Evaluation of Anticancer Activity of Polyherbal extract of Terminalia Chebula, Phyllanthus emblica and Dimocarpus Longan on Caco-2 Colorectal Cancer Cell Lines

Surya Prakash DV, Meena Vangalapati , T. Mohammad Munawar

Abstract*: Medicinal plants are the most essential wellsprings of medication or life sparing medications for most of the World& #39;s populace for a huge number of years. Many plant species are used to treat the cancer diseases. The ethanolic extract of composite medicinal herbs fruits of Terminalia chebula, Phyllanthus emblica and seeds of Dimocarpus longan are used as a potent natural source of anticancer agent. The colorectal cancer cell lines (Caco-2) are inhibited by ethanolic extract through MTT assay method. The percentage of Caco-2 colorectal cancer cell growth inhibition was increased from 1.5% to 62.9%.

***Keywords* : Anticancer, Caco-2 cell lines, Terminalia chebula, Phyllanthus emblica, Dimocarpus longan.**

I. INTRODUCTION

Colorectal disease is the development of growth from the colon and rectum of the biggest digestive tract. Colorectal tumors are because of hereditary issue and seniority. It every now and again begins as a dangerous tumor, now and again as a polyp and ends up plainly carcinogenic [1]. After lung growth, the WHO report it was second most regular malignancy affliction globally. It was estimated as 3% above 60 year old age people in Western Europe were evidence of colorectal tumor [2]. Medications utilized for colorectal growth may associate some combination of surgery, radiation treatment and chemotherapy [3]. Fecal occult blood test, Stool DNA test, Flexible sigmoidoscopy, Colonoscopy and MRI scan are the most well-known screening and indicative techniques for colorectal malignancy [4]. For a several decade, most number of home grown medications have been utilized are as yet used as a part of creating nations as the essential wellspring of medicinal treatment of different infections [5]. Medicinal plants and their active compounds were considered in medicine for their natural antiseptic properties. In this manner, examine has researching the employments of therapeutic plant separates for the readiness of medications for different sicknesses including tumor. Many plant species are as of now being utilized to treat or counteract improvement of disease [6]. The phenolic compounds cell reinforcement capability are play promising role in the treatment and counteractive action of growth and its consider as the most and commonly occurs in various medicinal plants [7]. In the present paper, In the present paper, the anticancer action of polyherbal formulation of ethanolic fruit extract of *Terminalia chebula* (TCf), *Phyllanthus emblica* (PEf) and *Dimocarpus longan* (DLs) seeds extracts against Caco-2 colorectal disease cells by utilizing MTT assay.

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II. MATERIAL AND METHODS

A. Plant Materials

TCf, PEf dry fruits and DLs seeds were collected in around market in Visakhapatnam, Andhra Pradesh state, India during April 2015. The plant species were identified and authorized as *Terminalia chebula*, *Phyllanthus emblica and Dimocarpus longan* by the Department of Botany, Andhra University and the herbarium was registered as AUH-7561, AUH-8565 and AUH-2461. The collected fruits and seeds were pre-prepared were cleaned and sliced into small pieces for the experiment later then make into dry powder form. The powder obtained was filtered with the 120 mesh size. The various size powders put away noticeable all around tight little covers.

B. Soxhlet extraction

The TCf, PEf and DLs dry powder were mixed in the ration of 3:1:2 respectively. About 24 grams of mixed powder (12.0gm of TCf, 4.0gm PEf and 8.0gm of DLs seeds) was placed with care in the thimble and fixed firmly to the condenser. 200ml of 80% ethanol was used as a solvent and taken in the round bottom flask [8]. The total apparatus was positioned in the heater and a temperature not more than the boiling point of the solvent was maintained. Using the soxhlet apparatus [9] facilitated the process of continuous extraction was for a period of 9hrs.

C. Cell culture

Caco-2 (Human cell lines) was purchased from the National Centre for Cell Science (NCCS), Pune, India. The experimental cells were grown in the Minimal essential medium (MEM, GIBCO) which was in addition supplemented with 4.5gm/L of glucose, 2mM/L of glutamine and 5% of foetal bovine serum (FBS for growth medium) and maintained at 37°C temperature in 5% CO_2 incubator.

D. Cell plating and MTT assay

The invitro inhibitory effects of test compounds on cell growth were done based on slight changes in the Mosmann MTT assay protocol [4] [10]. The trypsinized cancer cell lines (A549 and Caco-2) from T 25 flask were kept in 96 wells of tissue culture plate at 5 x 103 density of cells/well in the growth medium and cultured at 37°C temperature in the presence of 5% CO_2. Each wells, 20µl of fresh MTT (5mg/ml in PBS) were added and followed by 24hr incubation period at 37°C temperature, then the supernatant growth medium were separated from the each wells and restored with 100 µl of Dimethyl sulfoxide for solubilize the colored formazan substance. After 30 min incubation period, the absorbance OD was read at 570nm on an ELISA reader [11].

% of cell viability = (OD of test / OD of control) X 100

E. GC-MS Analysis

The GC-MS analysis was conducted at Venture labs, Hyderabad. 1µl of extract injected into the gas chromatography (Perkin-Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler) [12]. Separation of compounds was achieved by using Elite 5MS (5% diphenyl / 95% dimethyl poly siloxane) combined with a capillary column (40 × 0.25 µm ID × 0.25 µm df). Oven temperature was raised from 60°C- 250°C at 5°C/min and Helium gas pumped with flow rate of 1.0ml/min as a carrier gas. The total experiment time were set as 3.10 to 45.00 min. The Compounds were identified with the help of Wiley and NIST Libraries based on their molecular mass [13].

III. RESULTS

The anticancer activities of the polyherbal formulation of the selected plants were identified using MTT assay. Measurement of cell growth and the viability are the repressed basis for in vitro assays directed towards the quantization of a cell population response to external factors. Cell growth assays have used the uptake of radio labelled thymidine into the cellular DNA [14]. The MTT assay method is reduced by cell metabolic activity, in fraction by the action of dehydrogenase enzymes, to produce dipping equivalents like NADH and NADPH. The MTT cell proliferation assay method allows capacity of growth rate and equally, when metabolic events lead to apoptosis, the minimization in cell viability [15]. Each cell type the relationship between cell number and signal formed is established, as a result allowing a perfect quantization of changes in the rate of cell proliferation.

A. Effect of composition of herbal extract on Caco-2 (Colorectal cancer) cell inhibition

The MTT cell growth inhibition assay was done for ethanolic extracts of composition of medicinal herbs at different concentration doses of 6.25, 12.50, 25, 50, 100, and 200µg/ml was shown in Table 1. The Comparison of effect of composition of herbal extract and Standard sample on Caco-2 cancer cell growth inhibition was shown in Fig.1.

Table 2. Effect of composition of herbal extract on Caco-2 cancer cell line growth inhibition

Concentration of Test sample (µg/ml)	% of Caco-2 (Lung cancer) Cell inhibition
6.25	1.5
12.5	7.6
25	15.2
50	30.9
100	52.2
200	62.9
IC50 (µg/ml)	150.865 µg/ml

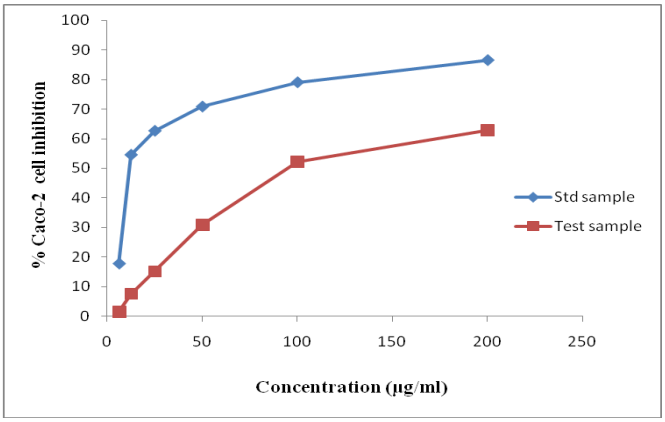


Fig. 1. Comparison of effect of composition of herbal extract and Standard sample on Caco-2 cancer cell growth inhibition

Table 1: Dose Response of Test sample on Caco-2 (Colorectal cancer) Cell line

Concentration (ug/ml)	OD of 5' fluorouracil (STD) at 570 nm	% Cell Survival	% Cell Inhibition	OD of Test sample at 570 nm	% Cell Survival	% Cell Inhibition
6.25	0.407	82.3	17.7	0.442	98.5	1.5
12.5	0.243	45.5	54.5	0.417	92.4	7.6
25	0.200	37.4	62.6	0.386	84.8	15.2
50	0.165	29.2	70.8	0.322	69.1	30.9
100	0.130	21.0	79.0	0.245	47.8	52.2
200	0.098	13.5	86.5	0.199	37.1	62.9

From the Fig.2 and Table 2, it was noticed that as the concentration of herbal extract from 6.25 to 200µg/ml, the percentage of inhibition of Caco-2 colorectal cancer cell as 1.5% to 62.9% respectively. This means, the plant extract inhibit the growth of cancer cell by induce cell arrest mechanism [16] [17].

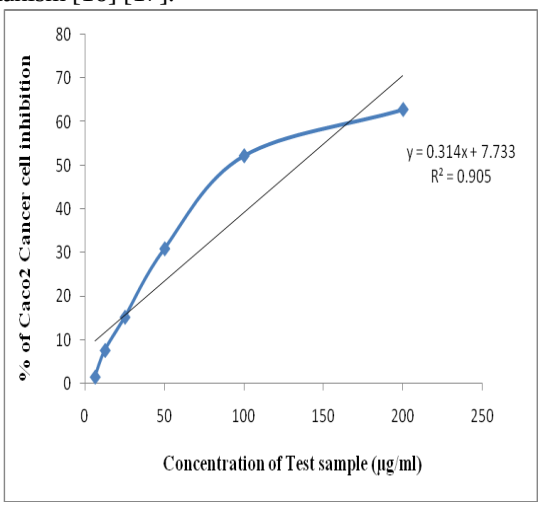
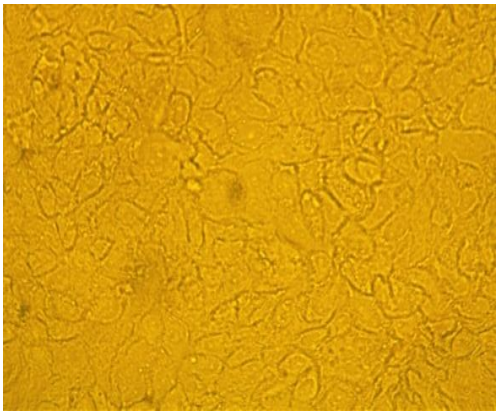
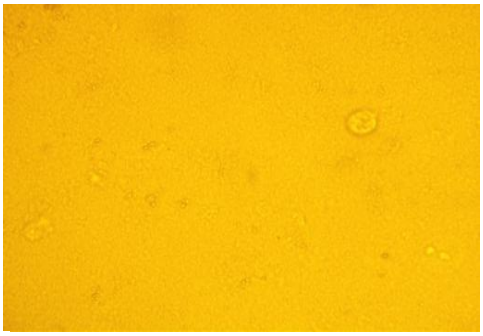


Fig. 2. Effect of composition of herbal extract on Caco-2 cancer cell growth inhibition



Before treated cell lines



After treated cell lines at 200 µg/ml

Fig. 3. Images of Caco-2 cancer cell lines observed under microscope

The images of Caco-2 cancer cell lines before treated and herbal extract of 200 µg/ml treated cell lines of Caco-2 cancer was observed under microscope was shown in Fig.3.

(1)

B. GC-MS analysis

The total phytochemical profiles of the selected plant extract were analyzed using GC-MS analysis. The results of the analysis were represented in Table 3. The chromatogram results expose the comparative concentrations of different phytocomponents concentration getting eluted as a result of retention time intervals in Fig. 4. The high peaks point specifies the presence of high concentration compounds present in the sample. The mass spectrometer analyzes expose the various compounds eluted at different time intervals as a fingerprint of that compound which can be identified from the data library.

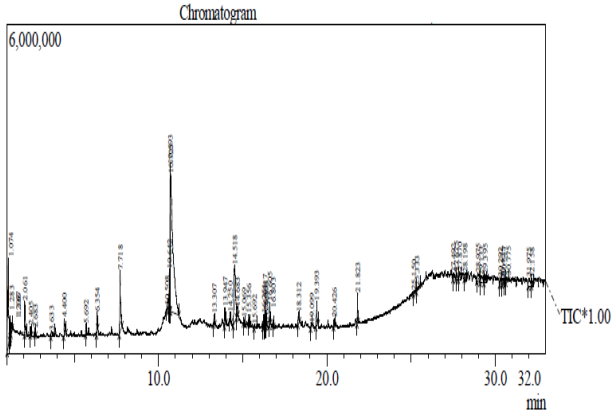


Fig. 4. GC-MS Chromatogram

Table 3. Phytocomponents identified in polyherbal formulation by GC – MS analysis

S.No	RT (min)	Compound Name	Molecular Formula	Molecular Weight	Peak area%
1	1.042	Cyclobutanol	C_4H_8O	72	1.49
2	1.092	Glycidol	$C_3H_6O_2$	74	2.13
3	1.425	2-Pentanone, 3-methyl-	$C_6H_{12}O$	100	0.68
4	1.558	Silane, trimethylpropyl-	C6H16Si	116	0.43
5	1.642	2,2'-[methylenebis(oxy)]bis-	$C_7H_{16}O_2$	132	0.65
6	1.750	4-methyl-3-penten-2-one	$C_6H_{10}O$	98	0.46

7	2.050	Furfural	$C_5H_4O_2$	96	1.40
8	2.150	4-hydroxy-4-methyl-2-penta tone	$C_6H_{12}O_2$	116	4.25
9	2.275	2,3-Hexadien-5-ol	$C_6H_{10}O$	98	0.31
10	2.492	Carbonic acid, bis(1-methylethyl) ester	$C_7H_{14}O_3$	146	0.27
11	6.350	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6 -methyl-	$C_6H_8O_4$	144	0.95
12	7.658	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	3.92
13	10.492	1,2,3-Benzenetriol	$C_6H_6O_3$	126	9.98
14	10.675	3,3,3-Trifluoro-N-(4-methyl -2-pyridyl)-2-(trifluorometh yl)propionamide	$C_{10}H_8F_6N_2O$	286	3.97
15	12.250	Sydnone, diphenyl- Sydnone, 3,4-diphenyl- 3,4-Diphenylsydnone	$C_{14}H_{10}N_2O_2$	238	1.60
16	12.350	Tricyclo[4.3.1.13,8]undecan e-1-carboxylic acid 1-Homoadamantanecarboxy lic acid	$C_{12}H_{18}O_2$	194	0.42
17	12.433	1,2,4-Benzenetricarboxylic acid, cyclic 1,2-anhydride, nonyl ester	$C_{18}H_{22}O_5$	318	0.59
18	13.375	Phenol	C_6H_6O	94	1.05
19	13.433	Acetic acid, phenyl ester	$C_8H_8O_2$	136	1.86
20	13.600	N-Phenyl-N'-furaldehyde hydrazone	$C_{11}H_{10}N_2O$	186	1.19
21	13.700	Phenol, 3-phenoxy-	$C_{12}H_{10}O_2$	186	2.34
22	13.933	Urea, N-[2-[1-piperidyl]cyclohexy l]-	$C_{12}H_{23}N_3O$	225	1.42
23	14.033	3-Pyrrolidin-2-yl-propionic acid	$C_7H_{13}NO_2$	143	1.98
24	14.142	(2,4-Dimethyl-3-pentyl)pyra zine	$C_{11}H_{18}N_2$	178	1.47
25	14.325	Hexahydropyrrolizin-3-one	$C_7H_{13}NO$	143	2.14
26	14.467	Isoglutamine	$C_5H_{10}N_2O_3$	146	4.90
27	14.567	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	$C_7H_{10}N_2O_2$	154	22.55
28	14.767	Trans-10-methyldecalone-1	$C_{11}H_{18}O$	166	0.65
29	15.442	2-Decene, 3-methyl-, (Z)-	$C_{11}H_{22}$	154	0.61
30	15.600	Carbonic acid, sec-butyl phenyl ester	$C_{11}H_{14}O_3$	194	1.31

31	15.942	Cis-6-nonenol	$C_9 H_{18} O$	142	1.23
32	16.042	exo-Norbornanyl propionate	$C_{10}H_{16}O_2$	168	0.63
33	16.308	2-Decene, 3-methyl-, (Z)-	$C_{11}H_{22}$	154	4.10
34	16.433	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane	$C_{11}H_{18}N_2O_2$	210	1.61
35	16.475	t-Butoxycarbonylleucylproline	$C_{42}H_{32}O_{27}$	328	0.32
36	16.550	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.26
37	16.658	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.85
38	16.858	4(3H)-Pyrimidinone, 3-ethyl-2,6-dimethyl-	$C_8H_{12}N_2O$	152	0.60
39	17.150	9-.alpha.-d-Arabinofuranosyladenine	$C_{10}H_{13}N_5O_4$	267	0.40
40	18.342	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	0.79
41	18.742	2-benzyl-3,6-dioxopiperazine	$C_{11}H_{12}N_2 O_2$	204	0.55
42	19.075	2-Trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	282	0.82
43	19.183	3-Methyl-cyclopentadecylamine	$C_{16}H_{33}N$	239	0.60
44	19.325	Staphylomycin S3 Virginiamycin S1, 5-(5-hydroxy-4-oxo-L-2-piperidinecarboxylic acid)-(CAS) Virginiamycin S3	$C_{43}H_{49}N_7O_{11}$	839	0.20
45	19.392	1-(2-Methylallyl)azetidine	$C_7H_{13}N$	111	3.86
46	20.075	Dihydroergocristine	$C_{35}H_{41}N_5 O_5$	611	1.01
47	20.433	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'.alpha.,10.alpha.)-	$C_{33}H_{37}N_5O_5$	583	0.82
48	20.492	Benzaldehyde phenoxycarbonylhydrazone	$C_{14}H_{12}N_2O_2$	240	0.26
49	21.692	6-methyl-1-hydroxypteridine	$C_7H_6N_4O$	162	1.25
50	21.825	Di-n-octyl phthalate	$C_24H_{38}O_4$	390	2.86

IV. DISCUSSION

In the present work, the extract comprises of the medicinal composition and was used for anticancer activity studies. This herbal extract shows promising anticancer activity by means of inhibit the cell growth mechanism of Caco-2 colorectal cancer cells from 1.5% to 62.9% by MTT assay method. Roy Karnati and Nilufar Nahar [16] reported that, ethanolic extract of *Terminalia chebula* and phyllanthus embilica inhibited the growth of Caco-2 colorectal cancer cells lines as 51.2% and 49% respectively. Mainly in vitro studies on plant phenolic compounds have suggested that they exhibit cell growth inhibited at various cell cycle phases (G1, S, and G2) by direct and indirect ways like down controlling cyclins and cdks, with the expression of p21, p27 and p53 genes by acting as prooxidants are reported by Lala et al [18]. The GC-MS result reveals fifty chemical compounds from the sample. In terms of percentage amount of hexadecanoic acid and 3-Pyrrolidin-2-yl-propionic acid shows hypocholesterolemic activity, antioxidant and lubricating activity [19]. Anticancer and antiproliferative are shown by 3-Pyrrolidin-2-yl-propionic acid and 6-methyl-1-hydroxypteridine, while Hexahydropyrrolizin-3-one and exo-Norbornanyl propionate and other compounds show antimicrobial and antiinflammatory activities [19]. Singh and Kumar [20] reported the ethanolic extract of Terminalia chebula has been subjected to GC-MS analysis. Twenty chemical constituents have been identified. Similarly

Balasubramanian et al [21] reported the ethanolic extract of Phyllanthus embilica.

Eugin Amala and Jeyaraj [19] revealed the ethanolic extract of Triphala for the presence of some of the important components resolved by gas chromatography analysis.

V. CONCLUSION

The study on the polyherbal formulation of ethanolic extract of TCf, PEf and DLs was assessed for its anticancer action against Caco-2 cell lines. The polyherbal extract have shown promising impact in suppress the cancer growth in in-vitro assay experiment. Henceforth, the polyherbal formulation of plant extract could be utilized as potential anticancer agent which is required further in vivo studies.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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