

In Vitro Anticancer Effects of a Traditional Sri Lankan Polyherbal Decoction and Its Individual Components Against Colorectal Cancer Cells

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Abstract

Background

Colorectal cancer is globally the third highest prevalent and second highest mortality malignancy. Modern treatments were failed to reduce the cancer deaths to the expected level via producing exceptional side effects. On the contrary, traditional herbal medicine is gaining more attention in cancer remedies mainly due to its high efficacy and fewer side effects. The current study was aimed at investigating the anticancer and antioxidant properties of a poly-herbal decoction prescribed in Sri Lankan traditional cancer treatments. A polyherbal decoction (PHD) prepared using the *Adenanthera pavonina*, *Thespesia populnea*, *Tinospora cordifolia*, and *Withania somnifera*, and decoctions prepared from these four individual plants were used commonly in Sri Lankan traditional medicine for colorectal cancer treatments.

Methods

investigated for their total polyphenolic content, anti-oxidant activity, in vitro anti-colon cancer and apoptosis inducing activity (in HCT116 cell line) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, Ferric Reducing Antioxidant Power (FRAP) assay, hydroxyl radical scavenging assay, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay, caspase activity assay and Real-time quantitative Polymerase Chain Reaction (RT-qPCR) respectively.

Results

Out of the five decoctions, *W. somnifera* decoction (WSD) exhibited strong anti-colon cancer activity ($EC_{50} = 83.1 \pm 1.1 \mu\text{g/mL}$) while PHD and other decoctions exhibited limited anti-cancer activity ($EC_{50} > 100 \mu\text{g/mL}$). WSD suppressed colony formation and induced caspase dependent programmed cell death, and lactate dehydrogenase (LDH) leakage. Up regulation of tumor suppressor p53 gene expression and down regulation of expression of key oncogenic and anti-apoptotic genes MMP-2, MMP-9, STAT3, JAK2, Cyclin D1, VEGF, and Bcl-XL were observed.

Conclusions

Overall findings validates the traditional use of Poly Herbal Decoction and *W. somnifera* decoction against colon cancer.

1.0 Introduction

Colorectal cancer is a significant global health concern globally. In 2020, approximately 1.9 million new colorectal cancer cases were diagnosed, accounting for 9.6% of all cancer incidences, making it the third most commonly occurring cancer worldwide (WHO, 2025). In the same year, colorectal cancer led to about 900,000 deaths, representing the second leading cause of cancer mortality globally (WHO, 2025).

Chemotherapy and radiotherapy remain the currently practiced treatments for colorectal cancer. However, these therapies often result in significant adverse effects. While short-term side effects such as nausea, fatigue, and stomatitis typically subside within a few months, long term complications including infertility, cardiac dysfunction, and secondary malignancies like leukemia can persist for years (Nurgali et al., 2018; Partridge et al., 2001; Yin et al., 2013). Given these limitations, there is an urgent need to explore alternative therapeutic strategies that are both effective and safe. Natural products, particularly plant-based compounds, have gained considerable attention in cancer research due to their diverse bioactive properties, including immune modulation and enhanced survival benefits (Kumar et al., 2016; Wang et al., 2017; Jafarian et al., 2014).

Sri Lanka has a long-standing tradition of herbal medicine, with historical records indicating the use of medicinal plant extracts and decoctions for over 3,000 years (Weragoda, 1980). Many of these formulations are reputed to have anticancer properties (Jayasinghe et al., 2017; Luo et al., 2010). Notably, a decoction derived from the bark of *Adenanthera pavonina* and *Thespesia populnea*, the stem of *Tinospora cordifolia*, and the tuberous root of *Withania somnifera* have been incorporated into Sri Lankan traditional medicine and have claimed to be effective in cancer therapy with particular emphasis against colorectal cancer (Kuruppu et al., 2019).

However clinical studies or other forms of *in vivo* or *in vitro* efficacy studies has not been conducted for poly herbal decoction (PHD). Present study aims to evaluate the anticancer, cytotoxic and apoptosis inducing activity of the PHD and its individual components in colorectal cancer cells. Additionally, the study seeks to determine the individual contributions of each plant component to the overall bioactivity of the decoction as well as total phenolic, flavonoid, and tannin content and the antioxidant properties. By providing scientific validation for the traditional use of this formulation, this research contributes to the growing body of evidence supporting herbal medicine as a complementary approach in cancer therapy.

2.0 Methodology

Collection and identification of plant materials

All plant species of *Tinospora cordifolia* (TC), *Thespesia populnea*(TP), *Adenanthera pavonina* (AP) were collected from Kotte, Western Province, Sri Lanka. *Withania somnifera* (WS) was purchased from a registered ayurvedic material vendor (Sridhara ayurvedic shop, Malabe, Western Province, Sri Lanka) in Sri Lanka. Each plant species was identified by a botanist at Bandaranaike Memorial Ayurvedic Research Institute (BMARI), Sri Lanka and voucher specimen were deposited at BMARI (voucher specimen numbers 2093, 2094 and 2095 for AP, TP and TC respectively).

Preparation of plant decoctions

The aerial parts of TC, the bark of TP, the bark of AP and the rhizome of WS were freeze-dried and the poly-herbal decoction was prepared as of the methods given by the traditional medical practitioners. The individual decoctions were prepared by using 15 g of each freeze-dried aerial parts of TC, the bark of TP, the bark of AP and the rhizome of WS. In brief, the plant material was added with 1600 mL of deionized water and was heated till the volume reduced to 1/8th (200 mL) of the initial volume (Lindamulage and Soysa 2016). The decoction was centrifuged (5000 rpm, 15 min) and the supernatant was freeze-dried to obtain the dried powder dissolved in deionized water/phosphate-buffered saline (PBS) to obtain known concentrations of plant extracts. Hereafter, decoctions of TC, TP, AP and WS will be indicated as TCD, TPD, APD and WSD respectively. Poly-herbal decoction will be indicated as PHD.

Estimation of Total phenolic, tannin and flavonoid contents

Estimation of Total Phenol Content (TPC)

TPC of the decoctions was determined by Falin-Ciocalteau method (Kaur and Kapoor 2002). Gallic acid was used as the standard and a standard curve was obtained at a concentration range of 5–65 µg/mL of gallic acid. The TPC of the decoctions were calculated from the standard curve (Fernando et al, 2015). All determinants were carried out in triplicates.

Estimation of Total Flavonoids Content (TFC)

The aluminium chloride colorimetric method was carried out to determine the total flavonoid contents of the decoctions (Fernando and Soysa 2015). Quercetin was used as the standard and the standard curve was obtained at a concentration range of 10-1000 µg/mL of quercetin. The TFC of the decoctions were calculated from the standard curve. All determinants were carried out in triplicates.

Estimation of Tannin content (TAC)

The tannin content (TAC) of the plant decoctions was determined according to the following method described (Pulipati et al. 2017). A volume of 500 µL of the decoction (20 mg/mL) was mixed with deionized water (500 µL) and 50 mg of polyvinyl polypyrrolidone (PVPP) and it was vortexed and refrigerated (4 °C, for 15 min). The reaction mixture was subjected to vortex again and was centrifuged at 3000 g, for 10 min. A concentration series was prepared at 70-5000 µg/mL from the supernatant to estimate the tannin content. The rest of the reaction method was performed according to the phenolic content estimation. Tannic acid was used as the standard and a standard curve was obtained at a concentration range of 20–73 µg/mL of tannic acid. The tannin content of the decoctions was calculated from the standard curve and all determinants were carried out in triplicates.

Determination of antioxidant properties

Free radical scavenging properties

DPPH radical scavenging assay (Soysa and Silva 2011), Ferric reducing antioxidant power assay (FRAP Assay) (Jemli et al. 2016) and Hydroxyl radical scavenging assay (Halliwell et al. 1987, Poorna et al. 2013) were performed according to the standard methods given in the literature. All determinations were carried out in triplicate.

Protein and lipid oxidation inhibition

Inhibition of Protein oxidation was carried out using protein oxidation inhibition assay (Poorna et al. 2013; Martnez et al. 2001) and inhibition of lipid oxidation was performed using egg yolk lipids (Kizil et al. 2010; Perera et al. 2016, Dhar et al. 2013) to the standard methods reported in previous study.

Cell cultures

HCT116 colorectal cancer cell line was used to determine the cytotoxicity of the targeted plant decoctions. HCT116 cell line was acquired from the Medical Research Institute, Colombo, Sri Lanka. It was cultured in Modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS), 3% L-glutamine, 7% Sodium bicarbonate, streptomycin (100 µg/mL), and penicillin (100 U/mL). Cells were incubated in humidified air at 37 °C. Exponentially growing (80% confluent) cells were passaged every 2–3 days using 0.25% trypsin–EDTA solution (Perera et al. 2008; Razak et al. 2019; Vichai and Kirtikara 2006; Fernando et al. 2015).

Cytotoxicity assays

Dimethylthiazol-diphenyltetrazolium bromide (MTT) assay

Cytotoxicity was determined using MTT cell viability assay after being subjected to the 24 h treatment of the PHD and individual decoctions to HCT116 cells (Wageesha et al. 2017). Experiments were carried out in triplicates. WSD decoction was subjected to 24 h, 48 h, and 72 h after the initial screening.

Preparation of cell lysate

Cell plates were prepared and treated according to the previously described method (Fernando et al. 2015). Each well was washed with PBS (100 µL, pH 7.4) three times. Thereafter, Triton-X-100 (100 µL, 0.1 v/v) was added to each well and subjected to sonication for 5 seconds. The cell lysates were then collected and centrifuged at 3000 rpm for 5 min to remove cell debris.

LDH leakage

Cells were treated with WSD decoction, assay was performed according to the manufacture's description (LDH SCE mod. liquiUV, Human Diagnostics, Germany) for both the treated medium and cell lysate. The percentage LDH leakage was determined by the equation given below;

$$\text{Percentage LDH leakage} = \frac{\text{LDH activity in the supernatant}}{\text{Total LDH activity}} \times 100 \text{ (Wageesha et al., 2017)}$$

Colony formation assay

Trypsinized cells were seeded in a culture dish and after 24 h cells were treated with the WSD as previously described. The colonies were stained with 6% glutaraldehyde and 0.5% crystal violet. The colonies were counted using stereomicroscope (Franken, 2006).

Caspase assay

Cells were seeded and treated with WSD as previously described, and the assay was performed according to the manufacturer's description (Caspase 3 Activity Assay Kit, China (E-CK-A311)).

$$\text{Caspase activity} = \frac{\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{blank}}}{\text{Absorbance}_{\text{negative control}} - \text{Absorbance}_{\text{blank}}} \times 100$$

Ethidium bromide/acridine orange (EB/AO) assay

Decoction-treated cells were stained with a 1:1 mixture of Ethidium bromide and acridine orange, 100 µg/mL, prepared in PBS and observed in UV under a fluorescent microscope (Wageesha et al., 2017). Morphological criteria were used to evaluate cell injury. Cells containing normal nuclear chromatin exhibit green nuclear staining. Cells containing fragmented nuclear chromatin due to apoptosis exhibit orange to red nuclear staining.

DNA fragmentation

Treated cells were trypsinized and were collected in microcentrifuge tubes (4×10^5 per microcentrifuge tube). Cells were pelleted by centrifuging (2000 rpm, 5 min, 4 °C). TES lysis buffer (20 µL) was added, and the pellet was carefully resuspended. RNase was added (10 mg/mL, 10 µL) added and incubated for 2 hrs at 37 °C. Thereafter, Proteinase K (20 mg/mL, 10 µL) was then added and incubated at 50 °C overnight. The sample (10 µL) was mixed with 2 µL of DNA loading dye and was run in 1.5% agarose gel (Kasibhatla et al., 2006) (Wageesha et al., 2017).

Gene expression analysis

Based on the cytotoxicity, oxidative, and morphological changes results, a hypothetical gene expression pathway that might have expressed in driving cell death (apoptosis) was postulated. In that gene expression pathway, 9 genes were targeted (with the house keeping gene, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and primers were designed to analyze the relative gene expression during cell death.

Some primers were manually designed following the standard guidelines (Bustin & Huggett, 2017; Dieffenbach et al., 1993). Either the RefSeq transcripts or all the transcript variants were considered in primer designing. A few primers were acquired from published literature. Primer sequence, alignment, position of annealing, nucleotide length, melting temperature and GC percentage of each primer are included in Table 1.

RT-qPCR and Gene expression analysis

RT-qPCR analysis of the genes was performed in Rotor Gene Q (Qiagen, Germany), following the given amplification parameters (40 cycles for Initial denaturation at 95 °C for 3 minutes, Denaturation at 95 °C for 30 seconds, Annealing at 60 °C for 30 seconds, Extension at 72 °C for 1.0-minute, Final extension at 72 °C for 1.0 minute, 4 °C, ∞). Reaction mixtures were prepared as follow GoTaq® qPCR Master Mix, 2X; 10.0 µL (1X), Forward Primer (20X); 1.0 µL (250 nM), Reverse Primer (20X); 1.0 µL (250 nM), Nuclease-Free Water; 5.0 µL, cDNA template; 3.0 µL. Melting curves

were obtained and the relative gene expression was evaluated. A small aliquot (10 μ L) of each RT-qPCR product was loaded along with the loading buffer. The gel was run in ethidium bromide stained, 0.8% w/v agarose gel at 90 V. It was observed under UV after running for 15 minutes and 30 minutes.

Statistical data analysis

The data were represented as mean $\pm$ standard deviation. For comparison, data were analyzed by regression and Pearson analysis. A value of $p < 0.05$ was considered statistically significant.

Table 1
Primers used in gene expression analysis

Target gene	Primer name	Primer sequence	Primer length	Annealing site	Melting Temperature	GC content (%)	Product Length (cDNA)	Reference
Matrix metalloproteinase 2	MMP2_F	5' TACAGGATCATTGGCTACACACC 3'	23	661–683	63.9	48	90	(Dai et al., 2017)
	MMP2_R	5' GGTCACATCGCTCCAGACT 3'	19	732–750	63.3	58		(Dai et al., 2017)
Matrix metalloproteinase 9	MMP9_F	5' TTGACAGCGACAAGAAGTGG 3'	20	1155–1174	62.5	50.0	179	(Uehara et al., 2017)
	MMP9_R	5' GCCATTACGTCGTCCTTAT 3'	20	1314–1333	62.1	50.0		(Uehara et al., 2017)
18 S (house-keeping gene)	18S_F	5' CATTCTGATTGCGCCGCTAG 3'	20	937–956	63.3	55.0	135	Newly designed
	18S_R	5' CTACGACGGTATCTGATCGTC 3'	21	1051–1071	61.1	52.4		Newly designed
B-cell lymphoma-extra large	BclxI_F	5' GCGTAGACAAGGAGATGCAG 3'	20	595–614	62.2	55.0	136	Newly designed
	BclxI_R	5' GCATTGTTCCCATAGAGTTCCAC 3'	23	708–730	62.3	47.8		(Taghavi et al., 2019)
Signal transducer and activator of transcription 3	STAT3_F	5' AGCAGTTTCTTCAGAGCAGGT 3'	20	755–774	60.7	50.0	134	Newly designed
	STAT3_R	5' GCGTGATTCTTCCACAGGC 3'	21	868–888	60.1	47.6		Newly designed
Cyclin D1	Ccnd1_F	5' GATACCAGAAGGGAAAGCTTC 3'	21	1141–1161	60.2	47.6	189	Newly designed
	Ccnd1_R	5' ATCTGTAGCACAACCCTCCTC 3'	21	1309–1329	62.3	52.4		Newly designed
Vascular endothelial growth factor	VEGF_F	5' CTACCTCCACCATGCCAAGT 3'	20	1084–1103	63.7	55.0	109	(Du et al., 2015)
	VEGF_R	5' GCAGTAGCTGCGCTGATAGA 3'	20	1173–1192	63.8	55.0		(Du et al., 2015)
p53	P53_F	5' TATGAGCCGCCTGAGGTTGG 3'	20	1653–1672	60.7	50.0	104	(Hübner et al., 2018)
	P53_R	5' CGCACCTCAAAGCTGTTCCG 3'	20	1738–1757	60.5	50.0		(X. M. Li et al., 2020)
Glyceraldehyde 3-phosphate dehydrogenase (house-keeping gene)	GAPDH_F	5' GGAGCGAGATCCCTCCAAAAT 3'	21	554–574	63.9	52.4	197	(Fu et al., 2019)
	GAPDH_R	5' GGCTGTTGTCATACTTCTCATGG 3'	23	728–750	63.0	47.8		(Fu et al., 2019)
Janus Kinase 2	JAK2_F	5' TTGGAGCTTTGGAGTGGTTC 3'	20	3784–3803	62.4	50.0	178	Newly designed
	JAK2_R	5' ATCTGGGCATCCATCTGGTC 3'	20	3942–3961	63.6	55.0		Newly designed

3.0 Results and Discussion

Estimation of total phenolic, flavonoid and tannin contents

Estimated Total phenolic (TPC), total flavonoid (TFC) and total tannin contents (TAC) of the poly herbal decoction and individual plant decoctions are summarized in Table 2. According to the results, APD and TPD have shown higher TFC and TAC contents. However, in TPC estimation, the

highest value (49.01 ± 4.9 mg GAE/g) was observed in PHD while TPD and WSD resulted in considerably lower values (1.9 ± 0.1 mg GAE/g, 0.4 ± 0.0 mg GAE/g respectively). The estimation of TPC considers flavonoids and tannins, which encompasses a broad range of compounds including other phenol-type derivatives, this may be the reason for the deviations in TPC estimation (Muflihah, 2021).

Table 2
Total Phenolic Content, Total Flavonoid Content and Tannin Content of the PHD and individual plant decoctions; APD, TPD, TCD and WSD. The values are mean of three independent experiments $\pm$ standard deviation (SD)

Decoctions	TPC (mg GAE/g)	TFC (mg QCE/g)	TAC (mg TAE/g)
PHD	49.1 ± 4.9	1.0 ± 0.1	24.7 ± 2.2
APD	44.6 ± 2.6	1.8 ± 0.4	64.9 ± 5.8
TPD	1.9 ± 0.1	7.8 ± 0.8	74.2 ± 7.6
TCD	5.1 ± 0.2	0.1 ± 0.0	1.1 ± 0.3
WSD	0.4 ± 0.0	0.1 ± 0.0	1.1 ± 0.3
Antioxidant assays			

In the present study, three different antioxidant assays are used to accurately confirm the antioxidant ability of each decoction.

DPPH assay

The present findings indicate that all decoctions induced a colorimetric transition of DPPH confirming the antioxidant capacity. PHD exhibited antioxidant activity with an EC_{50} value of 44.2 ± 0.7 μ g/mL, while TCD and WSD resulted with EC_{50} value of 1025.5 ± 11.6 μ g/mL 1011.9 ± 15.9 μ g/mL respectively. APD displayed significantly stronger antioxidant activity (EC_{50} value of 15.0 ± 0.5 μ g/mL) as given in Table 3.

Hydroxyl radical scavenging assay

All the decoctions produced a characteristics pink chromogen upon thermal treatment, indicating the presences of phenolic compounds. PHD exhibited antioxidant activity with an EC_{50} of 36.5 ± 0.2 μ g/mL and TPD had the most potent antioxidant activity (EC_{50} value of 13.2 ± 0.2 μ g/mL), while WSD resulted in weakest anti-oxidant activity with EC_{50} 858.1 ± 14.3 μ g/mL (Table 3).

Table 3
Comparative Antioxidant activities of PHD and individual plant decoctions. The values are mean of three independent experiments $\pm$ standard deviation (SD)

Decoction/ Standard	EC_{50} values (μ g/mL)	
	DPPH	Hydroxyl radical scavenging assay
PHD	44.2 ± 0.7	36.5 ± 0.2
APD	15.0 ± 0.5	13.2 ± 0.2
TPD	15.5 ± 0.2	12.4 ± 0.1
TCD	1025.5 ± 11.6	816.3 ± 16.6
WSD	1011.9 ± 15.9	858.1 ± 14.3
Ascorbic Acid	5.42 ± 0.10	

Ferric ion reducing power assay

The dose-response curves of ferric ion reducing power assay are presented in Fig. 1. TCD (200–600 μ g/mL of concentration range in between 0.300-1.000 of absorbance) and WSD (with 175–600 μ g/mL of concentration range in between 0.300–0.600 of absorbance) were excluded in the figure.

Results from all three antioxidant assays, confirmed significant anti-oxidant activities in all tested decoctions with varying potency. APD and TPD demonstrated strongest antioxidant properties whereas TCD and WSD with the weakest antioxidant properties PHD exhibited intermediate anti-oxidant properties due to its composition containing individual components, PHD was prepared as a mixture including all the individual components together where it can be expected to have the highest antioxidant properties as a collective of all the herbal decoctions. However, this effect may be affected by the interactions and reactions of different components which may not undergo when they are in separate individual extracts and also no correlation was observed between antioxidant (DPPH) and TPC, TFC and TAC.

Oxidation assays

Inhibition of Protein oxidation

The maximum inhibitory activity (lowest EC₅₀) was demonstrated in PHD at 223.7 ± 1.8 µg/mL, while TCD exhibited a marginal inhibitory effect at EC₅₀ value of 511.8 ± 4.5 µg/mL (Table 4).

Table 4
Inhibition of protein oxidation and lipid peroxidation shown by PHD and other individual plant decoctions; APD, TPD, TCD and WSD. The values are mean of three independent experiments (n = 3) ± standard deviation (SD)

Decoction	EC ₅₀ values (µg/mL)	
	Protein oxidation	Lipid peroxidation
PHD	223.7 ± 1.8	383.2 ± 8.7
APD	343.8 ± 0.9	206.4 ± 2.7
TPD	260.5 ± 2.2	207.6 ± 3.5
TCD	511.8 ± 4.5	523.7 ± 6.2
WSD	295.0 ± 10.1	156.9 ± 12.4

Inhibition of Lipid Peroxidation

Lipid peroxidation was quantified spectrophotometrically by measuring the chromogenic transition in the reaction medium. WSD demonstrated the maximal inhibitory activity (EC₅₀ 156.9 ± 12.4 µg/mL), while APD (EC₅₀ 206.4 ± 2.7 µg/mL) and TPD (EC₅₀ 207.6 ± 3.5 µg/mL) exhibited the minimal marginal inhibitory activity as given in Table 4. All decoctions exhibited a considerable anti-oxidant activity against both protein oxidation and lipid oxidation. However, no significant correlation was observed between these protective effects, suggesting distinct mechanisms of action for each oxidative pathway.

Cytotoxicity assays

MTT assay was employed in determining the cytotoxicity of the decoctions against the colorectal carcinoma. Level of cytotoxicity of the PHD and its individual decoctions against HCT 116 and HEK 293 cells were determined by MTT assay after 24 h of treatments (Table 5). As shown in Table 5, the highest cytotoxic effect was observed with WSD (EC₅₀ value; 83.1 µg/mL). However, no significant cytotoxic effect was detected in TCD decoction, while PHD (EC₅₀ 383.1 ± 2.7 µg/mL) resulted in a comparatively higher EC₅₀ value than TPD (EC₅₀ 268.3 ± 1.3 µg/mL) the least active individual component (Fig. 2). The EC₅₀ values of the individual components resulted in a wide range from 83.1 µg/mL to 268.3 µg/mL.

A positive correlation was observed in PHD, APD, TPD and TCD decoctions, however a significant correlation was not observed in WSD, which demonstrated a strong cytotoxicity comparatively to other decoctions. While WSD exhibited potent anti-cancer activity, due to its low total phenolic, flavonoid and tannin content a significant correlation was not observed. Comparatively WSD exhibited highest anti-cancer activity against HCT 116 cells than individual components decoction and PHD. Based on this activity, WSD was selected for further studies.

Table 5
Level of cytotoxicity of the PHD and its individual decoctions against HCT 116 and HEK 293 cells by MTT assay after 24 h of treatments, expressed as mean ± SD of three individual experiments (n = 3).
*n = 2

Decoctions	Cell line	EC ₅₀ values (µg/mL)
PHD	HCT 116	383.1 ± 2.7
APD	HCT 116	191.2 ± 2.7
TPD	HCT 116	268.3 ± 1.3
TCD	HCT 116	Not detected
WSD	HCT 116	83.1 ± 1.1
WSD	HEK 293	534.5 ± 16.0*

* The value is a mean of two independent experiments (n = 2) ± standard deviation (SD)

In consistent to our study, A prior research reported the dose dependent and time dependent anti-cancer activity in *Withania somnifera* crude extract against human malignant melanoma cells (Halder, 2015). Evidently a study performed with *Withania somnifera* fruit extract resulted in cytotoxicity against HCT 116 cells with a LC₅₀ value of 410.2 µg/mL, and HepG2 cells with a LC₅₀ value of 164.7 µg/mL as the highest cytotoxicity demonstrated (Abutaha, 2015).

Table 6
Cytotoxicity effects of the WSD
after 24, 48 and 72 h of
treatments.

Time	EC ₅₀ values (µg/mL)
24 h	81.2 ± 0.7
48 h	17.4 ± 0.1
72 h	3.1 ± 0.3

Figure SEQ Figure * ARABIC 3. At 24 hours, EC₅₀ of WSD was 81.2 ± 0.7 µg/mL. When the incubation period was extended for 24 and 72 hours, the EC₅₀ values decreased to 17.4 ± 0.1 µg/mL and 3.1 ± 0.3 µg/mL against HCT 116 cells respectively.

LDH leakage

Quantification of membrane integrity damage was demonstrated upon WSD treatment within 24 h, a significant LDH leakage (59.5 ± 2.1%) was exhibited resulting in dose dependent cytotoxicity as demonstrated in Fig. 4. LDH leakage and increased level of LDH release were previously reported in *Withania somnifera* extract-treated on androgen-independent prostate cancer 3 cells (PC3) exhibiting the anti-proliferative effect. (Balakrishnan et al., 2017). Similarly, in another study *Withania somnifera* root powder was reported to have a significant suppression in lysosomal and cytoplasmic enzyme release in polymorphonuclear leucocytes cells (Rasool, 2006).

Colony forming assay

As of the obtained data, the WSD decoction treatments have significantly reduced cell proliferation and differentiation into colonies (262 ± 20). The negative control resulted in higher number of colonies after 7 days of the treatments (595 ± 22), while the positive control resulted in 2 ± 2 colonies, as shown in Fig. 5.

Sumantran et al 2007 reported that aqueous extract of *Withania somnifera* roots had a dose dependent inhibition in colony formation with Chinese Hamster ovarian cell line. It also reported that *Withania somnifera* root extract induce long term growth inhibition of CHO cells which was cell density dependent upon the drug treatment (Sumantran et al 2007).

Ethidium bromide/acridine orange (EB/AO) assay

Acridine orange (AO) is a chromatin staining dye that emits green fluorescence upon intercalation and enters live cells with normal membrane potentials. Hence, it is important in live, healthy and proliferating cells. Ethidium bromide (EB) penetrates only the cells that have lost their membrane integrity and emits a fluorescence of red upon DNA intercalation. Further, it dominates over AO. This makes the live cells in green colour and apoptotic and dead cells in orange-red colour (Ribble et al., 2005).

Nuclei stained with green colour indicate live cells, while greenish yellow shows early apoptotic cells. Condensed orange red nuclei demonstrate late apoptotic cells, whereas red colour indicates dead cells. As shown in Fig. 6, the EB/AO assay resulted in prominent apoptotic morphology in the HCT 116 cells upon treating with WSD for 24 hours. As shown in Fig. 6A, orange colour and red colour cells were observed, indicating the late apoptotic cells and dead cells respectively. When the cells were observed in high power, fragmented nuclei were observed as in 6B. The images 6C and 6D indicate the negative and positive control respectively. The morphological changes of the images indicate the ongoing apoptosis in the cells upon treatment.

DNA fragmentation

As shown in Fig. 7, HCT 116 cells resulted in DNA fragments upon treatment after 24 hours. According to the gel image visualization, faint bands of DNA fragments provide evidence of active apoptosis. As the negative control is free of such DNA fragments, it can be concluded that the WSD treatment has initiated apoptosis and DFF function, resulting DNA fragments in the HCT 116 cells.

DNA fragmentation is a main feature of apoptosis and thus is considered as a marker of apoptosis. During apoptosis, double-stranded DNA is cleaved at A and T-rich regions by the DNA fragmentation factor (DFF). The 40 kDa catalytic subunit (DFF40) provides the endonuclease activity of

the DFF for DNA cleavage, while 45 kDa subunit (DFF45) provides the regulatory functions (Majtnerová & Roušar, 2018). At normal stages, DFF40 remains inactive, which is regulated by DFF45. Upon activation of Casapase3, the DFF40-DFF45 complex is cleaved. Active DFF40 is released, and hence, DNA fragmentation is started. It cleaves DNA in about 180 bp and its multiples (360 bp, 540 bp and 720 bp), resulting a ladder pattern upon agarose gel run (Majtnerová & Roušar, 2018).

Evidently in a previous study *Withania somnifera* extracts significantly induce in apoptosis against Hepatocellular carcinoma cells (Ahmed et al, 2018). Another study reported that caspase activity was increase on a time dependent manner against HL- 60 cells (Malik, 2009)**Figure 8. Caspase activity of decoction, negative control and positive control.**

Effect of WSD on MMP2, STAT3, JAK2, MMP9 ,Bcl-XL, Ccnd1, VEGF and p53

The effect of MMP2 and MMP9 in apoptosis and cell death

MMP-2 has a role in promoting tumour angiogenesis, which is initially supported by interleukin 8 (IL-8) (Quintero-Fabián et al., 2019). In this study both MMP-2 and MMP-9 were down regulated with negative fold changes of -14.7 ± 0.9 and -5.4 ± 1.1 respectively. This indicates that the WSD treatment effectively retards the angiogenesis and metastasis in the cancer. It was reported that *Withania somnifera* has great potential in down regulating MMP-2 and MMP-9, leading to the inhibition of cell migration in neuroblastoma (Kataria et al., 2013).

The effect of Cyclin D1 in cell cycle arrest

Cyclin D1 shows a negative fold change of -12.5 ± 0.7 where the expression is being downregulated. Considerably *Withania somnifera* has been reported in previous literature as an agent to reduce the Cyclin D1 level in human prostate cancer (Balakrishnan et al., 2017) cells and human neuroblastomas (Kataria et al., 2013).

The effect of VEGF in reducing angiogenesis

Withaferin A, the active component of *Withania somnifera* has been identified in a previous research study as a compound that reduces the expression of VEGF functioning as an anti-VEGF agent (Saha et al., 2013). In this study, VEGF has exhibited a negative fold change of 7.6, indicating the down-regulation of the expression. This indicates the effectiveness of the WSD in retarding angiogenesis and proliferation of the tumour.

The effect of STAT3 and JAK2 in apoptosis

According to the experimental results given in the Table 7, the expression of both JAK2 and STAT3 were down regulated in 13.4 ± 1.4 and 10.7 ± 1.2 folds respectively which exhibited significantly higher fold changes, collectively this study demonstrated that the WSD decoction promotes apoptosis involving the JAK2/STAT3 gene expression pathway, leading to the death of the tumour cells. The effect of *Withania somnifera* on STAT3 and JAK2 pathways was described in related to renal carcinoma (Um et al., 2012) and breast cancers (J. Lee et al., 2010) in the literature.

The effect of Bcl-XL in apoptosis

As evident by, the differential expression of Bcl-XL dropped in around 14 folds (-14.4 ± 1.1), indicating the effectiveness of the decoction in promoting apoptosis. The effect of the *Withania somnifera* on reducing the expression level of Bcl-XL in human Neuroblastomas was reported in a previous study (Kataria et al., 2013).

p53

the analysis demonstrate p53 relative expression has increased significantly (11.7 ± 1.3 folds). This can be an overall effect generated from the DNA damage due to the upregulation of p53 and STAT3 down regulation. The upregulation of p53 promoted by *Withania somnifera* has also been previously reported (Munagala et al., 2011).

Table 7
Names and short names of the genes with their fold change expressed as mean $\pm$ S.D of three independent experiments.

Name of the gene	Short name of the gene	Fold change
Matrix Metallopeptidase 2	MMP-2	-14.7 $\pm$ 0.9
Signal transducer and activator of transcription 3	STAT3	-10.7 $\pm$ 1.2
Janus kinase 2	JAK2	-13.4 $\pm$ 1.4
Matrix Metallopeptidase 9	MMP-9	-5.4 $\pm$ 1.1
B-cell lymphoma-extra large	Bcl-XL	-14.4 $\pm$ 1.1
Cyclin D1	Ccnd1	-12.5 $\pm$ 0.7
Vascular endothelial growth factor	VEGF	-7.6 $\pm$ 1.3
Tumor protein 53	p53	11.7 $\pm$ 1.3
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH	-1.5 $\pm$ 0.1

4.0 Conclusions

Poly-herbal decoction (PHD) is enriched with valuable anti-oxidative and anticancer properties which were differently and unevenly contributed by its individual components. Each component might be contributing in a balanced manner in such a way as to compensate any toxicity that might result from hyperactivities. As PHD is prepared by adding several herbs in a single mixture, each acts as a protective agent by reducing the effects of other components making it is tolerable and harmless to the normal cells and tissues. In summary, the poly-herbal decoction and all the individual plant decoctions that was used in this study have shown antioxidant and anticancer properties implying the medicinal value of traditional plant decoctions used in Sri Lanka. In addition, current study provides a scientific validation for the traditional use of this poly-herbal decoction in anti-cancer treatments. The findings of this study can be used as an initiation for performing more expanded cancer research using traditional plant decoctions towards a remedy for colorectal cancer.

Abbreviations

DPPH- 2,2-diphenyl-1-picrylhydrazyl

FRAP- Ferric Reducing Antioxidant Power

MTT- 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

RT-qPCR- Real-time quantitative Polymerase Chain Reaction

LDH- Lactate dehydrogenase

PVPP- Polyvinyl polypyrrolidone

MEM- Modified Eagle's medium

FBS- Fetal bovine serum

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Conflicts of Interest

The authors declare that they have no conflict of interest

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Author Contribution

N. U and N. N wrote the manuscript. M.D.M.F, B. G. D. N. K. D, P.S and H.K reviewed and edited the manuscript.

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Data Availability

The dataset supporting the conclusions of this article are included within the article.

References

1. Abutaha N. In vitro antiproliferative activity of partially purified *Withania somnifera* fruit extract on different cancer cell lines. *J BUON*. 2015;20(2):625–30.
2. Ahmed W, Mofed D, Zekri AR, El-Sayed N, Rahouma M, Sabet S. Antioxidant activity and apoptotic induction as mechanisms of action of *Withania somnifera* (Ashwagandha) against a hepatocellular carcinoma cell line. *J Int Med Res*. 2018;46(4):1358–69.
3. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *J Taibah Univ Sci*. 2015;9(4):449–54.
4. Balakrishnan AS, Nathan AA, Kumar M, Ramamoorthy S, Mothilal SK. *Withania somnifera* targets interleukin-8 and cyclooxygenase-2 in human prostate cancer progression. *Prostate Int*. 2017;5(2):75–83.
5. Bustin S, Huggett J. qPCR primer design revisited. *Biomol Detect quantification*. 2017;14:19–28.
6. Dhar P, Bajpai PK, Tayade AB, Chaurasia OP, Srivastava RB, Singh SB. Chemical composition and antioxidant capacities of phytococktail extracts from trans-Himalayan cold desert. *BMC Complement Altern Med*. 2013;13:1–5.
7. Dieffenbach CW, Lowe TM, Dveksler GS. General concepts for PCR primer design. *PCR methods appl*. 1993;3(3):S30–7.
8. Don N, Wageesha A, Soysa P, Atthanayake K, Choudhary MI, Ekanayake M. A traditional poly herbal medicine Le Pana Guliya induces apoptosis in HepG^a sub 2^a and HeLa cells but not in CC1 cells: an in vitro assessment. *BMC Chem*. 2017;11(1):1.
9. El Jemli M, Kamal R, Marmouzi I, Zerrouki A, Cherrah Y, Alaoui K. Radical-scavenging activity and ferric reducing ability of *Juniperus thurifera* (L.), *J. oxycedrus* (L.), *J. phoenicea* (L.) and *Tetraclinis articulata* (L). *Adv Pharmacol Pharm Sci*. 2016;2016(1):6392656.
10. Fernando D, Wijesundera R, Soysa P, De Silva D, Nanayakkara C. Strong radical scavenging macrofungi from the dry zone forest reserves in Sri Lanka. *Front Environ Microbiol*. 2015;1(2):32–8.
11. Fernando DM, Wijesundera RL, Soysa P, de Silva D, Nanayakkara CM. Antioxidant potential, in vitro cytotoxicity and apoptotic effect induced by crude organic extract of *Anthracyllum lateritium* against RD sarcoma cells. *BMC Complement Altern Med*. 2015;15:1–9.
12. Fernando CD, Soysa P. Extraction Kinetics of phytochemicals and antioxidant activity during black tea (*Camellia sinensis* L.) brewing. *Nutr J*. 2015;14:1–7.
13. Franken NA, Rodermond HM, Stap J, Haveman J, Van Bree C. Clonogenic assay of cells in vitro. *Nat Protoc*. 2006;1(5):2315–9.
14. Halder B, Singh S, Thakur SS. *Withania somnifera* root extract has potent cytotoxic effect against human malignant melanoma cells. *PLoS ONE*. 2015;10(9):e0137498.
15. Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: a simple test-tube assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem*. 1987;165(1):215–9.
16. Jafarian A, Zolfaghari B, Shirani K. Cytotoxicity of different extracts of arial parts of *Ziziphus spina-christi* on Hela and MDA-MB-468 tumor cells. *Adv biomedical Res*. 2014;3(1):38.
17. Jayasinghe S, Bandara BR, Wickramasinghe A, Karunaratne DN, Wijesundara DS, Karunaratne V. The importance of harnessing the rich diversity of Sri Lankan flora for their medicinal value. *Ceylon J Sci*. 2017;46(4).

18. Jiang D, Rasul A, Batool R, Sarfraz I, Hussain G, Mateen Tahir M, Qin T, Selamoglu Z, Ali M, Li J, Li X. Potential anticancer properties and mechanisms of action of formononetin. *Biomed Res Int*. 2019;2019(1):5854315.
19. Kaur C, Kapoor HC. Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int J food Sci Technol*. 2002;37(2):153–61.
20. Kataria H, Wadhwa R, Kaul SC, Kaur G. *Withania somnifera* water extract as a potential candidate for differentiation based therapy of human neuroblastomas. *PLoS ONE*. 2013;8(1):e55316.
21. Kintzios SE. Terrestrial plant-derived anticancer agents and plant species used in anticancer research. *CRC Crit Rev Plant Sci*. 2006;25(2):79–113.
22. Kizil M, Kizil G, Yavuz M, Ceken B. Protective activity of ethanol extract of three *Achillea* species against lipid peroxidation, protein oxidation and DNA damage in vitro. *Acta Aliment*. 2010;39(4):457–70.
23. Kuruppu AI, Paranagama P, Goonasekara CL. Medicinal plants commonly used against cancer in traditional medicine formulae in Sri Lanka. *Saudi Pharm J*. 2019;27(4):565–73.
24. Kumar A, Sharma PR, Mondhe DM. Potential anticancer role of colchicine-based derivatives: an overview. *Anticancer Drugs*. 2017;28(3):250–62.
25. Lee J, Hahm ER, Singh SV. Withaferin A inhibits activation of signal transducer and activator of transcription 3 in human breast cancer cells. *Carcinogenesis*. 2010;31(11):1991–8.
26. Lindamulage IK, Soysa P. Evaluation of anticancer properties of a decoction containing *Adenanthera pavonina* L. and *Thespesia populnea* L. *BMC Complement Altern Med*. 2016;16:1–8.
27. Luo M, Liu X, Zu Y, Fu Y, Zhang S, Yao L, Efferth T. *Cajanol*, a novel anticancer agent from *Pigeonpea* [*Cajanus cajan* (L.) Millsp.] roots, induces apoptosis in human breast cancer cells through a ROS-mediated mitochondrial pathway. *Chemico-Biol Interact*. 2010;188(1):151–60.
28. Malik F, Kumar A, Bhushan S, Mondhe DM, Pal HC, Sharma R, Khajuria A, Singh S, Singh G, Saxena AK, Suri KA. Immune modulation and apoptosis induction: Two sides of antitumoural activity of a standardised herbal formulation of *Withania somnifera*. *Eur J Cancer*. 2009;45(8):1494–509.
29. Martínez G, Giuliani A, Leon OS, Perez G, Núñez Selles AJ. Effect of *Mangifera indica* L. extract (QF808) on protein and hepatic microsome peroxidation. *Phytother Res*. 2001;15(7):581–5.
30. Muflihah YM, Gollavelli G, Ling YC. Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12 Indonesian indigenous herbs. *Antioxidants*. 2021;10(10):1530.
31. Munagala R, Kausar H, Munjal C, Gupta RC. Withaferin A induces p53-dependent apoptosis by repression of HPV oncogenes and upregulation of tumor suppressor proteins in human cervical cancer cells. *Carcinogenesis*. 2011;32(11):1697–705.
32. Muniyandi K, George E, Sathyanarayanan S, George BP, Abrahamse H, Thamburaj S, Thangaraj P. Phenolics, tannins, flavonoids and anthocyanins contents influenced antioxidant and anticancer activities of *Rubus* fruits from Western Ghats, India. *Food Sci Hum Wellness*. 2019;8(1):73–81.
33. Nurgali K, Jagoe RT, Abalo R. Adverse effects of cancer chemotherapy: Anything new to improve tolerance and reduce sequelae? *Front Pharmacol*. 2018;9:245.
34. Partridge AH, Burstein HJ, Winer EP. Side effects of chemotherapy and combined chemohormonal therapy in women with early-stage breast cancer. *JNCI Monogr*. 2001;2001(30):135–42.
35. Perera D, Soysa P, Wijeratne S. Polyphenols contribute to the antioxidant and antiproliferative activity of *Phyllanthus debilis* plant in-vitro. *BMC Complement Altern Med*. 2016;16:1–9.
36. Perera MG, Soysa SS, Abeyunga DT, Ramesh R. Antioxidant and cytotoxic properties of three traditional decoctions used for the treatment of cancer in Sri Lanka. *Pharmacognosy Magazine*. 2008;4(15):172.
37. Poorna CA, Resmi MS, Soniya EV. In vitro antioxidant analysis and the DNA damage protective activity of Leaf extract of the *Excoecaria agallocha* Linn Mangrove plant. *Agricultural Chemistry*. Volume 20. New York: InTech; 2013 Feb. pp. 155–66.
38. Pulipati S, Babu PS, Naveena U, Parveen SR, Nausheen SS, Sai MT. Determination of total phenolic, tannin, flavonoid contents and evaluation of antioxidant property of *Amaranthus tricolor* (L). *Int J pharmacognosy phytochemical Res*. 2017;9(6):814–9.
39. Quintero-Fabián S, Arreola R, Becerril-Villanueva E, Torres-Romero JC, Arana-Argáez V, Lara-Riegos J, Ramírez-Camacho MA, Alvarez-Sánchez ME. Role of matrix metalloproteinases in angiogenesis and cancer. *Front Oncol*. 2019;9:1370.
40. Rasool M, Varalakshmi P. Suppressive effect of *Withania somnifera* root powder on experimental gouty arthritis: an in vivo and in vitro study. *Chemico-Biol Interact*. 2006;164(3):174–80.
41. Razak NA, Abu N, Ho WY, Zambari NR, Tan SW, Alitheen NB, Long K, Yeap SK. Cytotoxicity of eupatorin in MCF-7 and MDA-MB-231 human breast cancer cells via cell cycle arrest, anti-angiogenesis and induction of apoptosis. *Sci Rep*. 2019;9(1):1514.
42. Saha S, Islam MK, Shilpi JA, Hasan S. Inhibition of VEGF: a novel mechanism to control angiogenesis by *Withania somnifera*'s key metabolite Withaferin A. *Silico Pharmacol*. 2013;1:1–9.

43. Silva IK, Soysa P. Evaluation of phytochemical composition and antioxidant capacity of a decoction containing *Adenanthera pavonina* L. and *Thespesia populnea* L. *Pharmacognosy Magazine*. 2011;7(27):193.
44. Sumantran VN, Boddul S, Koppikar SJ, Dalvi M, Wele A, Gaire V, Wagh UV. Differential growth inhibitory effects of *W. somnifera* root and *E. officinalis* fruits on CHO cells. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*. 2007;21(5):496–9.
45. Um HJ, Min KJ, Kim DE, Kwon TK. Withaferin A inhibits JAK/STAT3 signaling and induces apoptosis of human renal carcinoma Caki cells. *Biochem Biophys Res Commun*. 2012;427(1):24–9.
46. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc*. 2006;1(3):1112–6.
47. Wang WH, Hsuan KY, Chu LY, Lee CY, Tyan YC, Chen ZS, Tsai WC. Anticancer effects of *Salvia miltiorrhiza* alcohol extract on oral squamous carcinoma cells. *Evidence-Based Complement Altern Med*. 2017;2017(1):5364010.
48. Weragoda PB. The traditional system on medicine in Sri Lanka. *J Ethnopharmacol*. 1980;2(1):71–3.
49. Yin SY, Wei WC, Jian FY, Yang NS. Therapeutic applications of herbal medicines for cancer patients. *Evidence-Based Complement Altern Med*. 2013;2013(1):302426.

Figures

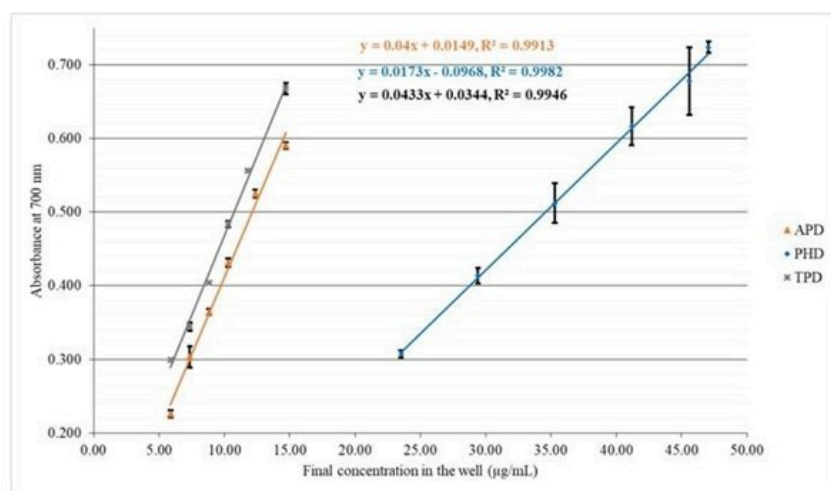


Figure 1
Dose responsive curves of ferric ion reducing power assay for PHD and two individual decoctions; APD and TPD. Each value is expressed as mean $\pm$ standard deviation (n=3)]

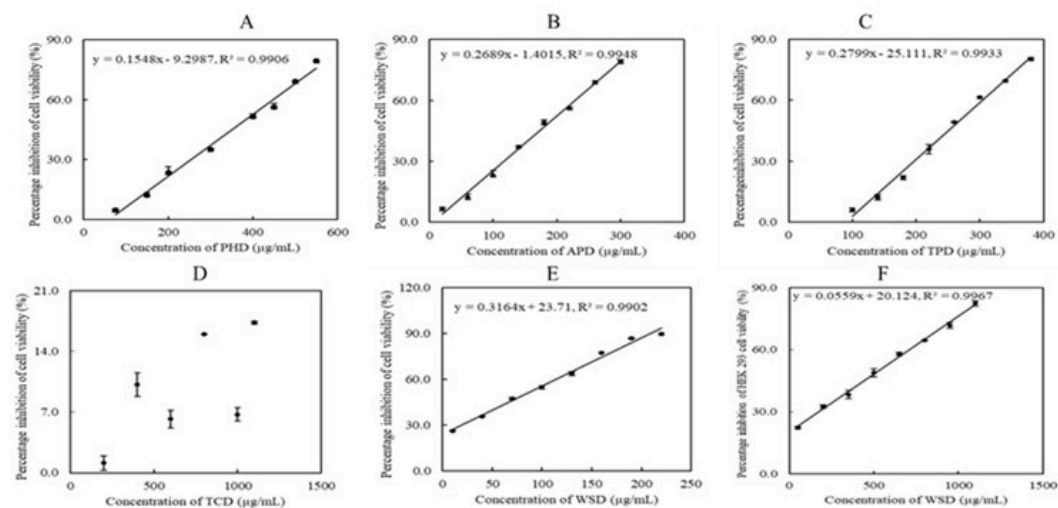


Figure 2

The cytotoxicity effects of polyherbal decoction and individual component decoctions. A) PHD, B) APD, C) TPD, D) TCD, E) WSD, F) WSD HEK293 cells.

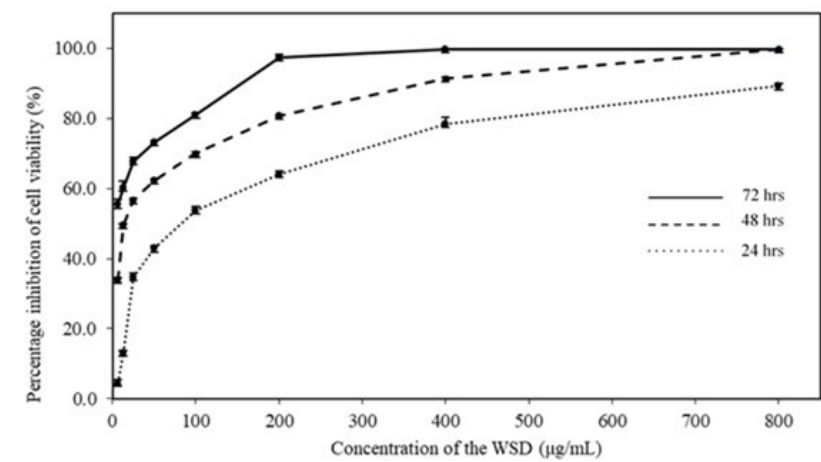


Figure 3

Cytotoxicity effects of WSD at 24 h, 48 h and 72 h against HCT 116 cells.

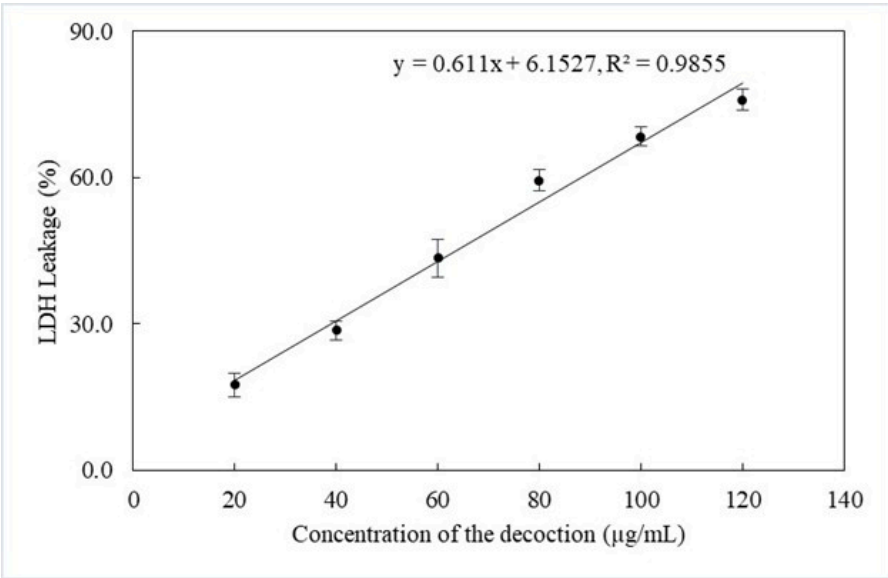


Figure 4

Dose dependent curve of LDH leakage.

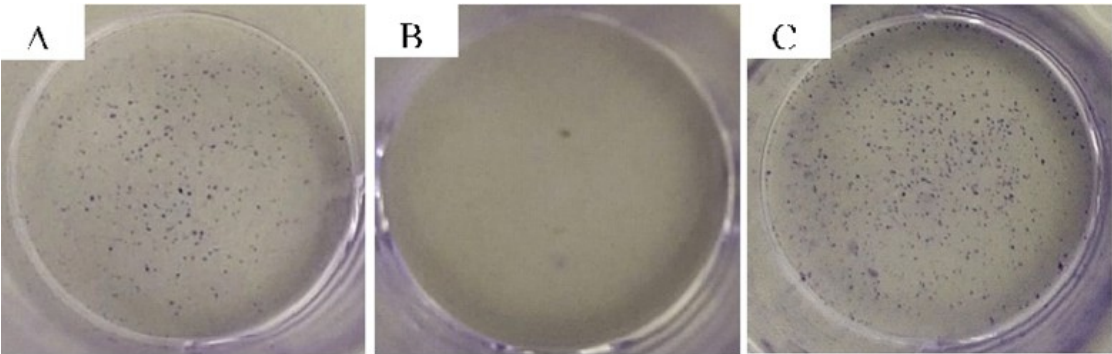


Figure 5

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Colony formation of HCT 116 cells upon treatment and after 7 days. A) WSD treated cells, B) Positive control, C) Negative control.

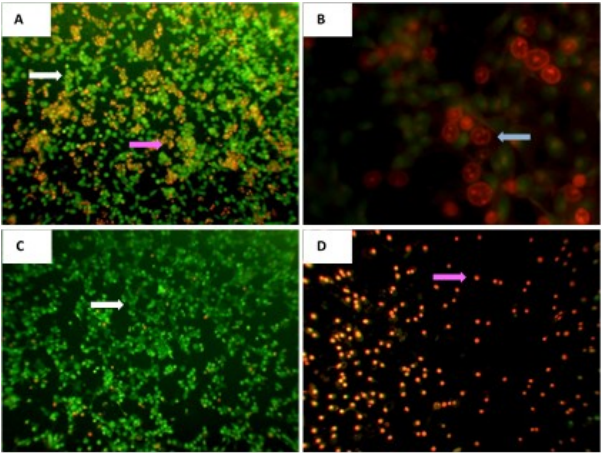


Figure 6

A) Emits orange-red fluorescence upon Ethidium bromide intercalation with DNA in HCT 116 cells; indicating late apoptotic cells and dead cells, B) Fragmented nuclei C) Negative control (untreated cells) D) positive control.

1 2 3 4 5 6 7 8

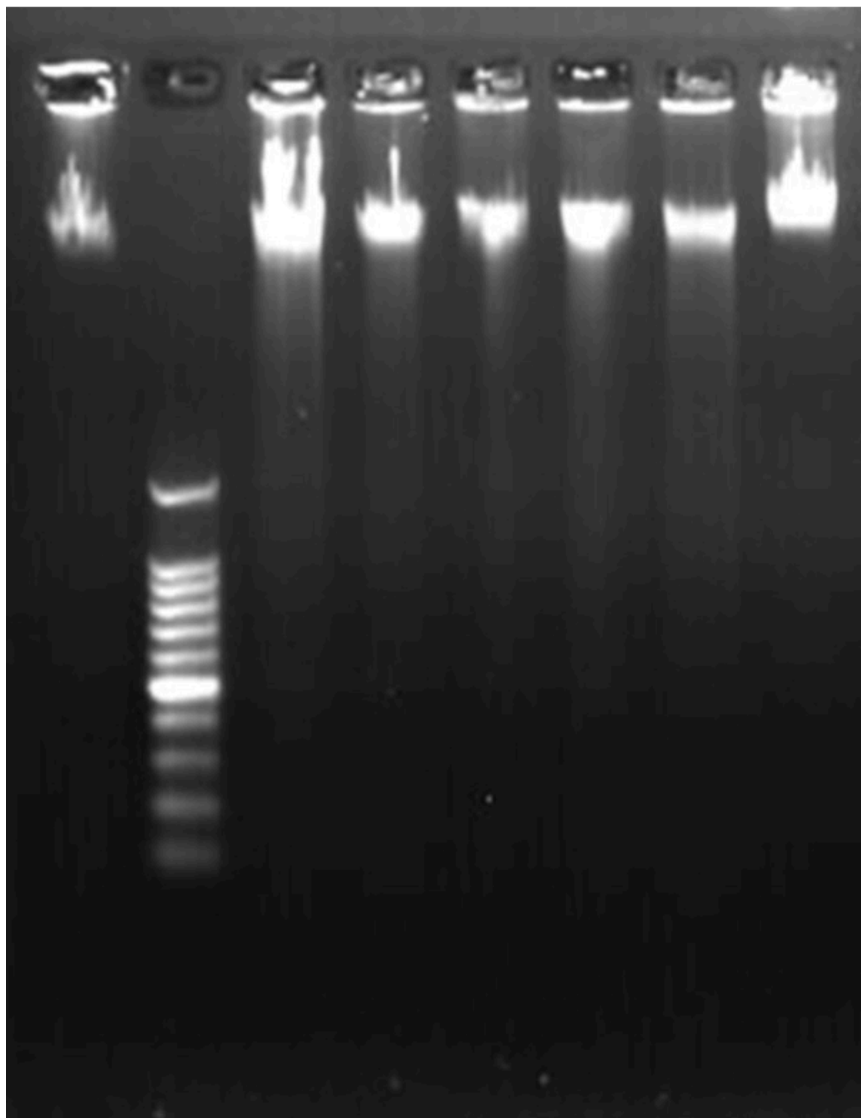


Figure 7

Analysis of genomic DNA fragmentation. DNA fragmentation was assessed on 2% agarose gel electrophoresis and ethidium bromide staining. Lane 1: Negative control (1), Lane 2: 100bp ladder, Lane 3: Positive control (1), Lane 4: Positive control (2), Lane 5: WSD treated cells (24 hrs)-(1), Lane 6: WSD treated cells (24 hrs)-(2), Lane 7: WSD treated cells (24 hrs)-(3), Lane 8: Negative control (2)

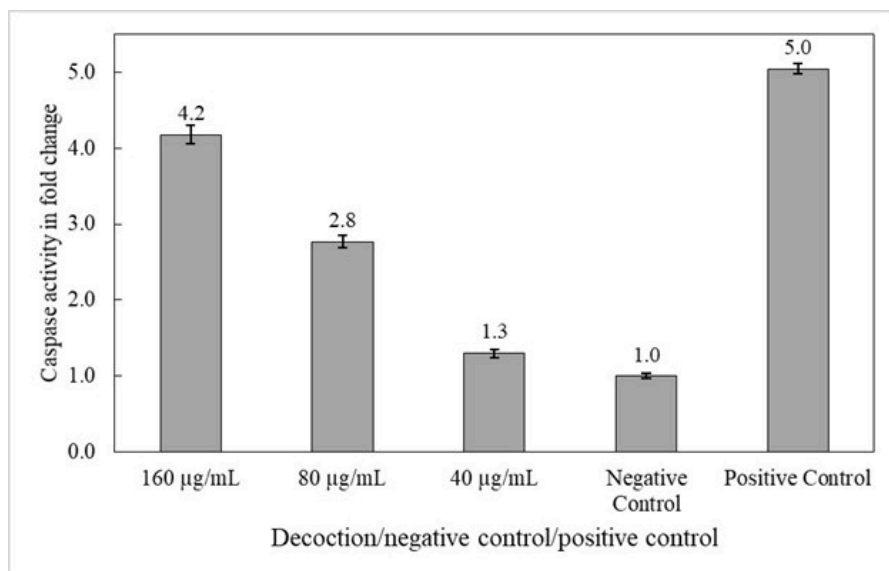


Figure 8

Caspase activity of decoction, negative control and positive control.

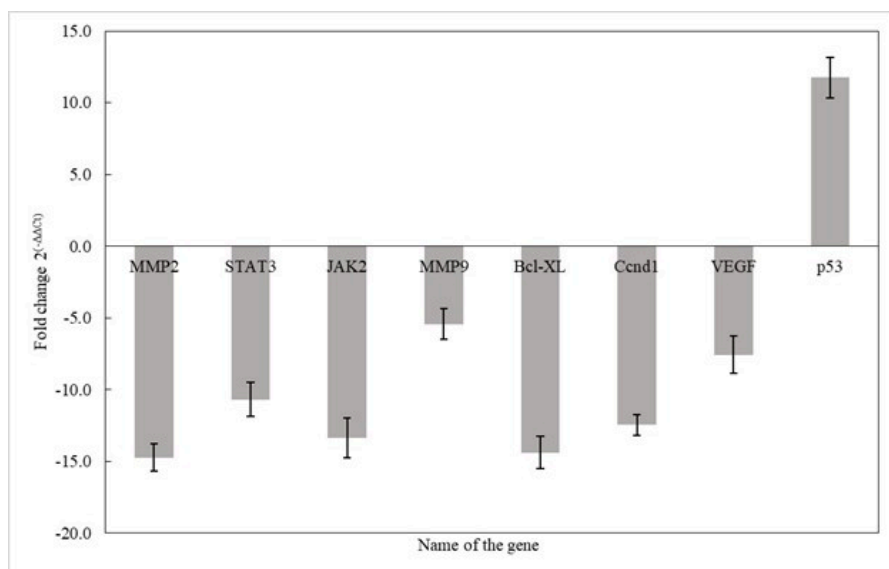


Figure 9

Expression of fold change in MMP2, STAT3, JAK2, MMP9, Bcl-XL, Ccnd1, VEGF and p53.

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