Preparation, Quality Evaluation and antibacterial activity of ready to serve beverage (RTS) from mint and *Aloe vera* gel

R. Mangalanayaki and S. Priyangadevi

Article History
Received: 19.09.2021
Revised and Accepted : 22.01.2022
Published: 23.03.2022

https://doi.org/10.56343/STET.116.015.003.005
www.stetjournals.com

Abstract

The demand of health beverage is growing in the soft drink industry. The functional properties and health benefits of *Aloe vera* are known world wide.So, present study is aimed to develop Mint and *Aloe vera* ready to serve (RTS) beverage by adding 0,5,10,15, 20% of *Aloe vera* gel and Mint juice. For all the treatments, physicochemical characteristics, microbiological count, photochemical contents as well as organo leptic attributes were evaluated at an interval of 21 and 45 days of storage at refrigerator temperature.

Key words: *Aloevera* gel, Microbiological count, Physicochemical properties, Mint juice, Phytochemicals contents.

INTRODUCTION

To improve the quality of life extensive investments have been made for the therapeutic applications of herbal plant resources. Herbal preparations have been known to treat various infectious diseases throughout the history of mankind (Mitscher *et al*., 1978). Wine represents one of the functional fermented foods (Soni *et al*., 2009) and consumption of wine has been reported to exhibit protective effect. As regards to the tendency to develop bacterial food infections (Bellido Blasco *et al*., 2002).It provides relaxation necessary for proper digestion and absorption of food and hence serves as a vital adjunct to the human diet (Joshi, 1997).

Strong antibacterial activity of wine is its essential biological function which has been verified under various experimental conditions (Sheth *et al*., 1988).Wine serves as a base for medicinal preparations compounded with a range of herbs adopted to treat various disorders (George *et al*., 1997). Functional botanical ingredients are more admired than ever in the beverage market. Many wines are made from herbs with perceived medicinal value and such wines have many additional health benefits.

The consumption of wine has also been reported to have antimicrobial effect against various pathogens. Moretro and Daeshel (2004), have shown that wine has bactericidal activity against *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli*, *Listeria monocytogenes*. Many wines are made from herbs with perceived medicinal value.

The protective and disease preventing potential of fruits and herbs like *Aloe vera,* amla, ginger, cranberry, blueberry etc. have given new dimension to then on-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 solid 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, enzymes, polysaccharides, phenolic compounds, minerals and organic acids (Boudreau *et al*., 2006). Aloe gel has bacteriostatic or bactericidal against a variety of wound-infecting bacteria including *Staphylococcus aureus, Streptococcus pyogenes, Serratia marcescens, Klebsiella pneumoniae, Pseudomona saeruginosa, E. coli, S. typhi* and *Mycobacterium tuberculosis* (Robson *et al*., 1982).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 throw-out the world.

Fruit wines are produced and consumed in large quantities in all advanced countries in the world. A few industries in our country produce wine but fruit

R. Mangalanayaki
email: *sivamangalamsree@gmail.com*

PG and Research Department of Microbiology, Sengamala Thayaar Educational Trust Women's College (Autonomous), Sundarakkottai, Mannargudi - 614 016, Tamil Nadu, India.

P - ISSN 0973 - 9157
E - ISSN 2393 - 9249

wine production at this time is in significant in spite of tremendous increase in the fruit production (Joshi, 2012).

Herbal is a traditional folk medicine practice based on the use of plants and plant extract. Herbs are staging a comeback and herbal 'resurgence' is ensuing all over the world. The herbal products today represent safety as these are compatible with human normal physiology. Natural products, obtained from dietary sources provide a huge number of antioxidants (Oluwaseun and Ganiyu,2008). Bioreactive constituents commonly found in herbs and other plants have been shown to have possible health benefits with anti-oxidative, anti-carcinogenic, anti-hypertensive, anti-mutagenic properties (Yen *et al*., 2002). Wine being one of the rich sources of antioxidants can be considered as an effective neutraceutical (Sharma *et al*.,2013). In addition antioxidants of herbal origins have been proved to be beneficial in reversing the hepatotoxicity and oxidative stress (Anita*etal.,*2005).

According to World Health Organization (Santos *et al.,* 1995). The medicinal plants would be the best source to obtain a variety of drugs. The use of plant extracts, with recognized antimicrobial properties, can be of great importance in the treatment of various microbial infections (Srinu*etal.,*2012).

MATERIALS AND METHODS

Sample Collection

Fresh and superior quality was bought from the local market. The *Menthaar vensis* leaves were washed and extraction was done using juice extraction machine. The juice was filtered using muslin cloth to remove unwanted particles and stored at refrigerated temperature until use. Fresh *Aloe vera* leaves were collected from local nursery and washed with tap water. Gel was prepare by cutting leaves vertically and blended in a juice blender to makes smooth and homogenized mixture, filtered and stored at refrigerated temperature until use (Ramachandra and srinivasa, 2008). The *Menthaar vensis* juice and *Aloe vera* gel were analyzed for pH. Brix and titrable acidity according to the methods described in (AOAC, 2000)

Microorganisms

Saccharomyces cerevisiae procured from local market, Thanjavur, India was used to carry out the fermentation. Mostly used as a source of *Lactobacillus sporogenes* is the lactic acid bacillus powder. It was procured from Madras scientific supplies, Trichy and was used as a probiotic organism in the study.

Inoculum Preparation

Saccharomyces cerevisiae was grown in sterilized glucose yeast extract (GEY) broth for overnight at 30C on a rotary shaker (159 rpm) and then separated by centrifugation at 10000 rpm(4^{o}C,5min).These were washed twice and suspended in normal saline to obtain a concentration of the cells was used as pre-inoculum. The inoculum was prepared by transferring 10mL solids of pre-inoculum in the 250ml conical flask having 100ml mixture of *Aloe vera* gel and 10% (w/v) *Menthaar vensis* 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 incubate overnight 30^{o}C in shaking incubator (159rpm).

Treatment

T0: RTS prepared without *Aloe vera* gel

T1: RTS prepared with 95% Mint juice and 5% *Aloe vera* gel

T2: RTS prepared with 90%Mint juice and10% *Aloe vera* gel

T3: RTS prepared with 85% Mint juice and 15% *Aloe vera* gel

T4: RTS prepared with 80%Mint juice and20% *Aloe vera* gel

Physico-Chemical Analysis of Mint-*Aloe vera* RTS

pH, total soluble solids (TSS), titrable acidity, sugar / acid ratio were determined by methods explain in AOAC(2000).

Phytochemical Analysis of Mint- *Aloe vera* RTS

Total phenolic content was estimated by using Folincioclitеu reagent .And results were expressed as gallic acid equivalents (GAE) per 100g of samples. Standard calibrated curves were made by ascorbic acid and trolox and results are shown as microgram of ascorbic acid per gram of sample. Reducing power was determined by method and results were shown as micro gram of ascorbic acid per100 g of sample.

Sensory Evalution

Sensory evaluation of Mint-*Aloe vera* RTS were carried out by using 9 point hedonic scaleon 0.21 days and 45 days of storage Meilgaard *et al.,*(2007).for color, flavor, taste, consistency and over all acceptability.

Fermentation of *Aloe vera* Gel and Mint Extract

Aloe vera gel and 10% *Menthaar vensis* extract was taken in a ratio of 1:1, supplemented with sugarcane for adjusting TSS to 20 Brix. One liter of the mixtures prepared was taken in two liter Erlenmeyer flask and seeded with 10% (v/v) DAP, Megnesium sulphate and potassium di hydrogen ortho phosphate, respectively. 100ppm of sodium meta bi sulphate was added to it and the flask was incubated to stationary state in a

BOD incubator at 25ÚC 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 analyzing total soluble solids (TSS), pH and ethanol content. The wine was clarified after completion of fermentation by siphoning it four times with a repeated sedimentation period of 3 days.

Antibacterial Activity of Wine Against Food Borne Pathogens

The agar well diffusion treatment based on the method of Deans and Riychie (1987) was used to determine *in vitro* inhibitory effect of Mint-Aloe and probiotic supplemented Mint-Aloe wine against common food borne pathogens including *E.aerogines, S.aureus* and *E.coli*. The actively grown respective cultures in nutrient agar plate to create a bacterial lawn.

Three wells with diameter of 6 mm were punched in each nutrient agar plate and the wines were added to the well under aseptic condition. Mint-Aloe extract and 10% v/v ethanol was also loaded in two different wells as a control to compare the antibacterial activity with Mint-Aloe wines. The plates were left for 30 minutes at room temperature for the diffusion of the test samples before being incubated at 30ÚC for 24 hour. The diameters of the zones of inhibition were measured after 24 hours. All analyses were carried out in triplicates.

Table 1. Chemical properties of mint juice and *Aloe vera* gel

Parameter	Mint Juice	*Aleo vera* gel
pH	5±0.03	5.2±0.20
Moisture Content	80±0.42	95.7±0.30
Acidity (%)	0..19±0.30	1.4±0.26
TSS(˚Brix)	6±0.21	20.18
Vitamin C	29.4±0.30	6.7±0.22

*Values are Mean ± Standard Deviation

Table 2. Zone of inhibition of Aloe Mint wine

Sample	Zone of Inhibition (mm)		
	S.typhimurium	*E.coli*	*S.aureus*
Aleovera – Mint Extract	1.8±0.05	1.9±0.08	1.6±0.02
10% ethanol Extract (v/v)	2.2±0.11	1.9±0.07	2.0±0.05
Aloe Mint Wine	2.5±0.11	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

RESULTS AND DISCUSSION

In the present study the herbal wine was prepared by using Mint and *Aloe vera*. After preparation the total acidity, the titrate acidity, titrable acid, pH, taste, odour, colour, sugar, phenolic content were estimated by standard procedures. Then to check the toxicity of Mint-Aloe wine against probiotic strain *Lactobacillus sporogenes*. Then the wine was fortified with probiotic *Lactobacillus sporogenes* and the antibacterial activity against food borne pathogens.

Chemical Properties of Mint Juice and *Aloe vera* gel

Mint juice and *Aloe vera* gel were analyzed for different chemical properties such as pH, titrable acidity and total soluble solids and the result are presented in Table 1. Total soluble solid of Mint juice was observed to be 12.33° Brix. It could be observed that Mint juice contributed total solid contents of the final beverage.

Physico-Chemical attributes of Mint- *Aloe vera* during storage

Freshly prepared Mint-*Aloevera* RTS samples exhibited non-significant change in pH within treatments and during storage (Table2).Although,there was slight increase in pH fromT0(4.0) to T4 (4.06) and decrease during storage from 0 day (4.05) to 45 days (3.99), the changes are not statistically significant. Various studies showed that pH of blended drink decrease during storage, increase in total soluble solids. Increase in TSS might be attributed in conversion of polysaccharides and other constituents of juice into sugar Yadav *et al.,*(2010), Tandon *et al.,* 1983. During storage, increase in total soluble solids was observed. Increase in TSS might be attributed on conversion of polysaccharides and other constituents of juice into a sugar (Yadav *et al.,* 2010), Tandon *et al.,* 1983, Tripathi *et al.,* 2013, Jakhar 2012 and salder 2012. The ratio of the TSS and titrable acidity contribute to the flavor and taste of the fruits and juice. The TSS is directly proportional to swetness index (ratio of TSS values during the storage periods have a great role in conserving the taste and flavor of the ERA blends during the storage period. Similar findings have been reported in the case of lime blended Ammasquash Rani *et al.,* 2014. In *Aloe vera* and sapota drink by Khan *et al.,*2012. In aloe melon juice Natha *et al.,* 2015. In prebiotic beverage made from *Aloe vera*, honey and soy milk Galani *et al.,* 2016.

Phytochemical Properties of Mint-*Aloe vera* during storage

Total phenolic contents are the most commonly found phyto-constituents present in medicinal plants with enormous biologial activity. Addition of *Aloe vera*

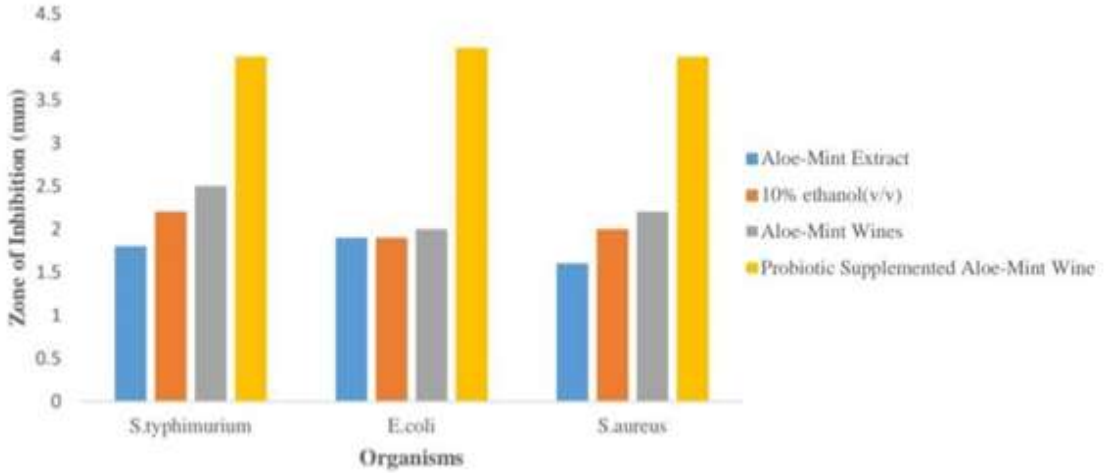

Fig. 1. Zone of inhibition (mm) of Aloe-Mint wine against selected food borne pathogens

increase the total phenolic content in Mint-*Aloe vera* blended RTS. It is an indicator of the antioxidant capacityof natural product. Results regarding reducing power of Mint-Aloe Vera RRD showed that addition of *Aloe vera* increased the reducing power from 207 to 334.This may be due to the presence of more antioxidant constituents in *Aloe vera* than Mint. The storage study showed that reducing power decreased with increase in storage time. These results were in accordance with the results of Gorsi*et al.,* (2019), who witness a remarkable decrease in reducing ability of different fruits and vegetables during storage at refrigeration temperature.

Microbial Load of Mint-*Aloe vera* RTS during storage

Total plate count of Mint-Aloe Vera RTS was performed at 0,21 and 45 days of storage at dilution (Table 4 and Fig 1).Maximum bacterial count was found in T0 at 45 days storage and minimum contamination occurred in sample. It is clear from the results that addition of *Aloe vera* is reported to exhibit antibacterial and antioxidant properties Rashid *et al.,* (2018). However, microbial load in all samples increased with storage time.

Antibacterial Activity against food borne pathogen

The *in vitro* anti-bacterial efficacy of Mint-Aloe wine and probiotic supplemented Mint-Aloe wine produced larger zones of inhibition as compared to Mint-Aloe extract, 10% v/v ethanol and Mint-Aloe wine. This can be attributed to the synergistic effect of ethanol, polyphenolic content of probiotic organisms 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 Mint-Aloe wine.

CONCLUSION

The wine does not kill the probiotic bacteria *Lactobacillus sporogenes*. So it does not have any toxic property. In antibacterial activity it should be able to kill all food borne pathogens effectively. The study revealed that the all the treatments were suitable up to 45 days of storage by physicochemical and sensory attributes analysis and remains microbiologically safe. This, development of blendedMint and *Aloe vera* RTS in an effective way of delivering the bioactive benefits of *Aloe vera* in atasty and refreshing way to the consumers. Functional foods contribute to the well-being, apartfrom providing basic nutrition. In this context, *Menthaar vensis* and well studied herbs for their nutritive and medicinal aspects and therefore, could beautiful good substrate for the production of wine, a functional beverage. Mint and Aloe both proved to be excellent substrates for wineproduction.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 invited anti-bacterial efficacy of probiotic supplemented wine than Mint- Aloe extract and Mint-*Aloe* wine, indicating it to be a better beverage in terms of medicinal functionality. Thus, value- additiin

probiotic wine from *Aloe vera* and *Menthaar vensis* can be a prospective contender of expanding class of health beneficial beverages.

REFERENCES

Bellido -Blasco J.B, Arnedo-Pena A., Cordero-Cutillas E, Canos-Cabedo M, Herrero-CarotC,Safont-Adsuara L. 2002. The protective effect of alcoholic beverages on the occurrence of a *Salmonella* food-borne outbreak. *Epidemiol.*2002; 13:2 28-230. https://doi.org/10.1097/00001648-200203000-00020

George, S.K, M.T.Dipu,,U.R. Merhra, P.Singh, A.K.Verma and J.S.Ramgaokar. 1997. Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. *J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci.*17: 832:134-137. https://doi.org/10.1016/j.jchromb.2005.10.051

Gruenwald, J. 2009. Novel botanical ingredients for beverages. *Clin. Dermatol*., 27:210-216. https://doi.org/10.1016/j.clindermatol.2008.11.003

Joshi,V.K.1997. Fruit Wines. 2nd Ed. Directorate of extension education, Dr.Y.S.Parmar Universityof Horticulture and Forestry, Nauni-Solan,India.

Mitscher, LA, Drake S,Gollapudi SR,Okwute SK.1978.A modern look at folkloric use of anti-infective agents. *J. Nat. Prod.* 50:1025-1040. https://doi.org/10.1021/np50054a003

Moretro, T. and Daeschel, M.A. 2004. Wine is bactericidal to food borne pathogens. *J.Food.Sci.*, 10:1365-2621.

Sheth, J. Gardne, Gardner, D and Garrate , D. 1998. Marketing theory: Evolution and Evaluation, John willey and sons Newyork, NY.

Soni, S.K.,Bansal,N and Soni,R.2009.Standardization of conditions for fermentation and maturation of wine from Amla (*Emblica officinalis*). *Nat. Prod. Rad.*, 8:436-44.

Tapsell, L.C., Hemphill, I., Cobiac, L., Patch, C.S., and Sullivan, D.R. 2006. Health benefits of herbs and spices: the past, the present, the future. *Med.J. Aust.*, 185:4-24. https://doi.org/10.5694/j.1326-5377.2006.tb00548.x

Wang, H., Chung, J., Ho, C., Wu, L. and Chang, S. 1998. Aloe - Emodin effects on Arylamin N-Acetyl transferase activity in the bacterium *Helicobacter pylori*. *Planta. Med.*,64:176-178. https://doi.org/10.1055/s-2006-957399