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Method of preparation and biochemical analysis of local tribal wine *Judima:* an indegenous alcohol used by Dimasa tribe of North Cachhar Hills District of Assam, India

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Abstract

In North Cachar Hills district of Assam, Dimasa, a type of tribal people prepared their indigenous rice beer called *Judima*. The fermentation of *Judima* is usually carried out in earthen/aluminum pots at room temperature and takes about 4-5 days for completion of the fermentation. The authors visited some of the rural areas where judima is prepared and the process of preparation was observed and documented. The Carbohydrate, protein, free amino acids and alcohol percentage of the particular tribal wine was estimated to evaluate their nutritional status. Antioxidant activity of *Judima* along with different plants used for the preparation of *Judima* was also studied. A short term cytotoxicity study on peripheral blood mononuclear cells (PBMC) was carried out to determine its cytotoxic effect on the cells keeping ethanol (50% and 100%) as a standard. The result of present study showed that *Judima* contains good amounts of protein, carbohydrate and free amino acids with high antioxidant activity.

Introduction

The preparation and consumption of traditionally prepared rice beer (wine) from different varieties of rice ingredients with plants, fruits, and flowers is a common practice among many tribal communities residing in the North-Eastern (N.E.) states of India and many of them have been preparing it since time immemorial. In addition each of the tribes also prepares their own unique starter cultures to carry out fermentation, and each type is a mixture of different parts of various plants (Das *et al*., 2012). The household liquor is associated with the regions rich indigenous knowledge system (Achaya, 1991). The traditional knowledge of wine making practice in N.E. region has been transferred from one generation to another through ages (Tanti *et al*., 2010; Das *et al.*, 2012). This suggests a sense of common ownership amongst different communities. The method for beverage and wine production amongst tribes differ to quite an extent and employing different starter cultures, although they use more or less similar substrates for fermentation (Das *et al.*, 2012). It also plays an important role in the socio-cultural life of the tribal people as it is found to be associated with many occasions like merry making, ritual ceremonies, festivals, marriages and even death ceremonies (Das *et al.*, 2012). Fermentation is the natural process in which carbohydrates are oxidised to alcohol and other compounds by anaerobic microbes. It is a biological oxidation process employed by certain microorganisms for their energy requirements (Kuwaki *et al*., 2012). Man has exploited micro-organism since time immemorial for the production of various types of foods and beverages and for making bread. The micro-organisms are the chief sources in connecting a particular food substance in fermented forms. Alcoholic beverages were produced individually in many countries even in 19th Century (Achaya, 1991).

Judima is the most popular homemade alcohol beverages of Dimasa Tribes of N.E. India (Das *et al*., 2012). The Dimasa are one of the earliest indigenous ethnic groups of India, they are mostly found in the North Cachar Hills of Assam and Dimapur in Nagaland (Das *et al.*, 2012). Present study was carried out in Dimasa residing North Cachar Hills (N.C. Hills) district of Assam, India. *Judima* is prepared by fermentation of worked rice mixed with plants leaves/ barks containing different phytochemicals broadly described as phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, polyphenols, flavonoids, isoflavonoids and anthocyanidins. They have tremendous impact on the health care system and may provide medical health benefits including the prevention and/ or treatment of diseases and physiological disorders. These phytochemicals, either alone and/or in combination, have tremendous therapeutic potential in curing various ailments (Prakash *et al.*, 2012).

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The starter material for the fermentation is a home made starch. Yeast culture maintained in a semi sterilized medium made of ground rice (most gluten) mixed with a number of plant parts are reported to carry medicinal properties (Das *et al.*, 2012). The fermentation temperature during indigenous process of wine making is generally maintained at around 30°C. Since the entire practice of beverage preparation has been traditional and consumption of the fermented forth is a crude product, it was therefore felt that the evaluation of the biochemical status of the consumed product is definitely inevitable. The present investigation was undertaken in an attempt to evaluate the biochemical and nutritional status of *Judima* after successive fermentation of alcoholic beverages. At the same time phytochemical analysis of plants used in *Judima* preparation and *Judima* itself was also undertaken. Cytotoxicity study of *Judima* on the PBMC cells was also evaluated to determine its toxic effect on the host if any, considering ethanol (50% and 100%) as standard.

Materials and Methods

Field survey

The field survey was performed by Dr. Jashodeb Arjun using semi-structure questionnaires, personal visits were made to homes, working place and wine selling markets. A face to face interview was performed in local language about the methods of preparation and preservation of *Judima.* The questionnaire consisted of information about the methods of preparation and preservation, market potential, taste, unwanted effect(s) and cultural significance of *Judima.* During field survey total 100 participants were interviewed, which included 79 females and 21 males. The age groups of participating persons was 30-60 years and all participants were given detailed information about the main aim/ objectives of the project, and a prior consent form was filled and duly signed before interviews. Some photographs collected during field survey are shown in Figure 1. This documentation will fully recognize the contribution of the local people on indigenous knowledge, protection of community biodiversity and intellectual property rights.

Preparation of Judima

The whole process for preparation of *Judima* is completed in two stages, (a) making of rice cakes and preparation of rice beer. The details about the methods of preparations are given below.

Preparation of rice cakes: The starter cake for preparing *Judima* is called as umhu or humao, it is a

Figure 1. Traditional method for preparation of *Judima.* (a) rice cake, (b) rice cake and plant bark of *Acacia pennata*, (c) mixture of cooked rice with rice cakes, (d) fermentation and filtration and (e) ripe *Judima* ready to drink.

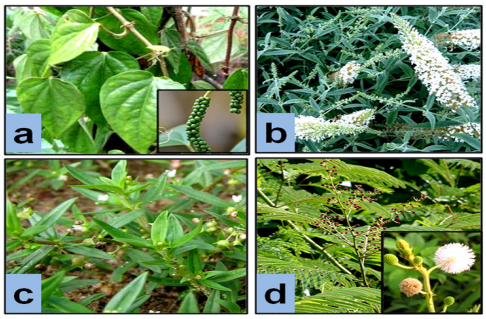

Figure 2. Photographs showing some selective plants used for the preparation of *Judima.* (a) *Piper betle*, (b) *Buddleja asiatica*, (c) *Hedyotis scandens* and (d) *Acassia pennata.*

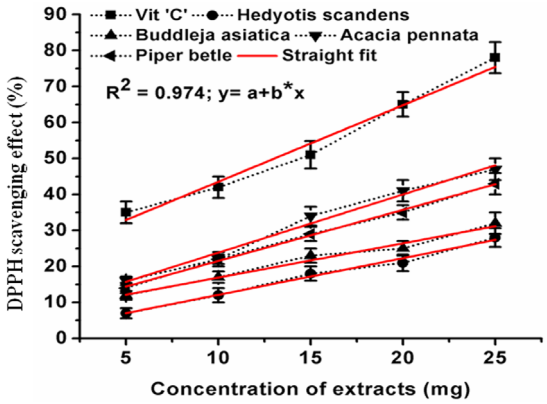

Figure 3. Flow chart for the preparation of rice cake (*umhu/humao*)

mixture of rice and bark of thempra (*Acacia pennata*) plant, for variation in test and flavor they use to mix leaves powder of *Piper betle* (leaves), *Buddleja asiatica* (leaves/twigs) and *Hedyotis scandens* (leave/ twigs) (Figure 2). The barks of *Acacia pennata* are cut into small pieces and dried in the sun. Glutinous rice (*Oryza glutinosa* Lour) is soaked in water until it is softened. It is then grinded in a wooden or metallic mortar and pestle called rimin along with the barks of thempra plant. A little water is added in order to make a paste; it is then made into cakes of appropriate sizes (radius: 5-7 cm; weght: 80-100 g). The old rice cake (15-20 g) was then powdered and sprinkled over the new rice cake. New rice cake was then kept for at

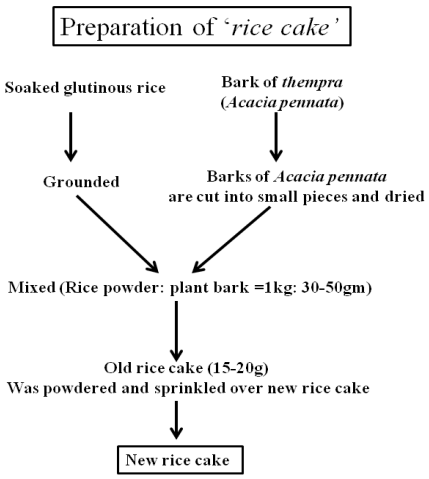

Figure 4. Flow chart for the preparation of rice beer (*Judima*)

least 24 hours, and then it is sun dried till it becomes hard and it can be stored for many months (Figure 3).

Preparation of rice beer: For preparing *Judima*, rice is boiled and allowed to cool. It is mixed with powdered humao one large sized humao is sufficient for 5 Kg of rice and kept in a large container which is covered with jute gunny bags. After 5-7 days, slightly yellowish juices come out from the fermented mass which indicates the completion of fermentation process. This can further be diluted with water and filtered for consumption. (Figure 4).

Estimation of alcohol

The decanted product of *Judima* was centrifuged at 5000 rpm for 25 minutes at 4°C and supernatant of which was used for estimation of alcohol. The ethanol content was estimated by chromic acid method (Williams and Darwin, 1950). A standard curve of 1% ethanol was used for determination of ethanol present in the sample. In brief 1.0 ml properly distilled sample and 4.0 ml chromic acid was incubated at boiling water for 10 minutes, cooled at room temperature and optical density (O.D.) was read at 600 nm (UV visible spectrophotometer, Beckman, Model DU 640) using chromic acid plus distilled water as blank.

Estimation of turbidity, carbohydrate and protein

The turbidity of the decanted product was determined against distilled water at 640 nm by UV visible spectrophotometer (Beckman, Model DU 640). The total carbohydrate of the decanted product was estimated by Anthrone reagent method by Morris (1948) using 1.0 mg/ml glucose solution as standard. The O.D. of reaction mixture was taken at 550 nm against reaction mixture as blank in which glucose solution was replaced by distilled water. The total protein content was determined by Folin-phenol method (Lowry *et al.*, 1951) and free amino acid estimation was carried out according to the method mentioned by Lee *et al.* (2003).

Collection of plants used for the preparation of Judima

Fresh parts of four plants, *Piper betle* (leaves), *Buddleja asiatica* (leaves), *Hedyotis scandens* (leaves) and *Acacia pennata* (leaves) (Figure 2) used for the preparation of fermentation starter cultures was collected from different locations of N.C. Hills district of Assam. The plant materials were taxonomically identified and authenticated by Dr. J. Arjun, Lumding College, Lumding and herbarium bearing voucher specimen no. JA-1, JA-2, JA-3 and JA-4 were deposited in the department of Zoology, Lumding College. Plant leaves (50 g) was dried from each plant in an oven (38-40°C), triturated and subjected to the maceration with 70% methanol (2L) for seven days, with daily agitation. The extract was filtered and concentrated under reduced pressure, in order to obtain methanol extract. The methanol extract of all plants were transferred into airtight culture vial with proper labeling for chromatographic and phytochemicals analysis.

Thin layer chromatography and Phytochemicals analysis

The phytochemicals analysis namely, Phenols/ tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids of selected plants (used as common growth supplements during the preparation of fermentation starter cultures) was carried out according to the methods mentioned by Yadav and Agarwala (2011).

Thin layer chromatography (TLC) of all plants extract (methanol) was conducted by analytical thin layer chromatography, using aluminum sheets of silica gel F254 (Merck®). All chromatograms were run in a saturated chamber. Mobile phases (Chloroform: methanol: 8:2) employed in this study was selected based on best separation. Aliquots (50 mg/ml) of the extract was spotted (~3 µl) onto a plate with capillary tubes, along a virtual line situated 10 mm from the bottom edge of the plate. The spots were applied at 5-mm intervals. The plate was developed at room temperature. After the chromatogram was developed, the plates were dried and the spots were visualized under iodine vapors. After keeping under iodine vapors for 8-10 minutes, the plate was removed and photographed. For analysis of TLC fingerprint in the form of spot intensity, the captured photographic image was imported into ImageJ software. Using ImageJ software each spot was converted into peak

based on spot intensity/thickness.

Scavenging of DPPH radicals

The scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was assayed following the method of Hatano *et al.* (1989). *Judima* of five different concentrations (30, 60, 90, 120 and 150 µl) were used for monitoring its possible antioxidant activity. The above mentioned *Judima* concentration was dissolved with 1:1 (v/v) of DPPH solution (0.1 mM). The mixture was shaken vigorously and left to stand for 50 min in the dark at room temperature. The reduction of the DPPH-radical was measured by continuous monitoring the decrease of absorption at 517 nm. Similarly antioxidant activity of all plants used for preparation of *Judima* was studied. DPPH scavenging effect was calculated as a percentage of DPPH discoloration using the equation: % scavenging effect = $[(A_{DPPH} - AS)/A_{DPPH}] \times 100$, where AS is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

Short term cytotoxicity study using MTT assay

Isolation of peripheral blood mononuclear cells (PBMC) for *judima* mediated cytotoxicity study was carried out according to the methods mentioned by Mallone *et al.* (2010). The PBMC cells was collected from mice (6 months old, body weight: 200 g). Cell growth inhibition was determined by *in vitro* MTT assay as described by Verma and Prasad (2012). Peripheral mononuclear blood cell (PBMC), collected from mice was used for cytotoxicity study. Briefly 1×10^6 cells in one ml were seeded on 24 well plates and the cells were treated with different concentration (30, 60, 90, 120 and 150 µl) of *Judima* for 12 hours. At the end of the incubation, MTT (5 mg/ml) was added and the cells were further incubated for 4 hrs after which the media was removed and DMSO (100 µl) was added in each well to solubilize the formazan crystals. The absorbance was read at a wave length 595 nm.

Statistical analysis

All values in the present study indicate mean ± standard deviation (S.D.), and all determinations were repeated three times. The one way analysis of variance (ANOVA) was used to evaluate the difference among multiple groups followed by a post hoc test (Tukey). Statistical significance was taken at a 95% confidence limit.

Results and Discussion

The traditional consumption of a variety of alcoholic beverages since time immemorial is still an integral part of different ethnic communities in the north-eastern region of India (Das *et al.*, 2012). The Dimasa Kacharis are one of the earliest indigenous ethnic groups of North-Eastern India. They believed that consumption of rice beer is good for health and act as a remedy for various ailments may be attributed to medicinal properties of the herbs used in the preparation of starter cultures. During the field study it is found that female candidates of the family are mainly involved in the preparation and selling of *Judima* for the betterment of their socio-economic conditions. A diverse knowledge system exists among the Dimasa women to prepare the nutritionally rich foods and fermented beverages, which play an important role in their day to day socio-cultural and spiritual occasions.

During the field study, it was found that different plant parts (leaves and twigs) are used for the preparation of *Judima* which includes *Piper betle*, *Acacia pennata*, *Buddleja asiatica* and *Hedyotis scandens* as common growth supplements during the preparation of fermentation starter cultures. The most frequently used species were *Buddleja asiatica* (leaves), *Hedyotis scandens* (leaves and twigs) and *Acacia pennata* (leaves and barks). The antioxidant activities of all the plants were evaluated and the result showed that all the plants are good source of antioxidant which could be due to the presence of high polyphenol, alkaloids and flavonoids (Figure 5 and Table 1). The thin layer chromatography (TLC) fingerprint of *Piper betle*, *Acacia pennata*, *Buddleja asiatica* and *Hedyotis scandens* was carried out and it was found that they contains 13, 14, 9 and 8 spots under iodine detection chamber respectively (Figure 6 and Figure 7). Such a chromatographic profile should feature the fundamental attributes of "integrity" and "fuzziness" so as to chemically represent the herbal medicines investigated. This suggests that the chromatographic fingerprint can also successfully demonstrate both "differences" and "uniformity" between various samples, and the authentication and identification of herbal medicines can be accurately conducted even if the number and/or concentrations of chemically characteristic constituents vary in different samples (Liang *et al.*, 2004). Therefore, the chromatographic fingerprint should be considered in evaluating the overall quality of herbal medicines,

Table 1. Phytochemical constituents of four plants used as common growth supplements during the preparation of fermentation starter cultures containing brewer's yeast

Plants	Proteins	Carbohydrates	Phenols/Tannins	Flavonoids	Saponins	Glycosides	Steroids	Terpenoids	Alkaloids
Acacia pennata	++ (0.33)	++ (0.44)	++++ (0.32)	++ (0.24)	+ (0.021)	++ (0.45)	+ (0.14)	+ (0.025)	+ (0.19)
Buddleja asiatica	+ (0.21)	+++ (0.48)	+++ (0.27)	+++ (0.29)	+ (0.024)	+ (0.32)	+ (0.11)	+ (0.027)	++ (0.28)
Hedyotis scandens	+ (0.23)	++ (0.43)	+++ (0.24)	++ (0.22)	+ (0.022)	+ (0.30)	+ (0.14)	++ (0.120)	+ (0.17)
Piper betle	++ (0.30)	++ (0.42)	+++ (0.24)	+++ (0.31)	+ (0.21)	++ (0.42)	++ (0.21)	+ (0.27)	+++ (0.38)
Judima	++ (0.28)	+ (0.18)	++ (0.23)	+ (0.16)	-	++ (0.20)	-	-	+ (0.11)

The values in bracket showing optical density (O.D.) recorded against water for respective test.

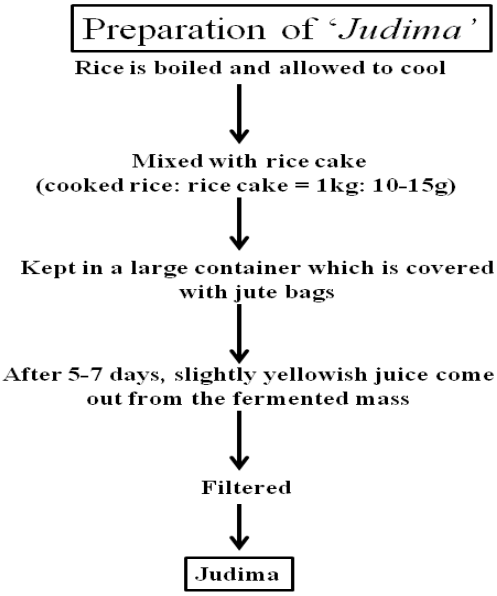

Figure 5. Antioxidant activity of some plants used for preparation of *Judima*

considering the multiple constituents that are present in medicinal plants and herbal drugs (Braz *et al*., 2012). Phytochemicals are bioactive constituents that sustain or promote health and occur at the intersection of food and pharmaceutical industries. Such substances may range from isolated nutrients, dietary supplements and specific diets to genetically engineered designer foods, herbal products, processed foods and beverages (Prakash *et al.*, 2012). Phytochemicals are broadly described as phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, polyphenols, flavonoids, isoflavonoids and anthocyanidins. They have tremendous impact on the health care system and may provide medical health benefits including the prevention and/ or treatment of diseases and physiological disorders. Majority of foods, such as whole grains, beans, fruits, vegetables and herbs contain phytochemicals of nutraceutical importance. These phytochemicals, either alone and/or in combination, have tremendous therapeutic potential

Table 2. Biochemical analysis of Judima, showing physicochemical, biochemical and microbial load

Sl no.	Parameters		Data
1	**Physico-chemical features**	State	liquid
		Colour	watery
		Opacity	clear
		Optical density (against water)	0.003
		Total solid contents	0.007mg/ml
		PH	5.1
2	**Biochemical features**	Total carbohydrates	32.43 ± 2.7 mg/ml
		Total protein	0.97 ± 0.18 mg/ml
		Free amino acids	3.21 ± 0.21 mg/ml
		The ethanol content	16 ± 2% (v/v)
3	**Microbial load**	Starter culture	632 x 10^7 cfu/gm
		First day of fermentation	732 x 10^7 cfu/gm
		Third day of fermentation	545 x 10^7 cfu/gm
		Fifth day of fermentation	422 x 10^7 cfu/gm
		Ripe (after filtration)	278 x 10^7 cfu/gm

Data are from the three different experimental groups.

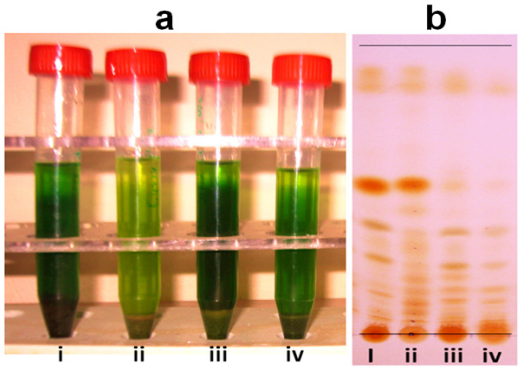

Figure 6. Methanol leaves extract (a) and TLC fingerprint (b) of some plants used for preparation of *Judima.* (i) *Piper betle* (ii) *Acacia pennata* (ii) *Buddleja asiatica* and (iv) *Hedyotis scandens*

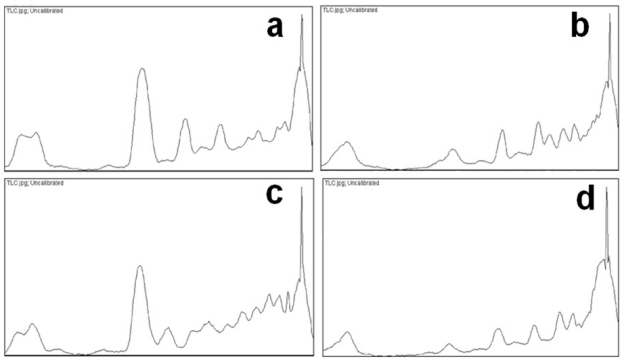

Figure 7. Thin layer chromatography (TLC) intensity profiles of (a) *Piper betle* (b) *Acacia pennata* (c) *Buddleja asiatica* and (d) *Hedyotis scanden* used for preparation of *Judima.*

in curing various ailments (Prakash *et al*., 2012). The respective health benefits are based on science and ethics, for health claims, functional foods and presence of certain phytochemicals. They play specific pharmacological effects in human health as anti-inflammatory, anti-allergic, antioxidants, antibacterial, antifungal, antispasmodic, chemopreventive, hepato-protective, hypolipidemic, neuroprotective, hypotensive, antiaging, diabetes, osteoporosis, DNA damage, cancer and heart diseases, induce apoptosis, diuretic, CNS stimulant, analgesic, protects from

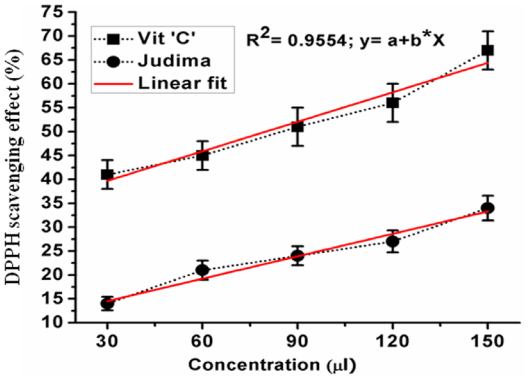

Figure 8. Antioxidant activity of *Judima* at different concentration

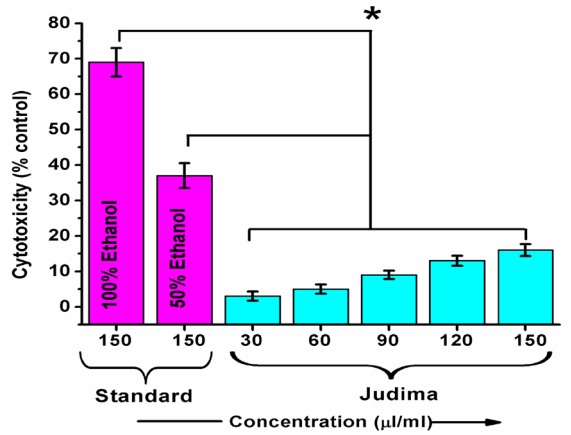

Figure 9. Cytotoxic effect of *Judima* at different concentration on normal PBMC cells. Ethanol (50% and 100%) are used as standard for comparison of cytotoxic effect of *Judima*. The results are expressed as mean ± S.D. ANOVA, n = 3, *P ≤ 0.001 as compared to standard ethanol (50% and 100%).

UVB-induced carcinogenesis, immuno-modulator and carminative (Prakash *et al*., 2012).

The antioxidant activity of *Judima* also showed concentration dependent antioxidant activity by the DPPH assay (Figure 8), which can be attributed due to high polyphenol, alkaloids and flavonoid contents in the plants used for preparation of starter culture (Table 1). The ideal decanted product of *Judima* is creamy white liquor with pH 4.2 at 22°C. It is consumable liquid characterized by suitable aroma. The ethanol content of decanted product was found to be 16% (v/v) (Table 2).

The total carbohydrates, protein and free amino acids content in the decanted product of *Judima* was estimated to be 32.43 ± 2.7 mg/ml, 0.97 ± 0.18 mg/ml and 3.21 ± 0.21 mg/ml respectively (Table 2). The presence of carbohydrate, protein and amino acids in the decanted product adds to the energy metabolism in the cell. The presence of these metabolites and energy processing substances in the decanted product would certainly changes the nutritional status of the beverage which is consumed as refreshing drink. The biochemical conversion of starch to alcohol during domestic alcohol fermentation occurs in two phases, viz. saccharification of rice to simple mono and disaccharification through hydrolysis of starch and conversion of monosaccharide to alcohol. The saccharification process is accomplished by the extracellular enzymes α-amylase and β-glucosidase (Bryzak, 2003) which has been observed to be in many yeast strains and molds. The- amylase involves in hydrolysis of 1-4 glycosidic linkage of starch to produce to maltose and other disaccharides (Bryzak, 2003). Alcoholic fermentation successively involves different microorganism although yeasts are the most prominent species. On the other hand, the use of plant materials such as *Buddleja asiatica* (leaves), *Hedyotis scandens* (leaves and twigs) and *Acacia pennata* (leaves and barks) as essential ingredients during preparation of starch for the fermentation of alcoholic beverage may contribute to additional of phenolics which in turn might have contributed changes in microbial population during perpetuation. Recent reports for alcoholic fermentation of Manipur shown Hamic as starter, traditional fermented foods of Naga tribes (Mao and Odoiso, 2007) and Sajan, a local rice beer of Deori tribe (Deori *et al*., 2007) suggests the use of starch in the fermentation of traditional wine.

It has also been reported that two types of yeasts are involved in fermentation. In Sikkim amylolytic yeasts from marcha degrade starch and produce glucose (Tsuyoshi, 2004). The alcohol producing yeasts then grows rapidly on the resultant glucose to ferment ethanol. It has been reported that a typical filamentous mould Amylomyces roucii and yeasts like Enclomycopses, Candida and Hensenuls are responsible for rice fermentation and preparation of "Tapeketan". The growth of various yeasts has been shown to be dependent on the amylolytic capacity of the mould given rise to typical flavor of tapeketan (Fleet and Heard, 1993). The people of Manipur use their traditional liquor (Yu) for medicine, relaxant and offerings which is a distilled product of fermented local rice. It is a strong solvent for many important active constituents of medicinal plants whose action plays a vital role in traditional medicine (Singh and Singh, 2006). Similar observation also been reported regarding the local liquor called 'Rokshi'of Sikkim (Singh *et al*., 1999; Singh and Singh, 2006).

The density of microbial load in decanted product of *Judima* is shown in Table. 1. The short term (12 h) cytotoxicity study of *Judima* on normal PBMC cells showed that there are significant (P ≤ 0.001) lower cytotoxicity as compared to 50% and 100% ethanol (Figure 9). Cytotoxicity result showed that there is negligible cell death after *Judima* treatment

for 12 h (Figure 9). Apart from the experiments performed and results obtained, it can be predicted that careful identification and selection of yeasts and bacteria and useful fungi from starter cultures employed in traditional brewing in North-East India can yield industrially important and beneficial microbes for the benefit of mankind. In addition to the above, the removal of deleterious metabolites from decanted product and preservation of beneficial wines is expected to enhance the nutritional status of traditional alcoholic drinks.

Conclusion

In North East India, diversity of ethnic fermented foods is related to diversity of ethnicity with unparallel food habits of each community. This study showed the antioxidant activity of the plants used for preparation of rice beer, may have contributed to the antioxidant activity of rice beer (*Judima*). Moreover, cytotoxic effect of *Judima* is found to be negligible, moreover it is rich in nutritional supplements which could be good for health. Many tribal fermented foods and beverages are unstable and spoiled very soon due to lack of suitable preservatives methods therefore; there is an urgent need of scientific ideas for its preservation and commercialization to different part. Some foods including different types of alcoholic beverages of North East India are popular and widely preferred by the consumers, such foods may be popularized to non-consumers in others part of the world too.

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