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A Study on Biopotentials of Fermented Tea

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The Brooke bond three roses tea packet and samples for the isolation of *Saccharomyces cerevisiae and Acetobacter sp.,* was collected from Dindigul, Tamil Nadu, India. The bacterial and yeast isolation was made with Glucose Ethanol Yeast Extract Agar and YPS medium. Predominant colonies were selected and pure cultured for further analysis. Microscopic observation and biochemical analysis was done to identify the pure cultured organism. After identification the identified organisms were inoculated in sugared Black Tea which was steeped, filtered and cooled. This set up was incubated for a period of 7-10 days. This post-fermented Black tea was used for the estimation of the tannin and it was found to be decreased in Tanin content than the normal Tea. The fermented Tea was used for Antibacterial activity assessment against *Vibrio cholerae, Staphylococcus aureus* and *Salmonella typhii* and it was found that higher inhibitory activity was there with *Staphylococcus aureus* followed by *Vibrio cholerae* and there was no effect with *Salmonella typhii.*

Key words: Fermented tea, *Saccharomyces cerevisiae, Acetobacter* sp., Tanin, Antibacterial activity.

Many types of tea come from the same plant *Camellia sinensis*. The type of tea depends on its manufacturing process and degree of fermentation (Elizabeth farell 2007). Fermented tea known as kombucha / kargosak tea is a popular drink in many Asian, European countries and in USA. (Blanc 1996) it is prepared by fermenting sugared black tea decoction with symbiotic culture of yeast *(Candida sp., Saccharomyces sp., Zygosaccharomyces sp., Pichia sp. and Brettanomyces sp.)* and acetic acid bacteria (A*cetobacter xylinum, Acetobaacter aceti* and *Gluconobacter oxupdaing)* for about 5-7 days at 21°c. Consuming kombucha has lots of beneficial roles (Haizhen M.O *et al.,* 2008) (C.Dufresne *et al.,* 2000). Kombucha shows a wide range of antibacterial activity against a number of bacteria such as *Staphylococcus aureus, Shigella sonnei and Escherichia coli*. But they have no Antimicrobial activity against yeast, it may due to its acidic nature.(Sreeramulu *et al.,* 1999*).* 1g/L of acetic acid of kombucha is enough to inhibit pathogenic and spore forming bacteria was reported by Levine *et al.,* (1940). Tanin is a pigment enrich imparts colour and characteristic

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flavor of Tea. Although Tanin has Antimutagenic, Anticarcinogenic, Antibacterial activity and effective for cardio vascular diseases and stroke, it should be consume in a limited amount (2 cups of tea/day) otherwise it can cause decrease intake of food, growth rate, feed efficiency, protein digestibility and anemia by preventing iron absorption of the body. The microbial fermented tea contains low level of tannin so that the tea lovers can take 3-4 cups of fermented tea/day. (Tabasum *et al.,* 2001).

MATERIAL AND METHODS

Brooke bond three roses Tea packet and samples (Grapes and spoiled Apples) were collected from Dindigul, Tamil Nadu, India.

Isolation of Bacteria and Yeast

The isolation of bacteria was carried out in Glucose Ethanol Yeast Extract $CaCo_3$ medium (Deley, 1961) , the isolation of yeast was carried out in YPS medium and was incubated at 30°C for 5 days for bacteria and at 28°C for 3-4 days for yeast. After incubation the zone of clearance around the colony in GEYE medium was observed and the colonies were collected for identification. The predominant colonies on YPS medium was collected and used for identification.

Identification of Bacterial isolate

The following biochemical tests were carried out for the identification of bacteria. Gram's staining, Motility, Indole production test, Methyl Red test, Voges-Proskauer test, Citrate utilization test, Gelatin hydrolysis, Catalase test and Growth in media containing Ethanol +$CaCO_3$ the results were tabulated in Table 1.

Identification of Fungal isolate

The colonies on YPS medium was collected and was identified based on colony morphology and Lactophenol Cotton Blue staining. The results were tabulated in Table 2.

Preparation of microbial fermented tea

Microbial fermented tea was prepared by adding 70g/L of commercial sucrose to tap water and after boiling, 5g/L of black Tea powder was added and steeped for 15 minutes and removed by filtration. After cooling to about 30°C the inoculum (*Acetobacter sp* and *Saccharomyces cerevisiae*) was added in an amount of 10% (v/v) and it was incubated under aerobic condition at 28^0C for 7-10 days. After 10 days it was pasteurized and used for further analysis.

Kirby Bauer Antimicrobial Susceptibility test

The antibacterial activity of the fermented Tea was assessed by Kirby Bauer disc diffusion technique (Bauer *et al.,* 1966).

Estimation of Tannin

Tannin content in the microbial fermented Tea was determined by Lowenthal's permanganate Oxidation process. (Pearson 1976, Barua and Roberts 1940).

RESULTS AND DISCUSSION

Tea is an infusion of flavourful leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties. According to Mbata, 2007, Ruxton, 2002 "Drinking Hot tea is actually better for you than drinking water. Water is essentially replacing fluid. Tea replaces fluids and contains anti-oxidants. So it has got two things going for it".

Isolation & identification of bacteria & yeast

Isolation of bacteria and yeast cultures were carried out using Glucose Ethanol Yeast Extract $CaCO_3$ medium and YPS medium, from these plates predominant colonies were choosen and pure cultured for further analysis. The results are tabulated in Table 1.

After isolation, identification of bacterial culture was done by biochemical tests and the results of the tests are tabulated in Table 2.

The yeast culture was identified by colony morphology and microscopic observation and the results are tabulated in Table 3.

With the above mentioned biochemical tests, the bacterial isolate was identified as *Acetobacter sp.* while the Yeast isolate identified was *Saccharomyces cerevisiae*. Acetic acid bacteria are known to exist in a wide variety of natural sources and a number of brewery products

Table 1. Isolation of bacteria and yeast

S.No	Organism	Dilution factor	CFU/ml
1	Bacteria	10^{-5}	4×10^5
		10^{-6}	3×10^6
2	Yeast	10^{-2}	6×10^2
		10^{-3}	5×10^3

Table 2. Identification of *Acetobacter sp.*

Name of the test	Results
Gram's Staining	Negative, Slightly curved rods
Motility	Motile
Indole production test	Negative
Metlyl red test	Positive
Voges- proskauer test	Negative
Citrate utilization test	Negative
Catalase test	Positive
Gelatin hydrolysis	Negative
Colony morphology in plates	Pale yellow colonies.
Growth in media containing	
Ethanol+$CaCO_3$	Growth observed
Methanol+ $CaCO_3$	No growth.

Table 3. Identification of *Saccharomyces cerevisiae*

Macroscopic Appearance	Microscopic Appearance	Results
Pale yellow colonies on YPS agar plate	Oval shaped budding cells was observed	*Saccharomyces cerevisiae*

Table 4. Antimicrobial activity of Microbial Fermented Tea

Organism	Zone of inhibition
Staphylococcus aureus	24mm
Vibrio cholerae	8mm
Salmonella typhii	No zone

(Kahlon and Vyas, 1971). *Saccharomyces cerevisiae* is a saprophyte found in sugary substances like flowers, fruits, soil, milk, sugar solutions and in fermented beverage. (Dubey and Maheswari, 2002). Microbial fermented tea was prepared and inoculated with *Acetobacter sp.* and *Saccharomyces cerevisiae* which was incubated for 7-10 days at 21°C and the following tests were done. The pH of the fermented tea was found to be 3.6.

Antimicrobial property of Microbial Fermented tea

The Antipathogenic assay of Microbial Fermented Tea was listed in Table 4.

According to the observation both *Vibrio cholerae* (8mm zone of inhibition) and *Staphylococus aureus* (24mm zone of inhibition) are sensitive to the microbial fermented tea. But *Salmonella typhii* shows no zone of inhibition it indicates that the organism was resistant to Microbial Fermented Tea.

Difference in antimicrobial property of tea have been found to be related with the kind and degree of fermentation (Chou *et al.,* 1999) Daily consumption of tea killed *Staphylococcus aureus* and other harmful bacteria (Sofawara 1984, Opara, 1992).

Estimation of Tannin

Tannin content in the Microbial Fermented Tea was determined by Lowenthal's permanganate Oxidation process and the results were tabulated in Table 5 and 6.

Elizabeth (2007) stated that tea tannin is actually called polyphenols. Polyphenols which include flavonoids, gives tea a boost of health benefits that help to prevent cancer, heart disease

Table 5. Estimation of tannin: Filterate A: (Total Titre)

Sample	Vol. of Tea (ml)	Burette reading		Concordant value (ml)	End Point
		Initial (ml)	Final(ml)		
Tea (Control)	10	0	9.2 10.2 10.5	9.9	Yellow with a faint pink at rim
Fermented Tea	10	0	8.9 9.0 8.9	8.9	

Table 6. Estimation of tannin: Filterate B: (Non - Tan Titre)

Sample	Vol. of Tea (ml)	Burette reading		Concordant value (ml)	End Point
		Initial (ml)	Final(ml)		
Tea (control)	25 ml	0	4.7 4.8 4.8	4.76	Yellow with a faintpink at rim
Fermented Tea	25 ml	0	4.6 4.6 4.7	4.6	

Control :
Total titre = (9.96-4)
Non- tan titre = (4.7-4.5)
Tannin titre = (9.96-4)-(4.76-4.5) =5.7 ml
Factor of converting 1ml of 0.008M KMnO4 in to gram tannin =0.001664g tannin.
i.e. 5.7ml of 0.008M KMnO4= 0.001664 x 5.76
= 0.009485g tannin/5gm of Black tea
= 9.5 mg tannin / 5gm of Black tea
Fermented tea:
Total titre = (8.9-4)
Non-tan titre = (4.6-4.5)
Tannin titre = (8.9-4)-(4.6-4.5)
=4.9-0.1
=4.8 ml.
Factor for converting 1ml of 0.008M KMnO4 tannin=0.001664g tannin.
1ml of 0.008M KMnO4= 0.001664g tannin
I.e. 4.8ml of 0.008M KMnO4 = 0.001664 X 4.8
= 0.007987g tannin.
= 7.9 mg tannin / 5g of Black tea.

and stroke.The present study shows that the Fermented Tea rather than the normal Tea has lots of beneficials like antibacterial property and decreased content of tannin which imparts the increased uptake of Tea it can be consume 4 cups/ day.. Uma devi *et al.,* (2004)estimated the tannin content of microbial fermented tea kombucha as 0.11±0.002 mg/L. Tabasum, *et al* (2001) analysed different Tea and compared for their tannin content. Supreme brooke bond tea has minimum (0.18%) quantity of tannin among the studied brand. So these brands are better as compared with other brands regarding the tannin content. Because tannin decrease food intake, growth rate, feed efficiency and protein digestibility. Therefore, foods which have high tannin content are harmful to health. Raymund yuen *(*2000) reported Kombucha drinkers all over the world have found great symptomatic relief from a wide range of disease such as Acne, Asthma, Arthritis, Chronic fatigue, Eczema, Lupus, Thyroid etc., Hedge (1995) , Dattatri (1994) reported that the consumption of this tea is popular in many countries including India mainly due to its refreshing power, low ethanol concentration and to speculative curative effects for many ailment including blood pressure, diabetics, increased lifespan, nervous weakness, digestive disorder etc.,

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