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INNOVATIVE VARIANT OF PINE TEA KOMBUCHA PREPARED FROM PINUS ROXBURGHII NEEDLES: A BOON FOR HEALTH

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ABSTRACT

In the present study new variant of kombucha has been prepared by using pine tea and Kangra green tea Pine tea was prepared by exploiting needles of *Pinusroxburghii*. Primary fermentation was carried out in presence of medicinal herb i.e. Banfsha. Kombucha fermentation process took place at 28^0C for 14 days. It was observed that pH decreased from 5.0 to 3.5 during fermentation period. Titratable acidity after 14^{th} days of fermentation exhibited a rise in acidity (0.02 to 0.16 %). The TSS of Kombucha steadily decreased from 4.85 to 3.10 ^{O}Brix. Kombucha showed antagonism against food borne pathogens viz. *Listeria monocytogenes, Staphylococcus aureus, Leuconostoc mesenteroides.* Sensory evaluation of kombucha was scored 8.0 by panelists for its overall acceptability. Kombucha prepared in this study is novel variant as the fermentation process carried out with pine tea Kangra green tea in presence of herb Banfsha, Kainth/ ground apple.

KEYWORDS: Kombucha, Pine Tea, Sensory, Banafsha, Green Tea.

INTRODUCTION

Kombucha is traditionally made by fermenting sweetened tea with a yeast and bacterium symbiotic culture (SCOBY). The yeast and bacterial components of this intricate fermentation process have a symbiotic connection. The sugars in the tea are fermented by the yeast into alcohol, which is then oxidized into a range of organic acids by bacteria. A variety of bioactive substances such as organic acids, vitamins, polyphenols and probiotics are added to the tart, fizzy beverage that is produces by this dual fermentation process.[1] These

substances give Kombucha its distinct flavor and solid reputation as a functional beverage with certain health advantages. Research on the health advantages of kombucha has grown over time, demonstrating its capacity to boost immunity, promote digestive health and offer antioxidant protection.[2] The probiotic microorganisms found in kombucha are thought to have these advantages because they are thought to improve gut health and general wellbeing. Additionally it is believed that Kombucha's health promoting properties are influenced by its bioactive ingredients, which include organic acids and polyphenols. Numerous studies exploring the health benefits, fermentation properties and potential applications of kombucha have emerges in response to the growing public interest in natural and functional foods. Because of this increased understanding of the link between diet and health, Kombucha has come to represent the contemporary movement towards healthy eating and living. With in the field of modern Food Science, Kombucha serves as link between historical customs and cutting- edge nutritional studies. Its changing function as a functional beverage highlights the value of investigating and verifying traditional foods within the parameters of scientific research, providing fresh chances to improve dietary habits and advance health.

New variants of Khombucha prepared in the present study from pine tea made up of *Pinus roxburghii* needles alone and in combination with green tea procured from North West Himalayan state Himachal Pradesh have shown promising results not only in terms of their organoleptic properties but also from therapeutic purpose. After primary fermentation, addition of valuable medicinal Himalayan – herb Banaksha has led to significant increase in health boosting properties of pine khombucha. For secondary fermentation, lesser known Himalayan fruits i.e. kainth/ ground apple are used to open new vistas in innovating entirely new variantsof pine tea kombucha.

MATERIALS AND METHODS

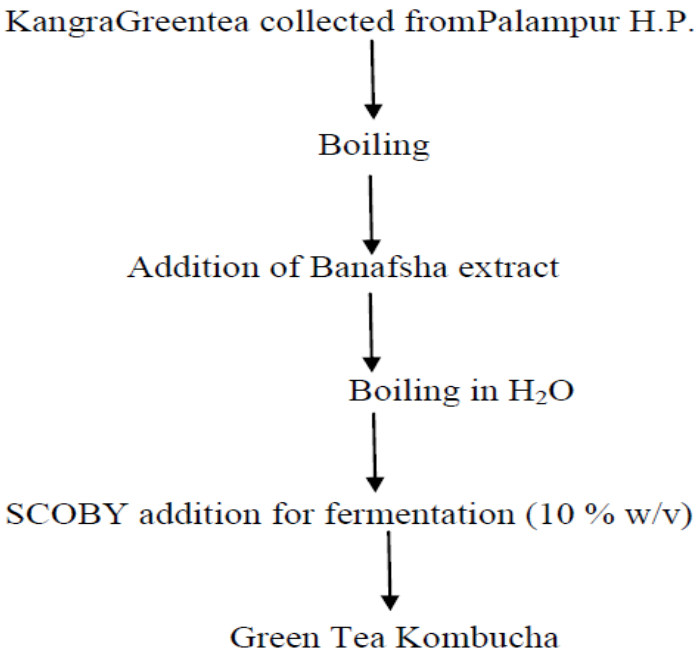
Substrates, Microorganisms and Chemicals

Pine needles were collected from near by areas of Dr Y. S. Parmar UHFNauni, Solan Himachal Pradesh. The Pine needles were brought to the laboratory grinded into small pieces in grinder and stored in clean air tight glass containers for further use. Table sugar was used in the study was procured from local market of solan. All materials were stored under specific conditions to maintain their quality. Sugar was kept in airtight containers in a cool, dry place to prevent moisture absorption and contamination. Green tea was purchased from, Palampur H.P. Live SCOBY (Symbiotic Culture of Bacteria and Yeast) Culture (Fig 1S) for Kombucha

tea preparation was purchased from Gutbasket from Gutbasket Foods Private Limited Ahmedabad, Gujrat, India.

Fermentation Conditions and Methodology

The fermentation process in the present study was initiated according to.[3] Briefly, to initiate the fermentation process, 100 g of sucrose and 1g of Pine needles were dissolved in 1000 mL of distilled water and steeping for 15 min at 98°C. Then, pasteurized at 95 °C for 12 min and was cooled to room temperature and filtered with autoclaved stainless steel strainer then, the above solution was aseptically transferred to 1.5L autoclaved glass jar. Kombucha culture [10% (w/v)] was SCOBY and procured from Gutbasket previously, aseptically inoculated into it and covered with clean muslin cloth and secured with a rubber band, in order to ensure that the colonies receive enough oxygen. Fermentation was monitored at 28 ±2 °C for 14 days (Fig 1). Following the fermentation process, samples were aliquoted and filtered using a sterile filter paper (Whatman Filter paper no.1) of pore size11µm, then physicochemical and therapeutic analysis was done. Microbiological examination was done prior to the filtration. The experiment was repeated thrice. The pictorial presentation for Kombuchaprepared from pine needles has been mentioned in Figure1a-1c.



1a) Green tea kombucha

Collection of *P. roxburghii* needles

↓

Boiling

↓

Addition of Banafsha extract

↓

Boiling in H_2O

↓

Pine Tea + Banaksha extract

↓

SCOBY addition for fermentation (10 % w/v)

↓

Pine TeaKombucha

1b) Pine tea kombucha

Green tea + Pine Tea + Banafsha extract

↓

Boiling in H_2O

↓

SCOBY addition for fermentation (10 % w/v)

↓

Kombucha

1c) Functional kombucha

Pine Needles

Banfsha

Green Tea

Sucrose (100 g)

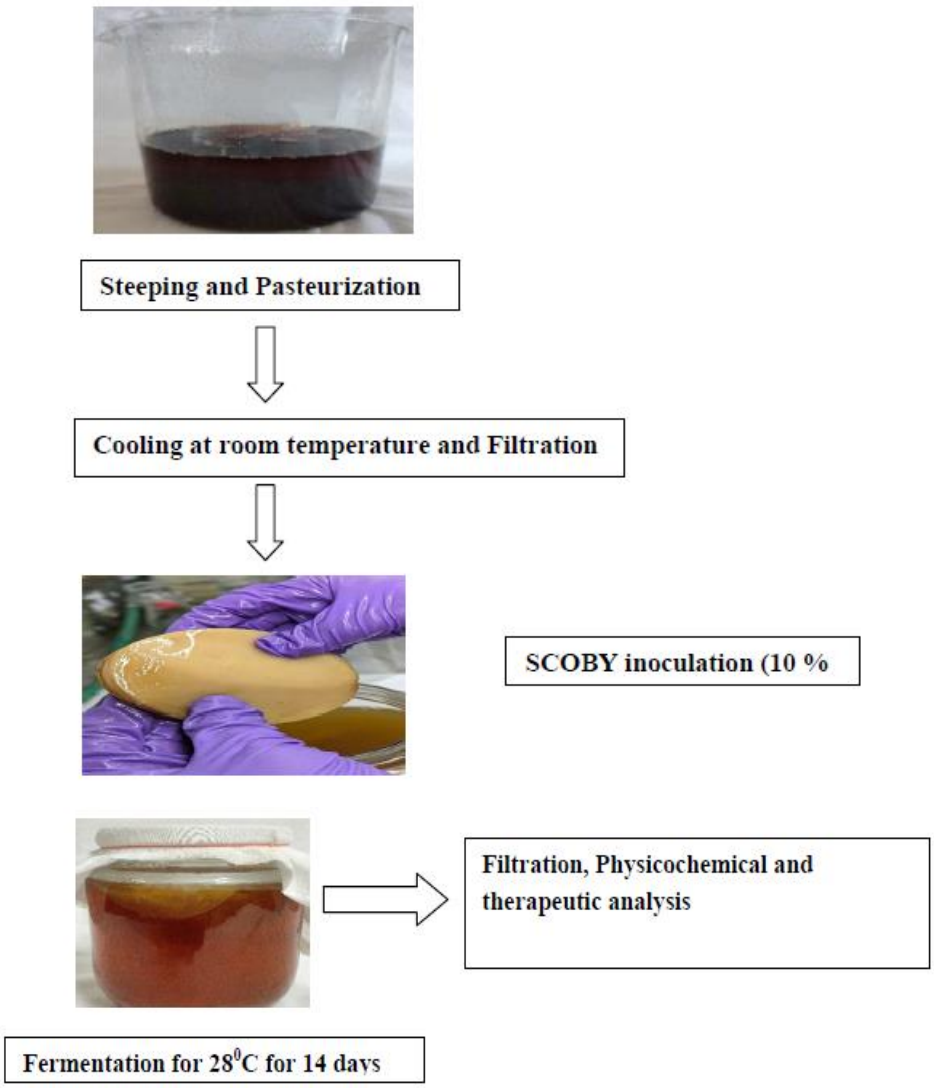


Figure 1: Flow diagram of pine needle kombucha preparation.

Physicochemical and Therapeutic evaluation of pine tea kombucha

Following physicochemical parameters were analyzed

- Total Phenol
- Total Sugar Content
- Antioxidant activity
- Acidity
- pH
- Ethanol concentration
- Sensorial analysis
- Microbiological analysis

TPC was determined using Folin- Ciocalteau method with little modifications. A sample (0.1 ml of extract) was mixed with 0.9 ml of water and then 2.5 ml of Folin- Ciocalteu reagent was added. After 5 min, 2ml of 0.7 N Na2CO3 solution was added. The mixture was incubated at room temperature for 2 h, then the absorbance was recorded at 765 nm using UV- VIS spectrophotometer against a blank. The amount of total phenols in both the extracts was expressed as gallic acid equivalent.

Total sugar

Total sugars were determined in culture supernatants by Phenol-sulphuric acid method as per[5] method with little modifications.

Reagents

1. Phenol 5%: redistilled Phenol (50 g) dissolved in water and diluted to 1000 ml.
2. Sulphuric acid: 96% reagent grade
3. Standard glucose: Stock- 100 mg 100 ml of water
4. Working standard: 10 ml of stock diluted to 100 ml.

According to the given methodology, 0.1 ml of extract was added to 0.9 ml of distilled water and 1ml of phenol solution. Shakenthe solution after adding 5ml of sulphuricacid. Incubated the tubes@25-30ºC for 20 min. Then the absorbance was recorded at 490 nm using UV- VIS spectrophotometer against a blank.

Free radical scavenging activity (FRSA)[6]

Free radical scavenging activiy was measured as per the method. DPPH (2,2-diphenyl-1-picrylhydrazyl) was used as a source of free radical An aliquot of 3.9 ml of $6x10^{-5}$ mol/l DPPH in methanol was taken in a cuvette with 0.1 ml of sample extract and the decrease in absorbance was measured at 515 nm for 30 min or until the absorbance become steady. The remaining DPPH concentration was calculated using the following equation:

$$\text{Free radical scavenging activity (\%)} = \frac{Ab_{(b)} - Ab_{(s)}}{} \text{X100}$$

$Ab_{(b)}$

Where, $Ab_{(b)}$= Absorbance of blank, $Ab_{(s)}$= Absorbance of sample.

pH determination

pH of sample was measured using pH meter.

Ethanol concentration

Ethanol content was determined by spectrophotometric method. 1 ml of sample and 29 ml of water was taken in a distillation flask. Volumetric flask of 50 ml containing 25 ml of potassium dichromate beneath the condenser was placed and tightly plugged with cotton. Distillation was carried out and 20 ml distillate was collected in receiving end. The content of the flask was heated at 60^0C in a water bath for 20 min and brought up to volume with distilled water. After mixing and cooling, the absorbance was taken at 600 nm. Ethanol standards were prepared by making ethanol water solutions containing 0,2,6,8 per cent ethanol by volume. The ethanol content in a given sample was estimated by referring to standard curve and expressed as %.

Calculations

OD x Dilution= Value of ethanol

(Standard curve reference)= Ethanol concentration

g/L= Ethanol concentration x0.79x10

0.79=Density of Ethanol

The ethanol was estimated in terms of g/L of fermented beverage

Sensorial analysis

Sensorial evaluation of Kombuchasample was done in terms of appearance, texture, flavor and overall acceptability. Ninepoint hedonic scale method as given by Joslyn and Amerine (1964) was followed for conducting the sensory evaluation of food products. The panel of 10 judges was selected to evaluate the products for sensory parameters such as appearance, flavor, texture, taste and overall acceptability depending upon the type of product. Efforts were made to keep the same panel for sensory evaluation throughout the course study.

Microbiological evaluation

Antagonistic activity was assessed against food borne pathogens viz. *Listeria monocytogenes, Staphylococcus aureus, Leuconostocmesenteroides Escherichia coli. Bacillsus cereus* by well diffusion assay.[7]

RESULTS AND DISCUSSION

Kombucha fermentation process took place at 28^0C for 14 days. Physicochemical analysis of Kombucha prepared from pine needles was done after 14 days of fermentation process. It was observed that pH decreased from 5.0 to 3.5 during fermentation period (Figure 2a-e). The

decrease in pH is due to increased concentration of organic acids produced during the fermentation process by bacteria and yeast. Our results are accordance to study of Elok Zubaidah, Yunita Khilyatun Nisak, Ike Susanti, Tri Dewanti Widyaningsih, Ignatius Srianta, Ihab Tewfik, Turmeric Kombucha as effective immunomodulator in Salmonella typhi-infected experimental animals.

Biocatalysis and Agricultural Biotechnology, et al., where pH of Kombucha decreases from around 5.0 to 2.5 within 6 days of fermentation. A pH range between 2.5 and 4.2 is considered desirable and safe for kombucha beverages.[8] Low acid beverages (<pH 4) are considered to be microbiologically safe as they can prevent the growth of pathogenic microorganisms.[9]

Titratable acidity after 14^{th} days of fermentation exhibited a rise in acidity (0.02 to 0.16 %) due to the presence of organic acids produced during fermentation process as depicted in (Figure 2c) Sucrose will be hydrolyzed into glucose and fructose during *kombucha* fermentation by yeast enzymes. After that yeast will metabolize glucose to ethanol and CO_2.Similar reports have been reported by[10] in turmeric kombucha.

The TSS of Kombucha steadily decreased from 4.85 to 3.10 ^{O}Brix throughout fermentation for 14 days (22 °C). The reduction in TSS may be due to the microbial metabolism of sucrose in the tea broth into a range of metabolites. During fermentation acidity increased and TSS decreased.[11] It has been observed in our study that Kombucha prepared from pine needle and kangra green tea has higher Free radical scavenging activity. This antioxidant properties of kombucha is more than vitamin C and vitamin E. Antioxidant activity[12] prevents body cells damage, inflammatory diseases, immune system failure, and cancerous tumor formation.

Figure (2e) depicting the rising of ethanol after 6^{th} day of fermentation. It was caused by the activity of SCOBY, that converted sugar to ethanol and CO2 during alcohol fermentation. Sugar concentration also contributes to the result of ethanol production. The added sugar in kombucha production is usually about 5-15% (w/v) In this research, sugar was added into the tea water by 10%. The level of ethanol has a positive correlation with the increase in sugar concentration until at a specific point.

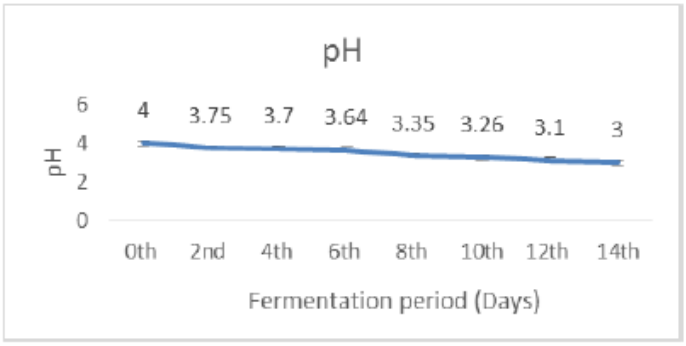
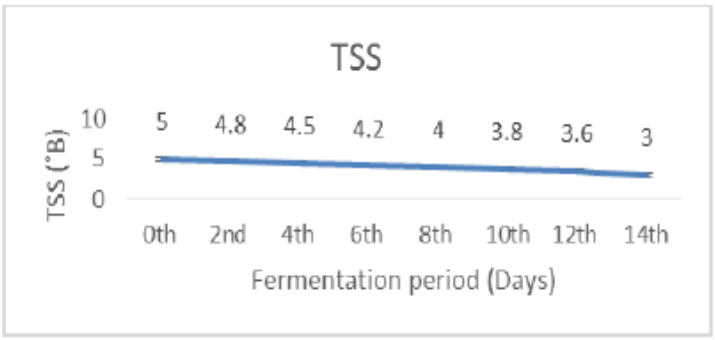
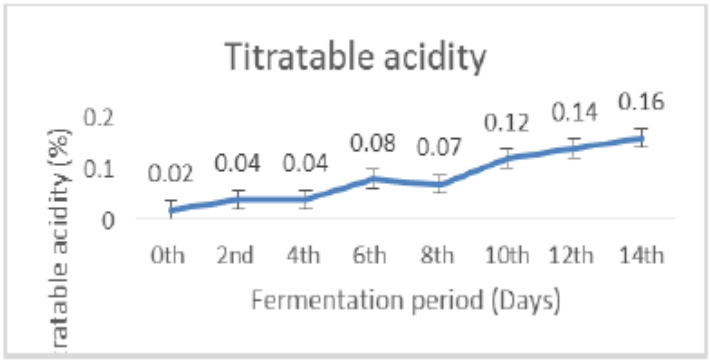


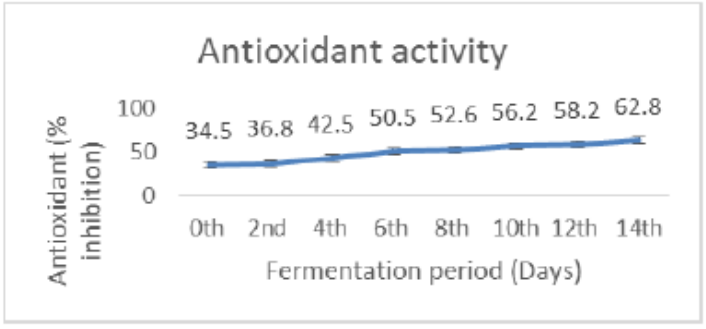
Fig. 2a): Effect of fermentation on pH.



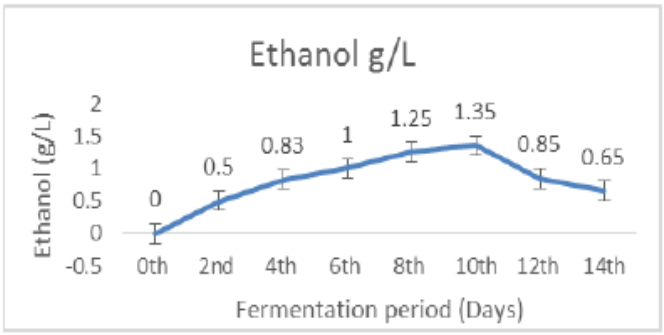
2b) Effect of fermentation on TSS.



2c) Effect of fermentation on Titratable acidity.



2d) Effect of fermentation on antioxidant activity.

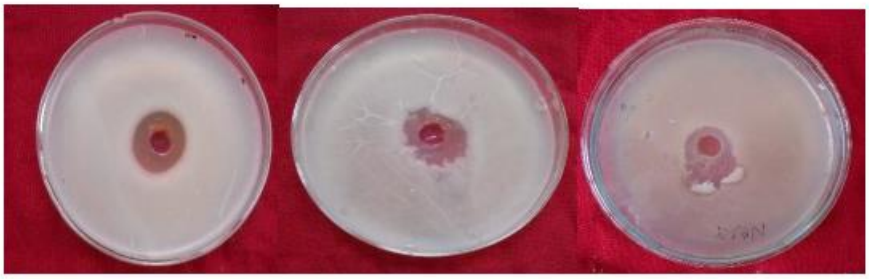


2e) Effect of fermentation on ethanol concentration.

Figure 2 (a-e): Physicochemical analysis of Kombucha fermentation.

Microbiology evaluation

Prepared Kombucha showed antagonism against serious food born pathogens. Antagonism was tested by well diffusion assay. Zone size so obtained were 12 mm against *Listeria monocytohenes*10 mm against *Leuconostocmesenteroides*, 8 mm against *Staphylococcus aureus*as depicted in Figure 3.



L. monocytogenes *L.mesenteroides* *S.aureus*

Figure 3: Antagonistic activity of kombucha against food borne pathogens.

Sensory evaluation of the product

It was revealed by 9 point hedonic scale. The product was scored 8.0 by panelists for its overallacceptability. Similar studies has been done by[13] where they reported overall acceptability for their Kombucha 8.3.

CONCLUSION

Novel variant of Khombucha prepared in the present study from pine tea made up of *Pinus roxburghii* needles and in combination with Kangragreen tea procured from North West Himalayan state Himachal Pradesh have shown promising results not only in terms of their organoleptic properties but also from therapeutic purpose. The beneficial bioactive

compounds present in pine needles that have not been present in kombucha fermentation so far may have a positive effect on the product properties, both sensory and health promoting activity. This is the first report of kombucha production by using pine needles, Kangra green tea and Banfksha. It is also believed that Kombucha prepared in this study is novel as the fermentation process critically dependent upon geographical region in which it is prepared and also on the type of plant material and additives. The scientific work to improve the product should be continued.

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