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General analysis and Antioxidant study of Traditional fermented drink Handia, its concentrate and volatiles

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Abstract – In this study, the antioxidant activity of Traditional fermented drink Handia, its concentrate and volatiles were determined in terms of total antioxidant, reducing capacity, free radical scavenging, metal chelating activity. The total phenolic compounds were also determined. Results indicated that Handia, its concentrate exhibited similar strong total antioxidant activity. This was also true for reducing capacity and free radical scavenging activity. For all the cases volatile components from fermented food Handia exhibits much lower antioxidant activity. Results indicated that active antioxidants were nonvolatile and resided in the concentrate after evaporation. In that particular drink other components rather than Phenolics compounds may play a very important role in the antioxidant function. However, identification of specific antioxidant components should be further investigated.

Keywords – Handia, Antioxidant activity; Reducing capacity; Scavenging activity; Chelating activity

1. Introduction

Handia, an indigenous alcoholic-fermented beverage is prepared from parboiled rice by the ethnic tribes Santal, Sabar, Bhumij, Paroja, Kondh, kolh, Mundari,Juang etc spread among eastern region specially in Orissa and West Bengal. It is made by mixing traditional fermenting culture- Bakhar with boiled rice and allowing them to ferment in an earthen pot (*Handia*) for 2-3 days with mouth slightly open. Bakhar holds the source of several microorganism prepared by mixing different plant ingredient with rice powder, water and giving batter to the shape of small round balls (Dhal et al., 2008), sometime Bakhar powder from previous batches are also added in batter (Hutchinson et al,1925). Then balls are wrapped with leaves, allowed to ferment and dry continuously in shade for 3 days.

After fermentation, the fermented slurry is filtered and weak cream-colored product is taken as drink. Handia is a staple beverage consumed by the people of mentioned ethnic group both men and women of all ages. It is used in day to day life both as a food and an intoxicant (Chowdhury et al., 2006).This practice of processing and drinking of Handia is the integral part of mentioned ethnic tribes. The consumption pattern increases on special occasions, like new born, weddings, worshiping god etc.

This low cost drink is claimed to be an energetic one which retards tiredness and offers uninterrupted sleep. Besides several therapeutic uses like relief from liver cancer, liver disorder and constipation, treating urinary infections (Dhal et al., 2008) and prevention of cancer and cardiovascular disease are also assigned with the drink consumer, but there is no epidemiological evidence.

We hypothesized that Handia contains some antioxidants and active biochemical that may partially contribute the claimed health effect.

The objective of this study was to evaluate the general composition and antioxidant properties of Handia, its concentrate and volatile compounds (VC) with the measurements including the reducing capacity, the scavenging activity on the free radicals, and the chelating activity on ferrous ions.

2. Materials and methods

2.1. Chemicals

All the chemicals were purchased from Sigma. All other chemicals used were of analytical grade.

2.2. Samples collection of traditionally prepared Handia

Traditionally prepared Handia was collected from a local village of the indigenous ethnic group Jhargram in Paschim Medinipur, West Bengal in India. Traditionally prepared Handia was centrifuged at 5000xg for 10 min, supernatant was collected and stored at 4°c for analysis.

2.3 Preparation of concentrated Handia and volatile compounds

The concentrate of Handia was obtained after it was concentrated to one-ninth of the origin volume by a vacuum evaporator at 45°C to remove the Volatile compound (vc). The concentrated sample (cs) was thus diluted 9 times with distilled water to compensate for the lost volume prior to analysis. The VC was collected after condensation of evaporated volatiles during concentration.

2.4 Determination of total antioxidant capacity by phosphomolybdenum method

The total antioxidant activity of the Handia, its concentrated sample (cs) and volatile components (vc) were evaluated by using phosphomolybdate method as described by Prieto et al. 1999. The assay is based on the reduction of Mo (VI)–Mo (V) by the test sample and subsequent formation of a green phosphate/Mo (V) complex at acid pH. 0.3 ml of fermented broth was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm using a spectrophotometer. The antioxidant capacity of each sample was expressed as ascorbic acid equivalent using the following linear equation established using ascorbic acid as standard: [A = 0.0037C + 0.0343; R^2 = 0.991] where A is the absorbance at 695 nm and C the concentration as ascorbic acid equivalent (µg/ml). The values are presented as the means of triplicate analysis.

2.5 Reducing Power Assay

The reducing capacities of Handia, its concentrated sample (cs) and volatile components (vc) were determined by (Oyaizu et al., 1986). Different volumes (50 µl, 100 µl -200 µl) of substrate were taken in 1 ml water. Then it was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide (w/v). The mixture was incubated at 50°C for 20 min. After cooling to room temperature 2.5 ml of 10% trichloroacetic acid (w/v) was mixed and mixture was centrifuged at 3000 x g for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml deionizer water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm. Higher absorbance indicates higher reducing power of the sample.

2.6 DPPH radical scavenging activity

The free radical scavenging activity of Handia, its concentrated sample(cs) and volatile components(vc) was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Que, et al., 1986 with slight modifications Roy, et al., 2012. DPPH solution (0.1 mmol/l) in ethanol was prepared. 2ml of this solution was added to equal volume of water solution containing different amount of sample. After 30 min absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The % inhibition of the antioxidant was measured as

$$\text{The \% inhibition } x = \frac{Abs\ (control) - Abs\ (sample)}{Abs\ (sample)} \times 100$$

Where Abs (control) is the absorbance of the control (blank, without extract) And Abs (sample) is the absorbance in the presence of the sample. From inhibition data, 50% inhibition or reduction amount (the amount of a particular concentration of antioxidant that reduces the 50% absorbance of DPPH solution) is calculated.

2.7 Chelating activity of hardia of Fe2+ ions

The chelating activity of the sample for Fe2+ is measured according to the methods described by Dinis et al. (1994). 0.5 ml of sample, 1.6 ml of de-ionized water and 0.05 ml of $FeCl_2$ (2 mM) is added, followed by the addition of 0.1 ml of ferrozine (5 mM) after 15 min gap. Then it was incubated 10 min at room temperature. The absorbance of the Fe2+ -ferrozine complexes with red or violet color was measured at 562 nm. One milliliter of distilled water, instead of Handia sample was used as a control. Lower absorbance of the reaction mixture indicated higher chelating activity. The chelating anti-oxidant activity for Fe2+ was calculated according to the following formula:

Chelating rate (%) = (Ac – As) / Ac × 100

Where, Ac is the absorbance of the control reaction and As is the absorbance of the sample extract.

2.8 Determination of total phenolic compounds

Total phenolic compound of the samples were estimated by the method Sadashivam and Manickam (2004). Different aliquots of the samples were pipette out and the volume in each tube was made up to 3.0ml with distilled water distilled water and 0.5 ml Folin –Ciocalteu reagent was added. After 3minutes, add 2ml of 20% Na2CO3 solution and place the tubes in boiling water bath for one min, cooled and the absorbance was measured at 765 nm. Standard graph was prepared by using different concentration of gallic acid.

3. Results and Discussion

3.1 Total antioxidant activity of Handia its concentrated sample (cs) and volatile components (vc)

The total antioxidant capacity determined after reduction of Mo (VI) to Mo (V) in presence of the antioxidant compounds and measuring the green phosphate/Mo (V)

complex at acidic pH, at 695 nm shows effective antioxidant activity. From the analysis, the total antioxidant content of the Handia was 520µg/ml, whereas concentrated compounds (cs) exhibited similar strong antioxidant activity (493 µg/ml), compared to Handia but the antioxidant activity of Volatile components was much weaker (21 µg/ml). For all the three cases a linear equation using ascorbic acid as standard was taken for analysis. [A = 0.0037C + 0.0343; R^2 = 0.991where A is the absorbance at 695 nm and C the concentration as ascorbic acid equivalent (µg/ml)]. The values are taken as the means of triplicate analysis.

3.2 Reducing Power Assay

Figure 1 shows the reducing power of the Handia, its concentrated sample (cs) and volatile components (vc) using potassium ferricyanide reduction method. Reducing power is associated with the antioxidant activity (Amarowicz et al., 2004). The yellow color of the test solution changes to various shades of green and blue, depending upon the reducing power of sample. The presence of ductant (Antioxidants) in the fermented broth causes the reduction of Fe^{3+}/ Ferric cyanide complex to ferrous form. Therefore Fe^{2+} complex is monitored by measuring the formation of Perl's prussian blue at 700nm (Hancock et al., 2007). The reducing capacity of Handia and concentrated sample (cs) increased with increasing amount, of the substrate and the reducing capacity of Handia was seen to be slightly higher than that of concentrated samples. However, volatile compounds (vc) showed little reducing capacity.

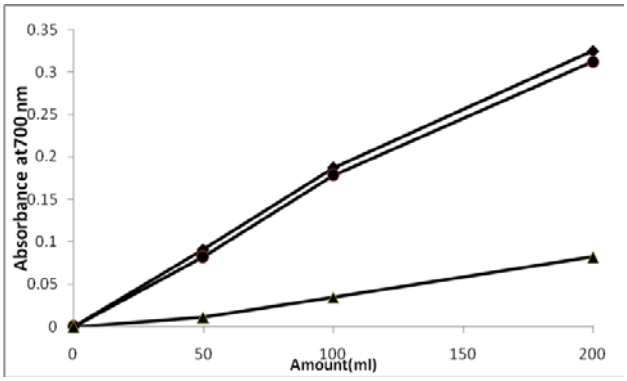

Figure 1. Reducing capacity of different amounts of Handia, its concentrated sample (cs) and volatile components (vc) by potassium ferricyanide method. Handia(◆), its concentrated sample(●) and volatile components(▲)

3.3 DPPH radical scavenging activity

Handia and its concentrated sample (cs) showed similar significant effects on the DPPH radical scavenging at all amounts (Fig. 2), but volatile compounds (vc) of Handia had limited DPPH radical scavenging activity. These results were consistent with total antioxidant activity (Fig. 1). 50% inhibition or reduction amount (the amount of a particular concentration of antioxidant that reduces the 50% absorbance of DPPH solution) of Gallic acid is 0.3827 ml (concentration 100µg/ml) where as 0.7781 ml is for Handia and 0.8297 ml for its concentrated sample (cs). The 18.73 ml volatile component (vc) of Handia offers 50% inhibition or reduction amount.

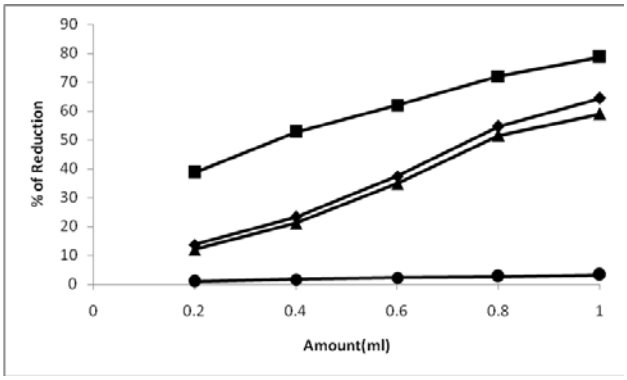

Figure 2. Free radical scavenging activity of different amounts of Handia, its concentrated sample (cs) and volatile components (vc) by DPPH radicals. Gallic acid (■), Handia (◆), its concentrated sample(▲) and volatile components(●)

3.4 Metal chelating activity

Ferrozine form complexes with Fe_2+. Presence of chelating agents disrupts the complex formation, resulting in a decrease in the red color of the complex. Measurement of color reduction therefore allows estimating the metal chelating activity of the coexisting chelator (Yamaguchi et al., 2000; Gulc ın et al., 2003).
Handia, its concentrated sample (cs) and volatile components (vc) showed limited chelating activity on Fe2+ ions. The standard EDTA (100 µg/ml) of 0.5 ml volume shows a chelating rate of 39.119% where as Handia, its concentrated sample (cs) and volatile components (vc) of 0.5 ml volume yields only 3.66%.3.25 and 0.12 chelating rate.

3.5 Total phenolic compounds

In this study, 3.92 and 3.21 µg of phenolic compounds were detected in 1ml of Handia, its concentrated sample (cs).Total phenolic compound in Volatile components (vc) of Handia was seen to be 0.4µg, consistent result with the total antioxidant and reducing capacity.
For all cases total phenolic content was measured as gallic acid equivalent using the following linear equation established using gallic as standard: [A = 0.210C + 0.292; R^2= 0.997] where A is the absorbance at 765 nm and C the concentration as gallic acid equivalent (µg/ml).

4. Conclusions

The antioxidant property of Handia, its concentrated sample (cs) and volatile components (vc) results a similar trend as compared to Chinese yellow wine, its concentrate and volatiles as reported by Que., et al. Results showed a similar result that Handia and the concentrate owned effective antioxidant activity, but the VC had little antioxidant activity for all indicative cases of total antioxidant activity, reducing capacity and free radical scavenging activity. This might indicate that the antioxidant components resided in the nonvolatile parts of the Handia, its concentrated sample. It can Ethanol is a

volatile component, and thus the ethanol in the Handia should be evaporated during concentration. That signifies that ethanol had little antioxidant capacity. Handia, its concentrated sample (cs) and volatile components (vc) had little metal chelating activity. That might be due to the less iron binding capacity of this fermented drink. The total phenolic component of Handia is minute as compared to the Chinese yellow wine. That might be due to the source of fermenting substrate. The raw material for Handia fermentation is parboiled steamed rice rather than any fruit juice (not like wine fermentation-whose raw material is grape juice). It also signifies that the phenolics are not the key component responsible for antioxidation properties of Handia, its concentrated sample (cs). So the focus should be on the identification of the specific antioxidant components of rice fermented Handia.

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