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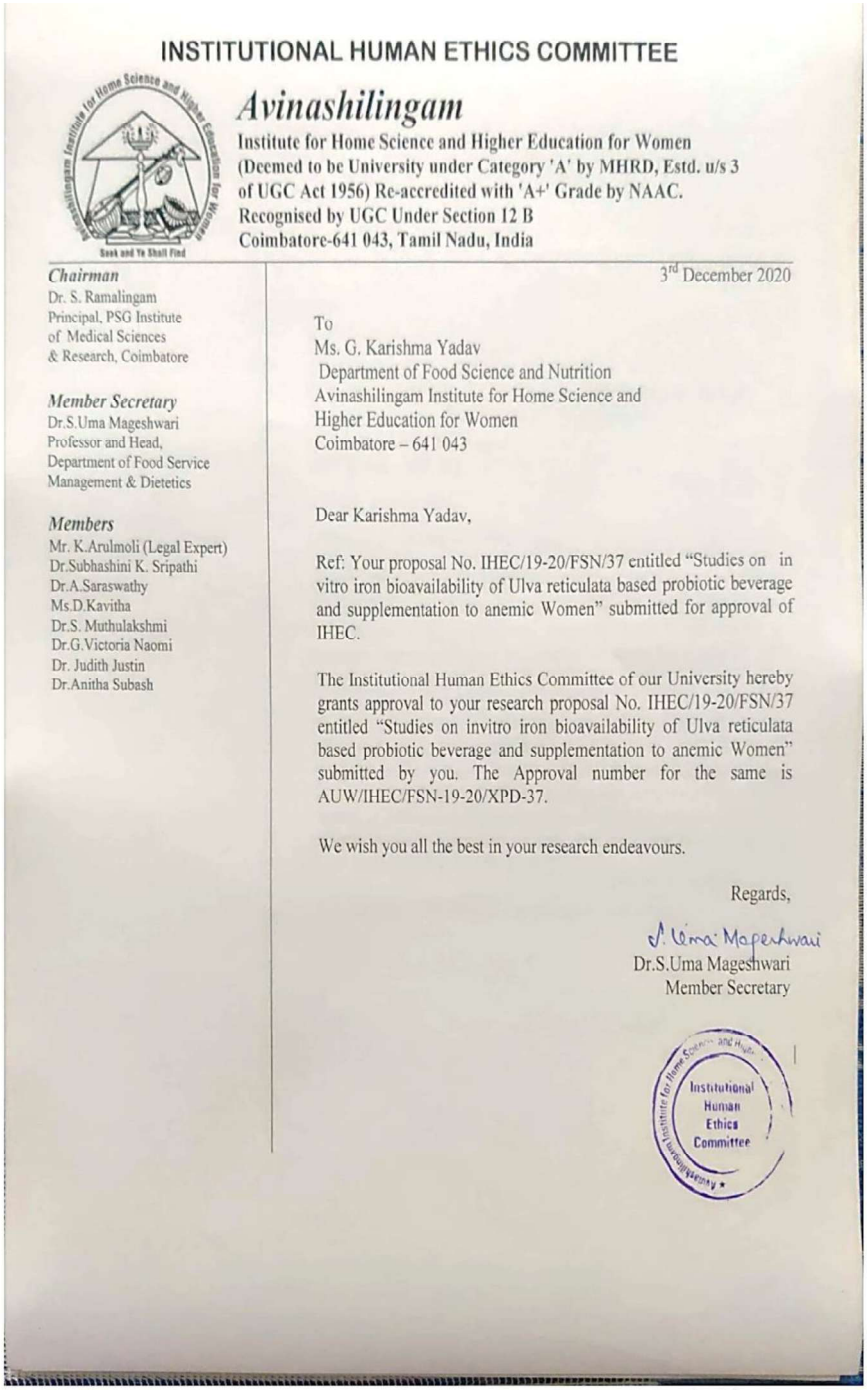
Appendices

APPENDIX I

INSTITUTIONAL HUMAN ETHICS COMMITTEE



INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam

Institute for Home Science and Higher Education for Women
(Deemed to be University under Category 'A' by MHRD, Estd. u/s 3 of UGC Act 1956) Re-accredited with 'A+' Grade by NAAC.
Recognised by UGC Under Section 12 B
Coimbatore-641 043, Tamil Nadu, India

Chairman
Dr. S. Ramalingam
Principal, PSG Institute of Medical Sciences & Research, Coimbatore

Member Secretary
Dr.S.Uma Mageshwari
Professor and Head,
Department of Food Service Management & Dietetics

Members
Mr. K.Arulmoli (Legal Expert)
Dr.Subhashini K. Sripathi
Dr.A.Saraswathy
Ms.D.Kavitha
Dr.S. Muthulakshmi
Dr.G.Victoria Naomi
Dr. Judith Justin
Dr.Anitha Subash

3rd December 2020

To
Ms. G. Karishma Yadav
Department of Food Science and Nutrition
Avinashilingam Institute for Home Science and Higher Education for Women
Coimbatore – 641 043

Dear Karishma Yadav,

Ref: Your proposal No. IHEC/19-20/FSN/37 entitled "Studies on in vitro iron bioavailability of Ulva reticulata based probiotic beverage and supplementation to anemic Women" submitted for approval of IHEC.

The Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No. IHEC/19-20/FSN/37 entitled "Studies on invitro iron bioavailability of Ulva reticulata based probiotic beverage and supplementation to anemic Women" submitted by you. The Approval number for the same is AUW/IHEC/FSN-19-20/XPD-37.

We wish you all the best in your research endeavours.

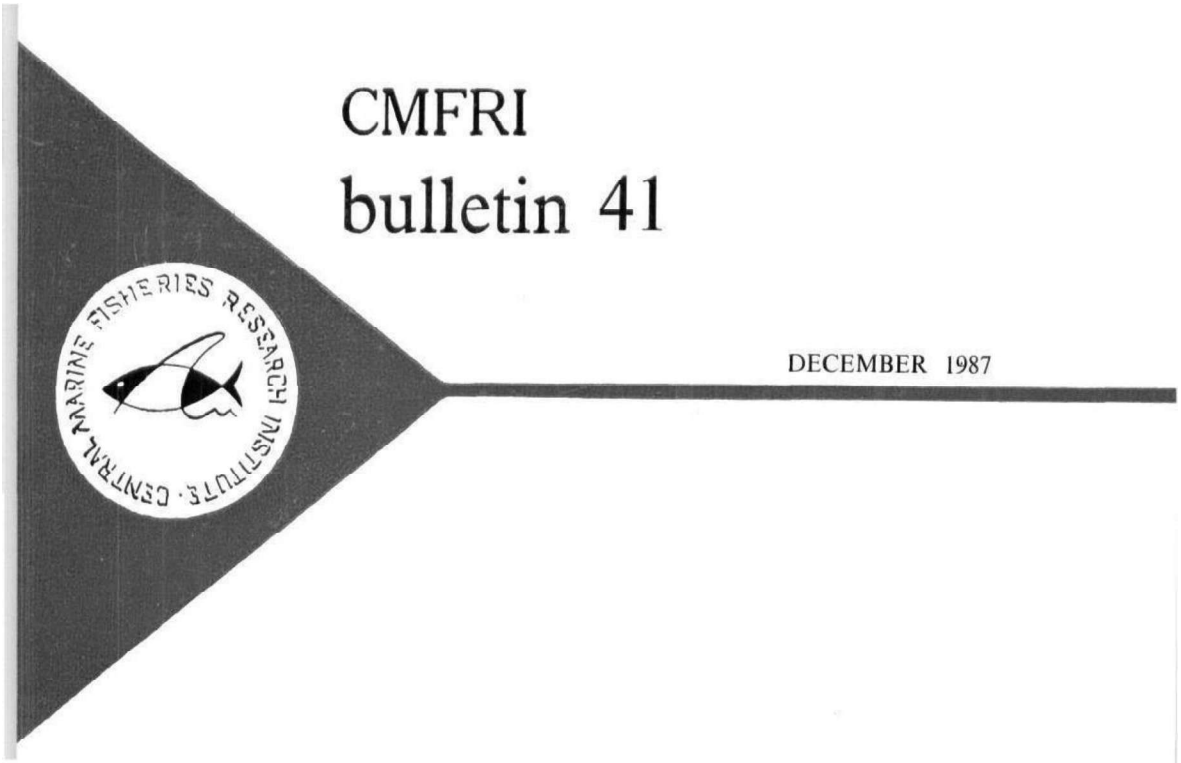
Regards,

Dr.S.Uma Mageshwari
Member Secretary

Institutional Human Ethics Committee

APPENDIX II

AUTHENTICATION OF SEAWEEDS – CMFRI BULLETIN 41.



SEAWEED RESEARCH AND UTILIZATION IN INDIA

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
P. B. No. 2704, E. R. G. Road, Cochin 682 031, India

which are presently found useful either as directly edible materials or as industrial raw materials are being dealt with in the classification given in this chapter following that of Fritsch (1935). The distribution of these economically important algae along the Indian coast are given along with a few other species commonly occurring in India in the Appendix III.

Classification

Following the classification of Fritsch (1935), a systematic list of the important and common Indian seaweeds is given below:

I CLASS : CHLOROPHYCEAE

Order : Ulotrichales

a. Family : Ulvaceae

1. *Ulva fasciata*
2. U. *lactuca*
3. *U. rigida*
4. *U. reticulata*
5. *Enteromorpha compressa*

Order : Cladophorales

b. Family : Cladophoraceae

6. *Chaetomorpha antennina*

Order : Siphonales

c. Family : Caulerpaceae

7. *Caulerpa racemosa*
8. *C. sertularioides*
9. *C. taxifolia*

d. Family : Codiaceae

10. *Codium adharens*
11. *C. decorticatum*
12. *C. tomentosum*

II CLASS : PHAEOPHYCEAE

Order : Dictyotales

a. Family : Dictyotaceae

13. *Dictyota dichotoma*
14. *Padina commersoni*
15. *P. gymnospora*
16. *P. tetrastromatica*

Order : Punctariales

b. Family : Punctariaceae

17. *Colpomenia sinuosa*
18. *Hydroclathrus clathratus*
19. *Rosenvingea intricata*
20. *Chnoospora minima*

Order : Fucales

c. Family : Sargassaceae

21. *Cystoseira trinodis*
22. *Hormophysa triquetra*
23. *Sargassum johnstonii*
24. *S. myriocystum*
25. *S. swartzii*
26. *S. tenerrimum*
27. *S. wightii*
28. *Turbinaria conoides*
29. *T. ornata*

III CLASS : RHODOPHYCEAE

Sub-Class : Bangioideae

Order : Bangiales

a. Family : Bangiaceae

30. *Porphyra vietnamensis*

Order : Gelidiales

b. Family :Gelidiaceae

31. *Gelidiella acerosa*

Order : Cryptonemiales

c. Family : Grateloupiaceae

32. *Halymenia floresia*
33. *Grateloupia filicina*
34. *G. lithophila*

Order : Gigartinales

d. Family : Gracilariaceae

35. *Gracilaria corticata*
36. *G. crassa*
37. *G. foliifera*
38. *G. edulis*
39. *G. verrucosa*

e. Family : Solieriaceae

40. *Sarconema furcellatum*

f. Family : Hypneaceae

41. *Hypnea musciformis*

g. Family : Gigartinaceae

42. *Gigartina acicularis*

Order : Rhodymenials

h. Family : Rhodymeniaceae

43. *Rhodymenia dissecta*

Order : Ceramiales

I. Family : Ceramiaceae

44. *Centroceras clavulatum*
45. *Spyridia filamentosa*
46. *S. fusiformis*

j. Family : Rhodomelaceae

47. *Acanthophora spicifera*
48. *Laurencia papillosa*
49. *L. obtusa*

APPENDIX III

1. Estimation of Crude Fibre

To estimate crude fibre, weigh a known amount of food sample, then boil it in dilute acid (e.g., 1.25% H2SO4) followed by dilute alkali (e.g., 1.25% NaOH) to remove soluble components. Filter the residue, dry it at 105°C, and then ignite in a muffle furnace at 550°C to burn off organic matter. The remaining ash is weighed, and the crude fibre percentage is calculated as:

Crude Fiber (%) = (Weight of residue/Sample weight) x 100

2. Estimation of Dietary Fibre

Weigh the food sample and treat it with a series of enzymes (e.g., amylase, protease) to digest starches and proteins. After enzymatic digestion, filter the fibre fraction, wash thoroughly, and dry at 105°C. The dietary fibre content is calculated similarly to crude fibre using the formula:

Dietary Fiber (%) = (Weight of fibre residue Sample weight) x 100

3. Estimation of Calcium

To estimate calcium, weigh a known sample of food and ash it at 550°C to remove organic matter. Dissolve the ash in dilute hydrochloric acid, and precipitate calcium as calcium oxalate by adding ammonium oxalate. Filter, wash, and ignite the precipitate to calcium oxide, then calculate the calcium content using the formula:

Calcium (%) = (Weight of CaO/ sample weight) x 100

4 Estimation of Phosphorous

For phosphorus, dissolve the food sample in hydrochloric acid and treat with ammonium molybdate and ascorbic acid. The resulting yellow complex is measured colorimetrically at 660 nm. Phosphorus is calculated using a standard calibration curve.

5 Estimation of Iron

To estimate iron, dissolve the sample with hydrochloric acid and treat with a reducing agent (e.g., ascorbic acid) to convert iron to ferrous form. The solution is then reacted with 1,10-phenanthroline, and the iron content is measured spectrophotometrically at 510 nm. The formula used is:

(Absorbance of sample/Absorbance of standard) x concentration of standard.

6 Estimation of Phytates

The total phytate content is typically determined using the colorimetric method, based on the reaction of phytate with iron (III) chloride. A sample is first extracted with hydrochloric acid and then treated with a ferric chloride solution. The absorbance of the resulting blue complex is measured at 500 nm. The phytate content is calculated using the formula:

Phytate [(mg/g) = (A x Vx f)]/weight of sample (g)

where A is the absorbance, V is the volume of extract, and f is the dilution factor.

7 Estimation of Oxalates

Oxalates are quantified by titration with a standard solution of potassium permanganate. The sample is boiled with dilute sulfuric acid to extract oxalates. After filtration, the extract is titrated against potassium permanganate until a pink color persists. The oxalate content is calculated as:

Oxalate (mg/g) = [(V×N×0.126×1000)]/ weight of sample (g)

where V is the volume of permanganate solution used, N is the normality of the permanganate, and 0.126 is the molecular weight of oxalic acid.

8 Estimation of Phenols

Phenolic compounds are estimated using the Folin-Ciocalteu method. A sample is mixed with Folin-Ciocalteu reagent and sodium carbonate, followed by a color change measured at 765 nm. The phenol concentration is determined using a calibration curve, and results are expressed as gallic acid equivalents (GAE):

Phenol (mg/g)=[(A×V×C)]/ weight of sample (g)

where A is the absorbance, V is the volume of the extract, and C is the concentration of the gallic acid standard.

9 Estimation of Flavonoids

The flavonoid content is determined using the aluminum chloride colorimetric method. The sample extract is treated with aluminum chloride, and the absorbance is measured at 430 nm. The flavonoid content is calculated using a standard quercetin curve:

Flavonoid (mg/g) = [(A×V×1000)]/weight of the sample (g) x molar extinction coefficient

where A is absorbance, V is the volume of extract, and the molar extinction coefficient refers to the standard's known value.

10 Estimation of Alkaloids

The alkaloid content is typically determined by gravimetric methods or colorimetrically using a reagent like Mayer‘s or Dragendorff‘s. The sample is first extracted with a solvent like ethanol or methanol, followed by precipitation with a reagent. The alkaloids are quantified by measuring absorbance or weighing the precipitate:

$$\textbf{Alkaloids (mg/g)} = [\ (A \times V \times F)] / \textbf{ weight of the sample (g)}$$

where A is the absorbance, V is the volume of extract, and F is a factor related to the standard concentration.

11 Simulated Gastric Juice Tolerance

The methodology proposed by Koh *et al.,* 2019 and Wu *et al.,* 2021 was adopted with minor modifications to assess the simulated gastric juice tolerance of the LAB isolate. The strain was centrifugally agitated at 5,000 revolutions per minute for 15 minutes at 5 °C after being cultivated in an MRS medium with 0.1% ascorbic acid for a 48-hour period at 37 °C. To conduct the test, gastric juice with a pH of 3.0, comprising 125 millimolar NaCl, 45 millimolar NaHCO3, 7 millimolar KCl, and 3 g/L pepsin was made. For the control, 1 M of HCl and sodium hydroxide were added to make the solution pH 7 (Archer *et al*., 2015). For both the assay and control groups, the bacterial pellet was initially resuspended in 10 ml of phosphate-buffered saline (PBS) at pH 7.4, followed by incubation in simulated gastric juice. The viability of the bacterial strain was subsequently assessed by enumerating colony formation over a period ranging from 0 to 3 hours.

$$\text{Bacterial viability (\%)} = \frac{CFU_{assay} \times 100}{CFU_{control}}$$

Bile Juice Tolerance

The procedure outlined by Nakkarach *et al*., 2018 was followed for conducting the bile tolerance test. 100 µL of bacterial culture that had been cultivated overnight was centrifuged and resuspended in PBS solution with 0.3% bile, adjusted to pH 8.0. Additionally, as a control, test strain isolate was inoculated in MRS broth solution without

bile. Resistance was measured for both test tubes by counting live colony counts after incubation at 37 °C. To establish bile salt tolerance, the absorption value of MRS broth at 600 nm was determined. This was done because the usual duration for meal assimilation in the small intestine is between 0 and 4 hours. By outspreading a hundred microliters of the isolate on top of the MRS agar plate after the organism had been incubated for four hours, the survivability of the same in 0.3% bile was measured as well.

Pancreatin Tolerance

0.1 ml of bacterial isolate from the 24-hour culture was extricated by centrifuging at 10,000×g for 5 min at 4 °C. They were briefly suspended in PBS (pH 7.2) and resuspended in the same solution of pH 8.0 containing 0.5% pancreatin. The control group received no treatment. For 48 hours at 37 °C, inoculated test tubes containing the bacterial isolate were incubated in a shaker incubator. By taking measurements of the absorbance at 600 nm at intervals of 24h for two days starting from 0h, pancreatin tolerance was ascertained. After 48 hours of incubation, the cell viability of the test and control specimen in MRS agar plates was also calculated as per the protocol suggested by Khagwal *et al.,* 2019 and Gebre *et al.,* 2023.

Determination of Surface-Hydrophobicity Index

The bacterial specimen was centrifuged for 10 min at 10,000 rpm. Using phosphate urea magnesium buffer, the cell-containing precipitate was washed twice. One part of n-hexadecane was incorporated into three parts of the LAB broth before it was incubated at 37 degrees Celsius for an hour. At 600 nm, the well-separated aqueous phase's absorbance was obtained (Reuben Roy *et al.,*2019).

$$\text{Hydrophobicity index (\%)} = \frac{OD_i - OD_f \times 100}{OD_i}$$

OD_i = Initial Absorbance; OD_f = final absorbance.

NaCl Tolerance

LAB cultured for 24h (1% v/v) were added to MRS culture medium containing varying amounts of NaCl (0%, 0.5, 2.0, 4.0, 7.5 and 10%) and incubated at ambient temperature for 12h. The extent of absorption at 600 nm was used to gauge the viability of the strains against the effect of Sodium Chloride concentrations (Yépez *et al.,* 2019)

Auto Aggregation Assay

The method proposed by de Oliveira Coelho *et al.,* in 2019 and Byakika *et al.,* in 2020 was slightly adapted to quantify the phenomenon of auto-aggregation, which represents a distinct interaction between the cells. To achieve this, a 12-hour bacterial suspension was subjected to centrifugal agitation at 5000 rotations per minute for a duration of 10 minutes to isolate cell pellets. Subsequently, these pellets were briefly suspended in PBS buffer at regular intervals, and upon redissolution, their initial absorbance was measured at 600 nm. Following incubation of the culture at 37 °C, the final absorbance of the supernatant was measured. The percentage of cellular auto-aggregation was determined using the equation provided below.

$$\text{Rate of Auto Aggregation (\%)} = \frac{OD_i - OD_f \times 100}{OD_i}$$

Determination of Antibiotic Susceptibility of *L. Reuteri* on Common Enteropathogens

With a few minimal alterations, the disc agar diffusion suggested by Cui *et al.,* 2018 and Wang *et al.,* 2021, was applied to determine susceptibility to antibiotics. On an MRS agar plate, 100µL of LAB cells (107 CFU mL^{-1}) were plated. The plates containing discs that had been infused with antibiotics were left to incubate at a temperature of 37°C for 24-48 hours. Amikacin –AK30mcg, Gatifloxacin – GAT 5mcg, Moxifloxacin –MO 5mcg, Amoxicillin sulbactam- AMS 30/15 mcg, Penicillin – P 10mcg, Amoxiclav– AMC 10mcg and Chloramphenicol – C 30 mcg, Vancomycin – VA 30mcg, Methicillin – MET 5mcg, procured from ‗hi-media' were used.

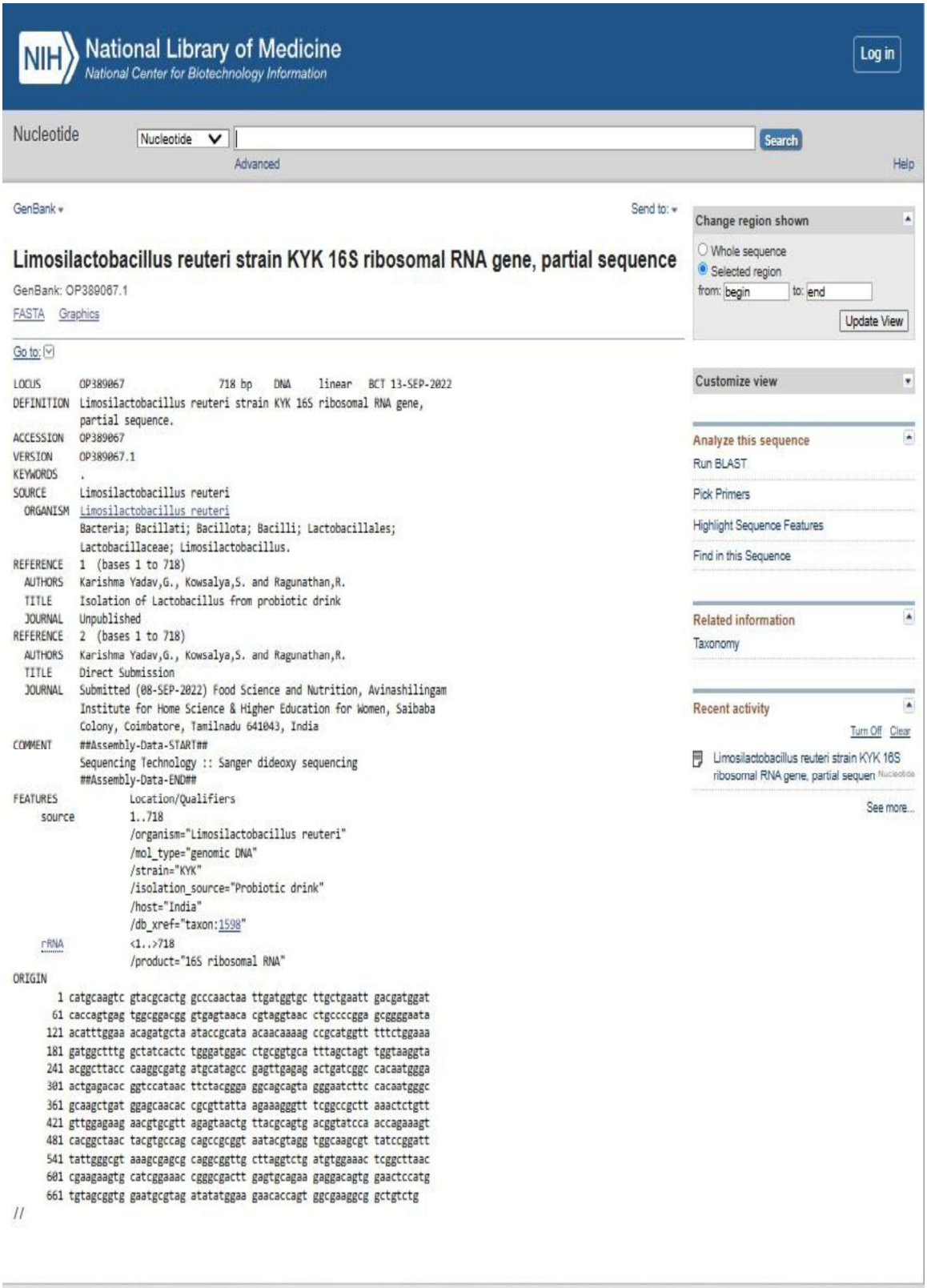
Antagonistic Activity of Extracted Antibacterial Agents Against Enteropathogens

Equal amounts of bacterial isolate were cultured for 48 hours at 37 ℃ with 5 mL of ethyl acetate added, and the composition was agitated in a rotary shaker for 10 minutes at 20 rotations per minute, and the supernatant was then transferred into a fresh tube. An antagonistic effect on the enteropathogens was carried out with the rest of the contents. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Salmonella typhi*, and *Escherichia coli* were the enteropathogens tested via well diffusion method to study the growth inhibitory effects of *L. reuteri* against the fore-mentioned test pathogens. According to Zhang *et al.,* 2020, the size of the growth-inhibiting zones was scaled and categorized as sensitive (18±2 mm), intermediately sensitive (10-18 mm), and resistant (>10 mm).

Haemolytic Activity

Determining the pathogenicity of isolates of bacteria is a crucial factor for prophylactic microorganisms in general. On the contrary, the absence of the action shows that non-virulent strains are present. The bacterial culture was scattered onto sheep blood agar plates that contained 5% sheep blood and incubated thereafter at ambient temperature for a 48-hour period (Romero-Luna *et al.,* 2020 Mousanejadi *et al.,* 2023). A prominent, colourless zone encircling the colonies, showing complete RBC lysis, indicated the possibility of beta (β) haemolysis and served as a visual representation of its presence. A small section of the media when discoloured from green to brown signifies alpha (α) haemolysis, due to the reduction of haemoglobin to met-haemoglobin. No change in the medium is indicated by gamma (γ) haemolysis (Vaithilingam *et al.,* 2016).

APPENDIX IV



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Limosilactobacillus reuteri strain KYK 16S ribosomal RNA gene, partial sequence

GenBank: OP389067.1

FASTA Graphics

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            Lactobacillaceae; Limosilactobacillus.
REFERENCE   1  (bases 1 to 718)
  AUTHORS   Karishma Yadav,G., Kowsalya,S. and Ragunathan,R.
  TITLE     Isolation of Lactobacillus from probiotic drink
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 718)
  AUTHORS   Karishma Yadav,G., Kowsalya,S. and Ragunathan,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2022) Food Science and Nutrition, Avinashilingam
            Institute for Home Science & Higher Education for Women, Saibaba
            Colony, Coimbatore, Tamilnadu 641043, India
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Change region shown
Whole sequence
Selected region
from: begin to: end
Update View

Customize view

Analyze this sequence
Run BLAST
Pick Primers
Highlight Sequence Features
Find in this Sequence

Related information
Taxonomy

Recent activity
Turn Off Clear
Limosilactobacillus reuteri strain KYK 16S ribosomal RNA gene, partial sequen Nucleotide
See more...

APPENDIX V

DEPARTMENT OF FOOD SCIENCE AND NUTRITION
SCHOOL OF HOME SCIENCE

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN, COIMBATORE

SENSORY EVALUATION SHEET

Name : **Date :**

Product : **Time :**

You are provided with..... number of sample/samples. Please evaluate these samples for acceptability and allot a score from the hedonic scale as below :

Liked extremely : 9

Liked very much : 8

Liked moderately : 7

Liked slightly : 6

Neither like nor dislike : 5

Dislike slightly : 4

Dislike moderately : 3

Dislike very much : 2

Dislike extremely : 1

Product Code	Colour	Flavour	Consistency	Appearence	Taste	Overall acceptability

Remark :

Signature

APPENDIX VI

PUBLICATIONS

Avinashilingam Institute for HomeScience and Higher Education for Women
(Deemed to be University Estd. u/s 3 of UGC Act 1956, Category 'A' by MHRD
Re-accredited with A++ Grade by NAAC. CGPA 3.65/4, Category I by UGC
Coimbatore - 641 043, Tamil Nadu, India

Appendix L2

(Item No 5 of Check List)

Details of Research

Publications

S. No	Article	Journal	Other Details Vol/No/Page No/ Year	Published in UGC-CARE / Scopus Indexed/ Web of Science
1	**Formulation and Evaluation of Nutritional and Bioactive Compounds in a Probiotic Beverage Containing *Ulva Sp.***	***The Indian Journal of Nutrition and Dietetics*** **ISSN: 2348-621X E-ISSN: 0022-3174**	**Volume-59; Issue-4; Page No.: 467-477**	**UGC-CARE 1 (Sciences – S.No-447)**
2	**Probiotic Properties and Safety Assessment of *Lactobacillus reuteri* in a Beverage Containing *Ulva sp.***	***The Indian Journal of Home Science*** **E-ISSN: 0970-2733**	**Volume-36; Issue-1; Page No.-330-341**	**UGC-CARE 1 (Social Sciences – S.No-405)**

*Proof of list of Journals from Internet to be attached along with copies of reprints.

Scholar : Karishma.

Supervisor : S. Kamalam 14/05/24

The scholar Miss. Guntiboina Karishma Yadav (18PHFNF003) has published her articles in the following journals:
1. The Indian Journal of Nutrition and Dietetics - indexed in UGC care Gp.I from Jan. 2021 to present and
2. The Indian Journal of Home Science - indexed and active in UGC Care Gp.I from July 2020 to present.
This may be considered.

Checked By:
14/5/24
HoD/Dean of Respective School
14.05.2024

Avinashilingam Institute for Home Science and Higher Education for Women
(Deemed to be University Estd. u/s 3 of UGC Act 1956, Category A by MHRD)
Re-accredited with 'A++' Grade by NAAC.CGPA 3.65/4, Category I by UGC
Coimbatore – 641 043, Tamil Nadu, India

PLAGIARISM CHECK REPORT (THESES)

1.	Name of the Research Scholar	Guntiboina Karishma Yadav
2.	Roll No. and Year of Registration	18PHFNF003, 2018
3.	Department	Food Science and Nutrition
4.	Name of the Research Guide	Dr. S. Kowsalya
5.	Title of the Thesis / Dissertation	Development and Evaluation of *Ulva lactuca* based Probiotic Beverage and *in vitro* Bioavailability of Iron using Caco-2 Cell Model
6.	Similarity Content (%) Identified	**6%**
7.	Software Used	Turnitin
8.	Date of Verification	11-11-2024

Note : The report is excluding 14 Consecutive words, Review of Literature and Quoted Materials.

Checked by :

11/11/24
Information Scientist

Karishma
Research Scholar

11-11-2024
Assistant Librarian

S. Kowsalya 11/11/24
Research Guide

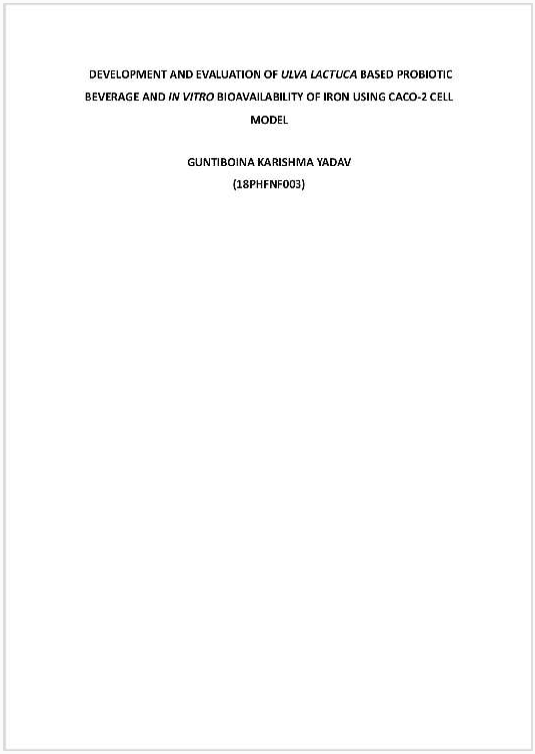
Date: 11-11-2024

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Central Library Avinashilingam
Assignment title:	Paper 2024
Submission title:	Development and Evaluation of Ulva lactuca based Probiotic …
File name:	ND_IN_VITRO_BIOAVAILABILITY_OF_IRON_USING_CACO-2_CE…
File size:	14.89M
Page count:	118
Word count:	32,213
Character count:	186,903
Submission date:	11-Nov-2024 11:31AM (UTC+0530)
Submission ID:	2402229779



DEVELOPMENT AND EVALUATION OF *ULVA LACTUCA* BASED PROBIOTIC BEVERAGE AND *IN VITRO* BIOAVAILABILITY OF IRON USING CACO-2 CELL MODEL

GUNTIBOINA KARISHMA YADAV
(18PHFNF003)