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## Review Paper

# A Phytochemical Study in Betel Leaf Wine

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## Abstract

*Piper betel* crop is extensively grown in India. In Kodagu district of Karnataka, India, wines are made from betel leaves too. Betel leaf wine was prepared using wild yeast and commercially available yeast. Wild yeast was isolated in the laboratory using spoiled grapes. Some of the phytochemical components were estimated in the betel leaf wine made from both wild yeast and commercially available yeast. Alcohol level of betel leaf wine was increased by 15-21% on the final day of fermentation. Total sugar levels in all the samples were gradually decreased, indicating the utilization of total sugar by yeasts. Polyphenols were present in all the samples in a minute quantity initially and as the days passed, the levels were increased. pH was decreased gradually in all the samples from the initial day of fermentation and there was a slight increase in antioxidant activity when compared to initial day level. There was a decrease in protein level in sample containing more betel leaf (01:10 and 01:15). Glucose levels were decreased in all the samples. With the present study, it is identified that commercially available yeast yields better phytochemicals than wild yeast in betel leaf wine production. Further, betel leaf wine is suitable for consumption.

**Keywords:** Alcohol, betel leaf, fermentation, glucose, pH, polyphenol, protein, total sugar, wine.

## Introduction

Wine is a psychoactive drug, like all alcoholic beverages, commonly used for its intoxicating effects today and throughout history. Wines made from produce besides grapes are usually named after the product from which they are produced and are generically called fruit wine. Wines are also made from some leaf such as oak leaf whereas in Kodagu district which is located in Karnataka, India, wines are made from betel leaves, ginger, pineapple, passion fruit, rice and banana. In addition, there are wines made of dates, figs and star fruits too. Two primary species of yeasts found in wines are *Saccharomyces bayanus* and *Saccharomyces cerevisiae* which ferment glucose, sucrose and raffinose and assimilate glucose, sucrose, maltose, raffinose and D- gluconate<sup>[1]</sup>.

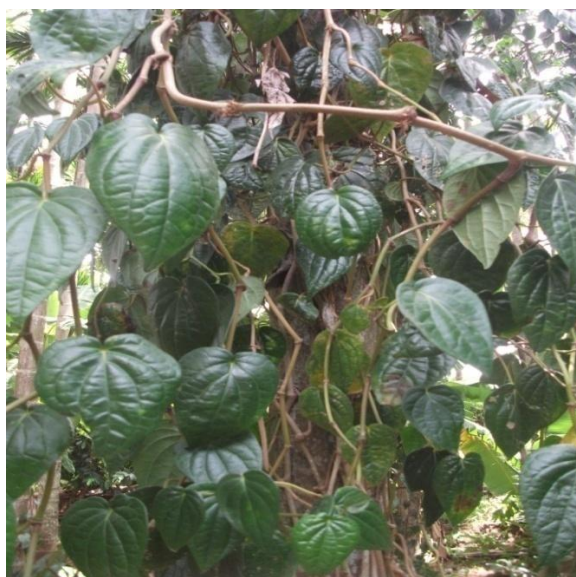
*Piper betel* leaves are widely used as a post meal mouth freshener and the crop is extensively grown in India and other Southeast Asian countries. Due to strong pungent aromatic flavor betel leaves are used as masticators by the Asian people. Betel leaves are grown abundantly in many parts of India<sup>[2]</sup>. 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<sup>[3]</sup>.

Sugar beet roots were used to produce ethanol using *Saccharomyces cerevisiae*. The ethanol from sample was converted to ethanolic acid by dichromate solution. Remaining unused dichromate solution was then estimated with thiosulphate solution to determine ethanol percentage<sup>[4]</sup>. Sugar levels influence certain aspects, including formation of adducts with polyphenols and antioxidant activity. In addition, the winemaking philosophy and, consequently, the phenolic and biochemical qualities (color, bitterness, sweetness, flavor, longevity, antioxidant properties, etc.) can influence the

acceptance of the products by consumers around the world<sup>[5]</sup>. Different authors estimated Polyphenol in grape wine by various methods<sup>[6,7]</sup>. Protein precipitation in wines is increasingly regarded as a multifactorial process, with factors such as pH, phenolic compounds and possibly others directly involved or modulating wine haze formation. The Arinto wine was found to contain 280 mg protein/L<sup>[8]</sup>. pH is a fundamental element of the wine-making industry. pH values range from 2.9 to 4.2 in wine. Chemical and biological stability of wine are dependent on pH value. Lower pH values are known to improve the stability, so winemakers usually prefer a pH range of 3.0 to 3.5.

So far, a precise study was not conducted in betel leaf wine and hence this study was intended at estimating various phytochemical compounds found in betel leaf wine such as alcohol, total sugar, total polyphenols, protein and glucose. Further, it was aimed at checking the pH of betel leaf wine at different time intervals to ensure whether it is suitable for consumption.

## Materials and Methods



**Figure 1: Betel leaves**

The betel (*Piper betle*) is the leaf of a vine belonging to the Piperaceae family (Figure1), which includes pepper and *kava*. It is valued both as a mild stimulant and for its medicinal properties. Betel leaf contains 85.4 % moisture, 3.1 % protein, 0.8% fat, 2.3% minerals, 2.3% fiber and 6.1% carbohydrates. Its mineral and vitamin contents are calcium, carotene, thiamine, riboflavin, niacin and vitamin C. Its calorific value is 44<sup>[9]</sup>.

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<sup>[10]</sup> 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. Thus obtained pure culture 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 conical flask, then 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 01:20 (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.

Ethanol concentration in wine sample was determined by potassium dichromate method (Outreach College of Science University of Canterbury New Zealand). Total sugar estimation was done by the method of Scot and Melvin<sup>[11]</sup>. Total polyphenol concentration was determined by the method of Vernon *et al*<sup>[12]</sup>. Total protein was estimated by Lowry's method<sup>[13]</sup> and glucose by DNS method<sup>[14]</sup>. pH was checked using the digital pH meter (Degisum Electronics, Hyderabad, Model No. 7007). During the fermentation of betel leaf wine, most phytochemicals were tested for every 3 days.

## Results and Discussion

### Alcohol

Alcohol level was tested for every ten days. It was increased from initial day in all the samples of betel leaf wine (Figure 2). Initially the alcohol level was ranged between 0- 4 %. After 21 days, the alcohol level was increased to the range of 15-21%. But in control there was less amount of alcohol. In sugar beet, the alcohol was estimated by modified dichromate method and it was found to be 20- 25%<sup>[4]</sup>. In Charmat sparkling wines the alcohol level was found to be  $11.54 \pm 0.15\%$  v/v<sup>[15]</sup>. It was found to vary in different wine samples.

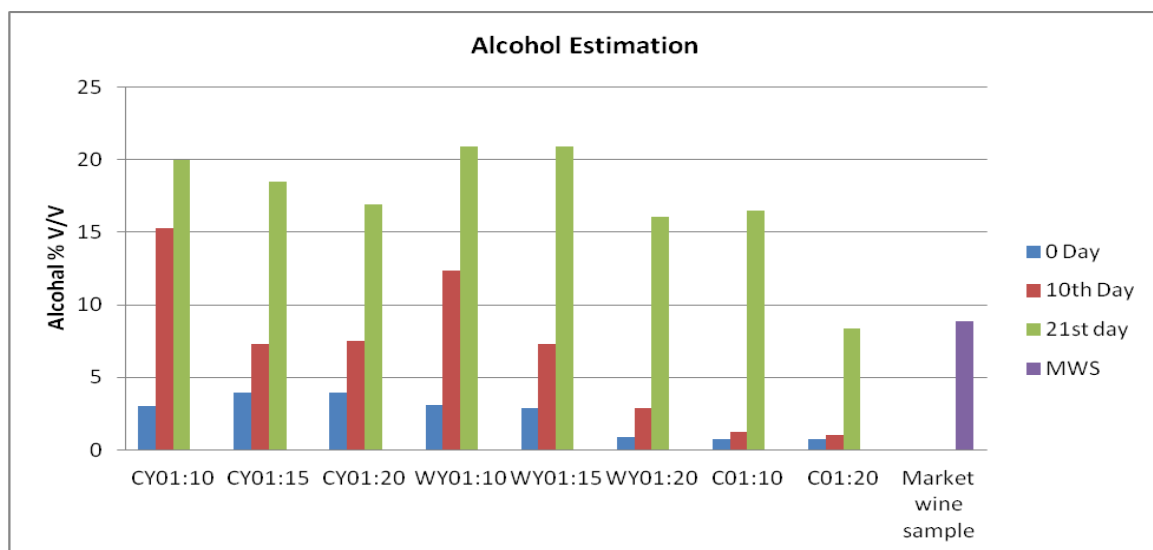
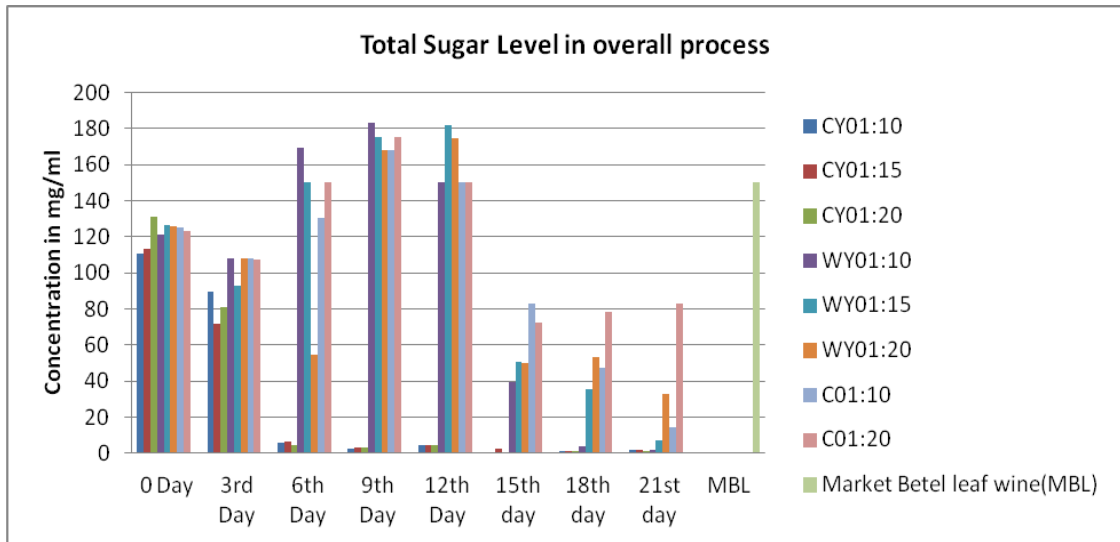


Figure 2: Percentage of Alcohol in different concentrations of betel leaf wine samples

**Total sugar**

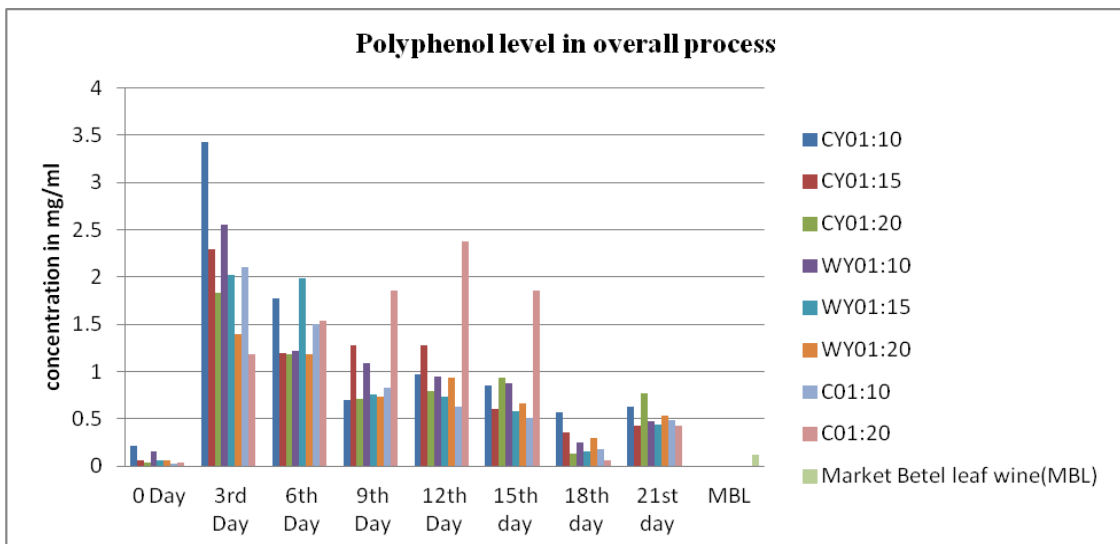
Total sugar was checked in the betel leaf wine sample for every three days. It was found that betel leaf wine sample containing commercial yeast (CY01:10, CY01:15, CY01:20), there was a decrease in total sugar level up to 1-2 mg/ml from initial day because sugar was utilized by the yeast. In the sample that contained white wild yeast (WY01:10, WY01:15, WY01:20), sugar level was increased from initial day to 12<sup>th</sup> day up to 170-185 mg/ml. It may be because of the release of exo-pectinase and endo-pectinase enzymes by the yeast which broke down the pectin that would have been present in leaves (Figure 3). So, there was an increase in sugar level until 12<sup>th</sup> day. Then, it was decreased because total sugar was utilized by the yeast to produce alcohol.



**Figure 3: Total sugar levels in different concentrations of betel leaf wine samples**

**Total polyphenols**

Total polyphenol was checked in the betel leaf wine sample for every three days. All the samples of betel leaf wine have minute quantity of total polyphenols on the zero day. In all the samples, there was an increase in total polyphenol level ranging between 1- 4 mg/ml on 3<sup>rd</sup> day except in C01:20(control). Then it was decreased and fell in the range of 0-0.8mg/ml (Figure 4).



**Figure 4: Polyphenol levels in different concentrations of betel leaf wine samples**

In C01:10, total polyphenol level was decreased as expected. However, in C01:20, the level was increased till 12<sup>th</sup> day up to 2 mg/ml and then it was decreased. This aspect has to be further

investigated. Even though, there is a presence of total polyphenols in all the samples at 21<sup>st</sup> day, the concentration seems to be comparatively low.

The three monovarietal wines present different amounts of total polyphenols, the highest amount being registered in red wine (727.4 mg GAE/l). Romanian variety Royal Maiden is also a wine with high content of total phenolics (585.06 mgGAE/l) and Chardonnay wine presents the amount of total phenolics (454.13 mgGAE/l) by Folin-Ciocalteu method<sup>[16]</sup>.

**Total Proteins**

Total proteins level was tested on the initial day and on the 21<sup>st</sup> day. It was observed that on final day, in betel leaf wine there was a slight decrease in total protein level in all the samples compared to initial day (Figure 5). This may be because of decrease in pH level and increase in the alcohol content in all the samples which denatured and precipitated proteins respectively.

At low pH (e.g., 2.8 and 3.0), the greater the pKa of the organic acid, the greater is the repulsion between organic acid (positive net charge) and wine proteins dissolved in water (positive net charge) and the smaller is the stabilizing effect of the organic acid on protein haze formation<sup>[8]</sup>.

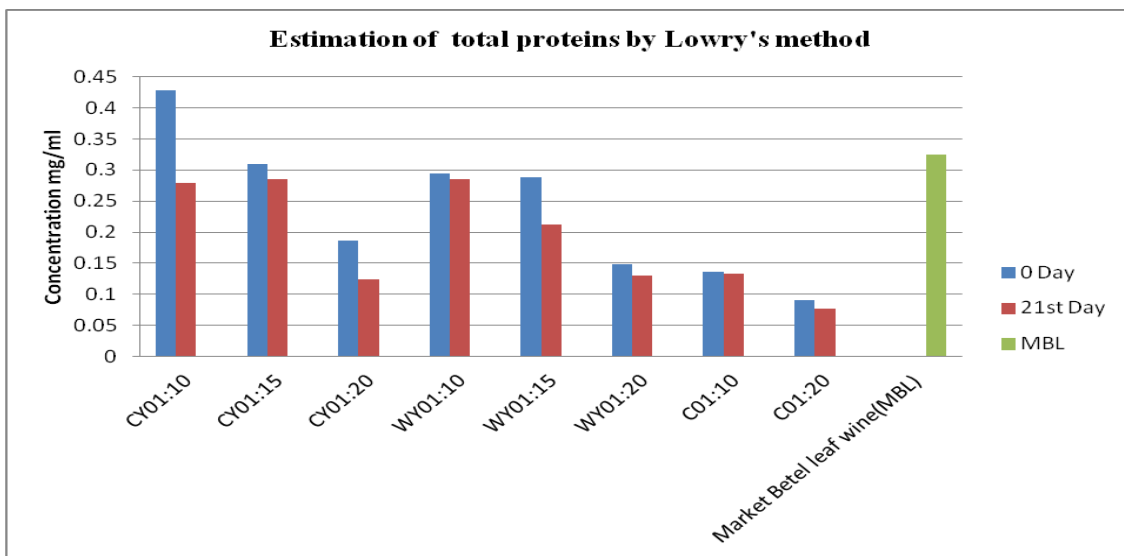


Figure 5: Total protein levels in different concentrations of betel leaf wine samples

**Glucose level**

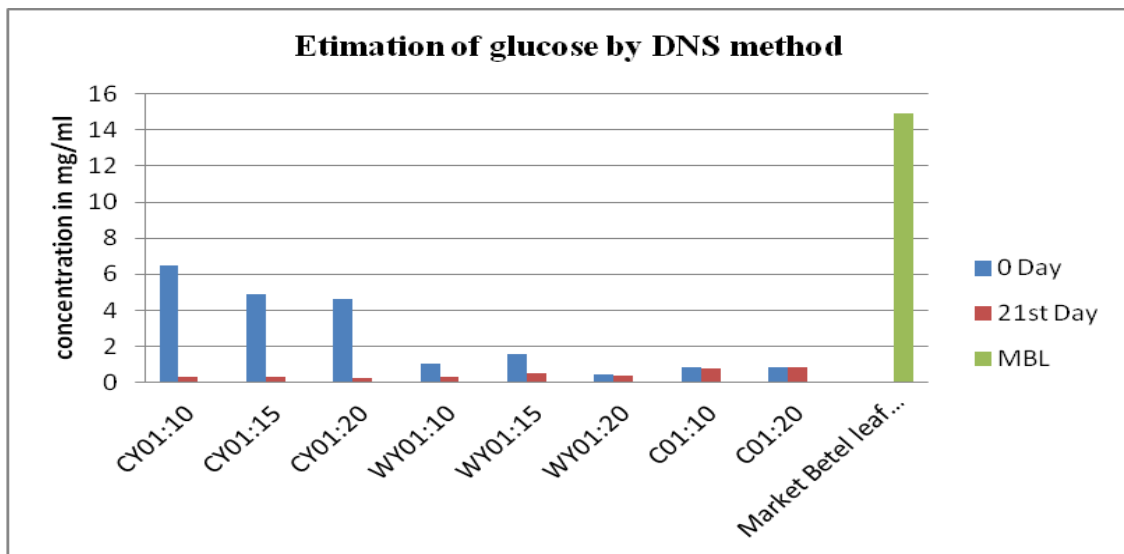
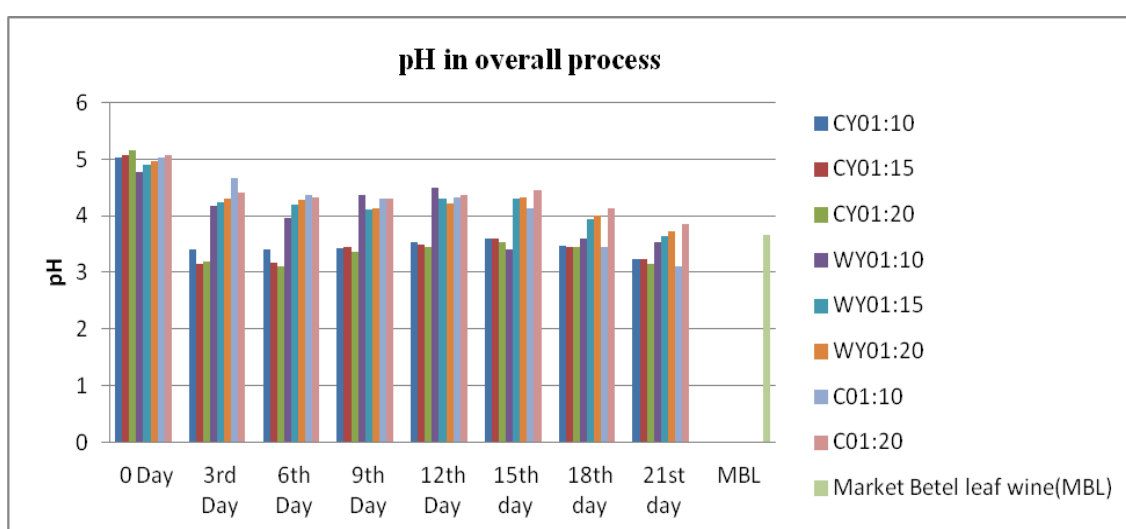


Figure 6: Glucose levels in different concentrations of betel leaf wine samples

Glucose level was tested on the initial day and on the 21<sup>st</sup> day. It was observed that glucose levels in all the samples of betel leaf wine were decreased because glucose was utilized by the yeast, where as in C01:10 and C01:20 it is almost same (Figure 6), because yeast was not added and so glucose was not utilized. This shows that yeast is essential for glucose utilization while preparing betel leaf wine. In market betel leaf wine, glucose content was around 15mg/ml due to the addition of excess sugar to increase the taste. Similar result was found in wine samples in which the glucose level was in the range of 1.5-1.75 mg/ml<sup>[17]</sup>.

## pH

pH was checked in the betel leaf wine sample for every three days. pH level of all the samples of betel leaf wine were decreased from initial day because of growth of organisms during fermentation. CY01:10, CY01:15, CY01:20 samples show rapid decrease in pH due to the presence of *Saccharomyces cerevisiae* while in other samples presence of wild yeast delayed the process. On the last day, pH level of all the samples was ranged between 3- 4 (Figure 7). Similar results were also found in sugar beet, which was close to a pH 4.5<sup>[4]</sup>.



**Figure 7: pH in different concentrations of betel leaf wine samples**

Thus, betel leaf wine was prepared using both commercial and white wild yeast. The present findings show that the betel leaf wine contained around 15- 21 % alcohol, 2-15mg/ml total sugar and 0-0.5mg/ml glucose level at the end of 21 days of fermentation process. It also contained 0.3-0.8 mg/ml total polyphenols and 0.1 – 0.3 mg/ml total proteins. pH was decreased up to 3- 3.8 as the reactions of microorganisms increased in wine. This pH range is appropriate to drink the betel leaf wine.

## Conclusion

With the present study, it is confirmed that commercially available yeast yields better phytochemicals than wild yeast in betel leaf wine production and so, betel leaf wine is suitable for consumption. Further analysis can be carried out in betel leaf wine for some more phytochemicals.

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