International Journal of Home Science 2020; 6(2): 13-16

International Journal of Home Science

ISSN: 2395-7476
IJHS 2020; 6(2): 13-16

www.homesciencejournal.com
Received: 10-03-2020
Accepted: 12-04-2020

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Phytochemical studies and antibacterial activity of herbal wine produced from *Aloe vera* and *Ocimum tenuiflorum*

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Abstract
Herbs have many positive effects on health of human beings. Many wines are made from herbs with perceived medicinal value and such wines have many additional health benefits. During fermentation, these bioactive compounds are released into wine, making the polyphenols and bioactive components vulnerable and increasing their bioavailability. *Aloe vera* Linn. is a well-known herbal plant and possesses strong antibacterial, anti-inflammatory, anti-tumor and immune stimulatory properties. *Ocimum tenuiflorum* Linn. (Holy Basil) plays an important role as anti-diabetics, anti-oxidant, cardiac activity, gastroprotectant and general vitalizer. Wine was prepared in the laboratory from the leaves of *Aloe vera* and *Ocimum tenuiflorum* leaves using *Saccharomyces cerevisiae*. Various parameters like colour, pH, acidity, brix, alcohol content was noted. Phytochemical screening of both the extracts and wine samples were carried out. Evaluation of its antibacterial property against the test organisms like *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* showed the widest zone of inhibition in wines as compared to ethanol, Aloe extract and Basil extract. This paper shows that *Aloe vera* and Basil leaves can be used as a valuable substrate for the production of herbal wines as its important phytochemical constituents are retained in the wine. It can be produced on a large scale as Herbal Wine or Medicinal Wine and can also be taken as a Health tonic.

Keywords: Fermentation, phytochemical, antibacterial, screening, organoleptic

Introduction
Before the revolution in medicinal area and rise of modern medicines, people were treated using herbal formulations that were derived from plants (Jackson, 1992) [6]. Herbs have many positive effects on health of human beings. Many well-known herbal plants have been demonstrated to possess strong anti- bacterial, anti- inflammatory, anti- tumor and immune stimulatory properties. They have been utilised extensively in the preparation of health drinks (Trivedi *et al.*, 2015) [17].

Herbal wine serves as an excellent vehicle for extracting some of the beneficial components of plants because of the solvent properties of its alcohol paired with the acidity that typically characterizes wines of a particular terroir. Many wines are made from herbs with perceived medicinal value and such wines have many additional health benefits. The most important of these bioactive constituents of herbs present in the wine are alkaloids, tannins, flavonoids, saponins and phenolic compounds (Hill, 1952) [5]. Wine contains different types of polyphenols and other constituents such as bioactive compounds (bioactive peptides) which aids in health maintenance of a consumer. During fermentation, these bioactive compounds are released into the wine (aqueous ethanolic solution) making the polyphenols and bioactive components vulnerable and increasing their bioavailability (Shahidi, 2009) [13].

Wine reduces risk of type 2 Diabetes, lowers risk of stroke, cataracts, colon cancer and slows brain decline. It also serves as the important adjunct to the human diet as it is necessary for proper digestion and absorption of food (Joshi, 1997) [8]. Red wine contains tannins and pro-cyanidins, which reduces the risk of heart disease. It also contains flavonoids which are anti-oxidants that help to prevent free-radicals from damaging the cells and helps to prevent hardening of the arteries.

Wine also contains a substance called resevatrol. This substance has been shown to boost the immune system, block cancer and protect against cardiovascular diseases. These substances are found in all wines but they are present more in red wine than in white wine (Rai, 2009) [10].

Herbal wine prepared with incorporation of herbs possesses many health benefits. Herbs have natural anti-bacterial constituents. Most of the herbs have anti-cancerous, anti-diabetic, anti-microbial and anti-inflammatory properties (Rathi, 2018) [11]. Herbal wine is prepared either solely from single herb like amla, holy basil etc. or it could be prepared by adding a mixture of multiple herbs like aloe-amla and aloe-ginger wine (Soni *et al*, 2009) [14].

Aloe vera Linn. is a succulent herb with long fleshy leaves belonging to Family Liliaceae. It is the most widely used herbal plant for its medicinal properties, as it possesses strong antibacterial, anti-inflammatory, anti-tumor and immune stimulatory properties (Jamwal *et al.,* 1959) [7]. Hence it is used as a valuable ingredient for the production of herbal wine with all the important properties of wine (Trivedi *et al.,* 2012) [16].

Ocimum tenuiflorum Linn. (Holy Basil, Tulsi), the queen of herbs, is one of the holiest plant and is renowned for its religious and spiritual sanctity, as well as for its important role in traditional ayurvedic and herbal medicine. It is an erect much branched softly pubescent undershrub, with red or purple sub quadrangular branches and simple leaves belonging to Family Lamiaceae. The therapeutic activities of tulsi include its role in anti-diabetics, anti-oxidant, cardiac activity, gastroprotectant and general vitalizer (Shiradhonkar *et al.,* 2014) [12].

The objective of this study was to combine the beneficial properties of Aloe and Basil to produce a new class of herbal wines. To screen the phytochemical constituents present in both the wines and to evaluate the antibacterial activity of Aloe and Basil wine on common food borne bacteria.

Materials & Methods

Two medicinal herbs *Aloe vera* and *Ocimum tenuiflorum* (basil) were selected for preparation of herbal wines. Healthy leaves of *Aloe* was collected from Velguem in Sanquelim and Basil leaves was collected from Kasarverne in Pernem, both places in North Goa**.**

Test organisms used: The pure cultures of *Escherichia Coli*, *Staphylococcus aureus and Salmonella typhi* were procured from Department of Biotechnology and Department of Microbiology, St. Xavier's College, Mapusa, Goa. Yeast (*Saccharomyces cerevisiae*) was obtained as wet cake from Mapusa market.

Production of Aloe and Basil wine

1000g of Aloe leaves were thoroughly washed with water. The horny prickles were removed, small pieces were made and put in a glass jar with 3L of boiling water and KMS (Potassium metabisulphite). Cane sugar was added at 20° brix along with raisins, activated baker's yeast, and citric acid. It was kept for fermentation for 21 days at 25 °C.

250 g of Basil leaves were washed thoroughly with water and put in a glass jar with 3L of boiling water and KMS. Cane sugar was added at 20° brix along with raisins, activated baker's yeast, and citric acid. It was kept for fermentation for 21 days at 25 °C. Both the wines, i.e., Aloe and Basil were then separated from its sediments and kept for maturing. Phytochemical tests were carried out using standard procedures. The organoleptic (sensory) evaluation of the wines was done by prescribed performa. The anti-bacterial activity was carried out by disc diffusion method (Bauer *et al*, 1966) [3].

Inoculum preparation

The pure cultures of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* were maintained by inoculating a loop full of culture of the respective organism on a Nutrient Agar medium using T-Streak culturing technique and incubated for 24 hours at 37 °C. Loop full of test organism grown on media were suspended in sterile saline and was used to determine the anti-bacterial activity.

Physico- Chemical analysis of wines

The total soluble solids (TSS) of the wines was checked using a hand refractometer (ERMA) having a range of 0-32°Brix. pH was measured by digital pH meter. The total titratable acidity was expressed as per cent citric acid (g/100 ml of sample). The alcohol content of the wines was measured by using Amber Hydrometer (0 - 20).

Phytochemical tests

Qualitative phytochemical tests of Aloe and Basil extract and the wines were carried out using standard procedures as described below (Edeoga *et al*. 2005; Tiwari *et al*. 2011; Banu & Cathrine 2015) [4, 15, 2].

Phytochemical tests

Tests	Observation	Inference
Test for Tannins To 1ml of wine sample and extracts a few drops of 0.1% ferric chloride was added.	The appearance of brownish green or a blue black coloration.	Tannins are present.
Test for Flavonoids Wine samples and extracts were treated with a few drops of sodium hydroxide solution.	Formation of intense yellow colour, which becomes colourless on addition of dilute acid.	Flavonoids are present
Test for Alkaloids Wine samples and extracts were treated with few drops of Wagner's reagent.	Appearance of reddish brown coloration.	Presence of alkaloids.
Test for Terpenoids (Salkowski test) 2ml of wine samples and extracts were mixed in l of Chloroform and 1ml of concentrated sulphuric acid was added carefully to form a layer.	A reddish brown coloration was formed at the interface.	Terpenoids are present.
Test for Carbohydrates (Molish test) To 2 ml of wine samples and extracts two drops of alcoholic solution of alpha naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube.	A violet ring is formed	Presence of carbohydrates.
Test for Proteins To 1ml of wine samples and extracts 2ml of sodium hydroxide was added followed by few drops of 1% copper sulphate solution.	Formation of bluish violet coloration.	Proteins are present.

Test for Phenols 1ml wine samples and extracts + 2ml distilled Water + few drops 10%FeCl3.	Green coloration	Phenols are present
Test for Saponins Wine samples and extracts were diluted with distilled water to 10 ml and this was shaken in a graduated cylinder for 15 minutes.	Formation of 1 cm layer of foam.	Presence of saponins.

Organoleptic (Sensory) evaluation of Wines

The organoleptic evaluation of Aloe and Basil wine was carried by a panel of five judges on the basis of appearance, colour, aroma, bouquet, vinegar, acidity, sweetness, body, flavour, astringency and general quality by numerical scoring method of twenty point scale as per the prescribed performa (Amerine and Ough, 1980) [1] (Table 3a &b).

Antibacterial activity of wines

The disc diffusion test was performed using standard procedure by Kirby–Bauer method (Bauer *et al*, 1966) [3]. Nutrient agar plates were prepared and 0.1 ml of inoculum suspension of each test organism was spread evenly. Paper discs of Whatman No.1 filter paper approximately 6 mm in diameter were cut and sterilised. Disc saturated with 5% ethanol, Aloe leaf extract & Aloe wine were placed aseptically using sterile forceps in the centre of each media plate, similarly plates were also prepared for Basil leaves extract, ethanol and Basil wine. These plates were incubated for 30 min at room temperature for the diffusion of the test samples before being incubated at 37° c for 24 h. The diameter of the zones of inhibition (mm) was measured after 24 h. All analysis were carried out in triplicates.

Results & Discussion

The pH of Aloe and Basil wine was 3.77 and 3.71 (Table 1). Most undesirable bacteria are inhibited at low pH and for yeast a pH range between 3 to 6 is most favourable for growth and fermentation activity. Acidity is also an important factor as yeast will not ferment properly without acid. Citric acid is normally added as it assists in fermentation and also improves the flavour of the wine. Temperature also has an effect on wine as it affects the yeast and consequently the course of wine fermentation. Brix of the wine after 21 days was recorded as 8.0 for Aloe and 8.5 for Basil. The *Aloe vera* wine was yellow in colour and the alcohol content was 10.0. The Basil wine was Pale Yellowish Pink in colour and the alcohol content was 11.0.

Table 1: Analysis of Wine

Name of wine	pH	Acidity (%)	Alcohol concentration	Brix°	Colour
Aloe	3.77	0.064	10.0	8.0	Yellow
Basil	3.71	0.045	11.0	8.5	Pale Yellowish Pink

Table 2: Qualitative analysis of Phytochemicals

Phytochemical constituents	Aloe Extract	Aloe Wine	Basil Extract	Basil wine
Tannins	-	-	+	-
Flavonoids	+	+	+	+
Phenols	-	-	+	+
Alkaloids	-	-	+	+
Terpenoids	-	-	+	+
Carbohydrates	+	+	-	+
Proteins	+	+	+	+
Saponins	+	+	+	+

Presence of constituent = +
Absence of constituent = -

Flavonoids which are important anti-oxidants that prevent free-radicals from damaging the cells were present in both Aloe and Basil wine. Phenols were present only in basil wine and tannins were absent in both the wines. Alkaloids and terpenoids were absent in Aloe extract and wine but was present in Basil extract and wine. Carbohydrates, proteins and saponins were present in both the wines (Table 2).

The total score obtained after organoleptic evaluation for Aloe wine was16.4 and was graded as standard wine (13-16) with neither an outstanding character nor defect. For Basil wine the score obtained was 17.3 and was graded as wines (17-20) with outstanding characteristics and no mark defect (Table 3a & b).

Table 3a: Sensory evaluation of *Aloe* wine

Characteristics	Max score	Scores by Tasters					Mean
		I	II	III	IV	V	
Appearance	2	1	2	2	1.5	2	1.7
Colour	2	2	2	2	2	2	2
Aroma	2	1	2	2	1.5	2	1.7
Bouquet	2	1	2	2	2	2	1.8
Vinegar	2	2	2	1	0.5	1.7	1.4
Total acidity	2	2	2	1	1	1.8	1.6
Sweetness	1	1	1	0.5	1	1	0.9
Body	1	1	1	0.5	1	1	0.9
Flavour	2	1	1	1	2	2	1.4
Astringency	2	2	1	1	1	1.4	1.3
General quality	2	1	2	1.5	2	2	1.7
Total score	20	15	18	14.5	15.5	18.9	16.4

Table 3b: Sensory evaluation of Basil wine

Characteristics	Max score	Scores by Tasters					Mean
		I	II	III	IV	V	
Appearance	2	2	2	2	2	2	2
Colour	2	2	2	2	2	2	2
Aroma	2	1	2	1	1.5	2	1.5
Bouquet	2	2	2	2	2	2	2
Vinegar	2	2	2	1	0.5	1.6	1.4
Total acidity	2	1	2	1	1	1	1.2
Sweetness	1	1	1	1	1	1	1
Body	1	1	1	1	1	1	1
Flavour	2	2	1	2	2	2	1.8
Astringency	2	2	1	2	1	1	1.4
General quality	2	2	2	2	2	2	2
Total score	20	18	18	17	16	17.6	17.3

Table 4a: Antibacterial activity of Aloe vera wine

Sr. No.	Test organisms	Zone of inhibition (mm)		
		Ethanol	Aloe Extract	Aloe Wine
1.	*Escherichia coli*	7.6	9.6	13.3
2.	*Staphylococcus aureus*	10.3	11.3	14.6
3.	*Salmonella typhi*	8.3	10.0	12.6

Values are expressed as mean of the three different observations.

Table 4b: Antibacterial activity of Basil wine

Sr. No.	Test organisms	Zone of inhibition (mm)		
		Ethanol	Basil Extract	Basil Wine
1.	*Escherichia coli*	8.3	9.3	11.0
2.	*Staphylococcus aureus*	8.6	9.0	11.6
3.	*Salmonella typhi*	9.3	10.6	14.0

Values are expressed as mean of the three different observations.

Moretro and Daeschel (2004) [9] have shown that wine has bactericidal activity against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*. A number of studies have established the effectiveness of wine against food borne pathogen (Trivedi*et al.*, 2012) [16]. With Aloe wine maximum zone of inhibition of 14.6mm was seen with *Staphylococcus aureus* followed by *Escherichia coli* 13.3 and 12.6 with *Salmonella typhi.* The least zone of inhibition was seen with ethanol. With Basil wine maximum zone of inhibition was seen in *Salmonella typhi* 14.0 mm followed by *Staphylococcus aureus* (11.6mm) and *Escherichia coli* (11.0 mm). The least zone of inhibition was seen with ethanol. Hence both the wines prepared from Aloe and Basil showed wider zone of inhibition (Table 4a & 4b) as compared to ethanol and extract.

Conclusion

Aloe vera and *Ocimum tenuiflorum* are recommended for making wine as they have valuable ingredients and important medicinal properties. Wine from Basil obtained a higher score and was better in taste and appearance as compared with Aloe wine. However both the wines exhibited good antibacterial activity against the food borne pathogens and hence can be widely used for the production of a new class of herbal wines. Not much work has been done in this area hence this paper can be very useful to the wine industry for the commercial production of Herbal Wine or Medicinal Wine which can also be taken as a Health tonic.

Acknowledgement

The Authors are grateful to the Ex Officiating Principal of St. Xavier's College, Mapusa, Dr. Fr. Jeromino D'Silva for providing all the facilities required to carry out the work.

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