IJPSR (2022), Volume 13, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 February 2022; received in revised form, 21 March 2022; accepted, 30 March 2022; published 01 April 2022

ANTI-CANCER EFFECT OF PANCHAGAVYA ON HUMAN COLON ADENOCARCINOMA (HCT-116) CELL LINE

S. Funde [1], S. Patil [*1] and H. Palep [2]

Principal K. M. Kunanani College of Pharmacy [1], Mumbai - 400005, Maharashtra, India.
Dr. Palep's Medical Research Foundation Pvt. Ltd. [2], Mumbai - 400013, Maharashtra, India.

Keywords:

Colon cancer, Anti-proliferation, Panchagavya, Freeze dried

Correspondence to Author:
Dr. Swati Patil
Associate Professor,
Principal K. M. Kunanani College of Pharmacy, Mumbai - 400005, Maharashtra, India.

E-mail: snehalfundecogno@gmail.com

ABSTRACT: The objective of the study was to investigate the *in-vitro* anti-cancer effect of *Panchagavya* on the Human Colon Adencarcinoma (HCT-116) cell line. *Panchayavya* was formulated using five products from Cows, namely; Cow dung, Cow urine, Cow milk, Cow curd and Cow ghee. The whole mixture was further freeze-dried and stored until further assay conduction. The growth inhibitory activity of the raw *Panchagavya* (PGS1) and formulated *Panchagavya* (PGS2) in HCT-116 colon cancer cells were investigated by 3-(4, 5 - dimethyl thiazolyl - 2) - 2, 5-diphenyl-tetrazolium bromide (MTT) assay. The results from MTT assay indicated that cell proliferation was significantly ($p < 0.05$) inhibited in a concentration-dependent fashion, especially by formulated *Panchagavya*. Thus, *Panchagavya* formulation has a potential anti-cancer activity. In this context, we investigated the anti-proliferative potential of Fresh *Panchgavya* preparation against the Human Colon Adenocarcinoma (HCT-116) Cell Line. To the best of our knowledge, this is the first report demonstrating *in-vitro* anti-proliferative efficacy of *Panchgavya*against Human Colon Adenocarcinoma (HCT-116) Cell Line.

INTRODUCTION: Colorectal cancer (CRC) being considered the third most commonly diagnosed cancer in males and the second in females, with 1.8 million new cases and almost 861,000 deaths in 2018, according to the World Health Organization. Patients with inflammatory bowel diseases like ulcerative colitis have been shown to have a higher risk of developing CRC [12]. The Indian scenario is further gloomier as India is projected to have one of the highest disease burdens of gut inflammatory diseases, including CRC, across the globe [3, 4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(4).1771-76

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(4).1771-76

Animal studies have shown that bacteria may potentially contribute to inflammatory diseases and CRC development through direct and indirect interaction with the host's simmune system, production of cancer-associated inflammatory metabolites, and release of genotoxic virulence factors [20, 34]. Recently, a growing number of studies indicated that gut microbiota may be a key factor contributing to the development of many pathological processes, including cancer [16, 17].

The gut microbiota (microbiome) comprises a large population of diverse microorganisms (bacteria, viruses, fungi, and protozoa) inhabiting the gastrointestinal tract of humans. In healthy people, the microbiome is involved in nutrient metabolism and absorption, drug metabolism, and elimination of xenobiotics. In addition, normal gut microbiota participates in maintaining intestinal barrier integrity, protects against pathogens and plays an

important role in immunomodulation. According to the latest research that explored the microbiome of individuals with colorectal cancer, alternation in the composition and functionality of the normal gut microbiota may lead to the initiation, promotion, and progression of this cancer [6-12, 37].

It was demonstrated that toxic metabolites of bacteria cause DNA damage, affect cell cycles, stimulate the immune response and disturb the intestinal barrier function. As a result, impaired intestinal microbiota homeostasis contributes to developing the microenvironment favourable to developing colorectal cancer [14].

Panchagavya involves the use of five by-products of cow viz. Cow-dung, -urine, -milk, -curd, and ghee. The Ayurveda, the ancient Indian system of medicine, has detail edmentions of the importance of cow's milk, curd, ghee and urine in treating various human ailments. The ancient ayurvedic literature (Charak Samhita, Sushrut, Gadanigrah) [21] suggests several pharmacological Applications of the substances obtained from Panchgavya [20]. The religious ritual of Practice of Panchagavya prashan has been in existence for ages in our country. This practice was expected to deliver a person from all the sins. Sin in Sanskrit means papma.

Papma is a synonym for disease. This ritual is practiced once every year during July and August, at which time the Vaccinia viraemia is at its peak in cows. The Faecal Microbial Transplant (FMT), a recently emerged technique is now popularly used, is difficult to treat gastrointestinal, metabolic, and many other diseases in western countries [22-24]. *Panchagavya* appears to be a traditional technique of cow faecal transplant therapy. Systematic work needs to be carried out on exploring the anti-cancer or chemoprevention activity of Panchgavya.

MATERIALS AND METHOD: HCT-116 - Human colorectal adenocarcinoma cell line (From NCCS, Pune), Cell culture medium: DMEM-High glucose media - (Cat No:2120785, Gibco), Adjustable multichannel pipettes and apipettor (Benchtop, USA), Fetal Bovine Serum (#RM10432, Himedia), MTT Reagent (5 mg/ml) (#4060 Himedia), DMSO (#PHR1309, Sigma), Cisplatin (#PHR1624, Sigma, D-PBS (#TL1006, Himedia), 96-well plate for culturing the cells (From Corning, USA), T25 flask (#12556009, Biolite -Thermo).

Equipment: Centrifuge (Remi: R-8oC), Pipettes: 2-10µl, 10-100µl, and 100-1000µl, Inverted microscope (Biolink), 37°C incubators with the humidified atmosphere of 5% CO_2 (Heal force, China), freeze dryer (Gen-Tech).

The HCT-116 (Human colorectal adenocarcinoma cell line) was purchased from NCCS, Pune, India. The cells were maintained in DMEM high glucose media supplemented with 10 % FBS and the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37 °C temperature in the CO_2 incubator and subcultured every two days [2, 3].

The Panchgavya formulation used in this study was prepared using a different method from the one practiced traditionally (which yields a fermented preparation). Fresh cow dung and urine, sourced from a cow fed on green grass, were mixed thoroughly in a glass beaker. This mix was allowed to stand for 10 min and subjected to filtration through a muslin cloth. To this filtrate, fresh cow's milk and fresh curd were added and mixed until a uniform mixture was formed. Finally, cow ghee was added to this mixture and mixed thoroughly. This Panchagavya mixture was then transferred to a copper vessel (covered with a muslin cloth) and allowed to rest for 30 min. This was followed by freeze-drying at -20 °C to convert the preparation in powder form, which was then used for MTT assay [1, 4]. The MTT assay is preferable to other methods because it arguably has a high reproducibility rate, is sufficiently safe and straightforward to use in laboratory conditions, is highly sensitive for cytotoxicity tests, and is suitable for high-throughput screening and miniaturization [35-37].

MTT Assay Protocol: MTT assay is a colorimetric assay used to determine cell proliferation and cytotoxicity based on the reduction of the yellow-colored water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibit purple color, the intensity of which is proportional to the number of viable cells and can be measured

spectrophotometrically at 570 nm 4. Assay controls were: (i) Medium control (medium without cells), (ii) Negative control (medium with cells but without the experimental drug/compound), (iii) Positive control (cells treated with 12.5ug/ml of Cisplatin).

Protocol of Study [1-3]: 200 µl cell suspension was seed Edina 96-well plate at required cell density (20,000 cells per well), without the test agent. The cells were allowed to grow for about 24 h. Concentrations (12.5ug/ml, 25ug/ml, 50ug/ml, 100ug/ml, 200ug/ml) of the PGS1 and PGS2 were added accordingly. The plate was incubated for 24 h at 37°C in a 5 % CO_2 atmosphere. After the incubation period, the plate was taken out from the incubator and MTT reagent to a final concentration of 0.5mg/Ml of total volume. The plate was covered with aluminium foil to avoid exposure to light and were incubated for 3 h. The MTT reagent was discarded and then 100µl of solubilization solution (DMSO) was added.

The absorbance reading on a spectrophotometer or an ELISA reader at 570 nm and 630 nm was used as reference wavelength. The IC_{50} value was determined by using linear regression equation *i.e.* Y = Mx+C. Here, Y= 50, M and C values were derived from the viability graph. % of cell viability is calculated using the below formula:

% cell viability = (Mean absorbance of treated cells / Mean absorbance of Untreated cells) × 100

Statistical Analysis: All experimental measurements were tested in duplicate. The results are expressed as mean ± standard deviation (SD). Statistical analysis of data was evaluated using a one-way analysis of variance (ANOVA). The significance level was set at <0.05.

RESULT AND DISCUSSION: Our preliminary results suggested that the Panchagavya shows an inhibitory effect on cell growth in HCT 116 cells. Freshly prepared PGS1 and PGS2 were assessed against HCT 116 colon cells for anti-proliferative activity in 24 h at the above-mentioned concentrations (Table 1 and Table 2 using MTT assay).

After 24 treatments, it was seen that HCT116 cell growth was significantly inhibited in a concentration-dependent fashion, especially when treated with PGS2 **Fig. 2**. The observations in Statistical data of the MTT cytotoxicity study suggesting us that against HCT-116 cell lines, Test Compounds, namely S1, S2 showing significant cytotoxic potential properties with the IC_{50} concentration sat 84.42 ug/ml and 62.56ug/ml respectively, used for the study. Std drug, Cisplatin showing IC_{50} value at 12.5ug/ml respectively **Fig. 3.**

TABLE 1: CELL VIABILITY OF PGS 1 TREATED HCT-116 CELLS

Concentration Unit: µg/ml		Incubation: 24 h						
Parameter	**Blank**	**Cell Control**	**Std Control**	**12.5µg/ml**	**25µg/ml**	**50µg/ml**	**100µg/ml**	**200µg/ml**
Reading 1	0.054	0.842	0.456	0.813	0.728	0.567	0.421	0.248
Reading 2	0.046	0.848	0.445	0.806	0.715	0.552	0.416	0.253
Mean	0.05	0.845	0.451	0.810	0.722	0.560	0.419	0.251
Mean OD (Sample-Blank)	-	0.795	0.401	0.760	0.672	0.510	0.369	0.201
Standard deviation	-	0.004	0.008	0.005	0.009	0.011	0.004	0.004
Standard error	-	0.003	0.006	0.003	0.007	0.007	0.003	0.003
Cell Viability%	-	100.000	50.377	95.535	84.465	64.088	46.352	25.220

TABLE 2: CELL VIABILITY OF PGS 2 TREATED HCT-116 CELLS

Concentration Unit: µg/ml		Incubation:24hrs						
Parameter	**Blank**	**Cell Control**	**Std Control**	**12.5µg/ml**	**25µg/ml**	**50µg/ml**	**100µg/ml**	**200µg/ml**
Reading 1	0.054	0.842	0.456	0.754	0.642	0.531	0.356	0.194
Reading 2	0.046	0.848	0.445	0.746	0.657	0.524	0.349	0.187
Mean	0.05	0.845	0.451	0.750	0.650	0.528	0.353	0.191
Mean OD (Sample-Blank)	-	0.795	0.401	0.700	0.600	0.478	0.303	0.141
Standard Deviation	-	0.004	0.008	0.006	0.011	0.005	0.005	0.005
Standard error	-	0.003	0.006	0.004	0.008	0.004	0.004	0.004
Cell Viability%	-	100.000	50.377	88.050	75.409	60.063	38.050	17.673

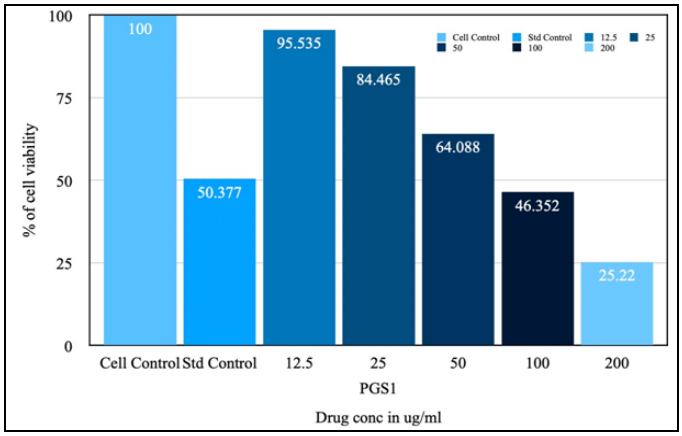


FIG. 1: COMPARATIVE % OF CELL VIABILITY OF PGS 1 TREATED HCT-116 CELLS

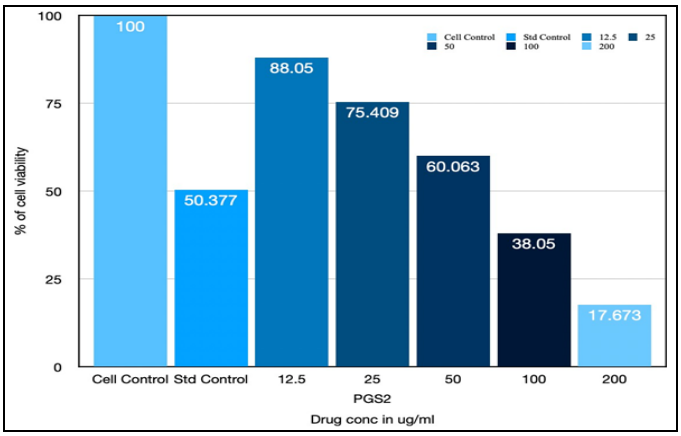
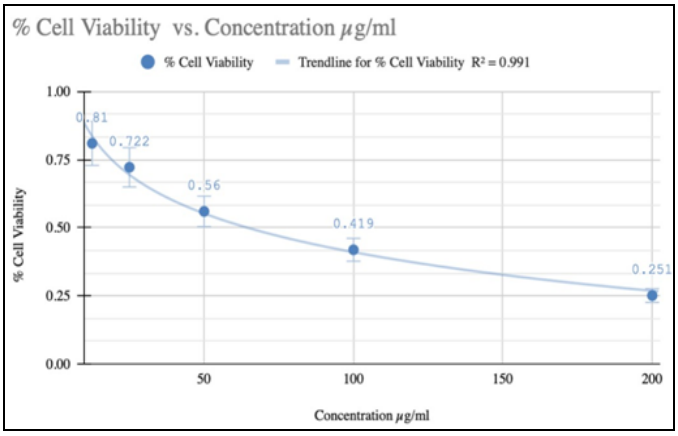
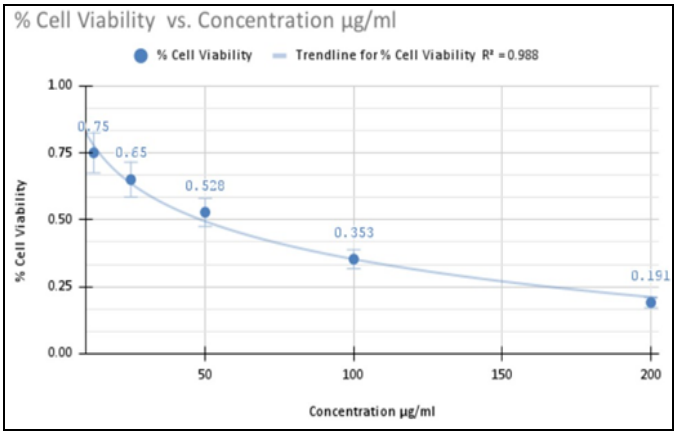


FIG. 2: COMPARATIVE % OF CELL VIABILITY OF PGS 2 TREATED HCT-116 CELLS



GRAPH 1: DENOTING IC_{50} VALUE FOR PGS1



GRAPH 2: DENOTING IC_{50} VALUE FOR PGS2

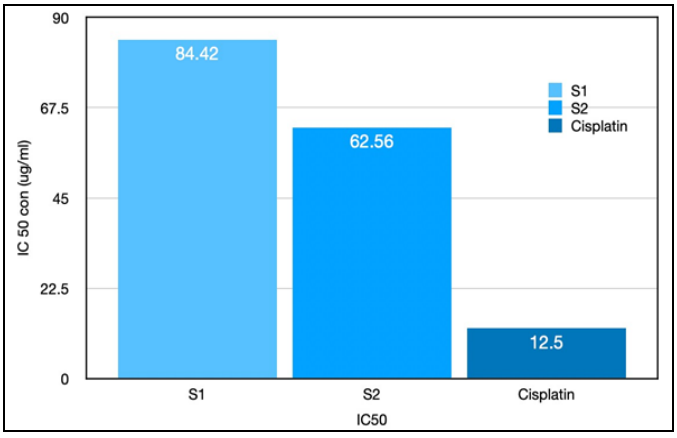


FIG. 3: COMPARATIVE IC_{50} VALUE OF PGS 1 AND PGS 2 ON HCT-116 CELL

CONCLUSION: Colon cancer is considered the most common cancer in men and the third in women [10, 16-18]. The development and invasion process of cancers, including colon cancer, is correlated with dysbiosis in gut microbiota. Among the compounds, PGS1 shows moderate cytotoxicity and PGS2 shows significant cytotoxicity and may be considered a potent anti-cancer agent due to its low IC_{50} value on HCT-116 cells. Further studies like Cell Cycle Study by PI staining, Apoptosis study by Annexin V/PI staining, Apoptotic Protein expressions like Caspase 3, 7, 9, Bcl 2, p 53, and ROS study to evaluate the mechanism of action of test compounds *viz.*, PGS1 and PGS2 behind the anti-cancer potential in *in-vitro* conditions.

ACKNOWLEDGMENT: Authors thank Prime Minister's Research Fellowship for financial support.

CONFLICTS OF INTEREST: The author declares no conflicts of interest.

REFERENCES:

1. Arthur JC, Perez-Chanona E and Mühlbauer M: Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 2012; 338: 120-3.
2. Brandt LJ and Aroniadis OC: An overview of fecal microbiota transplantation: techniques, indications, and outcomes. Gastrointest Endosc 2013; 78: 240–249.
3. Chin SM, Sauk J, Mahabamunuge J, Kaplan JL, Hohmann EL and Khalili H: Fecal microbiotatrans plantation for recurrent Clostridium difficile infection in patients with inflammatory bowel disease: asingle-center experience. Clin Gastroenterol Hepatol 2017; 15: 597–599.
4. Cipe G, Idiz UO and Firat D: Relationship between intestinal microbiota and colorectal cancer. World J Gastrointest Oncol 2015; 7: 233-40.
5. Jain NK, Gupta VB, Garg Rajesh and Silawat N: Efficacy of cow urine therapy on various cancer patients in Mandsaur District, India - A survey. International Journal of Green Pharmacy 2010; 29-35.
6. Kostic AD, Chun E and Robertson L: Fusobacteriu mnucleatum potentiates intestinal tumor genesis and modul a test he tumor-immunemicro environment. Cell Host Microbe 2013; 14: 207-15.
7. MTT Cell Proliferation Assay Instruction Guide–ATCC, VA, USA www.atcc.org
8. Rani Rita and Kansal Vinod: Effects of cow ghee (clarified butter oil) & soybean oil on carcinogen-metabolizing enzymes in rats. Indian Journal of Medical Research 2012; 136: 460-465.
9. Rani Rita and Kansal Vinod: Study on cow ghee versus soybean oil on 7, 12-dimethylbenz (a)-anthracene induced mammary carcinogenesis and expression of cyclooxygenase-2 & peroxisomepro life rators activated receptor-γ inrats. Indian Journal of Medical Research 2011; 133: 497-503.
10. Rubinstein MR, Wang X and Liu W: Fusobacterium nucleatum promotes color ectalcarcinogenesis by modulating E-cadherin/ β-cateninsignaling viaits Fad Aadhesin. Cell Host Microbe 2013; 14: 195-206.
11. US Patent 6410059. www.patft.uspto.gov.USPatent6896907.www.patft.uspto.gov.
12. Wrzosek L, Ciocan D, Borentain P, Spatz M, Puchois V, Hugot C, Ferrere G, Mayeur C, Perlemuter G and Cassard AM: Transplantation of human microbiota into conventional mice durably reshapes the gut microbiota. Sci Rep 2018; 8(1): 6854.
13. Zackular JP, Baxter NT and Iverson KD: The gut microbiome modulates colon tumorigenesis. M Bio 2013; 4: 0692-13.
14. Manosroi J and Dhumtanom P: Colon tumorigenesis Monosroi A: Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Lett 2006; 235: 114-120.
15. Khoogar R, Kim BC, Morris J and Wargovich MJ: Chemoprevention in gastrointestinal physiology and disease. Targeting the progression of cancer with natural products: a focus on gastrointestinal cancer. Am J Physiol Gastrointest Liver Physiol 2016; 310.
16. Neumann J, Boerries M, Köhler R, Giaisi M, Krammer PH, Busch H and Li-Weber M: The natural anti-cancer compound rocaglamide selectively inhibits the G1-S-phase transition in cancer cells through the ATM/ATR-mediated Chk1/2 cell cycle checkpoints. Int J Cancer 2014; 15(134): 1991-2002.
17. Palep HS: Scientific foundation of Ayurveda. Chaukhamba Sanskrit Pratishthan, Delhi 2004; 170-172.
18. Benson AK, Kelly SA, Legge R, Ma F, Low SJ, Kim J, Zhang M, Oh PL, Nehrenberg D and Hua K: Individual I tyin gut microbiota composition is a complexpolyg enictraits haped by multiple environmental and host genetic factors. Proc Natl Acad Sci USA 2010; 107: 18933–18938.
19. Blaser M: Antibiotic overuse: Stop the killing of beneficial bacteria. Nature 2011; 476: 393–394.
20. Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, KcW, Carrero JA and Hunt S: Akey role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature 2008; 456: 259–263.
21. Cadwell K, Patel KK, Maloney NS, Liu TC, Ng AC, Storer CE, Head RD, Xavier R, Stappen beck TS and Virgin HW: Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg 16 L1 phenotypesin intestine. Cell 2010; 141: 1135–1145.
22. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A and Lambert DM: Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009; 58: 1091–1103.
23. Knights D, Costello EK and Knight R: Supervised classification of human microbiota. FEMS Microbiol Rev 2011; 35: 343–359.
24. Peterson DA, McNulty NP, Guruge JL and Gordon JI: IgA response to symbiotic bacteria as a mediator of gut homeostasis. Cell Host Microbe 2007; 2: 328–339.
25. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT and Ley RE: Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci USA 2011; 108(1): 4578–4585.
26. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global patterns and trends in colorectal cancer in cidenceand mortality. Gut 2017; 66: 683–691.
27. Navarro M, Nicolas A, Ferrandez A and Lanas A: Colorectal cancer population screening programs world widein 2016: Anupdate. World J Gastroenterol 2017; 23: 3632–3642.
28. Marley AR and Nan H: Epidemiology of colorectal cancer. Int J Mol Epidemiol Genet 2016; 7: 105–114.
29. KellyC, Bhuva N, Harrison M, Buckley A and Saunders M: Use of raltitrexed as an alternative to 5-fluorouracil and capecit abine in cancer patients with cardiac history. Eur J Cancer 2013; 49: 2303–2310.
30. Kanterman J, Sade-Feldman M, Biton M, Ish-Shalom E, Lasry A, Goldshtein A, Hubert A and Baniyash M: Adverse immunoregulatory effects of 5FU and CPT11 chemotherapy on myeloid-derived suppressor cells and colorectal cancer outcomes. Cancer Res 2014; 74: 6022–6035.
31. Qasim M, Lim DJ, Park H and Na D: Nanotechnology for Diagnosis and Treatment of Infectious Diseases. J Nanosci Nanotechnol 2014; 14: 7374–7387.
32. Van Tonder A, Joubert AM and Cromarty AD: Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) assay when compared to three

commonly used cell enumeration assays. BMC Res Notes 2015; 8.

33. Hamid R, Rotshteyn Y, Rabadi L, Parikh R and Bullock P: Comparison of alamar blue and MTT assays for high through-put screening. Toxicol Vitr 2004; 18:703–710.
34. Aslantürk ÖS: *In-vitro* Cytotoxicity and Cell Viability Assays: Principles, Advantages and Disadvantages. In Genotoxicity A Predictable Risk to Our Actual World; Intech Open: London UK 2017.

How to cite this article:
Funde S, Patil S and Palep H: Anticancer effect of panchagavya on human colon adenocarcinoma (HCT-116) cell line. Int J Pharm Sci & Res 2022; 13(4): 1771-76. doi: 10.13040/IJPSR.0975-8232.13(4).1771-76.