CONTENTS

Dedication		
Abstracts		
Declaration		
Certificate of supervisor		
Certificate of co-supervisor		
Acknowledgements		
List of Tables		
List of Figures		
List of Abbreviations		

Chapter 1	Introduction	1-26
1.1	Introduction	1-2
1.2	Review of literature	2
1.2.1	Rice beer in general	2-4
1.2.2	Plants involved in rice beer starter preparation and their properties	4-7
1.2.3	Microbes involved in rice beer fermentation	7-13
1.2.4	Preparation of beer from alternative sources and sensory evaluation	14-15
1.2.5	Phenolic esters and anti-inflammatory compounds	15-16
1.3	Objectives	16-17
	References	18-26
Chapter 2	Biochemical and microbiological characterization of rice beer and starter cakes produced in different regions of Assam and Northeast India	1-48
2.1	Introduction	1-2
2.2	Materials and methods	2
2.2.1	Materials	2-3
2.2.2	Field survey	3
2.2.3	Biochemical analysis of the collected rice beer samples	3
2.2.3.1	Colour measurement	3-4
2.2.3.2	Sample preparation for biochemical analysis	4
2.2.3.3	pH, acidity measurement Alcohol content estimation	4
2.2.3.4	HPLC analysis of organic acids	4

i-viii

2.2.3.5	HPLC analysis of carbohydrates	5
2.2.3.6	HPLC analysis of amino acids	5 5-6
2.2.3.7	GC-MS analysis of aromatic components	5-0 6
2.2.3.8	Mineral elements analysis by atomic absorption spectroscopy	0 7
2.2.3.8	Effect of the microbial starters cakes on some quality attributes of	7 7
2.2.4	rice beer	1
2.2.4.1	Physical analysis	7
2.2.4.1	Volume and density measurement of the starters cakes (SC)	7 7-8
2.2.4.1.1	•	8
2.2.4.1.3	Texture analysis of the SC Colour measurement	8
2.2.4.1.5		8 8
2.2.4.2	Production of rice beer (RB) in the laboratory	8 8
	Biochemical analysis	8 8
2.2.4.3.1	Sample preparation for biochemical analysis	
2.2.4.3.2	pH, acidity and alcohol content estimation	8-9
2.2.4.3.3	Total soluble solids (TSS) measurement	9
2.2.4.3.4	Estimation of proximate composition, reducing sugars, starch and	9
	amylose	-
2.2.4.3.5	Total polyphenols content (TPC) estimation	9
2.2.4.3.6	Radical scavenging activity estimation	9
2.2.4.4	Microbial analysis	9-10
2.2.5	Statistical analysis	10
2.3	Results and discussions	10
2.3.1	Observations on the methodologies followed by different tribes in	10
	the preparation of rice beer	
2.3.1.1	<i>Hor-alank</i> – Karbi tribe	10-11
2.3.1.2	Sujen – Deori tribe	11-12
2.3.1.3	<i>Xajpani</i> – Ahom community	12-13
2.3.1.4	Apong – Mising tribe	13-14
2.3.1.5	Joubishi – Bodo tribe	15-16
2.3.1.6	Judima - Dimasa tribe	16-17
2.3.1.7	Zutho – Angami tribe	17-18
2.3.1.8	<i>Opo</i> - Adi-Galo tribe	18-19
2.3.1.9	Sadhiar - Khasi tribe	19
2.3.2	Biochemical analysis of the rice beer samples	20
2.3.2.1	General characteristics of rice beer samples	20-21
2.3.2.2	Analysis of organic acids	22-23
2.3.2.3	Analysis of carbohydrates	23-25
2.3.2.4	Analysis of amino acids	26-27
2.3.2.5	Analysis of aromatic compounds	28-30
2.3.2.6	Presence of different mineral elements	31-32
2.3.3	Effect of the microbial starters on the quality of rice beer	32
2.3.3.1	Physical properties of the SC	32-33
2.3.3.2	Colour in CIELAB expression	33-34
2.3.3.3	Proximate composition	34-36
2.3.3.4	Biochemical attributes of the RB	36-38
2.3.3.5	Microbiological profile of the samples	38-40
	<u> </u>	

2.4	Conclusion	40-42
	References	43-48
Chapter 3	Evaluation of the antioxidative and antimicrobial properties of	1-86
3.1	plants used in rice beer starter culture preparation Introduction	1-3
3.2	Materials and methods	4
3.2.1	Materials	4
3.2.2	Phytochemical constituents, ATR-FTIR analysis and	5
3.2.2	antimicrobial activity of leaves of <i>Artocarpus heterophyllus</i> Lam.,	5
	Cyclosorus extensa (Blume) Ching, Oldenlandia corymbosa L.	
	and Alpinia malaccensis (Burm. f.) Roscoe	
3.2.2.1	Phytochemical analysis of the leaves	5
3.2.2.2	Preparation of solvent extracts (SEs)	5-6
3.2.2.3	FTIR analysis of the SEs	6
3.2.2.4	Antimicrobial activity of the SEs	6-7
3.2.2.5	Determination of minimum inhibitory concentration (MIC)	7
3.2.3	In vitro antioxidant activity of polyphenols purified from leaves	7
	of Artocarpus heterophyllus Lam., Cyclosorus extensa (Blume)	
	Ching, Oldenlandia corymbosa L. and Alpinia malaccensis	
	(Burm. f.) Roscoe	
3.2.3.1	Extraction and purification of phenolic compounds	7-8
3.2.3.2	Estimation of phenolic compounds in the PPEs by HPLC	8
3.2.3.3	Estimation of antioxidant activities	8-9
3.2.3.3.1	DPPH free radical scavenging activity assay	9
3.2.3.3.2	ABTS radical cationdecolourisation assay	9
3.2.3.3.3	Hydroxyl radical (•OH) scavenging assay	10
3.2.3.3.4	Hydrogen peroxide (H2O2) scavenging activity assay	10
3.2.3.3.5	Superoxide anion (O2) scavenging activity assay or NBT assay	10
3.2.3.3.6	Nitric oxide radical (NO•) scavenging activity assay	11
3.2.3.3.7	Ferrous ion (Fe2+) chelating assay	11
3.2.3.3.8	Ferric reducing antioxidant power (FRAP) assay	11
3.2.3.3.9	Ferric thiocyanate (FTC) assay	12
3.2.3.3.10	Thiobarbituric acid (TBA) assay	12
3.2.3.4	IC ₅₀ value calculation	12-13
3.2.4	Storage study of rice beer under accelerated temperature	13
	condition by incorporation of bioflavonoids from Artocarpus	
2 2 4 1	heterophyllus and Cyclosorus extensa leaves	10
3.2.4.1	Extraction and purification of bioflavonoids	13
3.2.4.2	HPLC estimation of flavonoid content	13-14
3.2.4.3	Preparation of rice beer	14
3.2.4.4	Storage of the rice beers	14
3.2.4.5	Analysis of the stored rice beers	15
3.2.4.5.1 3.2.4.5.2	Aerobic plate counts (APCs)	15 15
	pH Total phanolic content (TPC)	15 15
3.2.4.5.3	Total phenolic content (TPC)	13

22454	Dedical commune estimiter (DCA)	15
3.2.4.5.4	Radical scavenging activity (RSA)	15
3.2.4.5.5	Total proteins	15
3.2.4.5.6	Thiobarbituric acid reactive substances (TBARS) assay	16
3.2.4.5.7	Peroxide value (POV)	16
3.2.4.5.8	Colour measurement	16
3.2.5	Optimization of the extraction of phenolic compounds from	17
01210	<i>Cyclosorus extensa</i> with solvents of varying polarities	17
3.2.5.1	Drying and grinding of plant materials	17
3.2.5.2		17-19
	Experimental design	
3.2.5.3	Extraction procedure	19
3.2.5.4	Estimation of total phenolic compounds (TPC)	19
3.2.5.5	Estimation of radical scavenging activity (RSA)	19
3.2.5.6	Estimation of antibacterial activity (ABA) and antifungal activity	20
	(AFA)	
3.2.6	Statistical analysis	20
3.3	Results and discussions	20
3.3.1	Phytochemical constituents, ATR-FTIR analysis and	20
0.011	antimicrobial activity of leaves of Artocarpus heterophyllus Lam.,	20
	Cyclosorus extensa (Blume) Ching, Oldenlandia corymbosa L.	
	•	
2 2 1 1	and Alpinia malaccensis (Burm. f.) Roscoe	20.22
3.3.1.1	Various phytochemicals in the leaves	20-22
3.3.1.2	Functional groups detected in the SEs	22-26
3.3.1.3	Antimicrobial activity of the SEs	27-30
3.3.1.4	MIC values of the SEs	30-31
3.3.2	Antioxidant activity of the polyphenols purified from leaves of	31
	Artocarpus heterophyllus Lam., Cyclosorus extensa (Blume)	
	Ching, Oldenlandia corymbosa L. and Alpinia malaccensis	
	(Burm. f.) Roscoe	
3.3.2.1	Content of phenolic compounds in the leaves	31-35
3.3.2.2	DPPH and ABTS scavenging activity	35-36
3.3.2.3	•OH, H_2O_2 , O_2 •- and NO• scavenging activity	36-38
3.3.2.4	Fe2+chelating activity and FRAP assay	39-41
3.3.2.5		41-45
	FTC and TBA assay	
3.3.3	Storage of rice beer under accelerated temperature condition by	45
	incorporation of bioflavonoids from Artocarpus heterophyllus	
	and Cyclosorus extensa leaves	
3.3.3.1	Content of various flavonoids in the purified extracts	45-48
3.3.3.2	Effect of storage on various indicator parameters of rice beer	48
3.3.3.2.1	APC	49-50
3.3.3.2.2	pH	50-51
3.3.3.2.3	TPC	51-52
3.3.3.2.4	RSA	52-53
3.3.3.2.5	Protein content	53-54
3.3.3.2.6	Colour	54-55
3.3.3.2.7	TBARS and POV	56-57
3.3.4	Optimization of the extraction of phenolic compounds from	58

	Cyclosorus extensa with solvents of varying polarities	
3.3.4.1	Statistical analysis and model fitting	58-61
3.3.4.2	Effect of the process variables on various responses of the extracts	62-66
3.3.4.3	Optimization of parameters	66-67
3.3.4.4	Effect of the solvents and optimized extraction conditions on TPC	68
3.3.4.5	Effect of the solvents and optimized extraction conditions on RSA	68-69
3.3.4.6	Effect of the solvents and optimized extraction conditions on ABA	69-70
0.01.10	and AFA	07 70
3.4	Conclusions	70-72
	References	73-86
Chapter 4	Identification of fungal and lactic acid bacteria isolates from rice	1-101
	beer and starter cultures and evaluation of their functional	
	properties	
4.1	Introduction	1-3
4.2	Materials and Methods	3
4.2.1	Materials	3
4.2.2	Identification and studies on amylolytic properties of moulds	4
	isolated from rice beer starter cakes	
4.2.2.1	Isolation of moulds from starter cakes	4
4.2.2.2	Microscopic observation	4
4.2.2.3	Identification of the isolates	4-5
4.2.2.4	Construction of phylogenetic tree	5
4.2.2.5	Test for starch degradation	5-6
4.2.2.6	Assay for glucoamylase and α -amylase activity	6-7
4.2.2.7	Purification of glucoamylase enzyme and its molecular size determination and study of their enzymatic activity	7
4.2.2.8	Test for the production of mycotoxins	8
4.2.3	Identification and studies on physiological properties of yeast	8
	strains isolated from rice beer and starter cakes	
4.2.3.1	Isolation of yeasts strains from starter cakes	8
4.2.3.2	Microscopic observation	8
4.2.3.3	Biochemical characterization of the isolates	9
4.2.3.3.1	Diazonium blue B test	9
4.2.3.3.2	Urea hydrolysis	9
4.2.3.3.3	Production of extracellular starch like compounds	9
4.2.3.3.4	Growth in presence of cycloheximide	9
4.2.3.4	Genetic identification of the isolates	9-10
4.2.3.5	Construction of phylogenetic tree	10
4.2.3.6	Determination of ethanol tolerance	10
4.2.3.7	Ability to grow at different temperatures	11
4.2.3.8	Growth at high osmotic pressure	11
4.2.3.9	Ability to use sugars anaerobically	11
4.2.3.10	Ability to use sugars as sole source of carbon for aerobic growth	11
4.2.3.11	Determination of alcohol dehydrogenase (ADH) activity	12
4.2.3.12	Determination of alcohol producing capacity of the yeast strains	12-13

4.2.3.13	5 5 C I	13
4.2.4	(VOCs) Identification and studies on functional properties of lactic acid	14
4 0 4 1	bacteria isolated from rice beer and starter cakes	1.4
4.2.4.1	Isolation of lactic acid bacteria	14
4.2.4.2	Genetic identification of the isolates based on 16s rRNA sequencing	14-15
4.2.4.3	Construction of phylogenetic tree	15
4.2.4.4	General characteristics of the strains	15-16
4.2.4.5	Growth characteristics of the strains at different pH, salt	16
1016	concentration and temperature	16
4.2.4.6	Carbohydrate fermentation tests	16
4.2.4.7	Antibiotic susceptibility test	16
4.2.4.8	Antibiosis activity tests	17
4.2.4.9	Test for antioxidant activity	17-18
4.2.4.10	Test for acid tolerance of the isolates LAB strains	18
4.2.4.11	Test for bile tolerance of the isolated LAB strains	18-19
4.2.4.12	Test for cellular aggregation of the isolated LAB strains	19
4.2.4.13	Test for microbial adhesion to solvents (MATS) of the isolated LAB	19-20
	strains	• •
4.2.4.14	Surface associated adhesion proteins of two Lactobacillus casei	20
	strains	• •
4.2.4.14.1	Preparation of cellular extracts	20
4.2.4.14.2	Adhesion properties with and without the surface proteins	20
4.2.4.14.3	Adherence to Caco-2 and HT-29 intestinal epithelial cells	21
4.2.4.14.4	Imaging of the cell surfaces by transmission electron microscopy (TEM)	21-22
4.2.4.14.5	Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS- PAGE) to determine the surfaces associated proteins	22
4.2.4.15	Utilization of different carbohydrates for production of organic acids	22
	by the LAB strains	
4.2.4.15.1	Growth of the LAB strains in formulated media	23
4.2.4.15.2	HPLC analysis for the production of various organic acids	23
4.3	Results and discussions	24
4.3.1	Identification and studies on amylolytic properties of moulds	24
	isolated from rice beer starter cakes	
4.3.1.1	Identification of the isolates	24-26
4.3.1.2	Starch hydrolysis activity	26-27
4.3.1.3	Enzymatic activity of the strains	27-28
4.3.1.4	Molecular weight and enzymatic activity of the extracellular	28-29
	enzymes	
4.3.1.5	LC-MS/MS analysis to test the production of mycotoxin by the	30-33
	fungal strains	
4.3.2	Identification and studies on physiological properties of yeast	34
	strains isolated from rice beer and starter cakes	
4.3.2.1	General biochemical characteristics and identification of the yeast isolates	34-38

4.3.2.2	Construction of phylogenetic tree	38-39
4.3.2.3	Ethanol tolerance of the yeast strains	39-42
4.3.2.4	Growth characteristics of the strains at different temperatures	42-43
4.3.2.5	Tolerance of the yeast strains to osmotic stress	44-45
4.3.2.6	Anaerobic sugar fermentation by the yeast strains	45-46
4.3.2.7	Utilization of different carbon sources by the yeast strains	46-48
4.3.2.8	ADH activity of the yeast strains	49
4.3.2.9	Change in pH and total sugars of culture media and alcohol production by the yeast strains	50-52
4.3.2.10	Formation of volatile organic compounds (VOCs) by the yeast strains	52-55
4.3.3	Identification and studies on functional properties of lactic acid	56
1.5.5	bacteria isolated from rice beer and starter cakes	50
4.3.3.1	The LAB strains identified in the starter cakes	56-60
4.3.3.2	General characteristics of the LAB strains	60-61
4.3.3.3	Growth a different pH, NaCl concentration and temperature	61-63
4.3.3.4	Utilization of various sugars by the LAB strains	64-65
4.3.3.5	Antibotic susceptibility of the LAB strains	66
4.3.3.6	Antibiosis activity of the LAB strains	66-68
4.3.3.7	Antioxidant activity of the LAB strains	68-69
4.3.3.8	Tolerance of the LAB strains to acid	69-71
4.3.3.9	Tolerance of the LAB strains to bile salts	72
4.3.3.10	Cell Aggregation and microbial adhesion to solvents (MATS)	72-75
4.3.3.11	Surface associated adhesion proteins of two <i>Lactobacillus casei</i> strains	75
4.3.3.11.1	Adhesion properties with and without the surface proteins	75-76
4.3.3.11.2	Adherence of the <i>L. casei</i> strains to CaCo-2 and HT-29 intestinal	76-78
	epithelial cells	
4.3.3.11.3	Imaging of the cell surfaces by transmission electron microscopy (TEM)	78
4.3.3.11.4	Determination of the surfaces associated proteins by SDS-PAGE	79
4.3.3.12	Utilization of different carbohydrates for production of organic acids by the LAB strains	80
4.3.3.12.1	Production of various organic acids by utilizing D-glucose as carbon source	80-81
4.3.3.12.2	Production of various organic acids by utilizing maltose as carbon source	82-83
4.3.3.12.3	Production of various organic acids by utilizing lactose as carbon	84-85
4.4	source Conclusion	86-87
4.4	References	88-101
Chapter 5	Laboratory scale optimization of rice beer making process and sensory evaluation of the products	1-42
5.1	Introduction	1-2
5.2	Materials and methods	2

5.2.1	Raw materials	2-3
5.2.2	Microbial inoculums	3
5.2.3	Preparation of plantain and cassava chips	3 3-4
5.2.4	Substrates used for preparation of beer	3- 4
5.2.5	Biochemical analysis	4
5.2.6	Estimation of alcohol content	4
5.2.7		
	Total polyphenols content (TPC) analysis	4 5
5.2.8	pH and acidity Total soluble solids (TSS) measurement	5
5.2.9	Total soluble solids (TSS) measurement	
5.2.10	Colour measurement	5 5
5.2.11	Microbial count	
5.2.12	Optimization of fermentation parameters	6
5.2.12.1	Experimental design	6-8
5.2.12.2	Fermentation procedure	8-9
5.2.13	Sensory evaluation of the products	10
5.2.13.1	Obtaining sensory evaluation response score from panellists	10-11
5.2.13.2	Fuzzy analysis	11
5.2.13.2.1	Triplets associated with linguistic scale	12-13
5.2.13.2.2	Triplets for linguistic score of beer samples	13-14
5.2.13.2.3	Triplet for linguistic score of the quality attributes	14
5.2.13.2.4	Triplets for relative weightage of quality attribute	14
5.2.13.2.5	Triplets for overall linguistic score of different beer samples	14-15
5.2.13.2.6	Calculation of membership function on standard fuzzy scale	15-16
5.2.13.2.7	Calculation of overall membership function of linguistic scores on	16-17
	standard fuzzy scale	
5.2.13.2.8	Estimation of similarity values for beer samples	17
5.2.14	Statistical analysis	17
5.3	Results and discussions	18
5.3.1	Biochemical composition of the substrates	18
5.3.2	Experimental runs and the responses	18-19
5.3.3	Statistical analysis and model fitting	19-22
5.3.4	Effect of the process variables on various responses of rice beer	22
5.3.4.1	Protein content	22-23
5.3.4.2	Alcohol content	23-24
5.3.4.3	Lactobacillus plantarum count	24-25
5.3.4.4	Total polyphenols content (TPC)	25-26
5.3.4.5	Reducing sugars content (RSC)	26-27
5.3.4.6	Titratable acidity.	27-28
5.3.5	Optimization of fermentation parameters for rice beer	28-29
5.3.6	Attributes of the final products	29-31
5.3.7	Colour of the different beers	31-32
5.3.8	Sensory evaluation	32
5.3.8.1	Triplets for linguistic score of beer samples	32-34
5.3.8.2	Triplets for linguistic score of quality attribute	34-35
5.3.8.3	Triplets for relative weightage of quality attribute and overall	35-36
	linguistic score of different beer samples	
	-	

5.3.8.3 5.4	Similarity value of beer samples Conclusions References	36-37 37-38 39-42
Chapter 6	Studies on anti-inflammatory role of compounds derived from rice beer in <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> models	1-51
6.1	Introduction	1-3
6.2	Materials and methods	3
6.2.1	Materials	3
6.2.2	Synthesis of novel ester and structural validation	4
6.2.2.1	Zn(OTf) ₂ -catalyzed selective esterification of salicylic acid and	4
	phenylethyl alcohol	
6.2.2.2	NMR and FTIR study for structural validation of the synthesised compound	4
6.2.3	<i>In silico</i> studies with newly synthesized ester and related compounds	4
6.2.3.1	Chemical structure generation	4-5
6.2.3.2	Absorption, distribution, metabolism, excretion and toxicity (ADME-Tox) studies	5
6.2.3.3	Molecular docking against COX-2 (PDB ID: 4PH9) enzyme	5
6.2.3.3.1	Cavity prediction	5-6
6.2.3.3.2	Docking computation	6
6.2.3.4	Molecular dynamics (MD) simulation study of the SAPE-COX-2 docked complex	6
6.2.4	Test for <i>in vitro</i> cytotoxicity of SAPE in animal cell line models	7
6.2.4.1	Isolation and culture of human primary peripheral blood mononuclear cells (PBMC) and RBCs	7
6.2.4.2	Membrane stability assay of SAPE	7
6.2.4.3	Test for cytotoxic effect of SAPE by MTT assay	7-8
6.2.4.4	Test for cytotoxic effect of SAPE in CaCo2 cells by Alamar Blue® assay	8
6.2.5	In vivo study for anti-inflammatory role of SAPE in animal model	8
6.2.5.1	Experimental design	8-9
6.2.5.2	Pre-test in paw oedema model	9
6.2.5.3	Administration of drug and induction of colitis	9-10
6.2.5.4	Measurement of change in body weight	10
6.2.5.5	Evaluation of disease activity index (DAI)	11
6.2.5.6	Scanning electron microscope (SEM) study of intestinal morphological changes	11
6.2.5.7	Antioxidant activity assays	12-13
6.2.5.8	Pro-inflammatory biomarkers analysis by ELISA	13-14
6.3	Results and Discussions	14
6.3.1	Synthesis of SAPE and structural validation of the synthesised compound by NMR and FTIR study	14-17
6.3.3	<i>In silico</i> studies with SAPE and other anti-inflammatory drugs	17
6.3.3.1	Absorption, distribution, metabolism, and excretion (ADME) studies related to SAPE	17-21

6.3.3.2	Molecular docking study	21-26
6.3.3.3	Molecular dynamics simulation of protein-ligand docked complex	
6.3.4	In vitro cytotoxicity of SAPE in animal cell line models	27-32
6.3.5	In vivo study for anti-inflammatory role of SAPE in animal model	32
6.3.5.1	Pre-test in paw oedema model	32-33
6.3.5.2	Change in body weight of the different groups of rats and evaluation of disease activity index (DAI)	33-34
6.3.5.3	Scanning electron microscope (SEM) study of intestinal morphological changes	34-38
6.3.5.4	Antioxidative and anti-inflammatory role of SAPE on animal model	39
6.3.5.4.1	Antioxidative effects	39-43
6.3.5.4.2	Anti-inflammatory effects	43-45
6.4	Conclusions	45
	References	46-51