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Growth Inhibition of Common Food Spoilage and Pathogenic Microorganisms in the Presence of Brown Seaweed Extracts

Shilpi Gupta · Sabrina Cox · Gaurav Rajauria · Amit Kumar Jaiswal · Nissreen Abu-Ghannam

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Abstract The possibility of using extracts from brown seaweed, *Himanthalia elongata*, as a natural antimicrobial agent for food preservation is presented. The effect of different concentrations of seaweed extract on the growth kinetics of four common food spoilage (*Pseudomonas aeruginosa* and *Enterococcus faecalis*) and food pathogenic microorganisms (*Listeria monocytogenes* and *Salmonella abony*) was examined. Seaweed extract at a concentration of 6% inhibited the growth of all four of the studied organisms. Lower concentrations of seaweed extract prolonged the lag phase and reduced both the exponential growth rate and final population densities of the culture. Suitability of three kinetic models, Baranyi–Roberts, modified Gompertz and logistic, for describing the growth/survival of organisms in the presence of different concentrations of the extract, was evaluated. Root mean square error (RMSE) and correlation coefficient (R^2) were used to evaluate the model performance. The R^2 value was greater than 0.95 for most of the cases indicating that the models could provide a good fitting to the experimental data. The RMSE and residual sum of squares were very low for all the three models, and no significant difference was observed in the goodness of fit between the three models as indicated by the F test.

Keywords Seaweed · Non-thermal methods · Food preservation · Baranyi–Roberts · Modified Gompertz · Logistic

Nomenclature	39
A	The lower asymptotic line of the growth curve as t decreases to zero 42
B	The relative maximum specific growth rate (per hour) at time M 43
C	The difference between the upper asymptotic line of the growth curve (maximum population level N_{max}) minus the lower asymptotic line ($N_{max} - N_0$) (log colony-forming unit (CFU) per millilitre) 44
M	The time at which the specific growth rate is maximum (hours) 45
N_0	Initial population level at time $t=0$ (log CFU per millilitre) 46
N_t	The cell number at any time t (log CFU per millilitre) 47
N_{max}	Maximum population level 48
R^2	The coefficient of determination 49
RSS	Residual sum of squares 50
RMSE	Root mean square error 51

Greek Letters	62
γ	Log ₁₀ maximum population density for Baranyi–Roberts model (log CFU per millilitre) 63
λ	Lag time (hours) 64
μ	Exponential specific growth rate for Baranyi–Roberts model (per hour) 65
μ_{max}	Maximum specific growth rate (per hour) 66

Introduction	82
Preservatives are required to maintain the quality, extend shelf life and ensure safety of fresh and processed food products. Although chemical preservatives form an essential part in food preservation, legislation has restricted their	83

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87 use in different foods (Brul and Coote 1999). At the same
 88 time, consumer preferences are moving towards foods that
 89 contain lower levels of chemical preservatives, exhibit
 90 characteristics of fresh or natural products and are micro-
 91 biologically safe as well.

92 Non-thermal methods such as the addition of naturally
 93 occurring compounds having antibacterial activity (Hayes
 94 et al. 2010), high pressure carbon dioxide (Garcia-Gonzalez
 95 et al. 2009), high intensity pulsed electric field (Mosqueda-
 96 Melgar et al. 2008), irradiation (Alighourchi et al. 2008) or
 97 ultrasound (Schenk et al. 2008; Salleh-Mack and Roberts
 98 2007) are increasingly gaining attention for preservation of
 99 minimally processed foods. In recent years, the use of
 100 naturally occurring antimicrobial agents to inhibit pathogen
 101 growth and prevent food spoilage has received special
 102 attention (Hayes et al. 2010). Useful antimicrobial phyto-
 103 chemicals can be divided into several categories such as
 104 phenolics and polyphenols; quinines; flavones, flavonoids
 105 and flavonols; tannins; alkaloids and lectins; coumarins and
 106 polypeptides. Nowadays, minimal preservation processes
 107 based on the combined factors technology are also gaining
 108 importance for food preservation. Char et al. (2010) studied
 109 the response of *Listeria innocua* to combined treatments
 110 involving moderate temperatures and the addition of
 111 different levels of citral to obtain a minimally processed
 112 orange juice.

113 Seaweeds are considered a source of bioactive com-
 114 pounds as they are able to produce a great variety of
 115 secondary metabolites characterised by a broad spectrum of
 116 biological activities. Although seaweeds grow in a harsh
 117 environment, they seldom suffer any serious photodynamic
 118 damage during metabolism. This fact implies that seaweed
 119 cells have some protective compounds and mechanisms
 120 (Matsukawa et al. 1997). Since seaweeds are a good source
 121 of antimicrobial compounds, ω 3 fatty acids, antioxidants
 122 and other bioactive compounds, there is an interest to
 123 utilize these products as nutraceuticals and in functional
 124 foods (Yuan 2008). Compounds, such as polyphenols,
 125 flavonoids and polysaccharides, having antioxidant and
 126 antimicrobial activities have been detected in brown, red
 127 and green algae (Cox et al. 2009; Zaragoza et al. 2008). Ara
 128 et al. (2002) reported brown algae to be active against a
 129 number of Gram-positive and Gram-negative organisms.
 130 Nagayama et al. (2002) reported that phlorotannins, brown-
 131 algal phenolic compounds, such as eckol and eckol-related
 132 compounds, from *Ecklonia kurome*, have strong bactericid-
 133 al activity. A series of polyphenolic compounds such as
 134 catechins, flavonols and flavonol glycosides have been
 135 identified from methanol extracts of red and brown algae
 136 (Hosokawa et al. 2006) and found to have antioxidant and
 137 antimicrobial activity. Horie et al. (2008) isolated sargaqui-
 138 noic acid derivatives from the brown alga *Sargassum*
 139 *sagamianum* having antibacterial properties.

Traditional microbial enumeration techniques are time- 140
 consuming, and therefore, mathematical microbial models 141
 are used to assess the potential for growth of micro- 142
 organisms in foods during processing and storage (Bovil et 143
 al. 2001). Empirical sigmoidal type models such as the 144
 modified Gompertz and logistic models or the semi- 145
 mechanistic model of Baranyi–Roberts have been used for 146
 fitting bacterial growth (Xiong et al. 1999). However, data 147
 on the use of actual plant extract for inhibiting microbial 148
 growth and modelling the resulting kinetics are scarce. 149
 Most of the studies done till date use either thermal 150
 treatments or purified compounds having antimicrobial 151
 activity for studying growth inhibition. 152

This study was conducted to determine the effect of 153
 different concentrations of brown seaweed (*Himanthalia* 154
elongata) extract against *Listeria monocytogenes*, *Salmo-* 155
nella abony, *Enterococcus faecalis* and *Pseudomonas* 156
aeruginosa. There are some reports available wherein the 157
 antimicrobial effect of seaweed extract has been studied on 158
 different organisms (Taskin et al. 2010; Cox et al. 2009; Ely 159
 et al. 2004; Nagayama et al. 2002), but no studies are 160
 available where the growth inhibition has been modelled. 161
 Hence, the present study investigates the utilization of 162
 methanolic extract from brown seaweed as a natural 163
 antimicrobial agent for food preservation by examining its 164
 effects on the growth kinetics of four common food 165
 spoilage and food pathogenic microorganisms. In order to 166
 describe growth inhibition in the presence of seaweed 167
 extract, performance of three commonly used primary 168
 models, namely the Baranyi–Roberts, modified Gompertz 169
 and logistic models, was evaluated. 170

171 Materials and Methods

172 Seaweed Material

Brown seaweed *H. elongata* (Pheophyta) was purchased 173
 from Quality Sea Veg., Co. Donegal, Ireland. Samples were 174
 received in September 2009 and washed thoroughly with 175
 freshwater to remove epiphytes and salt. 176

177 Preparation of Seaweed Extracts

The extraction of seaweed was carried out with 60% 178
 methanol under nitrogen atmosphere at 40 °C and 179
 100 rpm in a shaker incubator (Innova 42, Mason 180
 Technology, Ireland). Samples were filtered and centrifuged 181
 at 10,000 rpm (8,720×g) for 15 min (Sigma 2K15, Mason 182
 Technology, Ireland). Resulting extracts were evaporated to 183
 dryness using vacuum polyevaporator (Buchi Syncore 184
 Polyvap, Mason Technology, Ireland) at 60 °C. A pressure 185
 gradient programme was designed for evaporation of the 186

187	solvents with vacuum conditions of 33,700 and 7,200 Pa	The plate was incubated in the microtitre reader for	233
188	for methanol and water, respectively.	24 h at respective temperature for each organism.	234
189	Antimicrobial Activity	Microbial growth was recorded every 2 h on a Power-	235
190	<i>Microbial Culture</i>	wave microplate spectrophotometer (Powerwave, Biotek)	236
191	Two species of common food pathogenic and food spoilage	driven by Gen5 reader control and data analysis	237
192	bacteria selected for this study were <i>L. monocytogenes</i>	software. Turbidity was measured as absorbance at	238
193	(ATCC 19115), <i>S. abony</i> (NCTC 6017), <i>E. faecalis</i> (ATCC	600 nm, with 20 s agitation before each OD measure-	239
194	7080) and <i>P. aeruginosa</i> (ATCC 27853), respectively	ment. The OD values were converted to log CFU per	240
195	(Medical Supply Company, Dublin, Ireland). All cultures	millilitre by the standard curve as described in “ Relation	241
196	were maintained at -70 °C in 20% glycerol stocks and	Between Turbidity and Viable Count ” section.	242
197	grown in Tryptic Soy Broth (TSB; Scharlau Chemie,	Growth Curve	243
198	Barcelona, Spain) at 37 °C, except for <i>P. aeruginosa</i> which	To describe the inhibition of bacterial growth in the	244
199	was incubated at 30 °C, to obtain sub-cultures. A final cell	presence of seaweed extract, three primary growth models,	245
200	concentration of 1×10^6 colony-forming units (CFU)/ml	namely modified Gompertz, logistic and Baranyi–Roberts	246
201	was used for the experiments.	model, were fitted to the data, and their performance was	247
202	<i>Relation Between Turbidity and Viable Count</i>	comparatively evaluated. Growth curves were plotted to	248
203	Before the kinetics study, a relationship between optical	evaluate the antibacterial activities of the seaweed extract.	249
204	density at 600 nm and viable count was determined for all	<i>Baranyi–Roberts Model</i>	250
205	of the bacteria studied. A volume of 200 µl of bacterial	A programme implemented in Microsoft Excel (DM-Fit;	251
206	suspension containing 6 log CFU/ml was dispensed into	Institute of Food Research, Norwich, UK) was used to fit	252
207	50 wells of the 96-well microtitre plate (Sarstedt Ltd., UK).	the equation of Baranyi and Roberts (1994) to the growth	253
208	Every hour, the optical density (OD) of the microtitre plate	data. To evaluate the effect of different extract concen-	254
209	was read. At the same time, an aliquot of 100 µl from one	trations main kinetic parameters such as exponential	255
210	well was transferred into 900 µl of maximum recovery	specific growth rate (μ), Log_{10} maximum population	256
211	diluent (Scharlau Chemie, Barcelona, Spain) to determine	density (γ), lag time (λ) and the coefficient of determination	257
212	the viable cell count. Spreading was carried out on Tryptic	(R^2) were calculated.	258
213	soy agar (Scharlau Chemie, Barcelona, Spain) plates by	<i>Modified Gompertz Model</i>	259
214	taking 100 µl of relevant dilution. Plates were incubated at	The modified Gompertz model (Gibson et al. 1987) is given	260
215	37 °C, with the exception of <i>P. aeruginosa</i> (30 °C), for 24 h	by Eq. 1,	261
216	before determining the CFU per millilitre. A standard curve	$N_t = A + C \times \exp \left[- \exp \left\{ -B \times (t - M) \right\} \right] \quad (1)$	
217	(OD _{600nm} vs. log CFU per millilitre) was drawn from the	where N_t is the cell number (log CFU per millilitre) at any	262
218	results obtained. This curve was later used for conversion	time t , A is the lower asymptotic line of the growth curve as	264
219	of the OD values to log CFU per millilitre for respective	t decreases to zero (that is N_0 : initial population level at	265
220	bacteria in the presence of seaweed extract.	time $t=0$ (log CFU per millilitre)), C is the difference	266
221	<i>Antimicrobial Activity Assay</i>	between the upper asymptotic line of the growth curve	267
222	The influence of varying concentrations of extract on	(maximum population level, N_{max}) minus the lower	268
223	efficacy was assessed against the four organisms using	asymptotic line (for example, $N_{\text{max}} - N_0$ (log CFU per	269
224	96-well microtitre plates. Extract (300 mg) obtained	millilitre)), B is the relative maximum specific growth rate	270
225	from 5 g fresh seaweed was dissolved in TSB (2.5 ml),	(per hour) at time M and M is the time at which the specific	271
226	and 200 µl was added to the first row of each plate. All	growth rate is maximum. Equations 2, 3 and 4 can then be	272
227	other wells were filled with 100 µl of TSB, and 100 µl	used for the calculation of maximum specific growth rate	273
228	from the first well was serially diluted into 2-fold along	(μ_{max} (per hour)), lag phase duration (λ , hours) and	274
229	each column. Finally, 100 µl of bacterial suspension	maximum cell population (N_{max}), respectively:	275
230	containing 6 log CFU/ml was added to the wells. The	$\mu_{\text{max}} = \frac{B \times C}{e} \quad (2)$	
231	last row was used for bacterium and media controls.		
232	Sample blanks were also prepared for all of the extracts.		

276 where $e=2.7182$

$$\lambda = M - \frac{1}{B} \quad (3)$$

279

$$N_{max} = A + C \quad (4)$$

282

283 *Logistic Model*

284 The logistic model used for defining bacterial growth as a
 285 function of time at constant environmental conditions, such
 286 as temperature, pH, water activity etc. is given by Eq. 5
 287 (Gibson et al. 1987):

$$N_t = A + \frac{C}{1 + \exp[-B \times (t - M)]} \quad (5)$$

289 where N_t , A , B , M and C have the same meaning as given
 290 for the modified Gompertz equation. The μ_{max} and λ
 291 parameters can be calculated by Eqs. 6 and 7, respectively,
 292 as follows:

$$\mu_{max} = \frac{B \times C}{4} \quad (6)$$

295

$$\lambda = M - \frac{2}{B} \quad (7)$$

296

298 *Model Comparison*

299 *Root Mean Square Error* The smaller the root mean square
 300 error (RMSE) values, the better the fit of the model to the
 301 data.

$$RMSE = \sqrt{\frac{\sum (\text{predicted} - \text{observed})^2}{n - p}} \quad (8)$$

303 where n is the number of observations and p the number of
 304 parameters to be estimated.

305

306 *Curve Fitting*

307 A plot of microbial count versus time for each extract
 308 concentration was used to derive the starting values for the
 309 parameters, N_0 and N_{max} , for all three models evaluated.
 310 The lag time was obtained from the raw data by noting the
 311 time when exponential growth started. The experimental
 312 data were fitted to equations described above by nonlinear
 313 regression with a Marquardt algorithm using the software
 314 Statgraphics Centurion XV (StatPoint Technologies, Inc.,
 315 Warrenton, VA, USA). The aim of the fitting procedure was

to find each model's parameters that best described the data 316
 by minimizing the sum of the squares of the differences 317
 between the model simulated and experimental values. 318

Statistical Analysis 319

All experiments were performed in duplicate and replicated 320
 at least three times. All statistical analyses were carried out 321
 using STATGRAPHICS Centurion XV. Statistical differ- 322
 ences between extract activities were determined using 323
 ANOVA followed by least significant difference testing. 324
 Differences were considered statistically significant when 325
 $p < 0.05$. 326

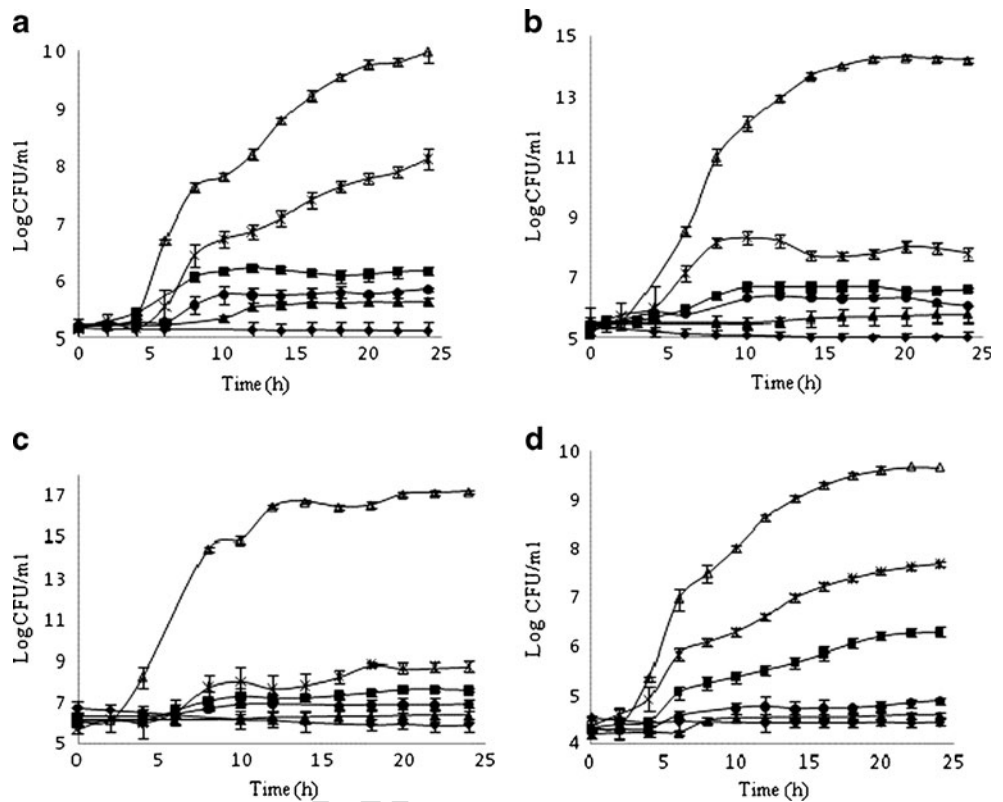
Results and Discussion 327

Antimicrobial Effect of Different Concentrations of *H.* 328
elongata Extracts 329

In our previous study (Cox et al. 2009), we had reported the 330
 antioxidant capacity of six species of Irish seaweeds and 331
 found the methanolic extracts from *H. elongata* to be the 332
 richest in terms of antioxidant properties. The selection of 333
 the pathogenic microbes (*L. monocytogenes* and *S. abony*) 334
 was made after discussions with the Food Safety Authority 335
 of Ireland as these were found to be the most challenging 336
 organisms for the safety of food products in Ireland. The 337
 other two (*E. faecalis* and *P. aeruginosa*) are the most 338
 widespread food spoilage microorganisms. Since the yield 339
 of the extract obtained was only 60 mg/g seaweed, growth 340
 inhibition was checked by measuring the OD by a micro- 341
 titre plate-based assay rather than by the conventional 342
 spread plate method. As expected, control samples showed 343
 a rapid and prolific growth, as the populations were 9.6, 10, 344
 14.1 and 17.2 log CFU/ml after 24 h for *S. abony*, *L.* 345
monocytogenes, *P. aeruginosa* and *E. faecalis*, respectively 346
 (Fig. 1). The incorporation of seaweed extract resulted in 347
 variable levels of inhibition in the growth of the different 348
 organisms. Resistance to extract was not correlated with 349
 taxonomy, since *E. faecalis* (Gram positive) and *P.* 350
aeruginosa (Gram negative) were the most sensitive to all 351
 of the different concentrations of the extract followed by *S.* 352
abony and *L. monocytogenes*. Figure 1 shows the influence 353
 of the different concentrations of crude extracts obtained 354
 from *H. elongata* against the four studied organisms. The 355
 extract had a strong antagonizing effect on the food 356
 spoilage and pathogens studied, showing a remarkable 357
 dose-response relationship with an increase of the lag 358
 phase duration and decrease of the exponential growth rate. 359
 In addition, a reduction in the maximum number attained or 360
 a complete suppression of growth was observed. The 361
 addition of *H. elongata* extracts resulted in complete 362

Q1

Fig. 1 Growth kinetics of food spoilage and pathogenic bacteria in presence of different concentrations of seaweed extract: **a** *L. monocytogenes*, **b** *P. aeruginosa*, **c** *E. faecalis* and **d** *S. abony*. Different concentrations of extract used: diamond 6%, black triangle 3%, circle 1.5%, square 0.75%, asterisk 0.375% and white triangle 0%



363 growth inhibition of all the studied organisms at the highest
 364 extract concentration (6%) used (Fig. 1). The bactericidal
 365 activity can be attributed due to the presence of phenolic
 366 compounds such as bromophenols and phlorotannins,
 367 produced by brown algae (Nagayama et al. 2002). Phenolic
 368 compounds from other plant sources have also been
 369 reported to inhibit various foodborne pathogens (Plaza et
 370 al. 2010; Kim et al. 2005; Prashanth et al. 2001).
 371 Polyphenols, such as tannins and flavonoids, are important
 372 antibacterial substances. Halogen-containing terpenoids,
 373 acetylenes and phenols have also been identified in several
 374 seaweed species as biologically active compounds having
 375 antibacterial and anti-tumoural activities (Cardozo et al.
 376 2007; Vairappan et al. 2001; Carvalho and Roque 2000).
 377 Plaza et al. (2010) identified volatile compounds like fatty
 378 acids, alkanes, phenols and compounds such as phytol (2-
 379 hexadecen-1-ol, 3,7,11,15-tetramethyl) and neophytadiene
 380 in the ethanol extracts from *Synechocystis* sp. and *H.*
 381 *elongata*. These compounds have been already proposed to
 382 have antimicrobial activity (Alagić et al. 2006).

383 The cell density in the presence of 6% extracts upon
 384 completion of the assay (24 h) was lower for all four
 385 bacteria than the initial bacterial density. Similar effect was
 386 seen in earlier studies on the effect of seaweed extracts on
 387 growth of marine and fish pathogenic extracts (Dubber and
 388 Harder 2008) where it had been anticipated the reason for
 389 this frequently observed result could be associated with the
 390 complete disappearance of the bacterial DNA upon incu-

391 bation with algal extract components. Therefore, the
 392 extracts presumably evoked not only a bacteriostatic but
 393 also bacteriolytic mode of action. Studies by Ceylan et al.
 394 (1998) revealed that addition of 1% spice (garlic, clove and
 395 cinnamon) to salami mixed with starter culture and
 396 *Escherichia coli* O157:H7 resulted in slight reduction of
 397 the pathogen; however, the addition of 7.5% garlic and
 398 clove killed 99% of the pathogen. Similar results were
 399 obtained in the present study as well wherein addition of
 400 6% extracts resulted in growth inhibition and extract
 401 concentrations lesser than that caused a reduction in the
 402 cell numbers.

403 As the extract concentration was serially diluted, the
 404 inactivation effect was reduced. Although the addition of
 405 extracts at a concentration of 3% did not result in a complete
 406 inactivation of bacteria, the growth kinetics was highly
 407 altered. An increase in the cell number, after 24 h of
 408 incubation with 3% extract, for each of the four bacteria
 409 was in the range of 0.21–0.43 log CFU/ml. A lag phase much
 410 longer than the control was observed, and the specific growth
 411 rate was significantly reduced for all the organisms. At the
 412 same time, a reduction of 98% (*E. faecalis*), 97% (*P.*
 413 *aeruginosa*), 93% (*S. abony*) and 91% (*L. monocytogenes*)
 414 in the stationary level growth was observed as compared to
 415 the control. There was a significant difference ($p < 0.05$) in
 416 the stationary phase growth (24 h) of *P. aeruginosa*, *E.*
 417 *faecalis*, *L. monocytogenes* and *S. abony* upon the addition
 418 of 3%, 1.5% and 0.75% extract. The increase in the cell

419 number upon the addition of 1.5% extract, after 24 h
 420 incubation, was in the range of 0.6–0.8 log CFU/ml. Thus, it
 421 can be said that addition of these concentrations of extracts
 422 resulted in an extended lag phase. Despite the fact that
 423 reducing the extract concentration to 0.75% resulted in a lag
 424 phase similar to that of the control, the specific growth rate
 425 was highly suppressed. Hence, an increase of 1.5-, 1.6-, 2.2-
 426 and 2.3-fold in the stationary phase growth of *S. abony*, *L.*
 427 *monocytogenes*, *P. aeruginosa* and *E. faecalis*, respectively,
 428 was seen in the control (0% extract) as compared to samples
 429 containing 0.75% extract. Thus, the bacteria started to grow
 430 at almost similar times, but the presence of the extract
 431 suppressed the maximum cell number attained. This was
 432 further evident from a slight increase of 1–1.9 log CFU/ml
 433 for each of the four bacteria after 24 h growth. Reducing the
 434 extract concentration further to 0.35% and 0.18% resulted in
 435 growth patterns very similar to the control in case of *L.*
 436 *monocytogenes* and *S. abony*.

437 The present study utilized methanol as a solvent for
 438 extraction of compounds responsible for the observed
 439 effect. Studies are also available wherein different solvents
 440 have been utilized for the extraction of biologically active

441 compounds from seaweeds. Earlier reports on the effective-
 442 ness of extraction methods evidenced that methanol
 443 extraction yielded higher antimicrobial activity than other
 444 solvents such as *n*-hexane and ethyl acetate (Sastry and Rao
 445 1994; Paul and Puglisi 2004). It is well documented that
 446 using organic solvents always provides a higher efficiency
 447 in extracting compounds for antimicrobial activities as
 448 compared to water-based methods (Masuda et al. 1997;
 449 Lima-Filho et al. 2002).

450 A significant finding of the present study was the
 451 potency of the extract against Gram-negative bacteria (*P.*
 452 *aeruginosa*). Gram-negative bacteria are more resistant
 453 pathogens compared to the Gram-positive bacteria. They
 454 have an additional lipopolysaccharide layer on the outer
 455 surface which prevents certain drugs and antibiotics from
 456 penetrating the cell thus accounting for the high resistance
 457 of these bacteria to antibiotics (Dowling 2004). Therefore,
 458 the present study brings out a new insight towards the
 459 development of antimicrobial agents against Gram-negative
 460 bacteria from seaweeds. In recent years, the use of non-
 461 thermal techniques for preservation of food has been
 462 gaining importance. The use of ozone, irradiation or

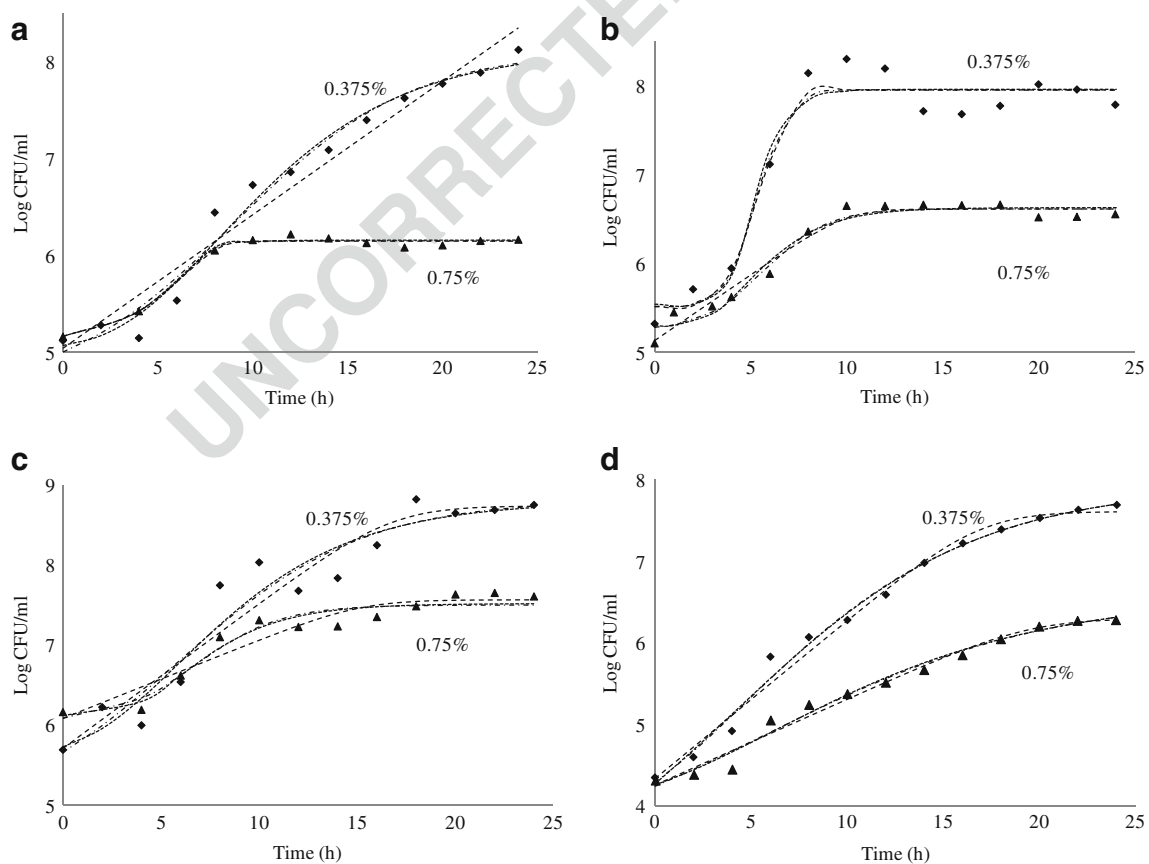


Fig. 2 Fitting of the three models to the inactivation of the four organisms by extract at a concentration of 0.75% and 0.375%. **a** *L. monocytogenes*, **b** *P. aeruginosa*, **c** *E. faecalis* and **d** *S. abony*. Different concentrations of extract used: triangle 0.75% and diamond

0.375%. Different models: dotted line modified Gompertz model equation, dashed line Baranyi–Roberts model equation and dash-dotted line logistic model equation. Points represent experimental data

463 ultrafiltration is better than thermal sterilization but can
 464 have a detrimental impact on the bioactive compounds
 465 present in foods if used at higher levels (Tiwari et al. 2009;
 466 Alighourchi et al. 2008; Zárate-Rodríguez et al. 2000). In
 467 this regard, the use of natural antimicrobials in foods to
 468 prevent spoilage might also provide the additional benefit
 469 of preserving the bioactive properties of foods. Thus, the
 470 addition of extracts from seaweeds can not only impart
 471 microbiological safety to food products as they are rich in
 472 bioactives (Cox et al. 2009) but can also provide foods with
 473 antioxidants in order to prevent oxidative spoilage.

474 Comparison of Kinetic Models

475 Generally, the models that can be used for describing the
 476 kinetics of survival curves are either empirical or based on
 477 biological assumptions. Three primary growth models
 478 (modified Gompertz (empirical), logistic (empirical) and
 479 Baranyi–Roberts model (semi-mechanistic model)) were
 480 used to analyse the delay or inhibition of growth against the
 481 four different organisms. In most of the cases, the R^2 values
 482 for all the models were greater than 0.9 (except when

extract at a concentration of 1.5% was added to *P.* 483
aeruginosa), indicating a good fit to the experimental data. 484
 Examples of the fit of the three models to the inactivation 485
 of the four organisms at an extract concentration of 0.75% 486
 and 0.375% are depicted in Fig. 2. All the parameters 487
 obtained for the three mathematical models were directly 488
 related to the extract concentration. Analyses of variance 489
 indicated that the maximum specific growth rate, μ_{max} , was 490
 significantly reduced ($p < 0.05$) with increasing extract 491
 concentration suggesting that the cells became more 492
 sensitive (Table 1). The estimated values for the lag phase 493
 for all the three models tended to increase as the extract 494
 concentration increased. In individual model analysis, it 495
 was found that all the three models were capable of fitting 496
 the experimental data very reasonably and produced almost 497
 similar curves; however, no model could produce consis- 498
 tently best fit to all the growth curves analysed. RMSE 499
 (Table 2) was used as a statistical measure for comparison 500
 of the experimental and model simulated values. There was 501
 no significant difference between the RMSE ($p > 0.05$) for 502
 the three models. One way to discriminate the goodness of 503
 fit among different models is to compare them statistically 504

t1.1 **Table 1** Estimations of the kinetic parameters using the logistic, modified Gompertz and the Baranyi-Roberts models

		Logistic		Baranyi–Roberts			Gompertz			
		Conc. (%)	μ	Lag	μ	Lag	<i>A</i>	μ	Lag	<i>A</i>
t1.4	<i>L. monocytogenes</i>	6	-0.0062	-	-0.0032	-	-	-0.007	-	4.94
t1.5		3	0.101	8.75	0.104	8.83	5.62	0.109	8.83	5.63
t1.6		1.5	0.195	6.24	0.18	6.106	5.77	0.179	6.02	5.77
t1.7		0.75	0.212	2.8	0.173	2.56	6.15	0.256	2.99	6.15
t1.8		0.375	0.191	-	0.137	-	-	0.209	2.77	8.13
t1.9		0	0.336	-	0.287	-	9.89	0.364	1.74	10.12
t1.10	<i>S. abony</i>	6	-0.163	1.73	-0.026	-	4.43	-0.177	1.75	4.43
t1.11		3	0.169	6.4	0.154	6.24	4.55	0.144	6.02	4.55
t1.12		1.5	0.223	0.47	0.191	4.49	4.76	0.148	4.05	4.77
t1.13		0.75	0.116	-	0.105	-	6.31	0.12	-	6.57
t1.14		0.375	0.22	-	0.193	-	7.61	0.226	-	7.95
t1.15		0	0.471	-	0.399	-	9.56	0.515	1.1	9.71
t1.16	<i>P. aeruginosa</i>	6	-0.005	-	-0.0415	-	5.03	-0.053	-	5.02
t1.17		3	0.035	10.3	0.0275	9.59	5.74	0.035	10.43	5.74
t1.18		1.5	0.129	2.5	0.102	-	6.25	0.141	0.74	6.25
t1.19		0.75	0.213	-	0.148	-	6.61	0.218	2.59	6.62
t1.20		0.375	0.764	3.74	0.669	3.54	7.94	0.808	3.65	7.95
t1.21		0	0.991	2.06	0.8127	1.49	14.118	1.09	3.1	14.25
t1.22	<i>E. faecalis</i>	6	-0.055	-	-0.049	-	5.92	-0.057	-	5.85
t1.23		3	0.017	6.4	0.0104	-	-	0.019	7.38	6.37
t1.24		1.5	0.167	5.55	0.147	5.33	6.88	0.173	5.47	6.88
t1.25		0.75	0.171	2.51	0.098	-	7.56	0.183	3.22	7.52
t1.26		0.375	0.216	-	0.18	-	8.73	0.235	0.985	8.79
t1.27		0	1.54	2.19	1.29	1.87	16.78	1.63	2.58	16.9

t2.1

Table 2 Values of the statistical indices, RMSE and RSS, for the three models against four different bacteria at six different extract concentrations

	Conc. (%)	RMSE			RSS			
		A	B	C	A	B	C	
<i>L. monocytogenes</i>	6	0.01	0.0	0.01	0.0	0.0	0.0	t2.4
	3	0.015	0.018	0.012	0.0018	0.0027	0.0012	t2.5
	1.5	0.035	0.036	0.035	0.0113	0.012	0.0113	t2.6
	0.75	0.044	0.043	0.046	0.014	0.013	0.0146	t2.7
	0.375	0.197	0.27	0.181	0.351	0.659	0.295	t2.8
	0	0.273	0.310	0.251	0.673	0.865	0.567	t2.9
<i>S. abony</i>	6	0.01	0.010	0.01	0.0	0.0	0.0	t2.10
	3	0.023	0.023	0.021	0.004	0.004	0.004	t2.11
	1.5	0.057	0.057	0.056	0.026	0.026	0.025	t2.12
	0.75	0.109	0.116	0.108	0.108	0.121	0.104	t2.13
	0.375	0.126	0.156	0.13	0.147	0.219	0.153	t2.14
	0	0.238	0.306	0.206	0.51	0.842	0.388	t2.15
<i>P. aeruginosa</i>	6	0.01	0.026	0.01	0.0	0.01	0.0	t2.16
	3	0.019	0.021	0.015	0.0024	0.003	0.001	t2.17
	1.5	0.146	0.133	0.149	0.148	0.124	0.155	t2.18
	0.75	0.106	0.106	0.121	0.112	0.112	0.147	t2.19
	0.375	0.332	0.233	0.257	0.994	0.488	0.595	t2.20
	0	0.235	0.337	0.15	0.44	0.909	0.181	t2.21
<i>E. faecalis</i>	6	0.027	0.031	0.027	0.004	0.006	0.004	t2.22
	3	0.019	0.026	0.017	0.002	0.005	0.002	t2.23
	1.5	0.021	0.017	0.027	0.003	0.002	0.005	t2.24
	0.75	0.155	0.185	0.139	0.192	0.272	0.156	t2.25
	0.375	0.337	0.357	0.332	1.026	1.147	0.994	t2.26
	0	0.455	0.559	0.374	1.65	2.51	1.12	t2.27

A logistic, B Baranyi–Roberts, C modified Gompertz equation

Zwietering et al. (1990). The models were statistically validated with the use of *F* test. The calculated *F* values were lower than the *F* table values, indicating that there was no significant difference in the goodness of fit between the three models, except for 6% extract concentration against *P. aeruginosa*. In this case, fitting by Gompertz and logistic were found to be better than Baranyi–Roberts model (data not shown). Although the performance of the modified Gompertz model was better than the logistic and the Baranyi–Roberts model, the use of one primary model or the other in case of inactivation curves should be guided by specific requirements (Geeraerd et al. 1997). However, based on the RMSE and residual sum of squares (RSS) values, it can be said that all the three model equations were effective for describing sigmoidal curves as previously reported by Xiong et al. (1999), who modelled the thermal inactivation of *L. monocytogenes*. The model kinetic parameters such as the λ , μ and N_{max} estimated using the experimental data with the modified Gompertz, logistic and Baranyi–Roberts model are summarized in Table 2. As there was no difference in the goodness of fit of the three models, the parameters of modified Gompertz were further analysed, as an example, to study the effect of extract

concentration on the bacterial growth. The most prominent effect of the extract was an increase in the lag phase duration. The increase in lag phase due to the addition of extract at a concentration of 3% was more than 3-fold for *L. monocytogenes* and *P. aeruginosa* as compared to the control. A delay in, or inhibition of, microbial growth is particularly useful in terms of food safety. The extension of the lag phase is probably the most widely used parameter to describe the inhibitory effects of antimicrobial compounds, and a slight delay in the lag phase may have an important

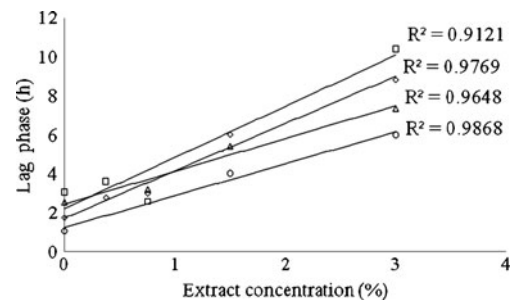


Fig. 3 Relation between lag phase and concentration of extract against the four different organisms (diamond—*L. monocytogenes*, circle—*S. abony*, square—*P. aeruginosa* and triangle—*E. faecalis*)

538 influence on the shelf life of food products. Thus, the
 539 prolonging of lag phase could be used as an appropriate
 540 index for evaluation of the activity of the antimicrobial
 541 compounds. In the present study, a linear positive correla-
 542 tion (R^2 ranging between 0.912 and 0.986) was obtained
 543 between the lag phase and the concentration of seaweed
 544 extract for the different organisms (Fig. 3). The seaweed
 545 extracts were potent even at an extract concentration as low
 546 as 0.75% resulting in 42% and 20% increase in the lag
 547 phase of *L. monocytogenes* and *E. faecalis*, respectively, as
 548 compared to the control. Extract concentration of 3%
 549 increased the lag phase in a range of 65% to 81% for all
 550 of the organisms. In an early report on microbial growth
 551 modelling of fresh filled pasta stored at different temper-
 552 atures by Giannuzzi (1998), it was observed that the ratio of
 553 specific growth rate to generation time was nearly constant
 554 which suggests a linear relationship between lag phase and
 555 the reciprocal of the maximum specific growth rate. Similar
 556 observations were confirmed in the present study during the
 557 inactivation of the four organisms at different extract
 558 concentrations.

559 The concentration of extract also had a strong effect on
 560 the maximum specific growth rate. A reduction of 99% and
 561 96.8% was observed in the maximum specific growth rate,
 562 as compared to control at 3% extract concentration (*E.*
 563 *faecalis* and *P. aeruginosa*, respectively).

564 It has been reported in the literature that flavonoids,
 565 polysaccharides, sesquiterpenes and phlorotannins can be
 566 obtained from seaweeds. These active ingredients produce
 567 varied pharmacological effects such as anti-angiogenic,
 568 anti-inflammation, disinfection and anti-tumour. The appli-
 569 cation of the extracts of *H. elongata* in food industry may
 570 contribute to such pharmacological activities as food anti-
 571 oxidation, health care and in addition as food nutrient.
 572 Therefore, these extracts could be applied as natural
 573 additives with extensive market prospect.

574 **Conclusion**

575 *H. elongata* can be considered as a promising marine plant
 576 in the development of bioactive ingredients for functional
 577 foods, nutraceuticals and other applications. The extracts
 578 showed an evident antimicrobial effect against the micro-
 579 organisms used in the present study in a dose-dependent
 580 manner. Complete growth inactivation of all of the studied
 581 organisms was observed at a concentration of 6%. Addition
 582 of extracts at a concentration less than that resulted in an
 583 extension of the lag phase and significantly reduced
 584 maximum specific growth rate. A reduction of 91–98% in
 585 the stationary level growth as compared to the control was
 586 also observed. The findings suggest that seaweed extracts
 587 have a good potential as natural antibacterial substances in

food preservation. It might be possible that high concen- 588
 589 trations of these extracts may adversely affect the organo-
 590 leptic properties of food; however, lower concentrations
 591 may be sufficient for food safety in situations where
 592 bacterial load is low. 593

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