FINAL PROJECT COMPLETION REPORT (PCR)

(F.No. Q-11/22/2019-R&D Date 18.10.2019)

(Under the Scheme of Research & Development in Processed Food Sector)

Development of Ready to Eat Anti-inflammatory Functional Food from 'Norabogori' (*Prunus persica*) of Assam

Submitted to

Ministry of Food Processing Industries
Government of India
Panchsheel Bhawan
August Kranti Marg
New Delhi - 110 049

Submitted by
Professor S. C. Deka, (Former Head)
Principal Investigator
Department of Food Engineering and Technology
Tezpur University(A Central University)
Tezpur-784028, Assam

ANNEXURE-C

FINAL PROJECT COMPLETION REPORT (PCR)

Notes:

1. 10 copies of the Final Project Completion Report (PCR) should be sent within one month of the completion or termination of the project.
2. The PCR should be in bound form.
3. Cover page should include the title of the project, file number, names and addresses of the investigation.

1. Title of the project: **Development of Ready to Eat Anti-inflammatory Functional Food from 'Norabogori' (*Prunus persica*) of Assam**

2. Principal Investigator(s) and Co-Investigator(s):

Principal Investigator: Prof. Sankar Chandra Deka
Department of Food Engineering & Technology
Tezpur University

Co-Investigator: Dr. Anupam Nath Jha
Molecular Biology and Biotechnology
Tezpur University

3. Implementing Institution(s) and other collaborating Institution(s): Tezpur University

4. Date of commencement: 18.10.2019

5. Planned date of completion: 18.10.2021

6. Actual date of completion: 31.06.2022

7. Objectives as stated in the project proposal:

i. To study the extraction of different phytochemical compounds from peel, pulp, and seeds of *Prunus persica*
ii. To study the extracted phytochemicals for anti-inflammatory effect by *in silico* method
iii. To study the *in vitro* efficacy and anti-inflammatory mechanism of the screened phytochemical compound.
iv. To study the incorporation of the tested anti-inflammatory molecule in food model and sensory test

8. Deviation made from original objectives if any, while implementing the project and reasons thereof:

No

9. Experimental work giving full details of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs:

Attached

10. Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject:

Attached

11. Conclusions summarising the achievements and indication of scope for future work:

Attached

12. S&T benefits accrued:

i. List of Research publications

S No	Authors	Title of paper	Name of the Journal	Volume	Pages	Year
1	Urbashi Neog, Sankar C Deka	Extraction, characterization and anti-inflammatory effects analysis of norabogori (*Prunus persica* L. Batch) extract and its application (Drafted)	Manuscript under preparation	-	-	-
2	Urbashi Neog, Sankar C Deka	Nutritional and therapeutic potential of *Prunus persica*: A review (Drafted)	Manuscript under preparation	-	-	-
3	Urbashi Neog, Sankar C Deka, Anupam N Jha	In silico exploration of anti-inflammatory activity of norabogori extract and encapsulation (Drafted)	Manuscript under preparation	-	-	-

ii. Manpower trained on the project

a) Research Scientists or Research Associates: 0
b) No. of Ph.D. produced: 1 (Ongoing)
c) Other Technical Personnel trained: 0

iii. Patents taken, if any: Fruit leather from norabogori (*Prunus persica* (L.) Batsch) of Assam with anti-inflammatory properties (Prepared)

13. Financial Position:

No	Financial Position/ Budget Head	Funds Sanctioned (in Rs.)	Expenditure (in Rs.)	% of Total cost
I	Salaries/ Manpower costs	7,23,127.00	7,22,767.00	28.62
II	Equipment	11,00,000.00	10,99,980.00	43.56
III	Consumables	5,04,000.00	5,04,130.00	19.96
IV	Contingencies	0	0	0
V	Travel	28,000.00	0	0
VI	Institutional Charges	1,99,425.00	1,98,528.00	7.86
	Total	25,54,532.00	25,25,405.00	**100%**

14. Procurement/ Usage of Equipment

a)

S No	Name of Equipment	Make/ Model	Cost (FE/ Rs)	Date of Installation	Utilisation Rate (%)	Remarks regarding maintenance/ breakdown
1	Refrigerated Incubated Shaker	Eppendrof; Model: Innova 42R	10,99,980.00	09.06.2020	95%	Working efficiently

b) Plans for utilising the equipment facilities in future: The refrigerated incubated shaker purchased under this project will be utilized for the future work on Norabogori. It will also be an important and valuable asset for the department as it maintains a controlled environment so that biological samples can grow continuously, in biochemical extractions, microbiology, bacteriology and cell and tissue culture experiments.

Name and Signature with Date

a. _____ 25/7/2022 (Principal Investigator)

b. _____ (Co-Investigator)

26/7/22

Head of Institute/Organization

Registrar
Tezpur University
Napaam, Tezpur

Date: 26.7.2022

Note: Final project Completion Report (PCR) is expected to be self-contained complete report of the work done. Please do not leave any column unanswered.

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Acknowledgements

We would like to extend our sincere gratitude to the Ministry of Food Processing Industries, New Delhi for funding the project.

The project timeline ended on 18.10.2021 but we lagged behind with the work as the pandemic forced us to stop for a crucial period of more than 7 months (March – September, 2020). So, the completion of the project took more than the allocated and planned time period. We are extremely grateful for giving us the opportunity and allowed us to complete our proposed work in an adequate manner. The financial assistance in the form of Junior Research Fellowship been of a great help for the PhD scholar employed in the position in completing the stipulated work smoothly. The chemicals and equipment funded made the experiments described in the report come through.

We would also like to thank Tezpur University for giving the permission and for availing all facilities needed to complete the research works.

Introduction

Norabogori is a sparsely available fruit in Assam. It is a deciduous plant with drupe fruit belongs to the Rosaceae family, Amygdaloideae subfamily (Ceccarelli et.al., 2016). It is one of the five most important and widely cultivated deciduous fruit along with apple and peas (Wu et. al.,2011). Characteristically it is a peach fruit variety having slight color difference than popular peach variety. Peaches and nectarines production has an important place in the world (21.8 million metric tons in 2020-21) with a cultivated area of around 1.5 million ha. China, Japan, Korea, Spain, USA, Greece etc. are the prime producer of peach. In India, it is primarily grown in Jammu and Kashmir, Himachal Pradesh, Punjab, Uttarakhand, Nilgiri hills, Jharkhand and North Eastern States.

From ancient Chinese time to present *P. persica* is being used for its high nutritional value and medicinal properties as well. It is known to be one of the most important and popular commodities consumed worldwide due to its delicious flavor and attractive appearance as well as exhibiting a high nutritional value because of its high levels of major antioxidants and anti-carcinogenic compounds, including vitamins (A, C and E), carotenoids and phenolics (Gil et al., 2002). Extensive studies of the chemical components of *Prunus persica* have resulted in the identification of different classes of phytochemicals phenolic acids, flavonoids, triterpenoids etc.

Apart from the uses as high nutritional food, it is proven to be effective for its antioxidant (Altemimi et.al.,2016), chemopreventive (Aubert et.al.,2014) etc. properties. *Prunus persica* has been included in the traditional Chinese medicine prescription to treat blood stasis. Fukuda et al. reported that several isolated glycosides from *Prunus persica* seeds can inhibit *in vitro* and *in vivo* tumorigenesis (Fukuda et al., 2003). A finding showed recently that the polyphenolic extract of *Prunus persica* fruit can suppress breast cancer cell proliferation, tumor growth (Noratto et.al.,2014; Vizzotto et.al.,2014) and lung metastasis (Noratto, et.al, 2014).

The bioactive components of fruit species vary with respect to the species and to the cultivars (Gil et al., 2002). The characterization of the phenolic profile in *P. persica* fruit could represent a useful tool for authenticity control (Scordino et al., 2012). Some studies have been done on Indian *P. persica* variety for its quality assessment, but not for its phytochemical profile or any disease related characteristics. So, to meet the discrepancy of knowledge, the project

intended to be an initiative to fill the gap with the exploration of *P. persica* based bioactive compounds from locally available variety in Assam, for their anti-inflammatory characteristic.

Objective 1:

To study the extraction of different phytochemical compounds from peel, pulp, and seeds of *Prunus persica*

Methodology:

Materials:

Chemicals and reagents:

Chemicals and reagents used in the project were purchased from HiMedia Laboratories Pvt. Ltd. and Sigma-Aldrich (USA). The Norabogori was collected from homestead garden of Napaam, Tezpur, Assam, India (latitude 26.651218, longitude 92.783813).

Sample identification and authentication:

Norabogori is a sparsely available fruit in Assam. Characteristically it is a peach fruit variety having slight color difference than popular peach variety. It has been identified to be *Prunus persica* (L.) Batsch of Rosaceae family. The data have been authenticated by The Herbarium of Botany Department of Gauhati University (Acronym: GUBH) with reference no. Herb./GUBH/2020/141 and an accession 19001 (Collection No. UN1) has been given.

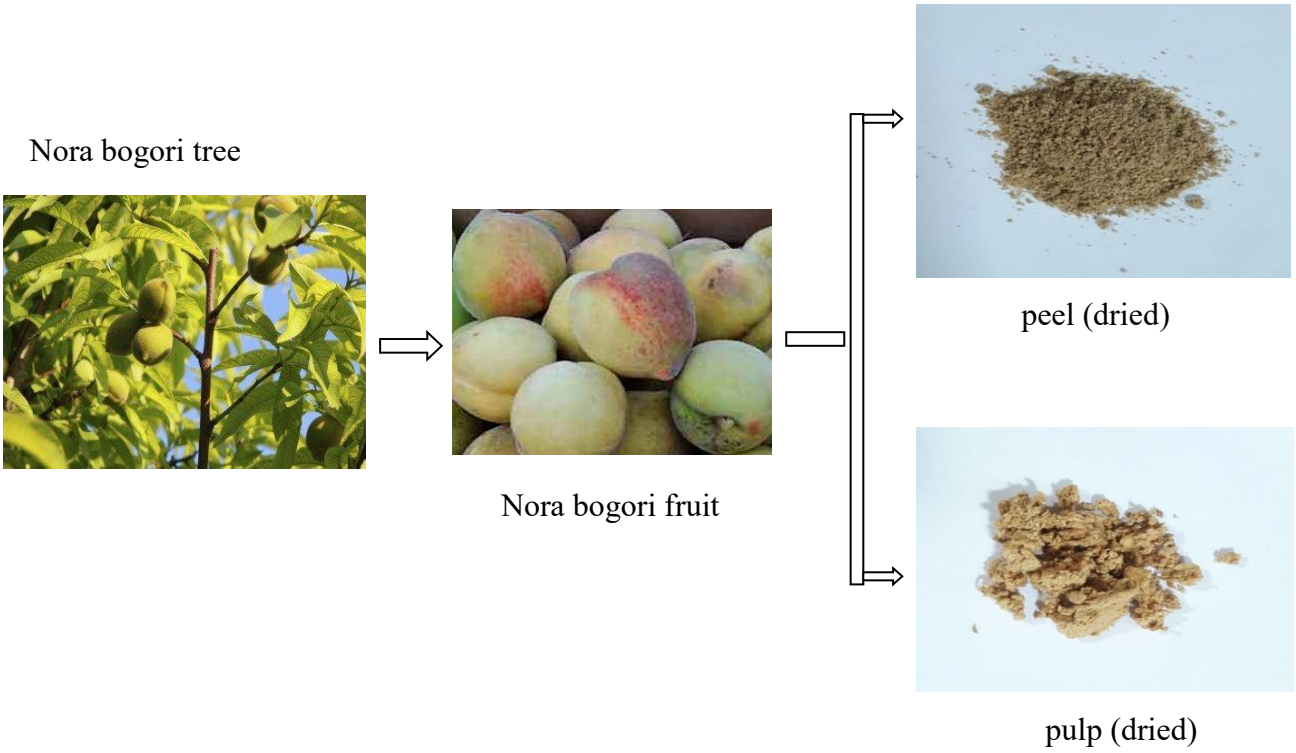


Fig 1: Plant, Fruit and dried Fruit sample of Nora bogori ((*Prunus persica* (L.) Batsch)

Physiochemical properties: The chemical composition of fruit is determined by proximate analysis according to AOAC method.

The moisture, ash, protein, and crude fat contents were determined according to methods described in AOAC. Ash content was determined by ignition in a muffle furnace (Optic Ivymen System, SNOL 8, 2/1100, Utena, Lithuania) at 550°C for 6 h. Nitrogen content was determined using the Kjeldahl apparatus (KelPlus, Pelican Equipment, Chennai, India). Crude fat was determined using the Soxhlet extractor (SocsPlus, Pelican Equipment, Chennai, India) with n-hexane as solvent. Crude fiber was determined following the acid and alkali treatment as described by Maynard (Maynard. et. al., 1970) and Sadasivam and Manikam (2008). The carbohydrate content was measured by hydrolyzing the polysaccharides (acid hydrolysis) into simple sugars and estimating the resulting monosaccharide by anthrone method (Hodge, et. al.,1962; Ranganna, et. al., 2008).

Total phenolic content (TPC)

Total phenolic content was determined with the Folin-Ciocalteu (F.C.) colorimetric method of Malick and Singh (1980) Phenols were reacted with phosphomolybdate which is an oxidizing agent in F.C. reagent under alkali conditions and result in the formation of a blue coloured complex which was measured at 725 nm colorimetrically. The sample has been taken 1mg/ml extract in water. Briefly, 400 µl of Na_2CO_3 solution was added to 100 µl of the extract and 500 µl of FCR and incubated at 22°C for 2 h. The phenolic content was calculated from gallic acid standard curve and finally expressed as mg/g gallic acid equivalent (GAE).

Total flavonoid content (TFC)

Total flavonoid content was determined with $AlCl_3$ following Chang et al. (2002) protocol. The sample extract was dissolved in 95% ethanol at 1 mg/ml concentration in a test tube. 10% aluminum chloride solution and 1 M potassium acetate was added to the sample and then incubated for 40 min at room temperature (25°C) in a dark chamber. The absorbance was read at 415 nm and the TFC was expressed in catechin equivalent (mg Catechin/100 g) (Sultana, et. al., 2008).

Antioxidant activity

DPPH free radical scavenging activity

The DPPH radical scavenging activity of the nora bogori extract was measured with the method of Brand-Williams et al. (1995) and the assay is based on the ability of antioxidant to scavenge the DPPH cation radical. This method determines the hydrogen donating capacity of molecule and does not produce oxidative chain reactions or react with free radical intermediates. The percentage inhibition of the DPPH radical scavenging activity was calculated colorimetrically at 517 nm after 30 minutes of incubation in dark using following formula

% DPPH radical scavenging activity = (Abs control−Abs test sample)/Abs control X 100%

Ferric-reducing antioxidant power (FRAP) assay:

The Ferric-reducing antioxidant power (FRAP) analysis involved the method of Benzie and Strain (1996) and was expressed in mg ascorbic acid equivalent/1g dry weight. FRAP assay is a singlet

electron transformer method which is based on the reduction capability of ferrous ion (Fe3+) to ferric ion (Fe2+) of a test sample. FRAP reagent formed by assimilation of the acetate buffer (0.3M, pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in HCL (40 mM) and $FeCl_3 \cdot 6H_2O$ (20 mM) in 10:1:1 ratio. The sample extracts (10 µL) were allowed to react with 290 µL of the FRAP reagent, the reaction mixture was kept in room temperature for 15 min and the absorbance was recorded at 595 nm. (Parit, et.al., 2018)

Extraction optimization:

Sample preparation:

The norabogori fruits were washed, dried, removed seeds. The remaining part freeze-dried in a lyophilizer at -80^0c overnight and homogenized in a blender before being kept at 4°C prior to analysis.

Extraction of phytochemicals:

After an extensive and careful literature review, a number of extraction methods like microwave assisted extraction, ultrasound assisted extraction, Supercritical fluid extraction etc. found to be advanced and emerging for effective extraction of phytochemical. From these, microwave assisted extraction has not been much explored for this specific sample. To gain an extensive insight and efficient extraction it has been chosen to use this method on Nora bogori.

At first five solvents (Methanol, Acetone, Ethanol, Hexane and Chloroform) were screened for maximum phenolic content in the extract. The dried fruit was extracted in different solvents at a fixed concentration (50%) first in 1:40 ratio (solid: solvent) at room temperature conditions in a shaking incubator (Certomat 1S, Sartorius) for 7 h. After completion of the extraction time, the crude extract was centrifuged at 5000 rpm (Hettich-Zentrifugen, Germany) for 20 min. The supernatant was collected and analysed for apparent phenolic content and selected one solvent based on that (V. González de Peredo, et.al., 2018).

Experimental design for optimisation using Box-Behnken design (BBD) by response surface methodology (RSM)

For the optimisation of the extraction process, the response surface methodology (RSM) based on a Box-Behnken design (BBD) model was applied. Four independent variables were taken viz., microwave power(watt) (400-900), solvent concentration (%) (50-100), solid-liquid ratio(ml/gm) (20-40) and time(min) (5-15) (Table 1). The design consisted of 29 experiments including independent variables were microwave power (X_1, watt), solvent concentration (X_2%), solid-liquid ratio(X_3, ml/gm) and time(X_4, min) while the dependent or response variables were apparent phenolic content (TPC, mg GAE/100g) (V. González de Peredo, et.al., 2018).

Table 1. BBD variables (microwave power, solvent concentration, solid-liquid ratio and time) and responses

Sl no.	Microwave Power (watt)	Ethanol concentration (etoh%)	solid liquid ratio (ml/gm)	time (min)	TPC (mg GAE/100g)
1	650	75	30	10	4113.2
2	400	75	20	10	3810
3	650	50	30	15	4600
4	650	50	20	10	2543
5	650	75	30	10	4613.2
6	650	100	20	10	3250
7	900	75	30	15	3749
8	650	100	40	10	2380
9	400	75	40	10	5370
10	650	75	40	5	3196
11	900	100	30	10	2132.5
12	650	75	40	15	6700
13	900	50	30	10	2975
14	400	75	30	15	4567
15	400	75	30	5	4967.5
16	650	75	20	5	4146
17	650	75	30	10	4513.2
18	900	75	40	10	2600
19	650	75	20	15	2654
20	650	75	30	10	4335.2
21	400	100	30	10	3256
22	650	100	30	15	4354
23	650	50	40	10	5135
24	900	75	20	10	3132
25	900	75	30	5	2787

26	650	75	30	10	4246.2
27	400	50	30	10	4665
28	650	100	30	5	4183
29	650	50	30	5	4786

The developed quadratic models in terms of coded values of all the factors are as follows:

$TPC = +4364.20 -771.67\ x_1 -429.04\ x_2 +487.17\ x_3 +213.21\ x_4 -511.47\ x_1^2 -480.41\ x_2^2 -398.35\ x_3^2 +323.34\ x_4^2 +141.63\ x_1 x_2 -523.00\ x_1 x_3 +340.63\ x_1 x_4 -865.50\ x_2 x_3 +89.25\ x_2 x_4 +1249.00\ x_3 x_4$

Design expert 7.0 software was used to generate response surfaces and the plot. The data were statistically analysed by ANOVA. The *p* values of $\leqslant 0.01$ were considered to be statistically significant. Response variables viz. apparent phenolic content (Slinkard & Singleton, 1977) were determined spectrophotometrically.

RP - HPLC study of the polyphenols

For comparison of polyphenols in the extract of Norabogori fruit, RP-HPLC study was conducted. RP-HPLC (Waters) gradient elution method was used to identify the major bioactive compounds composition whole extract. Symmetry 300™ C_{18} (5 μm, 4.6 × 250 mm) column with a binary pump (Waters, 1525) and a UV–vis detector (Waters, 2489) was used. The compounds were extracted from the extract under optimised conditions; the ethanolic extract was evaporated in hot air oven, and then redissolved in 1 mL methanol and then filtered through a membrane filter (0.22 μm) before injection.

Mobile phases used were acidified ultrapure water (0.1% acetic acid, pH 3.2, mobile phase A) and methanol (mobile phase B). The gradient method: 80% A (0–8 min), 65% A (9–12 min), 45% A (13–16 min), 30% A (17–20 min), 20% A (21–30 min), 10% of A (31–34 min) and then washing of the column with 65% A (35–39 min) and lastly, 80% A (40–45 min) was followed.

Sample volume of 20 µL was used. The flow rate was maintained at 0.8 mL/min and wavelengths used for UV–vis detector were 254 nm and 325 nm. The standards used for comparison and identification were (+) catechin, caffeic acid, gallic acid, rutin, quercetin and ferulic acid. (Muchahary, et.al.,2021)(Saikia, et.al., 2015)

Results and discussions:

Sample processing and store

Nora bogori fruits were washed thoroughly under running water followed by distilled water and spread out on absorbent tissue papers to remove surface moisture. The pulp and peel samples were separated using a stainless-steel knife. The pulp samples were cut into ~6 mm thick slices and dried in a freeze drier (Lyolab Freeze Lab, Lyophilization Systems Inc., USA) at -80ºC for 12 h. Dried samples were ground using mechanical grinder (Fritsch, Germany), sieved, and stored at ambient temperature (25±2^{o}C) in airtight containers till the time of analyses.

Physiochemical properties

Table 2. Proximate composition of the Nora bogori fruit

Part of the fruit	**Crude fibre (g/100g dry weight)**	**Protein (mg/g dry weight)**	**Fat (g/100g dry weight)**	**Total carbohydrates (g/100g dry weight)**
Pulp	10.9 ±0.76	13.0±0.26	0.62 ±0.09	68±3.24
Peel	18.1 ±0.23	7.4±0.11	0.56±0.06	59.31±2.87

Table 3. Total phenolic and flavonoid content of norabogori

Part of the fruit (Dry)	Total phenolic content (mg GAE/g)	Total Flavonoid content (mg catechol/100g)
peel	5120 ± 0.61	58.67 ± 0.14
pulp	45.83± 0.45	69.05± 0.23

The proximate composition, total phenolic and flavonoid content of pulp and peel from norabogori nora bogori fruit was giving nearly similar values and both could be taken as potential source of bioactive compounds. So, after the preliminary analysis, peels and pulp are kept together and treated as one sample for the next part of the study.

Table 4. Antioxidant activity of Norabogori

DPPH Assay	FRAP assay (mg ascorbic acid equivalent/1g dry weight)
41%	2.8

Optimization of phytochemical compounds extraction from Norabogori (*Prunus persica*)

Extraction of phytochemicals

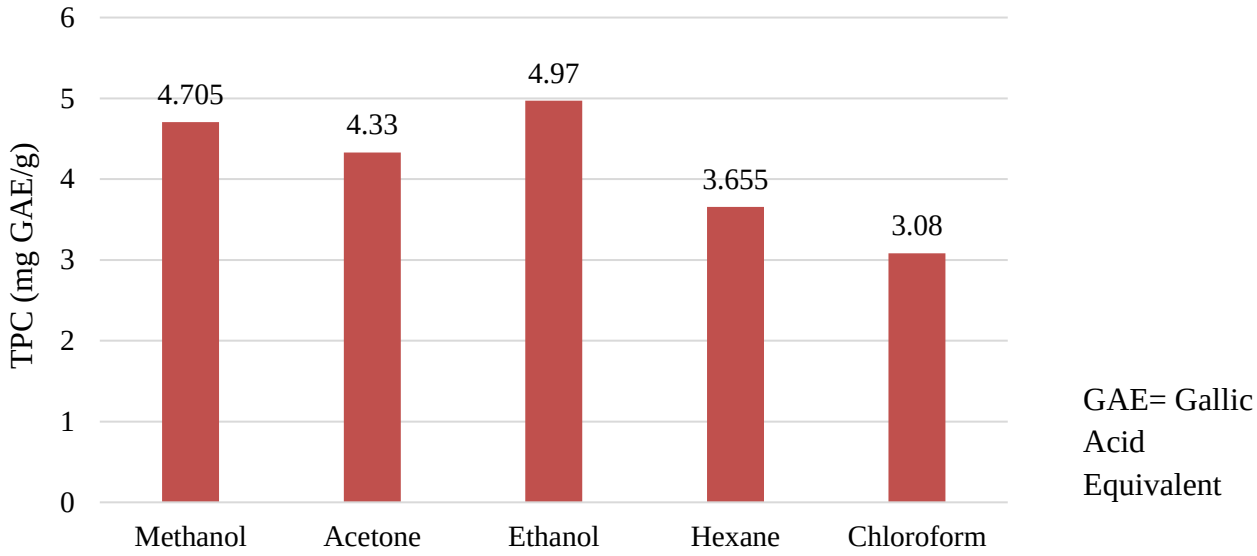


Fig 2. Effect of different solvents on Total Phenolic Content of the sample

Among five solvents screened, ethanol (4.97 mg GAE/g) is found to be highest yielding extraction solvent and so taken for the optimization procedure.

Generally, acetone is the best solvent for extracting proanthocyanidins and tannins; ethanol efficiently extracts flavonoids and their glycosides, catechols and tannins, whereas phenolic acids and catechin were better extracted with methanol. These facts are in agreement with polarity of the solvent used for the extraction and solubility of phenolics in them (Altemimi et. al., 2016). Therefore, there is no single solvent able to extract all of the classes of phenolic compounds from a sample, simultaneously. In the nora bogori sample, ethanol and methanol showed the highest TPC value and so they can be considered for the further extraction process. Between ethanol and methanol, due to the toxicity of methanol is more for any use like consumption, ethanol has been taken forward to the next sets of reactions.

Optimisation and fitting of the model

RSM was applied to determine the effect of microwave power, solvent concentration, solid-liquid ratio and time on total phenolic content of the norabogori extract. The results of the experiments performed for BBD of the variables along with the responses are given in Table. The regression coefficients obtained for the different responses and ANOVA results for the model responses are

presented in Tables, respectively. For the good fit of a model, the R^2 value should be >=0.80 (Joglekar & May, 1987). In the present study, R^2 values for the three responses were higher than 0.80 which implies the adequacy of the applied regression model. The lack of fit for all fitted models was found to be significant (p =< 0.05). Myers and Montgomery (2002) suggested that lack of fit measures the failure of the model to represent data in the experimental domain at points which are not included in the regression. Therefore, it can be assumed that the selected model can be used for the simulation and optimisation of variables for the phytochemicals extraction from norabogori.

Table 5. ANOVA (Analysis of variance) result of the fitted model for the response variable.

Variables	DF	Estimated Variables	F value
		TPC	TPC
Model	14	4364.20	11.40 a
x1	1	-771.66	39.89 a
x2	1	-429.04	12.33 b
x3	1	487.16	15.90 b
x4	1	213.20	3.04
$x1^2$	1	-511.47	9.47 b
$x2^2$	1	-480.41	8.35 c
$x3^2$	1	-398.35	5.74 c
$x4^2$	1	323.33	3.78
x1x2	1	141.62	0.44
x1x3	1	-523.00	6.10 c
x1x4	1	340.62	2.59
x2x3	1	-865.50	16.72 b
x2x4	1	89.25	0.17
x3x4	1	1249.00	34.83 a
Lack of fit			*0.052*
R^2			0.91

*P value, significance of difference a <0.001; b<0.01; c<0.05

Effect of ethanol concentration and solid liquid ratio on apparent phenolic content:

From the response plot (Figure 3), it was observed that initial rise in solid liquid ratio up to 30ml/g resulted in increased apparent phenolic content value but beyond 30ml/g slight drop occurred. However, ethanol concentration had imparted greater positive effect compared to temperature. The interaction effect between the two variables showed a positive effect on the response. The regression equation obtained for apparent phenolic content is given below:

$$Y=+2255.80+4.17X_1+42.03X_2-53.21X_1^2-40.09X_2^2+55.63X_1X_2$$

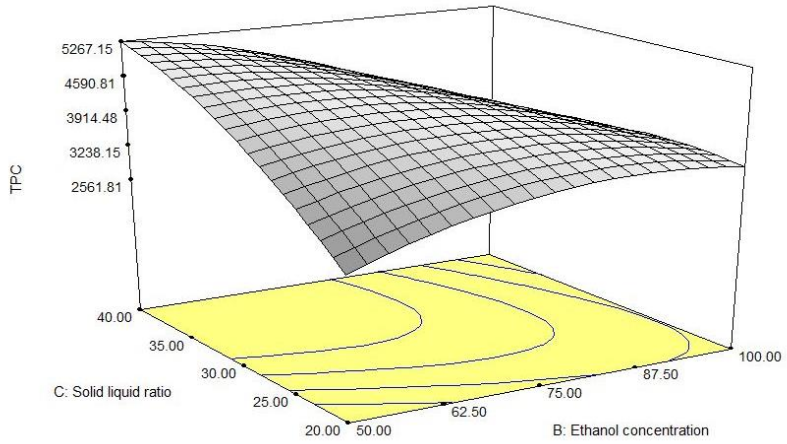


Fig 3. Effect of ethanol concentration and solid liquid ratio on apparent phenolic content

Effect of ethanol concentration and microwave power on apparent phenolic content:

In response plot for variables on phenolic content (Figure 4), it was seen that high microwave power had a detrimental effect on the total phenolic content of the extract. However, increase in ethanol concentration showed a gradual rise and then slight drop in apparent phenolic content value. A positive interaction effect between the microwave power and ethanol concentration was observed. The regression equation for the response TPC is given below:

$$Y=+15108.73-314.99X_1+264.13X_2-403.19X_1^2-308.4622+611.81X_1X_2$$

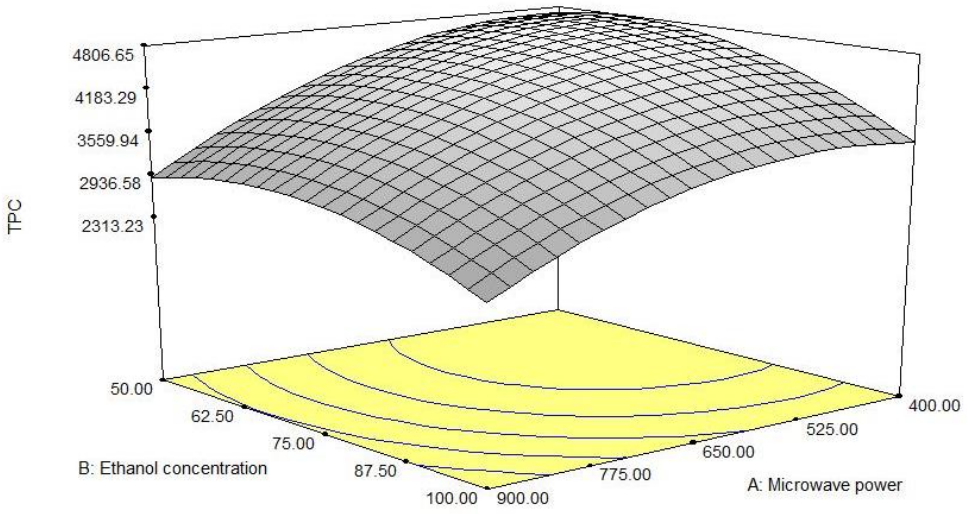


Fig 4. Effect of ethanol concentration and microwave power on apparent phenolic content

Effect of microwave power and time on apparent phenolic content:

From the response plot (Figure 5), it was observed that initial rise in microwave power up to 650 watt resulted in increased apparent phenolic content value but beyond 775watt slight drop occurred. However, ethanol concentration had imparted greater positive effect compared to time. The interaction effect between the two variables showed a positive effect on the response. The regression equation obtained for apparent phenolic content is given below:

$$Y=+2255.80+4.17X_1+42.03X_2-53.21X_1^2-40.09X_2^2+55.63X_1X_2$$

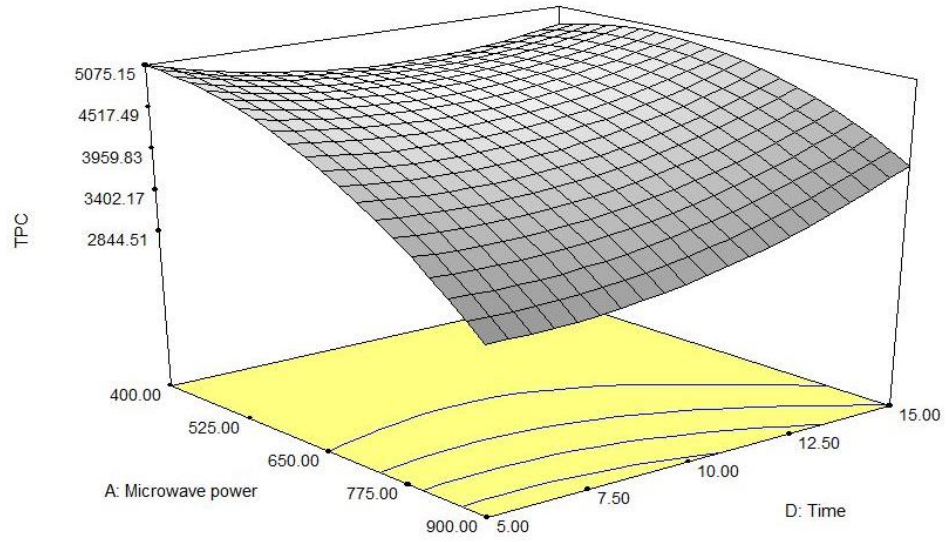


Fig 5. Effect of microwave power and time on apparent phenolic content

Effect of time and ethanol concentration on Total Phenolic Content

The selected variable conditions had positive effect (Figure 6) on TPC. With increase in time and ethanol concentration, increased TPC activity was observed. In this case also, interaction effect was found to be positive on TPC activity. The regression equation for the response TPC is given below:

$$Y=+97.33+0.73X_1+0.49X_2-0.65X_1^2-0.36X_2^2+0.82X_1X_2$$

The extraction of polyphenols from the plant sources depends on number of factors like polarity of the solvent used, solubility of the phenolic acids present, the interaction of these phenolic acids with other plant compounds which consequently could lead to development of newer complexes that may be soluble or insoluble in a given solvent. Therefore, it is quite difficult to develop a standard extraction method that would be suitable for all the phenolic compounds (Naczk & Shahidi, 2006). The apparent phenolic content of a particular sample may vary with the use of different solvent and extraction conditions.

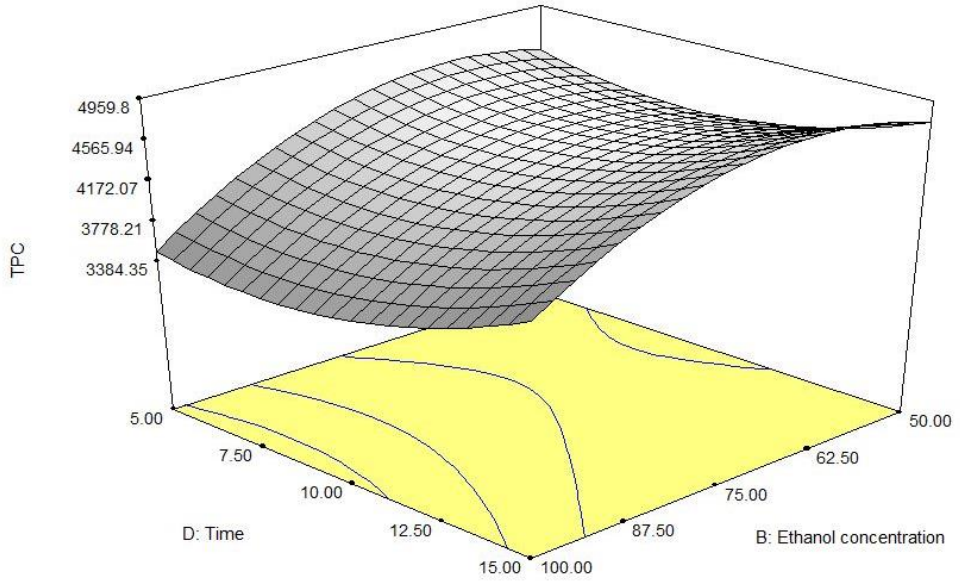


Fig 6. Effect of time and ethanol concentration on Total Phenolic Content

Verification of the predictive model

The suitability of the model for the responses was determined under the optimum conditions of 1g sample in 40ml 67% ethanol extracted with a microwave power of 570 watt for 15min the maximum yield (6824.38 µg GAE/g) is obtained. The experimental values for the responses were found to be quite comparable and in agreement with that of the predicted value (Table).

Table 6: Optimized solution obtained using the response optimizer

	Microwave power (watt)	Ethanol concentration (%)	Solid liquid ratio (ml/g)	Time (min)	TPC
Predicted values	571.82	66.55	39.48	14.79	6701.58
Experimental value	570	67	40	15	6824.38

The study showed that the microwave power, ethanol concentration and sample-solvent ratio influence the yield of phenolics. Also, the combination of microwave power and solid-liquid ratio, ethanol concentration and solid-liquid ratio and time and solid-liquid ratio shows efficient effect on the yield. When 1g sample in 40ml 67% ethanol extracted with a microwave power of 570 watt

for 15min the maximum yield is obtained. The values are predicted theoretically and experimentally found to be true.

RP - HPLC study of the polyphenols

RP- HPLC (Waters)
Solvent A: 0.1% acetic acid in Milli Q water
Solvent B: 100% Methanol
Standards were prepared in methanol
Sample volume: 20µl, 10mg/ml
Flow rate: 0.8 ml/min
Wavelengths: 254 nm
Column: 5µm, 4.6 ×250mm, Binary pump: Waters, 1525, UV- vis detectors: Waters, 2489

Table 7: Gradient method

Time (min)	A (%)	B (%)
0-8	80	20
9-12	65	35
13-16	45	55
17-20	30	70
21-30	20	80
31-34	10	90
35-39	65	35
40-45	80	20

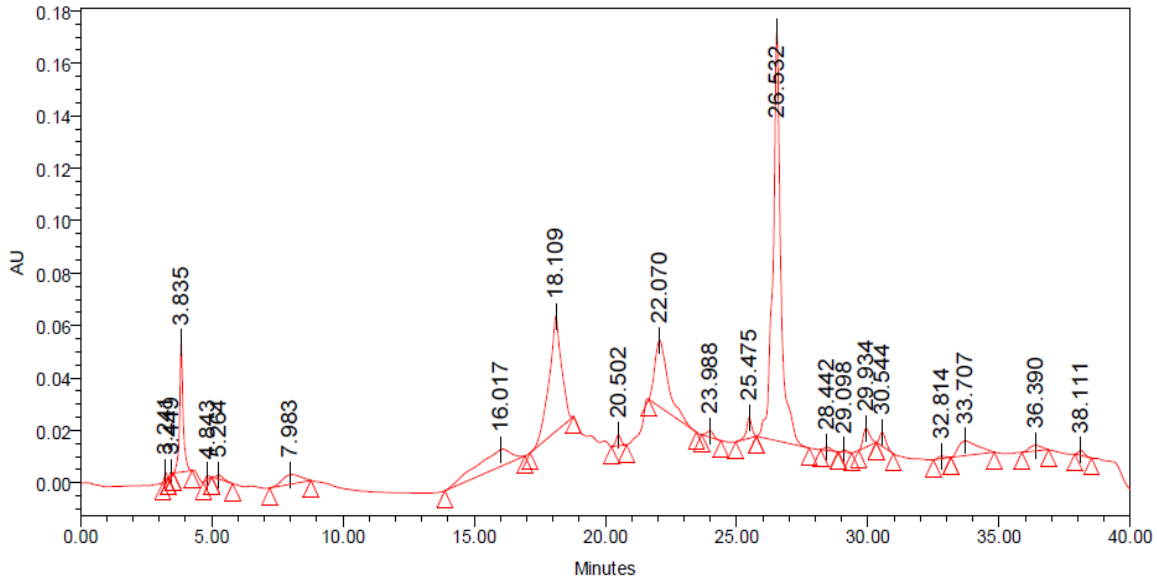


Fig 7. RP-HPLC chromatogram in 254 nm wavelength

Table 8: Identification of phenolics in RP-HPLC

Phenolics	**µg/g of dried sample**
Gallic acid	1800
Rutin	8287.095
Catechin	ND
Ferulic acid	ND
Quercetin	2355.499
Caffeic acid	2112.3

The chromatograms of the polyphenol extract separated by RP-HPLC detected Rutin and number of phenolic acids (Table 8 and Figure 7). As the peak intensity of the sample at 325 nm was not that high and clear, only the peaks obtained at 254 nm were considered. The obtained peaks were compared with the retention time of their respective standards and identified. The phenolic acids identified in crude extract were, gallic acid (RT = 3.8 min), catechin (RT = 7.9 min), chlorogenic acid (RT = 16.1 min), caffeic acid (RT = 22.1 min), ferulic acid (RT = 20.5), rutin (RT = 26.5 min), quercetin (RT = 18.1 min). The rutin content was found to be

52.87 ± 0.07 mg/100 g. Among the phenolic acids in the crude extract, the concentration of caffeic acid (21.12 ± 0 mg/100 g) and quercetin (23.55± 0.02 mg/100 g) was highest followed by gallic acid (18.0 ± 0.05 mg/100 g). The concentration of chlorogenic acid (16.25± 0.02 mg/100 g), catechin (8.41 ± 0.02 mg/100 g) and ferulic acid (10.54 ± 0.04 mg/100 g) were relatively low in the extract.

Objective 2

To study the extracted phytochemicals for anti-inflammatory effect by *in silico* method

Using the previously determined optimized conditions, extract from the sample has been prepared for High Resolution Liquid Chromatograph Mass Spectrometer (HR-LCMS) analysis. The HR-LCMS of the selected extract was carried out by using Q-Exactive Plus Biopharma-High Resolution Orbitrap Liquid Chromatograph Mass Spectrometer (6550 iFunnel Q-TOFs, Agilent Technologies, USA) in SAIF, IIT Bombay, Mumbai, India. For the detection of compounds, Electrospray Ionization (ESI) followed by the MS spectra of the analyzed samples were compared with the spectra from the library mzCloud.

Lipinski rule of five:

Lipinski rule of five gives away the drug likeliness property of bioactive compounds. According to the Rule of Five, a molecule would not be orally active if it violates two or more of the four rules (Lipinski) (6). The values of the concerned properties were estimated from the databases like PubChem and Chemspider. The rules are

1. Molecular weight (MW) ≤ 500
2. Octanol/water partition coefficient ($A \log P$) ≤ 5
3. Number of hydrogen bond donors (HBDs) ≤ 5
4. Number of hydrogen bond acceptors (HBAs) ≤ 10.6.

Veber's Rule:

Similar to Lipinski rule, Veber's rule evaluates the oral bioavailability of bioactive compounds. The properties were estimated from PubChem and Chemspider databases (7). Based on Veber's rule, orally bioavailable drugs should have

1. The number of rotatable bonds below or equal to 10 and
2. Topological polar surface area (TPSA) values ≤ 140 $Å^2$

ADME and Toxicity Profiles

The physicochemical and pharmacokinetics properties of the selected compounds were estimated using ADME (absorption, distribution, metabolism and excretion) descriptors by a SwissADME

online server (**http://www.swissadme.ch/**). Chemical absorption, distribution, metabolism, excretion (ADME), play key roles in drug discovery and development. A high-quality drug candidate should not only have sufficient efficacy against the therapeutic target, but also show appropriate ADME properties at a therapeutic dose (Khan et.al., 2022).

To explore the toxicity profiles of the compounds like Ames toxicity, carcinogenic properties, acute oral toxicity, rat acute toxicity are taken as a basis for screening.

Molecular docking evaluation:

Docking was carried out using AutoDock Vina (version 1.1.2) to predict the binding affinity of the identified compounds to the active site of the enzyme. From Protein Data Bank (PDB), the 3D structure of anti-inflammatory marker protein Tumor Necrosis Factor a was (PDB code: 5UUI) identified and collected from the RCSB PDB database as *.pdb file. The 3d structure of bioactive compounds was collected from Pub Chem in *.sdf format. All the structures of the identified compounds were changed to *.pdb format by Open Babel (version 2.3.1). Then AutoDock Tools (version 1.5.6) was used to convert the compounds in *.pdb format to *. Pdbqt, prior to docking. Gasteiger charges and hydrogen atoms were added. The rotatable bonds were set using AutoDock Tools. The top-levelled docking conformations were compared with the actual crystallographic conformation based on the values of root mean square deviation (RMSD). The grid box parameters were centered at the coordinate of 21.272, −0.751 and 18.634. The sizes of the grid box for X, Y, and Z were 20 Å, 26 Å and 22 Å, respectively (Hari, 2019). The docking was performed in triplicate. All docking outcomes were analyzed by AutoDock Vina Visualizer.

Tumor Necrosis Factor a:

It is the cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is primarily secreted by macrophages and can induce cell death of certain tumor cell lines. Thus it participates in inflammatory mechanism.

RCSB PDB - Crystal Structure of Spin-Labeled T77C TNFa

RCSB PDB entry- 5UUI

Sequence length – 158 Amino acids

Results and discussion:

Lipinski rule of five:

Table 9: Lipinski rule of five analysis of phytochemicals

Name	Molecular Weight	Xlogp	Hydrogen Bond Donor Count	Hydrogen Bond Acceptor Count
(±)-Malic Acid	134.09	1.3	3	5
Kojic acid	142.11	0.9	2	4
Adipic acid	146.14	0.1	2	4
4-Hydroxycoumarin	162.14	1.3	1	3
Gallic acid	170.12	0.7	4	5
Caffeic acid	180.16	1.2	3	4
Citric acid	192.12	1.7	4	7
D-(-)-Quinic acid	192.17	2.4	5	6
(E)-parinaric acid	276.4	5.9	1	2
Chlorogenic acid	354.31	0.4	6	9
Asiatic acid	488.7	5.7	4	5
Quercetin 3β-rutinoside	610.5	1.3	10	16
Hydroxycinnamic Acids	164.16	1.5	2	3
Catechin	290.27	0.4	5	6
Quercetin	302.23	1.5	5	7

Veber's Rule:

Table 10: Veber's rule analysis of phytochemicals

Name	Rotatable Bond Count	TPSA (Å²)
(±)-Malic Acid	3	94.8
Kojic acid	1	66.8
Adipic acid	5	74.6

4-Hydroxycoumarin	0	46.5
Gallic acid	1	98
Caffeic acid	2	77.8
Citric acid	5	132
D-(-)-Quinic acid	1	118
(E)-parinaric acid	12	37.3
Chlorogenic acid	5	165
Asiatic acid	2	98
Quercetin 3β-rutinoside	6	266
Hydroxycinnamic Acids	2	57.5
Catechin	1	110
Quercetin	1	127

Table 11. ADME analysis of phytochemicals

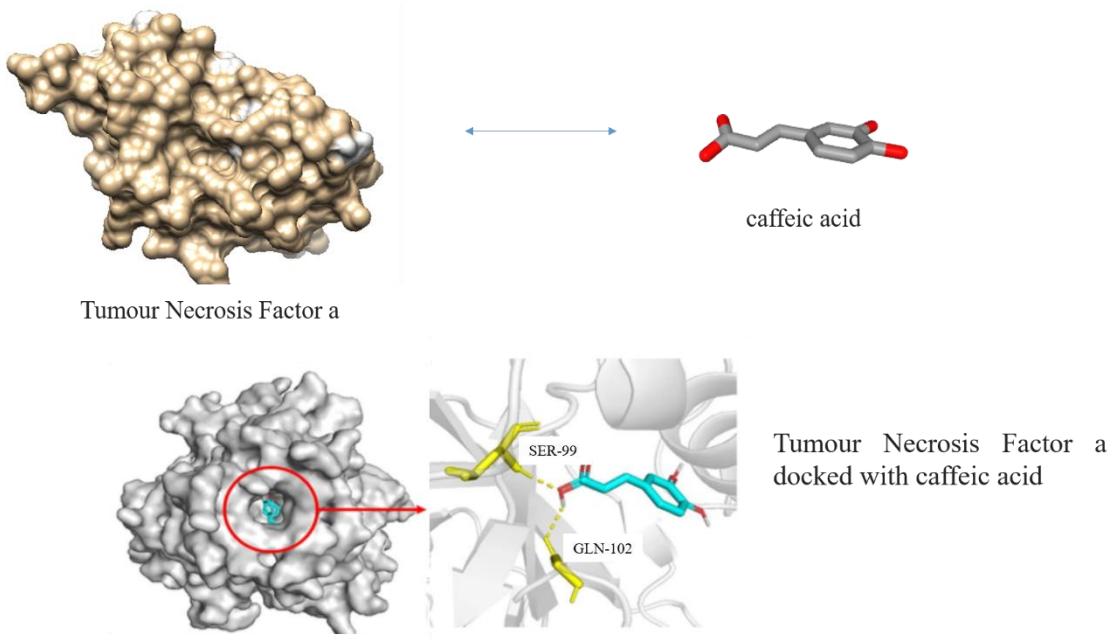
Molecule	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Bioavailability Score
(±)-Malic Acid	High	No	No	No	No	No	No	No	0.56
Kojic acid	High	No	No	No	No	No	No	No	0.55
Adipic acid	High	No	No	No	No	No	No	No	0.85
4-Hydroxycoumarin	High	Yes	No	Yes	No	No	No	No	0.55
Gallic acid	High	No	No	No	No	No	No	Yes	0.56
Caffeic acid	High	No	No	No	No	No	No	No	0.56
Citric acid	Low	No	No	No	No	No	No	No	0.56
D-(-)-Quinic acid	Low	No	Yes	No	No	No	No	No	0.56
(E)-parinaric acid	High	Yes	No	Yes	No	Yes	No	No	0.85
Chlorogenic acid	Low	No	No	No	No	No	No	No	0.11
Asiatic acid	High	No	Yes	No	No	No	No	No	0.56
Quercetin 3β-rutinoside	Low	No	Yes	No	No	No	No	No	0.17
Hydroxycinnamic Acids	High	Yes	No	No	No	No	No	No	0.85
Catechin	High	No	Yes	No	No	No	No	No	0.55
Quercetin	High	No	No	Yes	No	No	Yes	Yes	0.55

Toxicity evaluation

Table 12. Toxicity evaluation of Phytochemicals

Molecule	AMES Toxicity	Carcinogens	Rat Acute Toxicity (LD50 mol/kg)	Biodegradation	Caco-2 Permeability (LogPapp, cm/s)
(±)-Malic Acid	Non AMES toxic	Non-carcinogens	1.61	Yes	-0.9145
Kojic acid	AMES toxic	Non-carcinogens	2.0673	Yes	0.7808
Adipic acid	Non AMES toxic	Non-carcinogens	1.4221	Yes	0.4043
4-Hydroxycoumarin	Non AMES toxic	Non-carcinogens	2.2141	No	1.3028
Gallic acid	Non AMES toxic	Non-carcinogens	1.867	Yes	0.0595
Caffeic acid	Non AMES toxic	Non-carcinogens	1.4041	Yes	0.3485
Citric acid	Non AMES toxic	Non-carcinogens	1.7748	Yes	-0.7274
D-(-)-Quinic acid	Non AMES toxic	Non-carcinogens	1.7528	No	-0.1052
(E)-parinaric acid	Non AMES toxic	Non-carcinogens	1.4895	yes	1.3712
Chlorogenic acid	Non AMES toxic	Non-carcinogens	2.5685	No	-0.6124
Asiatic acid	Non AMES toxic	Non-carcinogens	2.0501	No	0.3846
Quercetin 3β-rutinoside	Non AMES toxic	Non-carcinogens	0.5327	No	2.6133
Hydroxycinnamic Acids	Non AMES toxic	Non-carcinogens	1.2009	No	1.3698
Catechin	Non AMES toxic	Non-carcinogens	0.5189	yes	1.87
Quercetin	Non AMES toxic	Non-carcinogens	0.2245	No	3.02

Molecular Docking Evaluation:



Caffeic acid act on TNF; Binding affinity = -17.882 kcal/mol

Fig 8. Example of molecular docking pose of phytochemical with anti-inflammatory marker

Table 13. molecular docking evaluation of phytochemical with anti-inflammatory marker

Compounds	**Binding affinity (kcal/mol)**	**No. of H-bond**	**H-bond length (Å)**	**Binding residues**
(±)-Malic Acid	-13.639	2	3.07	Gly-68, Cys-69, Pro-100, Arg-103, Thr-105, Glu-110, Asn-112, Pro-113, Trp-114
Kojic acid	-16.724	3	2.98	Gly-24, Ser-65, Gly-66, Gln-67, Asp-140, Tyr-141, Leu-142, Asp-143, Phe-144
Adipic acid	-16.604	2	2.79	Gly-68, Cys-69, Pro-100, Arg-103, Thr-105, Asn-112, Trp-114

4-Hydroxycoumarin	-15.359	1	3.01	Ala-22, Glu-23, Gly-24, Ser-65, Gly-66, Gln-67, Pro-139, Asp-140, Thr-141, Leu-142, Asp-143, Phe-144
Gallic acid	-14.923	1	3.23	Thr-77, Thr-79, Ser-81, Ile-83, Pro-90, Asn-92, Ser-133, Glu-135, Ile-136, Asn-137
Caffeic acid	-17.882	2	2.56	Ser-65, Ser-99, Gln-102, Pro-139, Asp-140, Tyr-141, Leu-142, Asp-143, Phe-144, Ala-145
Citric acid	-15.337	2	2.89	Ser-9, Val-13, His-15, Leu-57, Tyr-59, Tyr-119, Tyr-151, Ile-155
D-(-)-Quinic acid	-17.459	3	3.20	Gly-68, Cys-69, Pro-100, Arg-103, Glu-110, Asn-112, Pro-113, Trp-114
(E)-parinaric acid	-14.241	2	2.95	Pro-20, Ala-22, Glu-23, Gly-24, Ser-65, Pro-139, Asp-140, Tyr-141, Leu-142, Phe-144, Ala-145
Chlorogenic acid	-15.845	1	3.03	Ser-65, Gly-66, Gln-67, Gly-68, Pro-70, Asp-140, Tyr-141, Leu-142, Asp-143, Phe-144, Ala-145
Asiatic acid	-15.669	2	3.12	Gly-24, Ser-65, Pro-139, Asp-140, Tyr-141, Leu-142, Asp-143, Phe-144, Ala-145
Quercetin 3β-rutinoside	-16.232	3	2.91	Ser-65, Pro-139, Asp-140, Tyr-141, Leu-142, Asp-143, Phe-144, Ala-145
Hydroxycinnamic Acids	-17.375	2	3.19	Pro-20, Ala-22, Glu-23, Gly-24, Leu-142, Phe-144, Ala-145
Catechin	-16.945	3	2.88	Ser-65, Gly-66, Gln-67, Ile-83, Pro-90, Asn-92, Ser-133, Glu-13
Quercetin	-17.138	3	2.86	Cys-69, Pro-100, Arg-103, Thr-105, Asn-112, Trp-114, Leu-142, Phe-144, Ala-145, Tyr-151, Ile-155

Conclusion:

Compounds like Caffeic acid, Hydroxycinnamic Acids, Catechin etc. present in the extract optimized in objective 1 showed potential drug likability with their structure. These compounds also showed good interaction towards anti-inflammatory marker protein in docking studies.

Table 14. Overall *in silico* anti inflammatory analysis of phytochemicals present in norabogori

Lipinski Rule	**Veber's rule**	**ADME Evaluation**	**Toxicity Evaluation**	**Molecular Docking Evaluation**
(±)-Malic Acid	(±)-Malic Acid	(±)-Malic Acid	(±)-Malic Acid	(±)-Malic Acid
Kojic acid	Kojic acid	Kojic acid	Kojic acid	Kojic acid
Adipic acid	Adipic acid	Adipic acid	Adipic acid	Adipic acid
4-Hydroxycoumarin	4-Hydroxycoumarin	4-Hydroxycoumarin	4-Hydroxycoumarin	4-Hydroxycoumarin
Gallic acid	Gallic acid	Gallic acid	Gallic acid	Gallic acid
Caffeic acid	Caffeic acid	Caffeic acid	Caffeic acid	Caffeic acid
Citric acid	Citric acid	Citric acid	Citric acid	Citric acid
D-(-)-Quinic acid	D-(-)-Quinic acid	D-(-)-Quinic acid	D-(-)-Quinic acid	D-(-)-Quinic acid
(E)-parinaric acid	(E)-parinaric acid	(E)-parinaric acid	(E)-parinaric acid	(E)-parinaric acid
Chlorogenic acid	Chlorogenic acid	Chlorogenic acid	Chlorogenic acid	Chlorogenic acid
Asiatic acid	Asiatic acid	Asiatic acid	Asiatic acid	Asiatic acid
Quercetin 3β-rutinoside	Quercetin 3β-rutinoside	Quercetin 3β-rutinoside	Quercetin 3β-rutinoside	Quercetin 3β-rutinoside
Hydroxycinnamic Acids	Hydroxycinnamic Acids	Hydroxycinnamic Acids	Hydroxycinnamic Acids	Hydroxycinnamic Acids
Catechin	Catechin	Catechin	Catechin	Catechin
Quercetin	Quercetin	Quercetin	Quercetin	Quercetin

Objective 3:

To study the in-vitro efficacy and anti-inflammatory mechanism of the screened phytochemical compound

Materials and methodology

Materials

The THP-1 cell line purchased from NCCS Pune, India. DMEM (Dulbecco's Modified Eagle Medium) and FBS (Fetal Bovine Serum) were purchased from Life Technologies, USA. MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) was purchased from Sigma Aldrich and other chemicals were purchased from HiMedia, India.

Cell culture

The THP-1 cell line was routinely maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco), and ZR-75-1 in RPMI1640 supplemented with 10% fetal bovine serum and 1% antibiotic. Cell lines were kept in a CO_2 incubator at 5% CO_2 and 37° C temperatures.

Cytotoxicity assay

In this assay, cells (5000 each) were plated in a 96 well plate and incubated for 48 hr. Cells were treated with different concentrations (0–125 µg/ml) of Norabogori extract up to 48 hr. Following incubation, cells were treated with MTT and incubated for 3.5 hr. The media was removed carefully and MTT dissolving solution was added and absorbance was taken at 590 nm wavelength using UV-Vis spectrophotometer (Multiscan Go, ThermoScientific).(Bisht et. al.,2020)

Effect of Norabogori extract on pro-inflammatory marker:

Cell Treatments

THP-1 cells were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C in 5% CO_2. THP-1 cells were treated with Norabogori extract in two concentrations (100 and 200 µg/ml). The cells, in the presence of bioactive compounds, were then incubated with LPS (100 ng/mL) for another 4 h to induce an inflammatory response. Cells were pre-treated with extract to ensure full uptake and equilibration prior to LPS stimulation, helping to rule out effects, due to kinetic differences in their absorption versus LPS action. The cell monolayer was immediately extracted for subsequent qRT-PCR analysis.

Isolation of total RNA and RT-PCR

Total RNA was isolated from LPS-stimulated cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Reverse transcription was

conducted for 5 min at 70°C and 5 min at 4°C with total RNA (1 µg) and GoScript Reverse Transcriptase (Promega Corporation). cDNA was subsequently amplified by RT-PCR with specific primers and GoTaq DNA polymerase (Promega Corporation). The PCR primer sets were as follows: COX-2 (sense, 5′-TGAGTGGTAGCCAGCAAAGC-3′; antisense, 5′-CTGCAGTCCAGGTTCAATGG-3′), TNF-α (sense, 5′-AGGGAGAGTGGTCAGGTTGC-3′; antisense, 5′-CAGCCTGGTCACCAAATCAG-3′), IL-1β (sense, 5′-CAAGGAGAACCAAGCAACGA-3′; antisense, 5′-TTGGCCGAGGACTAAGGAGT-3′). Thermal cycling was performed as follows: Initial denaturation (95°C for 2 min), amplification (95°C for 1 min; 55°C for 1 min and 72°C for 1 min, 35 cycles), and final extension (72°C for 5 min). After amplification, the PCR products were resolved by agarose gel electrophoresis on 1% agarose gel containing 1X TAE (Biosesang, Inc.) and 1X Midori green advance (Nippon Genetics Europe GmbH), and visualized using a LAS-3000 device (FujiFilm). The band intensity was determined using ImageJ software (version 1.38; National Institutes of Health).(Xue et.al., 2021)

Results:

Norabogori extract treatment did not show adverse cytotoxicity in the THP-1 cell-line in dose dependent manner (Fig.). Treatment with 25, 50, 100 and 125 µg/ml extract induced cell death by 98.54%, 89.89%, 91.77% and 82.28% in 48 hr incubation, respectively. The sample did not show 50% toxicity and it can be used for further anti-inflammatory activity analysis.

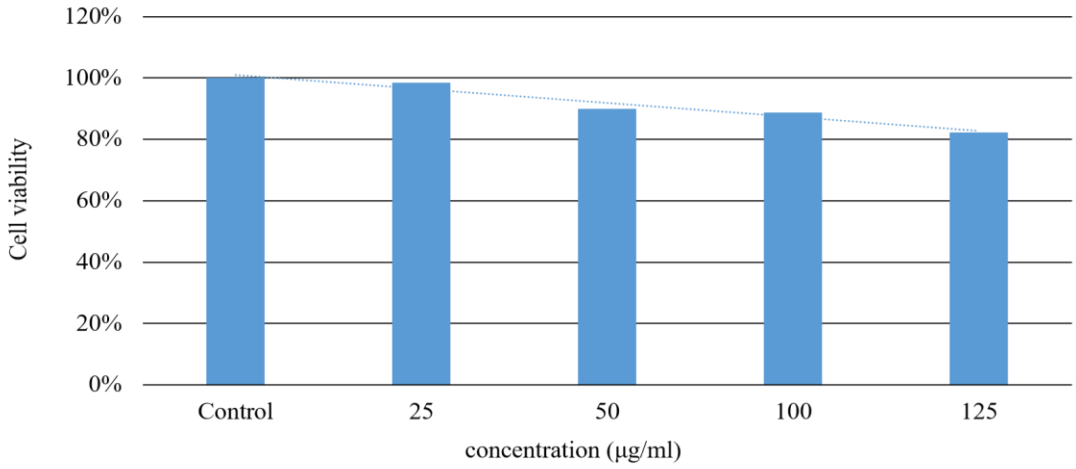
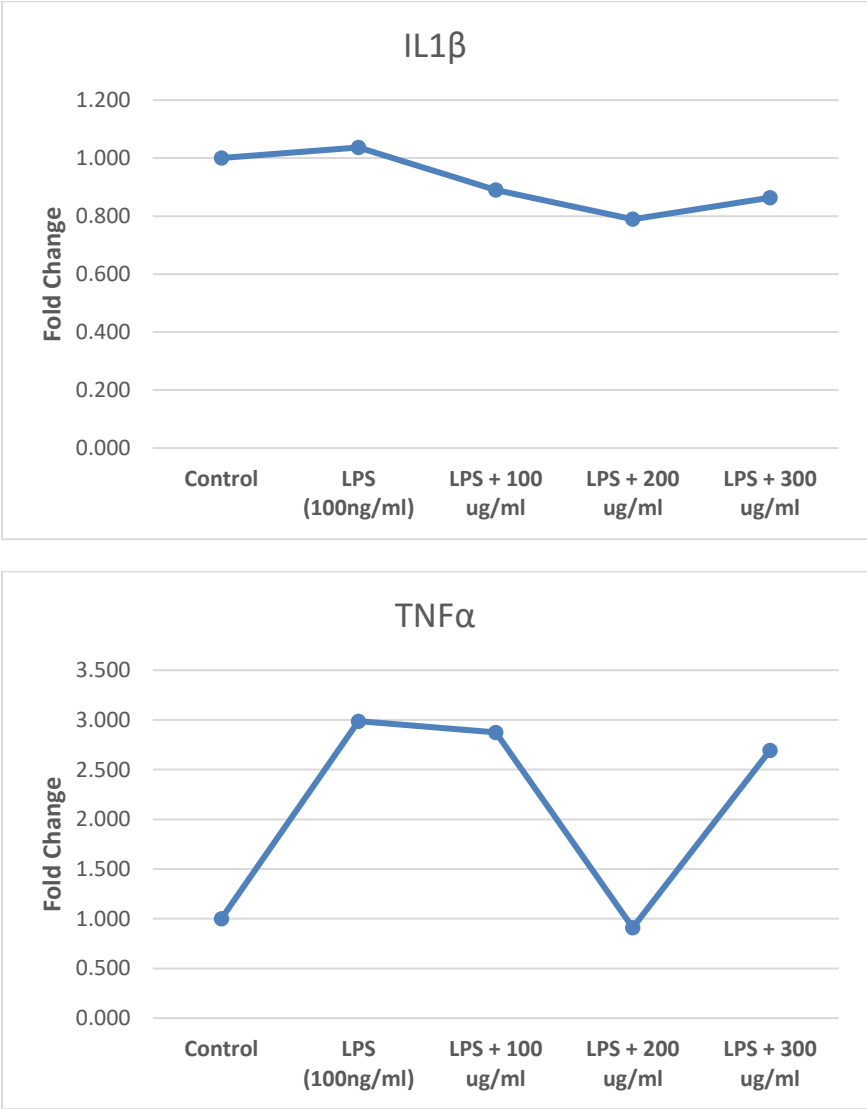


Fig 9. Cytotoxicity study of norabogori extract by MTT analysis

Effect of norabogori extract on pro-inflammatory genes

To investigate the molecular mechanisms by which the norabogori extract might exert its anti-inflammatory effects, we examined the mRNA expression of 3 pro-inflammatory genes (COX-2,

TNF-α and IL-1β) associated with inflammation. The relative mRNA levels of the genes were measured by qRT-PCR in THP-1 cells treated with the extract. Treatment of the THP-1 cells with the extract was able to downregulate the expression of subjected genes COX-2, TNF-α and IL-1β. Expression of the pro-inflammatory cytokines, was strongly suppressed by the extract treatment in different concentrations (Fig 10)



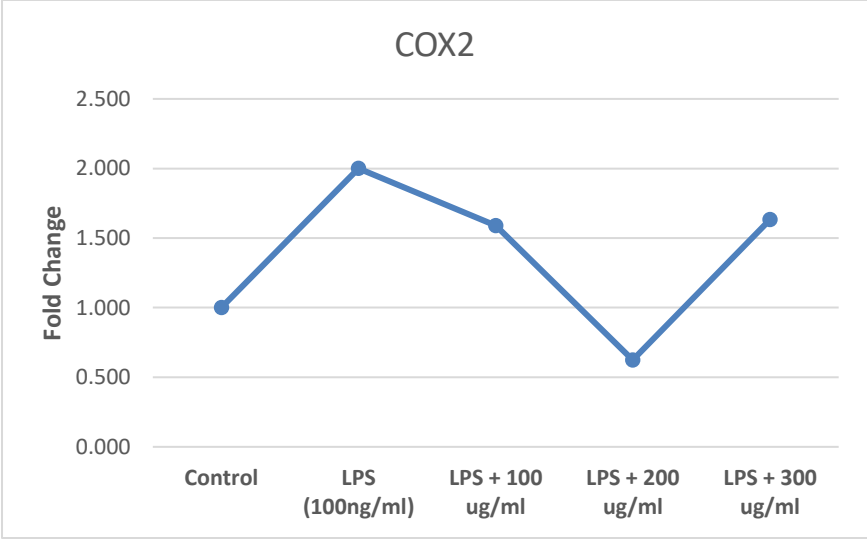


Fig 10. Effect of norabogori extract on pro-inflammatory genes

Objective 4:

To study the incorporation of the tested anti-inflammatory molecule in food model and sensory test

Materials and methodology
Preparation of microspheres

In the microsphere preparation studies, ionic gelation method and was used to create empty microspheres with 1/1, 1/2, 1/4, 1/8 ratio as extract/NaAlg ratio. The microspheres formed at different ratios were incubated with cross-linker for 30 min. In microencapsulation, 2% (w/w) NaAlg prepared with distilled water and 5% (w/w) $CaCl_2$ prepared with distilled water were used. 10 mL of NaAlg (2%) solution was mixed with sample extract in the proportions given above and the mixture was homogenized in a magnetic stirrer for 30 min. To the mixture, 10 mL of a solution of $CaCl_2$ (5%) was added as a complexing agent. The mixture, which was exposed to 45 minutes of crosslinking time, was washed with propanol, hexane and distilled water, respectively. The resulting microspheres were collected on glass for 24 h. Incubated in a 37°C oven (Celep et.al., 2020).

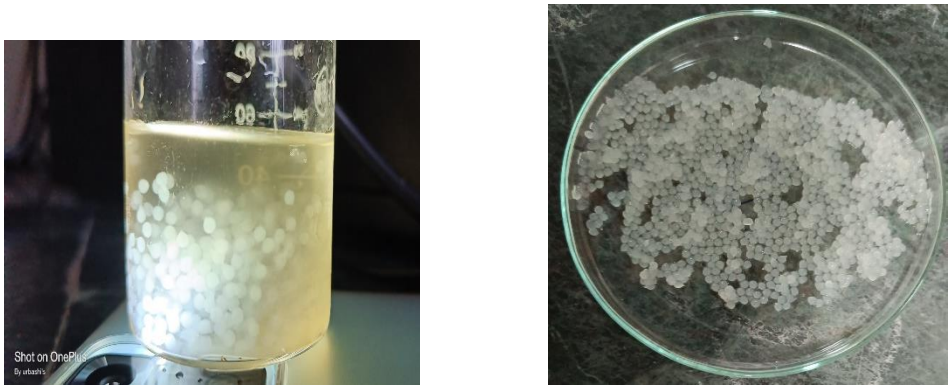


Fig 11. Microspheres prepared using ionic gelation method

***In vitro* release study**

Appropriate release medium was formed for the evaluation of *in vitro* release of encapsulated extract. Two release medium representing different digestion and absorption environment of different pH was created. The simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared as follows. For SGF preparation, 2.0 g of sodium chloride was mixed with 3.2 g of pepsin (porcine stomach mucosa) and 7 mL of hydrochloric acid was added to the mixture and volume made up to 1000 mL with water maintaining pH at 1.2. For the study, 1.4 mL of SGF was

added to 100 mg of sample in a 10mL test tube and incubated at 37^{o}C for 2 h at 80 rpm. After that, the solution was filtered and neutralized by adding 0.2M sodium hydroxide solution.

Similarly, SIF was prepared using monobasic potassium phosphate (6.8 g) dissolved in 250 mL water. To this solution 77 mL of 0.2N sodium hydroxide was added. Finally, 10g of pancreatin was added and the volume of the mixture was made up to 1000 mL and pH 6.8 was maintained. For the simulation study, 2.4 mL of SIF was taken in a 10 mL test tube and incubated with 100 mg of sample at 36.6^{o}C for 2 h with no shaking. At the end of experiment, the solution was filtered and enzyme activity was inhibited by decreasing pH to 1.2 using 100 µL of 3 M hydrochloric acid to 2 mL filtrate.
After 15 min, solution was neutralized (pH 7.0) by adding 900 µL of 0.2 N sodium hydroxide (Saikia et.al., 2015). Lastly, both the samples of SGF and SIF were analyzed for apparent phenolic content by Folin-Ciocalteau method (Slinkard & Singleton, 1977) and antioxidant activity by DPPH assay.

Product development

Fruit leather, also called a fruit bar or a fruit slab, is a dehydrated fruit-based confectionery dietary product. It is commonly favored as a popular fruit snack since it is chewy and flavorful, naturally low in fat and high in fiber and carbohydrates; it is also lightweight and can be easily stored and packed. Consuming fruit leather is an economic and convenient value-added substitute for natural fruits as a source of various nutritional elements like dietary fibers, carbohydrates, minerals, vitamins, and antioxidants. Furthermore, fruit leather has less than 100 kcals per serving, than many other snacks.

To prepare fruit leather, fruit pulps, puree or powder is mixed with appropriate quantities of sugar, pectin and acid and then dried into sheet-shaped products. Addition of sugar gives the product an increased the solids content, low hardness and sweeter taste for higher consumer acceptability in highly acidic fruit leather.

Compounds of higher molecular weight like pectin, starch, gelatin, alginate are added as gelling agents which generally improves the texture, ensure retention of the shapes, reduce drying rate and increases the product shelf life and rupture energy.

Browning of leather is a major problem in fruit leather making process. Addition of browning inhibitors like sulfite, citric acid, extract of maqui berry resulted in decreased browning reactions in different fruit leathers.

Preparation of Norabogori leather:

Freeze dried Nora bogori powder + finely ground table sugar (20%) + hydrocolloids (1%) (xanthan gum, starch, guar gum, and pectin)

↓

Spread evenly over the glass Petri dishes

↓

Dried in a tray dryer at 40 ± 2°C

↓

Stored at ambient temperature in a dry place, away from sunlight

Leather color

Color of the leather was measured using a Hunter Lab colorimeter (UltraScan VIS, Hunter Lab. Inc., USA) with an illuminance value of D65/10 equipped with a CR-400 measuring head color was expressed in terms of L^* (darkness/whiteness), a^* (greenness/ redness), and b^* (blueness/yellowness). The color difference (ΔE) between the leathers was calculated using Equation (1) (Lalnunthari et al., 2019).

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \qquad (1)$$

where L_2, a_2, b_2 are the color measurements of control leather and L_1, a_1, b_1 are the color measurements of leather developed using hydrocolloids.

Textural properties

Tensile properties of the norabogori leathers were measured with the help of Texture Analyzer (TA-HD Plus, Stable Micro, UK). The leather samples were cut into rectangular strips of 50 × 10 mm from the center of the leathers. The leather was clamped on two sides of the Tensile Grip (A/TG). The measurements were carried out at room temperature (24 ± 1°C), leaving an initial distance between clamps of 50 mm. The norabogori leathers were pulled apart at a speed of 50 mm/min until it got ruptured, using a cell load of 100 N. The extension force-displacement curve was obtained and from this, the rupture strength (N), tensile strength (MPa), extensibility (mm) and percentage elongation at break (%Elongation) were all obtained using the Equations (2) and (3).

Tensile strength = Force / leather thickness * length of the leather (2)

% Elongation = (Elongation at rupture/ initial length of the leather) * 100 (3)

Chewiness and Adhesiveness of leather were also performed using Texture Analyzer with a load cell of 25 kg weight. The leather was placed at the center of the probe in a flat form on the sample table. A two-cycle compression force versus time program was used to compress the samples to 70% of the original sample thickness, return to the original position, and again compress. A force versus time curve for a two-cycle compression was measured, with a disk probe (of 35 mm diameter) with pretest speed of 5 mm/s, test speed of 1 mm/s, and posttest speed of 5 mm/s. In-built software of the texture analyzer was used for analyzing the data generated. Parameters recorded from the test curves were chewiness and adhesiveness. This process was carried out in triplicate (Barman et.al., 2021).

Incorporation of encapsulate

The best found hydrocolloid from previous assay is taken forward for the incorporation of encapsulates in the product. In the norabogori fruit leather mixture optimized before, 2.5% and 5% of encapsulated extract were incorporated. The previous constituation and method of production kept intact. The resulted products are evaluated for leather color and texture obeying above mentioned conditions.

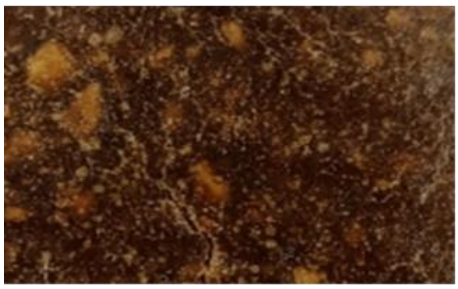
Results and discussion

***In vitro* release study**

Table 15. *In-vitro* release study of encapsulates

Extract/NaAlg	TPC (mg GAE/g)		Antioxidant activity (%)	
	simulated gastric fluid (SGF)	simulated intestinal fluid (SIF)	simulated gastric fluid (SGF)	simulated intestinal fluid (SIF)
1:02	8.14 ± 0.22	39.03 ± 0.74	16.85	48.55
1:04	7.42 ± 0.30	31.14 ± 0.53	12.34	39.75
1:08	7.08 ± 0.09	29.01 ± 0.32	5.95	23.87

Product Development



Control (S_0)

Guar gum (S1)

Starch (S3)

Pectin (S2)

Xanthan gum (S4)

Fig 12. Developed product with different hydrocolloids

Color and texture analysis

Table 16. Color and texture analysis of the product

Sample	Color			Texture			
	L*	a*	b*	Gummines s(N*mm)	Chewiness (N*mm)	springin ess	Cohesive ness
Control (S_0)	47.43± 0.43	5.86±0.17	5.08±0.31	57.71± 0.5	33.90± 0.3	5.87± 0.4	0.99± 0.01
Guar gum(S1)	46.23± 1.94	4.65±0.08	3.84±0.43	68.67± 0.7	42.60± 0.4	6.20± 0.5	1.61± 0.01
Pectin (S2)	44.87± 1.11	3.94±0.35	2.63±0.43	110.58± 0.2	65.56± 0.2	5.93± 0.4	1.87± 0.02
Starch (S3)	44.68± 0.84	4.83±0.08	2.81±0.62	118.48± 0.9	71.61± 0.5	6.04± 0.3	1.35± 0.02
Xanthan gum(S4)	44.23± 0.88	5.85±0.35	5.35±0.54	138.45± 0.3	92.14± 0.2	6.66± 0.5	1.59± 0.01

The texture analysis of norabogori fruit leather showed significant differences in the evaluated parameters. It was observed that with the change in the hydrocolloid in the fruit leather showed an important impact on the leather properties. The leather prepared using xanthan gum as hydrocolloid showed the best texture profile. Also, the color analysis of the leather indicates more acceptable attributes.

Incorporation of encapsulate

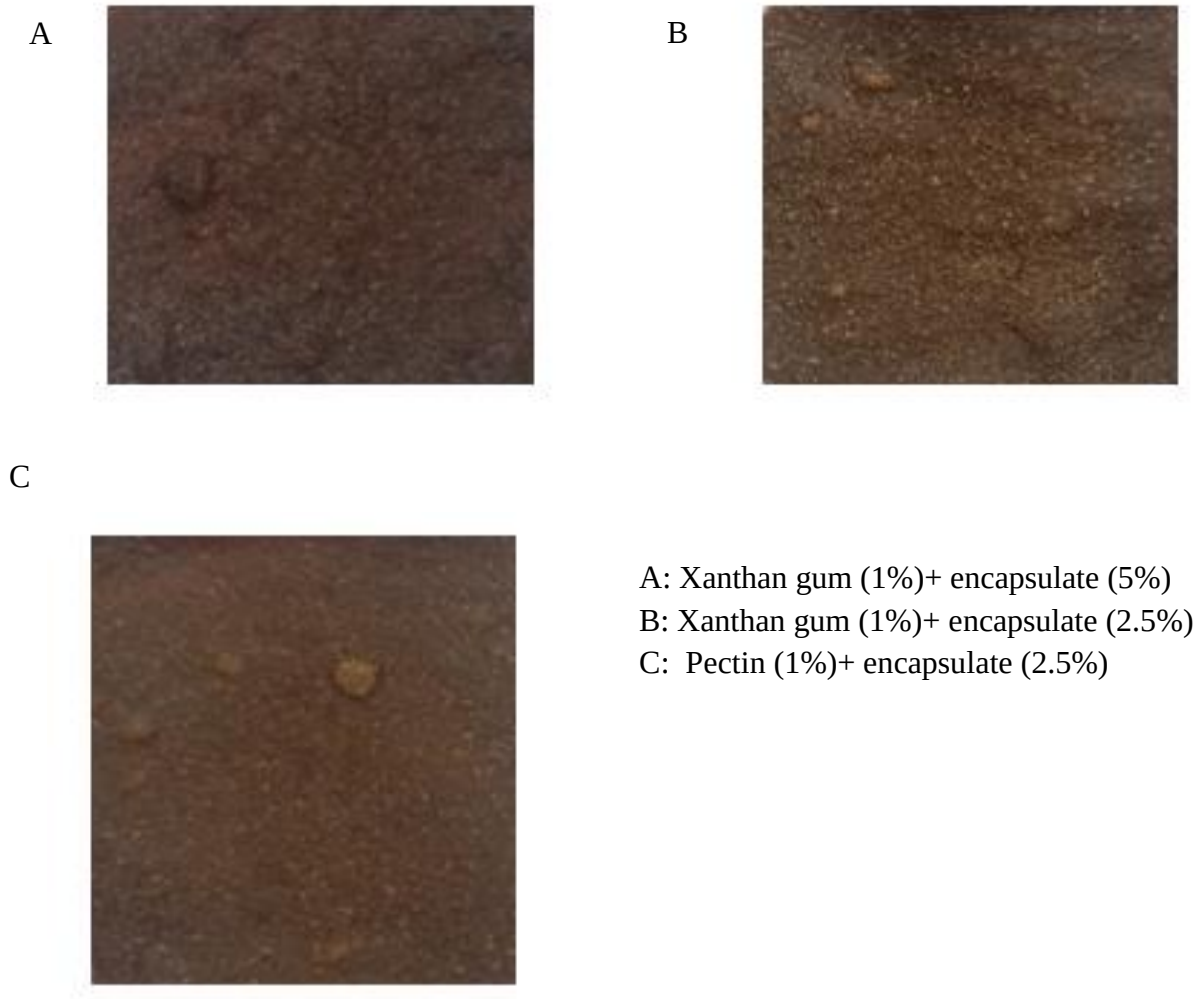


Fig 13. Product incorporated with encapsulate

Texture analysis

Table 17. Texture analysis of encapsulate incorporated developed product

Sample	Gumminess (N*mm)	Chewiness (N*mm)	Springiness	Cohesiveness
Control: sugar(10%)+norabogori powder+water	57.71± 0.5	33.90± 0.3	5.87± 0.4	0.99± 0.01
A: Xanthan gum(1%) +encapsulate(5%)+sugar(10%)+ norabogori powder+water	97.42± 0.8	66.07± 0.9	6.85± 0.4	1.27± 0.02
B: Xanthan gum(1%) +encapsulate(2.5%)+ sugar(10%)+norabogori powder+water	107.35± 2.1	79.41± 0.2	7.93± 0.2	1.37± 0.01
C:Pectin(1%)+encapsulate(2.5%)+ sugar(10%)+norabogori powder+water	83.53± 1.3	48.58± 1.1	6.04± 0.1	1.23± 0.02

Conclusion

In microencapsulation study, variation in the physical properties between the variants of microencapsulates was observed. Highest encapsulating efficiency was obtained in the sample containing 1:8 concentration of sample: NaAlg ratio. In vitro gastrointestinal simulation study showed greater release of phenolic compounds in the intestinal fuid of pH 6.8. So, it can be concluded that this condition is optimum for encapsulating norabogori extract and incorporation in product.

This study revealed the effects of different hydrocolloids (xanthan gum, guar gum, starch and pectin) on the physico-chemical properties of the different fruit leathers. The application of hydrocolloid affected the tensile strength, color and various other chemical characteristics of the norabogori fruit leather. The increase in the concentration of hydrocolloids helps to increase the color acceptability, tensile strength and elongation of norabogori fruit leather and the fruit leathers developed using xanthan gum showed the highest overall acceptability. During final product development, incorporation of 2.5% of encapsulated extract gave the most acceptable norabogori fruit leather.

Conclusions summarising the achievements and indication of scope for future work:

- The study revealed that the Norabogori fruit is a rich source of total carbohydrate (68±3.24 g/100g) and very less amount of fat (0.62 ±0.09 g/100g) and protein (13.0±0.26mg/g).
- The Response surface methodology (RSM) based on a Box-Behnken design (BBD) optimized microwave assisted extraction system resulted in an increased yield (Total Phenolic Content, TPC= 68.24 mg GAE/100g) in comparatively very less time (15min) than conventional extraction method (TPC= 49.70 mg GAE/100g, 48h).
- Phytochemical profile of Norabogori has been known and from them the drug likable compounds got sorted out.
- Potential anti-inflammatory bioactive compounds of Norabogori found out. Compounds like Caffeic acid, Hydroxycinnamic Acids, Catechin etc. present in the extract are most drug likable and good anti-inflammatory compound.
- The fruit leather with xanthan gum as hydrocolloid and 2.5% encapsulate incorporated gave away the most acceptable product in sensory analysis with best texture and color combination.
- The norabogori extract has been found out to have a high phenolic content, antioxidant activity and anti-inflammatory effect. The encapsulate released the extract efficiently in target site. So from the experimental steps it can be said that the product is an efficient source of phenolic compounds, antioxidant in nature and has enhanced anti-inflammatory effect.
- The product extract can farther be analyzed for other chronic diseases and can apply in animal model.

Technology transfer to industry partner:

The product resulted from the project which is a ready to eat anti-inflammatory functional food from 'Norabogori' (*Prunus persica*) of Assam, has been proceeded to an industry partner. For that, an enterprise named Quality Bakery has been associated and they primarily manufactures bakery product. A non-discloser agreement has been signed between Tezpur University and the enterprise and a "Draft of Agreement" has been prepared thereafter.

Nomenclature

g	Gram
mg	Milligram
c	Celsius
Min	Minute
uv	Ultra violet
MW	Microwave
ml	Milliliter
µL	Microliter
FCR	Folin Ciocalteu Reagent
GAE	Gallic acid equivalent
nm	Nanometer
h	Hour
ANOVA	Analysis of variance
M	Molar
mm	Millimeter
RP-UHPLC	Reversed Phase-Ultra High Performance Liquid Chromatographic
HR-LCMS	High resolution liquid chromatography- mass spectroscopy
Abs	Absorbance
RPM	Revolutions per minute
RT	Retention time
RMSD	root mean square deviation
Å	Armstrong

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FORM GFR 12 A

UC ID: 18241222

FORM GFR 12-A
[See Rule 238(1)]

Form of Utilization Certificate FOR AUTONOMOUS BODIES OF THE GRANTEE ORGANIZATION

UTILIZATION CERTIFICATE FOR THE YEAR 2019-2020 in respect
of Recurring/non-recurring
GRANTS-IN-AID/SALARIES/CREATION OF CAPITAL ASSETS

1.Name of the Scheme : **PRADHAN MANTRI KISAN SAMPADA YOJANA-HUMAN RESOURCE AND INSTITUTIONS-3612**

2. Whether recurring pr non-recurring grants :N/A

3.Grants positions of the beginning of the Financial year :
(i.) Cash in Hand/Bank : **0.00**
(ii.) Unadjusted advances : **0.00**
(iii.) Total: **0.00**

4. Details of grants received,expenditure incurred and closing balances:(Actuals) :

Unspent Balances of Grants received yrs(1)	Interest Earned there On(2)	Interest deposited back to the Government(3)	Sanction Number	Sanction Date	Amount(Rs) (4)	Total Available funds(5)= (1+2-3+4)	Expenditure incurred(6)	Closing Balances(7)
0.00	0.00	0.00	Q-11/22/2019-R&D	06-12-2019	1935470.00	1935470.00	396899.00	1538571.00

Grant-in-aid-General	Grant-in-aid-Salary	Grant-in-aid-creation of capital assets	Total
396899.00	0.00	0.00	396899.00

Details of grants position of the end of the year

3.Grants positions of the End of the Financial year :
(i.) Cash in Hand/Bank : **1538571.00**
(ii.) Unadjusted advances : **0.00**
(iii.) Total : **1538571.00**

Certified that I have satisfied myself that the condition on which the grants-in-aid was sanctioned have been duly fulfilled/are
being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

1. The main accounts and other subsidiary accounts and registers(including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors.
The figures depicted above tally with the audited figues mentioned in financial statements/accouns.

2. There exist internal controls for safeguareding public funds/assets , watching outcomes and achiwevemnets of physical targets against the financial inputs,ensuring quality in asset creation etc. & the periodic evaluation internal controls is exercised to ensure their effectiveness.

3. To the best or our knowledge and belief . no transactions have been enterd that are in violation of relevant Act/Rules/Standing instructions and scheme guidelines.

FORM GFR 19

UC ID: 18241222

FORM GFR 19-A
[See Rule 212(1)]
Form of Utilization Certificate

Sl No.	Sanction Number	Sanction Date	Amount(Rs)
1	Q-11/22/2019-R&D	06-12-2019	1935470.00

Certified that Out Of Rs. **1935470.00** Grants-in-aid Sanctioned during the year **2019-2020** in Favour Of **Tezpur University** under this Ministry/Department Letter No. given in the margin and Rs.**0.00** on account of unspent balance of the previous year,a sum of Rs. **1887518.00** has been utilized for the purpose of for which it was sanctioned and that the balance of Rs **47952.0000** remaining unutilized at the end of year has been surrenderd to Government (vide No dated)/ will be adjusted towards the grants-in-aid payable during the next year **2020-2021** Interest earned is **0.00** and Additional expenditure of Rs **0.00** has been incurred from internal resources and will be adjusted against next release.

2.Certified that I have satisfied myself that the condition on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds Of checks exercised

- Cash book verified regularly
- Bank Pass Book verified
- Stock Register verified
- Charted Accountant checked the expenses and certified
- Ledger
- Payment vouchers
- Bank Reconciliation
- Inventory
- Vouchers
- Account audited by competent authority
- Committed expenditure calculated w.r.t salary of staff ,travel and various other liabilities
- Allotment Register
- Bills
- Placing order generally on lowest quotation basis for supply after verification of approved heads A/C
- Passing of Bills with entries in Budget Register
- Checking of Bidget Register before making any payment
- Keeping of Assests Register
- Keeping of Salary Register
- Any other as applicable

Signature.....
Designation.....
Registrar
Date..... Tezpur University

FORM GFR 12 A

UC ID: 18241222

FORM GFR 12-A
[See Rule 238(1)]

Form of Utilization Certificate FOR AUTONOMOUS BODIES OF THE GRANTEE ORGANIZATION

UTILIZATION CERTIFICATE FOR THE YEAR 2019-2020 in respect
of Recurring/non-recurring
GRANTS-IN-AID/SALARIES/CREATION OF CAPITAL ASSETS

1.Name of the Scheme : **PRADHAN MANTRI KISAN SAMPADA YOJANA-HUMAN RESOURCE AND INSTITUTIONS-3612**

2. Whether recurring pr non-recurring grants :N/A

3.Grants positions of the beginning of the Financial year :
(i.) Cash in Hand/Bank : **0.00**
(ii.) Unadjusted advances : **0.00**
(iii.) Total: **0.00**

4. Details of grants received,expenditure incurred and closing balances:(Actuals) :

Unspent Balances of Grants received yrs(1)	Interest Earned there On(2)	Interest deposited back to the Government(3)	Sanction Number	Sanction Date	Amount(Rs) (4)	Total Available funds(5)= (1+2-3+4)	Expenditure incurred	Balance
0.00	0.00	0.00	Q-11/22/2019-R&D	06-12-2019	1935470.00	1935470.00	1887518.00	47952.00

Grant-in-aid-General	Grant-in-aid-Salary	Grant-in-aid-creation of capital assets	Total
391771.00	395767.00	1099980.00	1887518.00

Details of grants position of the end of the year

3.Grants positions of the End of the Financial year :
(i.) Cash in Hand/Bank : **47952.00**
(ii.) Unadjusted advances : **0.00**
(iii.) Total : **47952.00**

Certified that I have satisfied myself that the condition on which the grants-in-aid was sanctioned have been duly fulfilled/are
being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

1. The main accounts and other subsidiary accounts and registers(including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors.
The figures depicted above tally with the audited figues mentioned in financial statements/accouns.

2. There exist internal controls for safeguareding public funds/assets , watching outcomes and achiwevemnets of physical targets against the financial inputs,ensuring quality in asset creation etc. & the periodic evaluation internal controls is exercised to ensure their effectiveness.

3. To the best or our knowledge and belief . no transactions have been enterd that are in violation of relevant Act/Rules/Standing instructions and scheme guidelines.

4. The responsibilites among the key functionaries for execution of the schema have been assigned in clear terms and are not general in nature.

5. The benefits were extended to the intended beneficiaries and only such areas/districts were guidelines and terms and conditios of the gaints-in-aid.

6. It has been ensured that the physical and financial performance under PRADHAN MANTRI KISAN SAMPADA

FORM GFR 12 A

UC ID: 18242525

FORM GFR 12-A
[See Rule 238(1)]

Form of Utilization Certificate FOR AUTONOMOUS BODIES OF THE GRANTEE ORGANIZATION

UTILIZATION CERTIFICATE FOR THE YEAR 2020-2021 in respect
of Recurring/non-recurring
GRANTS-IN-AID/SALARIES/CREATION OF CAPITAL ASSETS

1.Name of the Scheme : **PRADHAN MANTRI KISAN SAMPADA YOJANA-HUMAN RESOURCE AND INSTITUTIONS-3612**

2. Whether recurring pr non-recurring grants :N/A

3.Grants positions of the beginning of the Financial year :
(i.) Cash in Hand/Bank : **0.00**
(ii.) Unadjusted advances : **0.00**
(iii.) Total: **0.00**

4. Details of grants received,expenditure incurred and closing balances:(Actuals) :

Unspent Balances of Grants received yrs(1)	Interest Earned there On(2)	Interest deposited back to the Government(3)	Sanction Number	Sanction Date	Amount(Rs) (4)	Total Available funds(5)= (1+2-3+4)	Expenditure incurred(6)	Closing Balances(7)
0.00	0.00	0.00	Q-11/22/2019-R&D	06-12-2019	1538571.00	1538571.00	1489389.00	49182.00

Grant-in-aid-General	Grant-in-aid-Salary	Grant-in-aid-creation of capital assets	Total
389409.00	0.00	1099980.00	1489389.00

Details of grants position of the end of the year

3.Grants positions of the End of the Financial year :
(i.) Cash in Hand/Bank : **49182.00**
(ii.) Unadjusted advances : **0.00**
(iii.) Total : **49182.00**

Certified that I have satisfied myself that the condition on which the grants-in-aid was sanctioned have been duly fulfilled/are
being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

1. The main accounts and other subsidiary accounts and registers(including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors.
The figures depicted above tally with the audited figues mentioned in financial statements/accouns.

2. There exist internal controls for safeguareding public funds/assets , watching outcomes and achiwevemnets of physical targets against the financial inputs,ensuring quality in asset creation etc. & the periodic evaluation internal controls is exercised to ensure their effectiveness.

3. To the best or our knowledge and belief . no transactions have been enterd that are in violation of relevant Act/Rules/Standing instructions and scheme guidelines.

4. The responsibilites among the key functionaries for execution of the schema have been assigned in clear terms and are not general in nature.

5. The benefits were extended to the intended beneficiaries and only such areas/districts were guidelines and terms and conditios of the gaints-in-aid.

6. It has been ensured that the physical and financial performance under PRADHAN MANTRI KISAN SAMPADA YOJANA-HUMAN RESOURCE AND INSTITUTIONS-3612 (name of the scheme has

Finance Officer
Tezpur University

been according to the requirements , as prescribed in the guidelines issued by Govt. of india and the. performane/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure- I duly enclosed.

7. The utlization of the fund resulted in outcomes given at Annexure -2 duly enclosed(to be formulated by the Ministry/Department concerned as per their requirements/specifications.)

8. Details of various schemes executed by the agency through grants-in-aid received from the some Ministry or from other Ministries is enclosed at Annesxure-2 (to be formulated by the Minisry/Departments concerned as per their requirements/specifications).

Date : 06/2/2024
Place :

Signature

Name.....

Chief Finance Officer
(Head of the Finance)

Signature

Finance Officer
Tezpur University

Name.....

Head of the Organisation

(Strike out in inapplicable terms)

FORM GFR 19

UC ID: 18242525

FORM GFR 19-A
[See Rule 212(1)]
Form of Utilization Certificate

Sl No	Sanction Number	Sanction Date	Carry Forward Amount
1	Q-11/22/2019-R&D	06-12-2019	1538571.00

Certified that Out Of Rs. **Nil** Grants-in-aid Sanctioned during the year **2020-2021** in Favour Of **Tezpur University** under this Ministry/Department Letter No. given in the margin and Rs.**1538571.00** on account of unspent balance of the previous year,a sum of Rs. **1489389.00** has been utilized for the purpose of for which it was sanctioned and that the balance of Rs **49182.0000** remaining unutilized at the end of year has been surrenderd to Government (vide No dated)/ will be adjusted towards the grants-in-aid payable during the next year **2021-2022** Interest earned is **0.00** and Additional expenditure of Rs **0.00** has been incurred from internal resources and will be adjusted against next release.

2.Certified that I have satisfied myself that the condition on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds Of checks exercised

- Cash book verified regularly
- Bank Pass Book verified
- Stock Register verified
- Charted Accountant checked the expenses and certified
- Ledger
- Payment vouchers
- Bank Reconciliation
- Inventory
- Vouchers
- Account audited by competent authority
- Committed expenditure calculated w.r.t salary of staff ,travel and various other liabilities
- Allotment Register
- Bills
- Placing order generally on lowest quotation basis for supply after verification of approved heads A/C
- Passing of Bills with entries in Budget Register
- Checking of Bidget Register before making any payment
- Keeping of Assests Register
- Keeping of Salary Register
- Any other as applicable

Finance Officer
Tezpur University

Signature.....

Designation.....06/04/2021

Date.....

Finance Officer
Tezpur University

YOJANA-HUMAN RESOURCE AND INSTITUTIONS-3612 (name of the scheme has been according to the requirements , as prescribed in the guidelines issued by Govt. of india and the. performane/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure- I duly enclosed.

7. The utlization of the fund resulted in outcomes given at Annexure -2 duly enclosed(to be formulated by the Ministry/Department concerned as per their requirements/specifications.)

8. Details of various schemes executed by the agency through grants-in-aid received from the some Ministry or from other Ministries is enclosed at Annesxure-2 (to be formulated by the Minisry/Departments concerned as per their requirements/specifications).

Date :
Place :

Signature

Name.....

Chief Finance Officer
(Head of the Finance)
Finance Officer
Tezpur University

Name.....

Head of the Organisation
Registrar
Tezpur University
(Strike out in inapplicable terms)

FORM GFR 12 A

UC ID: 18252818

FORM GFR 12-A
[See Rule 238(1)]

Form of Utilization Certificate FOR AUTONOMOUS BODIES OF THE GRANTEE ORGANIZATION

UTILIZATION CERTIFICATE FOR THE YEAR 2021-2022 in respect
of Recurring/non-recurring
GRANTS-IN-AID/SALARIES/CREATION OF CAPITAL ASSETS

1.Name of the Scheme : **PRADHAN MANTRI KISAN SAMPADA YOJANA-HUMAN RESOURCE AND INSTITUTIONS-3612**

2. Whether recurring pr non-recurring grants :N/A

3.Grants positions of the beginning of the Financial year :
(i.) Cash in Hand/Bank : **49182.00**
(ii.) Unadjusted advances : **0.00**
(iii.) Total: **49182.00**

4. Details of grants received,expenditure incurred and closing balances:(Actuals) :

Unspent Balances of Grants received from Govt.(1)	Interest Earned thereon (2)	Interest deposited back to the Government(3)	Sanction Number	Sanction Date	Amount(Rs) (4)	Total Available funds(5)= (1+2-3+4)	Expenditure incurred(6)	Closing Balances(7)
49182.00	0.00	0.00	Q-11/22/2019-R&D	19-01-2022	619062.00	668244.00	639117.00	29127.00

Grant-in-aid-General	Grant-in-aid-Salary	Grant-in-aid-creation of capital assets	Total
639117.00	0.00	0.00	639117.00

Details of grants position of the end of the year

3.Grants positions of the End of the Financial year :
(i.) Cash in Hand/Bank : **29127.00**
(ii.) Unadjusted advances : **0.00**
(iii.) Total : **29127.00**

Certified that I have satisfied myself that the condition on which the grants-in-aid was sanctioned have been duly fulfilled/are
being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

1. The main accounts and other subsidiary accounts and registers(including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors.
The figures depicted above tally with the audited figues mentioned in financial statements/accouns.

2. There exist internal controls for safeguareding public funds/assets , watching outcomes and achiwevemnets of physical targets against the financial inputs,ensuring quality in asset creation etc. & the periodic evaluation internal controls is exercised to ensure their effectiveness.

3. To the best or our knowledge and belief , no transactions have been enterd that are in violation of relevant Act/Rules/Standing instructions and scheme guidelines.

4. The responsibilites among the key functionaries for execution of the schema have been assigned in clear terms and are not general in nature.

5. The benefits were extended to the intended beneficiaries and only such areas/districts were guidelines and terms and conditios of the gaints-in-aid.

6. It has been ensured that the physical and financial performance under PRADHAN MANTRI KISAN SAMPADA

YOJANA-HUMAN RESOURCE AND INSTITUTIONS-3612 (name of the scheme has been according to the requirements , as prescribed in the guidelines issued by Govt. of india and the. performane/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure- I duly enclosed.

7. The utlization of the fund resulted in outcomes given at Annexure -2 duly enclosed(to be formulated by the Ministry/Department concerned as per their requirements/specifications.)

8. Details of various schemes executed by the agency through grants-in-aid received from the some Ministry or from other Ministries is enclosed at Annesxure-2 (to be formulated by the Minisry/Departments concerned as per their requirements/specifications).

Date :
Place :

Signature

Name.....

Chief Finance Officer
(Head of the Finance)

Finance Officer
Tezpur University

Signature

Name.....

Head of the Organisation

Registrar
Tezpur University

(Strike out in inapplicable terms)

FORM GFR 19

UC ID: 18252818

FORM GFR 19-A
[See Rule 212(1)]
Form of Utilization Certificate

Sl No	Sanction Number	Sanction Date	Amount(Rs)
1	Q-11/22/2019-R&D	19-01-2022	619062.00

Certified that Out Of Rs. **619062.00** Grants-in-aid Sanctioned during the year **2021-2022** in Favour Of **Tezpur University** under this Ministry/Department Letter No. given in the margin and Rs.**49182.00** on account of unspent balance of the previous year,a sum of Rs. **639117.00** has been utilized for the purpose of for which it was sanctioned and that the balance of Rs **29127.0000** remaining unutilized at the end of year has been surrenderd to Government (vide No dated)/ will be adjusted towards the grants-in-aid payable during the next year **2022-2023** Interest earned is **0.00** and Additional expenditure of Rs **0.00** has been incurred from internal resources and will be adjusted against next release.

2.Certified that I have satisfied myself that the condition on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds Of checks exercised

- Cash book verified regularly
- Bank Pass Book verified
- Stock Register verified
- Charted Accountant checked the expenses and certified
- Ledger
- Payment vouchers
- Bank Reconciliation

Signature.....
Designation.....
Date..... 23/6/22
Finance Officer
Tezpur University