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Extraction Of Caffeine From Tea Waste And Its Analysis

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ABSTRACT: Caffeine is a chemical found in coffee, tea, cola, gaurana, maste, and other products .Caffeine is one of most commonly used stimulants among the athelets. Taking caffeine, within limit, is allowed by the National Collegiate athletic Association (NCAA).It takes most people about 8 cups of coffee providing 100mg/cup to reach this urine concentration. The study also focused on whether we can extract significant amount of caffeine using different content in tea sample. The caffeine is bitter, white crystalline xanthine alkaloid and a stimulant drug. It is found in varying quantities in the seeds, leaves and fruits of some plant. Part of the reason caffeine is classified by the Food and Drug Administration as GRAS (Generally Recognized as safe) is that toxic doses (over 10 grams for an average adult) are much higher than typically used doses(less than 500 milligrams).In this work the processes like extraction of caffeine from tea waste, caffeine detection by TLC, likewise HPLC of caffeine is performed and DPPH was conducted to check antioxidant activity of caffeine. By TLC the Rf value calculated. By the extraction of caffeine from different tea waste sample the extraction yielded 2-3 mg caffeine molecule.

KEYWORDS: caffeine, TLC, HPLC, Antioxidant activity.

I. INTRODUCTION

Caffeine is a filter substance, obtained from tea waste or coffee, or from the dried leaves of *Camelliasinesis.* It is the principal alkaloid constituent of tea, coffee and some other beverages, caffeine (1,3,7-trimethyxanthanine) exists as an odourless compound which appears as white powder silky glistening needles which has a bitter taste. Caffeine is naturally occurring chemical formula is $C_8H_{10}N_4O_2$. Caffeine is most commonly used to improve mental alertness. Caffeine acts on the central nervous system facillating performance of muscular work. This also acts on cardiac muscles of kidneys [1].

For centuries, tea and coffee have been regarded as the most popular beverages because primarily they are easily available but they contain a stimulant caffeine which stimulate respiration , the central nervous system of human body and also knows to be diuretic (promotes urination). Excessive use of caffeine can be addicted and could result in breast cancer , coronary diurination and myocardial infraction. However, the long term health effects of moderate use of caffeine have not been associated with any medical problems in the absence if other risk factors smoking and alcohol.

Tea waste can be used at broad level to recover the residual caffeine. They are good antioxidant agents and have been used to enhance the shelf life of food products. Therefore, these waste materials can be utilized for production of valuable chemicals such as caffeine and catechins. But an economical and Eco-friendly extraction method should be used for separations of these chemicals from tea waste.

Tea waste is virtually as rich in effective antioxidant, like catechins. Chagra, the used tea leaves, is used for various purposes. The remaining brewed tea can be cooled and use4d to water plants, and infused tea leaves may be scattered in the garden flower as an nutrient enricher . The caffeine content of tea leaves varies with tea type, but the normal range goes from 2.5% (dry weight w/w) together with small amount of the estimated that caffeine consumption will raise even further , due to widening spread of the cultures, the supposition of caffeine thermogenic properties and ever increasing consumption of energy drinks. Extraction time and consumed energy is an important issue to process the waste materials at large quantities. For extraction purpose firstly the sources from where the tea is generated has to be identified [2].

Caffeine is called with different names: guaranine hen found in gaurana, mateine when found in mate, and theine when found in tea. The organic solvent dichloromethane is used to extract caffeine from an aqueous extract of tea powder. The caffeine is used as growth stimulant for plants. It was observed that the rapid growth of plants. Extraction of caffeine from tea waste is environmentally safe practice which leads to the caffeine generation as well as waste management and minimization. Caffeine can be an astonishing thing, specially in the area of fitness It can increase mental acuteness, performance, and stamina. Caffeine delivery through the skin to achieve systematic effect of a drug is commonly known as transdermal drug delivery and differs from traditional topical drug delivery. Various transdermal caffeine patches have been used as weight loss aids and energy sources [3].

Today caffeine is used much as stimulant drug. It provides aboost of drinks or a feeling of heightened alertness. Many workers can drink tea or coffee frequently in order to get briskness. Likewise, drivers on long road trips often fill their cup holders with energy drink or convenience store coffees to help them push through to there destinations. Overall , Caffeine is present in the beans, leaves and fruits of over 60 plants, where it acts as a natural pesticide that paralyses and kills certain insect feeding on them. Caffeine is used in broncho pulmonary dysplasia of premature infants for treatment it may stimulate the weight gain during therapy and reduced the incidence of cerebral pain as well as reduced language and cognitive delay. A Caffeine transdermal patches can prove to be good choice for people who get upset stomachs when they take caffeine drinks among with exercise. It is not delivered into your body system al at once. The purpose of this work was to use waste tea leaves from various sources for the extraction of caffeine. The extracted caffeine was checked for its antioxidant activity.

This study shows that tea waste should be utilized for production of valuable products using nontoxic solvent and substances at extremely short extraction periods. Caffeine concentration in urine over 15 mg/ml is prohibited. The aim of this study is to determine the concentration of caffeine in different tea samples. TLC, HPLC methods are use for analysis the amount of caffeine in different sample.

2. MATERIALS AND METHODS

2.1 Sample Material Collection

The different Tea waste sample were collected from college campus and local stalls in Aurangabad. Sugarless tea waste sample and without milk tea waste sample were collected from household means. The normal tea waste sample containing milk + sugar + tea powder and water was collected from local stall.

2.2 Preparation of samples

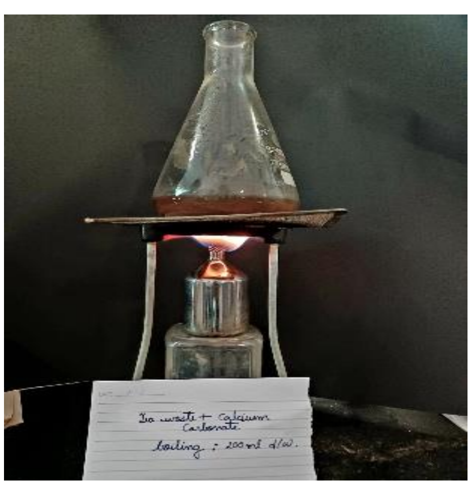


Fig No. 1: Boiling of sample mixture

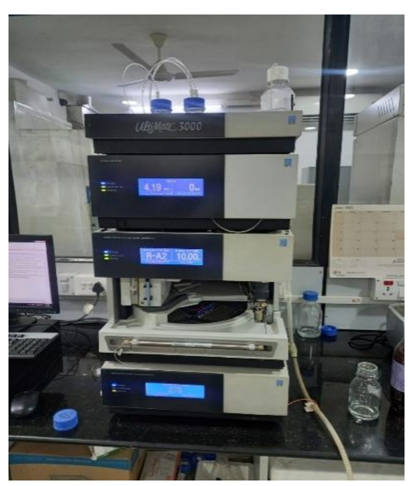


Fig No.2: HPLC set up for analysis of sample.

Extraction of Caffeine from Tea waste

Tea waste of 20grams + Calcium carbonate 1gram + distilled water 200ml and mix well

Keep this mixture on burner (burner = heating purpose)

This mixture was filtered in conical flask by using funnel and muslin cloth or filter paper.

Chloroform 10ml was taken in test tube and add this chloroform and the filtrate in separating funnel.

Shake slightly (not vigorously)

Let the solution get steady.

After 1 min the tap of separating funnel opened very carefully and gently. Therefore the Chloroform present at bottom of separating funnel was carefully removed and collected in beaker.

Then this beaker was placed in waterbath till the Chloroform gets evaporated.

After evaporation of Chloroform the Greenish substance remains at bottom of the beaker i.e the crude caffeine.

Then water and Charcoal was added to it. After application of charcoal if any impurities are present in crude caffeine gets remove.

Filtered substance will be caffeine(needle like structure).

3. Characterization of extracted caffeine.

3.1 Thin layer Chromatography :

There are different types of chromatographic methods such as paper chromatography, thin layer chromatography, column chromatography, gas chromatography, etc. They have same principle:

Different solutes have different solubility in a solvent /different solutes have different degrees of tendency to be dissolved in the same solvent.

As the solution moves along a stationary solid surface, different solutes absorbed onto the solid surface in different extent as they have different degree of adsorption characteristics.

Different solutes will then be separated on the different position of the solid surface.

In this experiment Purity of Caffeine compound was checked by using Thin Layer Chromatograph and compared with standard caffeine. The test sample was dissolved in chloroform and spotted on a TLC plates with Silica Gel (stationary phase) using solvent system Chloroform: acetone: methanol (4:3:3 V/V). The plates were observed in iodine vapor.

3.2 High performance liquid chromatography analysis :

High performance liquid chromatography (HPLC) analysis was performed in department of Biosciences and Technology MGM University to determine amount of caffeine present in the tea waste sample. The HPLC type was of Reverse Phase HPLC. The mobile phase used was HPLC grade Methanol: Water (25: 75). The flow rate was adjusted to1ml/min and detection was done at 276 nm. Extracted yields were calculated by comparing with standard caffeine.

Injection volume=20µl

Column=C_{18}

Solvent system=Methanol: water in 25;75

Flow rate of mobile phase =1ml/ min

Retention time=10 min

Wave Length =276 nanometer

Temperature =37ºC

Umber color vials / glass vials= Glass Bottle (sample required used vials)

Standard stock=5ml

Caffeine=5mg/5ml chloroform (stock)

Syringe filters and HPLC Water was used.

HPLC reagent bottle were prepared and was placed in RA_1, RA_2, RA_3, RA_4, RA_5, RA_6 column of HPLC.

3.3 Antioxidant Activity analysis of caffeine by DPPH method.

DPPH (2 ,2-diphenylpicrylhydrazyl). DPPH is a free radical, crystalline purple in colour which is insoluble in water. Therefore organic solvent like methanol, ethanol etc. are used. DPPH is light sensitive in nature .Antioxidant caffeine react with DPPH and the colour of solution changes to clear, transparent solution.

Absorbance was measured at 517nm.

Standard used Gallic acid in d/w

Sample caffeine was dissolved in chloroform.

0.04 mg of DPPH used in solvent methanol.

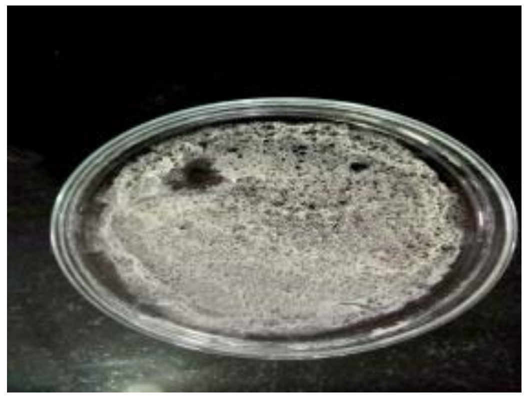
Blank= Methanol

Control= DPPH + methanol

4. RESULT AND DISCUSSION

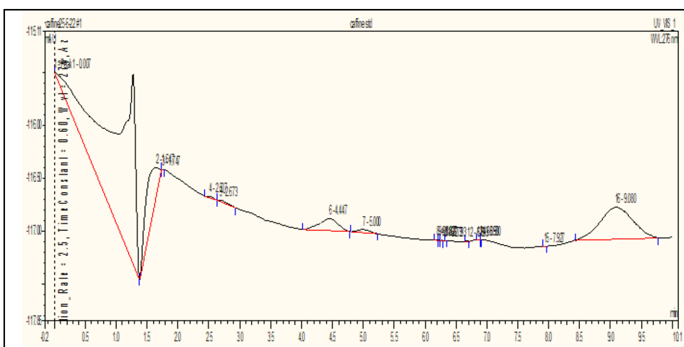
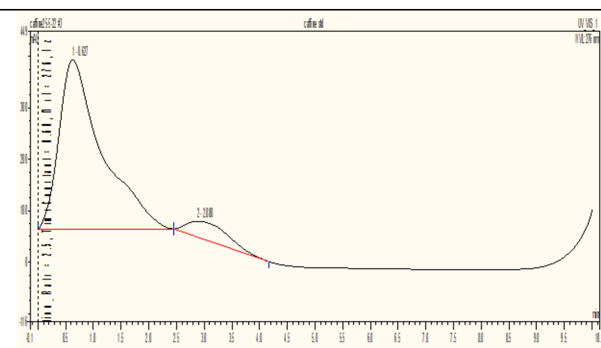
4.1 Result of TLC

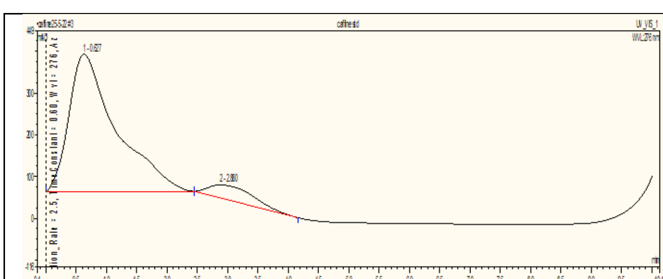
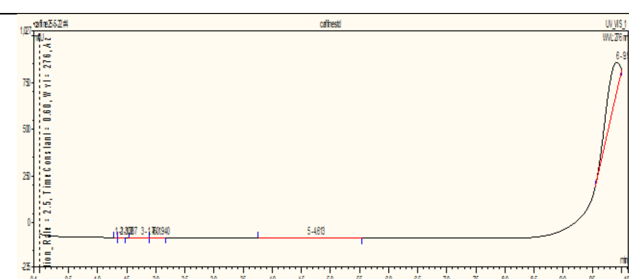
Rf value = Distance travelled by sample/distance travelled by solvent = 4.5/5=0.9

	
Fig no.3 TLC of Caffeine	**Fig no. 4 Extracted Caffeine**

All the Three types of extracted caffeine sample had the similar retention factor. Value ranging between 0.8-1

4.2 Result of HPLC: Caffeine, Solvent for caffeine sample used is chloroform

	
Graph no.1. **Sample 1: 200 µl sample + 800µl chloroform**	**Graph no.2.** **Sample 2: 400µl sample+600µl chloroform**

	
Graph no.3. **Sample 3: 600µl sample + 400µl chloroform**	**Graph no.4.** **Sample 4: 800µl sample + 200µl chloroform**

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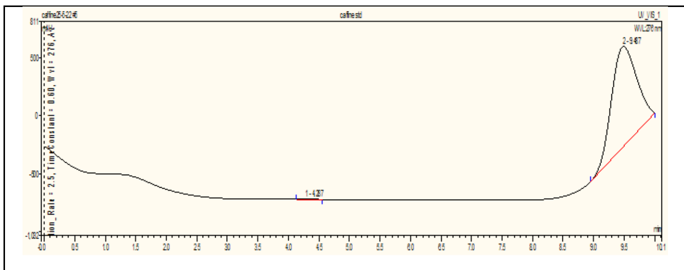
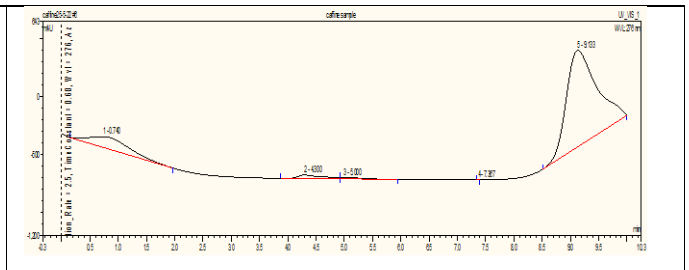
	
Graph no. 5 **Sample 5: 1ml stock solution**	**Graph no. 6** **Sample 6: 1ml sample**

Sample 1:

Table 1:integration

No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
		min	mAU*min			mAU	%	
1	Peak 1	0.007	0.7799	1	BMB	0.009	69.28	n.a.
2	n.a.	1.647	0.1069	n.a.	BMb	0.276	9.49	n.a.
3	n.a.	1.747	0	n.a.	bMB	0.001	0	n.a.
4	n.a.	2.507	0.0017	n.a.	BMB	0.014	0.15	0.64
5	n.a.	2.673	0.003	n.a.	BMB	0.013	0.27	3.91
6	n.a.	4.447	0.0403	n.a.	BMB	0.113	3.58	1.16
7	n.a.	5	0.0066	n.a.	BMB	0.029	0.59	5.52
8	n.a.	6.187	0.0001	n.a.	BM	0.004	0.01	0.94
9	n.a.	6.227	0.0001	n.a.	MB	0.003	0.01	1.02
10	n.a.	6.273	0.0001	n.a.	BMB	0.002	0	1.59
11	n.a.	6.333	0	n.a.	BMB	0.002	0	7
12	n.a.	6.693	0.0002	n.a.	BMB	0.004	0.01	2.66
13	n.a.	6.853	0.0001	n.a.	BMb	0.004	0.01	n.a.
14	n.a.	6.9	0	n.a.	bMB	0.001	0	n.a.
15	n.a.	7.927	0.0001	n.a.	BMB	0.002	0.01	2.21
16	n.a.	9.08	0.1867	n.a.	BMB	0.301	16.58	n.a.
Total:			1.1256	1		0.777	100	

Table 2: calibration

No.	Peak Name	Cal.Type	#Points	Rel.Std.Dev.	Coeff.Det.	Offset	Slope	Curve
				%	%			
1	Peak 1	Lin	1	0	100	0	0.7799	0
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Average:			1	0	100	0	0.7799	0

Table 3: peak analysis.

No.	Peakname	Ret.Time	Peak Width	Height	Type	Resol.	Asym.	Plates
n.a.	n.a.	min	min	mAU		(EP)	(EP)	(EP)
1	Peak 1	0.007	n.a.	0.009	BMB	n.a.	n.a.	n.a.
2	n.a.	1.647	0.34	0.276	BMb	n.a.	0.66	203

3	n.a.	1.747	n.a.	0.001	bMB	n.a.	1.53	n.a.
4	n.a.	2.507	0.25	0.014	BMB	0.64	1.36	2975
5	n.a.	2.673	0.25	0.013	BMB	3.91	3.85	1017
6	n.a.	4.447	0.63	0.113	BMB	1.16	0.87	963
7	n.a.	5	0.33	0.029	BMB	5.52	1.12	2716
8	n.a.	6.187	n.a.	0.004	BM	0.94	n.a.	277019
9	n.a.	6.227	n.a.	0.003	MB	1.02	n.a.	418067
10	n.a.	6.273	n.a.	0.002	BMB	1.59	0.9	222072
11	n.a.	6.333	n.a.	0.002	BMB	7	0.89	1281807
12	n.a.	6.693	n.a.	0.004	BMB	2.66	0.71	110004
13	n.a.	6.853	n.a.	0.004	BMb	n.a.	1.32	471170
14	n.a.	6.9	n.a.	0.001	bMB	n.a.	0.98	n.a.
15	n.a.	7.927	n.a.	0.002	BMB	2.21	1.21	354550
16	n.a.	9.08	0.99	0.301	BMB	n.a.	1.03	1333
Average:			0.4639	0.0486		2.67	1.26	241838

Table 4: summary

Sample Name	Ret.Time	Area	Height	Amount	Type	Plates
	min	mAU*min	mAU			(EP)
	Peak 1	Peak 1	Peak 1	Peak 1	Peak 1	Peak 1
	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
caffinestd	0.007	0.7799	0.0091	1	BMB	n.a.
caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
caffine sample	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Sample 2:

Table 5. Integration

No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution	
		min	mAU*min			mAU	%		
1	n.a.	1.673	142.0605	n.a.	BMb	123.675	90.29	1.63	
2	n.a.	5.007	15.2782	n.a.	bMB	10.261	9.71	n.a.	
Total:			157.3387	0		133.936	100		

Table 6: Calibration

No.	Peak Name	Cal.Type	#Points	Rel.Std.Dev.	Coeff.Det.	Offset	Slope
				%	%		
n.a.	Peak 1	Lin	1	0	100	0	0.7799
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Average:			1	0	100	0	0.7799

Table7: Peakanalysis

No.	Peakname	Ret.Time	Peak Width	Height	Type	Resol.	Asym.	Plates

n.a.	n.a.	min	min	mAU		(EP)	(EP)	(EP)
1	n.a.	1.673	1.43	123.675	BMb	1.63	1.32	22
2	n.a.	5.007	2.12	10.261	bMB	n.a.	1.75	56
Average:			1.772	66.9682		1.63	1.54	39

Table 8: summary

Sample	Sample Name	Ret.Time	Area	Height	Amount	Type	Plates
No.		min	mAU*min	mAU			(EP)
		[1.63 - 1.72]	[1.63 - 1.72]	[1.63 - 1.72]	[1.63 - 1.72]	[1.63 - 1.72]	[1.63 - 1.72]
		UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	caffinestd	1.647	0.1069	0.2758	n.a.	BMb	203
2	caffinestd	1.673	142.0605	123.6754	n.a.	BMb	22
3	caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	caffine sample	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Average:		1.66	71.0837	61.9756	n.a.		113
Rel.Std.Dev:		1.14%	141.21%	140.79%	n.a.		113.77%

Sample 3:

Table9: integretion

No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
		min	mAU*min			mAU	%	
1	n.a.	0.627	296.1288	n.a.	BMb	329.528	90.89	1.64
2	n.a.	2.88	29.6795	n.a.	bMB	30.901	9.11	n.a.
Total:			325.8082	0		360.428	100	

Table 10: callibration

No.	Peak Name	Cal.Type	#Points	Rel.Std.Dev.	Coeff.Det.	Offset
				%	%	
n.a.	Peak 1	Lin	1	0	100	0
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Average:			1	0	100	0

Table 11: peak analysis

No.	Peakname	Ret.Time	Peak Width	Height	Type	Resol.	Asym.	Plates
n.a.	n.a.	min	min	mAU		(EP)	(EP)	(EP)
1	n.a.	0.627	1.22	329.528	BMb	1.64	1.89	4
2	n.a.	2.88	1.32	30.901	bMB	n.a.	1.84	57
Average:			1.2703	180.2142		1.64	1.87	31

Table 12: summary

No.	Peakname	Ret.Time	Peak Width	Height	Type	Resol.	Asym.	Plates

n.a.	n.a.	min	min	mAU		(EP)	(EP)	(EP)
1	n.a.	0.627	1.22	329.528	BMb	1.64	1.89	4
2	n.a.	2.88	1.32	30.901	bMB	n.a.	1.84	57
Average:			1.2703	180.2142		1.64	1.87	31

Sample 4:

Table 13. Integration

No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
		min	mAU*min			mAU	%	
1	n.a.	1.307	0.0008	n.a.	BMB	0.024	0	0.91
2	n.a.	1.387	0.0323	n.a.	BMB	0.445	0.06	1.86
3	n.a.	1.76	0.0326	n.a.	BMb	0.18	0.06	0.56
4	n.a.	1.94	0.0054	n.a.	bMB	0.023	0.01	4.74
5	n.a.	4.613	0.3285	n.a.	BMB	0.563	0.61	8.39
6	n.a.	9.927	53.1341	n.a.	BMB	149.483	99.25	n.a.
Total:			53.5337	0		150.719	100	

Table 14: callibration

No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution	
		min	mAU*min			mAU	%		
1	n.a.	1.307	0.0008	n.a.	BMB	0.024	0	0.91	
2	n.a.	1.387	0.0323	n.a.	BMB	0.445	0.06	1.86	
3	n.a.	1.76	0.0326	n.a.	BMb	0.18	0.06	0.56	
4	n.a.	1.94	0.0054	n.a.	bMB	0.023	0.01	4.74	
5	n.a.	4.613	0.3285	n.a.	BMB	0.563	0.61	8.39	
6	n.a.	9.927	53.1341	n.a.	BMB	149.483	99.25	n.a.	
Total:			53.5337	0		150.719	100		

Table 15: peak analysis

No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
		min	mAU*min			mAU	%	
1	n.a.	1.307	0.0008	n.a.	BMB	0.024	0	0.91
2	n.a.	1.387	0.0323	n.a.	BMB	0.445	0.06	1.86
3	n.a.	1.76	0.0326	n.a.	BMb	0.18	0.06	0.56
4	n.a.	1.94	0.0054	n.a.	bMB	0.023	0.01	4.74
5	n.a.	4.613	0.3285	n.a.	BMB	0.563	0.61	8.39
6	n.a.	9.927	53.1341	n.a.	BMB	149.483	99.25	n.a.
Total:			53.5337	0		150.719	100	

Table 16: summary

No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
		min	mAU*min			mAU	%	
1	n.a.	1.307	0.0008	n.a.	BMB	0.024	0	0.91
2	n.a.	1.387	0.0323	n.a.	BMB	0.445	0.06	1.86
3	n.a.	1.76	0.0326	n.a.	BMb	0.18	0.06	0.56
4	n.a.	1.94	0.0054	n.a.	bMB	0.023	0.01	4.74

5	n.a.	4.613	0.3285	n.a.	BMB	0.563	0.61	8.39
6	n.a.	9.927	53.1341	n.a.	BMB	149.483	99.25	n.a.
Total:			53.5337	0		150.719	100	

Sample 5:

Table 17: integration								
No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
		min	mAU*min			mAU	%	
1	n.a.	4.287	0.0587	n.a.	BMB	0.26	0.01	9.13
2	n.a.	9.487	409.008	n.a.	BMB	864.896	99.99	n.a.
Total:			409.0667	0		865.156	100	

Table 18: callibretion								
No.	Peak Name	Cal.Type	#Points	Rel.Std.Dev.	Coeff.Det.	Offset	Slope	Curve
				%	%			
n.a.	Peak 1	Lin	1	0	100	0	0.7799	0
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Average:			1	0	100	0	0.7799	0

Table 19: peak analysis								
No.	Peakname	Ret.Time	Peak Width	Height	Type	Resol.	Asym.	Plates
n.a.	n.a.	min	min	mAU		(EP)	(EP)	(EP)
1	n.a.	4.287	0.34	0.26	BMB	9.13	1.46	2175
2	n.a.	9.487	0.77	864.896	BMB	n.a.	1.09	2398
Average:			0.5546	432.578		9.13	1.27	2287

Table 20: summary									
No.	Peakname	Ret.Time	Peak Width	Height	Type	Resol.	Asym.	Plates	
n.a.	n.a.	min	min	mAU		(EP)	(EP)	(EP)	
1	n.a.	4.287	0.34	0.26	BMB	9.13	1.46	2175	
2	n.a.	9.487	0.77	864.896	BMB	n.a.	1.09	2398	
Average:			0.5546	432.578		9.13	1.27	2287	

Sample 6:

Table 21: integration								
No.	Peakname	Ret Time	Area	Amount	Type	Height	Rel area	Resolution
		min	mAU*min			mAU	%	

1	n.a.	0.74	80.5706	n.a.	BMB	94.196	13.1	3.17
2	n.a.	4.3	15.9729	n.a.	BM	31.264	2.6	n.a.
3	n.a.	5	4.8084	n.a.	MB	11.675	0.78	n.a.
4	n.a.	7.367	0.0002	n.a.	BMB	0.007	0	3.6
5	n.a.	9.133	513.729	n.a.	BMB	829.633	83.52	n.a.
Total:			615.0812	0		966.774	100	

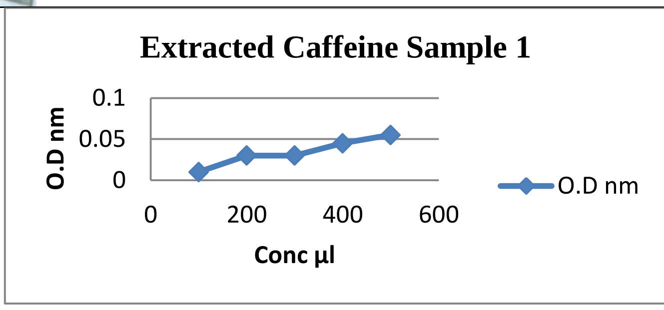
Table 22: peak analysis								
No.	Peakname	Ret.Time	Peak Width	Height	Type	Resol.	Asym.	Plates
n.a.	n.a.	min	min	mAU		(EP)	(EP)	(EP)
1	n.a.	0.74	1.31	94.196	BMB	3.17	1.48	5
2	n.a.	4.3	0.67	31.264	BM	n.a.	n.a.	372
3	n.a.	5	1.84	11.675	MB	n.a.	n.a.	n.a.
4	n.a.	7.367	n.a.	0.007	BMB	3.6	0.99	494118
5	n.a.	9.133	0.95	829.633	BMB	n.a.	1.45	1503
Average:			1.1927	193.3548		3.39	1.3	124000

Table 23: callibration									
No.	Peak Name	Cal.Type	#Points	Rel.Std.Dev.	Coeff.Det.	Offset	Slope	Curve	
				%	%				
n.a.	Peak 1	Lin	1	0	100	0	0.7799	0	
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Average:			1	0	100	0	0.7799	0	

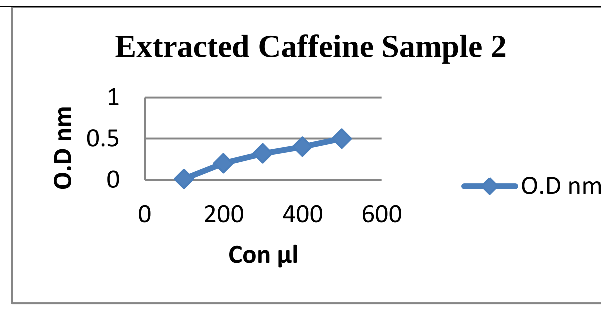
Table 24: summary								
Sample	Sample Name	Ret.Time	Area	Height	Amount	Type	Plates	
No.		min	mAU*min	mAU			(EP)	
		[0.72 - 0.76]	[0.72 - 0.76]	[0.72 - 0.76]	[0.72 - 0.76]	[0.72 - 0.76]	[0.72 - 0.76]	
		UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	
1	caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
2	caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
3	caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
4	caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
5	caffinestd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
6	caffine sample	0.74	80.5706	94.1955	n.a.	BMB	5	
Average:		0.74	80.5706	94.1955	n.a.		5	
Rel.Std.Dev:		n.a.	n.a.	n.a.	n.a.		n.a.	

4.3 Result of DPPH:

Graph of caffeine extracted and the std. taken for DPPH was in straight line.

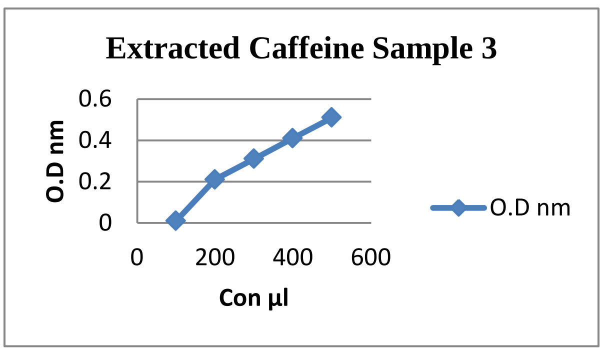


Graph no.7: sample 1



Graph no. 8: sample 2

Graph no. 9



Graph no.10

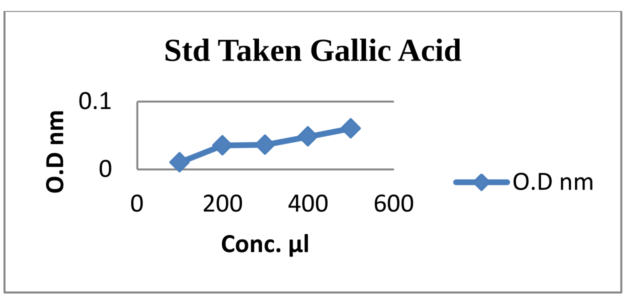


Fig no.5. DPPH of Caffeine

5. CONCLUSION

The purpose of the work was to use tea waste from various sources for extraction of caffeine. Tea is an antioxidant and widely used in beverages all over the world. It also has medicinal properties. This extracted caffeine can be used with combination of painkiller to cease the severity of pain in body. By performing DPPH we get to know that caffeine is having antioxidant activity, likewise qualitative and quantitative analysis of caffeine was done by HPLC.

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