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Original Article

GCMS ANALYSIS ON *ANDROGRAPHIS PANICULATA* SEED EXTRACT AND ITS ANTICANCER ACTIVITY

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ABSTRACT

Objective: *Andrographis paniculata* was an ancient medicinal herb recorded in Ayurvedic system of medicine from long times. The present study is to determine the bioactive phytocomponents present in *Andrographis paniculata* seed, extraction done using ethanol and Human Liver Cancer (HepG2) cells are used to prove the anticancer activity.

Methods: Gas Chromatography-Mass Spectroscopy (GCMS) analysis is used for determining the bioactive constituents of the *Andrographis paniculata* seed. To prove the *in vitro* anticancer activity, the MTT assay was done on Human Liver Cancer (HepG2) cells.

Results: GCMS analysis of *Andrographis paniculata* seed extract result determined the presence of 15 active bio components. These various potent bioactive compounds have been proved to possess various pharmacological activities, viz., antimicrobial, antidiabetic, antioxidant, anti-inflammatory and anticancer properties, which were supported by previous findings. The natural compounds with therapeutic effects on traditional medicine have paved the way for evaluating the *Andrographis paniculata* seeds for cytotoxic activity on HepG2 cell lines. The ethanol extract of *Andrographis paniculata* seeds was used on HepG2 to evaluate the cytotoxicity; Cisplatin (15 µg/ml) is used as standard with various concentration like 200, 100, 50 and 25 µg/ml were used. The bioactive compounds present in *Andrographis paniculata* seed extract revealed significant cytotoxicity activity against HepG2 cells with IC50 value of 103.03µg/ml.

Conclusion: The cytotoxic effect was found to be concentration-dependent and increased concentration of Andrographis *paniculata* seed extract showed increased cytotoxicity. The result suggest *Andrographis paniculata* seed extract have bioactive agents which when treated on hepatocellular carcinoma cells poses excellent anticancer activity.

Keywords: Bioactive components, GCMS, Anticancer, Hepatocellular carcinoma cells, Cytotoxicity

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INTRODUCTION

Medicinal plants have profound therapeutic applications and has become an integral part of human life, which could combat the sufferings due to various diseases. The pharmaceutical industries are now focusing on indigenous production of drugs using these medicinal plants. In Ayurvedic formulation, *Andrographis paniculata* or Kalmegh is one of the most widely used plants. Occurrence of *Andrographis paniculata* is common in most of the place in India, unlike other species. In hilly areas it grows up to 500m and its of wide use; communities in India have been using this herb for treating variety of diseases [1].

The phytocomponents likes diterpenoids, lactones, flavonoids, and flavonoid glycosides are found in aerial part of *Andrographis paniculata*'s extract [2]. *Andrographolide* is quantitatively the major bioactive secondary metabolite present in this herb. The antioxidant, anti-carcinogenic, antiparasitic, antiparasitic, antiHIV, antiHIV, anti-bacterial, anti-inflammatory, anticancer etc are the activities possessed by *Andrographis paniculata* which are reported in previous studies [3]. The death caused from Malaria, HIV/AIDS, Tuberculosis is less than the death caused by cancer and it rates about 8.8 million people every year. In 2035, the incidence of cancer would increase double times [4]. Among different cancer, rates of liver cancer in Africa and Asia are found to be high. The chronic infections with Hepatitis C virus (HCV) and Hepatitis B virus (HBV) are the causes of Hepatic Cellular Carcinoma (HCC) with a percentage of about 73.4% [5]. *Andrographis paniculata* leaf extract poses anticancer activity against H-29 cancer cell, which shows 50% inhibition at 200 µg/ml concentration for ethanol extract, proves to be an alternative medicine for cancer [6].

In Ovcar-5 cell line the inhibition was 51.12% when exposed to hydroalcoholic extract of *Andrographis paniculata.* In HepG2 cell the inhibition rate was 42.76% when exposed with a combination of *Andrographis paniculata* and *S. marianum* extract [7]. Ethanol extract of *Andrographis paniculata* can inhibit MCF-7 cell and studies are need on neoandrogpholide inhibition of adenylate kinase 2 in MCF-7 cells [8]. Due to the beneficial medical properties of *Andrographis paniculata* the demand is getting increased day by day. Many research report is available on the biological and pharmacological aspects but none of the reports deals with *Andrographis paniculata* seeds [9]. The present investigation is considered as important as it is the first of its kind. No previous reports are available on the medicinal use of *Andrographis paniculata* seeds.

MATERIALS AND METHODS

Extraction of bioactive components

The *Andrographis paniculata* seeds were purchased from local market, Avadi, Tamil Nadu. The seed samples were wild varieties, which are not macro or micro, propagated by human. The seeds were identified registration number of the certificate: PARC/2020/4258 which was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy research Centre, Tambaram. The seeds of *Andrographis paniculata* were dried in shade, cleaned and powdered with blender. In Soxhlet apparatus, 20 ml of ethanol (V/V) were taken and 50 g of powder sample of *Andrographis paniculata* were added. By using rota evaporator, the extract was filtered, and the content is evaporated for drying and finally, it is stored in the refrigerator at 2-8 °C. The final seed extract obtained are used for identification of phytoconstituents which are present in the extract [10].

Gas chromatography-mass spectroscopy (GCMS analysis)

The analysis in ethanol extract of *Andrographis paniculata* seeds for phytocompounds are performed using GCMS technology. The Perkin Turbo gold mass detector was equipped with a fused silica column containing BR-5MS (5% Diphenyl) with *Andrographis paniculata* seeds extract of 2 µl was used for GCMS analysis. The flow rate was

1.2 ml/min in the column and the carrier gas helium was used to separate the components. The National Institute of Standards and Technology mass library search was used to identify compounds in GCMS spectra. The compound name, the structure of the components, and molecular weight of *Andrographis paniculata* seed extract were tabulated [11].

***MTT assay for determination of in vitro* on cytotoxic effect**

The ethanol extract of *Andrographis paniculata* seeds were evaluated for the anticancer activity by MTT assay. The *in vitro* model of hepatocellular carcinoma cells HepG2 cells were used for cytotoxicity activity. From National Centre for Cell Sciences (NCCS), Pune, India the cell were procured. Using Cisplatin as a reference drug and different concertation like 100µg, 75µg, 50µg, 25µg of *Andrographis paniculata* seeds were treated on HepG2 cells. In tissue culture flask, the cell line were cultured in 25 cm^2 with supplement and are at 37 °C in a humidified 5% CO_2 incubator.

Using inverted phase-contrast microscope, the viability of cells was directly observed, followed by MTT assay method. In 96 well tissue plate a 100 µl cell suspension ($5x10^3$ cells/well) were added. A serial dilution of five times with concentration of 100µg, 75µg, 50µg, 25µg were prepared using 500 µl of 5% DMEM. The concentration of 100 µl is added to the cell wells in triplicates which are incubated at 37°C in a humidified 5% CO_2 incubator. The process is repeated for the non-treated control wells. Then using a microplate reader at a wavelength of 540 nm the absorbance (Abs) is measured.

% Cytotoxicity using the following formulas:

% Cytotoxicity= 100-[Abs (extract)/Abs (standard)] x100

% Cell Viability = [Abs (extract)/Abs (standard)] x100

Using Olympus CKX41 with Optika Pro5 CCD camera, inverted phase contrast tissue culture microscope after 24 h of treatment, the plates are examined, and images are recorded. The granulation, vacuolization, cell morphological changes, shrinking or rounding of cell, which are called the cytotoxicity indicators, are detected.

RESULTS AND DISCUSSION

GCMS analysis on *Andrographis paniculata* seed

The fig. 1 represents the GCMS spectra peak with the number of compounds from the GC fractions of the ethanol extract of *Andrographis paniculata* seeds is represented. In this observation, 15 bioactive compounds namely, 1,3,5-Triazine-2,4,6 triamine, Undecane, 4 H Pyran 4-one 2,3 dihyro 3,5 dihydroxy, 3,5 Dimethyl phenyl isocyanate, Bicyclo(5.3.0) dec-1 (7)–ene, 2,5 di one, Tetradecanoic acid methyl ester, Hexadecanoic acid, methyl ester, 9,12–Octadecanoic acid, (z,z) methyl ester, n-hexadecanoic acid, 9-octadecenoic acid methyl ester, Dodecanoic acid methyl ester, 9,12 octadecadienoic acid z,z, 9-octadecenoic acid 9(z,z), Octadecanoic acid and Methyl 20 methyl hemeicosanoate are the compounds identified. Table 2 shows the identified biomolecules and their previously reported biological activities.

The above-identified bioactive photo components' pharmacological activities are found from previous literature reports as anti-inflammatory, anticancer, antimicrobial etc (table 1).

Table 1: Pharmacological activity of photo components in the ethanolic extract of *Andrographis paniculata* seeds

Peak #	Name of the compound	Molecular formula	Molecular weight	Retention time/min	Peak area %	Pharmacological activity
1	1,3,5-Triazine-2,4,6 triamine	$C_3H_8N_6O_4S$	126.12 g/mol	5.050	1.45	Antagonists, Cytotoxicity against leukemia and adenocarcinoma, Inhibites glucocerobrosides, Antileismenial activity, Anti-HIV, Antimalarial, Antioxidant, Cancer preventive, Antimicrobial [12]
2	Undecane	$C_{11}H_{24}$	156.313 g/mol	5.671	1,72	Acts as Pheormones [13]
3.	4 H Pyran 4-one 2,3 dihyro 3,5 dihydroxy	$C_5H_6O_4$	130.099 g/mol	6.856	2.14	Antioxidant, Antimicrobial, Anti-inflammatory, Cytotoxicity, Antidiabetic, Bronchitis, Anemia, Dyspepsia, Throat diseases, Tuberculosis and Elephantiasis [14]
4.	3,5 Dimethyl phenyl isocyanate	C_7H_5NO	119.123 g/mol	14.160	2.12	Plays a role of hapten in Immunoloigcal reactions [15]
5.	Bicyclo(5.3.0) dec-1 (7)–ene, 2,5 di one	$C_{10}H_{16}$	136.238 g/mol	21.608	8.62	Antimicrobial, Anticancer, Anti-inflammatory [16]
6.	Dodecanoic acid methyl ester	$C_{13}H_{26}O_5S$	294.406 g/mol	24.040	2.65	Antimicrobial agent [17]
7.	Tetradecanoic acid methyl ester	$C_{14}H_{28}O_2$	228 g/mol	33.918	1.15	Antibacterial, Antifungal [18]
8.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	39.738	3.77	Antimicrobial, Anticancer [19]
9.	n-hexadecanoic acid	$C_{17}H_{34}ON$	270	40.533	10.34	Anticancer [19]
10.	9,12–Octadecanoic acid, (z,z) methyl ester.	$C_{19}H_{32}O_2$	292	42.831	8.38	Antimicrobial, Anticancer [20]
11.	9-octadecenoic acid methyl ester (E)	$C_{18}H_{34}O_2$	282	42.942	17.95	Cancer preventive, Anti-inflammatoryAllergenic, Anemiagenic, Antialopecic, Antiandrogenic, Antiinflammatory, Antileukotriene-D4 (Anti-platelet activating factor), Dermatitigenic [19]
12.	9,12 octadecadienoic acid z,z	$C_{18}H_{32}O_2$	280.452	43.374	19.14	Antimicrobial and Anticancer [21]
13.	9-octadecenoic acid 9(z,z)	$C_{18}H_{34}O_2$	282	43.466	17.44	Cancer preventive, Anti-inflammatory [22]
14.	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	43.784	2.47	
15.	Methyl 20 methyl hemeicosanoate	$C_{23}H_{46}O_2$	354.619	47.613	0.65	Pheromone [23]

The present investigation was aimed to determine the bioactive components which are present in ethanol seed extract of *Andrographis paniculata* seed and their anticancer activity on HepG2 cell lines. The GCMS yielded 15 different compounds like fatty acids, especially Fatty Acid Methyl Ester (FAME), namely, Dodecanoic acid, Hexadecanoic acid, Octadecenoic acid, 9,12-Octadecadienoic acid, Octadecanoic acid, Tetra decanoic acid, Heneicosanote and its respective methyl esters. The reported biological activities were discussed below. Antimicrobial

activities of the Dodecanoic acid methyl ester compound was confirmed by the work of Berrin Ozçelik *et al.* [17]. Tetradecanoic acid methyl ester belongs to the group of FAME which had potent antimicrobial agent tested against human pathogenic microorganisms viz., *Aspergillus fumigatus*, *Bacillus subtilis* and *Aspergillus niger* [23]. It has been recorded that hexadecanoic acid with anticancer activity against *in vitro* human colorectal carcinoma (HCT-116) cells are tested by MTT assay [19]. In one of the previous study, hexadecenoic acid was induced to the human gastric cancer cell, which showed good apoptosis results [20].

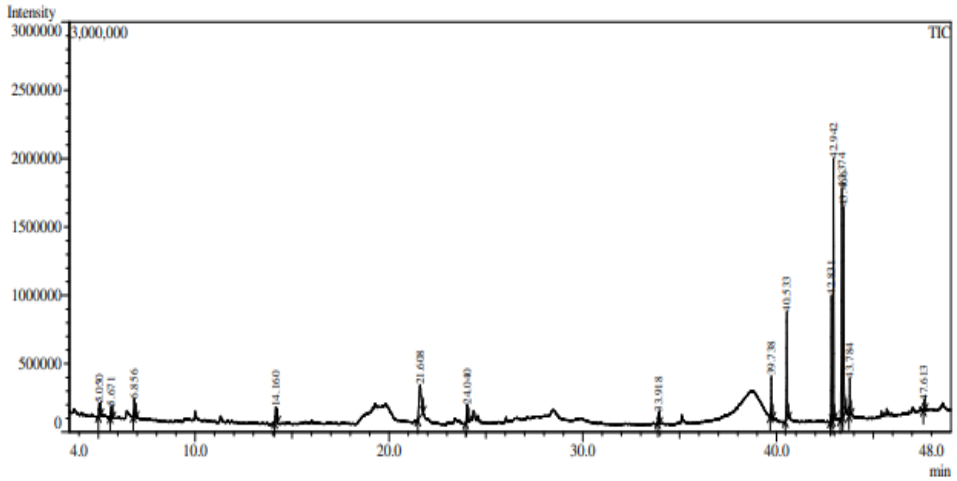

Fig. 1: GCMS Chromatographic profile of ethanol extract of *Andrographis paniculata* seed

The inhibitory activity of 9,12–Octadecadienoic acid and its methyl esters derivatives against cancer was studied Yu *et al.*, 2005 in SGC-7901 human gastric cancer cell line [20]. 9-Octadecenoic acid belongs to MUFA (Monounsaturated fatty acid) reported with anticancer activity *in vitro* mutant plantlets and reported for its cancer preventive [19], anti-inflammatory [20] and antimicrobial activity. The microorganism like *S. mutans, C. albicans, P. gingivalis,, A. actinomycetemcomitans, S. gordonii* and *F. nucleatum*, against which the antimicrobial activity was possessed by MUFA compound are reported and documented [24]. The findings of Yung Choon Yoo *et al.*, 2007, reported that octadecenoic acid, palmitic acid and (Z)-9-octadecenoic acid and the mentioned free fatty acids were identified in dichloromethane extract of Protaetia brevitarsis larva. The extract exhibits apoptosis by activation of caspase-3 in tumor cells [25]. Octadecanoic acid was reported to poses antibacterial activity against gram-positive bacteria *Bacillus cereus, Bacillus subtilis* and bacterium *Mycobacterium fortuitum* [22].

In a study it was reported that Methyl 20-methyl-heneicosanoate might be a promising antioxidant, anticancer, antimicrobial, and anticytotoxic activity. The activity like apoptosis, proliferation against various forms of cancer cells helps in drug formulation for replacing chemopreventive and chemotherapeutic agent for which further detailed research in this field are required. The compound 1,3,5-Trizane 2-4-6-triamine was reported to poses biological activity such as analgesic, antifungal, antibacterial, anti-viral activities and anti-malarial [12] The compound 4 H Pyran 4-one 2,3 dihyro 3,5 dihydroxy-6-methyl Tetradecane was reported for various biological activities viz., antioxidant, Antimicrobial [13], Anti-inflammatory, Antidiabetic, Dyspepsia, Bronchitis, Cytotoxicity, Anemia, Tuberculosis, Throat diseases and Elephantiasis.

Ruwona (2011) in his study, has confirmed that 3,5 Dimethyl phenyl isocyanate the compound, had been reported that when linked to carrier protein/antigen, capable of eliciting an immune response. The 3 Bicyclo (5.3.0) dec-1 (7)–ene, 2,5 di one poses properties like anti-inflammatory, anticancer and antimicrobial [15]. Thus most of these constituents identified by GCMS have been found to show interesting biological activity against certain illnesses and pathogens. The characterization studies by GCMS shows that the *Andrographis paniculata* seeds suggests that it possess promising medicinal properties and are found to be a rationale for employing in future plant-based drug formulations.

***In vitro* anticancer activity of *Andrographis paniculata* seed extract**

Table 2: Effect of different concentration of plant extract on viability and cytotoxicity against HepG2 cell line determined by MTT assay

Activity	HepG2 cell line					
	Cell control	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)	200 (µg/ml)	Standard (Cisplatin)
Viability (%)	100	84.12	70.47	37.89	20.82	18.48
Cytotoxicity (%)	0	15.88	29.53	62.11	79.18	81.52
IC_{50} Value (µg/ml)	103.03					

Andrographis paniculata seed extract reduced the HepG2 cell lines viability by does depend manner by MTT assay in table 2. The toxicity of the *Andrographis paniculata* seed are determined using different concentration like 25, 50, 100 and 200 µg/ml and standard as Cisplatin (15 µg/ml). It was observed that the *Andrographis paniculata* seed extract showed promising anticancer activity toward the cell lines (fig. 2). A significant increased in cytotoxicity was recorded with increasing concentration of the plant extract by MTT assay. 5-fluorouracil at a concentration of 25µg/ml was used as a positive control. The IC_{50} values of *Andrographis paniculata* seed in HepG2 was found to be 103.03µg/ml.

From the previous reports of Rajesh kumar (2015) [6] the *Andrographis paniculata* leaf extract poses anticancer activity against H-29 cancer cell showed 50% inhibition at 200 µg/ml concentration. In the present study, HepG2 cancer cell line was treated with ethanol leaf extract to determine the viable cells by MTT yellow colour into purple formazan. The number of living HepG2 cells decreases with an increase in the concentration of the *Andrographis paniculata* seed extract by 25, 50, 100 and 200 µg/ml and is standard as Cisplatin (15ug/ml) were used. A significant increased in cytotoxicity was recorded. The IC_{50} values of plant extract in HepG2 was found to be 103.03µg/ml. In previous studies

of Daryush, (2013) [26] it has been reported that ethanol extract of *Andrographis paniculata* leaf showed 50% inhibition at the concentration of IC 50 on IMR32 cells and HT-29 at 200 µg/ml, which is similar to the result obtained from the present study. The results of *Anthus sonachifolisu* leaf showed a potent inhibitory effect on HepG2 cell IC_{50} at (58.2 µg/ml) the effect on cell proliferation increases with increase in concentration in dose-dependent manner [27].

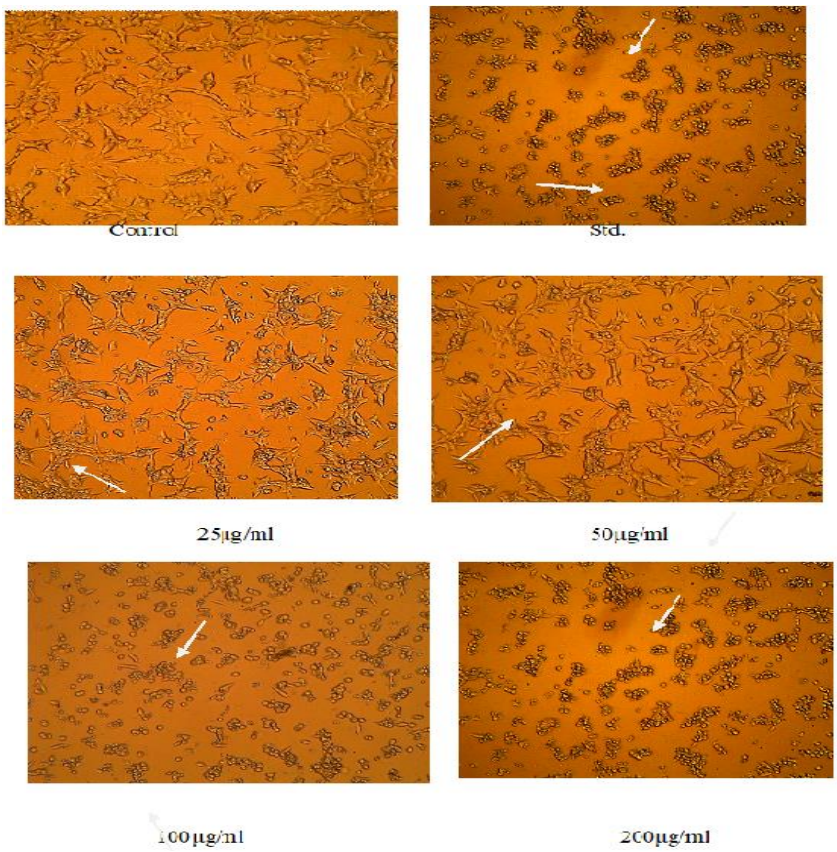

Fig. 2: Effect of different concentration of plant extract on cytotoxicity of HepG2 cell line as determined by MTT assay (Arrows indicate representative apoptotic cells)

CONCLUSION

The extract of *Andrographis paniculata* seed extract have shown good profile of many phytochemicals, which may account for numerous medicinal activities. From the results of GCMS, the *Andrographis paniculata* seeds strongly advocate the presences of various bioactive compounds. Few bioactive compounds which are identified are reported to have anticancer activity. The four different concentrations of (25, 50, 100 and 200 µg/ml) and standard as Cisplatin (15ug/ml) were used in HepG2 cell line to evaluate the cytotoxicity of the seed extract. The IC_{50} values of seed extract in HepG2 were found to be 103.03µg/ml with increased concentration of the extract, an excellent anticancer activity was noted. The outcome of the study would help to evaluate and assess the various therapeutic and anticancer activity of *Andrographis paniculata* seeds extract, which could pave the way for phytochemo-therapeutic drugs and can create awareness on *in situ* conservation of this medicinal plant.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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