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Evaluation of Antioxidant and Antimicrobial Activities in Betel Leaf Wine

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A B S T R A C T

Piper betel crop is extensively grown in India and the neighboring Southeast Asian countries. Piper betel leaves are used as a post meal mouth freshener. Wines are made from various other sources besides grapes. In Kodagu district of Karnataka in India, wines are made from betel leaves too. Alcoholic beverages act as an important adjuvant to the diet by increasing satisfaction. In our study, wild yeast was isolated by using spoiled grape and betel leaf wine was prepared by using wild yeast and commercially available yeast. Antioxidant activity was measured in the betel leaf wine by DPPH method. There was a slight increase in antioxidant activity when compared to initial day level in the betel leaf wine. Antimicrobial activity against human pathogenic bacteria such as *Salmonella species, Escherichia coli, Klebsiella* and *Bacillus subtilis* were checked using nutrient agar plates. Almost all the samples of betel leaf wine showed zone of inhibition against human pathogens whereas in control which contained less betel leaf (01:20), there was a complete absence of antimicrobial activity.

Introduction

The term 'wine' can also refer to starch-fermented beverages having higher alcohol content, such as barley wine, *huangjiu* or *sake*. Alcoholic beverages act as an important adjuvant to the diet by increasing satisfaction and contributing to the relaxation necessary for the proper digestion and absorption of food (Joshi *et al*, 1999). Wines are also made from some leaf such as oak leaf whereas in Kodagu which is located in Karnataka, wines are made from betel leaves, ginger, pineapple, passion fruit, rice and banana, which are available in the market. In addition, there are wines made from dates, figs and star fruit too.

The quantity of haze forming is dependent on the quantity of phenolics in the wine. As phenols are considered as free radical scavengers, their antioxidant activity depends upon the structure of polyphenols such as phenolic acids, flavonoids, proantocyanidins and anthocyanidins (Jordão *et al*, 2010). The content of phenols and antioxidant activity of wine depends on growth cultivation techniques, oenological techniques and ageing process (Yilmaz and Toledo, 2004). According to numerous authors, the antioxidant activity of wines is due to the total polyphenol concentration and also individual phenols (Satué-Garcia *et al.*, 1999; Sun *et al*, 2009). Various studies described the antimicrobial properties of wine against different relevant food-borne pathogens (Agarwal *et al.*, 2012).

Wines have a potent antimicrobial property against *Campylobacter* which has been demonstrated under various experiments. Various studies described the antimicrobial properties of wine against different relevant food borne pathogens. Some studies indicated that the strength of antimicrobial property of wine is attributed to different components of wines and a better antimicrobial proficiency associated with red wine than white wine because of high level of phenolic compounds in red wine (Chanyalew and Wiriya, 2013).

White and red wine alcohol-free extracts appear to contain compounds responsible for antimicrobial effects against Gram-positive and Gram-negative bacteria. The antimicrobial effect of the wine extracts was tested using the agar well diffusion method following the well-established method (Papadopoulou *et al.*, 2005).

However, there are no reports so far on the antioxidant activity and antimicrobial activity in betel leaf wine. Therefore, this study was aimed at checking the antioxidant and antimicrobial activities in the betel leaf wine samples prepared with different concentrations of betel leaves using White wild yeast and Commercial yeast.

Materials and Methods

Figure.1 Betel leaves

The betel (*Piper betle*) is the leaf of a vine belonging to the Piperaceae family (Figure 1), which includes pepper and kava. It is valued both as a mild stimulant and for its medicinal properties. Betel leaf contains moisture 85.4 per cent, protein 3.1 per cent, fat 0.8 per cent, minerals 2.3 per cent, fiber 2.3 per cent and carbohydrates 6.1 per cent per 100 grams. Its minerals and vitamin contents are calcium, carotene, thiamine, riboflavin, niacin and vitamin C. Its calorific value is 44 (Periyanayagam *et al.*, 2012).

Betel leaf is mostly consumed in Asia and elsewhere in the world by some Asian emigrants, as betel quid or *paan*, with or without tobacco, in an addictive psycho-stimulating and euphoria-inducing formulation with adverse health effects. The betel plant is an evergreen and perennial creeper, with glossy heart-shaped leaves and white catkin and it needs a compatible tree or a long pole for support. Betel requires high land and especially fertile soil.

Isolation, identification and pure culturing of wild yeast

One gram of spoilt grape was taken and serially diluted by using sterilized saline solution in test tubes. 100µl of inoculum were spread on YEPDA (yeast extract, peptone, dextrose and agar) media (Aneja, 2008) and incubated at 28°C for three to four days. Yeast was identified based on colony morphology and microscopic observations. A colony was picked, streaked on YEPDA slant to obtain pure culture and incubated. Pure culture which was obtained was stored in refrigerator for future use and it has been re-cultured for every ten days.

Preparation of wine

Inoculum preparation

In a clean and dried 250 ml conical flask, 100 ml of YEPD (yeast extract, peptone and dextrose) broth was taken, plugged using cotton, sterilized and then cooled. Two loops full of wild yeast was added to one conical flask and 0.05g commercial yeast (*Saccharomyces cerevisiae*) was added to another, incubated at 28°C on rotary shaker for 24 hrs. Betel leaves were taken and sterilized using 1% sodium hypochlorite and washed with distilled water. Betel leaves were cut into small pieces.

In clean and dried eight (250 ml) conical flasks, small pieces of betel leaf and distilled water were added with different dilutions 1:10 (15 g leaf in 150 ml distilled water), 1:15 (10 g leaf in 150 ml distilled water), 1: 20 (7.5 g leaf in 150 ml distilled water) and were labeled as given below:

Commercial yeast 01:10 (CY01:10); Commercial yeast 01:15 (CY01:15); Commercial yeast 01:20 (CY01:20); White wild yeast 01:10 (WY01:10); White wild yeast 01:15 (WY01:15); White wild yeast 01:20 (WY01:20); Control 01:10 (C01:10); Control 01:15 (C01:20). To each conical flask 20g of sugar was added and heated at 60°C for 30 minutes and it was allowed to cool at room temperature. 10% of commercial and white wild yeast inocula were added to the respective conical flasks and plugged using cotton in aseptic condition. The contents were stirred for 2 days and were kept under static condition at room temperature. After 20 days, the contents of the flasks were filtered, heated at 60°C for 30 minutes in water bath and then stored for future use.

Antioxidant activity was measured in the betel leaf wine by DPPH method (Shimada *et al.*, 1992). Antimicrobial activity against human pathogenic bacteria such as *Salmonella* species, *Escherichia coli*, *Klebsiella* and *Bacillus subtilis* were checked using nutrient agar plates (Aneja, 2008).

Results and Discussion

Antioxidant activity

In all the betel leaf wine samples there was a slight increase in antioxidant activity when compared to initial day. After 21 days the antioxidant activity of all the betel leaf wine samples was found to be the range of 32-38% (Figure 2). This is because of increase in total polyphenol and flavonoid levels in the sample. This shows that there was an increased antioxidant activity in all the betel leaf wine samples as the wine gets older.

The evidence for protective effects of polyphenols against diseases has generated new expectations for improvements in health, with great interest from the food and nutritional supplement industry regarding promotion and development of polyphenol-rich products (Scalbert *et al.*, 2005).

Antimicrobial activity

A comparative study of antimicrobial properties of Piper betel leaf wine

Wine was heated at 60°C for 30 minutes. Then the sample was allowed to cool at room temperature *and* 200µg/well was tested against human pathogenic microorganisms such as *Salmonella* Species, *Klebsiella*, *Bacillus subtilis* and *Escherichia coli* using agar well diffusion method (Figure 3–6; Agarwal *et al.*, 2012).

Antimicrobial study shows that almost all the samples show zone of inhibition against human pathogens whereas in control which contain less betel leaf (01:20) there was a complete absence of antimicrobial activity.

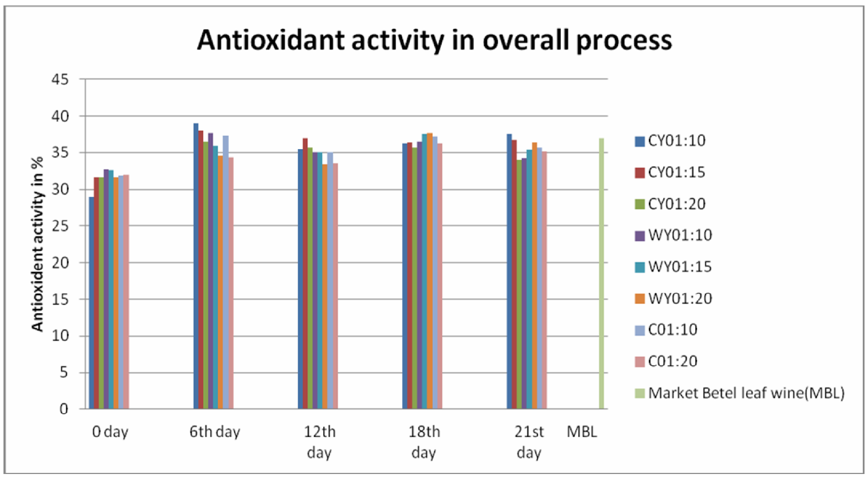

Figure.2 Antioxidant activity in betel leaf wine samples

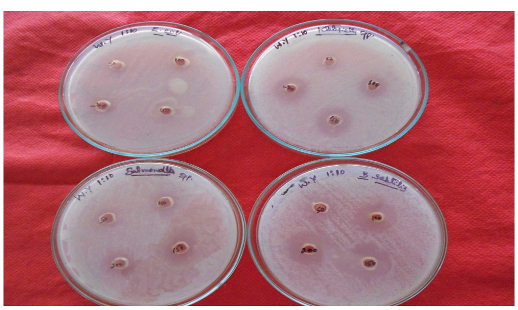

Figure.3 White wild yeast 01:10(WY01:10)

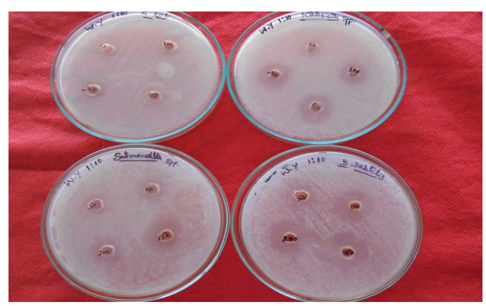

Figure.4 Commercial yeast 01:20(CY01:20)

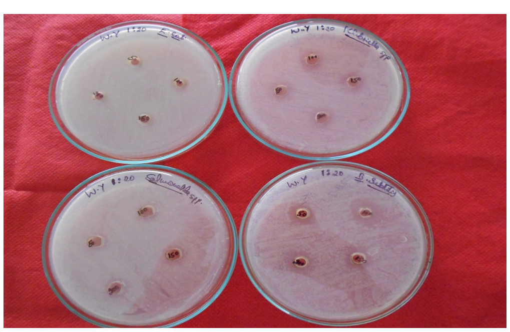

Figure.5 White wild yeast 01:20(WY01:20)

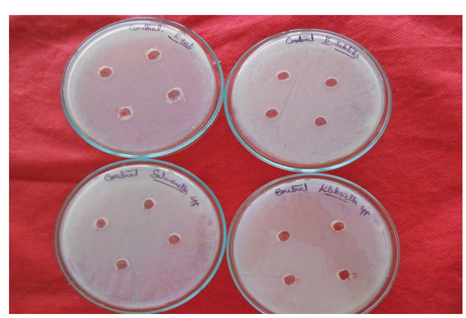

Figure.6 Control

Table.1 Zones of inhibition against different human pathogens

Test Samples	Zones of inhibition in centimeter			
	E. coli	*Salmonella sps*	*Klebsiella*	*Bacillus subtilis*
1. Commercial yeast 01:10(CY01:10)	1.5	1.7	1.6	2.0
2. Commercial yeast 01:10(CY01:15)	2.0	1.4	1.5	1.2
3. Commercial yeast 01:20(CY01:20)	1.5	1.3	1.4	1.6
4. White wild yeast 01:10(WY01:10)	1.5	1.4	1.3	1.9
5. White wild yeast 01:15(WY01:15)	1.8	-	1.1	1.0
6. White wild yeast 01:20(WY01:20)	2.2	-	-	1.3
7. Control 01:10(C01:10)	1.2	1.5	1.1	1.4
8. Control 01:20(C01:20)	-	-	-	-
Market wine sample	2.0	1.9	2.0	1.5

Almost all the samples showed antimicrobial activity (Table 1). It may be because of the presence of secondary metabolites like polyphenol, flavonoids etc., by the yeast from betel leaves after fermentation. While, in control C01:20 there is a complete absence of antimicrobial activity because of less amount of betel leaf and further, yeast was not added to this.

Similar results show that white and red wine with 11.5% ethanol exhibited significant ($p \leq 0.05$) antimicrobial activity against *C. jejuni* (Chanyalew and Loongyai, 2013). White wine shows a good antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. (Papadopoulou *et al.*, 2005).

Conclusion

Betel leaf wine was prepared using both Commercial and White wild yeast. The betel leaf wine showed antioxidant and antimicrobial activities. In our study, Commercial yeast (*Saccharomyces cerevisiae*) showed good antioxidant activity compared to White wild yeast wine. All the samples showed zones of inhibition around 1–2 cms against human pathogens which has proved the antimicrobial activity of betel leaf wine.

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