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PRODUCTION OF GINGER- AND ALOE VERA-BASED HERBAL WINES AND THEIR ANTIMICROBIAL PROFILING

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ABSTRACT

Herbal wines are rich decoction of active secondary metabolites of plants. The consumption of herbal wines imparts health benefits to the consumers because of their antioxidant and antimicrobial properties. In light of the increasing demand for value-added herbal products, the present study aimed to develop a production process for herbal wine from aloe vera and ginger juices. The prepared herbal wines were evaluated for their antimicrobial properties against food-borne pathogens. Aloe vera and ginger juices were adjusted to TSS level of 20° Brix by adding honey. The juices were subjected to fermentation using an active culture of *Saccharomyces cerevisiae* at 28°C. Various fermentation parameters like pH, TSS, reducing sugars, and ethanol concentrations were periodically measured. The wine samples were analysed for titratable acidity, total phenolic content, and antimicrobial potential by standard methods. The aloe-vera and ginger wines showed final titratable acidity of 0.09 and 0.16; a ºBrix to acid ratio of 66.6 and 56.2, respectively, in a fermentation period of 20 days. Total phenolic content was 34.5 ± 0.28 for aloe vera wine and 179.6 ± 0.56 for ginger wine. The alcohol content of aloe vera and ginger wine was 8.29 and 7.91 with fermentation efficiency of 97.8 and 96.2, respectively. Both herbal wines exhibited antimicrobial activity against food-borne pathogens, *E. coli* and *Bacillus cereus.* These wines can be accepted as safe and healthy drinks as they retained good phytochemical characteristics.

Keywords: Aloe vera, foodborne pathogens, ginger, herbal wine

INTRODUCTION

Before the rise of modern formulated medicines, people used to treat with herbal formulations derived from plants. Herbal-formulated medicines are the oldest and very widely used form of medicine (Watchel-Galor and Benzie, 2011). In a botanical sense, the herb is a plant lacking a permanent woody stem that produces flowers and seeds which die after its growing season. In traditional medicine, the herb is a small, non-woody plant valued for its medicinal, flavour or aromatic compounds. According to World Health Organization (WHO), 80% people worldwide prefer herbal medicine because they are natural or have no or fewer chances of side effects. Herbs show safe, gentle, and effective treatment as they offer medicinal properties like anticancer, anti-malarial, anti-hypersensitive antifungal, and antimicrobial (herbs are rich in phytochemicals and many more (Singh *et al.,* 2018).

Wine has always been seen as a safe and healthful beverage, as well as an important dietary supplement (Joshi *et al*., 2012). It is 6-20% ethanol on a volume basis, constitutes hundreds of compounds such as polyphenols, flavonoids, minerals, etc. extracted from the substrate during the process of fermentation, all of which have medicinal properties (Patil *et al*., 2005; Soni *et al*., 2009; Trivedi *et al.*, 2015). During fermentation, these bioactive compounds and polyphenols are relaesed in the aqueous solution increasing their bioavailability (Shahidi, 2009). Several studies in recent years have shown that moderate wine intake has a favourable influence on the cardiovascular system as well as the overall well-being of consumers. (Careina, 2011). Fruit wines are made and consumed in considerable amounts in all advanced countries across the world (Jangra and Jangra, 2021). Despite the good fruit supplies, fruit wine manufacturing in India is not up to the mark (Joshi *et al.*, 2012). Herbalism is a type of traditional folk medicine that involves the use of plants and plant extracts. Herbal products are considered safe nowadays since they are compatible with human natural physiology (Oluwaseun and Ganiyu, 2008). There are about 8000 species of herbs found in India and approximately 151 species are found in Punjab. Several herbs can be used for the preparation of wine including amla, tulsi, ginger, tea, lemongrass, aloe vera, bael fruit, leech lime, mentha and rosella flower, and many more. Herbs may be used solely as substrates or combinations (Singh *et al*., 2018). Bioactive elements found in herbs and other plants have been demonstrated to have anti-oxidative, anti-carcinogenic, anti-hypertensive, anti-mutagenic, and antibacterial characteristics, making them potentially beneficial to one's health (Kahkonen *et al*., 1999, Tapsell *et al*., 2006). The nutritional value of herbal wine also gets enhanced during the fermentation process due to the release of amino acids and other nutrients by the yeast (Opara *et al.*, 2019).

Aloe vera (*Aloe barbadensis* Miller), is a short-stemmed succulent herbal plant known for its strong antibacterial, anti-inflammatory, anti-tumour, and immune stimulatory properties (Christaki and Florou-Paneri, 2010), besides containing anti-inflammatory compounds like campesterol and β-sitosterol (Kyaw and Phyoe, 2019). As a traditional remedy, it is used to treat cough, wounds, ulcer, gastritis, headache, and arthritis since immemorial age (Sahu *et al*., 2013). It helps in regulating protein synthesis in the body and muscle tissues. About 23 polypeptides are found in its juice which can help fight against AIDS, boosts immune function, eliminate cancer, and tumours; and stabilize blood sugar. Aloe vera wine is known to have antimicrobial activities and improves probiotic bacteria colonization in the gut (Trivedi *et al*., 2012). Ginger (*Zingiber officinale*) is an herbaceous perennial that is known to have excellent antimicrobial and antioxidant properties (Ghasemzadeh *et al*., 2010). Ginger rhizome contains both volatile and non-volatile compounds. The volatile compounds impart aroma and taste to ginger e.g., sesquiterpene and monoterpenoid hydrocarbon while the non-volatile compounds impart pungency to ginger e.g., gingerols, shogaols, paradols, and zingerone (Mashhadi *et al*., 2013). The use of honey as an artificial sweetener is an attractive option as in many beverages it is used to mask the off flavours (Isabel *et al.*, 2012). The honey has also been reported to have antioxidant and anti-inflammatory benefits in both *in vivo* and *in vitro* studies (Alvarez-Suarez *et al*., 2013; Ranneh *et al.*, 2021). After evaluating the functional properties of aloe vera and ginger these herbs were chosen for the present study with the objectives to produce and characterize herbal wines supplemented with natural sweeteners like honey and evaluate the finished product in terms of sensory characteristics.

MATERIALS AND METHODS

Microorganism and their maintenance

Saccharomyces cerevisiae MTCC 11815 was used to carry out the fermentation. *Klebsiella pneumonia* MTCC 109, *Bacillus cereus* MTCC 430, and *Escherichia coli* were used to assess the antimicrobial potential of the wine. All the cultures were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Yeast strain, *Saccharomyces cerevisiae* was maintained on GYE (glucose yeast extract agar) media. The bacterial strains *Klebsiella pneumonia, Bacillus cereus,* and *Escherichia coli* were maintained on nutrient agar medium. These microorganisms were grown on petri-plates which were stored at 4°C and sub-cultured periodically. For long-term storage, glycerol stocks were prepared and kept at -70 ± 2°C.

Raw material for wine production

Aloe vera (*Aloe barbadensis* Miller) was collected from a local nursery located at Fatehgarh Sahib, Punjab (India). Ginger (*Zingiber officinale*) was collected from the local market of Khanna, Punjab (India). The raw material was washed thoroughly with water and dried in air.

Microbial inoculum

The sterilized glucose yeast extract (GYE) broth was inoculated with a loopful culture of freshly prepared *S. cerevisiae* broth. The flask was incubated at 30°C on a rotary shaker (150 rpm) for 24 h. The yeast cell concentration of 10^6 cells mL^{-1} was used as a pre-inoculum. The inoculum size of 10% (v/v) was used to inoculate 150 mL juices supplemented with 0.1% (w/v) magnesium sulphate and potassium dihydrogen orthophosphate each. The supplements act as nutritional factors for good alcohol production.

Extract preparation

Aloe vera leaves were collected and the transparent gel present in the inner part of fresh leaves was extracted. To prevent the loss of biological activity, aloe vera was processed within 24 h of harvesting the leaves. A knife was inserted into the mucilaginous layer beneath the green rind followed by the removal of upper and lower rinds. The gel obtained was then blended to obtain the aloe vera juice. To prevent the photo-oxidation of light-sensitive bioagents, the juice was stored in amber-coloured glass bottles. The upper layer of clean and dried ginger was removed followed by its cutting into small pieces. The pieces were blended to obtain the ginger paste. The paste was immersed in hot water for 15 min, followed by the extraction of juice, and storage in a clean glass bottle. The physicochemical properties of juice were determined i.e., total soluble solids (TSS), pH, and titratable acidity. The TSS of both aloe vera and ginger juices was set to 20°Brix by using honey.

Wine preparation and analysis

The aloe vera and ginger juice, pre-adjusted to 20°Brix were subjected to batch fermentation. The inoculated flasks were incubated in a BOD incubator at 28 ± 2°C. The contents of flask were gently mixed 2-3 times a day, and TSS and pH were recorded after 24 h intervals.

After the completion of fermentation, the wine was clarified by repeated siphoning. The wine was kept undisturbed under refrigerated conditions for a few days. The wine was siphoned off from the lees (dead yeast and other solids) which sedimented at the bottom. This process of racking was repeated 3-4 times with intermittent settling time of at least two weeks until no sediment was obtained. The clarified wine was bottled in sterile glass bottles, pasteurized at 65 ± 2°C for 10 min., and finally stored at room temperature. The bottles were cooled in a refrigerator before sensory evaluation.

The analytical procedures carried out were as follows: TSS (°Brix) of wine samples was determined by using an ERMA hand refractometer having a range of 0-32°Brix. The pH of samples was recorded by using digital pH meter. For calibration purposes, standard buffer solutions of pH 4.0 and 9.0 were used as a reference. The reducing sugar content was estimated by the method of Miller (1959). The titratable acidity was estimated by the method of Amerine *et al*. (1967) and data expressed as percent acidity. The ethanol was determined by the potassium dichromate method as described by Caputi and Wright (1969). The fermentation efficiency (%) of the process was determined by the following formula:

$$\text{Fermentation efficiency (\%)} = \frac{\text{Actual ethanol produced [\%] (v/v)}}{\text{Theoretical ethanol produced [\%] (v/v)}} \times 100$$

Theoretical ethanol produced (%) (v/v) = Sugars utilized x 0.64.

Total phenols were determined by the method of Malik and Singh (1980) using gallic acid as standard. The antibacterial efficacy of prepared herbal wines was evaluated against food-borne pathogens i.e. *Klebsiella pneumonia* MTCC 109, *Bacillus cereus* MTCC 430, and *E. coli* by performing the well diffusion assay (Deans and Ritchie, 1987).

Sensory evaluation

The acceptability of wines was ascertained by a panel of 3 judges wherein they assessed wine characteristics like i) visual appearance; ii) aroma and iii) flavour. Each characteristic carried ten marks. Evaluation was performed using the hedonic scale of 1-9 (Sharif *et al.*, 2017) with 1 = disliked extremely, 2 = disliked very much, 3 = disliked moderately, 4 = disliked slightly, 5 = neither liked nor disliked, 6 = liked slightly, 7 = liked moderately, 8 = liked very much, and 9 = liked extremely. All the experiments in present study were carried out in triplicate in one-way analysis of variance (ANOVA) design using SPSS software (IBM SPSS Statistics 20.0 version) and the data were analysed using the analysis of variance (Kaur, 2022). The values are expressed as mean standard deviations.

RESULTS AND DISCUSSION

In present study, two herbs, aloe vera and ginger, were chosen for the preparation of herbal wines. The juice recovery from aloe vera and ginger was 65 and 80%, respectively. Both the juices were supplemented with honey to adjust the TSS to 20° Brix. *Saccharomyces cerevisiae* was inoculated @ 10% (v/v). The fermentation was allowed to proceed for 20 days in a BOD incubator at 28 ± 2°C until constant TSS was obtained. The profile of fermentation was studied in terms of decrease in TSS, pH, changes in titratable acidity, and reducing sugars at regular intervals. After the completion of fermentation, the wines obtained from both herbs were analysed for their physicochemical properties and subjected to sensory evaluation.

Fall in TSS and pH during fermentation

The natural sugar content of herbs is less than the sugar content required for wine making. The juices are, therefore, supplemented with sugars from sources like jaggery, table sugar, or honey (Petrović. *et al.*, 2019). The initial TSS of aloe vera and ginger, and juices were adjusted by adding honey at 20°Brix. Shukla and Revis (1985) found that the TSS of musts should be maintained at 23°Brix for the preparation of citrus wines, while 18°Brix for fermentation of carrot juice has been reported by Lingappa and Naik (1997). The TSS and pH values of the fermenting samples were regularly recorded at 2 days interval. The results presented in Table 1 show the changes in TSS and pH of aloe vera- and ginger-based wines. The initial TSS of aloe vera and ginger juices dropped to 6.0 and 9.0°Brix, respectively, after 20 days of fermentation period. The maximum fall in TSS up to 8.4 and 9.8°Brix for aloe vera and ginger juices, respectively, occurred during the first 14 days of fermentation, thereafter there was a steady decrease in TSS. The amount of total soluble solids at the end of fermentation process help to categorize the wine as dry or sweet wines (Udeagha *et al.*, 2020). The herbal wines prepared in present study were sweet as the values >3.0°Brix are considered sweet wine. The pH of medium revealed gradual fall with passage of fermentation time, showing a final value of 4.20 and 4.13 for aloe vera and ginger juices, respectively. The pH decreases with progression in fermentation and it is a desirable trait as it leads to the suppression of contamination (Mbajiuka *et al.*, 2014). Conde *et al*. (2007) reported a final pH of aloe vera wine as 3.7 ± 0.02 after

Table 1: TSS and pH of aloe vera and ginger wine

Fermentation period (days)	Aloe vera wine		Ginger wine	
	TSS (°Brix)	pH	TSS (°Brix)	pH
0	20.0	5.11	20.0	5.38
2	16.6	5.06	19.0	5.38
4	15.6	4.97	18.6	5.37
6	14.8	4.89	17.8	5.29
8	13.2	4.41	15.6	5.16
10	11.2	4.12	14.0	5.03
12	9.2	4.14	11.6	4.73
14	8.4	4.15	9.8	4.54
16	8.0	4.17	9.6	4.25
18	7.2	4.18	9.2	4.19
20	6.0	4.20	9.0	4.13
$CD_{0.05}$	0.163	0.092	0.149	0.026

30 days. Rai (2009) reported the final TSS of ginger wine having initial TSS of 16, 20, and 24°Brix to be 6.0, 7.0, and 11.7°Brix, respectively. Trivedi *et al*. (2015) reported that the TSS of aloe vera juice reduced from 20°Brix to 4.1°Brix. Kyaw and Phyoe (2019) reported a final TSS of aloe vera wine to be 15.8°Brix after 30 days of fermentation, while Wu *et al.* (2020) reported a fall in pH from 5.3 to 3.5 in ginger wine within 14 days of fermentation.

Titratable acidity

Total acidity is a crucial wine ingredient not only for its acid taste but also to preserve the wine from spoiling and maintain its colour. Initial titratable acidity of aloe vera and ginger juices was observed to be 0.75 and 6.22%, respectively, which dropped to 0.09 and 0.16% at the end of fermentation. The TSS-acidity ratio for ginger and aloe-vera was found to be 66.2 and 56.2, respectively (Table 2). The permissible levels of percent tartaric acid in wine as per NAFDAC ranges in between 0.3 to 0.55% (NAFDAC, 2006). The low limits as observed in the present study can because the high yeast concentration decreases acid production (Erten *et al.,* 2006) and Ribeiro *et al*. (2015). Trivedi *et al*. (2015) reported the titratable acidity, in terms of tartaric acid, to be 0.89%. ± 0.07. The titratable acidity of Ginger was found at 7.4 g L^{-1} in a study by Kavitha and Kannahi (2018).

Table 2: Titratable acidity (%) and TSS (°Brix)-acid ratio of aloe vera and ginger wines

Fermentation period (days)	Titratable acidity (%)		TSS-acidity ratio	
	Aloe vera	Ginger	Aloe vera	Ginger
0	0.75	6.22	26.6	3.21
5	0.67	4.80	22.6	3.79
10	0.22	0.24	50.9	58.3
15	0.12	0.21	68.3	46.1
20	0.09	0.16	66.6	56.2
$CD_{0.05}$	0.04	0.03	0.09	0.06

Total phenolic content (TPC)

The phenolic contents in wines are regarded beneficial for human health since they show pharmacological activities such as anti-microbial, anti-carcinogenic, and antioxidant qualities. Wine's phenolic compounds work as antioxidants through methods that include both metal chelation and free radical scavenging (Tungmunnithum *et al.,* 2018). Anthocyanins, non-anthocyanin phenols, and polymeric pigments originating from anthocyanin and other phenol reactions are the key compounds that have a great sensory impact on wine. The colour of red wine is contributed by anthocyanins and polymeric pigments, while non-anthocyanin phenols, particularly flavan-3-ols, and flavonols, give wines their astringency, structure, and bitterness. Flavonols and stilbenes are primarily responsible for the anti-oxidant effects (Paixão *et al.*, 2007). The TPC of aloe vera and ginger wines was found to be 34.5 and 179.6 μg GAE mL^{-1}, respectively (Table 3). The aloe vera wine prepared by Lopez-Velez *et al.* (2003) had the total phenolic compounds of 1065 ± 10.11 μg GAE L^{-1}, while Kavitha and Kannahi (2018) reported a total phenol content of 0.42 μg mL^{-1} in ginger wine.

Table 3: Total phenolic content of aloe vera and ginger wines

Fermentation period (days)	Aloe vera wine (μg GAE ML^{-1})	Ginger wine (μg GAE ML^{-1})
1	22.7 ± 0.44	112.7 ± 0.88
10	27.6 ± 0.36	119.3 ± 0.83
20	34.5 ± 0.28	179.6 ± 0.56
$CD_{0.05}$	0.098	0.052

Ethanol production

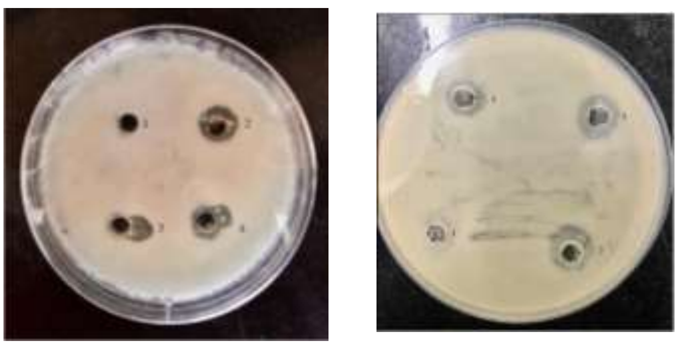
During the process of fermentation, yeast utilizes carbohydrates present in the mash for its growth and metabolic activities, resulting in the production of ethanol. The ethanol in wines is formed as a result of the utilization of carbohydrates present in mash for the growth and metabolic activities of yeast during fermentation. In a suitable environment and substrate. In a suitable environment and substrate, the amount of ethanol produced depends upon the amount of sugar present and the efficiency of yeast in converting the sugar to ethanol. The ethanol ~~or alcohol~~ content (% by volume) of both wines was determined by distillation. Table 4 presents the status of sugar contents and alcohol production from aloe vera and ginger wines. It was observed that the initial sugar content before inoculation for aloe vera and ginger juices was 15.0 and 13.6%, respectively. After the completion of fermentation in 20 days, the residual sugars recorded for aloe vera and ginger wines were 1.74 and 2.37%, respectively. The alcoholic content estimated for aloe vera and ginger wines was 8.29 and 6.91% (v/v), respectively. Based on the sugar utilized, the fermentation efficiency was calculated to be 97.8 % for aloe vera wine and 96.2% for ginger wine.

Table 4: Effect of sugar/honey concentration on the alcohol production during fermentation of aloe vera and ginger juices

Fermentation medium	Sugar/ honey added (%)	TSS (°Brix)	Available reducing sugars (%)	Residual reducing sugar after fermentation (%)	Sugar utilized (%)	Actual ethanol produced % (v/v)	Theoretical ethanol production % (v/v)	Fermentation efficiency (%)
Aloe vera wine	16.9	20	15.0	1.74	13.26	8.29	8.48	97.8
Ginger wine	18.0	20	13.6	2.37	11.23	6.91	7.18	96.2

Antimicrobial potential of aloe vera and ginger wine

To study the antimicrobial potential of aloe vera and ginger wine against known food-borne pathogens like *Klebsiella pneumonia*, *Bacillus cereus*, and *Escherichia coli*, well diffusion assay was performed for both wine samples. Table 5 and Fig. 1 show the recorded size of zone of inhibition against pathogens by aloe vera and ginger wines. Both wines samples showed remarkably higher efficacy of inhibition against *B. cereus* and *E. coli*, except for *K. pneumonia*. The inhibition zone due to the aloe vera wine against *B. cereus* and *E. coli* were 28.6 ± 0.03 and 21.6 ± 0.04 mm, respectively; while the inhibition zone with ginger wine against *B. cereus* and *E. coli* was 26 ± 0.03 and 25.2 ± 0.03, respectively, making ginger wine slightly more efficacious in terms of anti-bacterial activity as compared to its counter-part. Chauhan *et al.* (2015) reported 4.1 ± 0.02 mm zone of inhibition for *E. coli* in aloe vera wine. Trivedi (2015) reported 2.2 mm zone for *E coli* in aloe vera-ginger wine. The strong antimicrobial activity of wine is due to the synergistic action of alcohol, organic acids, and low pH, which show profound inhibitory action as compared to the individual effect of these components (Schmutzer *et al*., 2012).



(A) (B)

Fig. 1: Antimicrobial potential of aloe vera wine (A) against *Bacillus cereus* (1 - control and 2, 3 and 4 are aloe vera wine) and ginger wine (B) against *E. coli* (1 = control and 2, 3 and 4 are ginger wine)

Table 5: Antimicrobial potential of ginger and aloe vera wines

Bacterial culture	Zone of inhibition (mm)	
	Aloe vera wine	Ginger wine
Klebsiella pneumonia	-	-
Bacillus cereus	+ (28.6 ± 0.03)	+ (25.2 ± 0.03)
Escherichia coli	+ (21.6 ± 0.04)	+ (26.0 ± 0.03)

+ = Positive; - = Negative

Organoleptic evaluation of aloe vera and ginger wines

The acceptability of the prepared aloe vera and ginger wine was ascertained by a panel of 3 judges. The data on sensory evaluation of aloe vera and ginger based wine is presented in Table 6. The mean score of sensory evaluation out of 30 was 21.9 and 25.5, which can be adjudged as promising scores.

Table 6: Hedonic scale scores of various sensory parameters of wines prepared from aleo vera and ginger

Herbal wines	Visual appearance (10)	Aroma (10)	Flavour (10)	Total (30)
Aloe vera	7.3	8.0	6.6	21.9
Ginger	8.6	8.6	8.3	25.5

Table 7: Comparative assessment of aloe vera and ginger based herbal wines

Constituents	Aloe vera	Ginger
Juice (%)	65.00	80.00
TSS (° Brix)	6.00	9.00
pH	4.20	4.13
Titratable acidity (%)	0.09	0.16
Residual sugars (g L^{-1})	1.75	2.37
Alcohol content (%)	8.29	7.91
Fermentation efficiency (%)	97.80	96.20
Total phenolic content (μg GAE L^{-1})	34.5 ± 0.28	179.6 ± 0.56
Antimicrobial potential in terms of zone of inhibition (mm)	*Bacillus cereus* (28.6 ± 0.03) and *E. coli* (21.6 ± 0.04)	*Bacillus cereus* (25.2 ± 0.03) and *E. coli* (26.0 ± 0.03)
Hedonic scale score	21.90	25.50

Conclusion: Wine can be made from several fruit sources and its value addition can be done through use of medicinal herbs having therapeutic potential. The work has led to the development of a process methodology to produce aleo vera and ginger based herbal wines which showed antibacterial potential. Both herbal wines can be a tasty accompaniment to diet and prove to be a healthy drink with antibacterial potential against a range of food-borne diseases. These wines may serve as useful beverage having health benefits to the consumers because of their anti-microbial, antioxidant, and anti-mutagenic properties.

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