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Herbal Wine from *Aloe barbadensis*: A Restorative Approach

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ABSTRACT

In view of the increasing demand of value-added herbal products, an attempt was made to produce a functional fermented Aloe vera base herbal wine. Aloe vera gel supplemented with sugar found to be a good medium for the growth of *Saccharomyces cerevisiae* for making the Aloe vera wine. Disease preventing the potential of herbs like Aloe vera, Amla, Ginger, Cranberry, Blueberry have given new dimension to the non-grape wine or fruit wine. Aloe vera, a multifunctional herb, is being increasingly used in beverage applications including wines. Gas chromatography FID analysis reveals presence of alcohol in aloe vera wine. Antimicrobial activity of wine was screened against *Salmonella typhimurium* and *Pseudomonas species and zone of inhibition* were found to be 2.7cm and 1.3 -1.4cm respectively.

***Key words*:** Aloe vera, Fermentation, *Saccharomyces cerevisiae*, Medicinal value

Wine represents one of the examples of functional fermented foods. Wine, considered as a Phyto-complex to which ethanol has been added following fermentation, contains different constituents with strong antimicrobial properties, such as low pH (ranging from 3.0 - 4.0), relatively high ethanol concentrations (10 - 15%) and some bioactive components. There are several types of wines available around the world based on the type of fruit used, source of yeast inoculum, alcohol content, presence of carbon dioxide, color, processing of fruit prior to fermentation etc. To produce wines, a variety of raw materials including fruits, sugar, acid, nitrogen source, clarifying enzymes etc. are required. The fruit constitutes the most important raw material. Most of the wines are generally prepared from grapes but commercial wines are also made from several fruits other than grapes.

Aloe vera (*Aloe barbadensis*) is one such herbal plant with enormous medicinal value. It is an excellent example of functional food that plays a significant role in protection from oxidative stress. Banjare *et al*. [1] reported that Aloe vera has become a subject of interest because of its beneficial effects on human health. Aloe vera synthesizes aromatic substances / alkaloids including phenols, tannis and their derivatives which serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. These aromatic substances, herbs are useful for the maintenance of health in humans. Trivedi *et al*. [2] studied the protective efficacy of laboratory prepared Aloe-Amla wine against oxidative stress caused by the infection in a urine model.

Aloe vera is also popular in both traditional Chinese and Ayurvedic medicine (India). Aloe vera is used internally as a laxative, antihelminthic, hemorrhoids remedy and uterine stimulant (menstrual regulator). The bitter yellow exudates from Aloe vera leaves contain 1,8 dihydrooxyanthroquinine derivatives and their glycosides. Aloe vera gel consists of about 99.5% water, the remaining 0.5 - 1% solid material consists of a range of compounds including water soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids. Belda *et al*. [3] observed that Wine is a complex matrix that includes components with different chemical natures, the volatile compounds being responsible for wine aroma quality. This research article was undertaken to study development processes for the formation of Aloe vera wine and its standardization.

MATERIALS AND METHODS

Raw substrates for wine production

Aloe vera (*Aloe barbadensis*) leaves were collected from a local nursery from Nashik city, Nashik, Maharashtra. These were washed three times in sterile distilled water. The lower 1 inch of the leaf base, the tapering point (2-4inches) of the leaf top and the short sharp spines located along the leaf margins were removed by a sharp knife. The gel was then blended in a mixer and the resulting aloe vera juice is stored in amber colored glass bottles to avoid the effect of light on the sensitive bioactive agents [4].

Extraction and processing of aloe vera gel

2.5 liters of aloe vera gel with 1.5 liters of sterilized water (final volume of 4 liter) having 22°B TSS with 1020 gm sugar

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was taken in a Winture flask and the pH was adjusted to 4.5 using citric acid. ∘Brix was determined by using refractometer. Later Pectinase treatment was given to extracted juice and incubated for 4-5 hours at room temperature (25°C). Pectinase has the ability to speed up the breakdown of pectin molecules in the cell wall, allowing more juice to be released. After incubation, a pinch full of sodium metabisulphite was added in the mixture and refrigerated overnight.

Activation of yeast cells (Saccharomyces cerevisiae) by using 5% sucrose solution

A loopful culture of S. cerevisiae from a slant was inoculated in 25 mL of sterilized glucose yeast extract (GYE) broth (yeast extract 0.3 percent w/v, malt extract 0.3 percent w/v, peptone 0.5 percent w/v, and glucose 1 percent w/v, pH 4.5). The cells were separated by centrifugation at 10000 rpm (4°C, 15min) after an overnight incubation at 30°C on a rotary shaker (150 rpm). These were rinsed twice and re-suspended in normal saline to produce a pre-inoculum concentration of 10^8cells/ml.

The inoculum was made by adding 10 ml of the pre-inoculum to a 250 mL conical flask containing 100 mL of aloe vera juice supplemented with 5% sucrose and incubated for 24 hours [5].

Fermentation of aloe vera gel

Aloe vera gel supplemented with cane sugar was subjected to fermentation by seeding with prepared activated rehydrated *Sacchromyces cerevisiae* and plugging with cotton wool and kept at room temperature (25°C) for 15 days. The contents of the flask were mixed 2 – 3 times a day.

After fermentation, Bentonite powder was added for clarification of wine and allowed to stand for one week. Centrifugation for separation of solids from wine (at 5000 rpm at 4oC for 15 minutes) and sealed in clean pre sterilized bottles, then the bottles were stored in refrigerator. Clear wine was then used for biochemical and phytochemical analysis at the final for various constituents including TSS using, total acids, pH, residual sugars, soluble proteins, total phenolics and alcohol contents [5].

Qualitative Biochemical Analysis of aloe vera wine and aloe vera gel

Estimation of total soluble solids (°Brix): Brix (0B) reading of the wine samples was determined using refractometer.

Protein estimation: Was done by Bradford method. (HI media quantitative protein estimation kit).

Estimation of pH: pH of the samples was recorded by using the pH meter. Standard buffer solutions of pH 4.0, 7.0 and 9.0 were used as reference to calibrate.

Determination of alcohol content

Alcohol content in the Aloe vera wine was determined with the help of Gas chromatography in the RAP analysis laboratory at Nashik, District Nashik (Maharashtra). By using an Ebulliometer, the percentage of ethanol was determined [6]. A rapid and accurate method for determination of methanol in alcoholic beverages by direct injection capillary gas chromatography.

Estimation of titratable acidity

Titratable acidity was determined by titrating a known amount of wine sample (10 mL) against 0.1 N NaOH using a few drops of 1% phenolphthalein solution as indicator. The endpoint was the appearance of pink/purple color which should persist for 15-20 seconds [7] (AOAC 1990).

Estimation of carbohydrate

Carbohydrate estimation was done by Dinitro salicylic acid method by using a standard graph of glucose [8].

Qualitative analysis of phytochemicals in aloe vera wine

The qualitative screening for common phytochemicals including tannins, terpenoids, Flavonoids and polysaccharides was carried out in the aloe vera wine and juice by standard methods as described below:

Tannins: To 0.5 mL of test solution, l mL of water and 1-2 drops of 0.1% ferric chloride solution was added. Occurrence of a blue-black, green or blue green precipitate indicates the presence of tannins [9].

Terpenoids: 5 ml of test sample was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid was carefully added to the mixture to form a layer. An interface with a reddish-brown coloration was observed for terpenoids [10].

Flavonoids: 1 mL of test sample was treated with a few drops of 1% NH3 solution. Yellow color formation was an indication for the presence of flavonoids [10].

Polysaccharides: To 1 mL of test solutions, 2 drops of iodine solution was added. Blue coloured solution observed confirmed positive test for polysaccharides [9].

Gas chromatography - FID analysis of wine

Gas chromatography (GC) is a powerful tool in the analysis of alcoholic beverage products. Advantages of gas chromatography are minimal sample preparation is required, since the samples are in the liquid state in an alcohol or alcohol/water matrix. GC was equipped with HP of 30 cm length and 0.53 mm thickness. The carrier gas was hydrogen set to flow 0.2 µl/min. The injector was operated in split less mode at 100°C temperature. The chromatographic conditions were optimized for complete separation of the target compounds [4].

Antimicrobial activity of wine

According to CLSI recommendations, the antimicrobial evaluation of wine and aloe vera juice (10%) were screened against *Salmonella typhimurium* and *Pseudomonas* using agar well diffusion method using sterile Mueller-Hinton agar plates. McFarland standard was used to make the inoculums for each isolate. The inoculums size was determined using a $BaSO_4$ and sulfuric acid turbidity standard equivalent to 0.5 McFarland, which contained 2x 10^8 CFU/ml, as recommended by CLSI. The 6 mm cork borer was sterilized with alcohol and used to make wells on seeded Muller-Hinton agar plates for bacteria. 25 µL of wine sample is added to each well. The plates were then allowed to cool for 30 minutes to allow the wine and juice to diffuse into the agar plates. The inhibitory zones of diameter were measured in millimeters. The entire investigation was performed in triplicate and under sterile parameters in a laminar air flow [11].

RESULTS AND DISCUSSION

Fermentation of aloe vera gel

Aloe vera gel supplemented with cane sugar was subjected to fermentation (Fig 1).

Fermentation

Foaming

Aloe vera wine

Fig 1 Fermentation of Aloe vera gel

Table 1 Biochemical analysis of *Aloe vera* wine and *Aloevera* juice

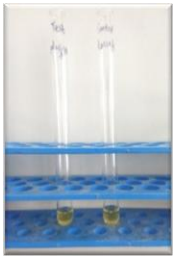
Biochemical analysis	Wine sample	Juice sample
Estimation of alcohol (by Ebulliometer)	7.4% V/V	-
Estimation of carbohydrates	2461.9 µg/ml	2545.2 µg/ml
Estimation of pH	3.8	4
Estimation of titrable acidity	4.5%	2.85%
Estimation of total soluble proteins	121.44 µg/ml	166.5 µg/ml

Table 2 Qualitative analysis phytochemical components of *Aloe vera* wine and *Aloe vera* juice

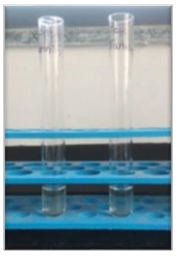
Phytochemical analysis	Wine sample	Juice sample
Tannin	Negative	Negative
Terpenoids	Positive	Positive
Flavonoid	Positive	Positive
Polysaccharides	Negative	Negative

Titrable acidity estimation

Terpenoids test



Polysaccharides test



Tannin test

Ebulliometer

Fig 2 Biochemical analysis of aloe wine and aloe juice

Biochemical analysis of aloe wine and aloe juice

(Table 1-2) shows the results of the biochemical and phytochemical analyses for Aloe vera wine and Aloe vera juice.

Gas Chromatography - FID analysis of wine

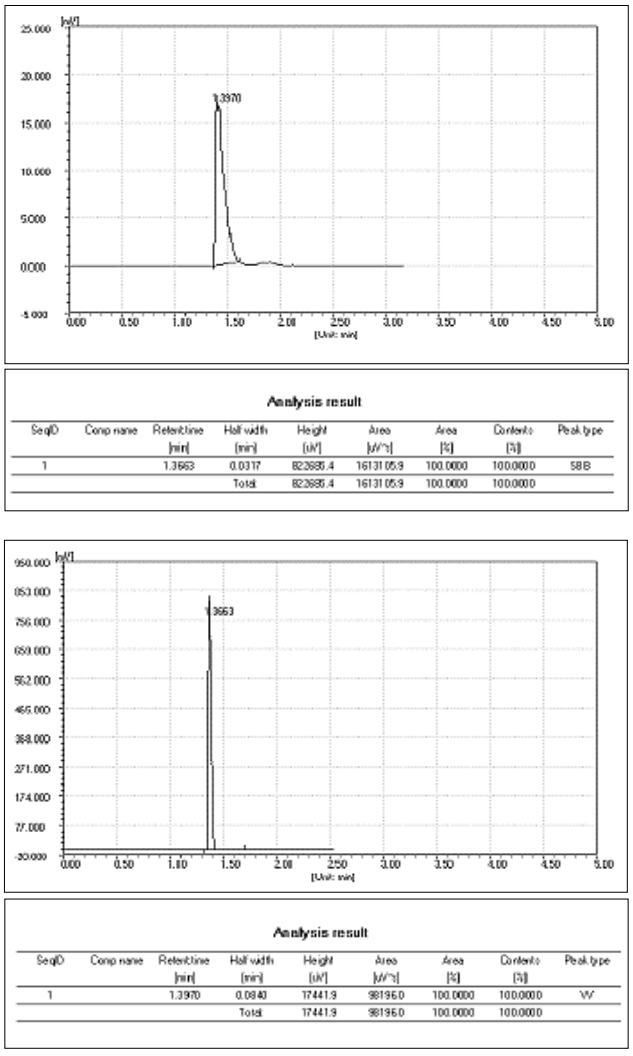


Fig 3 The GCFID chromatogram of Aloe vera wine and standard alcohol

To estimate the amount of alcohol in the sample a direct injection GC method with alcohol as standard was used. The retention time for sample and standard alcohol revealed by this method was at 1.3970 and 1.3663 min. respectively. The height of chromatogram of sample and test was found to be 17441.3 and 1613106.9 Area under the curve of sample matched 100 percent with standard as shown in (Fig 3).

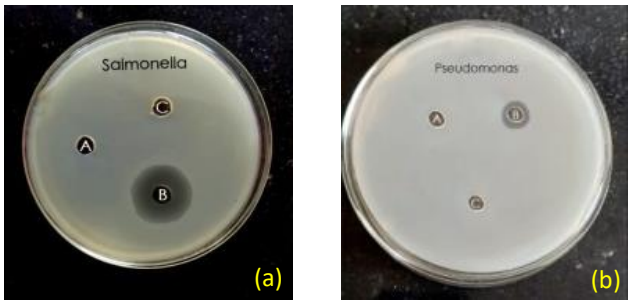


Fig 4 In-vitro study showing the effect of *Aloe vera* wine (1), Aloe vera gel (2) and 10% ethanol v/v on the growth of *Salmonella typhimurium* (a) and *Pseudomonas* (b) in agar well diffusion assay on nutrient agar plates. The clear zones around the well of *Aloe vera* wine indicate the antimicrobial effect against the test organisms

Antimicrobial activity of wine

Aloe vera wine and aloe vera juice revealed antibacterial activity against the common pathogens tested in the study. The zones of inhibition for *S. typhimurium* and *Pseudomonas* were found to be 2.7cm and 1.3-1.4cm respectively whereas no zone of inhibition was observed for *Aloe vera* juice and 10% v/v ethanol as assessed by agar well diffusion assay (Fig 4). Previously, several authors have noted that wine has a greater antibacterial effect compared with the same concentration of diluted absolute ethanol [12]. Wine possesses relatively high ethanol content in addition to other antimicrobial agents like organic acids, low pH, polyphenol compounds and preservatives [13] which may be responsible for the pronounced inhibitory effect. Marimon *et al*. [14] found similar results with ethanol and pH combinations against *H. pylori*. A combination of organic acids, ethanol and low pH has been reported to have significantly stronger antimicrobial activity than the effect of these components individually against various food-borne pathogens, indicating potential synergistic interactions between these components leading to an enhancement of antimicrobial activity [15]. A decrease in pH leads to an increase in the undissociated form of organic acids, which are considered to be the antimicrobials active species. Ethanol is known to damage the cytoplasmic membrane, causing an increase in permeability of the membrane. These changes in membrane permeability may lead to enhanced efficacy of organic acids and may partly explain the difference in antimicrobial activity between grape juice and wine [16-17]. The study also confirmed that *Aloe vera* juice alone is not able to inhibit the growth of pathogens as no zone of inhibition was observed (Fig 4).

CONCLUSION

Generally, Aloe vera juice is not often consumed by people because of its taste and odor. On the other side wine gives sweeter taste, fruity smell which can be easily consumed daily which promotes human health and exempts from diseases.

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