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Effect of Drying and Extraction Methods on Phytochemicals and Free Radical Scavenging Activity of Camellia assamica

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ABSTRACT

The results achieved from this research demonstrated that scavenging activity of ***Camellia assamica,*** tea increased significantly during freeze drying operation when comparing with vacuum dryer and oven dryer. Freeze drying is found as finest method and the product shows outstanding results regarding phenolic content and scavenging potency. Moreover among the three extraction methods we found that ethanol extraction was best regarding the determination of the phenolic content, flavonoid content and free antioxidant activity. Interestingly, the best rehydration capability of green tea was also found on freeze drying. Our study also demonstrated the presence of wide variation in caffeine content between different tea samples studied. In this research, we found that the caffeine content was approximately between 2.89% - 4.1%. The variation of caffeine content depending on some major factors like diversity of tea, location, time of cultivation, age and other climatological conditions for tea plantation, it is therefore reasonable that the previous factors might be responsible for the variation of caffeine present in tea samples. We also observed that maximum amount of caffeine was found in sample 1 i.e. Mithinga Tea Garden. Tea leaves lose their tannin contents with increase in age analyzed that tannin occurs at almost 4.5%. This difference may be by cause of different variety selected for analysis and also for different manufacturing process. Overall, our study reflects that ethanol extraction and freeze drying method was found to be the finest in terms of determining the constituients of tea.

Keywords: *Freeze drying; Vacuum drying; Oven drying; Phenolic content; Flavonoid; Radical scavenging; Tannin; Caffeine.*

1. INTRODUCTION:

Tea is among the most universaly consumed beverage after water. It is considerd as a refreshing drink consumed since ancient times. It also has huge beneficial effects on our health including antioxidant and anticancer effects. Assam tea is popular for its distinct flavour and assam is also one of the largest producer of tea in India. Assam tea is manufactured specifically from the plant *Camellia sinensis* Var. *assamica* (J. Masters) .Assam is considered as one of the largest tea producing region in the world. There are generally two types of tea is produced, green and black tea, both of the varaities contain caffeine (1-5%) along with little amount of xanthine alkaloids. The principal compounds are phenolic compounds, which maintains the quality of tea, well known as catechins, it considered as compounds belongs to the flavonoid ancestry. Catechins may contained in (5-27%) of the dried tea leaf (Leung and Foster,1996) which are divided into four primary compounds epigallocatechin(EGCG), epicatechingallate (ECG), epigallocatechin(EGC), epicatechin (EC) (Amra et al.,2006; salova et al.,2013). EGCG constituting (10-50%) of catechins and has the potential because of the degree of hydroxylation and gallation (Pellilo et al., 2002). It is also the main subject of scientific study regarding its potential health effects (Kanwar et al., 2012). Polyphenols in green tea

are also an outstanding free radical scavengers. Several clinical studies demonstrated that polyphenols has a good anticarcinogenic effect . Polyphenols have been acknowledged that it has antioxidant, anti-mutagenic, anticarcinogenic effects and also has protective effect for many health complications (Michael, 1999; John 2008). The drying process is a critical step in manufacturing of green tea in which several physical and chemical changes occurs which affect the product quality. Most important part of tea quality includes structural, optical, sensory, thermal, rehydration and nutritional properties (Liang, 2007). The heat pump drying can also be applied in tea production. 32% of energy can be saved by using water heat pump drying compared to hot air oven drying for tea leaves (Xie, Song and Yang, 2006). Spectrophotometric method is the best to determine the amount of components present in tea leaves.

2. MATERIAL AND METHODS

In this study, we collected tea leaves (samples) from Kokrajhar District such as Mithinga Tea Garden (sample 1), Greenland Tea garden (sample 2), Brahma Tea Garden (sample 3), Alongbar Tea Garden (sample 4). During this study, we report the method for the determination of free radicals (DPPH), Total Phenolic Content (TPC), Total Flavonoid Content (Chen YZ, Chan PT, Ma HM. Fung KP and Wang), Catechins, Caffeine and amount of tannins present. The study includes extraction of the samples with water from fresh tea leaves and to determine by Spectrophotometer. The absorption spectra were recorded on a Perkin-Elmer lambda 19 Spectrohotometer with a wavelength range of 170-3200nm.It consist of radiation source, monochromator, sample area, photometer and detection area.

2.1 Methods of drying:

We have carried out drying process in three different ways namely hot air drying, lyophilization (freeze drying), vacuum drying and then the changes in phenolic, flavonoid and antioxidant property has been noted.

2.2 Methods of extraction:

Extraction process has been accomplished in three different ways namely Hot water extraction, ethanol extraction and cold water extraction and the changes in phenolic, flavonoid and antioxidant properties has been noted. (DrazenkaKomes, et. *al*;2009)

2.3 Materials and methodology

The fresh tea leaf samples (*Camellia assamica*) used in this study were collected from assam. We have made the study in this sample because assam tea is famous throughout the world. The collected samples were performed in Oven Drying, Freeze Drying and Vacuum Drying at different temperatures with respect to time.

2.3.1 Determination of Moisture Content of Tea:

Water content or moisture is the quantity of water contained in a material. The moisture of the samples were determined in an apparatus called “Moisture Analyser” (Brand-Sartorius) for 10 min at 105ºC. Moisture content of food sample was calculated by using following formula- Percentage of

Moisture Content (wb)

$$= \frac{\text{Initial weight of the sample} - \text{Final weight of the sample}}{\text{Iinitial weight of the sample}} \times 100$$

2.3.2 Antioxidant assay:

Free radical Scavenging assay (DPPH):

Free radical scavenging activity of the extract was measured based on the process explained by Blois (Blois, M.S., 1985).100µl concentration of DPPH (µg) solution is prepared. To a known aliquot (0.1 ml) of the juice, 3.9ml of DPPH (1,1- diphenyl 2-picrylhydrazyl) solution in 100ml ethanol/methanol was added, followed by the incubation in a dark place for around 45 min at ambient temperature (30ºC). The reduction in absorbance (due to proton donating activity) was measured at 515nm using a spectrophotometer (EI-instrument, Model no 2375). For the accuracy of the results 2 replication of each sample was used. The radical scavenging activity calculated by

$$= \frac{Ab - As}{Ab} \times 100$$

Ab= absorbance of the blank

As= absorbance of the sample

2.3.3**Total Phenolic Content by the Folin-Ciocalteau's Assay:**

Materials and Methodology:

The research required a Sample (tea extract), Micropipette ,Test Tubes, Distilled water, Folin-Ciocalteau's phenol reagent, Sodium Carbonate (Na_2CO_3) and Spectrophotometer. The first step was to prepare an aliquot 1ml of tea extract was added into 25 ml volumetric flask containing 9ml of distilled water. Then a reagent blank using distilled water was also prepared. 1ml of Folin-Ciocalteau's reagent was put into the solution and mixed for 5 min, then 10 ml solution of 7% Na_2Co_3 was put into the solution. It was then diluted with 25 ml of water.Then incubation is done for 90 min at ambient temperature and the absorbance was noted at 784 nm against the blank sample with the help of spectrophotometer. The spectrum was taken by pouring the solution into 1cm cuvette and placed into the spectrophotometer. The value of the total phenolic contents of samples were expressed by the unit µgs of gallic acid equivalent (GAE) per gm dry mass (µg GAE/100 g dw)

2.3.4 Total Flavonoid Content by the Aluminium Chloride Assay:

Materials and Methodology:

For this experiment purpose, Micropipette, Test tubes, Distilled water, Sodium Nitrite($NaNo_2$), Aluminium Chloride($AlCl_3$), NaOH (1M),Spectrophotometer (EI- instruments, Model No 2375) have

taken along with the tea samples. The first step in the experiment was to prepare an aliquot (1ml) of extracts or standard solution of catechin was added into a 10 ml of volumetric flask containing 4ml of distilled water. Then 0.3 ml 5% $NaNO_2$ was taken into a flask. Then after 5 min 0.3ml 10% $AlCL_3$ was added. At the 6^{th} minute 2ml 1M NaOH was added into it to make the total volume up to 10ml with water. Then it was mixed well and the absorbance was measured against the blank sample at 570nm with an UV-VIS spectrophotometer. The spectrum was taken by pouring the solution into 1cm quartz cuvette and put into the spectrophotometer. The unit of total flavonoid contents is mg of catechin equivalents /100 gms of dry sample.

2.3.5 Estimation of Tannin percentage (Copper Acetate Method)

About 0.5 gm of powdered tea is added with 50 ml of water , then the solution is allowed to boil at about 50-60C for 1hr.Then the sample is filtered using a filter paper. Then, 7.5 ml of 5% copper-acetate solution is mixed with filtrate. The Cu-tannate precipitation is collected allowed to dry and weight is taken. The Tannin present in tea is calculated.

Note: For each1g of Cu-tannate is equal with the 0.964g of tannic acid.

2.3.6 Determination of Caffeine

Bunsen burner, separating funnel, Erlenmeyer flask, iron stand, evaporating dish, clamp, glass rod,unflavored tea bags, and sodium carbonate, dichloromethane were used for this experiment.The first step in the experiment was to weight 4.4 g of sodium carbonate which and pre-heated 100ml of distilled water was added in a small Erlenmeyer flask. Apart from that, 10 g of measured samples by the triple beam balance was kept into the mixture by putting into a tea bag. It was then boiled for 10 minutes on a low flame by covering the flask. After the boiling, the tea bags were removed from the mixture by squeezing to remove the excess liquid against the side of the flask with the help of a glass rod. This process should be done very carefully to prevent the bag from damage to avoid further purification. After that the mixture was cooled down for atleast 3 minutes and and transferred into a separatory funnel. Then the funnel was turned and the stop cock was opened which allows the pressure to be released. To mix, it was turned to release the inside pressure and the same process was repeated upto two minutes. After that the funnel was kept in a static condition until two separate layers was appears clearly. When the two seperate layers were evident, the bottom layer which was organic one drained into the Erlenmeyer flask. The solution (dichloromethane + caffeine) was decanted onto a pre weighed evaporating dish. It was evaporated until becomes powder and again the weight was taken along with the evaporating dish.

2.3.6 Quantitative Analysis of catechin:

For this process, 5gm sample and 100 ml of distilled water has been taken. Then put the tea sample in a beaker, then allowed to boil it for 10 min. After that filtration is done of the boiled solution by using funnel and watman paper to another beaker. Then Stored the residue aside and allowed to dry. Weight has been taken of the dry residue. Repeat the above step for another sample.

2.3.7 Determination of fluoride using ion-selective electrode method:

Preparation of fluoride standard solutions:

Atfirst, we repared a standard fluoride solutions whose concentrations vary by tenfold by using either the 0.1M, 1000ppm, or 100ppm fluoride standard. Then serial dilution techniques was used for this preparation. To a 150ml beaker, we have added 50ml of the lower value standard and 50ml of TISAB (Total ionic strength adjustment buffer). Then we placed the beaker on the magnetic stirrer at

a constant rate. Then we lowered the electrode into the sample. At this stage we have verified that the meter is on concentration mode. Then we adjusted the meter to the standard concentration and fix the value by following the instruction. After rinsing the electrodes by using distilled water it was allowed to dry completely. We have taken 150 ml beaker and put 50 ml of a higher value standard and 50 ml of TISAB into this and then we placed the beaker on the magnetic stirrer at a constant rate. Then we lowered the electrode into the solution and adjusted the meter to the standard concentration and set the value by control panel by following the instructions and maintained a slope of 90-100%.

3. RESULT AND DISCUSSION

3.1 Qualitative anyalysis of phytochemicals in TEA (Karanja M. simon, et al.)

3.1.1 Test for Flavonoids (Lead Acetate test)

Atfirst we have taken 1ml of each of the solution in separate test tubes and then 1ml of 5% lead acetate was added and the sample was allowed to rest at 25^0C temperature for two min. The presence of white precipitation in the samples indicated the presence of flavonoids.

3.1.2 Test for Phenolic Compounds (Lead Acetate)

For this test,5 ml of the extract was added into distilled water and 3ml of 10% lead acetate solution was put into the solution. The presence of dark green color was evidence of the presence of phenolic compound.

3.1.3 Test for Saponins (Foam Test)

For this test, in a graduated cylinder 2ml of sample solution was taken with 6ml of water. Then the solution was mixed properly by shaking and formation of persistent 2 cm foam was observed which ensures the it's presence.

3.1.4 Test for Steroids &Terpenoids (Salkowski test)

5 ml of the extract was treated with 2 drops of chloroform, acetic anhydride and conc.H_2SO_4.The mixture was appeared in a dark pink or red colour which proved the presence of Steroids & Terpenoids

3.1.5 Test for Tannin (Ferric Chloride test)

The determination of tannin in the test sample was carried out using ferric chloride test described by Harbone as reported by Osagie.2g powder of the sample was added into 10 ml of distilled water and the mixture was agitated for 30 min and the filtrate used as aqueous extract.2ml of the aqueous extract was kept in a test tube and 3ml of distilled water was put into it and agitated very well for homogenate, two drops of dil. FeCl3 was then put into the mixture. The development of a very dark precipitate ensures the existence of tannin in the sample.

3.1.6 Test for Glycoside (Legal's Test)

About 0.5 g of plant extract was taken with pyridine and sodium nitroprusside reagent in a test tube and make alkaline with the addition of NaOH solution and observed for the development of pink to red color, which ensures the existance of glycoside.

3.1.7 Test for lignin

About 2ml of 2% (w/v) furfuraldehyde was put into the test solution. Formation of red color proved the existance of lignin.

3.1.8 Test for anthraquinones (Modified Borntrager' test)

About 0.5g of the sample solution and 5ml of chloroform was taken in a test tube and mixed well for 5 min. Then the mixture was filtered and mixed well with same volume of 100% ammonia solution. Formation of pink, violet or red colour ensures the existance of anthraquinones in the sample.

3.2 Results

After oxidization, the tea leaves were dried by using hot air oven in different temperature with variation in time duration. The moisture present in tea samples performed under oven drying with respect to various temperatures were shown in Table1. In this study, it was observed that the moisture decreases with respect to temperature. Drying parameters was optimized on the basis of the defenseless parameters. In the experiment of caffeine two layers were formed the top and bottom layer. The top layer with less dense region is called aqueous layer and the bottom with more dense region which is organic. The organic layer constitutes of the dichloromethane and caffeine extracted and 4.4 % was computed after getting the ratio in between the weight of caffeine and weight of tea leaves used and was multiplied by 100, which is shown in Table 4.4. It means for every 10.00 grams of tea leaves, an amount of 0.04 grams of caffeine is contained, which in-accordence with the study done by (Bayquen, A.V., Cruz, C. T., et al. 2009).The result for tannins in the sample are presented in Table 4.4. Almost all analyzed samples contain tannins in the range of 3.4% - 16.38%. It was established that sample 3 has larger amount of tannins.Tea leaves lose their tannin contents with increase in age (Harller, 1964). Libert et al. (1999). In General, fluoride does not found in plants naturally and it comes from the insecticides and pesticides applied into it. After testing we found that the sample contained little amount of fluoride (1-5.96 ppm) given in Table 3 which on excessive consumption of tea beverages might create a risk for fluoride toxicity. The phenolic content or TPC measures the total phenols and polyphenols present in the sample, which is naturally obtained in tea. This has a great contribution for good flavor and taste and also has several benefits for health. After three types of drying (oven, vacuum and freeze) of the tea samples we found that the samples which was dryed by using vacuum dryer contain higher amount of phenolic compound, given in Table 4.4.Flavonoids are dietary compounds largly available in tea. They has significant contribution in taste and colour and also contribute to maintain the body function in a better way. It also helps to maintain healthy heart function. In our project work we found that the samples which was dryed by using in freeze dryer contain higher amount of flavonoid, given in Table 4.4. The samples were investigated for antioxidant potential using 2,2- diphenyl,1-picrylhydrazyl(DPPH). It works by reducing DPPH, which gives a highest absorption at 517nm with odd electrons. The reduction in absorbance is due to the antioxidants, which reacts with radicals, progresses and results in scavenging by donating hydrogen ions. The DPPH values were given in 4.4

Table 5:Phytochemical screening of Camellia assamica

Phytochemicals	**Test**	**Reagents**	**Positive results**	**Presence**		
				Vacuum	**Freeze**	**Oven**
Flavonoid	Lead Acetate Test	1ml of 5% lead acetate	Formation of white or creamy precipitate	+ve	+ve	+ve
Phenolic Compounds	Lead Acetate Test	3ml of 10% lead Acetate	Dark green colour	-ve	-ve	-ve
Tannin	Ferric Chloride	10% of alcoholic ferric chloride	Blue or green colour	+ve	+ve	+ve
Saponin	Foam Test	2ml of extract added to 6ml of Water	2cm presence of foam	-ve	-ve	-ve
Steroids &Terpenoids	Salkowski Test	2 drops of chloroform,acetic anhydride,conc.H2 SO4	Dark pink red colour, reddish brown	+ve	+ve	+ve
Glycoside	Legal's test	1ml of extract,3 drops of pyridine, sodium nitroprusside reagent, NaOH Solution	Pink to red colour	+ve	+ve	+ve
Anthocyanin	Sodium Hydroxide Test	1ml of sample,1ml of 2N NaOH	Bluish green colour	-ve	-ve	-ve
Anthraquinones	Modified Borntrager's test	0.5g of the extract was taken in a dry test tube and 5ml chloroform was taken and agitated for 5 min	A pink, violet or red colour in the ammonical layer	-ve	-ve	-ve
Lignin		2ml of 2% (w/v) furfuraldehyde was added	Formation of red color	+ve	+ve	+ve

Table 1.1: Moisture Content at various temperature. (Hot air oven):

Temperature (°C)	Time	Initial weight (g)	Final weight (g)	Moisture Content (%)
60	30	5	3.604	27.92
70	20	5	3.114	37.72
80	10	5	3.01	39.8

Table 1.2: Moisture Content at various temperature. (Vacuum drying):

Temperature (°C)	Time	Initial weight (g)	Final weight (g)	Moisture Content (%)
30	1hr 30 min	5	4.0069	19.86
45	1hr	5	3.125	37.5
70	45 min	5	2.99	40.2

Table 1.3:Moisture Content at 40°C temperature. (Freeze drying):

Temperature (°C)	Time	Initial weight (g)	Final weight (g)	Moisture Content (%)
-40	4hr	5	1.670	66.6

Table 2: Various data of Catechin present in tea Samples:

Samples	% of Catechin
Sample1	16.4
Sample2	29.2
Sample3	14.8
Sample 4	18.4

Table 3: Various Data for the Fluoride content in various tea samples :

Sample	pH	Fluoride (ppm)
Sample1	6.04	56.57
Sample2	6.17	41.13
Sample3	6.15	42.28
Sample 4	6.12	65.96

Table 4.1: Type of drying: Hot air oven dryer :

A. Ethanol extraction

Samples Name	Caffeine %	Tannin %	TPC (GAE µg/g)	TFC (CE µg/g)	DPPH %
Sample1	4.4±0.22	13.68±0.68	197.25±9.8	274.95±13.7	86.49± 4.3
Sample2	4.3±0.21	9.25±0.46	183.93±9.1	204.95±10.2	81.83±4.0
Sample 3	3.9±0.19	11.18±0.55	186.47±9.3	236.95±11.8	83.49±4.1
Sample4	3.1±0.15	14.07±0.70	171.37±8.5	242.95±12.1	84.28±4.2

B. Hot water extraction

Samples Name	Caffeine %	Tannin %	TPC (GAE µg/g)	TFC (CE µg/g)	DPPH %
Sample1	4.3±0.21	12.58±0.68	187.53±9.8	261.88±13.7	81.65± 4.3
Sample2	4.2±0.21	8.29±0.46	175.64±9.1	189.41±10.2	80.96±4.0
Sample 3	3.2±0.19	9.10±0.55	171.48±9.3	220.80±11.8	78.63±4.1
Sample4	2.8±0.15	11.17±0.70	163.60±8.5	232.05±12.1	76.90±4.2

Table 4.2: Type of drying: Vacuum dryer

A. Ethanol extraction

Samples Name	Caffeine %	Tannin %	TPC (GAE µg/g)	TFC (CE µg/g)	DPPH %
Sample1	4.3±0.21	16.96±0.84	203.72±10.18	441.95±22.0	89.96±4.4
Sample 2	4.9±0.24	16.58±0.82	164.31±8.21	349.45±17.4	89.05±4.5
Sample 3	4.1±0.20	16.77±0.83	168.23±8.4	355.45±17.7	89.26±4.6
Sample 4	4.6±0.23	14.46±0.72	167.84±8.3	350.45±17.5	88.74±4.5

B. Hot water extraction

Samples Name	Caffeine %	Tannin %	TPC (GAE µg/g)	TFC (CE µg/g)	DPPH %
Sample1	3.9±0.21	16.94±0.84	198.92±10.18	438.05±22.0	87.96±4.4
Sample 2	4.8±0.24	15.58±0.82	161.30±8.21	346.95±17.4	86.05±4.5
Sample 3	4.1±0.20	16.77±0.83	165.20±8.4	350.45±17.7	84.56±4.6
Sample 4	4.6±0.23	14.46±0.72	163.95±8.3	345.45±17.5	81.94±4.5

Table 4.3: Type of drying: Freeze dryer

A. Ethanol extraction

Sample Name	Caffeine %	Tannin %	TPC (GAE µg/g)	TFC (CE µg/g)	DPPH %
Sample1	3.6±0.18	17.35±0.86	219.21±10.96	469.45±23.47	94.99±4.7
Sample2	2.9±0.14	14.84±0.74	207.84±10.39	425.45±21.27	94.80±4.8
Sample 3	2.89±0.14	17.54±0.87	199.60±9.98	325.45±16.27	90.01±4.5
Sample 4	3.01±0.15	17.93±0.89	197.84±9.8	417.95±20.89	90.35±4.4

A. Hot water extraction

Sample Name	Caffeine %	Tannin %	TPC (GAE µg/g)	TFC (CE µg/g)	DPPH %
Sample1	3.5±0.18	16.35±0.86	211.31±10.96	463.45±23.47	91.99±4.7
Sample2	2.8±0.14	14.84±0.74	201.64±10.39	421.95±21.27	93.80±4.8
Sample 3	2.89±0.14	17.54±0.87	196.69±9.98	320.05±16.27	89.01±4.5
Sample 4	3.01±0.15	17.93±0.89	193.80±9.8	413.75±20.89	81.35±4.4

Table 4.4: For the last comparison

Types of Drying	Caffeine%	Tannin%	TPC(GAE µg/g)	TFC(CE µg/g)	DPPH%
Vacuum	4.1±0.20	16.96±0.84	203.72±10.18	336.95±56.43	89.96±4.4
Hot Air Oven	3.1±0.15	13.68±0.68	197.25±9.8	417.95±76.32	86.83± 4.3
Freeze	2.89±0.14	17.35±0.86	219.21±10.96	441.95±9.54	94.99±4.7

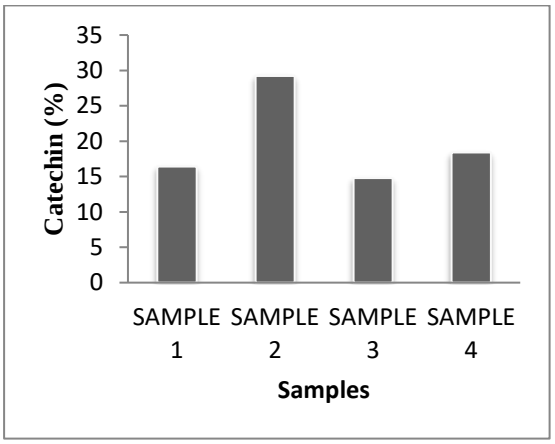

Fig1:Catechin present in tea samples

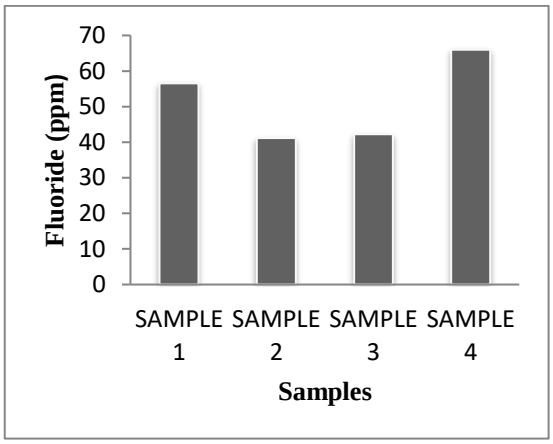

Fig2 :Fluoride present in tea samples

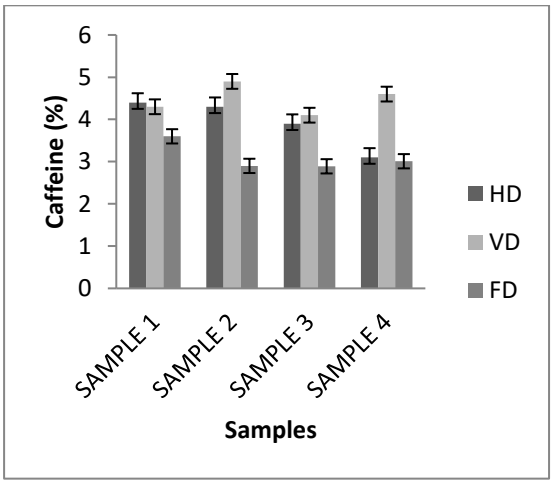

Fig 3: Caffeine content in Tea samples

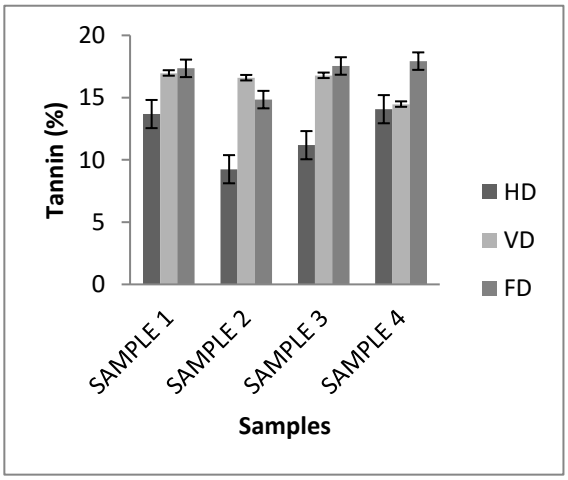

Fig 4: Tannin content in Tea samples

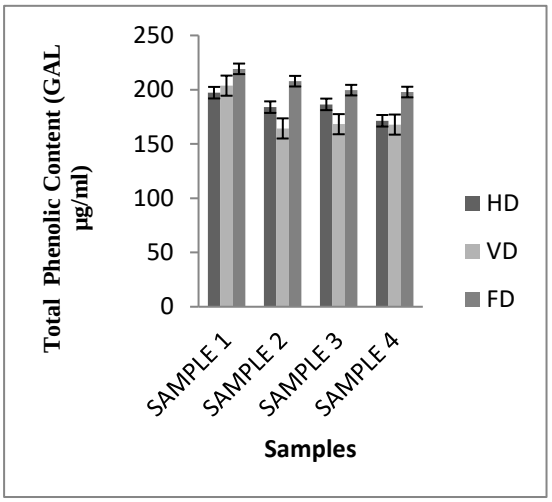

Fig 5: TPC in Tea samples

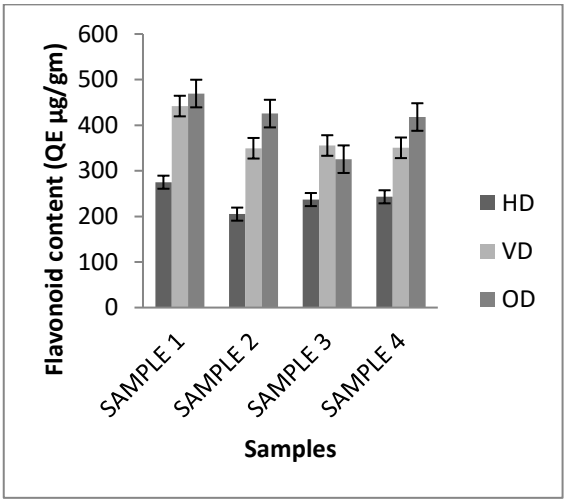

Fig 6: TFC in Tea samples

Discussion

Catechins (Fig 1)

Catechins are compound which helps to regulate blood pressure, increase weight loss and protect brain from illness. By this study we have found highest catechin content in sample 2 (29.2%) and 16.4%,14.8%, 18.4% in sample 1, sample 3 and sample 4 respectively. The sample 2 showed higher concentrations of catechin, when compared with the other samples. The significant difference obtained in catechin concentration between the samples by reconciling with the results reported in **Koch *et al*., 2018**. The difference is because of the variation in fermentation during production process. The processing of tea also involves natural fermentation, the autoxidation process has been catalyzed via polyphenol oxidase enzyme.

Caffeine Content (Fig 3)

Near 200 mg of caffeine has therapeutic effect and helps to stimulate central nervous system, help to reduce fatigue and to improvise our thoughts by increasing intellectual thoughts. Apart from that it also has a diuretic property which helps to maintain fluid balance in our body. In other side it also increase heartbeat, dilate blood vessels and increase the levels of free fatty acids and glucose in the plasma. 1 g caffeine can cause serious health issues like insomnia, nervousness, and nausea (**Wanyika *et al*., 2010**). By this study we found that higher caffeine in ethanol extracted samples compared with the hot water extraction of the same four tea samples. Steeping time is also a very important factor. According to **chin *et al*., 2008** , Increasing steeping time will increase caffeine extraction from tea leaves. We also found lower caffeine in freeze dryed samples (Fig 3). **Nan and Phu, 2012** have shown in their research that the caffeine extraction will increase with temperature, thus we found higher caffeine content in oven and vaccum dryed samples compared to freeze dryed samples. The difference in caffeine content between ethanol extracted samples and hot water extracted samples found by this study and according to **Koch *et al*., 2020** solvent has a very important role in extraction process.

TPC (Fig 5)

Air drying shows significant moisture loss in antioxidant activity and total phenolic content for all species **(Chan et al.2009)**. Drying process can release phenolic compounds by breaking down the cellular components of the sample **(Chang et al.2006; Arslan and Ozcan 2010)**.Another possibility of the low phenolic content in the fresh tea sample because of active enzyme which might be responsible for depredation of the compound. Because of minimum water activity in the dried sample, high phenolic compounds found in the sample as well as the destructive enzymes also get deactivated **(Hassain et al. ,2010)** By this study, we found that the phenolic content is higher in freeze dryed and vaccum dryed leaves as compared to the oven dryed samples (Fig 5)**. Ramirez *et al*., 2020** have shown in their research that the time and temperature combination in drying process might affect the polyphenol present in tea. Another factor might affect, which was the steeping temperature. According to the resear h done by **Rabeta and Lai,2013,** reported that vacuum dried samples had the higher amount of TPC as compared with the freeze dried and fresh samples.

TFC (Fig 6)

Total flavonoid was determined by using spectrophotometer. Several previous studies demonstrated that freeze-drying is the best methods to preserve the compounds of leaves and minimize the losses **(Arslan and Ozcan2008, 2012; Annegowda et al.2014)**. According to the **Prabha and Patwardhan, 1982** , the decrease in flavonoid and polyphenol after drying at temperature around 50°C might be due the activity of polyphenoloxidase and this statement also supported by **Mrkic *et al*., (2006), Buchner *et al*., (2006), Rohn *et al*., (2007) and Madrau *et al*., (2009).**

Free radical scavenging activity (Fig 7)

The decrease in the antioxidant activity of dried samples could be attributed not only to the same observed decrease in the antioxidant capacity in fresh leaves, thermal degradation is also an issue for the heat-sensitive phenolic compounds —TPC has a major contribution in antioxidant activity.**Kainama et al., 2020** showed a relationship between antioxidant activity and the TPC and TFC in their research by using Pearson coefficient. The relationship of antioxidant activity with TPC and TFC is inversely proportional.Thus, due to higher amount of TPC and TFC in freeze and vaccum dryed samples as compared to the oven dryed sample affects the free radical scavenging of the tea leaves.

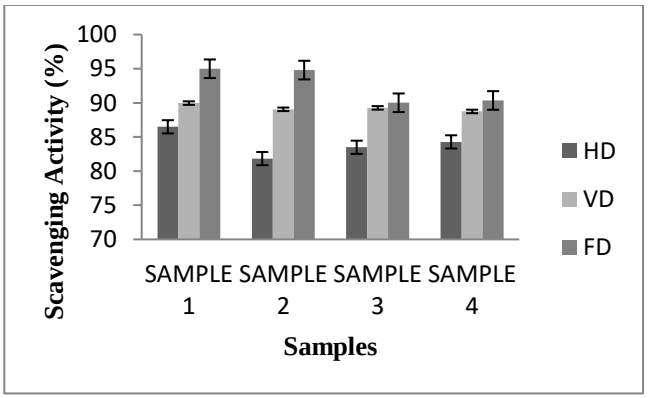

Fig 7: Free radical scavenging activity of Tea samples

4. CONCLUSION:

The results, which was obtained in this research demonstrated that scavenging activity of tea increased significantly during freeze drying operation when comparing with vacuum dryer and hot air oven. Freeze dried sample is the best in terms of phenolic content and scavenging potency also among the three extraction methods we found that ethanol extraction was best to determine the phenolic, flavonoid and free radical scavenging activity. Interestingly, the best rehydration capability was also found on freeze dried sample. Our study also shows the existence of wide variation in caffeine content between different sets of tea samples studied. It was also shown, the caffeine content in the sample was approximately between 2.89% - 4.1%. Since variability of caffeine also depends on other factors like the variety of tea, location, plucking time, age of the leaves and also on the climatic conditions. It is therefore reasonable that the above mentioned factors might be responsible for the variation in the caffeine present in all tea samples during this research. In this research, the high caffeine content was found on sample 1 (Mithinga Tea Garden). Tea leaves lose their tannin contents with increase in age analyzed that tannin occurs at almost 4.5%. This difference may be because of the difference of variety selected for analysis, difference in the manufacture process of tea.

Finally, we also can conclude that phytochemicals found present in the "*Camellia assamica*" indicates their potential as source of bioactive compounds that may supply novel medicines. Further studies are suggested to quantitatively determine these phytochemicals and ascertain their medicinal activities.Overall, our research reflects that Freeze Drying process and ethanol extraction process were the best in to determine the constituients of tea.

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