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RESEARCH ARTICLE

Effect Of Different Polyphenolic Fractions Of *Lycium barbarum* On The Expression Of DNMT1 And HDAC Gene.

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ABSTRACT

For a long time, the fruit of the Rubus species has been utilised as a meal in India. Polysaccharides, flavonoids, saponins, and alkaloids were isolated from the fruits and their contents determined in order to assess their active components. The antioxidant and anticancer effects of the four primary ingredients were examined *in vitro* against Hela cells (Cervical Cancer Cell Line). Total flavonoids were found to have significant antioxidant action. The highest phenolic content was observed in methanolic extract of *Lycium barbarum and Fragaria ananassa* (Strawberry)*;* 35.727937mg/gm and 35.3007307 mg/gm respectively. The MTT assay was used to determine cytotoxicity in HeLa cells, and the IC_{50} was reported in different fractions. The retention time was found to be maximum for Quercetin i.e., 18. 656. This was followed by HPLC analysis of fraction LBM70 for which reference gene for internal control in this study was β-Actin. Hence our study is in accordance with the previous research which clearly reveals that HDAC1 overexpression has been connected to a range of cancer prognoses. The results of this study reveal that treating Hela cells with a specific fraction of *Lycium barbarum* causes overexpression of the DNMT1 and HDAC genes.

Keywords: Flavanoids, Saponinns, DNMT1, HDAC, HPLC

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INTRODUCTION

Traditional knowledge of wild plant species with food and medicinal value is undoubtedly an important component of folk culture, and it plays a significant role in the revival of traditions [1]. Wild foods are quite popular in rural populations all over the world due to its cultural significance, effectiveness, and lack of availability of modern health services. Wild foods are not only beneficial to the environment, but they are also an important source of traditional ecology [2]. The main focus of investigators working in the field of functional foods and nutraceuticals has been the detection of health-beneficial secondary metabolites that act as antioxidants [3]. Fruit berries, in particular, have received a lot of attention since they are one of the best dietary sources of bioactive compounds (BAC) [4–7].

Berries, particularly those from the Rosaceae (strawberry, raspberry, blackberry) and Ericaceae (blueberry, cranberry) families, are among the finest sources of bioactive chemicals in the diet (BAC).

Phenolic components (phenolic acids, flavonoids such as anthocyanins and flavanols, and tannins) and ascorbic acid make up the majority of the bioactive compounds in berries. These substances are responsible for a variety of health benefits of berries, including the prevention of inflammatory disorders, cardiovascular diseases, and protective effects that reduce the risk of many cancers, either separately or in combination.

Cancer and Epigenetics

The heritable changes occurring in gene expression of DNA without changing its sequence itself is termed as epigenetics. There are three important types of Epigenetic mechanisms; DNA Methylation, Histone Modifications and RNA interference (RNAi) [8]. Among these three the most important Epigenetic Mechanisms is DNA methylation, which occurs primarily at CpG dinucleotide cytosine residues [9].

In case of mammals, there are three types of DNMTs which maintain the methylation pattern; DNMT1, DNMT3A and DNMT3B. DNMT1 is a majorly required for cell division, whereas DNMT3A and DNMT3B are essential for de novo DNA methylation during development [10].

Hypermethylation can also results in activation of certain oncogenes, which helps in initiation of tumorigenesis [11]. Frequent methylation patterns are seen in almost all neoplasms, which shows its importance as a molecular marker in cancer prevention, prognosis, and therapeutic approaches.

DNMTs

DNMTs are a very significant class of epigenetic regulatory enzymes. They catalyze DNA methylation, a normal endogenous modification of eukaryotic DNA, which is essential for life [12]. DNMT alterations have been generally observed by many researchers and scientists in different classes of tumors, which indicate that they often attend to the occurrence and growth of tumors. Therefore, analysis of DNMT abnormalities in tumors may discover a way to restore DNA stability, which may direct to new methods to treat tumors

Phenolic Compounds

Phenolic compounds are commonly known as polyphenols, which are present in all plants and, in the diet. There are 8,000 phenolic structures and identified their structure from being simple molecules (e.g., phenolic acids with a C6 ring structure) to being highly polymerized compounds (tannins).

The powerful activity of antioxidant in flavonoids may suggest a protective role for these compounds in carcinogenesis. Flavonoids act as antioxidant properties to interfere with carcinogenesis, such as protecting DNA from oxidative damage, deactivating carcinogens, and inhibiting the expression of mutated genes and the activity of enzymes that promote carcinogenesis, as well as promoting detoxification of xenobiotics [13, 14].

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MATERIALS AND METHODS

Method of extraction

The fruits/seeds were collected, rinsed with running tap water, surface sterilized with 0.1 percent mercuric chloride, and air dried in the shade for two to four weeks. The hot-extraction method, i.e., Soxhlet extraction, was used to extract the air-dried powder (5 g) using solvents of varied polarity, such as hexane, petroleum ether, chloroform, methanol, and water.

Phenolic compounds extraction from crude extracts

Samples were acidified to 2 pH with 2 M HCl prior to extraction process. Liquid -Liquid extraction of phenolic compounds was done and the fractions of similar compositions were collected together to give a total of ten pooled fractionated groups [A (I)–A (X)].

Group A (I) system of 100% chloroform.
Group A (II) system of 10% methanol: 90% chloroform.
Group A (III) system of 20% methanol: 80% chloroform.
Group A (IV) System of 30% methanol: 70 %chloroform.
Group A (V) System of 40% methanol: 60 % chloroform.
Group A (VI) system of 50% methanol: 50% chloroform.
Group A (VII) System of 60% methanol: 40% chloroform.
Group A (VIII) System of 70% methanol: 30% chloroform.
Group A (IX) System of 80% methanol: 20% chloroform.
Group A (X) System of 90% methanol: 10% chloroform.

Initial fraction of chloroform was discarded. TPC of all extracts were estimated and only the fraction of having maximum TPC was taken for further purification. Only the fractions of 70 to 80 percent of methanol were having the good phenolic content. Only 70 percent fraction of all samples having maximum phenolic yield were preceded for analysis.

TPC estimation of purified extracts

TPC was estimated according to the previous protocol followed for crude samples**.** The cytotoxicity of the materials was assessed using a modified MTT colorimetric test technique.

Determination of IC_{50} value

The IC_{50} value of cellular growth inhibition was calculated using the percent inhibitory activity of the tested material (concentration of the tested sample to inhibit 50 percent growth of the cells).

$$\% \text{ inhibition} = (1 - [A_1/A_0] \times 100)$$

where;
A_0 is the absorbance of the control and
A_1 is the absorbance of the extracts.

RESULTS

The extraction yield of various plant samples including RA: *Rubus ellipticus*(Himalayan raspberry), *LB: Lycium barbarum, FA: Fragaria ananassa*(Strawberry) ,*VB: Viburnum mullaha* (Indian cranberry) , *VC: Vaccinium corymbosum*(Blueberry*)* dissolved in different solvents was calculated.

The Initial weight of plant sample was 5 gm/20 ml i.e: 250 mg/ml. In addition to this the final dry weight was also calculated.

The final dry weight was maximum in REH Sample and extraction yield was maximum in FAH sample. The results have been shown in Table 1.

H: Hexane extract, P: Petroleum ether extract, C: Chloroform extract, M: Methanolic extract, W: Water extract

$$\text{Extraction yield/yield \%} = (W_0/W_t) *100$$

W_0 = weight of initial fruit/seed sample
W_t = weight of dried extract after Soxhlet extraction

This was followed by Phytochemical analysis of Plant samples.

Various phytochemical test was carried out for the analysis of Alkaloid, Flavonoid, Phenol, Glycosides, Tannins, Carbohydrates (Molisch, Fehling and Benedict test), saponins and steroids.

The results of phytochemical analysis have been shown in Table 2.

TPC (Total Phenol Content)

This was followed by calculation of total phenol content. The concentration was calculated in mg/ml as well as in mg/gm. TPC was found to maximum in LBM as shown in table 3. Based upon the graph of absorbance at 765 nm and gallic acid concentration was plotted and it was found to be a straight line, this shows that absorbance is directly proportional to increase in gallic acid concentration (Figure: 1). TPC Of Different Fraction have been shown in Figure 2. The Maximum was found to be in LBM as shown in table 3.

Cell viability assay was carried out before the treatment of Hela cell line with the fractions to determine IC_{50} value. The percentage cell viability is shown in Table 6.

IC_{50} (Inhibitory concentration) were calculated. This is the value at which 50 percent of cells get inhibited after the treatment with test compound.

The percentage cell viability of different concentration of REW70F3 along with the control is shown in figure 3.

The cell viability was calculated after the 48-h incubation period. The percent cell viability of at different concentration along with control of different fractions is shown in figures (4-6).

The morphological studies of the Hela cell were also carried out after treatment with the selected fraction and the IC_{50} value. The formation of apoptotic bodies indicated the apoptosis or programmed cell death taking place in Hela cells after treatment with selected fractions (REW70F3, REW70F4, LBM70F1, LBW 70F1, LBW70F3). The results of the morphological studies have been shown in Figures (7-12).

Samples were screened on the basis of their phenolic content percentage and TPC. The following samples were selected for HPLC purification. The HPLC analysis of selected fraction was carried out and in comparison, to the standards as shown in Figure 14.

The retention was found to be maximum for Quercetin i.e., 18. 656. This was followed by HPLC analysis of fraction LBM70 as shown in Figure 15.

Data were analyzed by DAGE (Data analysis Gene expression software)

All the primers set were tested as single plex real time PCR reaction. Optimization of annealing temperature and primers concentration were done and confirmed that primers set performed well as singleplex. Standard curve assay was performed to assess assay sensitivity, efficiency, reproducibility of the assay at various concentration of cDNA. Initial concentration (N_0) was 150 ng five 1:10 serial dilutions were subsequently generated resulting in range of DNA concentration. DNA dilution per reaction was 150 ng, 15 ng, 1.5 ng, 0.15 ng, 0.015 ng. Normal cell line was selected as biological control in standard curve assay.

Amplification curve of DNMT and HDAC gene shown in figure 16.

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Table 1: Extraction yield

Sample	Final dry weight	Extraction yield (%)
REH	1.924	38.48
REP	2.893	57.86
REC	2.4	48
REM	1.29	25.8
REW	1.891	37.82
LBH	1.7	34
LBP	3.78	75.6
LBC	2.90	58
LBM	2.20	44
LBW	3.97	79.4
FAH	3.98	79.6
FAP	3.21	64.2
FAC	2.87	57.4
FAM	2.08	41.6
FAW	2.92	58.4
VMH	2.18	43.6
VMP	2.74	54.8
VMC	2.10	42
VMM	2.432	48.64
VMW	2.83	56.6
VCH	3.9	78
VCP	2.7	54
VCC	2.1	42
VCM	3.9	78
VCW	2.2	44

Table 2: Phytochemical analysis of Plant samples:

Sample	Alkaloid			Flavonoid	Phenol	Glycosides	Tannins	Carbohydrate			Saponins	Steroids
	Mayer's test	Dragendorff's test	Wagner test					Molisch	Fehling's	Benedicts		
REH	-	-	-	+	+	-	+	++	+	-	-	-
REP	-	-	-	+	+	-	+	++	+	-	-	-
REC	+	+	+	++	+	+	+	++	+	-	-	-
REM	++	+	+	+++	++	+	++	+++	++	-	-	-
REW	++	++	+	+++	+++	+	++	+++	++	-	-	-
LBH	+	-	-	-	+	-	+	-	-	-	-	-
LBP	-	-	-	-	-	-	-	-	-	-	-	-
LBC	+	++	-	+	++	-	+	-	-	-	-	-
LBM	++	++	+	++	+++	-	++	-	-	-	-	-
LBW	++	+	+	-	+++	-	+	-	-	-	+	-
FAH	-	-	-	-	+	-	+	+	+	-	+	-
FAP	-	-	-	-	+	-	+	+	+	+	+	+
FAC	++	+	+	+	+	+	+	++	+	+	++	++
FAM	+++	++	+++	+	+++	++	++	+++	+++	++	+++	+++
FAW	++	++	+++	+	+++	++	++	++	++	+++	+++	++
VMH	-	-	-	-	-	+	-	-	-	-	-	-
VMP	-	-	-	+	-	+	-	-	-	-	-	+
VMC	+	-	-	++	-	++	+	+	+	-	++	+
VMM	+	+	-	++	+	+++	+	++	++	++	++	++
VMW	+	+	-	+++	+	+++	+	+++	+++	++	+++	+++
VCH	++	+	+	+	-	++	-	+	-	-	-	+
VCP	++	+	+	+	-	++	-	++	-	-	-	+
VCC	+	+	+	+	+	+	+	++	+	+	-	+
VCM	+++	++	++	++	++	+++	+	++	+	++	+	+++
VCW	+++	+++	+++	++	++	+++	+	+++	++	+++	+	+++

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Abbreviations: RA: *Rubus ellipticus* (Himalayan raspberry), *LB: Lycium barbarum, FA: Fragaria ananassa* (Strawberry), *VB: Viburnum mullaha*(Indian cranberry) , *VC: Vaccinium corymbosum* (Blueberry)
H: Hexane extract, P: Petroleum ether extract, C: Chloroform extract, M: Methanolic extract, W: Water extract
+++ abundant, ++ moderately presence, + present, – absence

Table 3: Total Phenolic Content of different fractions

Std. & Sample	Distilled Water (ml)	Gallic acid (ml)	Sample (µl)	FC reagent (ml)		Na_2CO_3 (ml)		OD at 765 nm	Conc. (mg/ml)	Conc. (mg/g)
Blank	0.5	0	-	2.5		**2**		0		
Standard1	0.495	0.005	-	2.5		**2**		0.059	0.01	
Standard2	0.49	0.01	-	2.5		**2**		0.123	0.02	
Standard3	0.485	0.015	-	2.5		**2**		0.306	0.03	
Standard4	0.48	0.02	-	2.5		**2**		0.405	0.04	
Standard5	0.475	0.025	-	2.5		**2**		0.511	0.05	
REH	0.45	-	50	2.5		**2**		0.068	0.01205734	4.82293423
REP	0.45	-	50	2.5		**2**		0.0873333	0.01368746	5.47498595
REC	0.45	-	50	2.5		**2**		0.086	0.01357504	5.43001686
REM	0.45	-	50	2.5		**2**		0.1803333	0.02152895	8.61157954
REW	0.45	-	50	2.5		**2**		0.8816667	0.08066329	32.2653176
LBC	0.45	-	50	2.5		**2**		0.2746667	0.02948286	11.7931422
LBM	0.45	-	50	2.5		**2**		0.9843333	0.08931984	35.727937
LBW	0.45	-	50	2.5		**2**		0.7843333	0.07245644	28.9825745
FAH	0.45	-	50	2.5		**2**		0.0536667	0.01084879	4.33951658
FAP	0.45	-	50	2.5		**2**		0.1043333	0.01512085	6.04834177
FAC	0.45	-	50	2.5		**2**		0.0693333	0.01216976	4.86790332
FAM	0.45	-	50	2.5		**2**		0.9716667	0.08825183	35.3007307
FAW	0.45	-	50	2.5		**2**		0.8256667	0.07594154	30.3766161
VMM	0.45	-	50	2.5		**2**		0.1033333	0.01503654	6.01461495
VCW	0.45	-	50	2.5		**2**		0.0313333	0.00896571	3.58628443
VMC	0.45	-	50	2.5		**2**		0.088	0.01374368	5.49747049
VMM	0.45	-	50	2.5		**2**		0.3773333	0.0381394	15.2557617
VMW	0.45	-	50	2.5		**2**		0.2666667	0.02880832	11.5233277

Incubate for 5 minutes

Incubate for 1.5 hours

Table 4: Response factor for different compounds

Response factor: Area of std./Conc. Of standard		
Gallic acid	2.0401254	7.092
Caffeic acid	1.5600121	7.124
Vanillic acid	6.5901654	9.968
Ellagic acid	0.7902451	12.588
Coumaric acid	19.200212	13.728
Benzoic acid	11.120121	14.852
Phenylacetic acid	16.540215	16.092
Quercetin	0.6220024	18.656

Sample	Fraction	Conc. (µg/ml)	Compound
REW70	**F1**	60.3597	Ellagic acid
	F2	4.47628	Coumaric acid
	F3	143.1674	Benzoic acid
	F4	103.3751	Phenylacetic acid
	F5	446.8639	Quercetin
LBM70	**F1**	53.61685	Vanillic acid
	F2	1.283152	Coumaric acid
LBW70	**F1**	82.84251	Caffeic acid
	F2	110.4171	Ellagic acid
	F3	141.891	Quercetin

Table 5: % Yield of different fraction in Methanol

Sample	Fraction(%methanol)	Initial TPC (mg/g)	TPC (mg/g)	Yield (%)
REW	**70%**	32.26532	29.495	91.41395
LBM	**70%**	35.72794	29.474	82.49567
LBW	**70%**	28.98257	24.494	84.51285
FAM	**70%**	35.30073	30.192	85.52797
FAW	**70%**	30.37662	27.911	91.88318

Table 6: Cell viability %

Sample/dilution	REW70F3	REW70F4	LBM70F1	LBW70F1	LBW70F3
Control	**100**	**100**	**100**	**100**	**100**
100	99.58124	96.48241	98.91122	98.99497	98.7437186
200	97.40369	94.89112	98.0737	98.65997	98.0737018
400	95.8124	93.21608	97.48744	98.24121	97.4874372
600	94.97487	92.46231	97.40369	98.32496	96.39866
800	93.46734	92.29481	96.1474	98.24121	95.561139
1000	93.04858	93.29983	95.8124	97.57119	94.8911223

Table 7: IC_{50} values

Sample	IC50 (µg/ml)
REW70F3	77.05083
REW70F4	136.9973
LBM70F1	162.1468
LBW70F1	416.4964
LBW70F3	124.4327309

Table 8: % Yield of different fraction in Methanol

Sample	Fraction(%methanol)	Initial TPC (mg/g)	TPC (mg/g)	Yield (%)
REW	**70%**	32.26532	29.495	91.41395
LBM	**70%**	35.72794	29.474	82.49567
LBW	**70%**	28.98257	24.494	84.51285
FAM	**70%**	35.30073	30.192	85.52797
FAW	**70%**	30.37662	27.911	91.88318

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Table 9: Retention time of different Phenolic Compound

Phenolic compound	Retention time	Area
Gallic acid	7.092	204.01254
Caffeic acid	7.124	156.00121
Vanillic acid	9.968	659.01654
Ellagic acid	12.588	79.02451
Coumaric acid	13.728	1920.02121
Benzoic acid	14.852	1112.01211
Phenylacetic acid	16.092	1654.02145
Quercetin	18.656	62.20024

Table 10: Amplification plot of Ct values of samples

Sample	B actin (Ct value)	DNMT1(Ct)	DNMT3A(Ct)	DNMT3B(Ct)	HDAC1(Ct)	HDAC2(Ct)	HDAC3(Ct)
Control	18.49	21.3	18.36	22.61	16.5	21.19	18.79
HelaS1	19.12838856	20.18787638	22.91837	24.92982	25.01	25.61	15.73
HelaS2	17.9382221	24.17087149	24.11	23.2827	24.918	25.82	15.26
HelaS3	18.97862727	24.72862727	23.94022686	20.272	25.79	22.1811	16.51
HelaS4	19.29087149	24.98434209	23.01	23.9392	25.69	25.41	16.19
HelaS5	20.45022686	26.43649905	23.11	20.2828	23.01	26.23	16.22

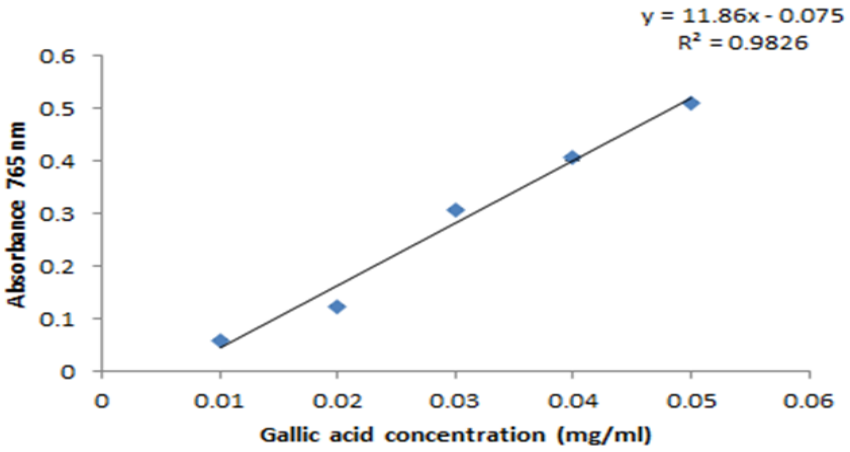

Figure 1: Graph between Absorbance at 765nm and Gallic acid Concentration

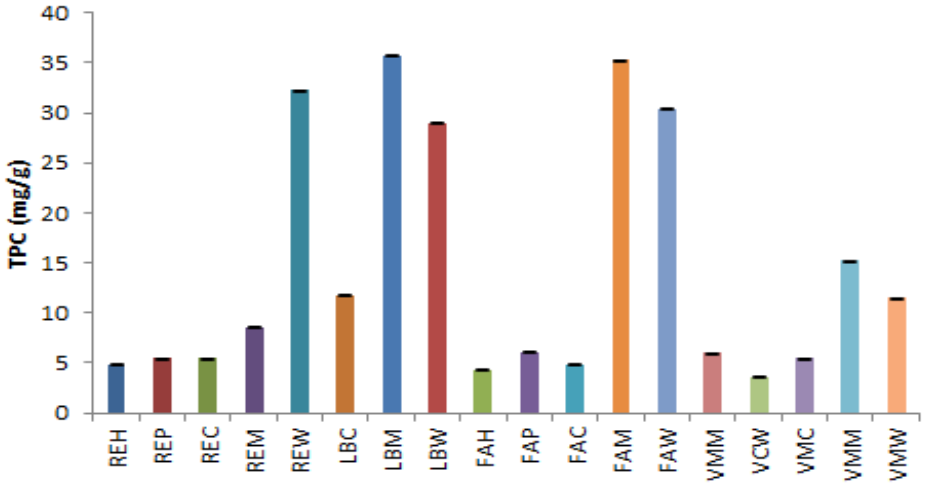

Figure 2: Graph between TPC values (mg/g) and different samples

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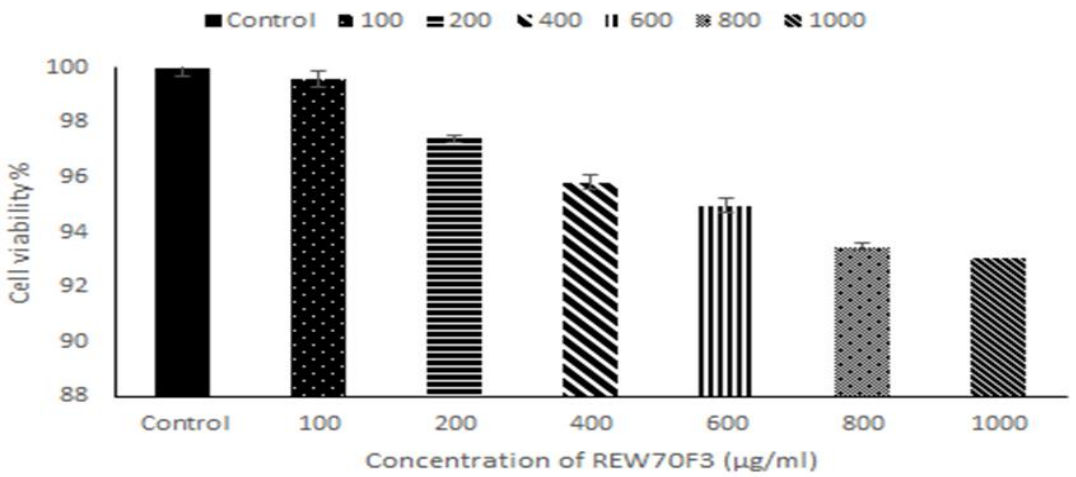

Figure 3: Cytotoxicity induced by REW70F3 on Hela cell viability after the 48-h incubation period. Cell viability was measured as MTT reduction%. Experiments were conducted in triplicates P < 0.001 compared to control.

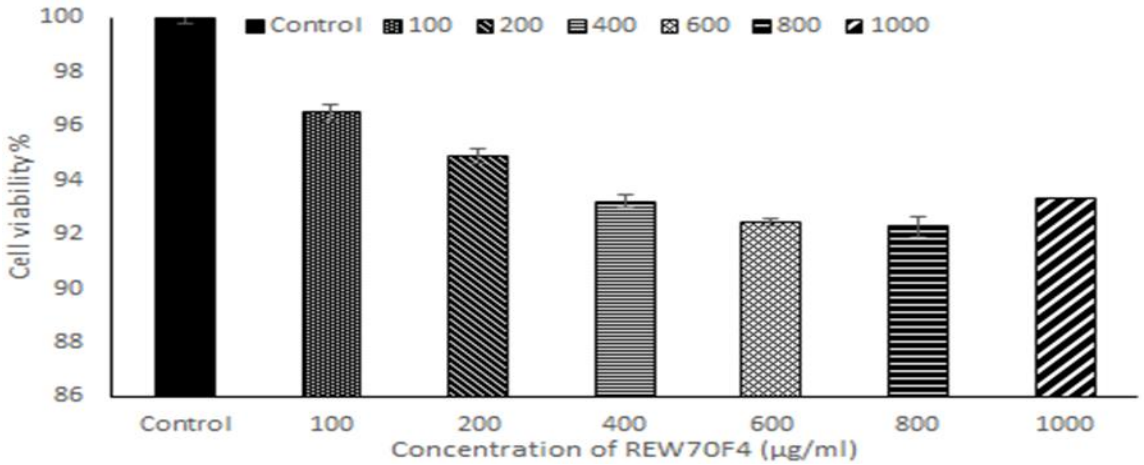

Figure 4: Cytotoxicity induced by REW70F4 on Hela cell viability after the 48-h incubation period. Cell viability was measured as MTT reduction%. Experiments were conducted in triplicates P < 0.001 compared to control.

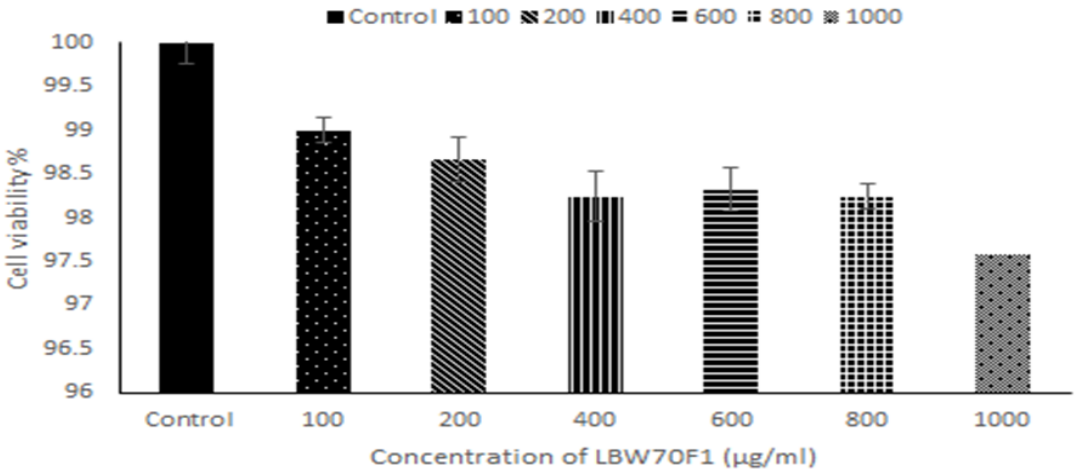

Figure 5: Cytotoxicity induced by LBW70F1 on Hela cell viability after the 48-h incubation period. Cell viability was measured as MTT reduction%. Experiments were conducted in triplicates P < 0.001 compared to control.

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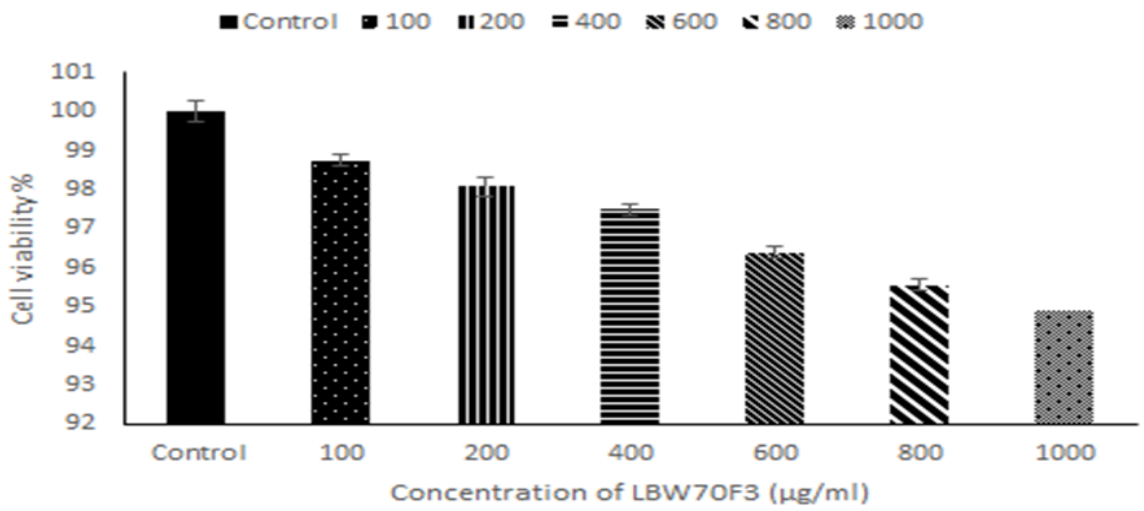

Figure 6: Cytotoxicity induced by LBW70F3 on Hela cell viability after the 48-h incubation period. Cell viability was measured as MTT reduction%. Experiments were conducted in triplicates P < 0.001 compared to control

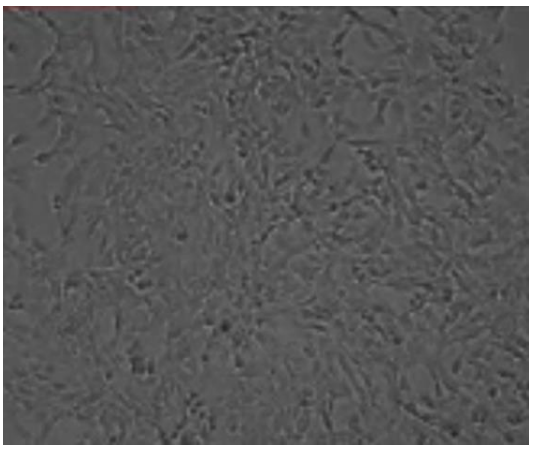

Figure 7: Control

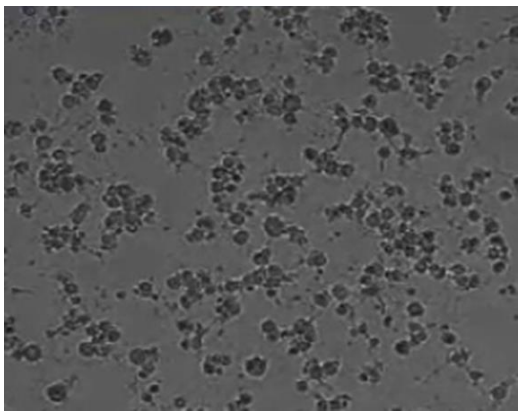

Figure 8: Hela with 100 µg/ml of REW70F3

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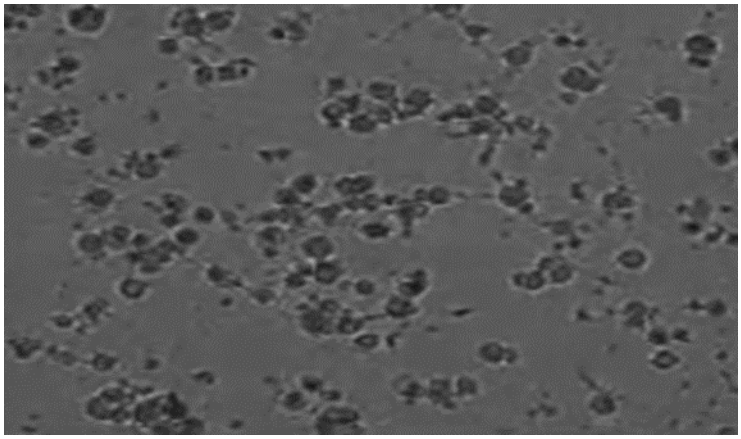

Figure 9: Hela with 100 µg/ml of REW70F4

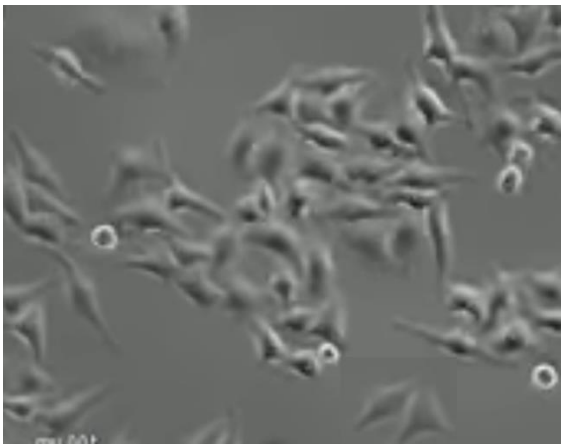

Figure 10: LBM70F1

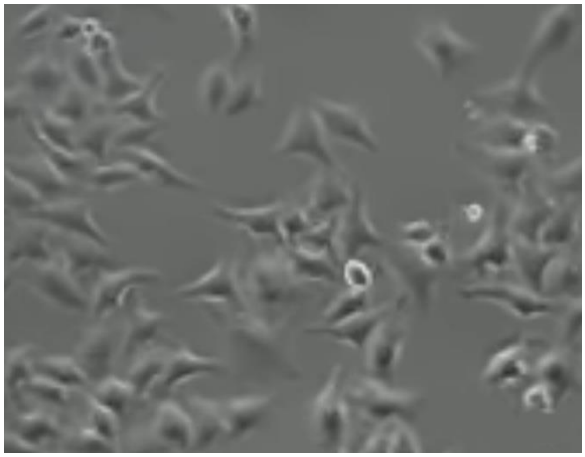

Figure 11: LBW70F1

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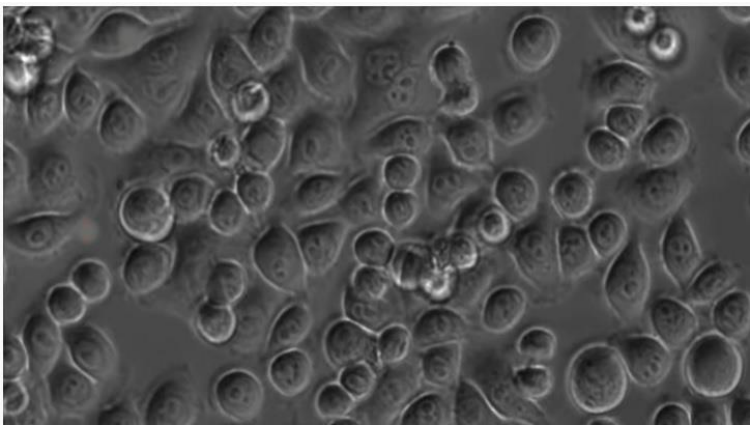

Figure 12: LBW70F3

The results of the morphological studies have been shown in Figures (7- 12)

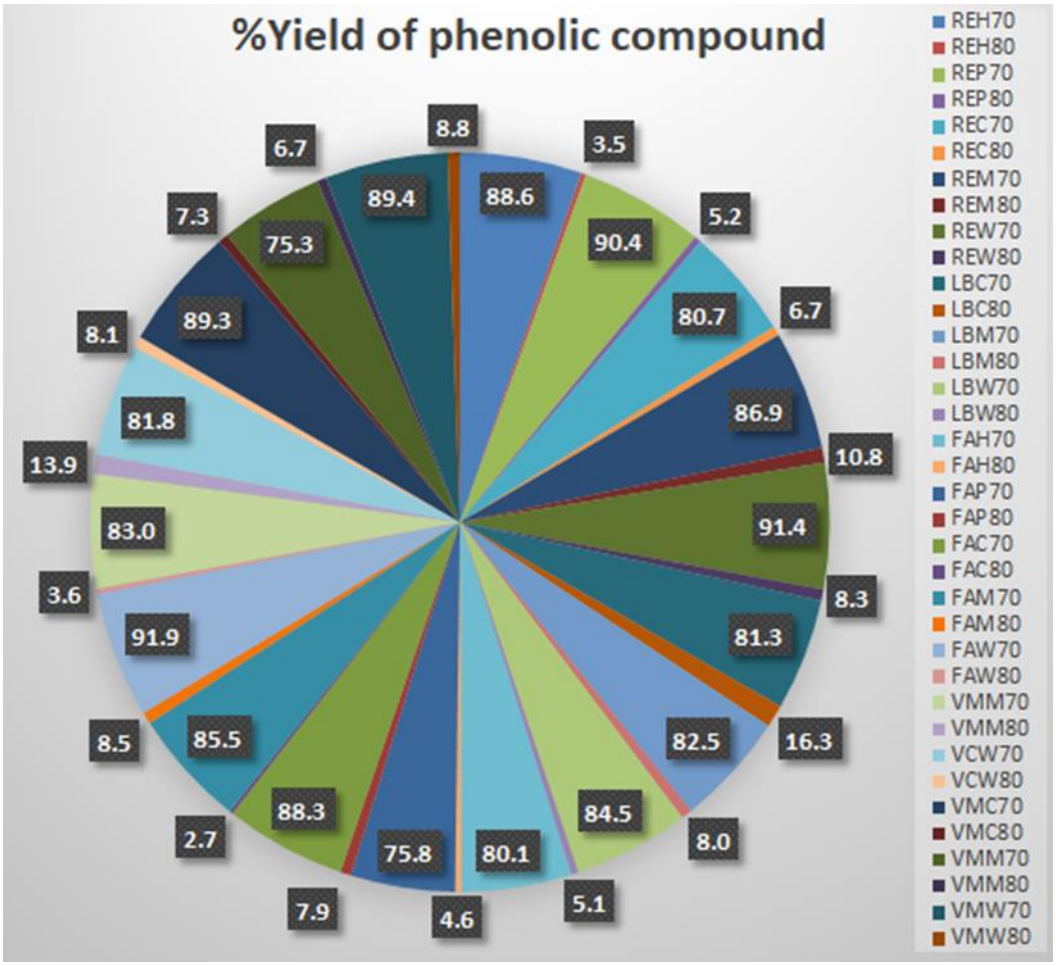

Figure 13: Percentage yield of phenolic compound in different samples

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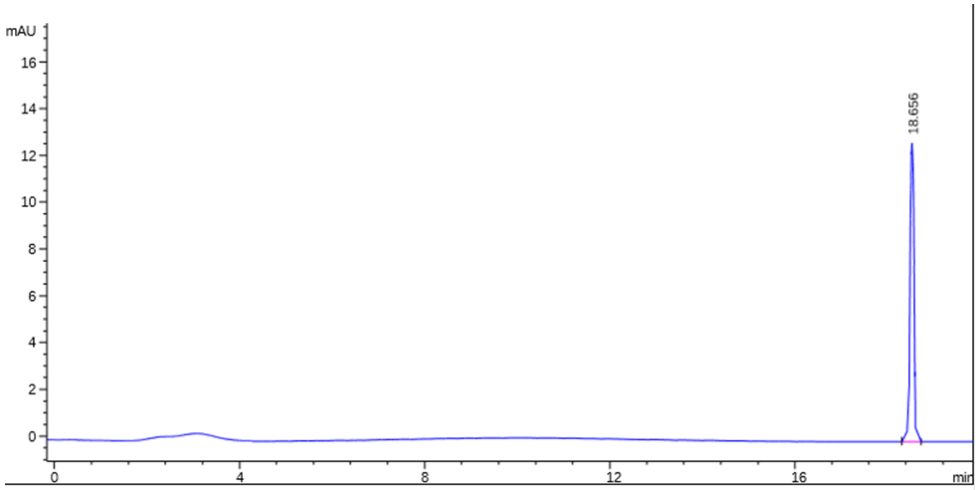

Figure 14: The HPLC analysis of Quercetin

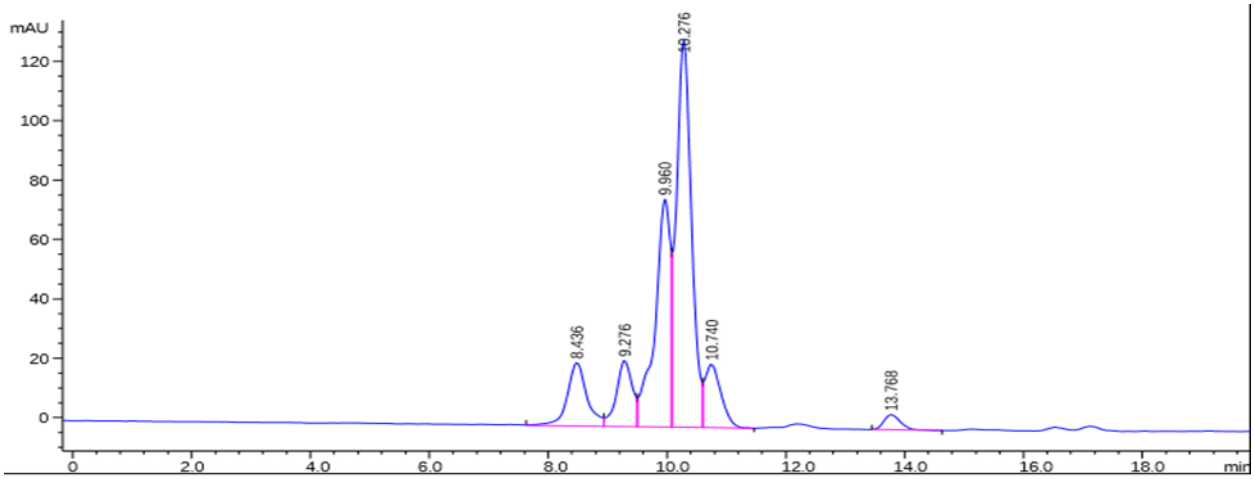

Figure 15: The HPLC analysis of LBM70

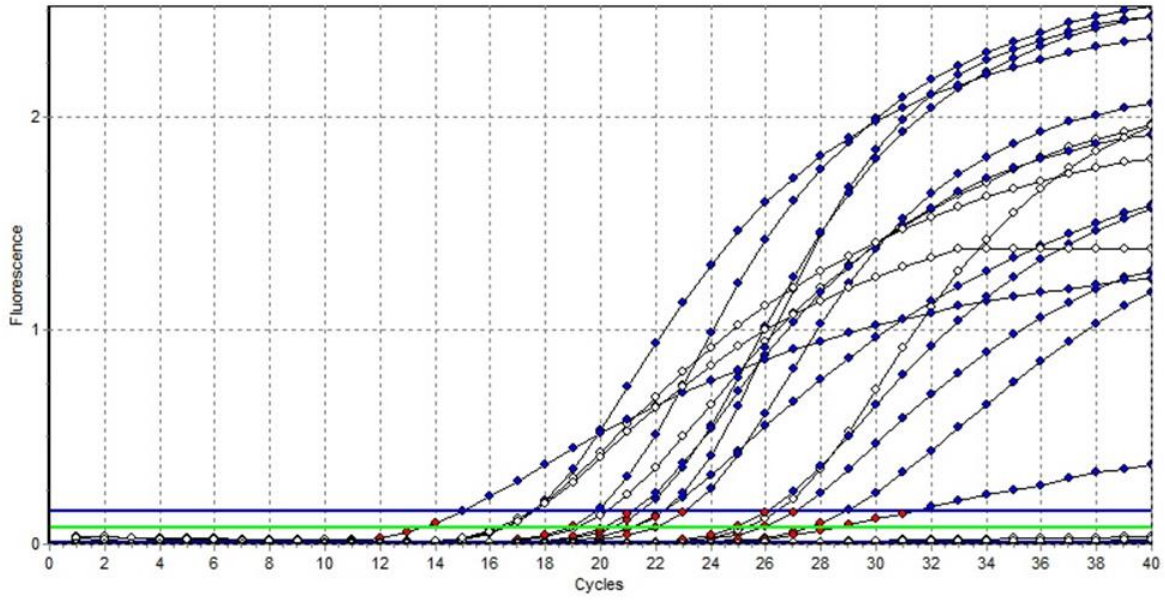

Figure 16: Amplification curve of DNMT and HDAC gene

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DISCUSSION

Natural resources have always been the primary source of food, protection, clothing, transportation, and remedies for humans since the time immemorial. Plants and natural products play an essential role in medicine, serving as prototypes for new drug development [15] They provide a rich source of compounds with a diverse range of biological activities and chemical structures. Because of the serious side effects and high expense of synthetic medications, the demand for innovative plant-derived drugs is growing.

However, a significant number of phytochemicals must still be identified in order to truly understand their health benefits. Secondary metabolism in plants produces phenolic compounds, which are one of the most common classes of chemicals. More than 8000.

Secondary metabolites are recovered from plant materials using a variety of extraction procedures that take into account their chemistry and uneven distribution in the complex plant matrix.

Results from the present study showed the presence of Alkaloid, Flavonoid, Phenol, Glycosides, Tannins, Carbohydrates, saponins and steroids that were analysed by various phytochemical test. The present investigation found that methanol and water extracts had the greatest total phenolic and flavonoid content of all the solvent extracts. This could be because phenolics are easily extracted in polar solvents [16]. The highest phenolic content was observed in methanolic extract of Lycium *barbarum and Fragaria ananassa* (Strawberry)*;* 35.727937mg/gm and 35.3007307 mg/gm respectively. The maximum flavonoid content was observed in *Rubus ellipticus* (Himalayan raspberry) and *Viburnum mullaha* (Indian cranberry); 3.3074713 mg/gm and 3.2873563 mg/gm respectively*.* There are no previous findings on the total phenolics and flavonoids in methanol extracts of the *Rubus species.*

The total phenolic content of methanol and aqueous extracts of R. chingii fruit (4.54 and 4.02 g/100 g extract, respectively) as well as the presence of phenolic components such gallic acid and ellagic acid have previously been reported. (17). The phenolic (4.52 mg GAE/g extract) and flavonoid (4.66 mg TE/g extract) contents of *R. sanctus* root extracts have been published (18), whereas Vadivelan et al. have reported the phenolic (21 to 225 mg GAE/g extract) and flavonoid (16 to 29 mg RE/g extract) contents of *R. ellipticus* root extracts (2009). The highest amounts of total phenolics and flavonoids were detected in the methanol extracts of *R. idaeus* and *R. fruticosus*

The Present study was focussed on the pharmacological examination of *Rubus ellipticus, Lycium barbarum, Fragaria ananassa, Viburnum mullaha, Vaccinium corymbosum with* special reference to inflammation and cancer, based on substantial evidence of traditional applications of this species. The extracts were tested for total phenolics, flavonoids, and *in vitro* antioxidant activity. The pharmacologically active extract was then subjected to compound isolation.

According to the present study *Lycium barbarum* with emerging as the best source of a wide range of chemicals with different chemical structures.

Furthermore, the present study is the first report on showing that *Lycium barbarum* has excellent pharmacological effects. To investigate the therapeutic efficacy against various disorders, medicinal uses of the most active chemicals recovered from the methanolic extract other parts of plant should be studied. In the present investigation the VMM extract depicted the highest ABTS radical cation scavenging activity (98.876275 % inhibition) and DPPH radical scavenging activity of methanolic extract of *Fragaria ananassa* (Strawberry) showed highest activity i.e., 339.8634µg/ml in comparison with all other extracts. Even before the emergence of tumours, changes in epigenetic events were detected during the development of cancers. The progression of DNMT1 overexpression with urethane exposure period from 1 to 36 weeks revealed that DNMT1 dysregulation and its downstream events were important both before and after the formation of tumours [20,21]. The overexpression of DNMT1 appeared to be time-dependent, peaking around 24 or 36 weeks after the development of well-defined tumours.

However, when DNMT1 is combined with DNMT3a and DNMT3b, it performs better [22] Our findings corroborated previous reports on established tumours [21,23] by demonstrating DNMT3b overexpression at early time points prior to tumour development.

Epigenetic modifications play a critical role in the pathophysiology and aetiology of cancer. The involvement of histone deacetylase (HDAC) among epigenetic enzymes is crucial in a variety of cancers, where HDAC overexpression contributes to malignant transformation. Indeed, as epigenetic cancer treatments, medicines targeting HDAC enzymes have been created and are now available in clinics.

High levels of HDAC1 have been seen in highly proliferating tissues, embryonic stem (ES) cells, and a variety of altered cell lines [26] . HDAC1 overexpression has been associated to a variety of solid tumour prognoses [24]. These findings point to HDAC1 as a possible target for tumour prevention and control, as its inhibitors are among the most widely utilised candidates for cancer control [25], potentially by triggering apoptosis, as HDAC1 plays an anti-apoptotic function in lung cancer growth [24,25].

After treatment with the specified fraction and the IC50 value, morphological investigations of the Hela cell were performed. Apoptosis, or programmed cell death, occurred in Hela cells after treatment with chosen fractions, as evidenced by the development of apoptotic bodies.

The qRT-PCR slope should be between -3.6 and -3.2; if the slope is -3.32, the PCR efficiency is said to be 100%. The PCR efficiency should be between 90 and 110 percent, but 100 percent is ideal. In the present study the efficiency of the target and selected controls were compared to see if they were nearly equal, which is a requirement for using the Ct method for quantification [27]. As analyzed through Amplification plot PCR efficiencies for β-Actin, DNMT1, DNMT3A, DNMT3B, HDAC1, HDAC2, and HDAC3 were 94.8 percent, 98 percent, 101 percent, 105 percent, 101 percent, and 117 percent, respectively, with amplification factors of 2.02, 1.98, 2.01, 2.05, 2.01, and 2.17, confirming their primers' singleplex efficiency. The present study shows the over expression of DNMT1 and HDAC genes by more than 2-fold based upon and fluorescence. The reference gene for internal control used in this study was β-Actin. Hence our study is in accordance with the research carried out by Bowman *et al*., 2006 which clearly shows that HDAC1 overexpression has been associated to a variety of solid tumour prognoses. Although Overexpression of DNMT1was shown by Beaulieu *et al*., 2002 by demonstrating DNMT3b overexpression at early time points prior to tumour development. The present study clearly shows that treatment of Hela cell line with selected fraction of *Lycium barbarum* leads to overexpression of DNMT1 and HDAC genes.

CONCLUSION AND INTERPRETATION

Results from the present study showed the presence of Alkaloid, Flavonoid, Phenol, Glycosides, Tannins, Carbohydrates, saponins and steroids that were analysed by various phytochemical tests. It can be concluded from the present study that methanol and water extracts had the greatest total phenolic and flavonoid content of all the solvent extracts. The highest phenolic content was observed in methanolic extract of *Lycium barbarum and Fragaria ananassa* (Strawberry)*;* 35.727937mg/gm and 35.3007307 mg/gm respectively. The maximum flavonoid content was observed in *Rubus ellipticus* (Himalayan raspberry) and *Viburnum mullaha* (Indian cranberry)*; 3.3074713 mg/gm and 3.2873563 mg/gm respectively.* There are no previous findings on the total phenolics and flavonoids in methanol extracts of the *Rubus species*. The retention time was found to be maximum for Quercetin i.e., 18. 656. This was followed by HPLC analysis of fraction LBM70 for which reference gene for internal control in this study was β-Actin. Hence our study is in accordance with the previous research which clearly reveals that HDAC1 overexpression has been connected to a range of cancer prognoses. The results of this study reveal that treating Hela cells with a specific fraction of *Lycium barbarum* causes overexpression of the DNMT1 and HDAC genes.

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