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Research paper

Production of a herbal wine from *Aloe vera* gel and evaluation of its effect against common food borne pathogens and probiotics

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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* based herbal wine. The wine was evaluated for effect on probiotics and food borne pathogens. *Aloe vera* gel, supplemented with sugar proved to be a good medium for the growth of *Saccharomyces cerevisiae* for making the *Aloe vera* wine. The wine was found to be similar to any other wine in terms of its composition, and sensory quantities. The wine exhibited bactericidal activity against common food borne pathogens (*Salmonella typhimurium, Staphylococcus aureus, Escherichia coli)*. However, its bactericidal effect was much faster in case of *Salmonella* as compared to other pathogens. Interestingly, the wine was not inhibitory to the selected probiotic strains and no significant difference in the viable count of lactobacilli was found in the fecal matter, hence, indicating their persistence in the gut of wine fed animal.

Keywords: *Aloe vera,* Wine, *Salmonella typhimurium, staphylococcus aureus, escherichia coli*, antibacterial activity.

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Wine represents one of the examples of functional fermented foods and have many health benefits including the anti-aging effects in red grape skins, improvement of lung function from antioxidants in white wine, reduction in coronary heart disease, development of healthier blood vessels in elderly people, reduction in ulcer-causing bacteria, destruction of cancer cells by protein in red grape skins, prevention of stroke by keeping the arteries clean by polyphenols in red grape skins, decreasing ovarian cancer risk in women and making the bones stronger (Altenburg and Zouboulis 2008). It also serves as an important adjunct to the human diet as it increases the satisfaction by providing relaxation necessary for proper digestion and absorption of food (Joshi, 1977). The consumption of wine has also been reported to have antimicrobial effect against various pathogens. Moretro and Daeschel (2004) have shown that wine has bactericidal activity against *Salmonella typhimurium, Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes.* Many wines are made from herbs with perceived medicinal value.

Many plants synthesize aromatic substances/ alkaloids including phenols, tannins and their derivatives which serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. These aromatic substances, herbs and spices are useful for the maintenance of health in humans and other animals (Tapsell *et al*. 2006). Herbalism is a traditional folk medicine practice based on the use of plants and plant extracts. The plant of *Aloe vera* and its usage as drug dates back to 6000 years B.C. The plates belonging to Sumer period during 2200 years B.C. exhibit the use of this plant as a drug (Sharrif and Verma 2011). *Aloe* is also popular in both traditional Chinese and Ayurvedic medicine

(India). The Chinese describe aloe's skin and the inner lining of its leaves as a cold, bitter remedy which is downward draining and used to clear constipation due to accumulation of heat (Bensky *et al.*, 1993); the gel is considered cool and moist. In Ayurvedic system of medicine, the traditional medicine of India, *Aloe* is used internally as a laxative, antihelminthic, hemorrhoid remedy, and uterine stimulant (menstrual regulator); it is used topically, often in combination with licorice root, to treat eczema or psoriasis. In Arabian medicine, the fresh gel is rubbed on the forehead as a headache remedy or rubbed on the body to cool it in case of fever, as well as being used for wound healing, conjunctivitis, and as a disinfectant and laxative (Ghazanfar 1994). The gel or mucilage obtained from the flesh of the leaf contains quite different compounds from the bitter latex extracted from the leaf lining (Klein and Penneys 1988). Many compounds with diverse structures have been isolated from both the central parenchyma tissue of *A. vera* leaves and the exudate arising from the cells adjacent to the vascular bundles. The bitter yellow exudate contains 1, 8 dihydroxyanthraquinone derivatives and their glycosides (Vazquez *et al.*, 1996).

The protective and disease preventing potential of fruits and herbs like *Aloe vera*, amla, ginger, cranberry, blueberry etc. have given new dimension to the non-grape wine or fruit wine (Gruenwald 2009). *Aloe vera*, a multifunctional herb is being increasingly used in beverage applications including wines. *Aloe vera* juice is also supplemented in fermented grape juice and is sold in some parts of the world as *Aloe vera* wine which is consumed because of the therapeutic properties of both *Aloe vera* and wine. *Aloe vera* gel consists of about 99.5% water (Eshun *et al.*, 2004), 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 (Boudreau *et al.*, 2006). *Aloe* gel is bacteriostatic or bactericidal against a variety of common wound-infecting bacteria including *Staphylococcus auerus, Streptococcus pyogenes, Serratia marcescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, E. coli, S. typhosa* and *Mycobaterium tuberculosis* (Robson *et al.*, 1982; Lorenzetti *et al*., 1964). *Aloe* - emodin also inhibits the growth of *Helicobacter pylori* in a dose dependent fashion (Wang *et al.*, 1998).

As wines and *Aloe vera* based juices constitute the highly acceptable classes of beverages throughout the world, the present study was planned to combine the useful properties of these two categories of the products with the aim to develop a new class of functional fermented *Aloe vera* based herbal wine and the evaluation of its bactericidal effect on some common food borne pathogens and probiotic bacteria.

Materials and Methods

Microorganisms

Saccharomyces cerevisiae MTCC 786 procured from Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India was used to carry out the fermentation. It had growth optima at 28±2°C and pH 4.75±0.25. *Salmonella enteric*a serovar Typhimurium (Virulent strain NCTC 74) was procured from the Central Research Institute (CRI), Kasauli (India), *Escherichia coli* NCIM 2065 was procured from National Collection of Industrial Microorganisms (NCIM), Pune (India). *Staphylococcus aureus* ATCC 9144, Standard probiotic strains of *Lactobacillus casei* MTCC 1423, *Lactobacillus plantarum* MTCC 2621 and *Lactobacillus acidophilus* MTCC 447, were procured from Microbial Type Culture Collection, IMTECH, Chandigarh, India.

Animals

Balb/c mice (20-25g) used in this study were procured from the Central Animal House of Panjab University, Chandigarh (India).The animals were housed under standard conditions of light and dark cycle with free access to food (Hindustan Lever Products, Kolkata, India) and water. The experimental protocols were approved by the Institutional Ethical Committee of Panjab University, Chandigarh, India.

Extraction and processing of **Aloe vera** *gel*

Aloe vera leaves were collected from local nursery in Chandigarh and the colourless gel contained in the inner part of the fresh leaves extracted by hand filleting method (Ramachandra and Srinivasa Rao, 2008) was used for the production of wine. Briefly, the lower 1 inch of the leaf base, the tapering point (2-4 inches) of the leaf top and the short sharp spines located along the leaf margins were removed by a sharp knife. The knife was then, inserted into the mucilage layer below the green rind followed by the removal of top and bottom rinds. The gel was then, blended in a mixer after adding cane sugar to adjust the TSS to 20% and then, supplemented with 0.5% $(NH_4)_2SO_4$, 0.1 % each of $MgSO_4$ and KH_2PO_4. The pH was adjusted to 4.75 to make it favourable for yeast growth, using citric acid.

Inoculum preparation

Twenty five ml of sterilized GYE broth (yeast extract, 0.3%, malt extract, 0.3%, peptone, 0.5% and glucose, 1%, pH 4.5), dispensed in 100 ml flask was inoculated with loopful culture of *Saccharomyces cerevisiae*. The flask was incubated at 30°C on a rotary shaker (150 rpm) for overnight and the cells were

separated by centrifugation at 10,000 rpm (4°C, 15 min). These were washed twice and re-suspended in normal saline to give a concentration of 10^8 cells/ml which was used as a pre-inoculum. The inoculum was prepared by transferring 10 ml of the pre-inoculum to 250 ml conical flask having 100 ml of *Aloe vera* juice supplemented with 5% sucrose and incubating overnight as a shake culture at 30°C.

Fermentation

Aloe vera gel supplemented cane sugar, $(NH_4)_2SO_4$ $MgSO_4$ and KH_2PO_4 with pH 4.75 was subjected to batch fermentation. One litre of medium containing 20% TSS with 19% w/v total sugar was taken in 2 L Erlenmeyer flask, seeded with 10% v/v yeast inoculum, supplemented with 100 ppm sodium metabisulphite and incubated in a stationary state after plugging with cotton wool in a BOD incubator at 28±2°C for 7 days. The contents of the flask was mixed 2-3 times a day and the progress in fermentation was noted at regular intervals of 24 h by analyzing TSS, sugar content, ethanol and pH. After completion of fermentation, the wine was clarified, by repeated siphoning which was carried out 4 times with a sedimentation period of 3 days between each siphoning and analyzed for various constituents including TSS, total acids, pH, residual sugars, soluble proteins, total phenolics, alcohol contents and various elements including Ca, Na, K, Mg, Fe, Cu, Zn, Mn.

Maturation of wine

Ageing of *Aloe vera* wine was done for one year in an oak wood barrel (2.5 L), procured from M/S Jagatjit Industries Limited, Hamira, Punjab (India). The containers were filled upto the brim and analyzed for various components after one year of ageing.

Physico-chemical analysis of wine

Total soluble solid (TSS) content was checked using a hand refractometer (Erma). pH was measured by digital pH meter. The total sugars as glucose (Dubois *et al*., 1956), titrable acidity as tartaric acid (Amerine *et al*., 1980), total soluble proteins as BSA (Lowry *et al.*, 1951), total phenolics as gallic acid (Rathee *et al.*, 2006), antioxidant activity (Benzie and Strain 1996) and ethyl alcohol (Caputi *et al*.,1968) were determined using standard procotols. Fermentation efficiency was calculated as:

$$\text{Fermentation efficiency (FE)} = \frac{\text{Alcohol content in wine}}{\text{Alcohol content obtainable from sugar utilized}} \times 100$$

Acetaldehyde, ethyl acetate, methanol, n-propanol, n-butanol, iso-amyl alcohol, iso-amyl alcohol and furfurals were detected and quantified by gas chromatograph (Hewlett Packard 5790) equipped with flame ionizing detector using a glass column (6´×1/4´) packed with carbowax-20M. The estimation of elements was done by atomic absorption spectrophotometer (Soni *et al.*, 2009).

Sensory evaluation of wine

The organoleptic evaluation of fresh and matured *Aloe vera* wine was done by a panel of five judges on the basis of scoring in terms of appearance, colour, aroma, bouquet, acescent, total acid, sugar, body, flavour, astringency and general quality as per the prescribed performa (Amerine *et al*., 1980).

Antibacterial activity against food borne pathogens

The agar well diffusion test based on the method of (Deans and Ritchie 1987) was used to determine *in-vitro* inhibitory effect of *Aloe vera* wine against the common food pathogens including *S. typhimurium* , *S. aureus* and *E. coli*. The actively grown cultures in nutrient broth were selected for plate assay with cell count of approximately 10^5 cfu/ml. 100µl of each of the liquid culture was spread on nutrient agar plate to create a bacterial lawn. Three wells with diameter of 6 mm were punched in each nutrient agar plate and 100µl of *Aloe vera* wine was added to the well under aseptic condition. 100µl *Aloe vera* juice as well as 10% v/v ethanol was also loaded in two different wells as a control to compare the antibacterial activity with *Aloe vera* wine. The plates were left for 30 min at room temperature for the diffusion of the test samples before being incubated at 37°C for 24 h. The diameters of the zones of inhibition were measured after 24 h. All analyses were carried out in triplicates.

Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of wine

MIC of wine was determined according to the method of (Moretro and Daeschel 2004). *Aloe vera* wine was diluted in brain heart infusion (BHI) to concentration in the range of 10% to 50% (5% interval, v/v), with a final volume of 3ml in test tubes. BHI medium of double strength together with sterile distilled water was used to make dilutions, resulting in BHI with normal strength in final dilutions. The tubes were inoculated with 30µl of culture grown overnight and incubated for 24 h at 37°C. Minimal inhibitory concentration was determined as the lowest concentration of *Aloe vera* wine that inhibited the visible growth of bacteria after overnight incubation. The MBC was determined by plating the contents of the tubes containing MIC and higher concentration of wine on nutrient agar plate and incubated at 37°C for 24 h MBC was defined as lowest concentration of wine resulting in >99.9% reduction of the initial inoculums. All analyses were carried out in triplicates.

Time dependent bactericidal assessment of wine against common food borne pathogens

Bactericidal activity of *Aloe vera* wine was determined against *S. typhimurium, S. aureus* and *E. coli* by the method of Moretro and Daeschel (2004). 50µl of 16 to 20h old culture of either of the pathogenic culture with approximately 10^9cfu/ml was added to 4.95 ml of wine. The final cell concentration during exposure was approximately 10^7cfu/ml. The suspension was mixed with a vortex mixer and plated on to BHI agar (Hi Media, India) after incubation at ambient temperature of 35°C for 0, 10, 20 and 30 min. Plates were incubated for 24 h at 37°C and bacterial count was taken. All analyses were carried out in triplicates.

Assessment of wine against probiotic organisms

The *in-vitro* effect of *Aloe vera* wine was evaluated against *L. casei, L. plantarum* and *L. acidophilus*, by agar well diffusion assay. 100µl actively grown culture with cell count of approximately 10^5cfu/ml was spread on De Mann Rogosa Sharpe (MRS) agar (Hi Media, India) plate to create a bacterial lawn. Well (6 mm dia) punched in each plate was filled aseptically with 100µl of *Aloe vera* wine. Thereafter, the plates were left for 30 min at room temperature before being incubated at 37°C for 24 h. The diameters of the zones of inhibition were measured after 24 h. All analyses were carried out in triplicates.

Health benefit potential of *Aloe vera* wine was assessed in terms of persistence of probiotic organisms in the gut by determining the microbial cell count in the fecal matter of the wine-fed animal group (3 in each group). Each mouse in the wine fed group was given standardized dose of 0.3 ml prepared *Aloe vera* wine once in a day for 4 weeks. To enumerate the lactobacilli, freshly voided feces were collected and pooled from each mouse (0.5 g/mouse) at 0, 7, 14, 21 days to confirm the non-inhibitory effect of *Aloe vera* wine on the probiotic strains within the gastrointestinal tract. The feces were homogenized in normal saline, serially diluted and plated on MRS agar for the enumeration of lactobacilli. The plates were incubated at 37°C for 24 h and the number of colonies appearing on the plates was recorded (Oyetayo *et al*., 2003). All analyses were carried out in triplicates.

Statistical analysis

All the results were expressed as mean±S.D. obtained from three trials. Comparisons between fresh wine and matured wine were made by Student's t-test and $p < 0.05$ was considered significant.

Changes during fermentation

Aloe vera gel supplemented with 0.5% $(NH_4)_2SO_4$, 0.1 % each of $MgSO_4$ and KH_2PO_4 along with cane sugar (TSS of 20%) proved to be a good growth medium for *Saccharomyces cerevisiae* and was converted into wine. The yeast strain grew well and utilized sugar at the rate of 110 mg/h/100 ml producing ethanol at the rate of 51 mg/h/100ml with over 90% fermentation efficiency (Figure 1) thus, yielding a yellowish orange alcoholic beverage. The fermentation started after about 12 h, as indicated by the start of CO_2 bubbles escaping out from the medium which picked up after 24 h and continued for 7 days. Total soluble solids and sugar levels decreased gradually after 1 day of incubation and the same continued for 7 days. The kinetics of the fermentation revealed that the rate of sugar utilization during the first 24 h was 42 mg/h/100 ml. This picked up to 167mg/h/100 ml during the next 24 h and remained constant from 24 to 96 h of fermentation, after which, it declined to 125 mg/h/100 ml from 96 to 120 h, 63 mg/h/100 ml during the next 24 h and ultimately to 42 mg/h/100 ml during the last 24 h of fermentation. On the other hand, the rate of alcohol production was just 13 mg/h/100 ml during the first 24 h of incubation which increased gradually to 42 mg/h/100 ml during the next 24 h, further rising to 58 mg/h/100 ml during 3rd day. Highest rate of alcohol production was noted at 83 mg/h/100 ml during the 4th day of fermentation followed by gradual decline to 75, 58 and 24 mg/h/100 ml during 5,6 and 7th day of fermentation respectively (Figure 1). The pH of the medium revealed a gradual fall with the progress in fermentation, showing a final value of 3.7±0.02 at the end of fermentation. Quantitatively, the ethanol content in the wine, as observed after 7 days of fermentation, was 8.5% w/v. Ethyl alcohol is the most important component present in all the alcoholic beverages (Amerine *et al*. 1980) and is associated with

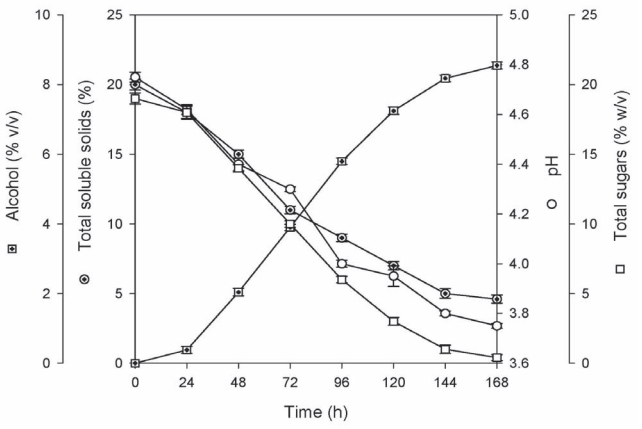

Figure 1: Pattern of sugar utilization, pH and alcohol production during the fermentation of *Aloe vera* gel. All the results represent the mean±S.D.of three independent experiments at 95% confidence level. For the assays of various parameters the S.D. was less than 5% with the samples from the same experiment. For the samples from different experiments, the S.D. was less than 10%.

stimulatory and intoxicating properties of these beverages (Gasteineau *et al*., 1979).

Composition of wine

The fermentation of *Aloe vera* gel yielded a clear yellowish orange wine, with pH of 3.7±0.02, at the end of 168 h of fermentation. The *composition of* wine is depicted in Table 1. It revealed the presence of various alcohols with ethyl alcohol as the main alcohol at 8.5±0.02% w/v in addition to methanol, n-propanol, n-butanol and iso-amyl alcohol at the levels of 122.5±2, 5.3±0.28, 107.5±3.5 and 1607.5±10.6 mg/l. It also revealed the presence of total acids (1.1±0.07 % w/v), phenolic compounds (180±1.8mg/l) acetaldehyde (18.0±1.2 mg/l), ethyl acetate (33.9±1.4 mg/l) and furfurals (1.5±0.70 mg/l). Ca, K and Na as the major elements amounting to 1153±1.4, 407.5±0.70, 124.5±0.70 mg/l with traces of Fe, Mg, Zn Mn and Cu in the range of 374.5±0.70, 80.7±0.35, 34.7±0.35, 13.4±0.07 and 5.1±0.075 µg/l respectively (Table 1).

Many odorous compounds and their precursors are present in wines. Relatively large amounts of volatiles, especially, acids, alcohols, ethyl acetate and acetaldehyde derivatives are produced during fermentation by yeasts whose growth and metabolism is affected by temperature. Total acids determine the amount of tartness, the wine delivers on the palate, and the resultant low pH may help in controlling the microbiological and chemical reactions (Vine *et al.* 1999). In addition to the ethyl alcohol, wines also contain several other alcohols including polyalcohols and cyclic alcohols, adding to the degree of sweetness and aroma of wines. *Aloe vera* wine also contained traces of methanol and higher alcohols, well within the desired level (Table 1). The level of higher alcohols upto 400 mg/l improves the aroma while the concentration higher than this level deteriorates the quality of wine (Gayon 1978). Of all the alcohols, the methanol is most undesirable and is toxic at 4% concentration, causing blindness or even death. It is generally formed as a result of the hydrolysis of pectic substances and is present in traces in wine (Lee *et al.*, 1975).

The phenolic compounds of wine are considered important, as they contribute to sensory characteristics, particularly color, astringency, and bitterness (Robichand *et al.*, 1990) and as they are also involved in biochemical and pharmacological effects, including antimicrobial, anticarcinogenic and antioxidant properties (Mazza *et al*., 1993). Esters(Table 1) are indispensable components of wine quality as they impart the

Table 1: Composition of fresh and matured *Aloe vera* wine

Constituent	Fresh wine	Aged inoak wood barrel
Colour	Pale yellowish	Yellowish orange
TSS (%)	4.1±0.41	4.3±0.10
Total Acids (g/100 ml)	1.1±0.07	0.85±0.06*
pH	3.7±0.02	3.8±0.02*
Soluble proteins (g/100 ml)	1.8±0.01	1.7±0.04
Ethanol (% w/v)	8.5±0.02	8.5±0.007
Methanol (mg/l)	122.5±2.1	112.8±3.32
n-propanol (mg/l)	5.3±0.28	1.3±0.07*
n-butanol (mg/l)	107.5±3.5	10.6±0.53*
iso-amyl alcohol (mg/l)	1607.5±10.6	1015.5±0.70*
Acetaldehyde (mg/l)	18.0±1.2	5.5±0.77*
Ethyl acetate (mg/l)	33.9±1.4	351.3±1.96*
Furfural (mg/l)	1.5±0.70	0.89±0.007
Phenolics (mg/l)Antioxidants (µM/l)	180±1.81408.47±7.2	800±0.62*4271.18±8.8*
Sugar (g/100 ml)	0.38±0.03	0.19±0.007*
Ca (mg/l)	1153±1.4	1155.5±0.70
Na (mg/l)	124.5±0.70	125.2±1.06
K (mg/l)	407.5±0.70	407.7±0.35
Fe (µg/l)	374.5±0.70	375.5±0.70
Cu (µg/l)	5.1±0.07	5.1±0.03
Mg (µg/l)	80.7±0.35	81.5±0.70
Zn (µg/l)	34.7±0.35	35.2±0.42
Mn (µg/l)	13.4±0.07	13.95±0.07

Values are expressed as mean±SD of the observations (in triplicate) from three independent experiments. *Shows significant difference (*$p<0.05$).

fruity flavour to the wines . Wines contain several inorganic constituents in the form of many anions and cations (Amerine *et al.,* 1980) amounting to the nutritive value of the wine and impart freshness to its flavour. The *Aloe vera* wine prepared in the present study also revealed the presence of Ca, K and Na as the major elements with traces of Fe, Mg, Zn Mn and Cu (Table 1). The salts amount to 0.2 to 0.4 % and are generally derived from mineral acids or organic acids. Amongst the minerals, K, Ca, Na and Mg are the major elements while Fe, Cu, Mn and Zn are the trace elements, generally found in wines (Amerine *et al*., 1980; Ough 1991).

Physico chemical changes during ageing of wine

The ageing of the wine in oak wood barrel improved the characteristics in terms of clarity, colour and proved to be very useful in bringing down undesirable components including acetaldehyde, methanol, n-propanol, n-butanol, iso-amyl alcohol, furfural which decreased to the levels of 5.5±0.77, 112.8±3.32, 1.3±0.07, 10.6±0.53, 1015.5±0.70, 0.89±0.007 mg/l respectively. It brought about an increase in desirable components including ethyl acetate and total phenolics which rose to the levels of 351.3±1.96 and 800±0.62 mg/l respectively in addition to an improvement in aroma, organoleptic characteristics and this wine was also adjudged to be the better after tasting by a panel of five judges (Table 2). Oak is the most commonly used wood for barrels used for aging quality wines and spirits because of its positive effects on improvement in wine characteristics and color stability (Sharma *et al.,* 2001).

Antibacterial activity against food borne pathogens

Aloe vera wine revealed antibacterial activity against the common pathogens tested in the study. The zones of inhibition for *S. typhimurium, S. aureus* and *E. coli* and were found to be 10.2±0.12 mm, 09±0.18 mm and 07±0.14 mm respectively whereas no zone of inhibition was observed for *Aloe vera* juice and 10% v/v ethanol as assessed by agar well diffusion assay Plate 1). Previously, several authors have noted that wine has a greater antibacterial effect compared with the same concentration of diluted absolute ethanol (Weisse *et al.,* 1995; Marimon *et al.,* 1998). Wine possesses relatively high ethanol content in addition to other antimicrobial agents like organic acids, low pH, polyphenol compounds and preservatives (Just and Daeschel 2003) which may be responsible for the pronounced inhibitory effect. Marimon *et al*., (1998) 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 (Moretro and Daeschel 2004). A decrease in pH leads to an increase in the un-dissociated form of organic acids, which are considered to be the antimicrobially 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

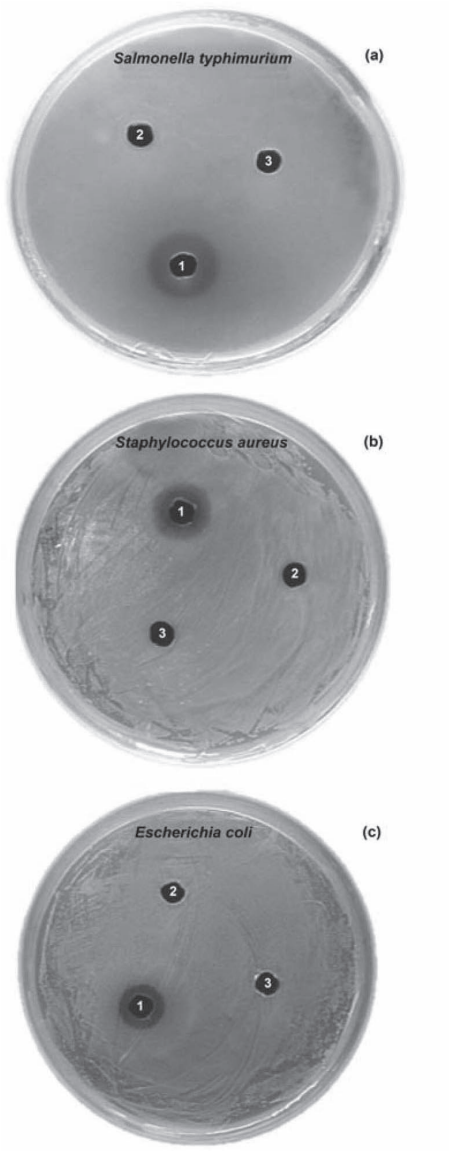

Plate 1: In-vitro study showing the effect of *Aloe vera* wine (1), Aloe vera gel (2) and 10% ethanol v/v on the growth of *Salmonella aureus* (a), *Staphylococcus aureus* (b) and *Escherichia coli* (c) in agar well diffusion assay on nutrient agar plates. The clear zones around the well of *Aloe vera*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

Table 2: Evaluation card of fresh and matured *Aloe vera* wine by five tasters

Characteristic	Max.score	Score by tasters									
		I		II		III		IV		V	
		F	M	F	M	F	M	F	M	F	M
Colour	2.0	0.5	2.0	0.5	1.5	1.0	1.5	0.5	2.0	1.0	2.0
Aroma	2.0	0.5	2.0	1.0	2.0	0.5	2.0	1.0	2.0	0.5	2.0
Bouquet	2.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0
Acescent	2.0	0.5	2.0	1.0	1.5	1.0	2.0	1.0	2.0	1.0	1.0
Total acid	2.0	0.5	2.0	1.0	2.0	1.0	2.0	1.0	1.0	1.0	1.5
Sugar	1.0	1.0	0.5	1.0	1.0	1.0	0.5	1.0	1.0	1.0	1.0
Body	1.0	1.0	1.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Flavour	2.0	1.0	2.0	1.0	1.5	1.0	2.0	1.0	2.0	1.0	2.0
Astringency	2.0	1.0	2.0	0.5	2.0	1.0	2.0	1.0	2.0	1.0	1.5
General quality	2.0	1.5	2.0	1.0	2.0	1.0	2.0	1.0	2.0	1.5	2.0
Total	20.0	8.5	17.5	8.5	16.5	9.5	17.0	9.5	17.0	10.0	16.0

F: Fresh wine, M: Matured wine.

juice and wine (Harding and Maidment 1996; Barker and Park 2001; Just and Daeschel 2003). 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 (Plate 1).

Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of wine

MIC and MBC of the *Aloe vera* wine for *S. typhimurium* was found to be 35% and 40%, respectively whereas for *S. aureus* and *E. coli* it was found to be 45% and 50% respectively (Table 3). *S. typhimurium* was found to be the most sensitive to wine, with 5 log reduction after 10 min exposure to wine, whereas more than 20 min of exposure was needed to obtain 5 log reduction for *S. aureus* and *E. coli* as determined by time dependent bactericidal assay. No viable organism could be detected in all the three cases after 30 min exposure (Figure 2). Just and Daeschel (2003) showed similar results when they treated *E. coli* O157:H7 and *Salmonella* spp. with Chardonnay and Pinot Noir, the grape wine varieties. It is also known that *E. coli* more readily survives at an acidic pH than *S. typhimurium* (Gorden and Small 1993, Lin *et al.*, 1995). Weisse *et al.*, (1995) reported significant reduction in the number of *E. coli* O157:H7, *Salmonella* serotype Typhimurium and *Shigella sonnei* using red and white wine from 10^6 cfu units/mL to undetectable concentrations within 20 min of exposure. Moretro and Daeschel (2004) detected that red wine was most potent against various foodborne pathogens tested compared to the white wines without added sulfites. Typically, a 3-6 log reduction in viable cell counts is expected after 10 min of exposure. Another study indicated that red wine led to a 4-5 fold reduction of the initial population of *Listeria innocua* in a model stomach system (Fernandes *et al.* ,2007). Phenolic fractions from wines showed marked antimicrobial activity, indicating some contribution of the phenolic compounds in inactivation of microorganisms by wine treatment. Efficacy of the phenolic compounds in the wine may be enhanced by the inherent environment present in the wine (that is, pH, ethanol concentration, and so on). Many researchers have investigated the impact of phenolic compounds on *H. pylori* inhibition. Mahady and Pendland (2000) and Mahady *et al.*, (2003) determined the MIC 50 value of 12.5 ìg/mL of resveratrol against *H. pylori* strains using an agar disk diffusion assay, while Chan (2002) determined the MIC of resveratrol against *S. aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* to be 171 to 342 µg/mL by a broth dilution assay. Additionally, Friedman *et al.* ,(2006) developed wine formulations containing plant essential oils and oil compounds that were significantly effective against *E. coli* O157:H7 and *Salmonella enterica*.

Table 3: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *Aloe vera* wine against selected pathogens

Name of pathogen	MIC	MBC
Salmonella typhimurium	35%	40%
Escherichia coli	45%	50%
Staphylococcus aureus	45%	50%

Values are based on results from 3 parallel experiments. The wine was tested at 5% intervals.

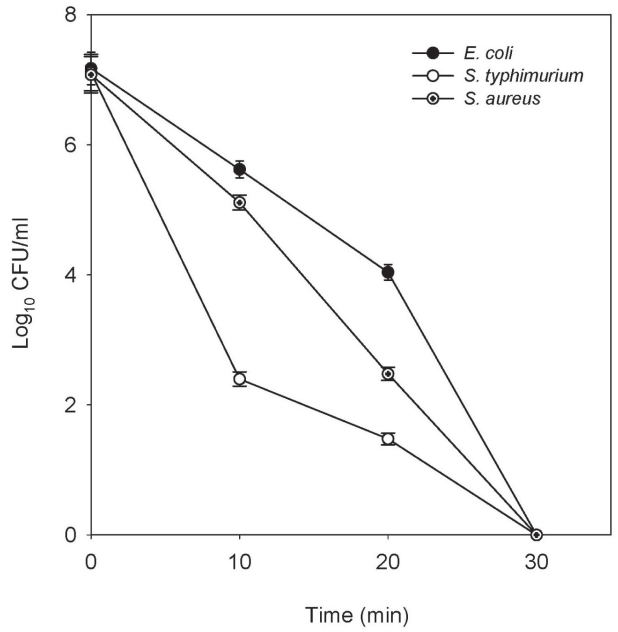

Figure 2: The pattern of reduction of S. *typhimurium* , S. *aureus* and *E. coli* counts in presence of Aloe uera wine indicating bactericidal activity. The data represents the means ± S.D. of three in dependent experiments at 95% confidential level. For the bacterial counts the S.D. was less than 5% .... The samples from the same experiment. For the samples from different experiments, The S.D. was less than 10%

According to Waite and Daeschel (2007), *S. aureus* was more resistant to wine treatment than was *E. coli* O157:H7. Our experiments also demonstrated different inhibition patterns for the pathogens tested against *Aloe vera* wine which can also be attributed to the fact that wine effectiveness in the inactivation of particular bacterium is strongly dependant on the specific wine composition as reaffirmed by Fernandes *et al.,* (2007). Volatile acidity may also impact the efficacy of a specific wine treatment against various microorganisms. Sugita-Konishi *et al.,* (2001) found that the majority of antibacterial effect of wine against *Salmonella enteritidis*, *E. coli* O157:H7 and *Vibrio parahaemolyticus* was due to the volatile components of wine.

Assessment of Aloe vera *wine against probiotic organisms*

Probiotics, which are considered the good bacteria, were also exposed to *Aloe vera* wine and the same were observed to survive at higher concentrations of *Aloe vera* wine. *Aloe vera* wine was found to be safe towards the probiotic bacteria including *Lactobacillus casei*, *L. plantarum* and *L. acidophilus*, which are important in the gut of the animal and the same were not inhibited. These bacteria continued to grow in the presence of wine without any discernible zone of inhibition around each well, indicating a higher tolerance to wine than the food borne pathogens tested. Furthermore, there was no significant difference in viable count of lactobacilli in the feces of mice fed with the wine for the period of 3 weeks (Table 4). The results show that the wine does have antimicrobial properties against food borne pathogens while not affecting health beneficial probiotic bacteria. Similor studies have reported that wine provides digestive benefits and intestinal health by encouraging the proliferation of friendly bacteria and simultaneously provides protection against foodborne pathogens (O'Connor 2012; Wells 2012). Drinking of wine increases the levels of gut probiotics, including bifidobacteria, *Enterococcus*, *Prevotella*, and *Bacteroides*, which are essential to digestive health. Probiotics also lower cholesterol, inhibit cancer, boost immunity, and protect against numerous other systemic conditions (Barron 1999). It is the polyphenols in wine that inhibit non-beneficial bacteria from the human microbiota and potentiate the growth of probiotic bacteria, promoting health benefits to the host (Cohen , 2012).

Table 4: Viable count of lactobacilli in feces of wine fed mice

Time(Weeks)	Log_{10} cfu/g faeces
0	7.6±0.29
1	7.4±0.39
2	7.7±0.16
3	7.8±0.11

Values are expressed as mean±SD of the observations (in triplicate) of samples from three independent experiments at 95% confidence level.

Conclusion

Aloe vera gel may be used as a valuable ingredient for the production of a herbal wine with all the important properties of wine. *Aloe vera* wine exhibited antibacterial activity against the pathogens along with the ability to maintain the persistence of lactobacilli in the gut of wine fed animal. This present study is probably the first study where the *Aloe vera* gel has been fermented for the production of a new class of functional wine.

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