International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 9, 2016

Original Article

TRIPLE OBJECTIVE OPTIMIZATION OF CYTOTOXIC POTENCY OF HUMAN CARCINOMA CELL LINES OF A MARINE MACROALGAE USING NON-SORTING GENETIC ALGORITHM–A THEORETICAL STUDY

J. SATYA ESWARI[a], SHADAB AHMED[b]

[a]Department of Biotechnology, National Institute of Technology Raipur, Raipur, Chhattisgarh, [b]Institute of Bioinformatics and Biotechnology, Savitribai Phule Pune University, Pune, Maharashtra
Email: satyaeswarij.bt@nitrr.ac.in

Received: 12 Apr 2016 Revised and Accepted: 22 Jul 2016

ABSTRACT

Objective: The prime objective of our work was to develop an acceptable model for optimizing the cytotoxic potency of human carcinoma cell lines of marine microalga which gives true indications when compared to experimental results.

Methods: The experimental result taken from Das *et al.*, 2014 [12] for cytotoxic potency against human colon cancer (HT-29), human hepatocyte cancer (Hep-G2), and human breast cancer (MCF-7) cell lines was used to carry out a multi-objective (triple objective) optimization. Thirty non-dominating solutions were considered for analyses of absorbance (y_1), % cell survival (y_2) and% cell inhibition (y_3) data. The multi-objective (triple objective) optimization study by using experimental data of Das *et al.*, 2014.

Results: The solutions obtained by non-dominated sorting genetic algorithm (NSGA) have been compared with data obtained experimentally, and the results were found to be significant. This method has distinct advantages over other methods which relied heavily on statistical-regression-models, single objective optimization methods in the sense that it does triple-objective optimization. The results were significant when compared with experimental data corroborating acceptability of the proposed model.

Conclusion: The solutions obtained by NSGA method, on comparison with experimental data, showed the applicability and suitability of the proposed model.

Keywords: Marine microalga, Carcinoma cells, Cytotoxic potency, Genetic algorithms, Triple-objective optimization, NSGA

© 2016 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4. 0/)
DOI: http://dx.doi.org/10.22159/ijpps.2016v8i9.12003

INTRODUCTION

Natural products can also be prepared by available routes or schematic organic synthesis processes (both semi-synthetic and synthetic) and they have been important in discovering or eliciting challenging synthetic targets based on cellular response [1]. Now, natural products can be a commercial product such as drugs, cosmetics, food supplements or food products having no added artificial ingredients [2-6]. Chemically derived drugs usually cause serious adverse effects, and hence the growing necessities for drugs from new sources of natural products are of paramount importance. Marine algae are not only major producers of carbon-based material in the sea; they also affect significant changes in the density and distribution of other inhabitants of the marine environment [7]. An understanding of the wide range of behavioral relationships that exist among different organisms would provide us with clues to produce substances of commercial or pharmaceutical importance. Marine secondary metabolites are set of diverse organic compounds, and may be obtained from microbes, sponges, seaweeds, and other marine organisms. The host organism synthesizes these compounds as secondary metabolites to protect themselves and it also helps them to maintain homeostasis in their environment [8].

Some of these secondary metabolites offer avenues for developing cost-effective, safe and potent drugs. Literature review suggested that many of the sea bio-products are being exploited for their industrial, chemical, food, pharmaceutical, medical and cosmetic applications [3-9].

Sea products named as seaweeds are taken by people because of nutritious value. Therefore, we can include food products from a marine organism or sea plants as a part of our daily meal or else to treat certain medical conditions such as inflammation, Herpes infection and sometimes even in cancer treatment [10-11]. One of the sea plant products from algae is used in the treatment of cancer. Das et al., [12] generated experimental data marine microalgae against human colon carcinoma (HT-29), human hepatocyte carcinoma (Hep-G2), and human breast carcinoma (MCF-7) cell lines. They developed a model based on response surface methodology (RSM) to get the various possible combinations of two independent variables so as to determine the optimum effect of cancer cells with the highest cytotoxicity. Hence, in our work we decided to perform a multi-objective optimization strategy for establishing the cytotoxic potency of an extract.

With the above objective, we focussed on the development of optimum condition using a genetic algorithm. Response surface methodology (RSM) is a practical, inexpensive and relatively easy method to adopt [12]. RSM approach is a combination of statistical and mathematical techniques useful for developing, improving and optimizing processes [12-16]. This method has been used by for prediction of optimization of copper reduction from solution using *Bacillus pumilus* PD3 isolated from marine water [17]. Kiran *et al.* [18] studied the anti-adhesive activity of polyhydroxy butyrate biopolymer from a marine *Brevibacterium casei* MSI04 against pathogenic shrimp vibrios. Chen, *et al.*, studied optimization of ultrasound-assisted extraction of Lingzhi polysaccharides using RSM and its inhibitory effect on cervical cancer cells [19]. But in our study, we used the advanced methodology for developing the optimum condition for this *micro algae* by using pareto technique. Since RSM has certain limitations and it may stick at local optima.

Remarkable advancement has been made as of late in the improvement of evolutionary algorithms for multi-objective optimization problems (MOPs) [20-25]. MOPs are designated by the presence of multiple conflicting objectives that must be optimized simultaneously and permit multiple best solutions [25, 26]. These multiple solutions are all optimal in the sense that there are no other solutions in the entire solution domain or search space that are superior to them when all objectives are considered simultaneously [20-22]. These “non-inferior” or non-dominated solutions are referred to as pareto-optimal solutions and collectively represent

the pareto set or front. The multi-objective evolutionary algorithms (MOEAs) populace based techniques for comprehending MOPs that have been created recently [25, 27]. These algorithms manage the pursuit process around the global pareto-ideal district while keeping up sufficient populace differences to catch, however, many results on the pareto-front as could be allowed. The idea behind the present work was to develop a multi-objective condition for the cytotoxic potency of *marine microalgae* based on the results assay against human colon carcinoma (HT-29), human hepatocyte carcinoma (Hep-G2), and human breast carcinoma (MCF-7) cell lines. The parameters which we optimized basically were the absorbance, cell survival (% of what) and cell inhibition (% of what), where we minimized the absorbance and enhanced the cell survival and the cell growth inhibition based on the response surface model obtained. This is a triple objective optimization, employed for the first time, as suggested by our literature survey, to develop the optimum condition for the cytotoxic potency *of marine microalgae* by using pareto technique.

MATERIALS AND METHODS

Experimental design procedures

Based on the Box-Wilson Central Composite Design (CCD) model, experimental data were conducted by Das *et al.*, [12] for three different cancer cell lines each at three levels of concentrations (low, medium and high) has taken from Das *et al.*, [12]).

Regression modelling

Regression modeling explores the relationships between several explanatory variables and one or more response variables [27, 28]. 'Explanatory' variable is the independent variable, whereas, 'Response' variably depends on independent variables. The objective is to optimize a response (output variable) which is influenced by several independent variables (input variables). The application of regression to design optimization is aimed at reducing the cost of expensive analysis methods and their associated numerical noise [28]. Response surface methodologies (RSM) are designs and models for working with continuous treatments when finding the optimal or describing the response is the goal. The first objective of RSM is to generate or predict the optimal response [29-31]. Since there are several responses, it becomes imperative to find the compromised optimum for such responses [29-31]. When there are constraints on the design data, the experimental model used must take into account such constraints [31-34]. The next important thing is to monitor and analyze how the response changes in a given direction, by fine-tuning the process variables [31-34]. In general, the response surface can be visualized graphically and response surface plots allow graphical visual observations to ascertain whether the regression analysis equations are significant or not. From the polynomial regression equations, the impact of varying concentrations on different cell lines was studied following the methodology adopted by Zhang *et al.*, [35]. All, highest negative influence was observed by the concentration of the drug. Whereas, % cell inhibition is greatly influenced by individual concentration or cell line, or their combined interaction, however, the role of concentration was found to be predominant. Using RSM, the relationship among the variables, i.e., the human cell lines and MEUF concentration were expressed mathematically in the form of the polynomial equation which gave the response of function of cell survival, cell inhibition and absorbance. This was done based on a central composite design to obtain a full second polynomial equation over a relatively broad range of parameters. The CCD took into account the combined effect of the both parameters and responses on the absorbance, % cell survival, and % cell inhibition (table 1). The polynomial equation is obtained from the regression analysis; the coefficient parameters were estimated by multiple linear regressions.

Table 1: CCD design inputs and outputs or responses used in polynomial equation

Inputs	Outputs
1. Concentration (µg/ml)	1. Absorbance at 492 nm
2. Cancer cell lines	2. % cell survival
	3. % cell inhibition

Genetic algorithm for optimization

Genetic algorithm (GA), which works on the principle of natural genetics, has the advantage of obtaining the pareto optimal set in a single run as it works with a population of points rather than with a single point [35, 36]. In the simplest form, the basic working principle of GA consists of a random selection of values for the variables which may be coded or real, constituting the population, calculation of the fitness value and then subjecting the population to reproduction, crossover and mutation so as to obtain a new population. This new population is also evaluated and checked to see if it meets the termination criteria.

Algorithm

GAs encodes the candidate solutions of a string of characters called as chromosomes, which are usually binary digits that are coded into a chromosome from substrings. The length of the string is usually determined according to the solution accuracy. A population size 'N' consists of the number of random strings for the variables or variations. The algorithm (GA) alters and appends the population iteratively, in each generation [35, 37]. The coding and decoding of sub-string is calculated and used in the next step function evaluation. The function value at the point *x* can be calculated by substituting the decoded x in the given objective function f(x). The function values for all the strings in the population are thus computed. A fitness function f(x) is derived from the objective function and used in successive genetic operations [37, 38].

Thus, the fitness of all individuals in the population is evaluated by using genetic operators such as reproduction, crossover and mutation to create a new population (http://in.mathworks.com/ help/gads/ some-genetic-algorithm-terminology.html). Reproduction selects best strings in the population and forms a mating pool. We first calculate the average fitness of the population F_{avg} obtained by finding the fitness values of all the strings and dividing the sum of the population size. Then we calculated the expected count 'A' of each string as $F(x)/F_{avg}$. Thereafter, we computed the probability of each string being copied into the mating pool, 'B' by dividing A with population size N; calculated the cumulative probability, 'C'; created random numbers between 0-1, and specified each string with a random number. If the random number falls near cumulative probability (C) of a particular string, that string is copied into the mating pool in a priority manner. During the crossover operation, new strings are created by exchanging information among strings in the mating pool. We chose a crossover probability P_c of around 0.8 and flip a coin that provides a random probability of yes or no condition. If the outcome is yes, crossing over is performed. Finally, the mutation operator changes 1 to 0 and vice versa with a small mutation probability, P_m of around 0.5. In every cycle, a new population is generated, evaluated and tested for termination. If this conditions, not met, the population repeatedly operates in a loop by the above three operators and assessed [34-41]. This process is routinely repeated until the termination conditions are achieved.

Multi-objective optimization

Invariably, most of the real-life problems require optimization of several objective functions and hence require the use of multi-objective optimization techniques. Traditionally, multi-objective functions are reduced to a single objective function by various methods and then saved as a single objective optimization technique [39-43]. All these optimization methods depend on the user's decision to specify weights to the different objective functions and therefore depend highly on the judgment of the user. The user may change the priorities and solve the problem to get a number of solutions. The set of all the solutions is known as the Pareto optimal set and the corresponding objective value vectors are known as pareto-optimal front, but the Pareto optimal set cannot be obtained simultaneously in a single run. Non-dominated Sorting Genetic Algorithm (NSGA) basically is different from the normal genetic algorithm in a way it selects. In the genetic algorithm, selection is unbiased in a way that, there are equal chances of processing of dominant and in dominant independent variables. However, in NSGA the sorting in ranking is allotted to the various groups after grouping and the maximum value is given to the non-dominant set hence it is

biased to favour the Pareto set of solutions. Doing this NSGA makes sure that there is a maximum number of copies of a non-dominant set of solutions in mating pool. So that output value obtained mostly using Pareto set to get the multi-objectives. The multi-objective optimization problem can be formulated as follows:

Minimize/maximize

$$f_i(x),\ I = 1, 2,3,\ldots, N_{\text{objectives}}$$

Subject to

$$gk(x) = 0,\ k = 1, 2, 3,\ldots, K$$

$$hl(x) \le 0,\ l = 1, 2, 3,\ldots, L$$

Where f_i is the i^{th} objective function, x is a decision vector, $N_{\text{objectives}}$ are the number of objectives, and K and L are the numbers of equality and inequality constraints, respectively. In the presence of conflicting objectives, optimizing x with respect to a single objective often results in unacceptable results with respect to the other objectives. A reasonable solution to a the multi-objective problem is to investigate a set of solutions, each of which satisfies the objectives at an acceptable level without being dominated by any other solution [12, 20, 43, 44, 46, 47, 48]. For a multi-objective optimization problem, any two solutions $x^1$and x^2can have one of two possibilities: one dominates the other or none dominates the other. In a minimization problem, without loss of generality, a solution x^1dominates x^2 if the following two conditions are satisfied:

$$\forall_i \in \{1,2,\ldots., N_{objectives}\}: f_i(x^1) \le f_i(x^2),$$

$$\exists_j \in \{1,2,\ldots., N_{objectives}\}: f_j(x^1) \le f_j(x^2).$$

If any of the above conditions is violated, the solution x^1does not dominate the solution x^2. If x^1 dominates the solution x^2, x^1 is called the non-dominated solution within the set $\{x^1, x^2\}$. The solutions that are non-dominated within the entire search space are denoted as Pareto-optimal and constitute the Pareto-optimal set or Pareto-optimal front.

Problem formulation

Minimize Absorbance (y_1), Maximize % cell survival (y_2), % cell inhibition (y_3).

The present work focuses on developing a multi-objective optimization methodology by integrating a response surface model (RSM) with genetic algorithms (GA) and evaluates its performance and applies it for Pareto optimization of cancer treatment. When GA is used to solve multi-objective optimization problems, it is denoted as non-sorted Genetic algorithm (NSGA). Here, the multi-objective optimization methodology that involves NSGA is referred to as a non-sorting Genetic algorithm. In cancer treatment study, the inputs were (x_1), concentration (µg/ml), (x_2) cancer cell line outputs are (y_1) absorbance at 492 nm, (y_2) % cell survival and (y_3) % cell inhibition, respectively. Now, the objective is to minimize the absorbance and at the same time, maximize the cell survival. No single optimal solution exists with respect to both objectives, as improving the performance of one objective deteriorates the performance of another objective. For optimal % cell survival, the best configuration of formulation conditions is to be chosen. Thus, a cancer treatment system with the conflicting objectives is considered to be a suitable test bed for multi-objective Pareto optimization. The performance of the NSGA strategies is evaluated with respect to this cytotoxicity study and the results are further compared with those of experimental results. The multi-objective Pareto optimization problem and its configuration to 3 objectives are described and executed in Matlab-2012.

RESULTS AND DISCUSSION

Development of multi-objective model from experimental data

From Das *et al.*, [12] the data taken were used to develop regression models, optimization by genetic algorithms and finally multi-objective optimization algorithms. According to CCD, 13 experimental trials (MTT assays) were conducted for three different cancer cell lines, each at three levels of concentrations (low, medium and high). These experiments were conducted by Das *et al.*, [12] published in 2014 were taken to develop multi-objective optimization strategies. The experimental versus predicted values for 3 responses are compared with multi-objective optimization and are depicted in fig. 1A, fig. 1B, and fig. 1C. From the fig. 1A, the absorbance (y_1) regression model, predicted versus experiments can be seen. In the same way, regression models of predicted versus experimental data for % cell survival (y_2) and % cell inhibition (y_3) shown in the fig. 1B and fig. 1C.

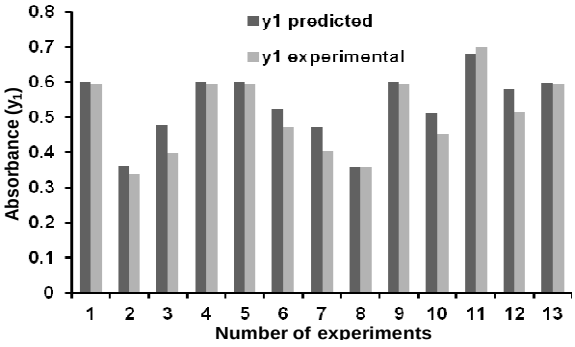

Fig. 1A: Regression model predicted (J. Satya eswari *et al.*, 2016) versus experimental (Das *et al.*, [12]) for absorbance (y_1), where x-axis represents number of experiments and y-axis absorbance

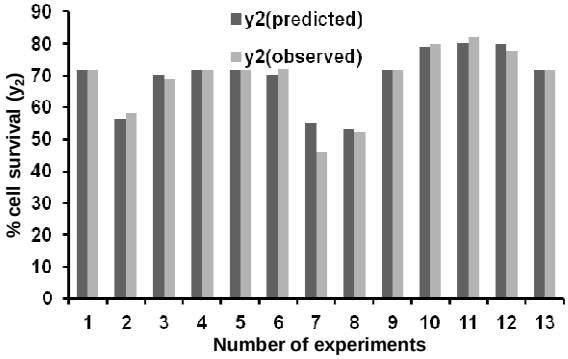

Fig. 1B: Regression model predicted (J. Satya eswari *et al.*, 2016) versus experimental (Das *et al.*, [12]) % cell survival (y_2), where, x-axis represents number of experiments and y-axis percentage (%) cell survival

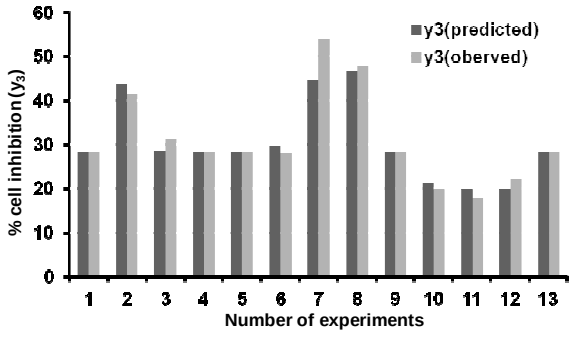

Fig. 1C: Regression model predicted (J. Satya eswari *et al.*, 2016) versus experimental (Das *et al.*, 2014) % cell inhibition (y_3), where, x-axis represents number of experiments and y-axis % cell inhibition

Regression modelling

In RSM based methods, it is always about finding a suitable approximation for the true functional relationship between the responses (y) and a set of independent variables. If the response is

well modelled by a linear function of the independent variables, then the approximation function is modelled. In a previous research study by Das *et al.*, [12] the regression equations were presented as response surface plots in showing the combined effect of independent factors X_1 and X_2 on the responses y_1, y_2, and y_3. However, in our study, we have used RSM, to establish a relationship between the variables, i.e. the human cell lines and MEUF concentration, expressed mathematically in the form of the polynomial equation, which gave the response of function of cell survival, cell inhibition and absorbance. The above was based on a central composite design utilized to obtain a full second polynomial equation over a relatively broad range of parameters (ref and justification). Whereas, % cell inhibition is greatly influenced by individual concentration or cell line, or their combined interaction and the role of concentration is predominant. It uses the combined effect of the both parameters and responses on the absorbance, % cell survival and % cell inhibition.

$$ß = \begin{pmatrix} = [(X^T*X]\ ]^{-1}\ [[X^T*Y] \\ 0.60 \quad 71.65 \quad 28.35 \\ -0.086 \quad -12.32 \quad 12.32 \\ 0.023 \quad -0.51 \quad 0.51 \\ -0.011 \quad -1.02 \quad 1.02 \\ 0.041 \quad -3.84 \quad 3.84 \\ -0.100 \quad -0.66 \quad 0.66 \end{pmatrix}$$

Regression equations

1) Absorbance at 492 nm (y_1) =

$$0.60-0.086A+0.023B-0.011AB-0.041A^2-0.100B^2$$

2) % cell survival (y_2) = $71.65-12.32A-0.51B-1.02AB-.4A^2-0.66B^2$

3) % cell inhibition (y_3) =

$$28.35+12.32A+ 0.51B+1.02AB +3.84A^2+0.66B^2$$

By using the above polynomial equations, we g*et al.* l the predicted values of response variables; they are similar to the observed values and after that we compare between the observed values and the predicted values by making a graph. The fig. 1A shows absorbance at 492 nm (y_1) experimental versus predicted. The fig. 1B shows % cell survival (y_2) experimental versus predicted, whereas, fig. 1C shows % cell inhibition (y_3) experimental versus predicted.

Genetic algorithms

The model-based genetic algorithm optimization was carried out in Matlab-2012. The first output for absorbance at 492 nm (y_1) is solved by a genetic algorithm. The optimization was running with the final objective function value 0.385. The termination criteria used for optimization by using tolerate function i.e. average change in the fitness value less than tolerance function. The population type is used double vector; the population size is 20 with the initial population given by Matlab-2012 GA default. The fitness scaling is scaling function by the ranking method. The selection criteria are a stochastic uniform method and in the reproduction, elite count is 2 with crossover function: 0.8. The mutation function is constraint dependencies. The stopping criterion is generations the crossover function is scattered: 50 and tolerance: 10^{-6}. The same conditions are maintained for solving the second and third objectives. The optimum output obtained for % cell survival (y_2) is by GA is 17.93, and the third objective % cell inhibition (y_3) is obtained by GA is 53.30. The fig. 2A, 2B, and 2C depicts the first, second and third objective functions solved by GA. From this fig. we can deduce the best fitness function along with the best Individual. The fitness of each individual and stopping criterion was also clearly observed looking at the above-mentioned figures.

Multi-objective optimization

The parameters used for NSGA (Non Sorting Genetic Algorithm) population type is double vector, the function creation dependent on constraint dependent, initial population used default, initial range in between 0 to 1, the selection criteria is tournament and the tournament size 2, in the reproduction the cross over fraction is 0.8, mutation is also constraint dependent, crossover function is intermediate type, migration is forward with the fraction 0.2, interval 20, multi-objective settings contain distance measure function and Pareto front population fraction is 0.35, stopping criteria is generations-200*number of variables, time limit =infinite, tolerance 10^{-4} [http://in.mathworks.com/help/gads/examples/multiobjective-genetic-algorithm-options.html; 45]. The objective functions were optimized fulfilling the constraints. The NSGA algorithm was used for obtaining the pareto optimal solutions. Real-parameter NSGA described in the earlier section has been used to optimize the parameters. A population size of 150 was chosen. The different operation was performed over 103 generations to obtain the non-dominated Pareto solutions. Average distance between two pareto solutions was found to be 0.0128. The lesser the observed or calculated distance is, better or significant the results are said to be. As many as thirty non-dominating solutions were obtained with respect to that absorbance (y_1), % cell survival (y_2) and % cell inhibition (y_3). The solutions obtained by non-dominated sorting genetic algorithm have been compared with data obtained experimentally and the results were similar and significant. The Pareto solutions are shown in fig. 3. The three objectives are shown by scattered plot in fig. 4.

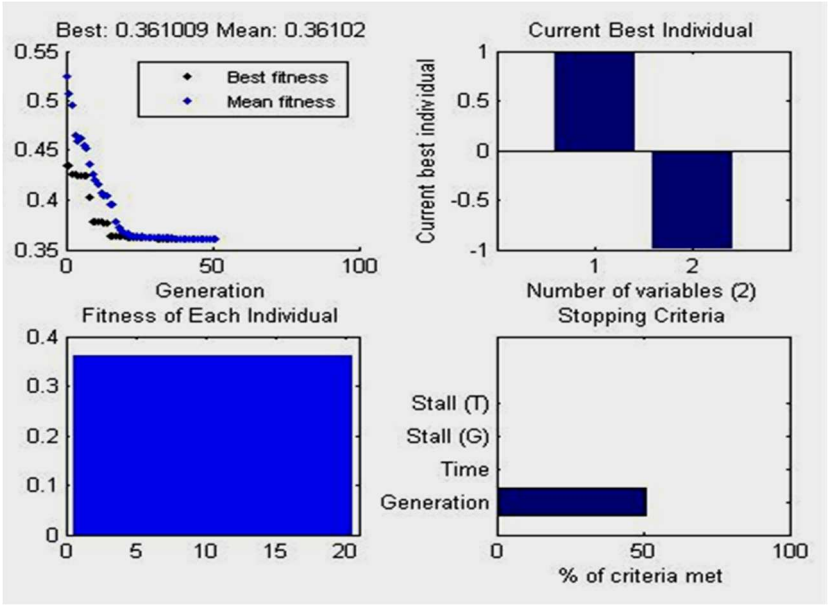

Fig. 2A: Genetic algorithm optimization for absorbance (y_1)

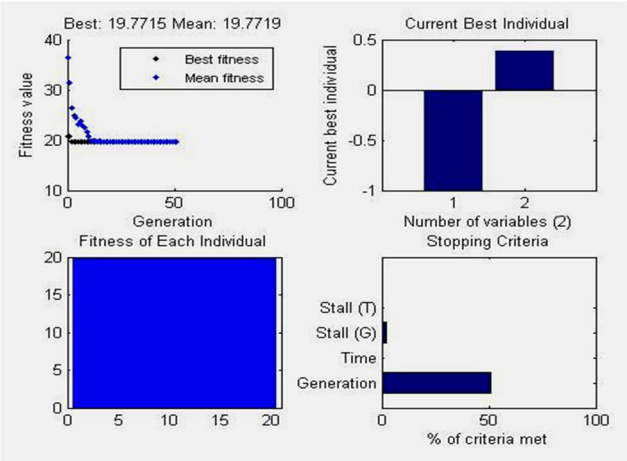

Fig. 2B: Genetic algorithm optimization for % cell survival (y_2)

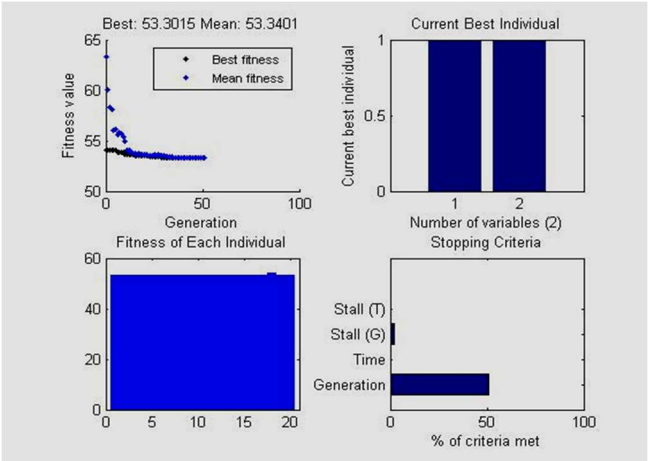

Fig. 2C: Genetic algorithm optimization for % cell inhibition (y3)

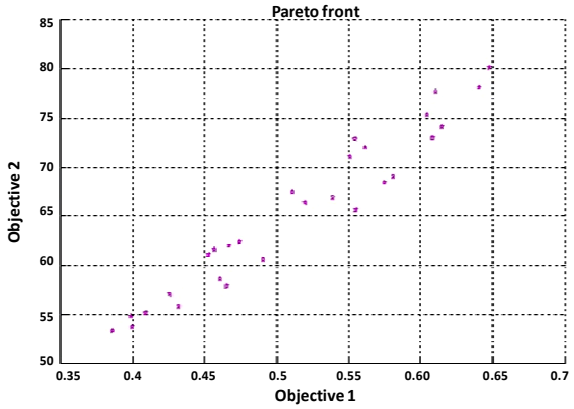

Fig. 3: Pareto solutions based on NSGA algorithm showing the mean and best fitness values

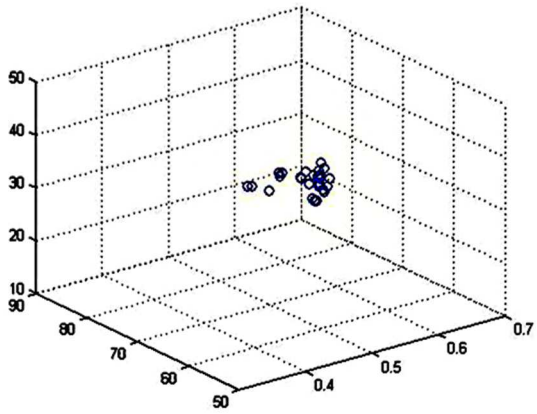

Fig. 4: 3D-scattered plot for triple objectives with 30 non-dominated solutions

CONCLUSION

The main objective of this work i.e., to solve multi-objective optimization for the establishment of cytotoxic potency of *marine microalgae* with the assay against human colon cancer (HT-29), human hepatocyte cancer (Hep-G2), and human breast cancer (MCF-7) cell lines was achieved and can be compared with the experimental results which established by Das *et al.*, 2014. The RSM based single optimal type of study using GA for each of the 3 objectives helped to minimize the absorbance and maximize cell survival and the cell growth inhibition. This is the first attempt for triple objective optimization, as far as we know, to develop the optimum condition for this *marine microalgae* by using Pareto technique. Thirty non-dominating solutions were obtained for absorbance (y_1), % cell survival (y_2) and% cell inhibition (y_3). The solutions obtained by non-dominated sorting genetic algorithm were compared with the experimental data from Das *et al.*, 2014 and the results were found to be significant which establishes the applicability of the proposed model.

ACKNOWLEDGEMENT

SJE is thankful to the National Institute of Technology Raipur (NITRR), for providing the necessary computational facility to analyze, prepare the manuscript and permission to publish it.

CONFLICT OF INTERESTS

The authors declare no conflict of interest for the publication of this manuscript.

REFERENCES

1. Cutler SJ, Cutler HG. Biologically active natural products: Pharmaceuticals. CRC Press: New York, NY; 2000. p. 279-96.
2. Hanson PC, Bade DL, Carpenter SR. Lake metabolism: relationships with dissolved organic carbon and phosphorus. Limnol Oceanogr 2003;48:1112-9.
3. Bhat ZA, Ansari SH, Mukhtar HM, Naved T, Siddiqui J, Khan N. Effect of Aralia cachemirica decne root extracts on blood glucose level in normal and glucose-loaded rats. Pharmazie 2005;60:712-3.
4. Thornburg CC, Zabriskie TM, McPhail KL. Deep-sea hydrothermal vents: potential hot spots for natural products discovery? J Nat Prod 2010;73:489-99.
5. Niehaus F, Bertoldo C, Kähler M, Antranikian G. Extremophiles as a source of novel enzymes for industrial application. Appl Microbiol Biotechnol 1999;51:711-29.
6. Vijayavel K, Martinez JA. *In vitro* antioxidant and antimicrobial activities of two Hawaiian marine Limu: *Ulva fasciata* (Chlorophyta) and *Gracilaria salicornia* (Rhodophyta). J Med Food 2010;13:1494-9.
7. Kolanjinathan K, Saranraj P. Pharmacological efficacy of marine seaweed *Gracilaria edulis* extracts against clinical pathogens. Global J Pharmacol 2010;8:268-74.
8. Sujatha S, Rajasree SR, Sowmya JD, Donatus M. Imminent intriguing acquired potential biological effect of marine seaweeds: a review. World J Pharm Res 2015;4:524-41.
9. Samuelsson G. Drugs of natural origin: A textbook of pharmacognosy, Swedish Pharmaceutical Press. first ed. Stockholm; 1999.
10. Yasuhara-Bell J, Lu Y. Marine compounds and their antiviral activities. Antiviral Res 2010;86:231-40.
11. Smith VJ, Desbois AP, Dyrynda EA. Conventional and nonconventional antimicrobials from fish, marine invertebrates and micro-algae. Mar Drugs 2010;8:1213-62.
12. Das MK, Sahu PK, Rao GS, Mukkanti K, Silpavathi L. Application of response surface method to evaluate the cytotoxic potency

of *Ulva fasciata* Delile, a marine macroalga. Saudi J Biol Sci 2014;21:539-46.
13. Satya EJ, Anand M, Venkateswarlu M. Optimum culture medium composition for Rhamnolipid production by *Pseudomonas aeruginosa* AT10 using a novel multi-objective optimization method. J Chem Technol Biotechnol 2013;88:271-9.
14. Satya EJ, Venkateswarlu C. Evaluation of anaerobic biofilm reactor kinetic parameters using ant colony optimization. Environ Eng Sci 2013;30:527-35.
15. Satya EJ, Anand P, Venkateswarlu C. Optimal state estimation and on-line optimization of a biochemical reactor. Chem Proc Eng 2013;34:449-62.
16. Satya EJ, Venkateswarlu C. Optimization of culture conditions for Chinese hamster ovary (CHO) cells production using differential evolution. Int J Pharm Sci 2012;4:465-70.
17. Ghosh A, Saha PD. Optimization of copper reduction from solution using *Bacillus pumilus* PD3, isolated from marine water. Pollution 2013;55:12910-4.
18. Kiran GS, Lipton AN, Priyadharshini S, Anitha K, *et al. the* Antiadhesive activity of polyhydroxy butyrate biopolymer from a marine *Brevibacterium casei* MSI04 against pathogenic shrimp vibrios. Microb Cell Fact 2014;13:114.
19. Chen XP, Wang WX, Li SB, Xue JL. Optimization of ultrasound-assisted extraction of Lingzhi polysaccharides using response surface methodology and its inhibitory effect on cervical cancer cells. Carbohydr Polym 2010;80:944-8.
20. Deb K. Multi-objective optimization using evolutionary algorithms, John Wiley and Sons Limited, New York; 2001.
21. Rajesh JK, Gupta SK, Rangaiah GP, Ray AK. Multi-objective optimization of industrial hydrogen plants. Chem Eng Sci 2001;56:999-1010.
22. Cheng SH, Chen HJ, Chang H, Chang CK. Multi-objective optimization for two catalytic membrane reactors-methanol synthesis and hydrogen production. Chem Eng Sci 2008;63:1428-37.
23. Burger J, Hasse H. Multi-objective optimization using reduced models in the conceptual design of a fuel additive production process. Chem Eng Sci 2015;99:118-26.
24. Oh PP, Rangaiah GP, Ray AK. Simulation and multi-objective optimization of an industrial hydrogen plant based on refinery off-gas. Ind Eng Chem Res 2002;41:2248-61.
25. Vandervoort A, Thibault J, Gupta Y. Multi-objective optimization in chemical engineering: Developments and applications. Eds. Rangaiah GP, Bonilla-Petriciolet A, John Wiley and Sons Ltd, Oxford, UK; 2013.
26. Zhou A, Qu BY, Li H, Zhao SZ. Multiobjective evolutionary algorithms: a survey of the state of the art. Swarm Evolutionary Computation 2011;1:32-49.
27. Vallerio M, Hufkens J, Impe JV, Logist F. Interactive multi-objective decision support for the optimization of nonlinear dynamic (bio) chemical processes with uncertainty. 12th International Symposium on Process Systems Engineering and 25th European Symposium on Computer Aided Process Engineering. 1st Ed. Elsevier; 2015. p. 1-2600.
28. Box GEP, Wilson KB. On the experimental attainment of optimum conditions. J Royal Statistical Soc Series 1951;B13:1-45.
29. Draper NR. Introduction to Box and Wilson (1951) On the experimental attainment of optimum conditions. In: Breakthroughs in statistics: methodology and distribution, Springer-Verlag New York; 199. p. 267-9.
30. Myers RH, Montgomery DC, Anderson-Cook CM. In: Response Surface Methodology: Process and Product Optimization Using Designed Experiments. 3rd ed. John Wiley and Sons, NY; 2009.
31. Khuri AI, Mukhopadhyay S. Response surface methodology. WIREs Comp Stat 2010;2:128-49.
32. Bradley N. The response surface methodology; 2007. Available from: https://www.iusb.edu/math-compsci/graduate-thesis.php. [Last accessed on 02 Mar 2016].
33. Oehlert GW. In: Design and analysis of experiments: response surface design. WH Freeman and Company, New York; 2000.
34. Hilla WJ, Huntera WG. A review of response surface methodology: a literature survey. Technometrics 2010;8:571-90.
35. Zhang M, Das C, Vasquez H, Aguilera D. Predicting tumor cell repopulation after response: mathematical modeling of cancer cell growth. Anticancer Res 2006;26:2933-6.
36. Mitchell M. In: An Introduction to Genetic Algorithms. Cambridge, MA: MIT Press; 1996.
37. Mitchell M. Can evolution explain how the mind works? A review of the evolutionary psychology debates. Complexity 1999;3:17-24.
38. Reev C, Rowe JE. In: Genetic Algorithms: Principles and Perspectives: A Guide to GA Theory. Springer; 2002.
39. Wang Y, Fan K. Applying genetic algorithms on pattern recognition: an analysis and survey. In: Pattern Recognition, International Conference on. IEEE Comput Soc 1996;2:740.
40. Cui FJ, Li Y, Xu ZH, Xu HY. Optimization of the medium composition for production of mycelial biomass and exopolymer by *Grifola frondosa* GF9801 using response surface methodology. Bioresour Technol 2006;97:1209-16.
41. Duta FP, Franca FP, de Almeida Lopes LM. Optimization of culture conditions for epoxy saccharides production in rhizobeumsp using the response surface method. Electron J Biotechnol 2006;9:391-7.
42. Anjum MF, Tasadduq I, Al-Sultan K. Response surface methodology: a neural network approach. Eur J Opera Res 1996;101:65-73.
43. Xia YG, Yang BY, Di Wang JL, Yang Q. Optimization of simultaneous ultrasonic-assisted extraction of water-soluble and fat-soluble characteristic constituents from *Forsythiae Fructus* Using response surface methodology and high-performance liquid chromatography. Pharmacogn Mag 2014;10:292-303.
44. Abraham A, Jain L, Goldberg R. Multi-objective evolutionary optimization: theoretical advances and applications, Springer Science+Business Media; 2004.
45. Jena S. Multi-objective optimization of design parameters of a shell and tube type heat exchanger using a genetic algorithm. Int J Curr Eng Technol 2013;3:1379-86.
46. Jan-Christer Janson T. Optimization of large-scale chromatography of proteins. Korean J Chem Eng 2001;18:2:149-58.
47. Kim K, Kim KS, Chung ES, Son SH. The manufacturing process of self luminou s glass tube utiliz ing tritium gas: optimization of phosphor coating conditions. Korean J Chem Eng 2005;22:6:899-904.
48. Choi YJ, Kwon TI, Yeo YK. Optimization of the sulfolane extraction plant based on modeling and simulation. Korean J Chem Eng 2000;6:712-8.
49. Lee JH, Lim HC. Development of nonsingular optimization algorithm and its application to chemical engineering systems. Korean J Chem Eng 1999;16:1:118-27.

How to cite this article

- J Satya Eswari, Shadab Ahmed. Triple objective optimization of the cytotoxic potency of human carcinoma cell lines of a marine macroalgae using non-sorting genetic algorithm–a theoretical study. Int J Pharm Pharm Sci 2016;8(9):79-84.