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In-Vitro Evaluation of Antioxidant Properties of Fermented Fruit Beer Samples

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Abstract: ***Alcoholic beverages conferred many advantages to the human species when consumed in moderation. It was the universal drug up until the advent of modern medicine. Beer industry is one the biggest market all over the world with a turnover of above $100 billion per year and its growing at a higher rate. Fruits have been used as a beer adjunct for centuries, especially with Belgian lambic styles. The use of botanical extracts has gained a lot of importance in the present as the ingredients are natural and have higher medicinal potential. In the present study the antioxidant property of few fruit derived beer samples where checked. The fermented beer from Guava and Apple samples were taken and their antioxidant property was checked by DPPH, reducing power and total antioxidant property. Both the samples showed that they had antioxidant property. The antioxidant activity increased with the increase in the sample concentration. Sample 1 and 2 showed DPPH scavenging activity of 47.28% and 56.64% for undiluted samples respectively. Sample 1 showed a better reducing power activity when compared to sample 2 whereas it was vice versa for total antioxidant assay.***

Keywords: Antioxidant, DPPH, Phytochemicals, Reducing Power, Total antioxidant, Fruit beer

1. Introduction

Antioxidants are molecules which inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers hydrogen from a substance to an oxidizing agent and these can produce free radicals. In turn, these radicals start chain reactions and these can damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such asthiols, ascorbic acid, or polyphenols [1].

The diet is one of the major sources of antioxidant and polyphenols are the most abundant antioxidants in our diet [2]. In addition to their antioxidant properties, polyphenols may have other specific biological activities affecting gene expression [3], cell signalling [4], and cell adhesion [5].

From ancient times, there has been an increasing interest in determining relevant dietary sources of antioxidant phenolics. Thus, red fruit juices such as grape and different berry juices have been investigated as they have high antioxidant activity. Pomegranate juice has become more popular because of the attribution of important biological actions [6]. Thus, the antioxidant and anti-tumor activity of pomegranate bark tannins (punicacortein) [7], [8] and the antioxidant activity of the fermented pomegranate juice (Schubert et al., 1999) have been reported in the recent years. Fruit wines have traditionally been popular with home winemakers and in areas with cool climates such as North America and Scandinavia; in Africa, India, and the Philippines, wine is made from bananas.

The berries high antioxidant activity has been indicated in both *in vitro* and *in vivo* studies [9]-[13]. Fruits have been used as a beer adjunct for centuries, especially with Belgian lambic styles. Cherry, raspberry, and peach are a common addition to this style of beer. One prominent brewer of fruit beer is Yanjing Beer, a large Chinese brewery, which widely markets Pineapple and Lemon beer. Früli is a Belgian fruit beer made from 70% wheat beer and 30% fruit juice.

The tradition of fruit to beer is practiced and commercialised in Belgium for the production of cherry limbic or raspberry lambic by adding, respectively, sour cherries (Prunus cerasus L.) or raspberries (Rubus idaeus L.) to fermenting lambic in casks [14-[15]. [16], investigated antioxidant and eicosanoid enzyme inhibition properties of *Punica granatum* fermented juice and seed oil flavonoids. The pomegranate fermented juice (pfj) showed strong antioxidant activity close to that of butylated hydroxyanisole (BHA) and green tea (*Thea sinensis*), and significantly greater than that of red wine (*Vitis itifera*).

2. Materials and Methods

2.1 Samples

Fermented fruit beer samples were collected from an up store in Bangalore being marketed for its low alcoholic content and health properties. The fruit beer samples were that of Guava and Apple samples.

2.2 Antioxidant activity

Antioxidant Capacity of the given Fermented Fruit Juice Sample was estimated using three different assays, DPPH Free Radical scavenging Activity Assay, Reducing Power Assay and Total Antioxidant Activity assay. Assays were performed for undiluted Sample, 20% diluted sample, 40% diluted sample, 60% diluted sample and 80% diluted sample according to standard protocols.

2.2.1 DPPH radical scavenging assay

The free radical scavenging activities of the given samples were measured using 2, 2-Diphenyl- 1-picrylhydrazyl (DPPH) as described by [17], with some modifications. 100 µl of sample solution (20% to 100%) was added to 3 ml of methanol solution of DPPH (24µg/ml). After shaking, the mixture was incubated at room temperature for 15 min in a

dark place. Then, the absorbance was measured against a blank at 517 nm with a spectrophotometer. All the samples were prepared in triplicate and the average of the three readings obtained was taken as the final absorbance. Radical scavenging activity (RSA) was calculated according to the following equation:

$RSA\% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$

Where A_{blank} was the absorbance of control reaction (containing all reagents except the test compound), and A_{sample} was absorbance of the test compound. L Ascorbic Acid was used for Standard Graph preparation. According to the graph, Antioxidant Activity was expressed in terms of % radical scavenging activity and Ascorbic Acid Equivalents (AAE).

2.2.2 Reducing Power Assay

Reducing capacities are generally associated with the presence of reductants in the antioxidant samples [18]. Reductants cause reduction of the Fe3+/ ferricyanide complex to ferrous form. Therefore, the amount of ferrous complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [19].

The reducing power of the given fermented fruit juice samples was determined according to the method described by [20]. 1 mL of sample solution (10% v/v) was mixed with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1 %). After incubation at 50˚C for 20 min, 2.5 mL of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 5000rpm g for 10 min. Finally, 2.5 mL of upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%). After 10 min, the absorbance was measured at 700 nm, against blanks that contained all reagents except the sample extracts. All the samples were prepared in triplicate and the average of the three reading obtained was taken as the final absorbance. A higher absorbance indicates a higher reducing power

2.2.3 Total Antioxidant Activity

Total antioxidant activity is a quantitative assay, since the antioxidant activity is expressed as the number of equivalents of Ascorbic acid. The assay is based on the reduction of Mo (VI) to Mo(V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH with the maximal absorption at 695nm (5µg/ml) as standard as at this concentration, absorbance becomes almost constant [21]. The basic principle is the scavenging ability of fruit juices on the phosphomolybdenum reagent. Different concentrations of juices were prepared in distilled water. 4.5 mL phosphomolybdate reagent was added. After incubation at 95°C temperature for 90 minutes, optical density was measured at 695nm. All the samples were prepared in triplicate and the average of the three reading obtained was taken as the final absorbance. Total antioxidant capacity was calculated by the formula.

Total antioxidant capacity (%) = [(As-Ac)/ (Aaa - Ac)] X 100
Where, Ac = control absorbance, As = sample absorbance, Aaa = ascorbic acid absorbance. Total antioxidant activity was also determined in terms of ascorbic acid (in mg/mL).

3. Results

3.1 Antioxidant Activity Analysis by DPPH Radical Scavenging Activity Assay

Radical scavenging activity and the presence of hydrogen donors in juice and beverage samples were examined by reduction of DPPH in methanol.

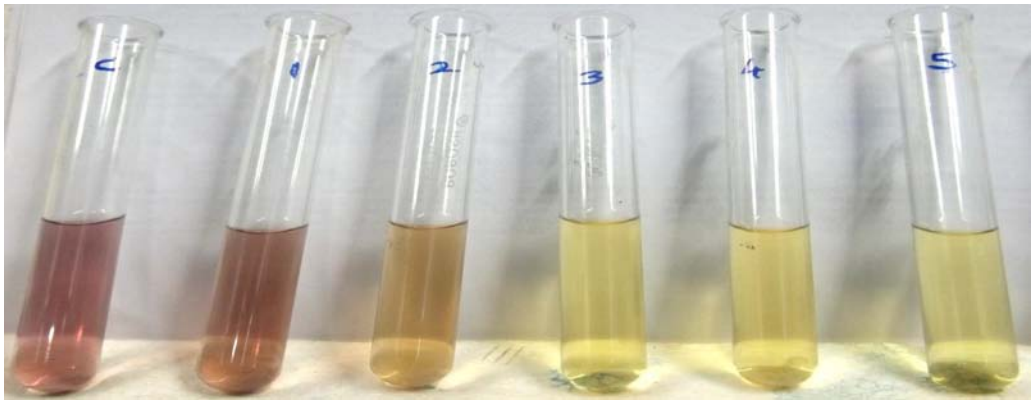

Figure 1: DPPH Radical scavenging activity assay – Standards

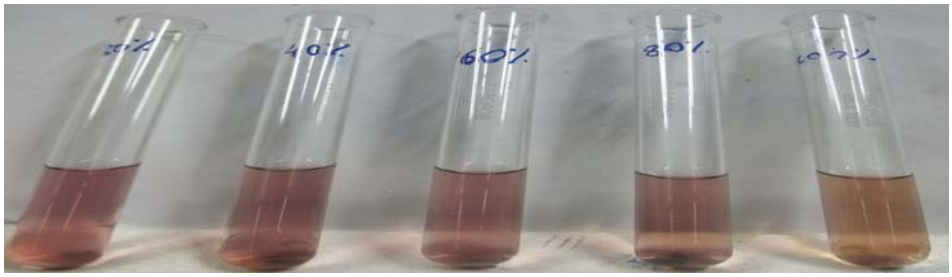

Figure 2: DPPH Radical scavenging activity assay of different dilutions of given sample

Figure 1 & 2 shows the decrease in intensity of colour of solution with increase in concentration as the Free radicals content of the solution decreases with increase in concentration of antioxidants in the solution. Absorbance at λ_{max} 514nm is due to presence of DPPH free radicals and therefore high Absorbance indicates low free radical scavenging activity whereas less absorbance of solution indicates high free Radical Scavenging activity

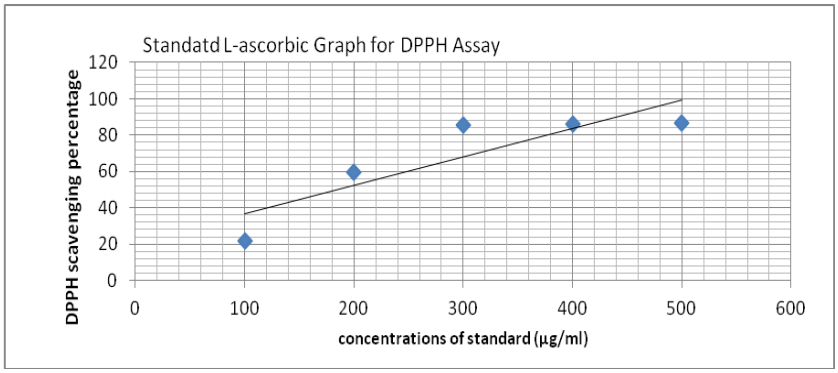

Figure 3: Standard Graph for DPPH Assay

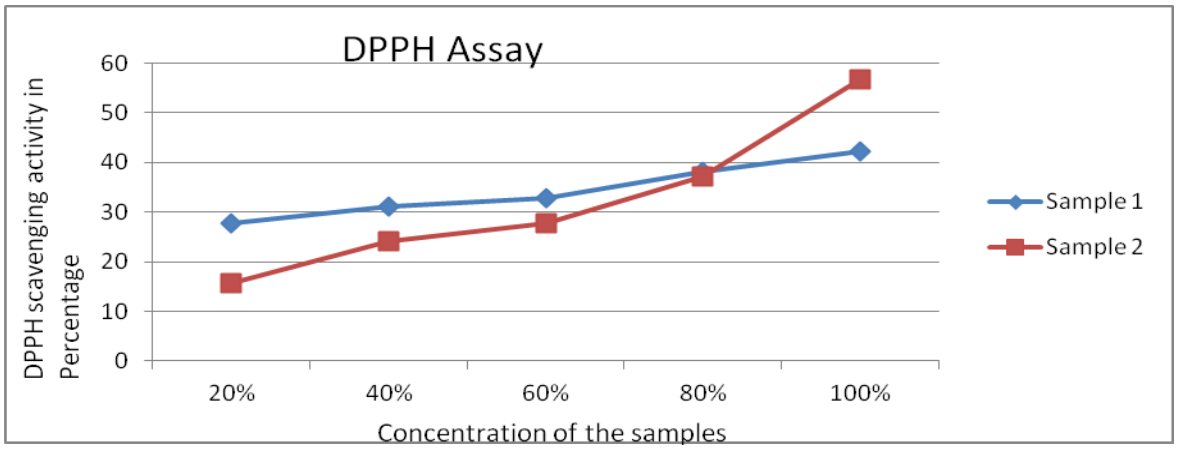

Figure 4: DPPH radical Scavenging activity in terms of percent radical scavenging activity.

Percentage Radical Scavenging Activity of the given sample 1 and its dilutions were estimated to be in range of 27.82% to 47.28% and for sample 2 it ranged between 15.68% to 56.64%. It can be concluded that sample has low DPPH radical scavenging Activity (Figure 3 and 4).

3.2 Reducing Power

In reducing power assay, the yellow color of the test solution is changed to various shades of green and blue color depending upon the reducing capacity of the sample

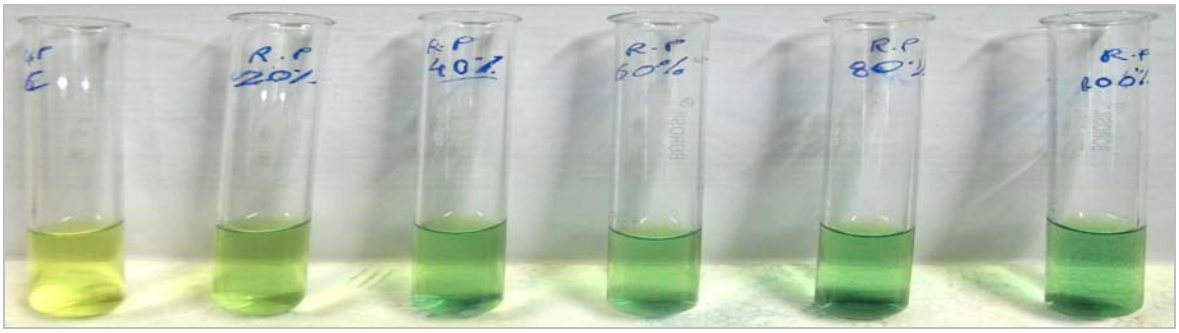

Figure 5: Reducing power assay of different dilutions of given sample

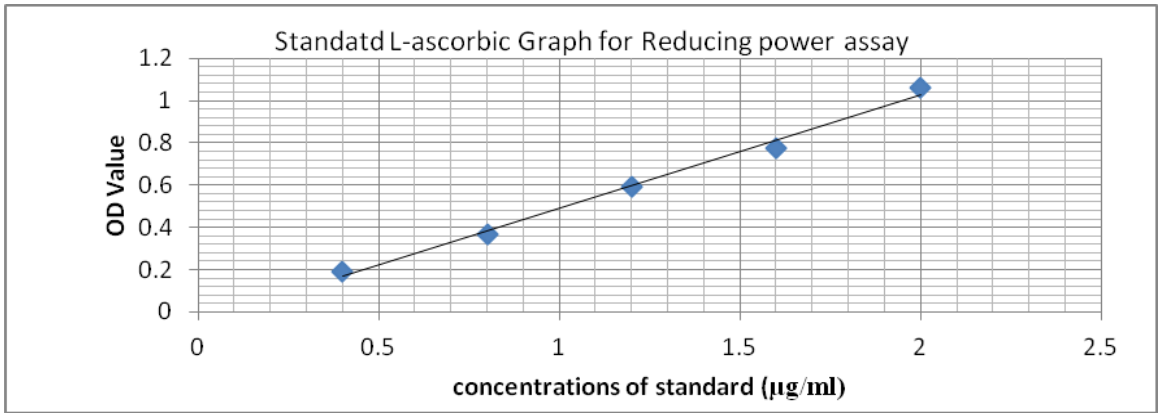

Figure 6: Standard Graph for Reducing Power Assay

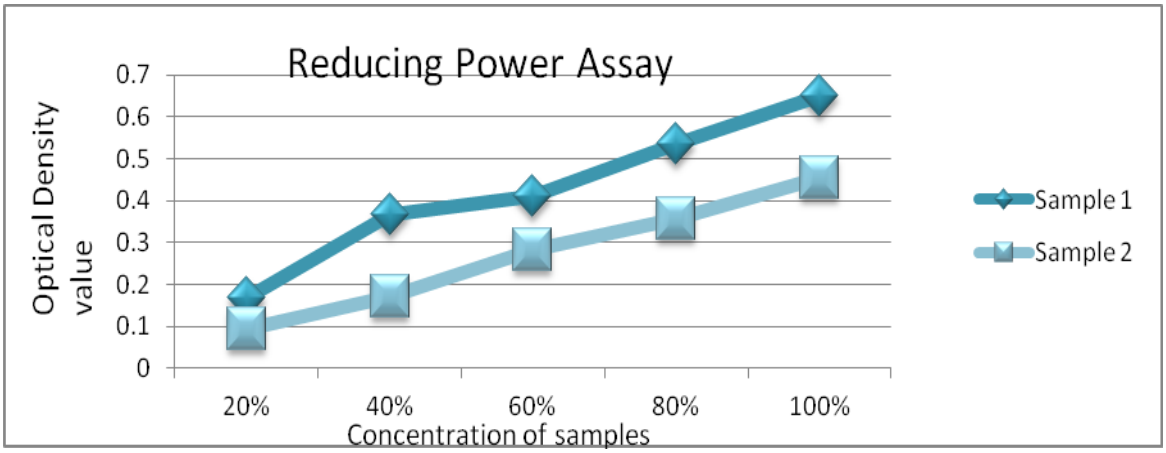

Figure 7: Antioxidant activity of given samples by Reducing power assay

Increasing absorbance at 700 nm indicates an increase in reducing capacity. Graph 2 indicates that OD value increased with the increase in the concentration of the samples. Sample 1 showed a better reducing power activity when compared to sample 2 (figure 6 and 7).

3.3 Total Antioxidant Activity

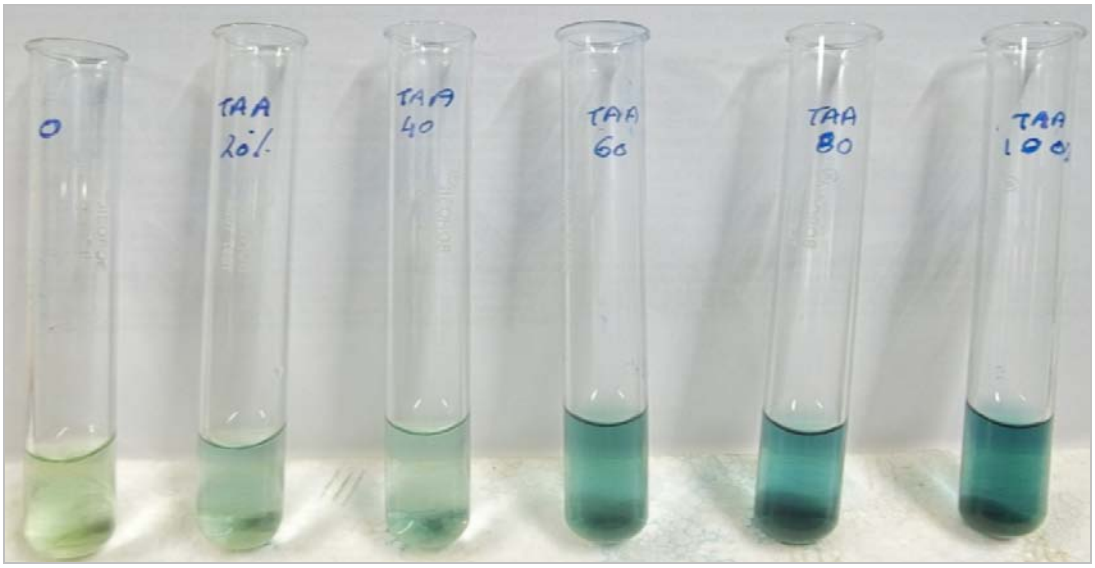

Figure 8: Total antioxidant Capacity assay of different dilutions of given sample

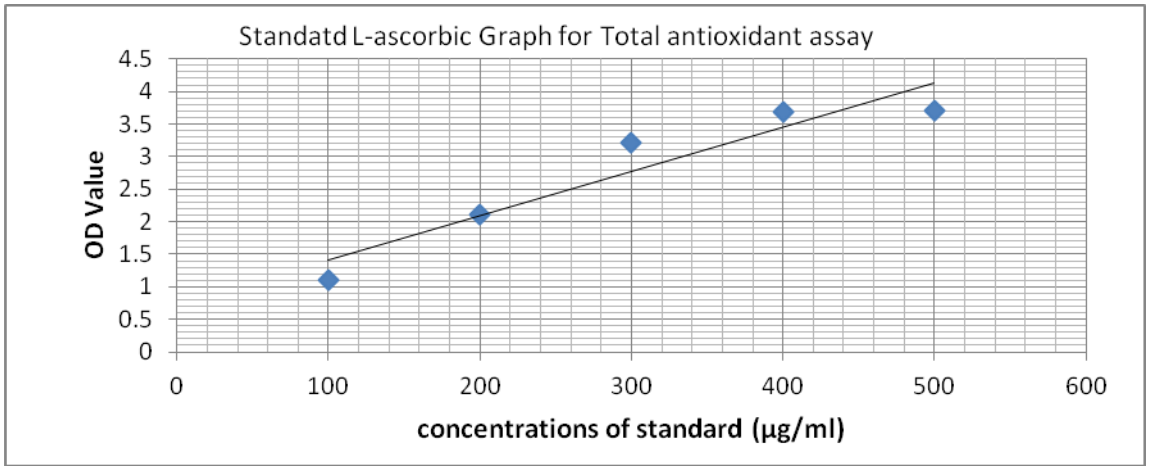

Figure 9: Standard Graph for Total Antioxidant Assay

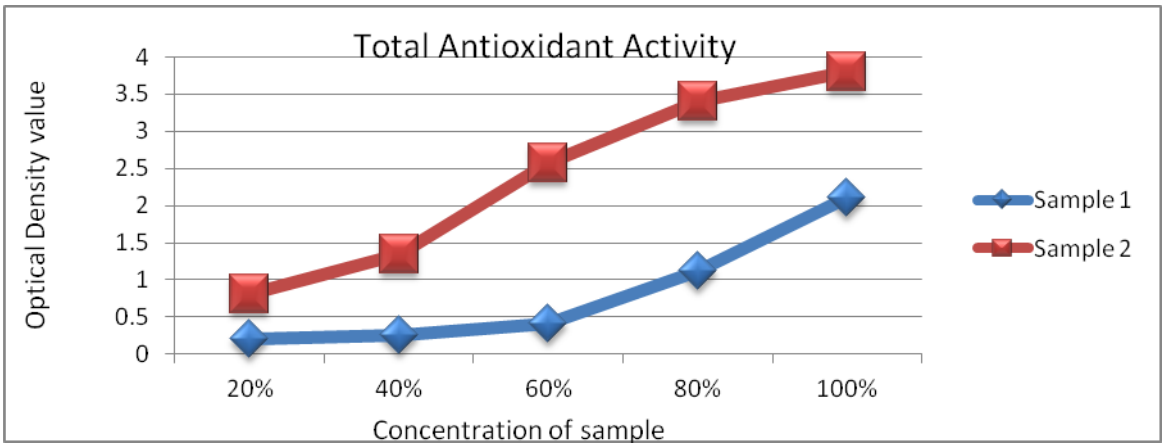

Figure 10: Total Antioxidant Activity Assay

Total Antioxidant assay was carried out for both the samples and was found that the optical density gradually increased with the concentration of the sample indicating that the samples had the capacity to reduce nascent atoms. The total antioxidant of sample 2 was found to be much higher than sample 1. Optical density of sample 2 showed OD value above 3.5 for undiluted sample whereas sample 1 showed OD value between 2-2.5 for undiluted sample (Figure 10).

4. Discussion

Alcoholic beverages conferred many advantages to the human species when consumed in moderation. It was the universal drug up until the advent of modern medicine, since its health benefits were obvious - alcohol relieved pain, stopped infection, and killed microorganisms and parasites in tainted water.

Fermented beverages, made from sweet fruits, honey and saccharified cereals, were likely discovered and utilized by humans at a very early date [22]. Hippophae rhamnoides recently have been proved to have a high medicinal property, because of its nutrient density of almost 200 active phytochemicals, among them unsaturated fats, carotenoids, and high amounts of vitamin C [23].

In many African countries, sorghum is used for local beer production. Sorghum has been proved to have phenolic acids like hydroxybenzoic and hydroxycinnamic acids [24]-[25] both free and bound as esters. Most of them are found in usual lager beers, issued either from barley malt or from hop [26], studied the aging and consequent changes in flavor molecules of a top-fermented beer and found that after 6 months of aging, the concentration changes were recorded for acetate esters, ethyl esters, carbonyls, Maillard compounds, dioxolanes, and furanic ethers.

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