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Haanj: The Rice Based Alcoholic Beverage of Assam, India as Dietary Antioxidant

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ABSTRACT

"Haanj", is an alcoholic beverage prepared by the Ahom community people of Assam, India, mainly based on rice. For preparation of the fermentation cakes (FC) of "Haanj", various medicinal plants and herbs are also mixed. Five "Haanj" samples were analysed for total phenolic content (TPC) and antioxidant activity (AOA). TPC was measured by Folin-Ciocalteu (FC) method expressing as mg gallic acid equivalents (GAE)/L of "Haanj" samples and AOA was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS {2,2'-azinobis-(3-ethylbenzothaiaziline-6-sulfonate)} and deoxy ribose method. All samples showed significant AOA and a good correlation was obtained between TPC and AOA. The amount of TPC was found in the range of 2.834 ± 0.005mg GAE/L to 4.997 ± 0.003 mg GAE/L. One (BR-4) of the samples showed highest AOA and TPC compared to the other tested "Haanj" samples. Electrochemical measurement of antioxidant activity of "Haanj" samples were also determined by monitoring the change of oxidation potential in the redox cycle of 1,4-diaminobenzene in presence of "Haanj" samples by using cyclic voltammetry (CV). The results of this study indicate that "Haanj" is an important dietary source of phenolic compounds with significant AOA.

Keywords: Haanj, Fermentation cake, Antioxidant.

1. Introduction

The alcoholic beverage, wine is a complex mixture of water, ethanol, saccharides, amino acids, phenolic compounds, pigments and trace metal [1, 2]. Epidemiological studies reveal that regular consumption of small amount of red wine reduces the risk of coronary heart disease (CHD), atherosclerosis, platelet aggregation and certain form of cancer [3, 4, 5]. The red wine is a very rich source of phenolics such as flavanols, phenolic acid, anthocyanins, oligomeric and polymeric proanthocyanidins and many others polyphenols which have multiple biological activities. It is assumed that the biological activities including anticarcinogenic, antiviral, cardioprotective, anti-inflammatory, antibacterial properties of wines are endorsed mainly to their antiradical and antioxidant activity [6, 7]. The phenolic composition of wines may vary depending upon factors like the grape variety, viticultural and environmental factors, wine making technique and ageing process [8-11]. However, some other factors like maceration, alcoholic fermentation, pressing, maturation, fining and bottle aging are also seemed to affect the phenolic composition of wine [12]. In the process of winemaking, particularly during ageing, different chemical reactions take place which are solely responsible for variation of both the phenolic composition and antioxidant activity of wine [8, 13, 14].

Recently, the area of studying the ability of wines, beverages and other foods to prevent free radical-mediated disease has been getting importance due to their interesting properties [15]. The remarkable health-promoting properties of wine and other alcoholic beverages are associated with the presence of phenolic compounds showing high antioxidant activity [16].

In view of the greater health benefit for human being in terms of their diet, the wine and other alcoholic beverage may play a significant role as dietary antioxidants. A traditional alcoholic beverage so called 'Haanj', used as dietary component by the people of Ahom community of Assam located in the north-eastern part of India is believed to have positive effect on human health. It was also reported that rice beverages are used as a source of drug [17] and effective against insomnia, expelling of worms, urinary problems, diarrhoea as well as for the treatment of cholera [18]. Various naturally available medicinal plants are used for the preparation of fermentation cakes used in the preparation of Haanj which leads to the importance of the consumption of Haanj. Therefore, it is necessary and beneficial to determine the polyphenols content and antioxidant activity of Haanj in order for the interpretation of epidemiological studies. In the present study, we have analysed different Haanj samples to fully elucidate a full profile of antioxidant capacity, different antioxidant capacity assays [19]. Since no single assay will perfectly reflect all antioxidants in a mixed or complex system because multiple reaction characteristics and mechanisms are usually involved, therefore various assays like ABTS, DPPH and deoxyribose method was applied for determination of antioxidant activities. Similarly, cyclic voltammetry [20] was used for electrochemical measurement of antioxidant activity of "Haanj" samples.

2. Materials and methods

2.1. Chemicals

The chemicals DPPH (2,2-diphenyl-1-picryl hydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), 1,4-diaminobenzene and Gallic acid, were purchased from Sigma. Folin-Ciocalteu reagent, Hydrogen peroxide, Potassium hydroxide and Potassium dihydrogen phosphate were purchased from Merck. Sodium carbonate, Potassium persulphate, perchloric acid and Disodium EDTA were obtained from Rankem. Deoxy ribose was purchased from Aldrich. Thiobarbituric acid (TBA), Trichloroacetic acid (TCA) and Ascorbic acid were purchased from Sigma-Aldrich. Tetra butyl ammonium bromide was obtained from Spectrochem. Ferric chloride was purchased from Fischer scientific and Methanol, Cyclohexane, Ethylacetate, N,N-dimethylformamide (DMF) and water were obtained from Merck.

2.2. "Haanj" samples

2.2.1 Traditionally prepared 'Haanj' samples

The three districts of Upper Assam, *viz*., Dibrugarh, Sivsagar and Tinsukia are densely inhabited by Ahom community people. From different places of these three districts, fifteen samples (five samples from each district) of 'Haanj' were collected. The substrate material for preparation of these samples is sticky or glutinous rice ('Bora' variety of *Oryzasativa* of Assam, India). Another five samples of 'Haanj' were collected from Dhemaji district (a flood affected area). The substrate material for preparation of these samples from Dhemaji is a flood tolerant variety of rice ('red Bao' variety of *Oryzasativa* of Assam, India). (Details are given in Table 1). All these samples were analysed, and here, we are reporting the results of only four samples, one from each district, which showed the best results within the lots of five samples.

For preparation of the fermentation cake, a large number of plants are used in traditional methods. Most of them are used as folk medicine for different diseases. An extensive field survey was done for documentation of the plant materials used for preparation of the fermentation cakes. It has been observed that the number and species of plants vary from place to place and also depends on their availability. All these plant species are wild in nature and not considered as edible otherwise.

These traditionally prepared samples were kept in glass sample bottles and stored in refrigerator until analysed. The quality, colour and taste depend on the rice variety, plants materials used for fermentation cake, preparation process and the container material used for storage.

The sample, BR-4, was prepared in laboratory using 'Bora' variety of *Oryza sativa*. The fermentation cakes used for the preparation of BR-4 was also prepared in laboratory, but by using only eight species of plants, selected as detailed in the next section.

Table 1: Description of 'Haanj' sample

Sl. No.	Sample Code	Substrate and the fermentation cake (FC), used	Place of collection
1	BR-1	Rice: Bora variety of *Oryzasativa*; FC: locally prepared*	Tinsukia, India
2	BR-2	Rice: Bora variety of *Oryzasativa*; FC: locally prepared*	Sivasagar, India
3	BR-3	Rice: Bora variety of *Oryzasativa*; FC: locally prepared*	Dibrugarh, India
4	BR-4	Rice: Bora variety of *Oryzasativa*; FC: Prepared in the laboratory (most commonly used eight plants are used)	Prepared in the Laboratory
5	RB	Rice: Red 'bao' variety of *Oryzasativa*; FC: locally prepared*	Dhemaji, India

FC = Fermentation cake,

*number of plant materials used in locally prepared F.C. varies according to availability of the plant at that place.

2.2.2."Haanj" sample prepared in the laboratory standardising the traditional method:

For preparation of "Haanj", the following steps are followed.

2.2.2a Preparation of the fermentation cake

To investigate the role of the large number of plant materials used for preparation of the fermentation cakes, a special emphasis was kept in mind to check whether all the plants are invariably required or not, as the availability of all the species at all places is not the same due to the dwindling status of various plant species. It was observed that only eight plants, *viz*., *Ficus bhotanica, Glochidion arborescens, Ipomoea cymosa, Lygodium microphullum, Melastoma malabathricum, Naravellia zeylanica, Rubu sellipticus and Selaginella sp.* are invariably used for preparation of FC in all places. In our earlier investigation, it was found that the plants are good sources of phenolic contents and have shown good antioxidant activity [2]. So, we have restricted the use of plant materials to these eight species only to check whether these eight

plants are enough for making FC or not. Our aim was to see whether unavailability of the other plants affect the quality of the beverage or not.

Leaves of the plants were dried under sun and coarsely ground. Rice flour is mixed with the dry leaf powder and using limited amount of water, a stiff dough is made. With the dough, small flattened balls are made and sun-dried, which are used as fermentation cakes.

2.2.2b Preparation of the rice substrate and fermentation

Rice was cleaned and soaked for 1 hour. Water was drained out and the rice was steam cooked. This steamed rice was used as the substrate and it was directly mixed with powered fermentation cakes and added a little amount of water just to wet the rice. This mixture was stored inside earthen vessels. The mouth of the vessel was loosely closed and allowed to remain untouched for 3-4 days until fermentation was completed. The 'Haanj' produced was collected by decantation, followed by filtration and kept in laboratory sample glass bottles.

2.2.3. Preparation of "Haanj" sample concentrates

100 mL of the "Haanj" samples was dealcoholated and concentrated by using rotary evaporator under reduced pressure at 70°C. The concentrate was then cooled at room temperature.

2.3 Determination of total phenolic content (TPC)

Spectrophotometric determination of the TPC was done by using the Folin-Ciocalteu reagent (FCR) method for the analysis of "Haanj" samples [21, 22]. To 1 mL of a "Haanj" sample, 1 mL of 10% dilute FCR, 2 mL of aq. Na_2CO_3(7.5%) and 2 mL of distilled H_2O were added and the resulting mixture was allowed to stand for 30 mins. Then the TPC were determined using an UV-Visible spectrophotometer at 760 nm. Gallic acid was used as standard for preparing calibration curve and the result was expressed as mg Gallic acid equivalents per litre of "Haanj" sample (mg GAE/L).

2.4 Determination of Antioxidant Activity (AOA)

The AOA of "Haanj" was studied by DPPH, ABTS and Deoxy-ribose method. The three methods, DPPH, ABTS and Deoxy-ribose method, are based on the measurements of the inhibiting ability of antioxidants towards the free $DPPH^{\cdot}$ radical [23], $ABTS^{\bullet+}$ radical cation [24-26] and OH^{-} radical [27] respectively.

2.4.1. DPPH method

The scavenging activity of DPPH by the different "Haanj" sample was determined by a slightly modified spectrophotometric method of Brand-Williams [23]. Firstly, 1 mM DPPH solution was taken as stock solution for UV measurement. For each measurement, 200 µL of DPPH solution was taken from the freshly prepared stock solution and its volume was made up to 3 mL by adding methanol to make a test solution. Again, the antioxidant activity of the "Haanj" samples was studied by preparing different concentrations (2, 4, 8 and 16 µL/mL) of "Haanj" samples and adding 0.1 mL from the respective solution of the "Haanj" to a test solution of DPPH. The solution was shaken and then kept in the dark for 30 mins. at room temperature. The reduced absorbance was measured at 517 nm and was compared with a control of DPPH in methanol in a UV-Visible spectrophotometer (Hitachi). The percentage inhibition of the "Haanj" sample was calculated by using the equation (1):

$$\%inhibition = \frac{[A_{control} - A_{sample}]}{A_{control}} \times 100 \qquad (1)$$

Where, $A_{control}$ = Absorption of DPPH solution without "Haanj" sample at (t=0 min)
A_{sample} = Absorption of DPPH solution in presence of "Haanj" sample at 30 min.

2.4.2. ABTS method

Radical scavenging activities of the "Haanj" samples were also measured by ABTS radical cation scavenging method [24-26]. Briefly, a stock solution of ABTS radical cation was prepared by dissolving equal amount of ABTS solution (7 mM) with potassium persulphate ($K_2S_2O_8$) (2.4 mM). The mixture was left to stand in the dark at room temperature for 16 h (the time required for formation of the radical) before use. For the evaluation of ABTS radical scavenging activity, the working solution was prepared by the stock solution and diluting it in methanol to obtain the absorbance 0.700 ± 0.02 at 734 nm (ABTS working solution was freshly prepared, because the free radical degrades easily). The "Haanj" samples (0.1mL) at different concentrations(2, 4, 8 and 16 µL/mL) were mixed with the ABTS working solution (2.9 mL) and the reaction mixture was allowed to stand at 30°C for 6 mins., then the absorbance was measured by using a UV-visible spectrophotometer at 734 nm, at which point the antioxidants present in the "Haanj" samples began to inhibit the radical, producing a reduction in absorbance, with a quantitative relationship between the reduction and the concentration of antioxidants present in the studied sample. The radical scavenging activity was given as ABTS radical scavenging effect that was calculated by the same equation (1).

2.4.3. Deoxy-ribose method

Hydroxyl radicals were measured by the Deoxy ribose method [27, 28]. Hydroxyl radicals generated by ferric- ascorbate- EDTA-H_2O_2, which attacks on deoxy ribose to form products called thiobarbituric acid reactive substances (TBARS), which in turn, upon heating with TBA at low pH yields a pink chromogen. The hydroxyl scavenger, when added, competes with deoxy-ribose for hydroxyl radical and decreases TBARS formation and the pink chromogen. The reaction mixture containing 0.36 mL of 10 mM deoxyribose, 0.01 mL of 10 mM ferric chloride, 0.1 mL of 1 mM EDTA, 1 mL of 1 mM ascorbic acid, 0.1 mL of 10 mM H_2O_2 and 0.33 mL of 50 mM Phosphate buffer of pH 7.4 was added 0.1mL of "Haanj" at different concentrations (2, 4, 8 and 16 µL/mL). After incubating for 30 min at 37°C, 1 mL of this reaction mixture, 1 mL of 10% TCA and 1 mL of 1% TBA were mixed to yield a final volume of 3mL. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm. Scavenging activity was expressed as percentage of inhibition of hydroxyl radical calculated by using equation (1).

2.5. Cyclic voltammetry (CV)

Electrochemical measurement of antioxidant activity was done by CV [29]. The cyclic voltammograms were recorded with an Electrochemical Analyzer CH Instrument (Model chi 600c) with three electrodes system comprising Ag/AgCl as reference electrode and two platinum electrodes as auxiliary and working electrodes respectively. The measurement was done in N,N-dimethyl formamide (DMF) with TBAP as supporting electrolyte with scan rate 0.05 V/sec. Before recording the voltammogram, pure nitrogen gas was passed through the solution.

2.5.1. Preparation of TBAP (tetra butyl ammonium perchlorate):

A saturated solution of 8.4 g of tetra butyl ammonium bromide (TBAB) in 18 mL of water was added with 2.1 mL of aqueous 70% $HClO_4$ to get insoluble perchlorate. The perchlorate so formed was filtered and washed with ice cold H_2O and dried. Recrystallization of the TBAP was done in cyclohexane-ethylacetate solution. To a saturated solution of TBAP in ethylacetate, cyclohexane was added to precipitate TBAP. Pure TBAP was dried at 100°C under vacuum.

At first, cyclic voltammogram was recorded by dissolving 4 mg of 1,4-diaminobenzene (1,4-DAB) in 3 mL DMF with 8 mg of TBAP as supporting electrolyte and to this solution, 0.1 mL of the "Haanj" sample was added and mixed well. Then the cyclic voltammogram of the resulting solution was recorded as the same procedure. The experiment was done separately with each of the "Haanj" samples, prepared to observe their effect on 1,4-DAB.

2.6. Statistical analysis

All measurements were done in triplicate and the results were expressed as mean value ± standard deviation.

3. Results and discussion

3.1. Total Phenolic Content: It is well known that phenolic compounds have potential antioxidant activity and possess good radical-scavenging activity. So, it was necessary and reasonable to determine the total amount of phenolic content present in the tested samples. The total phenolic content of the five "Haanj" samples were investigated by using the Folin-Ciocalteu reagent method and the results were expressed in terms of mg Gallic acid equivalents per litre of "Haanj" sample (mg GAE/L). The results were shown in table 2 and graphically presented in figure 1. The total phenolic content varied in all tested samples and ranged from 2.834 ± 0.005mg GAE/L to 4.997±0.003mgGAE/L. The highest TPC was found in BR-4 sample (4.997± 0.003mgGAE/L) and the lowest TPC was obtained in BR-3 sample (2.834 ± 0.005mg GAE/L). The order of TPC among the "Haanj" samples are as follow BR-4>BR-2>RB>BR-1>BR-3.

Table 2: TPC of the "Haanj" samples

Sl.No.	Sample code	Absorbance	Value of X	TPC	TPC(mean ± SD) (mgGAE/L)
1	BR-1	0.859	4084.5	4.0845	4.082 ± 0.003
		0.859	4084.5	4.0845	
		0.858	4079.5	4.0795	
2	BR-2	0.974	4659.5	4.6595	4.661 ± 0.003
		0.975	4664.5	4.6645	
		0.974	4659.5	4.6595	
3	BR-3	0.609	2834.5	2.8345	2.834 ± 0.005
		0.608	2829.5	2.8295	
		0.610	2839.5	2.8395	
4	BR-4	1.043	5004.5	5.0045	4.997 ± 0.003
		1.041	4994.5	4.9945	
		1.041	5004.5	5.0045	

5	RB	0.904	4309.5	4.3095	4.304 ± 0.005
		0.903	4304.5	4.3045	
		0.902	4299.5	4.2995	

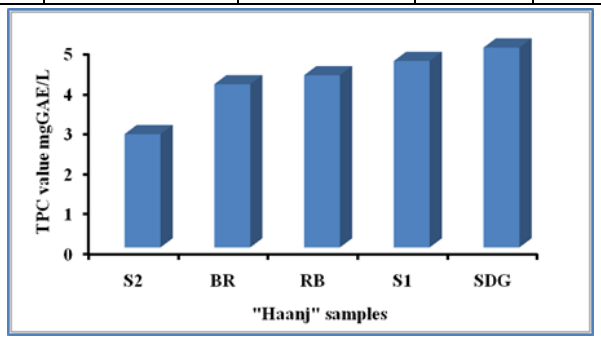

Figure 1: TPC of five "Haanj" samples expressed as mg GAE/L

3.2. DPPH method: In this method, the AOA was expressed as IC_{50}. IC_{50} values were calculated from plotted graphs of percentage inhibition vs the concentrations of samples. The IC_{50} values were presented in table 3 and Figure. 2. The term IC_{50} is defined as the amount of antioxidant required to decrease the radical concentration by 50%. It is known that lower the IC_{50} value higher is the antioxidant activity. The "Haanj" sample, BR-4, prepared in laboratory showed the highest antioxidant activity with IC_{50} value (43.213±0.181µL/mL) and the lowest antioxidant activity was exhibited by the sample BR-3 with IC_{50} value (60.656±0.655µL/mL), prepared traditionally by the local people of Dibrugarh district of Assam, India.

Table 3: IC_{50} values of five "Haanj" samples by DPPH method

Sl. No.	Sample code	Concentration (µL/mL)	% inhibition	IC_{50} value(µL/mL)
1	**BR-1**	2	2.060 ± 0.082	55.848 ± 0.059
		4	4.127 ± 0.073	
		8	8.217 ± 0.071	
		16	14.525 ±0.077	
2	**BR-2**	2	2.374 ± 0.098	52.625 ± 0.177
		4	4.347 ± 0.096	
		8	8.465 ± 0.196	
		16	15.528 ±0.069	
3	**BR-3**	2	1.59 ± 0.001	60.656 ± 0.655
		4	2.95 ± 0.110	
		8	5.77 ± 0.091	
		16	13.15 ± 0.164	
4	**BR-4**	2	3.423 ± 0.083	43.213 ± 0.181
		4	5.809 ± 0.087	
		8	11.267 ±0.090	
		16	19.137 ±0.066	
5	**RB**	2	1.354 ± 0.105	55.450 ± 0.571
		4	2.799 ± 0.104	
		8	6.114 ± 0.099	

		16	14.067 ±0.142	

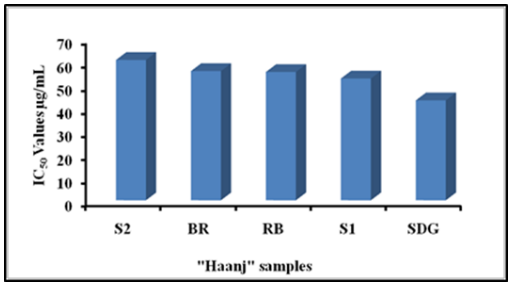

Figure 2: IC_{50} values of five "Haanj" samples by DPPH method

3.3. ABTS method

The AOA of this method also expressed as IC_{50}. ABTS method was based on the measurement of ABTS radical cation and was applicable for both lyophilic and hydrophilic antioxidants. The IC_{50} value of the tested "Haanj" samples was shown in table 4 and the graphical presentation was exhibited in figure. 3. The BR-4 sample has the highest ABTS radical cation scavenging activity with IC_{50} value 9.381±0.019µL/mL. The lowest ABTS radical cation scavenging activity was observed in BR-3 sample with IC_{50} value 12.129± 0.071µL/mL.

Table 4: IC_{50} values of five "Haanj" samples by ABTS method

Sl. No.	Sample code	Concentration (µL/mL)	% inhibition	IC_{50} value (µL/mL)
1	**BR-1**	2	14.67 ± 0.28	11.066 ± 0.037
		4	28.92 ± 0.23	
		8	37.95 ± 0.11	
		16	67.62 ± 0.19	
2	**BR-2**	2	10.77 ± 0.08	10.668 ± 0.033
		4	27.82 ± 0.09	
		8	46.52 ± 0.05	
		16	66.92 ± 0.25	
3	**BR-3**	2	17.66 ± 0.01	12.129 ± 0.071
		4	28.32 ± 0.08	
		8	41.21 ± 0.26	
		16	59.58 ± 0.23	
4	**BR-4**	2	14.14 ± 0.04	9.381 ± 0.019
		4	30.05 ± 0.05	
		8	48.35 ± 0.21	
		16	75.76 ± 0.11	
5	**RB**	2	15.39 ± 0.23	10.877 ± 0.004
		4	25.12 ± 0.03	
		8	36.87 ± 0.05	
		16	70.42 ± 0.05	

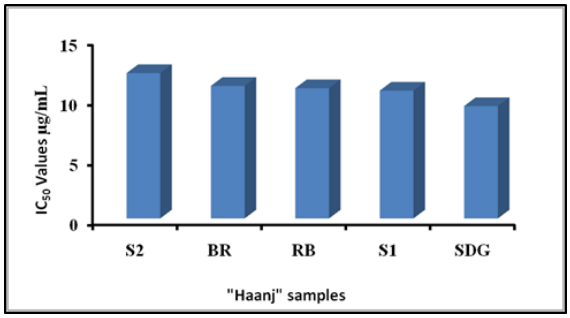

Figure 3: IC_{50} values of five "Haanj" samples by ABTS method

3.4. Deoxy-ribose method

Deoxy-ribose method was used to measure the hydroxyl radical scavenging capacity of "Haanj" samples by studying competition between deoxy-ribose and "Haanj" samples for hydroxyl radical generated from ferric-ascorbate-EDTA-H_2O_2 system. When a tested sample scavenges hydroxyl radical, the formation of TBARS is decreased. The IC_{50} value of the five "Haanj" samples was shown in table 5 and graphical presentation was shown in figure. 4. It was observed that the BR-4 sample has the highest Hydroxyl radical scavenging capacity among the other samples with IC_{50} value11.748 ± 0.087µL/mL The lowest hydroxyl radical scavenging capacity was found in BR-3 sample with IC_{50} value18.202 ± 0.172 µL/mL

Table 5: IC_{50} values of five "Haanj" samples by Deoxy-ribose method

Sl. No.	Sample code	Concentration (µL/mL)	% inhibition	IC_{50} value (µL/mL)
1	BR-1	2	8.922 ± 0.372	16.795 ± 0.087
		4	15.489 ± 0.214	
		8	31.970 ± 0.371	
		16	45.849 ± 0.214	
2	BR-2	2	10.285 ± 0.214	14.898 ± 0.120
		4	18.959 ± 0.372	
		8	36.555 ± 0.372	
		16	50.558 ± 0.371	
3	BR-3	2	6.939 ± 0.214	18.202 ± 0.172
		4	13.755 ± 0	
		8	24.287 ± 0.214	
		16	43.866 ± 0.372	
4	BR-4	2	9.789 ± 0.567	11.748 ± 0.087
		4	21.685 ± 0.214	
		8	41.264 ± 0.372	
		16	63.569 ± 0.372	
5	RB	2	9.913 ± 0.214	14.010 ± 0.095
		4	16.233 ± 0.214	
		8	31.351 ± 0.214	
		16	56.134 ± 0.372	

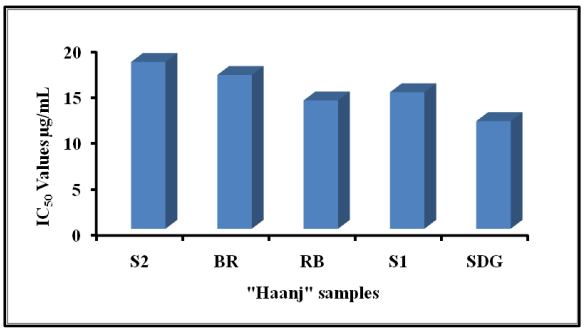

Figure 4: IC_{50} Values of five "Haanj" samples by Deoxy-ribose method

3.5. Cyclic voltammetry

The effect of "Haanj" samples on the electrochemical behavior of 1,4-diaminobenzene (1,4-DAB) was studied with the help of cyclic voltammetry. 1,4-DAB has well-defined redox cycle and if any change occurred to the redox behavior may be studied easily by cyclic voltammetry. 1,4-DAB can have benzene-benzenoid structure on electrochemical oxidation and reduction reaction. The first oxidation wave was observed at 264 mV and the second oxidation wave was observed at 910 mV. The first reversible cycle with $E_{1/2}$ at 208 mV is due to formation of a cationic radical and this radical in the second cycle with $E_{1/2}$ at 674mV transforms to a diimine. The overall redox reactions of 1,4-DAB in presence of the "Haanj" samples have been significantly affected.

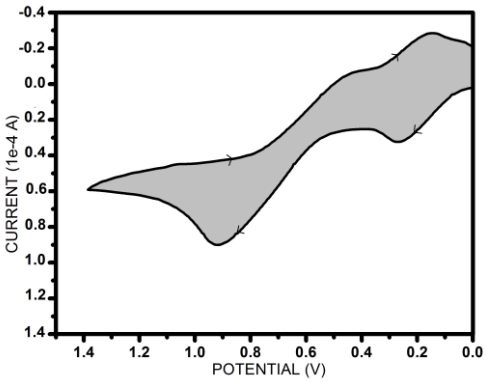

Figure 5.1 Cyclic voltammogram of 1,4-diaminobenzene (1,4-DAB)

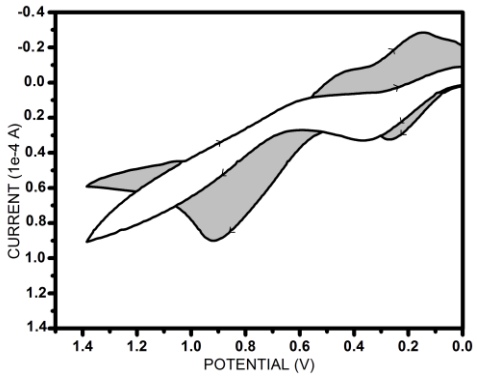

Figure 5.2 Overlaid CV of 1,4-DAB alone(shaded) and in presence of "Haanj" sample BR-1(not shaded).

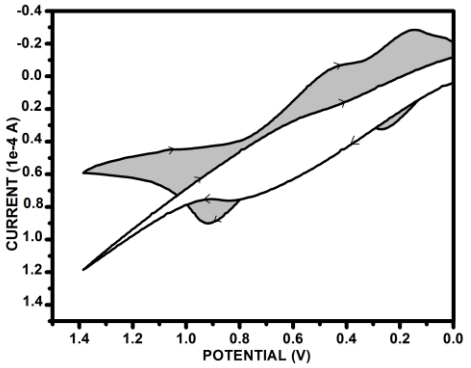

Figure 5.3 Overlaid CV of 1,4-DAB alone (shaded) and in presence of "Haanj" sample RB (not shaded).

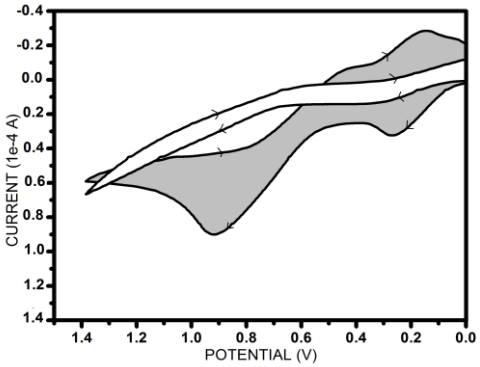

Figure 5.4 Overlaid CV of 1,4-DAB alone(shaded) and in presence of "Haanj" sample BR-4 (not shaded).

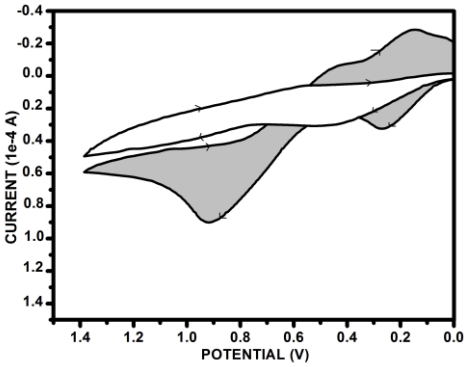

Figure 5.5 Overlaid CV of 1,4-DAB alone (shaded) and in presence of "Haanj" sample BR-2(not shaded).

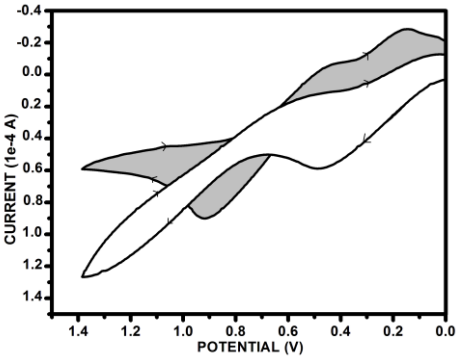

Figure 5.6 Overlaid CV of 1,4-DAB alone (shaded) and in presence of "Haanj" sample BR-3(not shaded).

Figure 5: CV of 1.4-diaminobenzene alone (figure 5.1) and that in presence of "Haanj" samples (Figure 5.2-Figure 5.6).

The effects of all "Haanj" samples were shown in figure 5 and the shifts and/or absence of anodic potential of the oxidation waves of 1,4-DAB is summarized in table 6. All "Haanj" samples delayed the first oxidation wave which indicates that the samples delay the oxidation process of 1,4- DAB to the radical cation probably by stabilizing 1,4-DAB. The second oxidation wave was not observed in all cases, indicating the complete inhibition of the oxidation and established the potential radical scavenging effect of the samples. Because once the cationic radical has been formed due to radical scavenging ability of the samples, the radical has become a non-radical and the second oxidation reaction was not possible.

Table 6: Values of anodic Potential of 1,4-DAB with and without "Haanj" samples

Entry	Value of anodic potential of 1,4-DAB	1st peak E_p(mV)	2nd peak E_p(mV)
1	1,4-DAB	264	910
In presence of Haanj samples			

2	BR-1	366	-
5	BR-2	455	-
6	BR-3	482	-
4	BR-4	348	-
3	RB	790	-

4. Conclusions

All the "Haanj" samples showed their capabilities to scavenge the DPPH radical, ABTS radical cation and hydroxyl radical indicating potential antioxidant activities. In all methods, it was found that BR-4 sample has highest scavenging activity and BR-3 sample has lowest scavenging activity. It is observed that use of only eight plants that are invariably used at all places for preparation of the FC seems to be sufficient for preparation of a good quality "Haanj". It may also be possible that even fewer plants may suffice the purpose, which may be investigated in future. Minimizing the number of plants with a view for commercial cultivation may also be a scope for future study. Since, the amounts of phytochemicals present in plants also vary depending on the season, soil quality and age of the plants which also affect the quality of the FC. So, the preparation of FC is one of the important factors for the quality of "Haanj".

In this paper, we used 'bora' and red 'bao' variety of *Oryzasativa* of Assam to examine the antioxidant activity and no significant difference in the properties, was observed in all cases. It was also observed that all five samples have good amount of phenolic content and a good correlation between TPC and AOA is obtained. In electrochemical measurement of antioxidant activities of "Haanj" samples, it was observed that all samples delay or scavenge the oxidation process of 1,4-DAB. Thus the "Haanj" can play as an excellent dietary antioxidant with high amount of phenolic content preventing a large number of diseases in human health.

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