

Production Of Herbal Wine From Fermentation Of Cymbopogon Citratus Decoction Using Saccharomyces Cerevisiae Strain Isolated From Baker's Yeast

Moin Kazi¹, Omkar Dere², Sayali Wagh³, Samrin Pathan⁴,
Saniya Pathan⁵, Shubham Pate⁶

^{1,2,3,4,5}Department of Microbiology, Samarth Institute of Pharmacy, Pune, India

⁶Department of Microbiology, Samarth Institute of Paramedical Science, Pune, India

Abstract:

The research concerns with the production of herbal wine from Cymbopogon citrate. Wine is an alcoholic drink which is made by fermentation of lemongrass with the use of baker's yeast which breaks sugar into Carbon dioxide, ethanol and heat.

Wine is one of the functional fermented food that have many health benefits. Wine also contains anti-oxidants that releases stress and decreases cellular aging. Lemongrass (Cymbopogon citrate) is a valuable source of vitamins, macro and microelements and essential oils. The concentration of Lemongrass in the wine show polyphenol content and anti-oxidant activity and it also increases alcohol content. Lemongrass is widely used a natural remedies for digestive issues, neurological problems and high blood pressure. It is a rich source of flavonoids and phenolic compounds which contains anti-oxidants. The fermentation process was carried out in the presence of baker's yeast (Saccharomyces cerevisiae) at room temperature for 10-15 days. Various fermentation parameters such as pH, specific gravity, alcohol content, TLC and preliminary tests. The yield of alcohol contents was found to be 0 to 9% and on addition of jaggery it .

These herbal wines were found to be better in quality and due to its various health benefits it can be widely used in medical applications for preventing and treating diseases such as stomachache, high blood pressure, rheumatism, etc.

This study showed that acceptable wine can be produced from Lemongrass with the yeast Saccharomyces cerevisiae isolated from baker's yeast.

The purpose of this research is to increased the concentration of alcohol. So as to get concentrated alcohol.

Keywords: Herbal wine, Cymbopogon citrate, Fermentation, Shelf-life, Baker's yeast (Saccharomyces cerevisiae), Jaggery, Anti-oxidants, Health benefits.

Introduction :

Herbal wine

Herbal wines are the wine having medicinal properties which is usually prepared with incorporation of different herbs and medicinal plants. Herbal wine has anti-cancerous, anti-microbial, anti-diabetic and

anti-oxidant properties. It has many health benefits like reduction in ovaries cancer, strengthening bones and overall skeletons, cancer cell deterioration, blood flow regulation of heart by keeping the coronary arteries clean, elevating lungs functionality.

India has rooted evidences of expertise in Ayurveda where herbs, herbal powder and liquid herbal formulations are proved effective against diseases from common alignment such as fatal diseases. Therefore this material provides good various health benefits and can be used to prevent diseases.

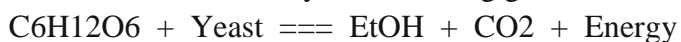
Cymbopogon citrate play a vital role for flavors enhancement and act as an preservative in wine. Lemongrass use in wine contains more tannins, polyphenols and lowers acidity. Tannins found in the Lemongrass are astringent in nature; they have aroma enhancing and anti-oxidant properties.

History of Herbal wine

When the contemporary medicine was not in use, herbal formulations were tried on persons to heal and for body soothing. The earliest evidence of plant additives in fermented beverages were reported in China and Middle East. Chemical analysis data of earthenware gave the proof of herbal incorporation in ancient alcoholic beverages. Also, addition of tree resin in wine was reported to protect the consumer against wine disease. Evidence of tree fragrance additives along with native species like rice, wheat and millets in the alcoholic formulation were reported in China. Vegetables, fruits and roots – bulbs like ginger, garlic and onion were subjected to fermentation in aqueous medium thus producing herbal formulation. These ingredients are macerated mixed together, steeped and strained for extracting herbal flavors. These ingredients are still part of Egyptian wine making and tradition and are effective against common alignment like cough, common cold and fever. Researchers have started to report chemical and botanical evidence like herbal concoctions in alcoholic drinks at the same time Abydos wine, herbal drink made of native rosemary and mint mix with thyme added to fermented emmer wheat barley beverages from Spain came into reflection.

The purpose of this research is to increase the concentration of alcohol in less time so as to get concentrated wine.

Cymbopogon citrate were shown to have free radical scavenging effects. The process of alcoholic fermentation requires careful control for the production of high quality wines. *Saccharomyces* is preferred because of its efficiency in converting glucose to alcohol.



Temperature is a vital part of fermentation in wine-making. Fermentation occurs when yeast convert glucose to alcohol and CO₂. Due to exothermic nature of fermentation, temperature increases as glucose is metabolized. Heat production can be managed with the usage of external temperature control.

The purpose of this study was to make herbal wine using lemongrass and strain *Saccharomyces cerevisiae* obtained from baker's yeast.

Methodology :

Material and methods

Collection of sample –

- Lemongrass (*Cymbopogon citratus*) aerial parts were collected from
- Organic jaggery purchase from local market of Narayangaon
- *Saccharomyces cerevisiae* obtained from baker's yeast

Preparation of must:

Aerial part of lemongrass were sorted and wash with water to remove dirt. Decoction of lemongrass were made with 1000 ml water at temperature 150 °c for 2-3 hours.

Preparation of yeast culture:

Media composition:

Ingredients	Quantity
Nutrient broth	2.5 gm
Potato fusion	100 ml
Agar	7 gm
Distilled Water	100 ml

Table no.1: Media composition for yeast culture

Medium and growth conditions for bacterial culture preparation:

The bacterial culture of *Saccharomyces cerevisiae* obtained from baker’s yeast. The baker’s yeast sample was inoculated in PDA with the help of sterile nichrome wire loop by using Streak – plate and Spread – plate method. Growth at 37 °C for 48 hrs.

Production of broth

Composition:

Ingredient	Quantity
Potato fusion	100 ml
Saccharomyces cerevisiae	2 to 3 loopful culture (20-30 µg)
Nutrient broth	2.3 g
Distilled Water	100 ml

Table no.2: Preparation of broth

Inoculation of Culture in Broth

Inoculated 2-3 loopful culture of *Saccharomyces serevisea* in the PDB kept in flask. Keep this flask for incubation for 2 days.

Preparation of must:

Fermentation

100 ml of must is added in 100 ml of PDB incubated the broth for 4-5 days at 28-30°C in an incubator. Added 80 g of sucrose in it.

Clarification:

After fermentation clarification of wine is important step. Clarification is done by using bentonite powder which removes unwanted particles and impurities. It has a excellent clarifying properties due to its ability to absorb and bind proteins and yeast cells and other haze forming substances.

Methodology:

- Bentonite preparation-
Firstly weigh the 2gm of bentonite powder and into a clean container. Gradually add the warm water and stir continuously until the slurry with smooth consistency is formed. Allow the slurry for rest for 12 hours.
- Bentonite addition-
After the preparation of slurry, gently stir it and slowly add this slurry into the fermented wine. During adding stir gently and mix the bentonite and wine mixture for few minutes.
- Settling and clarification-
Allow the wine to settle for a specific time period i.e. 1-2 weeks. During this time, the bentonite will gradually settle to bottom of container, taking suspended particles, unwanted substances and dead cells of microorganisms with it. This settling process helps to clarify the wine. The clarified wine is carefully separated from the sediment and transfer into clean container.

Aging:

After 12 days of incubation wine is prepared.

Result:**A. Collection:**

Collection of lemongrasses were done.



Figure 1:lemongrass

B. Preparation of must:

Decoction of lemongrass was done and the must was prepared.

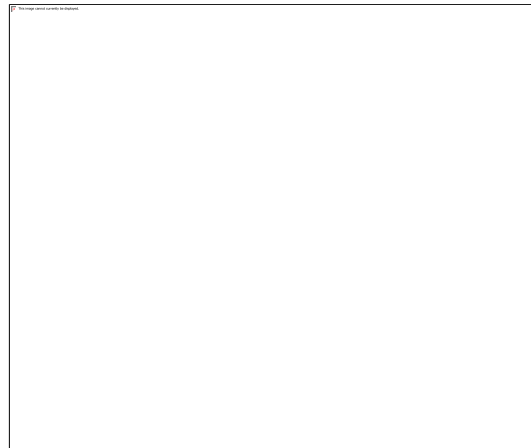


Figure 2: Decoction of lemongrass

C. Preparation of yeast culture

The yeast culture *saccharomyces cerevisiae* was prepared by using baker's yeast and this was inoculated into the potato dextrose agar with the help of streak plate method and spread plate method and incubate it for 48 hrs at 37 °c.

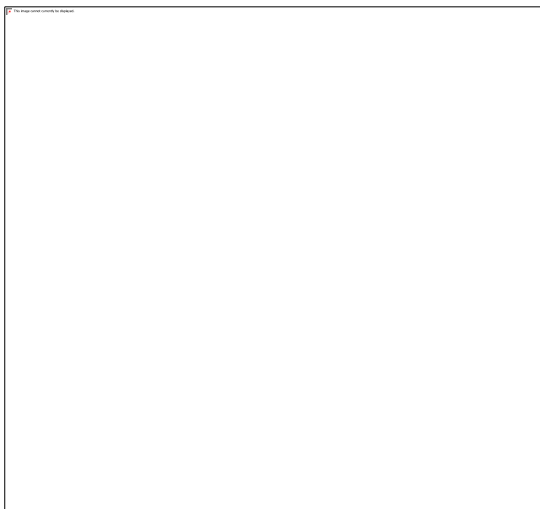


Figure 3: Inoculation of culture by streak plate method

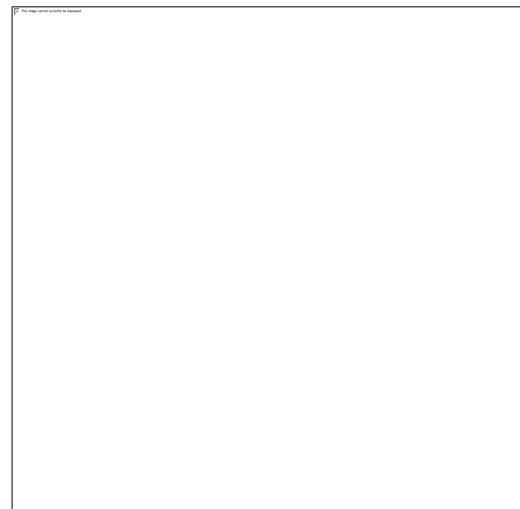


Figure 4: Inoculation of culture by spread plate method

D. Preparation of broth

Inoculation of culture in broth

Inoculation of culture in broth was done by using PDB in which 2-3 loopful of culture were inoculated and incubated that flask for 2 days at 27 °c.

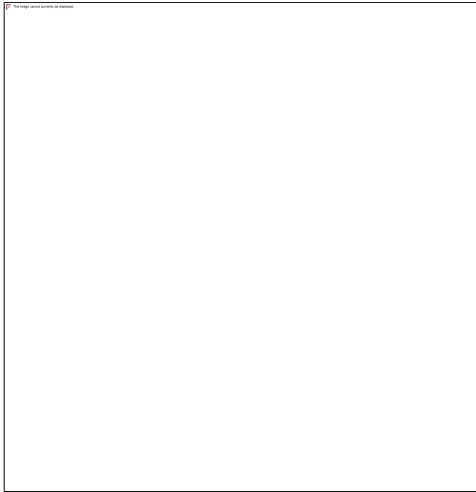


Figure 5: Prepared broth

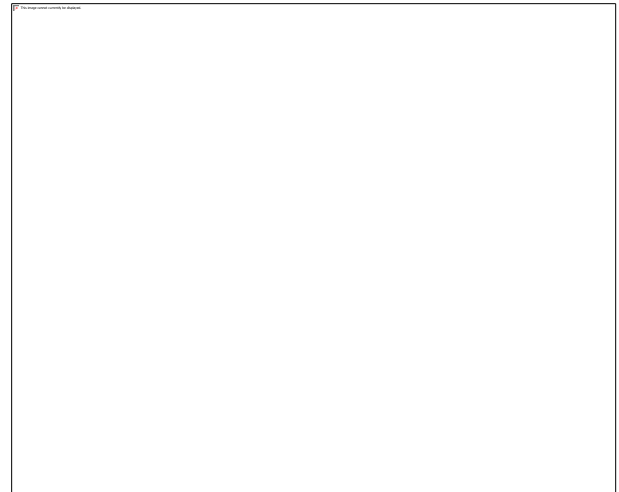


Figure 6: Inoculation of culture in broth

E. Test for pH

The pH measures with a pH meter if pH of wine is too high the wine becomes unstable. Low pH inhibits microorganism growth.

Time period (in days)	pH
1 st day	5.14
5 th day	4.12
12 th day	4.00

Table no.3: pH of wine at particular time period

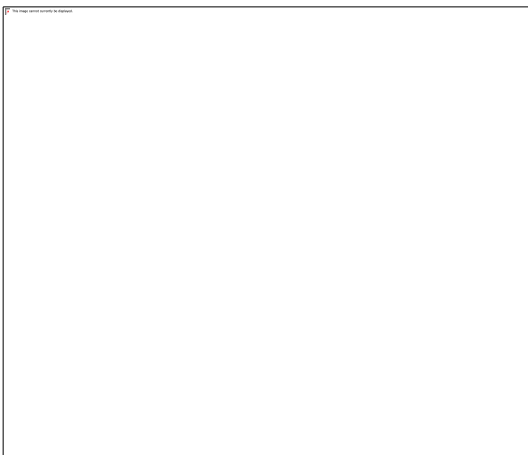


Figure 7: pH of wine at 1st day

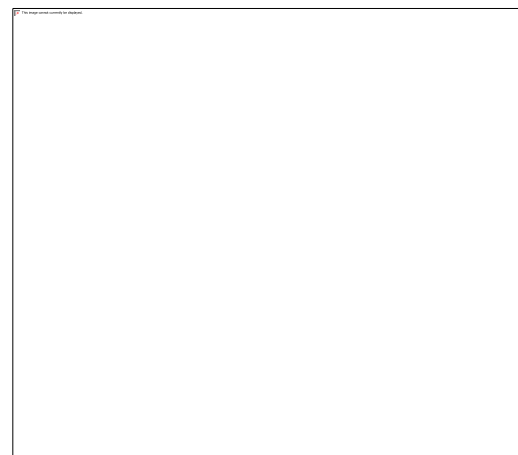


Figure 8: pH of wine at 5th day



Figure 9: pH of wine at 12th day

E. Preliminary test:

Test	Observation	Result	Contain Present /Absent
Seliwanoff’s test	Rose red colour	+	Presence of carbohydrates
Wagner’s test	Brown ppt	+	Presence of alkaloid
Fehlig’s test	Red ppt	+	Presence of reducing sugar
Benedict’s test	Yellowish red ppt	+	Presence of reducing sugar
Mayer’s test	Creamy yellow ppt	+	Presence of alkaloid
Dragendroff’s test	No reddish brown ppt	-	Absence of alkaloids
Barfoed’s test	No red ppt	-	Absence of carbohydrates

Table no.4: Preliminary test of wine

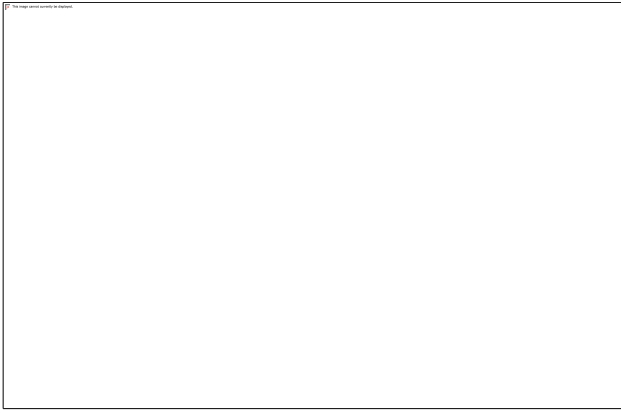


Figure 10:Wager's test

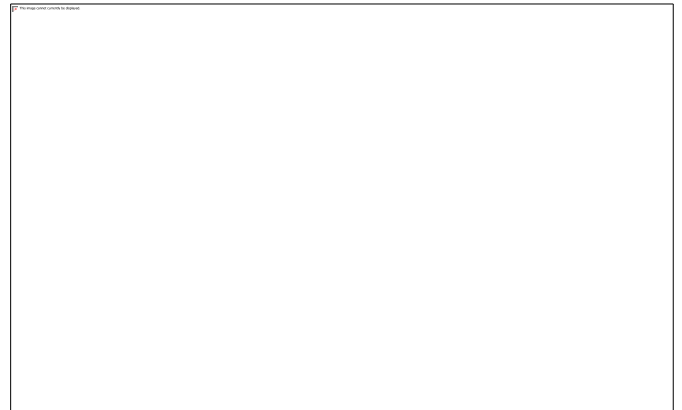


Figure 11:Benedict's test

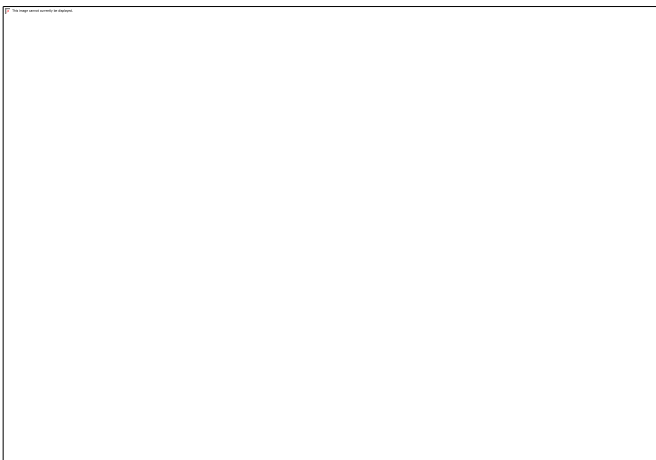


Figure 12:Fehling's test

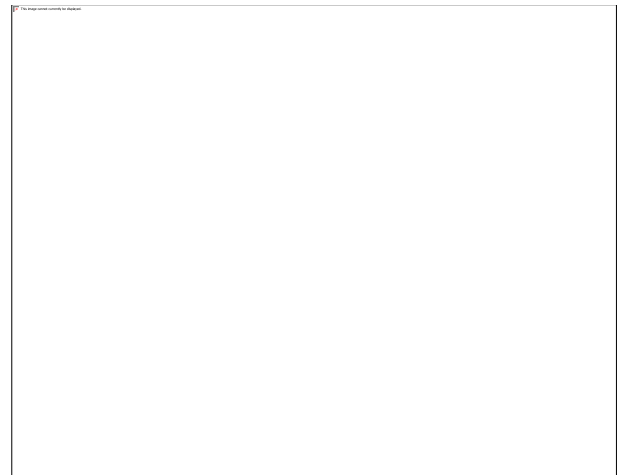


Figure 13: Seliwanoff's test

F. Fermentation

Fermentation is done by adding 100 ml of must into the 100 ml of PDB into a flask and incubate it for 4-5 days at 28-30 °c in an incubator. Total 80 gm of sucrose was added. Bentonite powder is added to fermented wine because of its has clarifying property, making it useful for removing unwanted particles and debris such as dead microbes from fermented wine.

Time period (in days)	Sugar concentration(%)	Ethanol concentration(%)
1	20	6
3	40	7
5	60	8
7	80	12
9	-	15
12	-	17

Table no.5: sugar conc. And alcohol conc. at specific time period

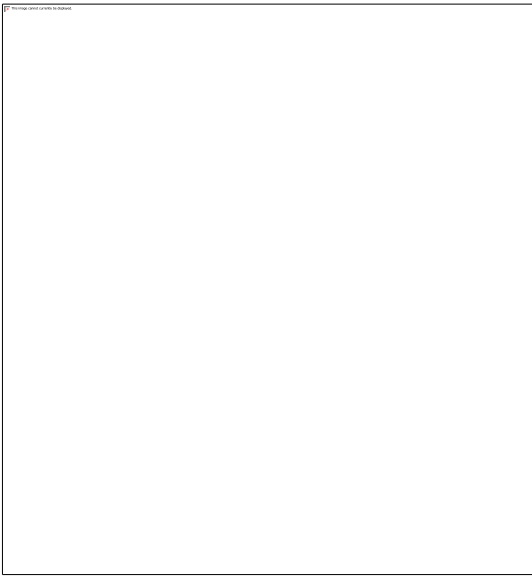


Figure 14: addition of sugar in flask

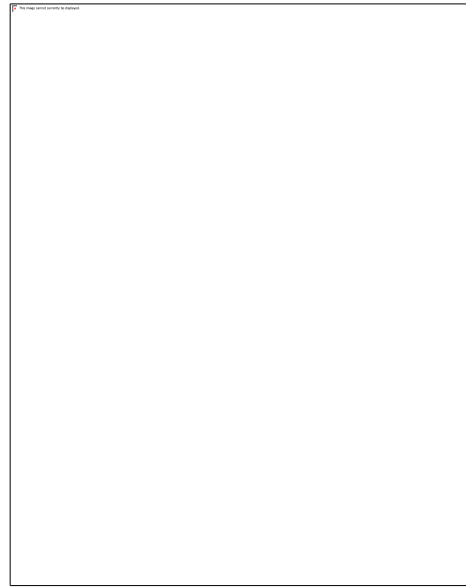


Figure 15: Fermentation flask

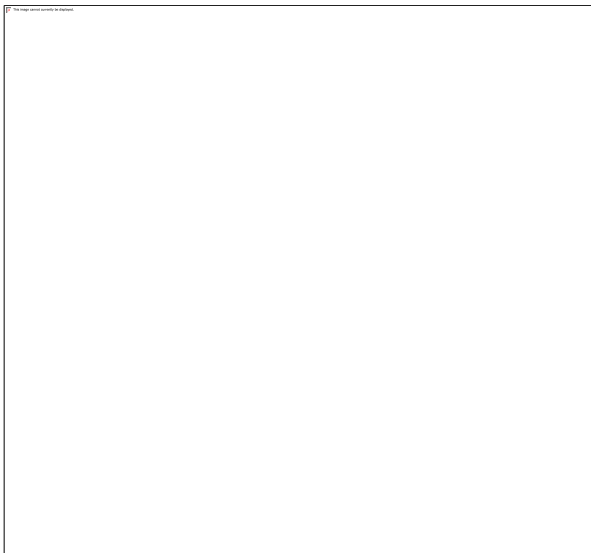


Figure 16: Bentonite powder

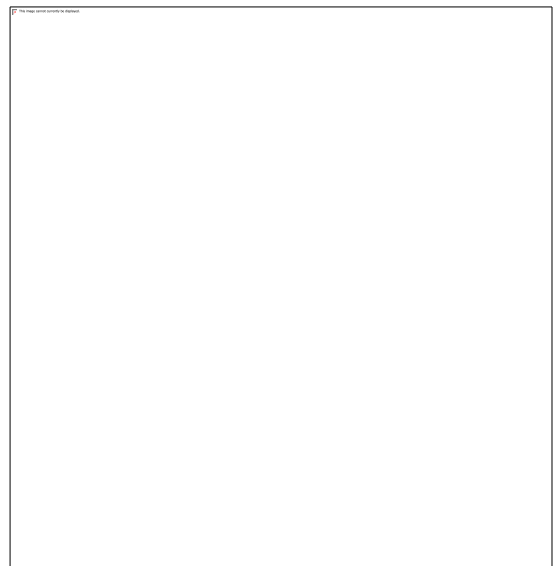


Figure 17: Addition of bentonite in fermented wine

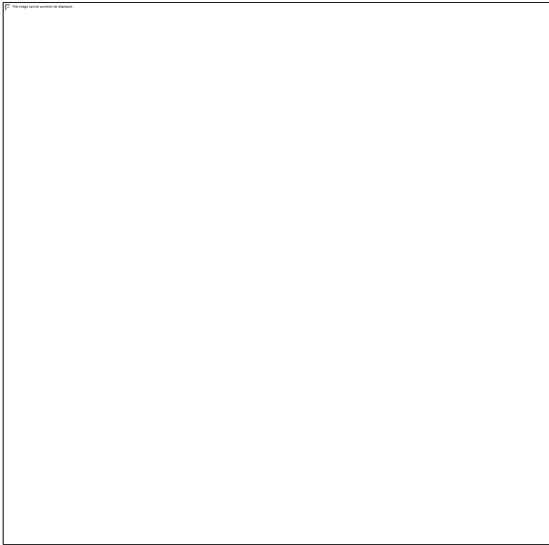


Figure 18: Clarified wine

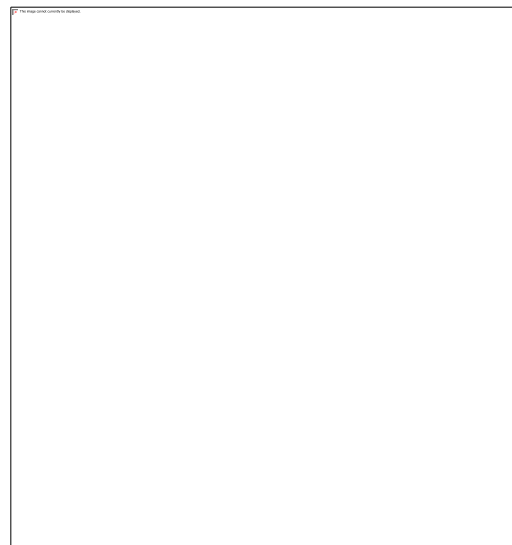


Figure 19: detection of conc. of alcohol by using hydrometer

Discussion and conclusion:

Herbal wines are the wine having medicinal properties which is usually prepared with incorporation of different herbs and medicinal plant. Normal wine Merlot, cabernet Sauvignon, Malbec etc. causes High blood pressure, hear disease, stroke, liver disease, cancer.

So, in our project we have overcome this problem by preparing herbal wine of Cymbopogon citrate which has anti-cancerous, antimicrobial antidiabetic, anti-oxidant properties. Normal wine required 5 to 6 months for reaching to the optimum concentration of alcohol, but our herbal wine of on required only 10-15 days for optimum alcohol concentration and its shown maximum concentration unto 17%.For preparation of this herbal wine we follow the methods such as decoction, must preparation, preparation of culture, fermentation, clarification, TLC, preliminary tests, pH, specific gravity.

Our aim as a future prospect is to demonstrate the clinical trial of also study the activity of SAR towards human being of lemongrass wine, such as its potential for innovation in the beverage industry or its integration into culinary experiences.

Bibliography:

1. Sharma, V., et al. (2017). "Herbal wines: A potential functional beverage for health promotion." *Food Chemistry*, 221, 1279-1289.
2. Alañón, M. E., et al. (2018). "Medicinal properties of grape and wine polyphenols: An update." *Trends in Food Science & Technology*, 81, 1-13.
3. Wang, J., et al. (2019). "Health benefits of fermented herbal beverages: An update." *Journal of Functional Foods*, 60, 103426.
4. Ru, Q. M., et al. (2019). "Research advances on traditional Chinese herbal medicine fermenting with *Aspergillus oryzae*." *Journal of Integrative Agriculture*, 18(4), 787-798.
5. Cardoso, S. M., et al. (2020). "Functional wines: A review of the effects of grapes and wine polyphenols on cardiovascular risk factors." *European Journal of Nutrition*, 59(1), 17-42.

6. Pandey, A., et al. (2020). "Traditional fermented beverages as functional foods: A review." *Journal of Ethnic Foods*, 7(1), 9.
7. Das, S., et al. (2021). "Herbal wines: An innovative approach for value addition to traditional fermented beverages." *Journal of Ethnic Foods*, 8(1), 26-38.
8. Sun, Y., et al. (2021). "Antioxidant and antimicrobial activities of herbal wines fermented by *Aspergillus oryzae* and *Saccharomyces cerevisiae*." *LWT*, 146, 111421.
9. Bavaresco, L., et al. (2021). "Herbal wines and functional beverages: An overview of their production, health benefits, and future perspectives." *Molecules*, 26(2), 543.
10. Cho, S. M., et al. (2021). "Fermented herbal medicinal products: Current status and future prospects." *Journal of Ethnopharmacology*, 272, 113909.
11. M. Koffi, N. Kouadio, R. N'Guessan, et al. (2019). "Optimization of the brewing process of herbal wines using response surface methodology." *Journal of Food Measurement and Characterization*, 13(3), 1610-1618.
12. P. Sz wajgier, M. Duda-Chodak, & J. Tarko (2019). "Production and sensory characteristics of herbal wines." *Molecules*, 24(10), 1851.
13. E. Frías-Cervantes, I. Medina-Pradas, P. González-Martín, et al. (2018). "Optimization of the formulation of herbal wines: Sensory, nutritional and functional properties." *Food Research International*, 105, 390-397.
14. L. Li, L. Zhang, C. Li, et al. (2020). "A novel approach to evaluate antioxidant activities of different herbal wine extracts." *Journal of Food Science and Technology*, 57(4), 1281-1291.
15. S. Nurrahma, I. Amalia, S. Mahfudh, et al. (2019). "Evaluation of antioxidant activity, total phenolic, and total flavonoid contents of different types of herbal wines." *Food Science & Nutrition*, 7(3), 899-904.
16. J. Sun, M. Fan, & Y. Nie (2020). "Effect of different plant materials on antioxidant activity, volatile composition, and sensory characteristics of herbal wines." *Food Chemistry*, 319, 126560.
17. M. Pérez-López, C. González-Rompinelli, J. M. Amigo-Benavent, et al. (2021). "A comprehensive study of the impact of yeast selection on the volatile profile of herbal wines." *Journal of the Science of Food and Agriculture*, 101(8), 3274-3282.