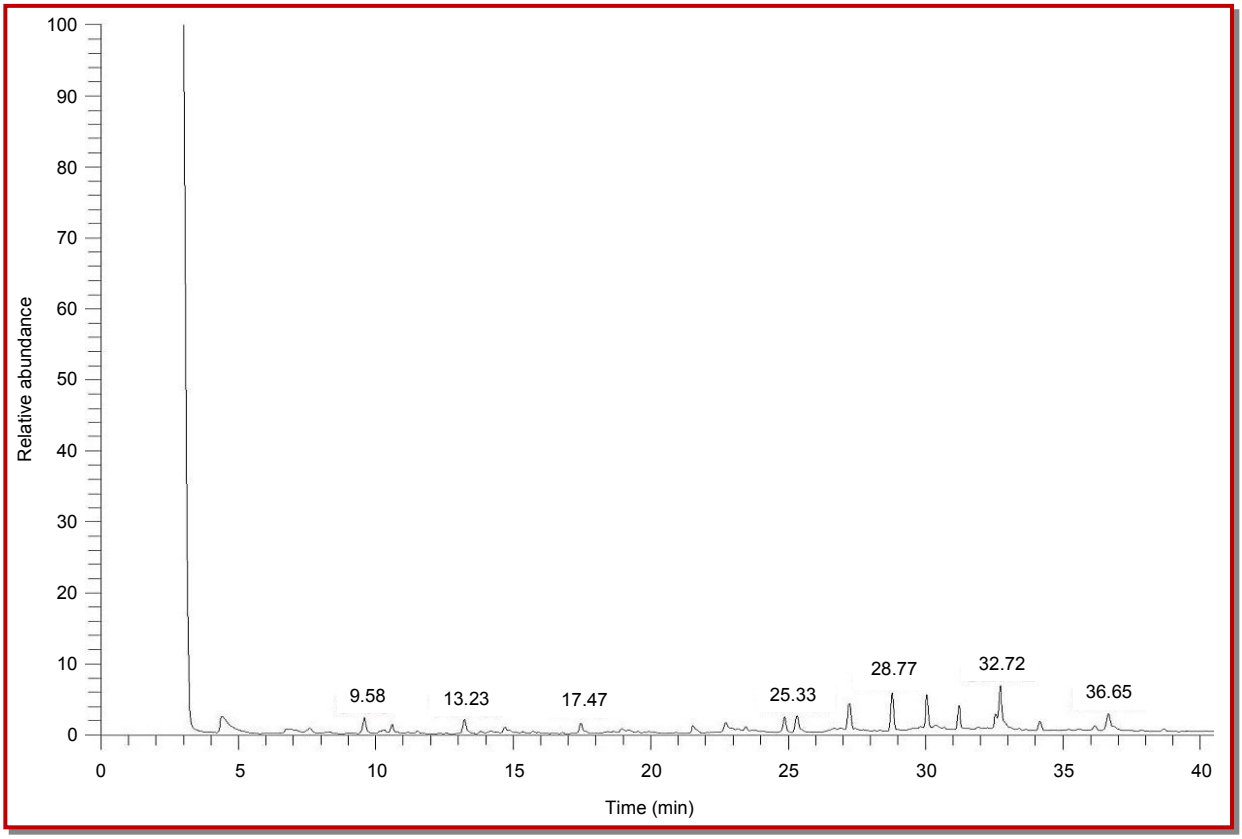

Figure 2: GC-MS chromatogram of EAE-B of *Drimia nagarjunae*

compared to ADR 33.1 µg/mL. GI50 of CHL-B, EAE-B and EAE-L extracts were found to be similar (<10 µg/mL) to the standard drug. Hence, EAE-B and CHL-B extracts were chosen for GC-MS analysis for identification of possible bioactive components.

GC-MS analysis showed the presence of ten and seven compounds in EAE-B and CHL-B extracts of *D. nagarjunae*. The active principles with their retention time (RT), molecular formula, molecular weight and concentration (peak area%) of EAE-B extract are depicted (Table II). Compound peaks of EAE-B extracts were determined from GC-MS chromatogram (Figure 2). The active principles and the compound peaks of CHL-B extract were also studied (data not shown).

Discussion

The present study was aimed to analyse the *in vitro* anti-cancer activity of *D. nagarjunae* against Colo205 Human Colon Cancer cell lines by SRB assay. Sulforhodamine B (SRB) assay is a high-throughput and sensitive method for evaluating cytotoxic activity against cancer and noncancerous cell lines. It has several advantages over other contemporary cytotoxicity assays; because SRB assay is independent of cell metabolic activity, not interfered by test compounds and easy to perform (Vichai and Kirtikara, 2006). Among the tested extracts, CHL-B, EAE-B and EAE-L have exhibited prominent and dose dependent anti-cancer activity when compared with standard drug, ADR. Low GI_{50} i.e. <10 µg/mL indicates the anti-cancer property of the plant extracts (data not shown). Similar studies related to anti-cancer activity has been reported for *D. indica* and *D. maritima*. The aqueous bulb extracts of *D. indica* showed 82% of anti-proliferative activity on breast cancer cell lines (Ganesh et al., 2012). The novel 29 KDa glycoprotein isolated from *D. indica* showed the anti-angiogenic and proapoptotic activity (Deepak et al., 2006). The EAE-B extracts showed LC50 of 61.5 µg/mL while ADR exhibited 76.0 µg/mL. The TGI of CHL-B, EAE-B and EAE-L have 32.1, 20.1 and 43.6 µg/mL respectively when compared to ADR (33.1 µg/mL). The results were in accordance with *D. maritima* extracts which has shown more cytotoxicity on lung cancer cell lines compared to standard chemotherapeutics (Bozcuk et al., 2011). GC-MS results revealed that the two extracts from *D. nagarjunae* are rich in bioactive compounds which might be responsible for anti-cancer activity.

The present study demonstrated that *D. nagarjunae* can be a promising candidate as a potential anti-cancer agent. Further studies will be carried out for the characterization of bioactive compound(s) responsible

for the activity. In the best of our knowledge, this is the first report on anti-cancer activity and GC-MS analysis of *D. nagarjunae.*

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