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Preparation, physicochemical analysis and antioxidant properties of *Rohi* and *Sepa*: traditional rice beers of the Sonowal Kacharis, Assam

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Sonowal Kacharis are a major tribal community of Assam. Their literacy rate is highest amongst the tribal communities in Assam. Their traditional alcoholic beverage, *Rohi* and *Sepa* are prepared from rice using a traditional fermentation process. The fermentation process is assisted by use of a starter culture, *Mod Pitha* that provides the necessary fermenting agents. Preparation of *mod pitha* involves use of rice and a good number of medicinal plants. *Rohi* and *Sepa* are integral parts of the life of Sonowal Kacharis, not only consumed regularly but also considered essential in all their social functions. Here in this report, we are reporting the preparation, physicochemical parameters and antioxidant properties of *Rohi* and *Sepa*.

Keywords: Mod pitha, Rice beer, Rohi, Sepa, Sonowal Kachari

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Sonowal Kachari is an indigenous tribe of Assam. They reside mainly in Dibrugarh, Lakhimpur, Dhemaji, Tinsukia, Jorhat and Golaghat districts of Assam but Dibrugarh district has the sizeable population. Their traditional brew, which is prepared by using their unique starter culture, plays an important role in their socio-cultural life. It is offered to deity in religious festivals, served to guests and invitees in social gatherings like marriage festivals, birth and death rituals, etc. Four densely populated Sonowal Kachari villages, namely Chaharikota village, Teporguri village, Bhoralibari village, Meslou Village- all located 25-35 km away from the Dibrugarh University campus, were visited during the period from June-2020 to March-2021 and various information collected from aged and experienced villagers. Traditionally, women folk of the family look after the whole preparation process of their brew. They use their brew for their family consumption, and some families sell it in local markets for a livelihood. Investigation on *Rohi* and *Sepa* will provide information that may help this community in deriving logical conclusions about their consumption pattern, health benefits or otherwise Greater emphasis has been given to put *Rohi* and *Sepa* in the international map of traditional alcoholic rice bears such as Japanese Sake.

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Materials and Methods

Preparation of the starter cake

The fermentation process for the preparation of *Rohi* or *Sepa* is kicked off by adding a home-made fermenting agent called the starter cake or the fermentation cake. It is known by different names in Assamese such as *mod pitha* or *saoul pitha* or *vekur pitha* or *Bakhar*. It is a collective source of fermenting enzymes, *Saccharomyces cerevisiae*. For the preparation of *mod pitha*, plant parts shown in (Fig. 1a-d) are washed and grinded with pre-soaked rice grains in a wooden mortar and pestle (details of the plants are listed in Table 1). The mixture is converted into a paste by adding requisite quantity of water, and rolled into small spheres. The spheres are covered with leaves of *Cyclosorus dentatus* (*Saldaokunwi* in local dialect, Fig. 1e) and left in a dark room for drying for about two weeks. After 2 weeks, these small spheres or small cakes are wrapped in leaves of *Casearia aculeate* (*Khorkhoria pat* in native language), put into a polythene bag and hung from the roof of the house (Fig. 1f) until used.

Preparation of rice beers

Sonowal Kacharis prepare two types of beer *i.e.*, *Rohi* and *Sepa* (Fig. 2a). *Rohi* (yellowish in colour) is considered the standard (superior) class of rice beer,

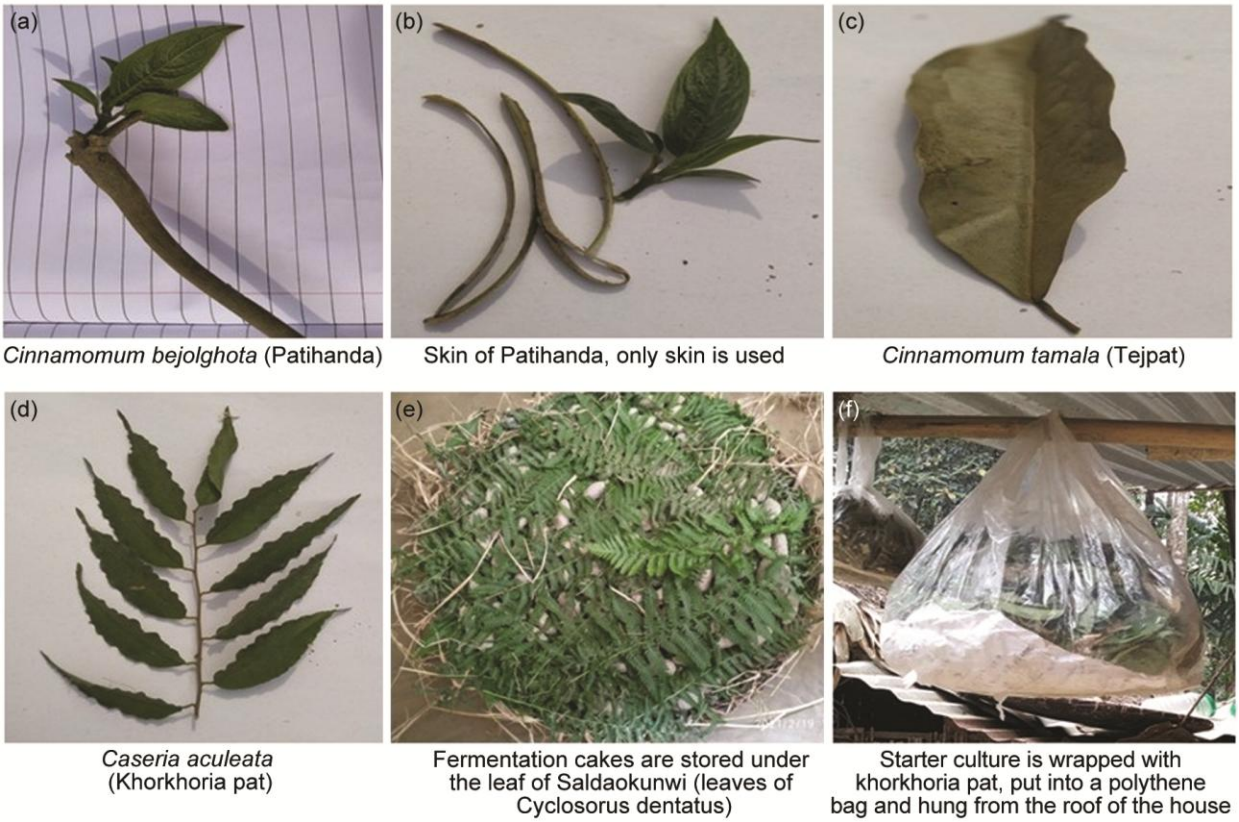

Fig. 1 — Plant parts used by Sonowal Kacharis for the preparation of fermentation cake

Table 1 — Plant parts used in the preparation of the starter culture

Local name of plants	Scientific Name[18,19]	Family[18,19]	Plant parts used	Medicinal properties[18-20]
1. Dhan	*Oryza sativa*	Poaceae	Rice grain	Rice-wash water used in diarrhea and dysentery.
2. Khorkhoria pat	*Casearia aculeata*	Salicaceae	Leaves	Used in diabetes, eye infection, jaundice, liver disease etc.
3. Patihanda	*Cinnamomum bejolghota*	Lauraceae	Skins	Used to cure stomach disorders, treatment of cough.
4.Saldaokunwi	*Cyclosorus dentatus*	Polypodiaceae	Leaves	Rhizome used as antibacterial agent.
5. Tezpat	*Cinnamomum tamala*	Lauraceae	Leaves	Leaves useful in gonorrhea, diarrhea rheumatism.

and *Sepa* (whitish in colour) is considered the regular (inferior) class of rice beer. To prepare *Rohi* and *Sepa*, half-cooked rice (called *Aaroi* in their dialect) is cooled down to ambient temperature, spread over a mat made of bamboo and mixed uniformly with powdered *mod pitha*. The mixture is then put into an aluminium pot (called *Handi*), water is added, sealed with banana leaves (Fig. 2b) and the whole container is left undisturbed for a few days, preferably in a dark corner inside the house. The time duration required for the rice beers to get ready depends on the weather conditions. Usually in summer, 4-5 days are necessary, but in winter, it may be 8-10 days to achieve the expected level of fermentation. After this requisite time, the fermented mass is filtered through a sieving arrangement made of a bamboo-made basket (called *Khorahi*) and perforated banana leaves (to function as the filter papers, Fig. 2c). The filtered liquor, which comes out of the fermented mass, appears yellowish in colour and contains both soluble and insoluble starchy ingredients. This is *Rohi* which can be stored in an earthen pitcher (called *Koloh*) for about a year (Fig. 2d). After the separation of *Rohi*, the residual fermented mass from the *Khorahi* is transferred into another container, water added and thoroughly mixed. The mixture is packed into a piece of clean cotton cloth and pressed hard to yield a kind of whitish liquor which is called *Sepa*. *Sepa* as an alcoholic drink is considered inferior to *Rohi*.

Sonowal Kacharis use two types of rice to prepare *Rohi* and *Sepa*, *i.e.*, general rice (*Baismuthi* rice in Assamese language) and sticky rice (*Bora* rice in

Fig. 2 — Accessories used in different steps in the preparation of rice beer

Assamese language). For special functions like wedding ceremony and other local celebrations, they use only sticky rice for the preparation of rice beer. According to them, rice beer from sticky rice tastes better than that from general rice.

Laboratory validation of traditional method: Preparation of traditional alcoholic beverage in the laboratory

For commercialization of the beverage, laboratory fermentation technique is considered important. To validate the traditional method in laboratory, 1 kg of sticky rice grain was washed with distilled water. The rice was cooked in an aluminium *Handi*, spread over a bamboo mat, covered with banana leaf and allowed to cool down to ambient temperature for 2 days. After 2 days, it was transferred to a large plastic bucket and mixed with grinded *mod pitha*, the fermenting agent (approximately 10 g procured from Sonowal Kacharis). Half a litre of distilled water was added to the bucket; its mouth was covered with banana leaves and kept undisturbed at room temperature for 4 days. After 4 days, *Rohi* and *Sepa* were collected following the same procedure as described above. Physicochemical analyses were then followed. Laboratory validation was strengthened through the use of internationally accepted analytical methods and recent literature on fermented beverages, bioactive constituents and traditional alcoholic drinks.

Experimental methods

All the parameters were estimated with reported procedures. The physiochemical analyses like ethanol content[1], bitterness[2,3], carbohydrate content[4], protein content[4], colour[5], pH[6], total solid content[7], turbidity[8,9] were determined by using standard methods. It can be noted that the colorimetric method was used to determine alcohol contents[1], Phenol-Sulfuric acid method was used to determine carbohydrate content[4], and Lowry's method was used for protein content (this method is recommended for protein estimation in low concentration)[4]. Antioxidant and phenolic properties of rice beer were determined as per reported method[10]. The analytical procedures used in this study were chosen based on well-established and internationally accepted methods reported in recent studies on fermented beverages, bioactive constituents and traditional alcoholic drinks[11,12]. Standardized and validated protocols were followed wherever applicable to ensure the accuracy, reproducibility and comparability of the findings with current food science research[11,12].

Experimental data and result analysis

In (Table 2), experimental data for physicochemical parameters of both homebrewed and lab brewed *Rohi* and *Sepa* are reported. Physicochemical parameters like carbohydrate content, ethanol content, protein content and pH of homebrewed and lab brewed *Rohi* and *Sepa* are compared in the bar diagram shown in (Fig. 3). In home-brewed and lab brewed samples, carbohydrate content is lower in *Rohi* (respectively 9.94 mg GLE/mL and 7.67 mg GLE/mL) as compared to that in *Sepa* (respectively 12.78 mg GLE/mL and 8.08 mg GLE/mL). But ethanol content (6.14v/v%) and pH (3.57) are both higher in home-brewed *Rohi* as compared to the corresponding values in home-brewed *Sepa* (respectively 5.36v/v% and 2.92). Similar to home-brewed beverages, for lab-brewed beverages also, % of ethanol content and pH both are higher for *Rohi* as compared to *Sepa*. Although the difference is not very significant, protein content of home-brewed *Rohi* (6.52 TSPC mg/mL) is higher as compared to that in *Sepa* (6.45 TSPC mg/mL), but protein contents of laboratory brewed samples are almost same. Higher alcohol content in *Rohi* is considered one of the contributing factors that make traditional *Rohi* superior to *Sepa*. Alcohol contents of both *Rohi* and *Sepa* are higher in laboratory brewed samples as compared to the corresponding values in the home-brewed samples. The unfermented starch molecules account for the total carbohydrate content and solid content. It is observed that for homebrewed *Rohi*, ethanol content (6.14v/v%) is higher than that for homebrewed *Sepa* (5.36v/v%). The carbohydrate content in *Rohi* (9.94 GLE mg/mL) is consequently lower than that of homebrewed *Sepa* (12.78 GLE mg/mL). Similar trend is also observed for total solid content. Bitterness level is almost same in both the types of beers. All the beer samples are turbid, and the turbidity originates mainly due to the presence of unfermented carbohydrates.

Study of antioxidant activity

The starter culture, which is mainly responsible for fermentation, has powerful antioxidant properties as it is prepared by mixing grinded rice with medicinal plants. So, Sonowal Kachari people demand that rice beer possesses antioxidant properties too. In this article, our main aim is to study the antioxidant properties and polyphenol and flavonoid content of *Rohi* which are not reported earlier. As *Rohi* from sticky rice is the main traditional alcoholic beverage of this community, so detailed analysis of *Rohi* is discussed.

Table 2 — Physiochemical analyses of traditional rice beers from sticky rice

Parameters	Homebrewed beverages		Lab-brewed beverages	
	Rohi	*Sepa*	*Rohi*	*Sepa*
Bitterness (EBU)	5.08±0.21	5.06±0.23	5.02±0.21	5.23±0.23
Carbohydrate content (GLE, mg/mL)	9.94±0.35	12.78±0.52	7.67±0.35	8.08±0.52
Colour (EBC)	Creamy Yellow	White	Creamy Yellow (light)	Whitish (light)
Ethanol content (v/v)%	6.14±0.28	5.36±0.22	7.04±0.28	6.72±0.22
pH	3.57±0.05	2.92±0.12	4.08±0.05	3.84±0.12
Protein content (TSPC, mg/mL)	6.52±0.51	6.45±0.48	4.07±0.51	4.05±0.48
Total Solid content (mg/mL)	18.65±0.74	20.54±0.72	14.65±0.74	16.32±0.72
Turbidity	Turbid			

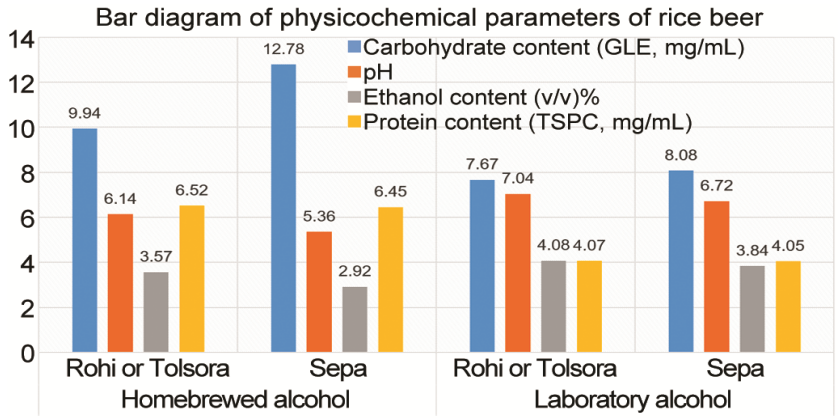

Fig. 3 — Bar diagram of physicochemical parameters of Sonowal Kachari's rice beer

Our body cells produce free radicals as waste substances in metabolic processes, which do harm body functions[13]. And therefore, we need to introduce antioxidants to our body to counter these free radicals[13]. So, foods and alcoholic beverages having antioxidant properties and rich in phenolic compounds are drawing attention of researchers. Antioxidants can remove reactive oxygen species (ROS) such as peroxides, superoxides, hydroxyl free radicals, singlet oxygen etc[13,14]. Thus, antioxidants help to reduce stress related diseases. Similarly, phenolic compounds have antispasmodic, antiviral, estrogenic, antidiarrhoeic, antibacterial, anti-inflammatory, immune stimulating properties[15,16]. Antioxidants scavenge reactive oxygen species from causing oxidative damage[15,16]. Many serious diseases like cancer, liver disease, ageing, Parkinson disease, diabetes, hypertension, heart diseases are caused because of oxidative damage[15,16]. Both natural and artificial antioxidants are available. Polyphenols are available in micronutrients, plants, fruits, vegetables, teas, and spices. There are about 8000 different types of polyphenols. Polyphenols are powerful antioxidants. They destroy the free radicals which are produced in our body during metabolic activity[15,16]. Excessive formation of free radicals causes oxidative stress[15,16]. Flavonoids like quercetin and catechin are available in fruits[17]. Food stuff richin flavonoids, polyphenols etc. are strong antioxidants, consequently rice beer demonstrates antioxidant potential due to the presence of these phenolic constituents[18]. Here in this article, we are reporting the presence of polyphenol and flavonoid content in rice beer and it is listed in (Table 3).

Statistical analysis

All determinations were done in triplicate. Values are expressed as mean ± standard deviation (n=3) and significant at $p < 0.05$.

Results

Rohi prepared from sticky rice is the most popular drink among the community, so detailed investigation was done for *Rohi*. From the results listed in (Table 4), rice beer *Rohi* has higher antioxidant activity as compared to *Sepa*. Detailed investigation was done for total polyphenol and flavonoid contents in *Rohi* as antioxidant activity depends upon both these parameters. We have prepared *Rohi* in the laboratory and its antioxidant property was found less as compared to *Sepa*. Home-brewed *Rohi* had higher % of RSE (27.86) as compared to *Sepa* (23.45) and lab-brewed Rohi (22.78). It means home-brewed *Rohi* provides essential nutrients like polyphenol, flavonoids etc. This variation between home-brewed and lab-brewed *Rohi* may happen due to preparation method, temperature and weather conditions. We have calculated TAA, TPPC and TFC for home-brewed *Rohi*, *Sepa* and Lab-brewed *Rohi*.

Table 3 — TAA, TPPC, TFC in homebrewed and lab brewed Rohi with different storage periods

Sl No.	Storage period	Home-brewed Rohi			Lab-brewed Rohi		
		TAA (ASAE, mg/mL)	TPPC (GAE, mg/mL)	TFC (QAE, mg/mL)	TAA (ASAE, mg/mL)	TPPC (GAE, mg/mL)	TFC (QAE, mg/mL)
1	2 months	0.53±0.02	2.17±0.08	5.12±0.19	0.29±0.02	1.13±0.03	3.01±0.13
2	4 months	0.58±0.01	2.84±0.05	5.76±0.20	0.31±0.02	1.19±0.02	3.09±0.14
3	6 months	0.64±0.03	3.20±0.05	6.32±0.23	0.35±0.03	1.23±0.05	3.13±0.10
4	8 months	0.69±0.02	3.33±0.06	6.84±0.24	0.38±0.02	1.28±0.04	3.16±0.09
5	10 months	0.72±0.03	3.54±0.09	7.02±0.26	0.39±0.02	1.29±0.04	3.17±0.05
6	12 months	0.75±0.03	3.59±0.10	7.05±0.23	0.40±0.01	1.29±0.03	3.18±0.13

- TAA: Total Antioxidant Activity in terms of Ascorbic Acid Equivalent (mg/mL)
- TPPC: Total Polyphenol Content (Gallic Acid Equivalent, mg/mL)
- TFC: Total Flavonoid Content (Quercetin Acid Equivalent, mg/mL)
- RSE: Radical Scavenging Effect

Table 4 — Antioxidant activity and phenolic contents of rice beer prepared from sticky rice

Name of the Tribe	Fresh beer samples	% RSE	TAA (ASAE, mg/mL)	TPPC (GAE, mg/mL)	TFC (QAE, mg/mL)
Sonowal Kachari	Rohi (Home-brewed)	27.86±1.13	0.46±0.01	1.63±0.06	4.04±0.15
	Sepa (Home-brewed)	23.45±1.14	0.38±0.02	1.24±0.04	3.92±0.14
	Rohi (Lab-brewed)	22.78±1.13	0.24±0.01	1.04±0.04	2.89±0.12

It is found that TAA, TPPC and TFC in home-brewed *Rohi* have higher values (respectively, 0.46 mg/mL ASAE, 1.63 mg/mL GAE, 4.04 QAE mg/mL) as compared to other beverages listed in (Table 4). Storing of alcoholic beverages is a common practice among Sonowal Kacharis, and as believed by them, as the storing period increases medicinal value does increase. They store rice beer specially *Rohi* for up to 11-12 months. But *Sepa* cannot be stored for longer. It contains higher level of carbohydrates. As per our information, if *Sepa* is stored for a greater number of days, its sweat taste becomes tangy due to bacterial contamination. In our investigation, for up to 12 months antioxidant properties of *Rohi* are estimated as per reported procedures. Freshly prepared home-brewed *Rohi* and lab-brewed *Rohi* were stored for up to 12 months and % RSE and TAA were estimated at an interval of 2 months. Initially % of RSE of homebrewed *Rohi* was 27.86 thereafter it increased continuously for up to 12 months. It needs to mention that % RSE increased sharply for up to 10 months, but after 10 months the values became steady. At 12 months % RSE is 44.98 whereas at 10 months of storage it is 44.95. It is observed that % RSE is increased by almost 3% in two months interval. But for lab-brewed *Rohi* % of RSE rise becomes steady as compared to homebrewed beverage. Initially % of RSE for lab brewed *Rohi* was 22.78 whereas after 12 months, it became 30.86 and this value is almost 14% less than that of home-brewed *Rohi*. In (Table 5), % RSE determination with 2 months interval have been shown for home-brewed and lab-brewed *Rohi*, and all these data have signified that up to 10 months, values are rising continuously but after 10 months it became steady. And at the same time, it can be mentioned that small microbes are born after 10 months in all the beer samples which is clearly visible from outside of transparent rice beer containers. It can be assumed that, as larger number of molecules are fermented in longer storage period, ethanol content increases and simultaneously radical scavenging effect and total antioxidant activity also increase. But ethanol content shows no direct relationship with antioxidant activity. % of ethanol is higher in lab-brewed beverages but they have poorer antioxidant activity. In (Fig. 4), plots are drawn to check the correlation between antioxidant activity *vs* polyphenol content and antioxidant activity *vs* flavonoid content for both home-brewed and lab-brewed beverages. Strong correlation constants (R^2 =0.9246 and 0.9675) for both the plots (Fig. 4a-b) signify that phenolic compounds are the main contributors towards antioxidant activity. Significant correlation between flavonoid content and antioxidant activity is also observed (R^2=0.9721 and 0.924).

Table 5 — Percentage of radial scavenging effect of Rohi with different storage period

Serial number	Storage period of Rice beer	%RSE *Rohi*	
		Home-brewed	Lab-brewed
1	2 months	31.05±1.24	25.78±1.12
2	4 months	34.86±1.31	27.65±1.15
3	6 months	37.95±1.34	28.46±1.18
4	8 months	41.09±1.42	29.34±1.19
5	10 months	44.95±1.44	30.85±1.21
6	12 months	44.98±1.28	30.86±1.22

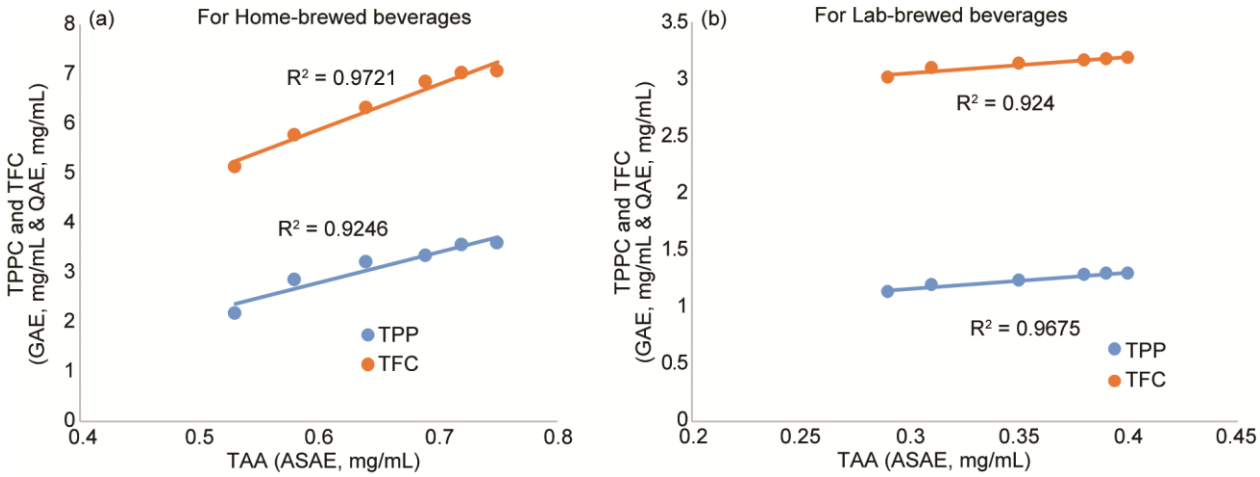

Fig. 4 — (a) Corelation between total polyphenol and flavonoid content versus antioxidant activity for home-brewed rice beers, (b) Corelation between total polyphenol and flavonoid content versus antioxidant activity for lab-brewed rice beers

Conclusion

Rice beer is an integral part of Sonowal Kacharis' food habits and it plays an indispensable role in their life style. From marriage ceremony to religious rituals, the role of rice beer is significant. Sonowal Kacharis strongly believe that rice beer is good for health and good for mental relaxation. From our study also, *Rohi* is found to have nutritional and therapeutic properties. There is further scope to study other parameters related to nutritional values. Comparison of *Rohi* with commercial alcohols may also be considered. *Rohi* may be evaluated alongside globally recognized traditional alcoholic beverages by comparing their substrates, fermentation practices, alcohol levels, physicochemical characteristics and bioactive constituents. Such a comparative perspective can establish *Rohi* within the international framework of traditional fermented beverages while emphasizing its unique scientific and cultural significance.

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Author Contributions

BB, the first author did field study, conducted the experiments, recorded and analysed the data, wrote the complete manuscript and it was checked by the second author. Both the authors read and approved the final version of the manuscript.

Conflict of Interest

Authors declare no conflict of interest.

Ethics Approval

Not applicable.

Prior Informed Consent

All participants were given detailed information about the aim, procedures, potential advantages and voluntary nature of the involvement. Authors declare that prior informed consent was obtained from all the participants including the individuals whose images are presented in this study.

Use of Artificial Intelligence (AI)

Author did not use any AI tool for data interpretation and development of original manuscript.

Data Availability

The dataset generated during this study is available from the corresponding author upon reasonable request. It is not publicly accessible due to privacy and ethical considerations involving human participants.

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