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Phytochemical screening and Antibacterial activity of Ginger wine

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Abstract
Wine an alcoholic drink is widely accepted, consumed and preferred due to its nutritive properties. To enhance the nutritional quality of wine, it can be fortified with certain additives that are potentially beneficial to health. Ginger rhizome is consumed worldwide as flavouring agent, extensively used in food, beverages, and confectionary industries. Numerous active ingredients are present in ginger including terpenes and oleoresin. An attempt was made to prepare wine in the laboratory from ginger rhizome using yeast, sugar and raisins. It was kept for fermentation for 21 days at 25° c. The wine was then separated from its sediments and kept for maturing for about 300 days. The colour of the ginger wine, pH, acidity, brix, alcohol content was noted down. Phytochemical tests of the wine sample was carried out and the various constituents like flavonoids, alkaloids, terpenoids, carbohydrates and proteins were found to be present. Evaluation of its antibacterial property against the test organisms showed the widest zone of inhibition in wine as compared to ethanol and ginger extract. Ginger can therefore be used as a valuable ingredient for the production of a herbal wine as its important phytochemical constituents are retained in the wine and hence can be produced on a large scale as a health beverage.

Keywords: wine, phytochemical, antibacterial, screening, organoleptic

1. Introduction
Herbal preparations have been known to treat various infectious diseases throughout the history of mankind. Over three quarters of the world population still rely on plants and plant extracts for health care (Sasidharan and Menon 2010) [9]. Various kinds of herbs and spices play an important role in beverage production and are used as enhancers, preservative and as antioxidant sources.

Wine an alcoholic drink is widely accepted, consumed and preferred due to its nutritive properties. To enhance the nutritional quality of wine, it can be fortified with certain additives that are potentially beneficial to health (Shiradhonkar *et al*. 2014) [11].

Wine promotes longevity and reduces the risk of heart-attack. Moderate drinkers suffering from high blood pressure are 30% less likely to have a heart attack than non-drinkers. It also lowers the risk of type 2 Diabetes, stroke, cataracts, colon cancer and slows brain decline. Wine also serves as an important adjunct in the human diet as it helps for proper digestion and absorption of food (Joshi 1997) [5].

Ginger (*Zingiber officinale* L.) is a herb, member of Zingiberaceae family and is a well-known spice used in the daily diet in many Asian countries. Moreover, it is consumed worldwide as flavouring agent, extensively used in food, beverages, and confectionary industries (Wang *et al*. 2011) [14].

Numerous active ingredients are present in ginger including terpenes and oleoresin. Ginger also constitutes volatile oils approximately 1% to 3% and non-volatile pungent components oleoresin. The major identified components from terpenes are sesquiterpene hydrocarbons and phenolic compounds which are gingerol and shogaol (Hasan *et al*. 2012) [4]. Gingerols help improve the intestinal motility, has anti-inflammatory, analgesic, nerve soothing anti-pyretic as well as anti-bacterial properties (Winston 1997).

Ginger compounds are active against specific type of diarrhoea which causes death in infants. It is also effective in treating nausea caused by sea sickness, morning sickness, chemotherapy and for post-operative nausea (Sebiomo *et al*. 2011) [10].

The objectives of this study was to combine the beneficial properties of Ginger to produce a new type of herbal wine. To screen the phytochemicals present in the wine and also to evaluate the antibacterial activity of Ginger wine on common food borne bacteria.

2. Materials & Methods

2.1 Collection of sample

Fresh rhizome of Ginger and Yeast (*Saccharomyces cerevisiae*) was procured from Mapusa market.

2.2 Methods of preparation of wine

250g of Ginger rhizome was thoroughly washed with water. The skin was peeled, cut it into small pieces and was put into a glass jar with 4 litres of boiled water. 90g of raisins was added and 2 g of KMS. It was kept for a day to infuse, and then 1 kg of sugar, 3g of citric acid and 15 g of activated yeast (*Saccharomyces cerevisiae*) was added. It was stirred and kept for fermentation for 10 days and then 1 kg of sugar was added. The fermentation continued for 21 days until the bubbling had ceased, stirring every alternate day. The wine was then separated from the sediments by siphoning. 2 g of KMS was added and bottled. It was kept for maturing for about 300 days.

2.3 Physico- Chemical analysis of Ginger wine

The total soluble solids (TSS) of the wine 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 Ginger wine was measured by using Amber Hydrometer (0 - 20).

2.4 Phytochemical screening of Ginger wine

Qualitative phytochemical tests were carried out with the ginger wine using standard procedures as described below.

- **Test for Tannins:** To 1ml of wine sample a few drops of 0.1% ferric chloride was added. The appearance of brownish green or a blue black coloration indicated the presence of tannins in the tests sample (Edeoga *et al*. 2005) [3].
- **Test for Flavonoids:** Wine sample was treated with a few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids (Tiwari *et al*. 2011).
- **Test for Alkaloids:** Wine sample was treated with few drops of Wagner's reagent. Appearance of reddish brown coloration indicated the presence of alkaloids (Tiwari *et al*. 2011).
- **Test for Terpenoids (Salkowski test):** 2ml of wine sample was mixed in l of Chloroform and ml of concentrated sulphuric acid was added carefully to form a layer. A reddish brown coloration was formed at the interface indicating a positive result for presence of terpenoids (Edeoga *et al*. 2005) [3].
- **Test for Carbohydrates (Molish test):** To 2 ml of wine sample, 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 indicates the presence of carbohydrates (Banu & Cathrine 2015) [2].
- **Test for Proteins:** To 1ml of wine sample 2ml of sodium hydroxide was added followed by few drops of 1% copper sulphate solution. Formation of bluish violet coloration indicated the presence of proteins.
- **Test for Saponins:** Wine sample was 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 indicates the presence of saponins (Tiwari *et al*. 2011).

2.5 Organoleptic (Sensory) evaluation of Wine

The organoleptic evaluation of Ginger wine was done 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 *et al*. 1980) [1].

2.6 Antibacterial activity of Ginger wine

Test organism 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. They 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 27°C. Loop full of test organism grown on media were suspended in sterile saline and used to determine the anti-bacterial activity.

Determination of anti-bacterial activity by Paper Disc method

The disc diffusion test was performed using standard procedure by Kirby–Bauer method. Nutrient agar plates were prepared and 0.1 ml of inoculum suspension of each test organism was spread on the entire surface of the plates. Paper discs of Whatman No. 1 filter paper approximately 6 mm in diameter were cut and sterilised. Disc saturated with 5% ethanol, ginger extract & ginger wine were placed aseptically using sterile forceps in the centre of each media plate. These 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 was measured after 24 h. All analyses were carried out in triplicates.

3. Results & Discussion

The pH of ginger wine was 3.45. 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 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 22.8. The wine was yellow in colour and the alcohol content was 16.0 (Table 1).

Table 1: Analysis of Wine

Name of wine	Yield of wine (litre)	pH	Acidity (%)	Alcohol concentration	Brix°	Colour
Ginger	4.3	3.45	0.051	16.0	22.8	Pale Yellow

Table 2: Qualitative analysis of Phytochemicals of Ginger wine.

Phytochemical constituents	Wine sample
Tannins	-
Flavonoids	+
Alkaloids	+
Terpenoids	+
Carbohydrates	+
Proteins	+
Saponins	-

Presence of constituent = +
Absence of constituent = -

The qualitative analysis of phytochemicals in the ginger wine showed the presence of flavonoids, alkaloids, terpenoids carbohydrates and proteins (Table 2). Ginger rhizome has the presence of terpenes which was also found to be present in the wine.

The organoleptic evaluation of wine by numerical scoring method of twenty point scale was done by a panel of five judges. The total score obtained for the wine was 16.8 (Table 3) and was graded as wines (17-20) with outstanding characteristics and no marked defect.

Table 3: Sensory evaluation of Ginger 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	2	1	2	1	1.5	1.5
Bouquet	2	2	2	2	2	2	2
Vinegar	2	2	2	1	0.5	0.5	1.2
Total acidity	2	2	2	1	1	0.5	1.3
Sweetness	1	1	1	1	1	1	1
Body	1	1	1	1	1	1	1
Flavour	2	2	1	2	2	1.6	1.7
Astringency	2	1	1	2	1	0.5	1.1
General quality	2	2	2	2	2	2	2
Total score	20	19	17	18	15.5	14.6	16.8

Table 4: Antibacterial activity of Ginger wine.

Sr. No.	Test organisms	Zone of inhibition (mm)		
		Ethanol	Ginger Extract	Ginger Wine
1.	*Escherichia coli*	9.0	10.0	14.0
2.	*Staphylococcus aureus*	8.6	9.6	14.6
3.	*Salmonella typhi*	9.6	10.6	13.6

Values are expressed as mean of the three different observations.

The results of antibacterial activity of ethanol, ginger extract and ginger wine on *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* is shown in table 4. Ethanol and ginger extract was taken as control to compare the antibacterial activity with ginger wine. The results indicates that ginger wine was the most effective on all the three types of bacteria as compared to ethanol and ginger extract. The widest zone of inhibition was seen with the wine in *S. aureus* (14.6mm), followed by *E.coli* (14.0 mm) and 13.6mm in *S.typhi*. Ginger wine contains ethanol an organic solvent which dissolves organic compounds better and hence liberate the active components present in ginger such as zingerone, gingerol and shogaol required for antimicrobial activity (Nelson *et al*. 2007) [8]. In addition to ethyl alcohol, wine also contains several other alcohols, adding to the degree of sweetness and aroma of wines. Many odorous compounds and their precursors are present in wine. Large amounts of volatiles, especially acids, alcohols, ethyl acetate and acetate derivatives are produced during fermentation by yeasts whose growth and metabolism is affected by temperature (Trivedi *et al*. 2012) [13]. A combination of organic acids, ethanol and low pH has been reported to have significantly stronger antimicrobial activity than the effect of these components indivisual against various food borne pathogens (Moretro & Daeschel 2004) [6].

4. Conclusion

Ginger can be used as a valuable ingredient for the production of a herbal wine as its important phytochemical constituents are retained in the wine. The prepared ginger wine also exhibited considerable antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* and hence can be produced on a large scale as a health beverage.

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