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Antibacterial Activity of Indigenous Fermented Rice Beverage of West Garo Hills, Meghalaya, India

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Abstract

Indigenous fermented beverage brewed from rice is an integral part of the rich tribal diet and culture in the region of West Garo Hills, Meghalaya. The beverage is fermented using locally available rice varieties and traditional rice starter cultures containing a mixed population of indigenous microbes (yeast, bacteria and moulds) and topical medicinal plants. The aim of the present study was to evaluate the antibacterial property of the traditionally fermented rice beverage with respect to five different medicinal plants used individually in the starter culture namely *Plumbago zeylanica, Thelypteris clarkei C.F. Reed*, *Clorodendrum D. Don*, *Leucas lavandulaefolia* and *Scoparia dulcis*. All the beverages exhibited good antibacterial property in agar well diffusion assay with the beverage brewed using *S. dulcis* and *L. lavandulaefolia* showing the highest inhibition zone of 39.50 ± 0.70mm against *B. cereus* Control beverage brewed from the starter without medicinal plants showed the least inhibition zones ranging from 8.75±0.21mm to 15.67±0.45mm. Hence the investigations testified a potential increase antibacterial property of the traditional rice beverage which can be attributed to the addition of medicinal plants thus plausibly contributing to its curative and health-promoting property claimed by the local tribal folks of West Garo Hills, Meghalaya.

Keywords: Fermented rice beverage, medicinal plants, antibacterial property, West Garo Hills.

Fermented beverages from cereals especially rice are very popular across the Asian countries namely China, Japan, Korea, Philippines, Vietnam and India in particular the central and north-eastern states of India. Across the various cultures around the world, they sustain a significant position spiritually (Dung, 2013) and are widely consumed during social and cultural events. Among the tribal communities, they are a part of offerings for a good harvest, traditional medicine and postnatal recovery (Chiang *et al*. 2006). The reason they are greatly relished and revered is because they possess high medicinal value and nutritional benefits besides having low alcohol content. The brewing process uses medicinal plants, herbs and spices containing natural antibacterial agents that apparently enhance the antimicrobial potency of the product (Kim *et al*. 2013). There have been several claims that the health-promoting effects of alcoholic rice beverages may be related to their antioxidant and anti-bacterial activity. Japanese rice wine *sake* has also been widely also known for its antimicrobial and anticancer properties. Similar to *sake*, the Korean rice wine has recently been reported to have beneficial health effects, with gastro-protective, antioxidant and antibacterial properties (Que *et al*. 2006). There are reports of the beverages fermented from rice being used as a drug and have been conceived to be effective against insomnia, headache, body ache, inflammation of body parts, diarrhoea, urinary problems, expelling worms and as a treatment for cholera (Deka and Sarma, 2010).

The present study on the rice beverages prepared by the different tribal communities of West Garo Hills, Meghalaya is the first of its kind in the region. The method of preparation of the fermented beverage from rice by the diverse groups of tribes is similar. The difference lies in the variety of rice and available medicinal plants used. Sticky red rice variety called *menil* is mostly preferred by the Garo tribes and is copiously available in all Garo populated regions. There are two important locally available medicinal plants that are used by the Garos in the preparation of the starter rice cake locally called *wanti*. They are leaves and flowers of *Plumbago zeylanica* and leaves of the fern *Thelypteris clarkei C.F. Reed.* The Boro and Hajong tribes mostly use the yellow boiled rice variety called *mirong* locally available in their inhabited regions. The plants used in their starter (*wanti)* include the leaves of the *Clorodendrum* species namely *Clorodendrum D. Don* and *Clerodendrum infortunatum L*, leaves or vernal stem of *Scoparia dulcis L.* (sweet broom) and *Leucas lavandulaefolia*. Although beneficial effects associated with medicinal plants and herbs have been widely studied, limited research has been conducted to determine the positive health effects associated with herbal ingredients when added to alcoholic beverages. Thus, the main objective of the present study was to evaluate the beverages with respect to its antibacterial property and understand the rationale that the curative properties of the beverages conceived by the local tribes could be due to the addition of medicinal plants. To establish the above claim, the antibacterial property of the traditional beverages brewed with and without medicinal plants added to the starter culture were assessed and compared.

MATERIALS AND METHODS

Sample collection, materials and reagents

Traditionally brewed rice beverages by the different tribes of West Garo Hills were collected in sterile culture bottles (200ml). Also rice beverage brewed with starter culture without any added medicinal plants was collected. All the beverage samples collected were refrigerated at 4°C in the laboratory for analysis. Nutrient agar and Mueller Hinton agar media were purchased from HiMedia laboratories, India. Ethanol was purchased from Merck Specialities Pvt., Ltd., India. All the materials and chemicals used in this work were of analytically pure grade.

Antibacterial assay

In vitro antibacterial activity of the rice beverages was carried out using the agar well diffusion method of Cheruiyot *et al*. (2009) with some modifications. Fermented rice beverages by and large contain alcohol in the rage of 7 to 15 %. Thus 15% ethanol was used as a positive control. The bacterial indicator strains used as test organisms included two gram positive bacteria (*Bacillus cereus and Staphylococcus aureus*) and two gram negative bacteria (*Salmonella typhi and Escherichia coli*). The antibacterial assay test was performed at the Department of Dairy Technology, Anand Agricultural University (AAU), Anand, Gujarat, India. The test pathogens are maintained by the department on nutrient agar slants (HiMedia, Mumbai) and stored at 4°C and in glycerol stocks. The strains were subcultured and cross checked for purity before use.

Agar well diffusion method

This method is based on the principle that the antibacterial constituents present in the rice beverages are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. If the test organisms are susceptible, it would result in formation of zones of inhibition that would be uniformly circular as there will be an affluent lawn of bacterial growth. The diameter of inhibition zones can be measured in millimetre (mm). Bacterial strains preserved in nutrient agar at 4°C were revived in nutrient broth and incubated at 37±1°C overnight, and the suspensions were checked to provide approximately 10^5 colony forming units (CFU)/ml. Active culture (100 µl) of each indicator strains were added to 100ml of nutrient agar (1.6 percent) and pour plated. The agar was allowed to solidify. The plates were refrigerated at 4°C for 10-15 minutes before several wells (6 mm diameter, 4 mm

deep and 2 cm apart) were punched out of the agar with sterile 6mm cork borer (HiMedia). 100 µl of filter sterilised rice beverages were filled into the wells and the plates were once again refrigerated at 5°C for 1-2 hours to facilitate the diffusion of supernatant after which they were incubated at 37°C for 24-48 hours. The inhibition activities of rice beverages on the test bacterial strains would be indicated by the presence of a clear zone surrounding the agar wells. In addition, minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of rice beverage samples were determined using the macro broth dilution method (Barchiesi *et al*. 1994). Bacterial cultures were prepared in the nutrient broth, incubated at 37°C for 24 h and were adjusted with sterilized saline to a concentration of 10^8 CFU/ml. The beverage samples were diluted to produce concentrations in the range from 10 to 100 percent. All mixtures were then incubated at 37 °C for 24 h to determine the MIC at which the growth of bacterial cells was fully inhibited. To determine MBC, 100µl of broth showing no visible growth was inoculated on Mueller Hinton agar (MHA) and incubated for 24 h at 37 °C. The MBC of beverage samples could be defined as the lowest concentration of the samples at which 99.9% of inoculated microorganisms were killed.

Statistical analysis

The experimental determinations were executed in triplicate. The data was recorded as Mean ± Standard deviation using Microsoft Excel Windows10. Comparison of means was performed by one-way ANOVA with post-hoc Scheffe's test and the differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Antibacterial activity of fermented rice beverages

The present study revealed that all rice beverages brewed with the medicinal plants showed good antibacterial activity against all the test organisms (Table 1). Plain rice beverage (RBP) showed minimal inhibition zones ranging from 8.57±0.21mm in case of *S. typhi* to 15.67±0.45 in case of *E. coli* and showed bactericidal effect only towards *E. coli* (MIC/MBC - 80/100) (Table 2). 15% ethanol positive control aseptically prepared showed the least inhibition zones ranging from 7.23±0.35mm in case of *S. typhi* to 12.13±0.55mm in case of *B. cereus*. The ethanol samples showed inhibitory effect in dilutions of 90% towards *B. cereus* and *E. coli* while undiluted samples showed an inhibitory effect towards *S. aureus* and *S. typhi* (Table 2). However, the ethanol samples did not show any bactericidal effect against any of the test strains. It is worthwhile to accredit the beverage brewed with *S. dulcis* and *L. lavandulaefolia* (RBSL) as it showed excellent inhibition zones in the range of 34.00±0.60mm (MIC/MBC - 50/80) against *E. coli* to 39.50±0.70mm (MIC/MBC-40/70) against *B. cereus*. It

Table 1: Antibacterial activates of traditional fermented rice beverages represented as inhibition zone diameter (mm)

Samples	*S. aureus*	*B. cereus*	*S. typhi*	*E. coli*
RBPZ	14.80 ± 0.35^{a}	26.87 ± 0.31^{b}	25.53 ± 0.31^{b}	30.33 ± 0.58^{b}
RBTC	34.00 ± 0.00^{b}	16.00 ± 0.20^{c}	15.40 ± 0.40^{c}	29.53 ± 1.36^{b}
RBCl	32.33 ± 0.5^{c}	14.80 ± 0.40^{dc}	8.47 ± 0.50^{d}	15.03 ± 1.08^{c}
RBSL	37.70 ± 0.61^{d}	39.50 ± 0.70^{a}	38.67 ± 0.38^{a}	34.00 ± 0.60^{a}
RBP	11.07 ± 0.60^{e}	14.00 ± 0.60^{d}	8.57 ± 0.21^{d}	15.67 ± 0.45^{c}
Ethanol (15%)	8.17 ± 0.35^{f}	12.13 ± 0.55^{e}	7.23 ± 0.35^{e}	11.93 ± 0.31^{d}
F value	2,346.52**	1,409.75**	3,454.09**	532.65**

The data are presented as the mean ± standard deviation (SD). Mean data with different superscript lower-case letters in the individual column are significantly different at p ≤0.05, according to Scheffe's test. The legends represent: RBSL, rice beverage brewed with S. dulcis and L. lavandulaefolia; RBPZ, rice beverage with P. zeylanica; RBCl, rice beverage with C.D Don; RBTC, rice beverage with T. clarkei C.F. Reed; RBP, plain rice beverage.

Table 2: Minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) of traditional fermented rice beverages

Samples	*S. aureus*		*B. cereus*		*S. typhi*		*E. coli*	
	MIC%	MBC%	MIC%	MBC%	MIC%	MBC%	MIC%	MBC%
RBPZ	80	100	60	90	70	80	50	80
RBTC	50	70	90	100	90	nd	60	80
RBCl	50	80	90	nd	90	nd	80	100
RBSL	40	70	40	70	40	70	50	80
RBP	90	nd	90	nd	90	nd	80	100
Ethanol (15%)	100	nd	90	nd	100	nd	90	nd

The legends represent: RBSL, rice beverage brewed with Scoparia dulcis and Leucas lavandulaefolia; RBPZ, rice beverage with Plumbago zeylanica; RBCl, rice beverage with Clorodendrum D. Don; RBTC, rice beverage with Thelypteris clarkei C.F. Reed; RBP, plain rice beverage; nd, not detected.

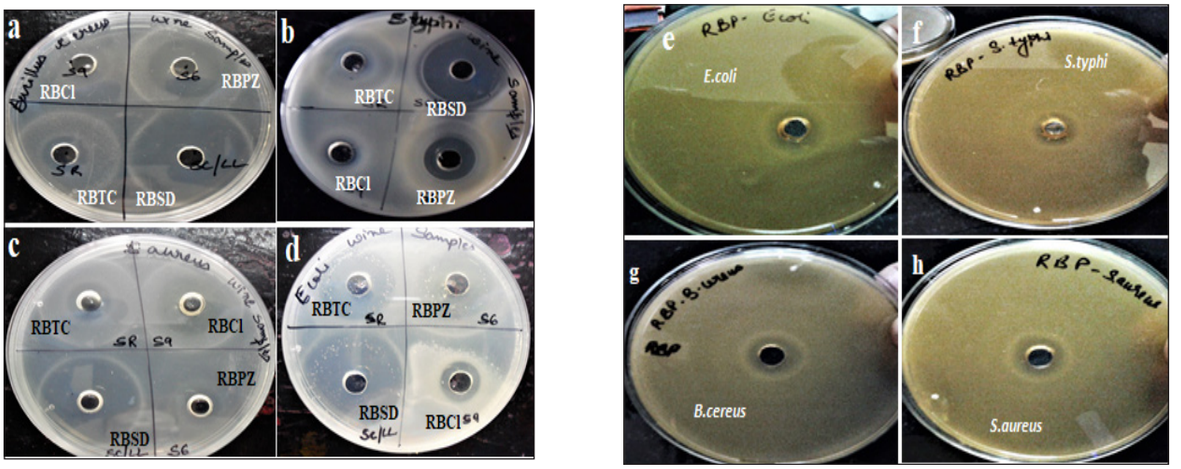

Fig. 1: a-d show the antibacterial activity of traditional rice beverage samples brewed with medicinal plants, **e-h** show the antibacterial activity of plain rice beverage

is worth mentioning here that all the different types of beverages tested showed a zone of inhibition higher than RBP and the positive control. They also exhibited bactericidal effect not detected in control samples. The F values in each case was found to be significantly very high (Table 1) which evidently depicts that the medicinal plants help to increase the antibacterial property of the rice beverage.

Very few literatures are available on antimicrobial assessments of fermented rice beverages. A study by Chang *et al.,* (2015) on the antimicrobial activities of commercial rice wine extracts of Taiwanese *Allium fistulosum* by using agar disc diffusion and tube dilution tests showed that all extracts exhibited antibacterial activities against *Bacillus subtilis and Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The antimicrobial property of ethanol in beer was studied by the prominent brewing microbiologist Shimwell (1935) who had noted that as low as 5% ethanol inhibits cell membrane functions, and inactivates bacteria by inducing cell membrane leakage leaving bacterial cells unable to maintain pH homeostasis. As a result the morphology and a range of cell functions may be affected. Jana *et al*. (2004) studied the antibacterial activity of *Handia* against five pathogenic bacterial

strains viz. *Vibrio cholerae, Escherichia coli, Micrococcus luteus, Staphylococcus faecalis, Staphylococcus aureus* to establish its ethnomedicinal property and found that the beverage showed average results against all test organisms which they conclude may be due to the presence of bacteriocin producing bacteria in it. Varma in 2016 had reported that fermentation facilitates the extraction of the active constituents contained in the plant material and reduces the toxicity of some of the toxic compounds present in plants. Mulay and Khale (2011) observed that fermentation ruptures the cells of herbs actively and that the bacterial enzymes breakdown the cell walls of herbal materials to further enhance the leaching process. Thus fermentation is known to enhance antibacterial activities of some plant product by releasing functional ingredients and enhancing various bioactive properties of the herbal and medicinal plants used (Wen *et al*. 2013).

Some scientific publications have reported on the antibacterial and other therapeutic properties of the medicinal plants used the starter culture *wanti*. A review by Makhija *et al*. (2011) gives accounts of various *in vivo* studies carried on *L. lavandulaefolia* that report the plant to possess hepatoprotectve, hypoglycemic, antipyretic, antidiarrheal, antitussive, wound-healing, psychopharmacological and antimicrobial properties. Likewise Pamunuwa *et al*. (2016) discussed the anti-diabetic, antioxidant, anti-inflammatory, analgesic, antimalarial, hepatoprotective, sedative, hypnotic, antiulcer, anti-sickling, and antimicrobial properties of S. *dulcis* and concluded that the plant could be used as an alternative and complementary to therapy for diabetes. Abera *et al*. (2015) investigated antimicrobial activities of crude extracts of *Plumbago zeylanica* and showed that the plant possesses significant antimicrobial activity against *S. aureus*, *S. pneumoniae*, *K. pneumoniae*, *S. boydii*, *E. coli* and antifungal activity against *C. albicans* and suggested that *P. zeylanica* plant may be used effectively as preventive agent in the pathogenesis of some diseases. Wang *et al*. (2017) in their review highlighted the phytoconstituents isolated and reported from the genus *Clerodendrum* anddiscussed the pharmacological activities (anti-inflammatory and anti-nociceptive, antioxidant, antihypertensive, anticancer, antimicrobial, antidiarrheal, hepatoprotective, hypoglycemic and hypolipidemic, memory enhancing and neuroprotective) of these compounds. Studies on antimicrobial activity of *Thelypteris* genus are still naive, hitherto in the present study, the beverage brewed with *T. clarkei C.F. Reed* showed good inhibition against *S. aureus* (In. z - 34.00 mm, MIC/MBC – 50/80) suggesting potential antimicrobial property possessed by the fern. Thus, we can adjudicate that the fermented rice beverages assessed in our study possess antimicrobial properties primarily due to addition of medicinal plants in the starter culture and also probably due to other components such as secondary metabolites produced by the fermenting yeast , fungi and bacteriocins produced by lactic acid bacteria present in the beverage samples.

CONCLUSION

The present study on the indigenous fermented rice beverages brewed in the region of West Garo Hills endorses the conception that the medicinal plants added in the starter rice cake (*wanti)* aid to increase the antimicrobial potential of the rice beverage. It has been noted that that the beverage brewed with *S. dulcis & L. lavandulaefolia* showed excellent antibacterial activity against all the test pathogens used in the study with the highest antibacterial activity against *B. cereus* (diameter of inhibition zone - 39.50±0.70mm). Hence it can be considered as a good source of natural potent antibacterial constituents. Nevertheless, all the medicinal plants used in the starter culture *wanti* exhibited good inhibition towards the test organisms used. Hence, it and could be ascertained that these plants emphatically contributed to increase the antibacterial property of the rice beverage thus plausibly conferring to its curative and health-promoting value claimed by the humble tribes of West Garo Hills, Meghalaya.

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