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RESEARCH PAPER

A Valorized Wine from *Aloe vera* and *Mentha arvensis* and its LC-Q-ToF-MS Metabolic Profiling

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Abstract

In the view of evidently proven medicinal benefits of *Aloe vera* and *Mentha arvensis* and growing consumer interest in functional foods, aloe and mint were chosen for the production of a functional beverage in the form of wine. The fermentation of exogenously cane sugar supplemented mixture of aloe gel and mint extract, yielded a pale yellowish wine with an ethanol content of 9.5% (v/v). The wine could be considered to be quite health promoting in terms of its total phenolic content, which was 1785 mg GAE/L. LC-MS studies of the same showed the presence of many bio-active compounds including aloin, myricentin, luteolin, quercitin. Value-addition of wine was done by adding the probiotic strain *Lactobacillus sporogenes.* The probiotic supplemented wine when evaluated against common food borne pathogens, proved to be an effective anti-bacterial therapy. *Aloe-Mentha* wine thus, can be a promising candidate of the expanding range of functional beverages.

Keywords: *Aloe vera*, *Mentha arvensis*, Functional food, Wine, Polyphenols, Probiotic

Recent studies in the field of food and medicine have shown the explosion of consumer interest in specific foods that help in the maintaining optimal health and thereby, act as functional foods. Functional foods provide physiological benefits beyond the basic nutrition. Plants are the major source of functional foods. More than a dozen classes of biologically active components called as phytochemicals have been identified in various plants, which have experimentally been proven to possess medicinal properties (Steinmetz and Potter, 1991). The past decade has witnessed an intense interest in herbal foods in which their phytochemical constituents impart medicinal benefits (Dillard and German, 2000). Phytochemicals present in food and beverages have been shown to reduce the risk of various ailments including cardiovascular problems, diabetes, neurodegenerative disorders, oxidative stress, cancer etc. (Dillard and German, 2000). In this context, wine is a beverage that has been discussed and investigated more than any other functional beverage. Advanced studies have proved that red wine polyphenolics mainly, resveratrol, quercetin, catechin and gallic acid. have protective effect against many diseases (Shahidi *et al.* 2008). Value addition of white wine by supplementation with polyphenols improve, its nutraceutical properties (Landrault *et al.* 2003). Polyphenolic profile depends upon the substrate used for wine production. Many groups are working on the development of new varieties of herbal wines, which can add to the growing pool of functional foods (Trivedi *et al.* 2012; Soni *et al.* 2009 and Joshi *et al.* 2012). *Aloe vera* and *Mentha arvensis* (mint) are two medicinally important plants with proven medicinal potencies. Mucilaginous gel from the parenchyma cells of *Aloe vera* have been used since early times for curative purposes. Aloe has been found to be capable of wound healing, burn healing, arthritis, anti-diabetic, immune boosting and anti-bacterial properties (Reynolds and Dweck,

1999). Similarly, the essential oils of mint, especially menthol have been found to exhibit many *in vitro* and *in-vivo* activities such as antimicrobial, anticancer and analgesic. (Kamatou *et al.* 2013). Taking into consideration, the functional properties of aloe and mint, they were chosen in the present study to product a new herbal wine. Further, studies were undertaken for the value-addition of the wine prepared by exogenous supplementation with Probiotic strain *Lactobacillus sporogenes.* To the best of our knowledge, this is a first study of its kind for the preparation of a probiotic supplemented wine.

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. Sporlac sachet of Sanzyme limited was used as a source of *Lactobacillus sporogenes* i.e. lactic acid bacillus powder. It was procured from a local chemist store in Chandigarh and was used as a probiotic organism in the study.

Extraction and processing of Aloe vera gel

Aloe vera leaves were collected from local nursery in Chandigarh and the parenchymatic gel of the fresh leaves was extracted by hand filleting method (Ramachandra and Srinivasa, 2008). The gel was, then blended in a mixer and used for the production of wine.

Extraction and processing of Mint extract

Mint (*Pudina*) was collected from the local vegetable market of Chandigarh. 10% w/v of aqueous extract of mint was prepared for the production of wine.

Inoculum preparation

Saccharomyces cerevisae was grown in sterilized glucose yeast extract (GYE) broth for overnight at 30ºC on a rotary shaker (150 rpm) and then, separated by centrifugation at 10000 rpm (4ºC, 15min). These were washed twice and suspended in normal saline to obtain a concentration of 10^8 cells/mL which was used as Pre-inoculum. The inoculum was prepared by transferring 10mL of pre-inoculum in the 250mL conical flask having 100mL mixture of *Aloe-vera* gel and 10% (w/v) Mint extract taken in a ratio of 1:1. The mixture was supplemented exogenously with cane sugar to adjust Total Soluble Solids (TSS) at 5°Brix and incubated overnight at 30ºC in shaking incubator (150rpm).

Fermentation of Aloe-vera gel and Mint extract

Aloe vera gel and 10% mint extract was taken in a ratio of 1:1, supplemented with sugarcane for adjusting TSS to 20°Brix. 1 L of the mixture so prepared was taken in 2 L Erlenmeyer flask and seeded with 10% (v/v) inoculum having a viable count of 1×10^8 cells/ml. It was followed by supplementation with 0.1% (w/v) DAP, Magnesium sulphate and Potassium dihydrogen orthophosphate, respectively. 100ppm of Sodium metabisulphate was added to it and the flask was incubated in stationary state in a BOD incubator at 25±2ºC for 15 days for batch fermentation. The contents of the flask were shaken 2-3 times a day. The progress in fermentation was noted at a regular interval of 2 days by analysing total soluble solids (TSS), pH and ethanol content. The wine was clarified after completion of fermentation by siphoning it 4 times with a repeated sedimentation period of 3 days.

Physico-chemical analysis of wine

TSS content was checked using a hand refractometer (Erma). pH was measured by a digital pH meter. Total sugars as glucose (Dubois *et al*. 1956), total soluble proteins as BSA (Lowry *et al.* 1951), titrable acidity as tartaric acid (Amerine *et al*. 1980), total phenolic content as gallic acid equivalents (Rathee *et al.* 2006), antioxidant activity as ferric reducing ability (Benzie and Strain, 1996) and ethanol (Caputi *et al*.1968) were determined using standard protocols.

LC-Q-ToF-MS metabolic profiling of wine

20µL of the samples was used for LC-Q-ToF-MS analyses. Metabolites were separated using a Waters

Micromass Q-Tof Micro equipped with electrospray ionization. A reversed-phase (RP) separation method was employed using a C18 column (5 µm, 250 mm × 5 mm i.d.). Buffer consisted of methanol (A) – 0.1% formic acid (B) under gradient elution. The linear gradient increased from 10 to 17% A in 7 min, and increased to 30% A in another 1 min. Then, the solution A was continuously increased from 30 to 90% in 12 min. Finally, the solution A was linearly decreased back to the initial condition of 10%. Analysis was carried out in total chromatography ion (TIC) mode with positive ionization.

In vitro evaluation of toxicity of wine against probiotic bacteria

The *in vitro* effect of Aloe-mint wine was evaluated against *Lactobacillus sporogenes* by agar well diffusion assay. 100µl actively grown culture of *L. sporogenes* 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) punched in each plate was filled aseptically with 100µl of Aloe-mint wine. The plates were left for 30 min at room temperature before incubating at 37±2°C for 24 h. The diameters of the zones of inhibition if any, were measured after 24 h. All analyses were carried out in triplicates.

Fortification of wine with Lactobacillus sporogenes

L. sporogenes solution was prepared in 100 ml sterilized MRS broth by suspending one sachet of commercial Sporolac powder (1 gram) containing 150 million spores per gram. The broth was then incubated at 37°C for 3 days. After the incubation, broth was centrifuged at 10000 rpm for 10 min at 4°C. The pellet obtained was washed twice and suspended in sterilized distilled water. This probiotic solution with a viable cell count of 10^6 cells/ml was added to the wine at the rate of 10% (v/v). Viability of *L. sporogenes* in wine was checked repeatedly after 7 days for 5 weeks by pour plating and counting the bacterial colonies.

Antibacterial activity of wine 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-mint and probiotic supplemented Aloe-mint wine against the common food pathogens including *S. typhimurium*, *S. aureus* and *E. coli*. The actively grown respective 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 the wines was added to the well under aseptic condition. 100µl Aloe-mint extract and 10% v/v ethanol was also loaded in two different wells as a control to compare the antibacterial activity with Aloe-mint wines. 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.

Results and Discussion

Aloe-Mint fermentation yielded a clear pale yellowish colored wine with an ethanol content of 8.5±0.05% (v/v) and a final pH of 3.67±0.02. Physico-chemical characteristics of the wine is described Table 1.

Table 1: Physico-chemical characteristics of Aloe-Mint wine

Constituents in Wine	
Color	Pale yellow
TSS (OB)	3.1±0.20
Total Acids (g/100ml)	1.5±0.03
pH	3.67±0.02
Soluble Proteins (g/100ml)	0.7±0.01
Ethanol % (v/v)	9.5±0.05
Total Sugar (g/100ml)	0.619±0.03
Total Phenolics (mg GAE/L)	1785±10.28
Total Antioxidants (AAmmol/ml)	12.825±0.08

LC-Q-ToF-MS metabolic profiling of wine

LC-MS was conducted to validate the presence of polyphenols and anthocyanins in the Aloe-mint wine. Diode array and TIC chromatogram are shown in Fig.1. The major polyphenolic compounds were identified by their molecular M^+ and literature data (Table 2). HPLC coupled to absorbance monitor has been widely used for the detection of phytochemicals and photo diode array detectors are most extensively used for their identification. Gradient RP-HPLC with absorbance detection and MS with an electron interface was used for the analysis.

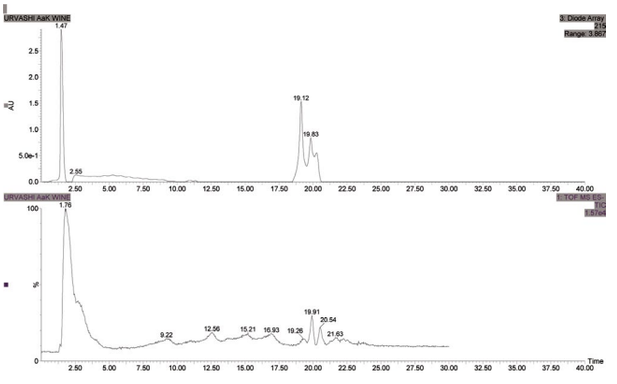

Fig. 1: Diode array and ToF MS ES-Total Ion Chromatograms of Aloe-mint wine

Evaluation of toxicity of Aloe-mint wine against probiotic strain Lactobacillus sporogenes

Aloe-mint wine did not produce any zone of inhibition against *L. sporogenes in vitro* in the well diffusion agar assay. As the wine did not infer any toxicity to the growth of the probiotic strain, therefore, it was considered as safe to add *L. sporogenes* to the wine for its value-addition.

Table 2: Polyphenolic profile of Aloe-mint wine

Compound	m/z
Ursolic Acid	191
Myricetin	317
Rosamarinic Acid	359
Gallic Acid	169
5-Hydroxyaloin	433
7-o-Methylaloesin	409
Luteolin	285
Malic Acid	133
Quercitin-3-o-rhamnopyranoside	487
Apigenin	117
Catechin/Epicatechin	289
Quercitin	301
6-methyl flavonol	275

Fortification of wine with probiotic Lactobacillus sporogenes

After addition of 10^6 cells/ml of *L. sporogenes* to aloe-mint wine @10% (v/v), the survival of the cells was evaluated regularly for 5 weeks. The cell count decreased significantly in first two weeks, but at the end of 5^{th} week, the final stabilized cell count was observed to be 10^3 cells/ml as shown in Table 3.

Table 3: Survival rate of *L. sporogenes* in Aloe-Mint wine

Time (days)	Cells/ml in wine
7	1×10^5
14	7×10^4
21	1×10^4
28	3×10^3
35	3×10^3

Antibacterial activity against food borne pathogens

Probiotic supplemented Aloe-Mint wine revealed better antibacterial efficacy than non-probiotic Aloe-Mint wine against the common pathogens tested in the study. The zones of inhibition for *S. typhimurium, E. coli* and *S. aureus* were found to be 4.0±0.18 mm, 4.1±0.02 mm and 4.0±0.07 mm respectively as compared to 2.5±0.2, 2.0±0.04 and 2.2±0.09 mm zones produced by non-probiotic Aloe-Mint wine as shown in Table 4.

Discussion

Aloe vera gel and mint extract mixture with added cane sugar for achieving a TSS of 20°Brix and supplemented with 0.1% (w/v) Diammonium

orthophospahte, Magnesium sulphate and Potassium dihydrogen orthophosphate proved to be an ambient medium for the growth of *Saccharomyces cerevisiae*.

Table 4: Zone of inhibition (mm) of Aloe-Mint wine against selected food borne pathogens

Sample	Zone of Inhibition (mm)		
	S. typhimurium	***E. coli***	***S. aureus***
Aloe-Mint Extract	1.8±0.05	1.9±0.08	1.6±0.02
10% ethanol (v/v)	2.2±0.11	1.9±0.07	2.0±0.05
Aloe-Mint Wine	2.5±0.2	2.0±0.04	2.2±0.09
Probiotic Supplemented Aloe-Mint Wine	4.0±0.18	4.1±0.02	4.0±0.07

The organism grew well and utilized sugars, yielding a clear pale yellowish colored beverage with an ethanol content of 9.5±0.05 % (v/v). pH of the medium gradually decreased and reached at a value of 3.67±0.02 from an initial pH of 4.5. Similar observations have been documented in case of naive *Aloe vera* wine whose fermentation yielded a clear wine with an ethanol content of 8.5% and pH of 3.7 (Trivedi *et al.* 2012). The sugar content of the wine decreased from 18 ±0.02 % to 0.619±0.053 % (w/v) demonstrating the fermentation efficiency of 85.97%. Peptides and proteins are non-utilizable for yeast metabolism and their solubility decreases with increase in alcohol content of wine.

This may lead to precipitation of proteins to form a visible amorphous haze (Weiss and Bisson, 2002). The total soluble proteins in the wine were recorded to be 0.7±0.01 % (w/v). Phenolic content which is now considered as the second most important constituent after ethanol, was 1785±10.28 mg GAE/L. Seabuckthorn wine has been reported to possess a phenolic content of 689 mg GAE/L followed by grape wine (647 mg GAE/L), black currant (310 mg GAE/L) and apple wine (290 mg GAE/L) (Negi and Dey, 2009). This shows that aloe-mint have higher polyphenols as compared to many other herbal wines and thus, can be presumed as more healthy and a better nutraceutical product. Similarly, the antioxidant potential of aloe-mint wine in terms of its ferric ions reducing ability was recorded to be 12.825±0.08AAmmol/L, depicting the free radical scavenging capacities of aloe-mint wine to be quite high.

In order to get a qualitative estimation of the nature of polyphenolics present in aloe-mint wine, LS-MS was performed with TOF-ESI and positive ionization mode. The diode array and total ion chromatograms of the same are shown in Fig. 1. Molecular weight of major ions detected was used for identification of the compounds (Table 2). Detection of *Aloe-vera* components 5-Hydroxyaloin (433) and 7-o-Methylaloesin (409) are in agreement with earlier reports of detection of these compounds at similar peaks (Dagne *et al.* 1997 and Bisrat *et al.* 2000). Both of them are associated with anti-microbial, wound healing and many other medicinal efficacies (Chen *et al.* 2012). Other compounds including myricetin (317), luteolin (285), quercitin (301), 6-methyl flavonol (275) and Catechin (289) etc., also have many proven medicinal benefits (Dillard and German, 2000; Shahidi *et al.* 2008).

With an appreciable amount of phenolic and anti-oxidative content, aloe-mint wine proved to be another potential candidate of the growing pool of functional foods. To further enhance its attributes, we added the probiotic strain *Lactobacillus sporogenes* to it. Recently, owing to the evidences of health beneficial qualities of the probiotic, there has been a tremendous boost in the manufacture and consumption of probiotic foods. There are many studies on addition of probiotic to peach, apple, orange, cashew and many other juices (Marhamatizadeh *et al.* 2012 and Pakbin *et al.* 2014). However, to the best of our knowledge this is the first study on the production of a probiotic supplemented wine. Therefore, to start with, we evaluated the toxicity of aloe gel and mint extract mixture against *L. sporogenes* by well diffusion agar assay test, both of which did not show any toxic effect to the growth of the organism. Hence, we added *L. sporogenes* to aloe-mint wine at a viable cell count of 10^6 cells/ml and checked the survival rate of the organism for a period

of 35 days. Expectedly, the cell count decreased to a final value of 10^3 cells/ml after 35 days (Table 3). This can be explained by the effect of ethanol content of the wine on the growth of organism. Further, low pH of the wine could have been a problem for the survival of the organism. The viability of the probiotic have also been found to associated with oxygen content, oxygen permeation and storage temperature of the product (Pakbin *et al.* 2014). Because of these stringent conditions in the wine, the organism might have not been able to grow and survive. But, a viable cell count of 10^3 cells/ml in wine proves that further optimization of conditions for the growth of *L. sporogenes* in wine may lead to production of a favorable environment for the sustainability of the same in wine.

The *in vitro* anti-bacterial efficacy of Aloe-Mint wine and probiotic supplemented Aloe-Mint wine revealed that the addition of probiotic organism improved the anti-microbial activity of the wine as probiotic supplemented Aloe-Mint wine produced larger zones of inhibition as compared to Aloe-Mint extract, 10% v/v ethanol and Aloe-Mint wine. This can be attributed to the synergistic effect of ethanol, polyphenolic content and probiotic organism present in the wine. Previously, several studies have demonstrated that wine has a better antibacterial efficacy as compared to 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. Further, recent studies have demonstrated the anti-microbial activity of both probiotic strains and probiotic foods (Anas *et al.* 2014). This may have boosted up the *in vitro* antimicrobial potency of probiotic supplemented Aloe-Mint wine.

Conclusion

Functional foods contribute to the well-being, apart from providing basic nutrition. In this context, *Aloe vera* and *Mentha arvensis* are well studied herbs for their nutritive and medicinal aspects and therefore, could be a good substrate for the production of wine, a functional beverage. Aloe and mint both proved to be excellent substrates for wine production. A desirable ethanol content and significant quantities of phenolics show that the wine is a new potential candidate of functional beverage class. Value-addition with probiotic strain *Lactobacillus sporogenes* led to a better *in vitro* anti-bacterial efficacy of probiotic supplemented wine than Aloe-Mint extract and Aloe-Mint wine, indicating it to be a better beverage in terms of medicinal functionality. Thus, value-added probiotic wine from *Aloe vera* and *Mentha arvensis* can be a prospective contender of expanding class of health beneficial beverages.

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