ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

(ISSN: 0975-8585)

RESEARCH ARTICLE

Analysis Of Various Extracts Of Berry Plants For Anti Cancerous Effects.

Oshin Chauhan[1], and SB Sharma[2]*.

[1]Research Scholar, Faculty of Science, Motherhood University, Roorkee, Uttarakhand, India.
[2]Supervisor, Professor and Dean, Faculty of science, Motherhood University, Roorkee, Uttarakhand, India.

ABSTRACT

Flavonoids are polyphenolic secondary metabolites having a wide range of pharmacological and biological properties, the most notable of which is their potential involvement as anticancer drugs. The presence of Alkaloid, Flavonoid, Phenol, Glycosides, Tannins, Carbohydrates, Saponins, and Steroids was detected in various extracts of berry plants study using various phytochemical tests. It can be concluded from the present study that methanol and water extracts had the greatest flavonoid content of all the solvent extracts. 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.*
Keywords: Flavanoids, Glycosides, Tannins, Ethanol

https://doi.org/10.33887/rjpbcs/2021.12.5.8

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Approximately 80-85% of the world's population relies on traditional plant-based medicines to meet their health-care needs. A variety of plant extracts, isolated chemicals, and analogues have been employed as efficient anticancer medicines, and the study of therapeutic capabilities of plant-derived compounds is gaining popularity [1]. A significant area of research is the characterisation and investigation of medicinal values of plant extracts and extracted bioactive chemicals. Plant-based diets have been shown to protect against a variety of diseases, including cancer, in epidemiological studies.

Plant bioactive chemicals such as phenolics and flavonoids have been shown to have cytotoxic capabilities against a variety of tumour cells while causing minimal damage to normal cells. Oxidative stress is a rather common occurrence.

Rubus L. (Rosaceae) berries have garnered considerable attention because of their nutritional and bioactive properties. This genus' raspberries and blackberries contain vitamins, minerals, proteins, carbohydrates, and polyphenols, among other nutrients and bioactive components. The antioxidant, anti-inflammatory, chemopreventive, and antimicrobial activities of Rubus berries, as well as their beneficial effects on blood lipids and atherosclerosis, demonstrated that these fruits are important sources of biologically active compounds, and their biological effects suggest potential applications for human health.

MATERIAL AND METHODS

Total Flavonoid content

Quercetin standard: 1mg/ml of quercetin standard was prepared in distilled water.

Aluminium chloride: 1.2 gm of aluminium chloride was weighed and dissolved in 100 ml of distilled water. 1M Potassium acetate solution: 0.9815 gm of potassium acetate was weighed and diluted to 10 ml of solution with distilled water.

All the chemicals and samples were added as tabulated.

Antioxidant assay of crude extract (ABTS)

Reagent

7mM ABTS:.180 gm of ABTS was weighed of ABTS and dissolved in 50 ml water.

2.45mM potassium persulphate: 0.033 gm of potassium persulphate was weighed and dissolved in 50 ml of water.

Procedure

In a nutshell, 7 mM ABTS in water was combined with 2.45 mM potassium persulphate (1:1) and incubated in the dark for 12-16 hours. After that, the mixture was diluted to achieve an absorbance of 0.7 at 734 nm. 5 litres of extract and 3.395 litres of reagent were combined and incubated for 10 minutes in the dark. At 734 nm, the absorbance was measured. The absorbance of ABTS and methanol was measured as a control. Ascorbic acid standard curves were created simultaneously with dilution ranges of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 g/ml.ABTS$^+$ scavenging effect (%) =(Ab-Aa/Ab) *100

Ab=Ab. Of ABTS+ Methanol

Aa= ABTS+ sample/standard

CEAC (Vitamin C Equivalents Antioxidant Capacity) or ascorbic acid content of all the extract will be estimated by standard curve of ABTS$^+$ scavenging effect (%) linear curve equation.

ISSN: 0975-8585

Antioxidant assay of crude extract (DPPH)

Based on the scavenging of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical, the crude extract's DPPH free radical scavenging activity was evaluated.

The samples were diluted to a concentration of 1 mg/ml conc. 1.0 ml of 0.1 mM DPPH in methanol and 0.1 ml of extract were added to the reaction mixture. The combination was kept at room temperature for 30 minutes in the dark. Monitoring the decrease in absorbance at 517 nm to determine the degree of DPPH inhibition. The positive control was ascorbic acid. The following formula was used to compute radial scavenging activity, which was expressed as a percentage of free radical inhibition by the sample:

$$\%Inhibition=((A0-At)/A0)*100$$

where A0 was the absorbance of control (blank without sample) and At was the absorbance in presence of sample. All the tests were performed in triplicate and graph was plotted with mean values.

Antioxidant assay of crude extract (FRAP assay)

The FRAP assay utilized was essentially the same as described earlier (Benzie and Strain, 1996). The samples were diluted to a concentration of 250 mg/ml. At 0 time and after 6 minutes of standing at room temperature, an aliquot (100 l) of the appropriately diluted extract was added to 3 ml of the standard reaction solution, and the absorbance was measured at 593 nm. The measurement was done three times. In the range of 200–1000 M, $FeSO4$ was utilized to create the standard curve. The standard and sample FRAP values were computed and represented as M Fe[II]/gm dry wt. Antioxidant assay of crude extract .

H_2O_2 assay

0.6 ml aliquot of 40 mM H_2O_2 solution was combined with 0.1 ml of crude extract that had been diluted. 2.4 mL phosphate buffer (0.1 M, pH 7.4) was added to the mixture, which was violently agitated before being incubated at room temperature for 10 minutes. The reaction mixture's absorbance was then measured at 230 nm. The positive control was ascorbic acid. The following formula was used to compute the H2O2 scavenging activity:

$$\%Inhibition= ((A1-A2)/A1)*100$$

Where A1 is the absorbance of the ascorbic acid, A2 is the absorbance of the sample.

TFC (Total Flavonoid Content)

Similarly, the total flavonoid content was also calculated for different fractions and the results have been shown in table 1. The absorbance was taken at 415nm and the graph between absorbance values and concentration of Quercetin was plotted as shown in Figure 4.3 as it can be observed in Figure 1, the maximum TFC was found to be in VMW fraction.

Antioxidant

ABTS ASSAY (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

Antioxidants were also studied using ABTS. The level of Ascorbic acid along with the CEAC and percent inhibition have been shown in Table 2. The percent inhibition in this case was found to be maximum in.REM as shown in Figure 3

DPPH ASSAY (2,2-diphenyl-1-picryl-hydrazyl-hydrate)

Antioxidants were also studied using DPPH. The level of Ascorbic acid along with the CEAC and percent inhibition have been shown in Table 3. The percent inhibition in this case was found to be maximum in FAM as shown in Figure 6

H_2O_2 assay

Antioxidants were also studied using H_2O_2 assay. The Absorbance of Ascorbic acid at 230 nm along percent scavenging have been shown in Table 4. The percent scavenging in this case was found to be maximum in REC as shown in Figure 9.

FRAP assay (Ferric Reducing Antioxidant Power Assay)

Antioxidants were also studied using FRAP assay. The FRAP value (μM Fe (II)/g dry wt.) along with FeSO4(μM) have been shown in Table 5. The absorbance was taken at 593 nm and the graph between absorbance values and concentration of FeSO4(μM) was plotted as shown in Figure 10. The FRAP value in this case was found to be maximum in REM as shown in Figure 11.

Phenolic purification

It was found to be maximum in FAM 70 fraction as shown in Table 6. The percent yield of phenolic compound has been shown in Figure 13. It can be clearly observed that it was found to be maximum in FAW at 70 % methanolic concentration with a yield of 91.9 percent.

DISCUSSION

Plant polyphenols, which are a varied collection of phenolic chemicals, have a perfect structural chemistry for scavenging free radicals. Polyphenols have antioxidative capabilities due to their high reactivity as hydrogen or electron donors, ability to stabilize and delocalize the unpaired electron, and ability to bind metal ions [9]. Flavonoids' ability to act as antioxidants is owing to a wide range of processes, including scavenging free radicals, chelation of metal ions like iron and copper, and inhibition of free radical-generating enzymes.

Flavonoids can scavenge virtually all known ROS depending on their structure [2] A lot of researchers have discovered a link between phenolic concentration and antioxidant activity (3].

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.

However, there have been reports of similar Rubus species exhibiting considerable DPPH radical scavenging activity.

The ability of the n-butanol extract of *R. parvifolius* to scavenge DPPH (IC50 52.20.9 g/mL) was investigated (10).When compared to Vitamin C (97.15%) and BHT (96.47%), *R. sanctus* was found to scavenge the DPPH radical by 83.27 percent [4]. *R. idaeus* extracts have DPPH scavenging capabilities ranging from 305 to 351 M TE/g, according to Zhang et al. (2010).. The antioxidant activity of R. ulmifolius (TEAC value: 3.80.3 mM TE; DPPH EC50 value: 5.100.5 g/mL) and total phenolic contents (2.760.08 mg/L GAE) have been reported .The antioxidant activity of R. ulmifolius (TEAC value: 3.80.3 mM TE; DPPH EC50 value: 5.100.5 g/mL) and total phenolic contents (2.760.08 mg/L GAE) have been reported. The antioxidant properties of extracts from black raspberry fruits and wines were studied by [6]. For the black raspberry wine containing seeds, the ethanol extracts of crushed seeds showed stronger antioxidant activity (DPPH• IC_{50} 130 g/mL) and the lowest ABTS•+ (IC_{50} 198 g/mL). Total phenol level in raspberry can be linked to DPPH radical scavenging values. The antioxidant activity of 11 cold-field fruits was studied in China. Total phenolic content was strongly linked with antioxidant activity of fruit extracts ($R2 > 0.7112$) (11). In China, It was investigated the antioxidant activity of 11 cold-field fruits. The antioxidant activity of fruit extracts was positively correlated with total phenolic content ($R2 > 0.7112$) (11). The extracts of *R. kamarowii* had the highest capacity for scavenging DPPH (EC_{50} 25.60.51 M TE/g) and ABTS+ (EC_{50} 63.61.67 M TE/g) among the 11 fruits. The antioxidant activity of a methanolic extract of R. ulmifolius fruits (93 percent at 42 g/mL) has been observed [12].

This investigation indicated promising radical scavenging capabilities, which may be attributed to the greater phenolic content, when compared to earlier studies of DPPH and ABTS radical scavenging activities of various allied Rubus species.

ISSN: 0975-8585

Table 1: Total Flavonoid Content of different fractions

sr. no	quercetin(mg /ml)	volu me	Fruit extra ct	metha nol	aluminium chloride (1.2%)	pot assi um acet ate		Absorbance (415 nm) Mean	Std.d ev.)	QE (mg/ml)	TFC (mg/ gm)
Blank	-	-	-	2	0.5	0.5	30-minute incubation at room temperature			-	-
t1	0.02	0.04	-	1.96	0.5	0.5		0.0792	0.02	-	-
t2	0.04	0.08	-	1.92	0.5	0.5		0.1	0.03	-	-
t3	0.06	0.12	-	1.88	0.5	0.5		0.349	0.08	-	-
t4	0.08	0.16	-	1.84	0.5	0.5		0.509	0.012	-	-
t5	0.1	0.2	-	1.8	0.5	0.5		0.615	0.018	-	-
t6	0.12	0.24	-	1.76	0.5	0.5		0.81	0.06	-	-
t7	0.14	0.28	-	1.72	0.5	0.5		1.034	0.023	-	-
t8	0.16	0.32	-	1.68	0.5	0.5		1.204	0.04	-	-
REH	-	-	0.5	1.5	0.5	0.5		0.103	0.02	0.031968391	0.7672414
REP	-	-	0.5	1.5	0.5	0.5		0.209	0.09	0.044659962	1.0718391
REC	-	-	0.5	1.5	0.5	0.5		0.407	0.01	0.068366858	1.6408046
REM	-	-	0.5	1.5	0.5	0.5		0.987	0.08	0.137811303	3.3074713
REW	-	-	0.5	1.5	0.5	0.5		0.832	0.09	0.119252874	2.862069
LBC	-	-	0.5	1.5	0.5	0.5		0.219	0.04	0.04585728	1.1005747
LBM	-	-	0.5	1.5	0.5	0.5		0.319	0.02	0.05783046	1.387931
FAC	-	-	0.5	1.5	0.5	0.5		0.08	0.03	0.029214559	0.7011494
FAM	-	-	0.5	1.5	0.5	0.5		0.129	0.08	0.035081418	0.841954
FAW	-	-	0.5	1.5	0.5	0.5		0.439	0.01	0.072198276	1.7327586
VMP	-	-	0.5	1.5	0.5	0.5		0.417	0.1	0.069564176	1.6695402
VMC	-	-	0.5	1.5	0.5	0.5		0.098	0.2	0.031369732	0.7528736
VMM	-	-	0.5	1.5	0.5	0.5		0.882	0.03	0.125239464	3.0057471
VMW	-	-	0.5	1.5	0.5	0.5		0.98	0.01	0.13697318	3.2873563
VCH	-	-	0.5	1.5	0.5	0.5		0.343	0.09	0.060704023	1.4568966
VCP	-	-	0.5	1.5	0.5	0.5		0.209	0.01	0.044659962	1.0718391
VCC	-	-	0.5	1.5	0.5	0.5		0.22	0.03	0.045977011	1.1034483
VCM	-	-	0.5	1.5	0.5	0.5		0.118	0.04	0.033764368	0.8103448
VCW	-	-	0.5	1.5	0.5	0.5		0.109	0.02	0.032686782	0.7844828

ISSN: 0975-8585

Table 2: Ascorbic acid concentration in (µg/ml) and CEAC (mg/ml) of different fractions

sample	Ascorbic acid conc. (µg/ml)	% Inhibition	CEAC (mg/ml)
control	0		
S1	1	42.32215	
S2	1.5	59.03533	
S3	2	63.29603	
S4	2.5	74.20363	
S5	3	84.08257	
S6	3.5	92.32248	
S7	4	96.67609	
S8	4.5	97.7532	
S9	5	98.73589	
REH	-	58.84872	0.001604
REP	-	64.74695	0.002019
REC	-	75.32709	0.002763
REM	-	97.56547	0.004327
REW	-	88.15555	0.003665
LBH	-	47.37873	0.000797
LBP	-	59.7842	0.00167
LBC	-	64.74695	0.002019
LBM	-	86.98533	0.003583
LBW	-	89.51296	0.00376
FAH	-	44.42936	0.00059
FAP		61.4237	0.001785
FAC		64.46657	0.001999
FAM		91.05778	0.003869
FAW		86.37658	0.00354
VMH		69.75698	0.002371
VMP		71.72274	0.002509
VMC		71.58229	0.002499
VMM		98.87627	0.004419
VMW		97.56561	0.004327
VCH		59.6444	0.00166
VCP		61.61024	0.001798
VCC		71.20822	0.002473
VCM		89.51362	0.00376
VCW		86.798	0.003569

Table 3: Ascorbic acid concentration in (µg/ml) and CEAC (mg/ml) of different fractions

sample	%Inhibition	Conc.(µg/ml)	CEAC (mg/ml)
blank			
S1	30.49743	10	-
S2	37.3959	20	-
S3	46.62857	40	-
S4	57.41542	80	-
S5	71.05691	160	-
S6	88.22519	320	-
REH	80.44607	249.4633	0.249463
REP	79.04516	241.4581	0.241458
REC	54.87295	103.3312	0.103331
REM	85.37346	277.6198	0.27762
REW	69.70832	188.1047	0.188105
LBH	73.13264	207.6722	0.207672
LBP	52.6434	90.59083	0.090591
LBC	43.6201	39.02917	0.039029
LBM	72.50918	204.1096	0.20411
LBW	80.08238	247.385	0.247385
FAH	80.55056	250.0604	0.25006
FAP	81.06881	253.0218	0.253022
FAC	94.39675	329.1814	0.329181
FAM	96.26609	339.8634	0.339863
FAW	95.69519	336.6011	0.336601
VMH	80.34199	248.8685	0.248868
VMP	43.7749	39.91371	0.039914
VMC	51.97229	86.75596	0.086756
VMM	79.19947	242.3398	0.24234
VMW	77.4389	232.2794	0.232279
VCH	44.86518	46.14387	0.046144
VCP	54.45952	100.9687	0.100969
VCC	51.71195	85.26829	0.085268
VCM	78.78555	239.9746	0.239975
VCW	85.27115	277.0351	0.277035

Table 4: Ascorbic acid concentration in (µg/ml) and CEAC (mg/ml) of different fractions

Sample	Absorbance at 230 nm	% Scavenging
REH	0.045	42.22222
REP	0.036	77.77778
REC	0.035667	79.43925
REM	0.045333	41.17647
REW	0.049	30.61224
LBH	0.046333	38.1295

ISSN: 0975-8585

LBP	0.042333	51.1811
LBC	0.052333	22.29299
LBM	0.042333	51.1811
LBW	0.044333	44.3609
FAH	0.043333	47.69231
FAP	0.056	14.28571
FAC	0.036667	74.54545
FAM	0.041	56.09756
FAW	0.044667	43.28358
VMH	0.048333	32.41379
VMP	0.052	23.07692
VMC	0.044333	44.3609
VMM	0.044667	43.28358
VMW	0.045667	40.14599
VCH	0.047333	35.21127
VCP	0.048333	32.41379
VCC	0.051	25.4902
VCM	0.055	16.36364
VCW	0.037	72.97297

Table 5: FRAP value (µM Fe (II)/g dry wt.) and FeSO4(µM) concentration

sample	FeSO4(µM)	FRAP value (µM Fe(II)/g dry wt.)
blank		
S1	200	
S2	400	
S3	600	
S4	800	
S5	1000	
REH	12.38095	1485.714
REP	2.857143	342.8571
REC	4.285714	514.2857
REM	38.57143	4628.571
REW	17.61905	2114.286
LBH	1.428571	171.4286
LBP	5.714286	685.7143
LBC	14.7619	1771.429
LBM	24.28571	2914.286
LBW	17.61905	2114.286
FAH	8.095238	971.4286
FAP	5.238095	628.5714
FAC	3.809524	457.1429
FAM	25.2381	3028.571
FAW	17.61905	2114.286

ISSN: 0975-8585

VMH	8.095238	971.4286
VMP	7.619048	914.2857
VMC	11.42857	1371.429
VMM	40	4800
VMW	26.19048	3142.857
VCH	8.571429	1028.571
VCP	3.333333	400
VCC	12.38095	1485.714
VCM	25.71429	3085.714
VCW	13.80952	1657.143

Table 6: % Yield of different fraction in Methanol

Sample	Fraction(%methanol)	Initial TPC (mg/g)	TPC (mg/g)	Yield (%)
REH	70%	4.8229342	4.275	88.63899
	80%	4.8229342	0.17	3.524825
REP	70%	5.4749859	4.948	90.37466
	80%	5.4749859	0.283	5.168963
REC	70%	5.4300169	4.383	80.71798
	80%	5.4300169	0.363	6.685062
REM	70%	8.6115795	7.484	86.90624
	80%	8.6115795	0.928	10.77619
REW	70%	**32.265318**	**29.495**	**91.41395**
	80%	32.265318	2.683	8.31543
LBC	70%	11.793142	9.584	81.26757
	80%	11.793142	1.928	16.34848
LBM	70%	**35.727937**	**29.474**	**82.49567**
	80%	35.727937	2.858	7.999342
LBW	70%	**28.982574**	**24.494**	**84.51285**
	80%	28.982574	1.485	5.123768
FAH	70%	4.3395166	3.474	80.055
	80%	4.3395166	0.1983	4.569633
FAP	70%	6.0483418	4.585	75.8059
	80%	6.0483418	0.476	7.869926
FAC	70%	4.8679033	4.298	88.29263
	80%	4.8679033	0.13	2.670554
FAM	70%	**35.300731**	**30.192**	**85.52797**
	80%	35.300731	2.99	8.47008
FAW	70%	**30.376616**	**27.911**	**91.88318**
	80%	30.376616	1.094	3.601454
VMM	70%	6.014615	4.992	82.99783
	80%	6.014615	0.839	13.94936
VCW	70%	3.5862844	2.933	81.78381

	80%	3.5862844	0.292	8.142132
VMC	70%	5.4974705	4.911	89.33199
	80%	5.4974705	0.4	7.276074
VMM	70%	15.25576	11.493	75.33547
	80%	15.25576	1.029	6.744993
VMW	70%	11.52333	10.304	89.41861
	80%	11.52333	1.009	8.756151

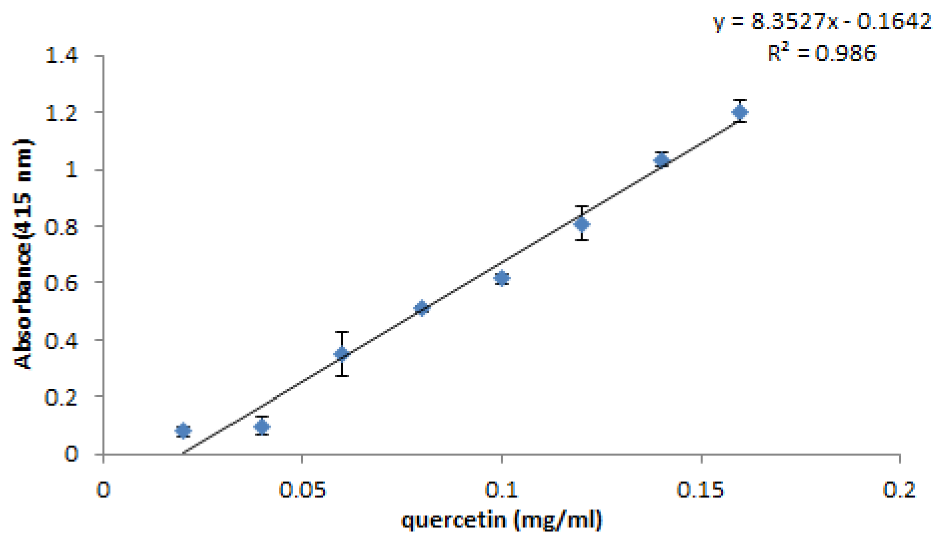

Figure 1: Graph between Absorbance and Quercetin Concentration

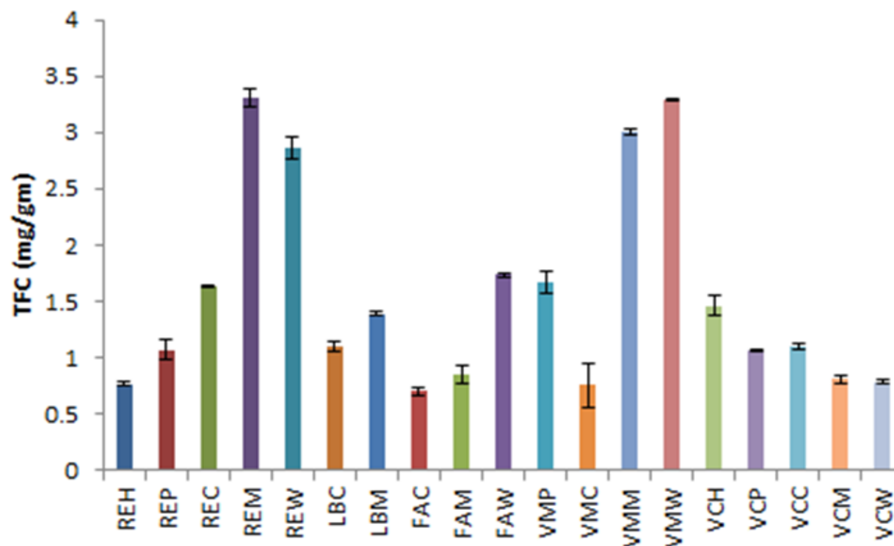

Figure 2: Graph between TFC values (mg/g) and different samples

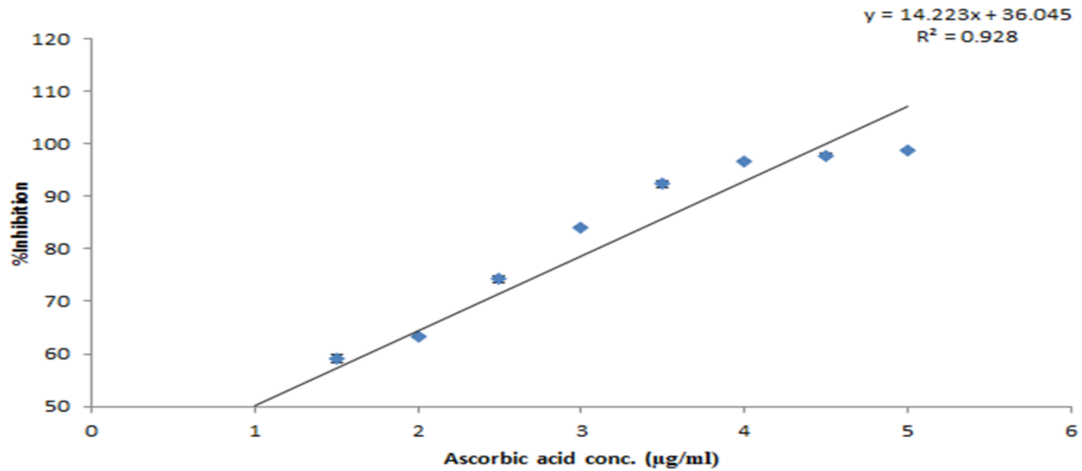

Figure 3: Graph between % inhibition and Ascorbic acid Concentration

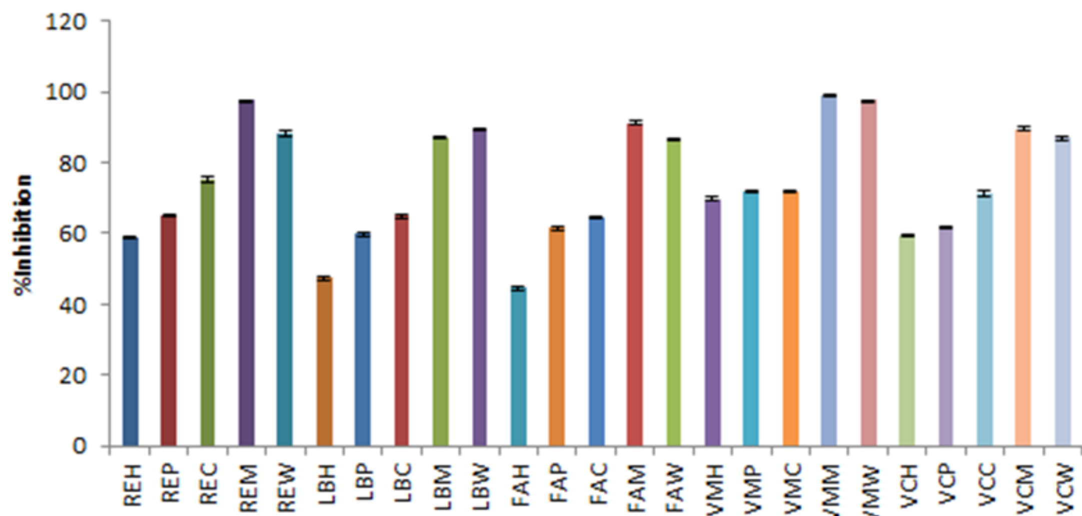

Figure 4 : Graph between % inhibition and different samples

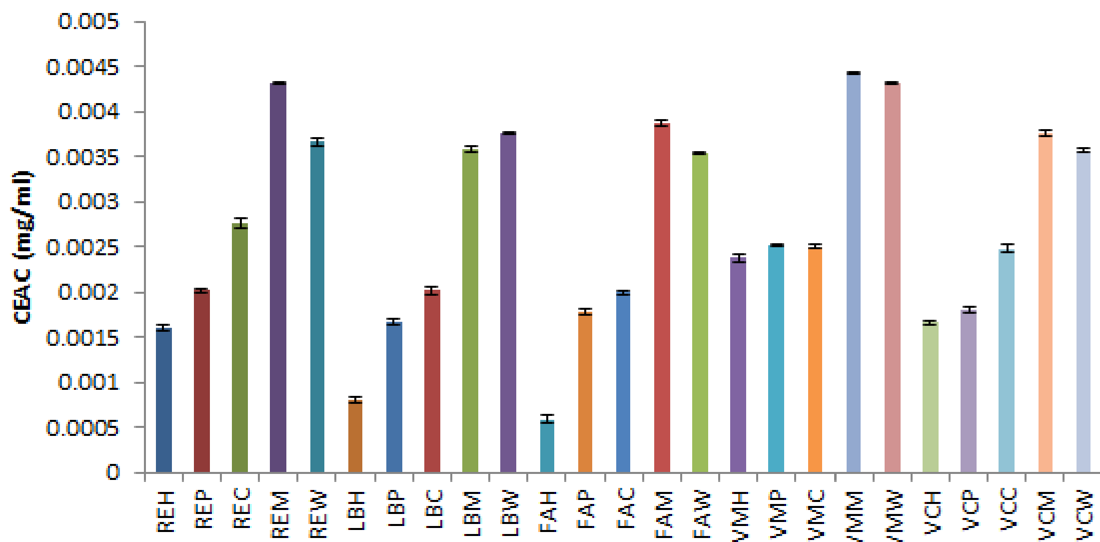

Figure 5: Graph between CEAC values (mg/g) and different sample

ISSN: 0975-8585

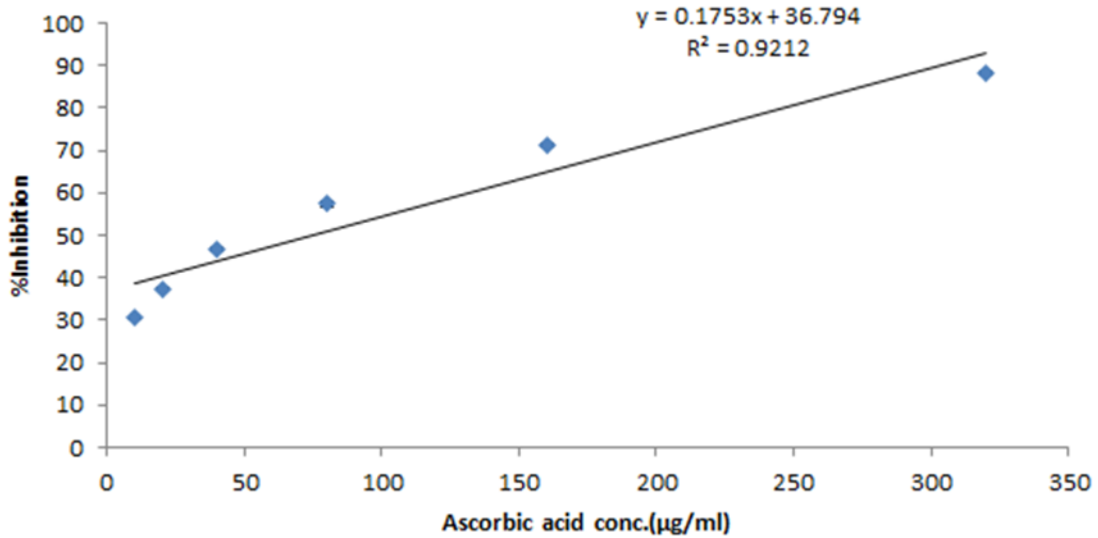

Figure 6: Graph between % inhibition and Ascorbic acid concentration

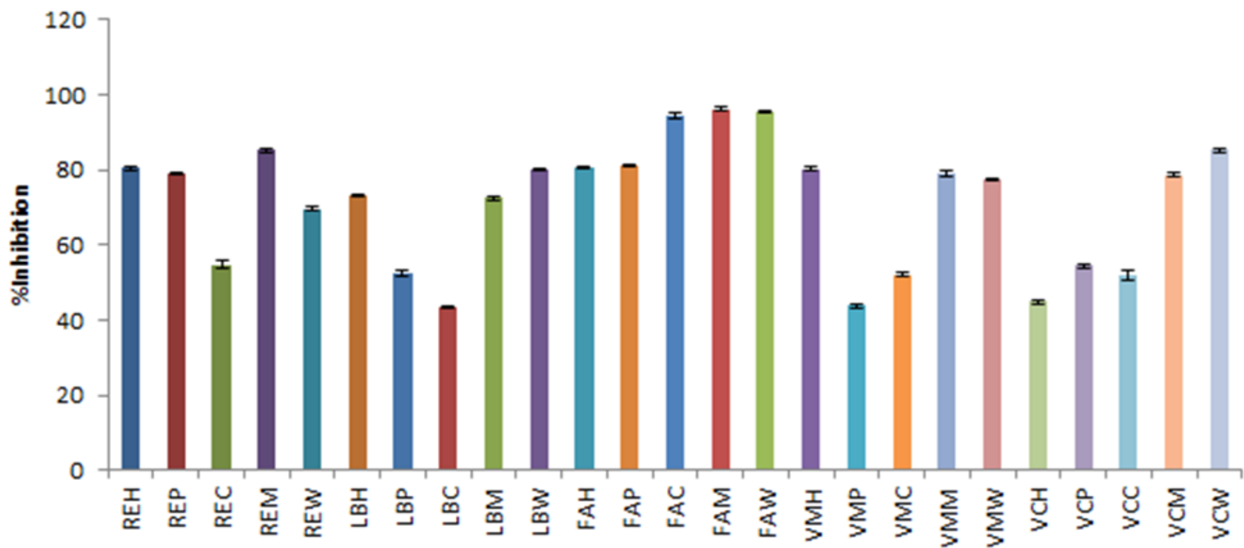

Figure 7: Graph between % inhibition and different samples

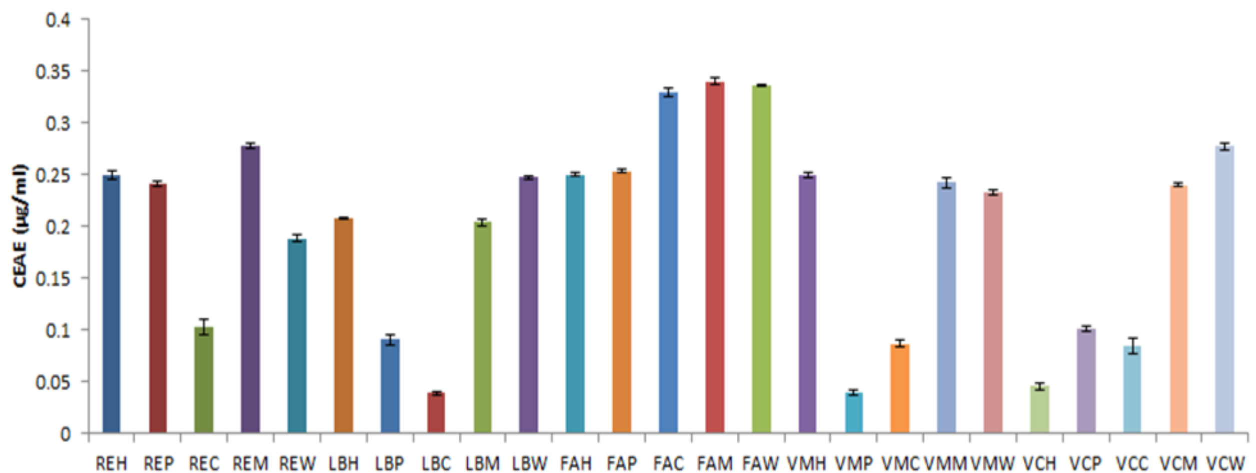

Figure 8: Graph between CEAC values (mg/g) and different samples

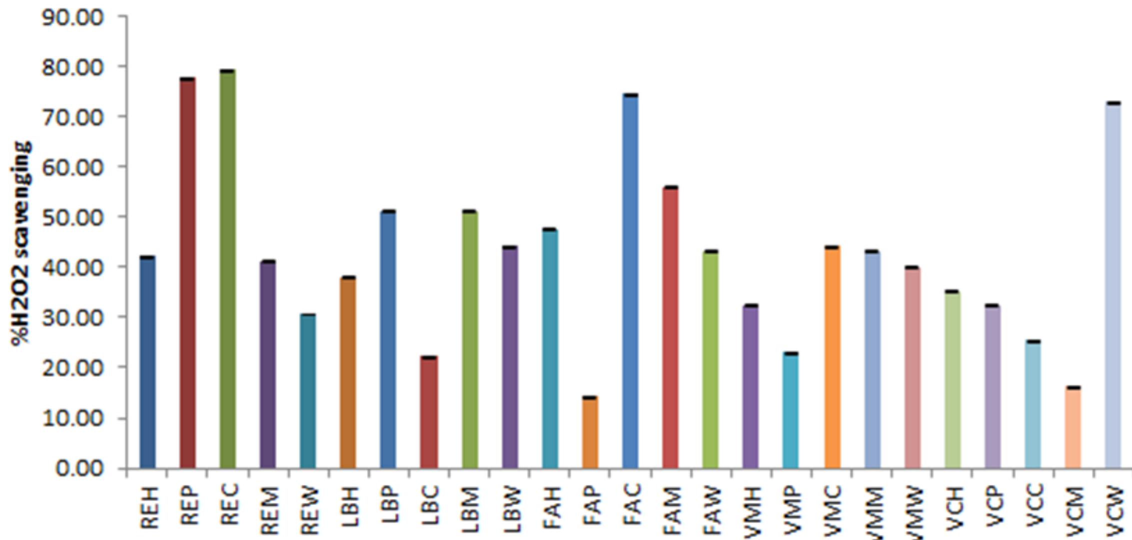

Figure 9: Graph between % H_2O_2 scavenging and different samples

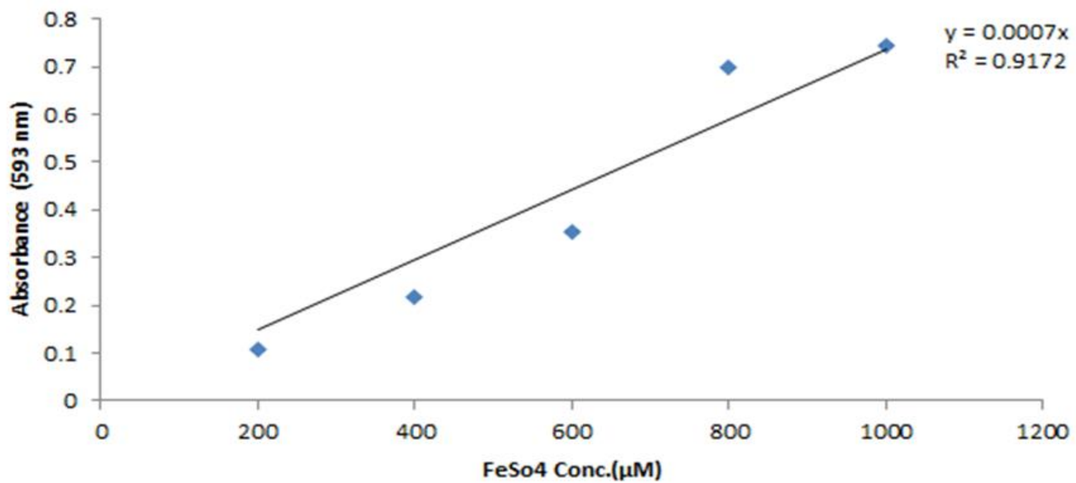

Figure 11: Graph between Absorbance (593 nm) and FeSO4 (µM) concentration

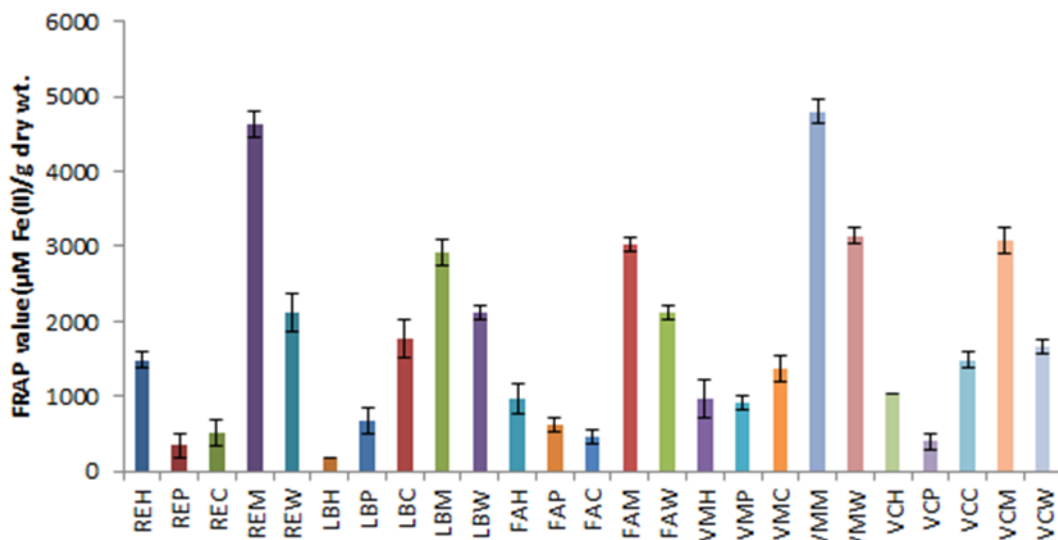

Figure 12: Graph between FRAP value (µM Fe (II)/g dry wt) and different samples

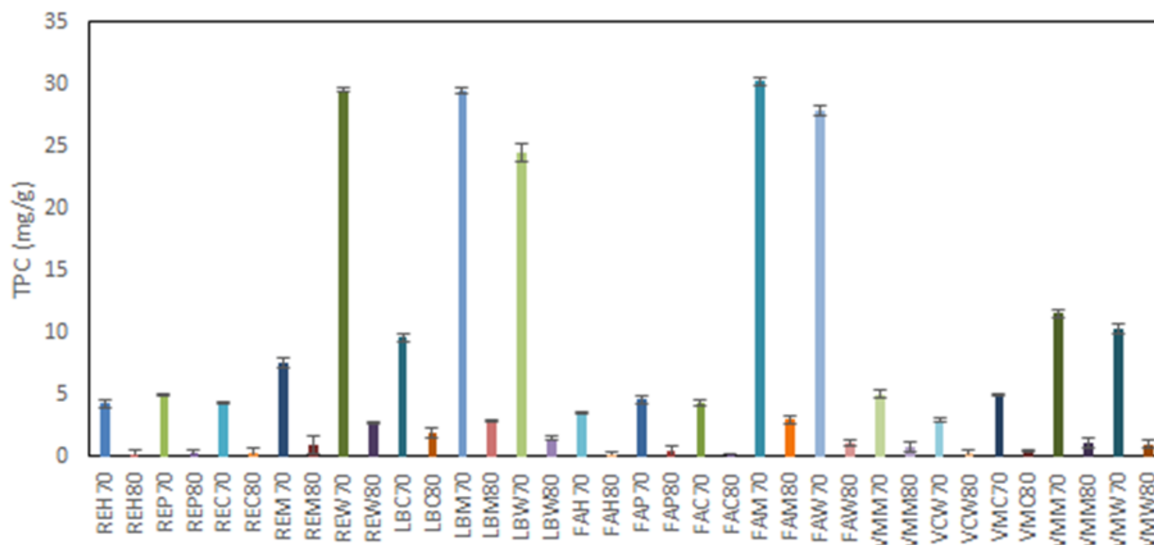

Figure 13: Graph between TPC value (mg/g) and different samples

CONCLUSION

It can be concluded from the present study that methanol and water extracts had the greatest flavonoid content of all the solvent extracts. 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.*

Nonetheless, because of their lack of selectivity for particular isoforms of epigenetic modulating enzymes like HAT and HDAC, it is unclear whether long-term exposure to "epigenetic diets" high in flavonoids or flavonoid supplements could cause undesired effects. Low specificity has been found to hinder the therapeutic applicability of epigenetic medicines like HDACi, and flavonoids target a variety of different pharmacological pathways despite their epigenetic action.

Future Perspectives

In most nations, traditional plant-based medicine, sometimes known as herbal medicine, is an important part of the health-care system. For their basic health care needs, the majority of individuals rely on the traditional medical system. Thousands of therapeutic plants can be found in nature, and a great number of contemporary medications have come from different natural sources. Plant-derived medicine has acted as a valuable source of alternative medicine due to the high cost and adverse effects of synthetic medications. Furthermore, combination therapy is occasionally employed to improve therapeutic efficacy, and a new generation of safer and more potent medications is desperately needed.

REFERENCES

[1] R. D. Lawania and A. Mishra. J Pharm Biomed Anal 2013;1(2).
[2] Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del Rio JA. J Agric Food Chem 1997;45: 4505–4515.
[3] Y Sedat Velioglu *et al*., J Agric Food Chem 1998;46(10):4113–4117.
[4] Motamed SM, Naghibi F. Food Chem 2010; 119:1637-1642
[5] Tang N, Zhang J, Du Y. Zhongguo Fei Ai Za Zhi 2010;13(4):301-6.
[6] Ji-Hyun Jeong Hana J *et al*., Food Chem 2010;123(2):338-344
[7] Zi-LuanFan, *et al*., Food Chem 2011;129(2):402-407
[8] Alessia Fazio *et al.,* Food Chem 2013;140(4):817-24.
[9] Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice Evans C. Arch Biochem Biophys. 1995; 322:339–346.
[10] Gao *et al.,* *Zhejiang Univ Sci B. 2011 Feb; 12(2): 135–142.*
[11] Fang *et al.,*(2011) *Sci Transl Med*. 3(75):75

[12] Fazio et al., *Food Chem.* 15;140(4): 817-24