International Journal of Dentistry, Diabetes, Endocrinology and Oral Hygiene, 6(1) pp,8-29, 2024

Print ISSN: ISSN 2631-567X

Online ISSN: ISSN 2631-5688

Website: https://www.eajournals.org/

Publication of the European Centre for Research Training and Development-UK

Release of Plant Based Insulin from Kinetics of *Dioscorea Dumetorum* for the Management of Type 2 Diabetes

Morris, T. Aloysius[1], Andreou, Eleni[2], Kyriacos, Felekkis[3], C. Petrou[4], Egwim Chidi Evans[5]

1. Department of Biochemistry, Federal Polytechnic, Nasarawa, Nasarawa State, Nigeria;
2. Programs Coordinator Nutrition and Dietetics,
3. Department of Life and Health Sciences, University of Nicosia, 46 Makedonitissas Ave, CY-2417, P.O. Box 24005, CY-1700, Cyprus
4. Department of Life and Health Sciences, Human Biology, University of Nicosia, Makedonitissas Ave, CY-2417, P.O. Box 24005, CY-1700, Cyprus. Department of Life and Health Sciences, Pharmacy, University of Nicosia, 46 Makedonitissas Ave, CY-2417, P.O. Box 24005, CY-1700, Cyprus;

5.Department of Biochemistry, Faculty of Sciences, Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria;

Corresponding Author: aloysius.m@live.unic.ac.cy; terfamorris@yahoo.com

doi: https://doi.org/10.37745/ijddeoh.18/vol6n1829 Published July 06, 2024

Citation: Morris, T. A., Andreou, E., Kyriacos, F., Petrou C.., Egwim C.E. (2024) Release of Plant Based Insulin from Kinetics of Dioscorea Dumetorum for the Management of Type 2 Diabetes, *International Journal of Dentistry, Diabetes, Endocrinology and Oral Hygiene*, 6(1) pp,8-29

ABSTRACT: *Glucose uptake and growth kinetics with extract fractions of Dioscorea dumetorum was studied employing standard procedures. Pure yeast cell line culture was used to estimate the capability of yeast cell to grow and take up glucose from the system facilitated by extract fractions from food plants. Result shows that kinetics of yeast cell growth with extract fractions at higher rates met up with the healing faster. Some of these extract fractions were very active at very low concentrations compared to standard drug; these can be drug candidates for formulating type 2 diabetes drug that may compete favourable with standard medications which are burdened with side effects and diverse levels of difficulties. The extract fractions being of food plant origin has no likely toxicity and are a natural antioxidant. Kinetic modeling of the extract fractions with yeast cells have suggest a 'Time-Bound Insulin Releasing System' (TBIRS) where uptake of glucose was possible at specific given time similar to a intial insulin release kinetics which may lead to prospects for the design of type 2 diabetes medications to support an all-embracing blood glucose control, which is really essential by diabetics in order to reduce the difficulty of drug systems as there is a high probability of the extract fractions effectiveness in the future as drug candidate for type 2 diabetes.*

KEYWORDS: glucose-uptake-kinetics, *dioscorea dumetorum*, yeast-cell-growth-kinetics, type 2 diabetes, plant based insulin, extract fractions, quadratic observed plot.

INTRODUCTION

Several plant species are employed in traditional treatment in various places as an alternative means for the management of diabetes (Mentreddy, 2007). Different molecules derived from food plants can decrease the plasma glucose levels and therefore posses possibilities for engaging it as a blood-glucose lowering therapy (Stud, 2000). In fact, seversl blood-glucose lowering therapies presently used to manage type 2 diabetes are linked to food plant constituents that were derived from investigations carried out toward establishing their relationship with the blood-glucose lowering actions as they are employed in the menagement of diabetes in traditional treatment. For instance, metformin, a therapy of the biguanide type that efficiently decreases sugar levels in diabetics, was initially formed from a guanidine derived from Galega officinalis, a plant employed as a healing herb in primitive Europe to manage signs associated with diabetes and other disorders (Bailey and Day, 1989; Bailey and Turner, 1996). Lesser molecules with elevated structural variety formed in the course of food plant secondary metabolism that support glycemic regulation, encourage insulin release, decrease insulin resistance and prompt insulin-like actions have been derived, comprising terpenes, alkaloids as well as flavonoids (Li *et* al; 2004). Since a lot of medications employed to manage diabetes exibit similar blood-glucose lowering ways of its activeness (Qaseem *et al*; 2012), there seem to be a close connection among likely molecules and the formation of novel therapies. Even though, there is a worthy recognition given to formed metabolites in encouraging the blood-glucose lowering activities of prepared food plant extracts, not much emphasis has been given to the subjective and methodical information that links to the anti-diabetic characteristics of food plant extracts to insulin-like food plant proteins (Xavier-Filho *et al*; 2003) and the prospect of their neutraceutical usage to manage diabetes. However, assumption in this area has been on. In 1923, immediately reseracher discovered that insulin was part of the ethanol extract of dog pancreas, James B. Collip - who was part of those who formulated this technique; revealed the existence of insulin-like components in leaves of green beans, wheat, lettuce as well as green onions. He as well discovered that food extracts from these plants stimulated substantial decreases in sugar levels in common rabbits also in dogs that their pancreas has been removed after the intravenous management (Banting *et al*; 1922; Collip, 1923). At the same time, the existence of insulin-like combinations in sprouted potato and rice seeds was revealed, and in 1924, statistical information about the blood-sugar lowering effect of glucose from sugar beet extracts was made available through a journal that was published (Best and Scott, 1923; Best, 1924).

After 50 years, an Indian investigator revealed the existence of insulin food plant was patented; the procedure of procuring that material through ethanol-acid extraction of fruits and seeds of the bitter melon Momordica charantia (Khanna *et al*; 1976).

Equally, not all the derived proteins exhibited the existence of insulin-like regions of protein that can activate a cellular immune response mediated through T or B cells. Consequently, we postulate that the food plant proteins that are more fundamentally similar to the mammalian insulin as well

as documented through anti-insulin antibodies they are more expected to trigger the insulin-like signals which spreads out since those pathways are stimulated by the interface with insulin as well as its membrane receptors (Menting *et al*; 2013; Hubbard, 2013; Wallis, 2015). Due to these concerns, maybe the word “plant insulin” maybe more suitable for similar plant proteins. As a result, proteins that lack several biological and chemical resemblances with mammalian insulin may exert a blood-glucose lowering influence through the selection of stimulating insulin-like channels. Similarlly, triggering of channels apart from the interface of insulin with receptors can as well be ultimately supporting glucose decrease, the protein or peptide may encourage b-cells to release insulin. In humans beings, there are two polypeptide hormones, glucose-dependent insulinotropic peptide as well as glucagon-like peptide 1, refers to as incretins, which are released through cells in the digestive region in reaction to the existence of starches and sugars in diets (Baggio and Drucker, 2007). polypeptide hormones, glucose-dependent insulinotropic peptide as well as glucagon-like peptide 1 support and encourage glycemic regulation immediately starches and sugars intake does not through openly encouraging the elimination of glucose from the vascular to the intracellular partition, as insulin does, but through encouraging the release of insulin through b-cells (Yabe and Seino, 2011).

MATERIALS AND METHODS

Chemicals and reagents

The chemicals and reagents utilized in this study were of analytical grade and products of Sigma Aldrich. Chemicals used include methanol, phosphate buffer, 2,2-diphenyl-1- picrylhydrazyl (DPPH), potassium hexacyanoferrate(III), ferric chloride, thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), ferrous sulfate, acetic acid (TCA), baker’s yeast, Ascorbic acid, and metronidazole.

Plant materials: Collection and identification

Dioscorea dumetorum (Bitter yam tubers) were obtained from a commercial source in Benue and the Nasarawa States of Nigeria; a botanist recognized the plants. The plants were peeled, washed, cut into small pieces, and air-dried at 37 ^{o}C for three days to scale down the moisture content.

Grinding process (pulverization)

The sample *Dioscorea dumetorum* (Bitter Yam or BYAM) for the study were grinded to powder using an Electronic Grinder model Nima Japan. The grinded samples were packed into polystyrene (nylon) bag sailed and placed in a desiccator with colloid (desiccant) to forestall samples from absorbing moisture from the atmosphere. The dried pulverized (powdered) plant sample material were stored in a desiccator until use.

Methanol extraction of plants sample

Fine powdered material was extracted in order to obtain active substances with a suitable solvent (methanol). For the preparation of methanol extract, 100g each of powdered *Dioscorea dumetorum* was weighed into a 1000ml beaker and was exhaustively extracted by adding 80% methanol for

eighteen hours at a sonicating temperature of (30 ºC) under shaking condition. For every six hours, the solution was sonicated for twenty minutes to get the precise antidiabetic agents (bioactive component) of the plant samples was followed by filtration to yield a final volume of 1litre (1000mL). The extract was filtered with Whitman filter paper No.1 and concentrated to dryness under reduced pressure and controlled temperature (40-50 ºC) in a digital controlled water bath and fractionalized one after the other (partitioning) by n-Hexane, Chloroform and Ethyl ethanoate (Ethyl acetate). n-Hexane, Chloroform, and Ethyl ethanoate extract fractions were evaporated under reduced pressure.

Filtration of extracted samples

After the sonication of the samples, there was a transparent separation of the supernatant from the residue cemented at the bottom of the conical flask. However, the filtration process forestalls tiny residues from entering the filtrate if decanted. Whitman paper No1 was folded twice into a plastic funnel and the funnel over a conical flask. The solution separated into the funnel with filter paper, gradually the filtrate was collected at the bottom of the conical flask and the residue was retained by the filter paper.

Concentration of the filtrate

The filtrate collected contains both methanol and water alongside the extract. In order to remove the methanol used for extraction, a digital controlled water bath, was used. The essence was to remove the extraction solvent (methanol) and concentrate the extract. The digitally controlled water bath allowed the evaporation of methanol at 40 ºC.

Freeze-drying

The concentrated extract contained water after the methanol was evaporated from the filtrate. The extracts were frozen to -20 ºC and dried in a vacuum-compressed system called the dryer to get rid of water using Freeze dryer; model LGJ-18 with fitted compressor pump.

Fractionation of crude extract (partitioning)

Fractionation of the methanol crude extracts (Partitioning), 10g of the extracts was dissolved in 100 mL of distilled water and partitioned into n-hexane, chloroform, and ethyl acetate fractions in increasing order of the solvent polarity (n-hexane < chloroform < ethyl acetate < distilled water) using separating funnel. The resultant fractions were dry at a reduced temperature of 40 ºC with the digital controlled water bath. The weight of every one of the fractions was taken. The fractions reacted with yeast cells for viability, kinetics of yeast cell-glucose uptake, and kinetics of yeast cell growth. The method of (Rajeswari and Sriidevi, 2014) was used to identify the bioactive fractions.

After fractionation of the extracts BYAM, a total of six (6) fractions were partitioned (Table 1).
The coded extract fractions employed two letters and a number. Where the prefix is the name of the food plant, the suffix is the name of the solvent used for extraction of that particular fraction

while the number is fractions partitioned numbered according to their separation from the separating funnel (Table 1).

Table 1: Showing the fractions from the partitioning of crude extracts with n-Hexane, chloroform, ethyl acetate, and aqueous solution

Samples	Hex	$CHCl_3$	EtOAc	Aqueous
Bitter Yam (BYAM)	BH_1, BH_2	BC_1, BC_2	BE	BA

Kinetics analysis of yeast cell growth and glucose uptake.

Kinetic analysis of yeast cell growth and glucose uptake was carried out with the technique of (Kabir *et al*; 2005) with slight modifications. The trypan blue dye exclusion assay was used with a hemocytometer to estimate cell density, and viability. Two extract fractions of BC1, and BC2, were selected that showed high glucose uptake at 10mM/L glucose solution, and were treated in a similar way as the standard drug metronidazole (MET) as control. Nevertheless, the standard one-hour incubation period which yeast cells utilized in taking up glucose was changed to an interval of ten minutes each for each setup starting with zero minute.

The two extract fractions, and the standard drug metronidazole were placed in seven different setups, with each having its control. Initially, 0.0005g of the extract fraction, and also the standard drug was dissolved in 100ml methanol as the stock solution, and a serial dilution of the stock solution was carried out at the concentrations of 500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 3.91µg/mL in the sample tubes respectively. Next, 1mL extract fraction was added to the test tubes followed by 1mL of 10mM/L glucose solution, and was incubated for 10 minutes at 37 ^oC. After which 1mL of 1% yeast suspension was added and incubated at 0, 10, 20, 30, 40, 50, and 60 minutes respectively.

At the end of each setup time, DNSA reagent was added. This was to allow the yeast cell to react according to the pattern of yeast cell growth, and at the same time take up glucose; the tubes were heated in boiling water for five minutes - without the content of the test tube boiling. Consequently, there was a colour change from yellow to red. The absorbance of each setup was taken employing a spectrophotometer (UV - 1800 SHIMADZU) at 540nm. The percentage increase in glucose uptake was calculated with the formula: Increase in glucose uptake = absorbance of control minus absorbance of the sample divided by absorbance of control multiplied by 100.

Statistical analysis

Data were collected using a one-way, and two-way analysis of variance (ANOVA) as well as independent T-test, and paired T-test analysis. Groups were considered significant if $P<0{\cdot}05$ and, a F-value was significant for ANOVA; the differences between all pairs were carried out using Ducan Post Hoc Test; SPSS version 26, and Microsoft excel windows 10 were used for statistical analysis, and data figure generation.

RESULTS

International Journal of Dentistry, Diabetes, Endocrinology and Oral Hygiene, 6(1) pp,8-29, 2024
Print ISSN: ISSN 2631-567X
Online ISSN: ISSN 2631-5688
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development-UK

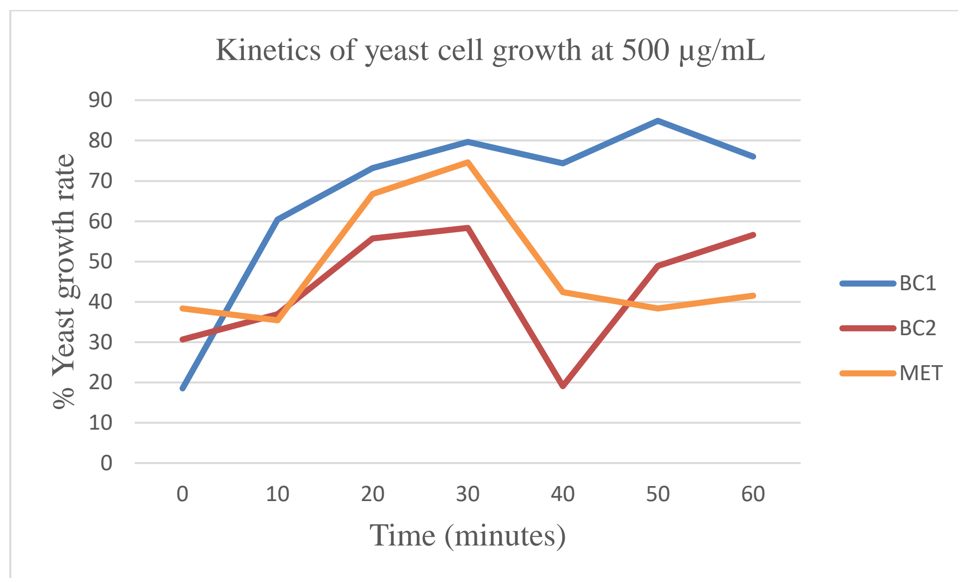


Figure 1: Rate of yeast cell growth at a concentration of 500.0 µg/mL; showing unstructured growth pattern. Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P < 0.05$.

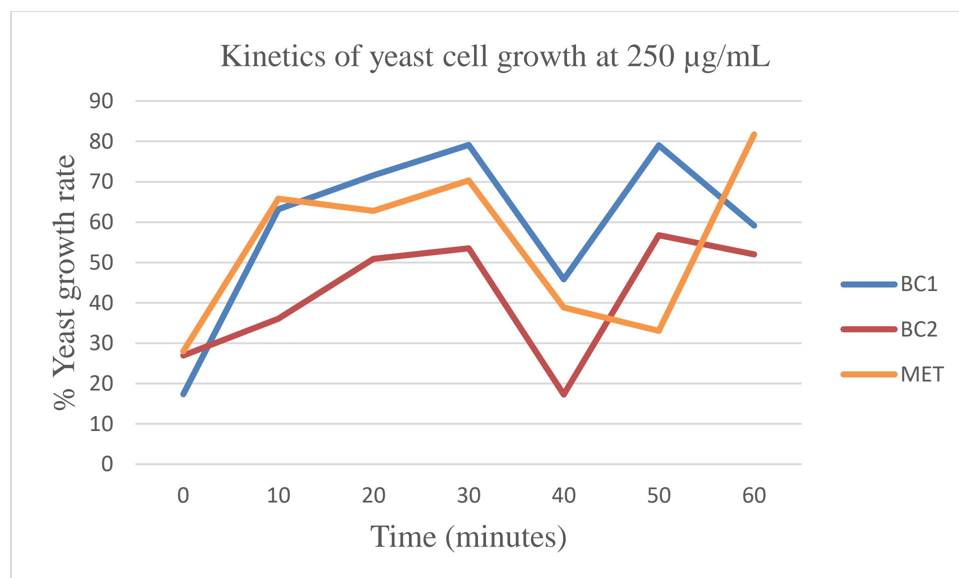


Figure 2: Rate of yeast cell growth at a concentration of 250.0 µg/mL; showing unstructured growth. Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P < 0.05$.

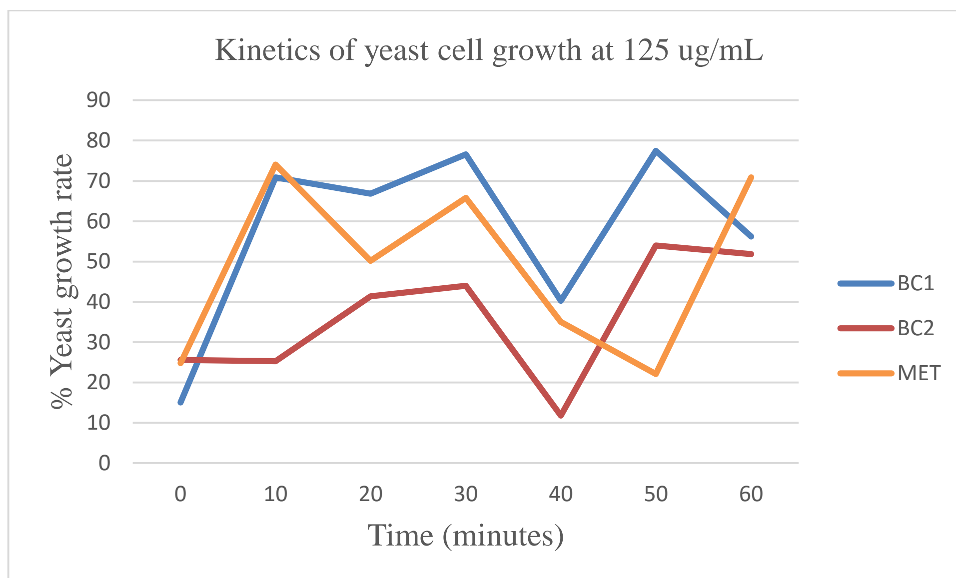
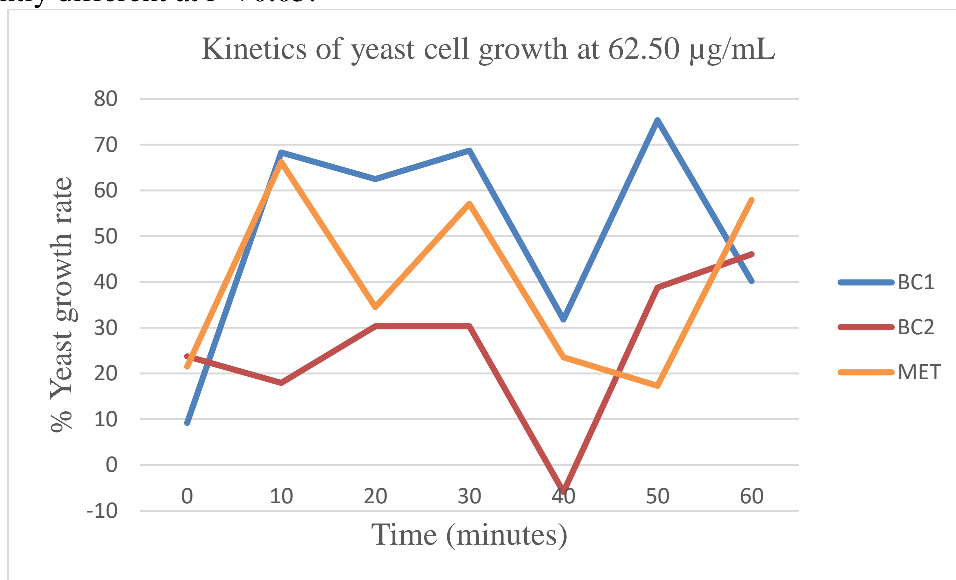


Figure 3: Rate of yeast cell growth at a concentration of 125.0 µg/mL; showing unstructured growth. Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P< 0.05$.



.

Figure 4: Rate of yeast cell growth at a concentration of 62.5µg/mL; showing unstructured growth pattern. Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P< 0.05$.

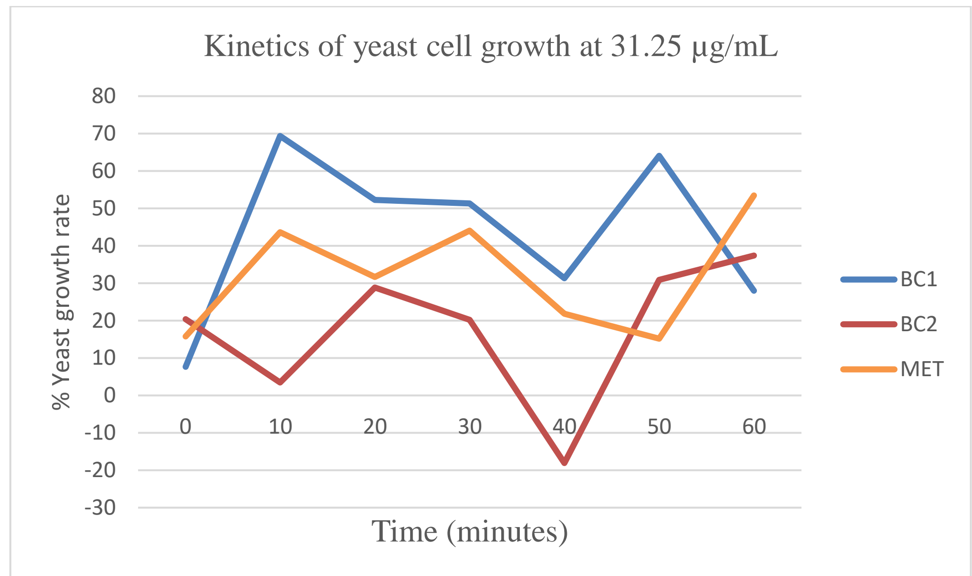


Figure 5: Rate of yeast cell growth at a concentration of 31.25/mL; showing unstructured growth pattern. Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P < 0.05$.

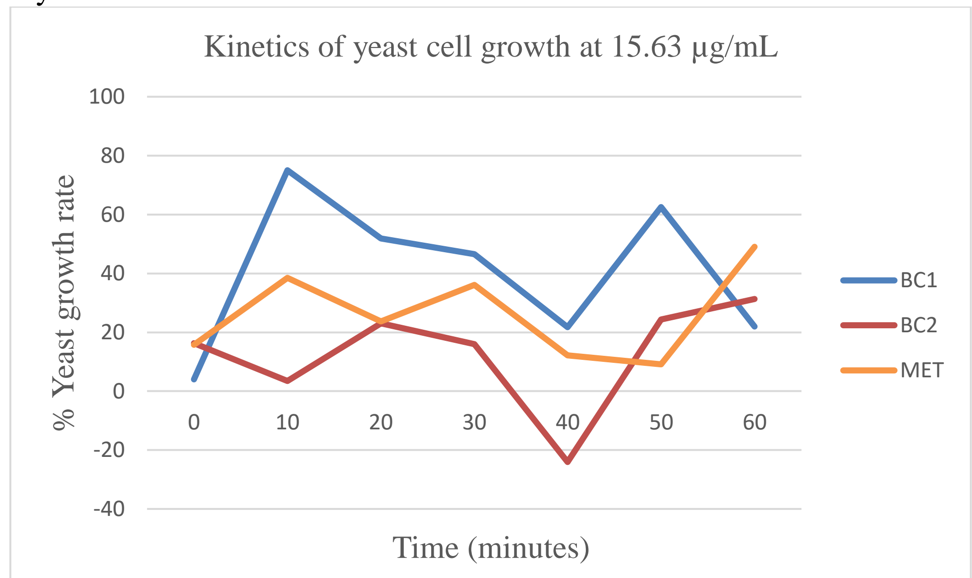


Figure 6: Rate of yeast cell growth at a concentration of 15.63µg/mL; showing unstructured growth pattern. Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P < 0.05$.

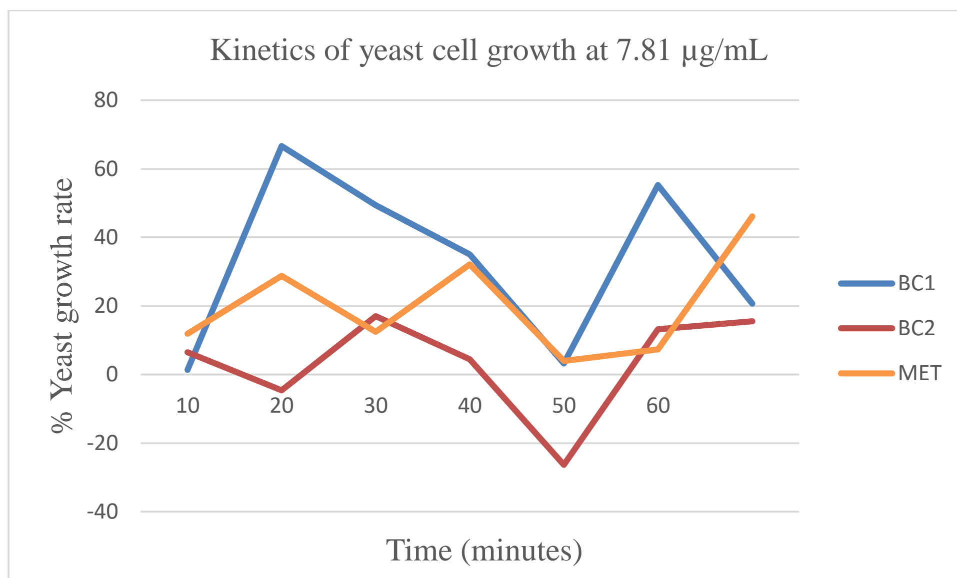


Figure 7: Rate of yeast cell growth at a concentration of 7.81µg/mL; showing unstructured growth pattern. Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P < 0.05$.

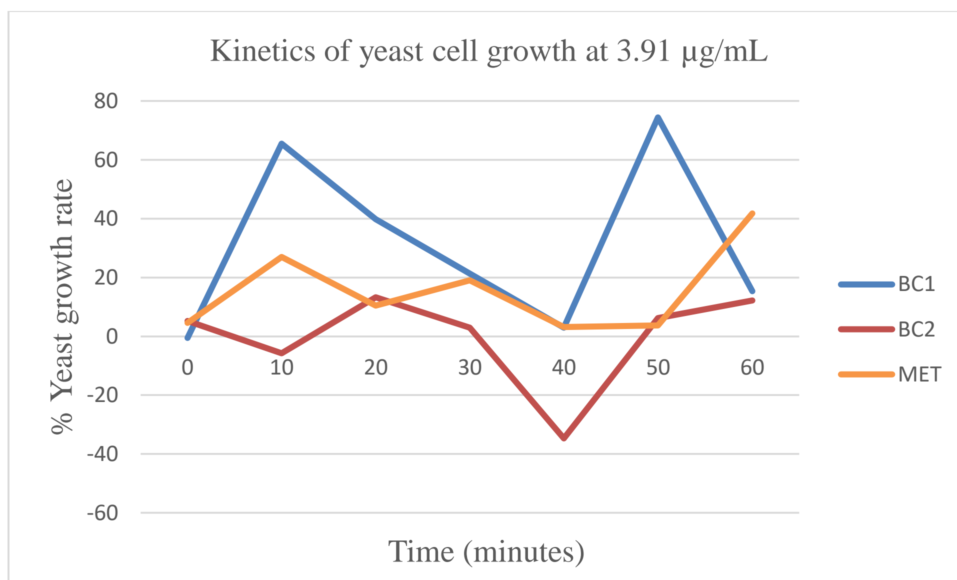


Figure 8: Rate of yeast cell growth at a concentration of 3.91µg/mL; showing unstructured growth pattern. Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P < 0.05$.

The rate of yeast cell growth at concentration of 500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 3.91µg/mL (Figures 1-8) respectively showed cell growth which utilized extract fractions for

enhanced process multiplication of cells. The extract fractions were higher compared with the standard drug in enhanced use of nutrient (glucose) energy for yeast cell growth.

The growth pattern was unstructured. This warranted the use of polynomial-order equations for best fit to model the unstructured nature. Again, the growth followed established microbial growth patterns – Lag (initial) phase, exponential (growth) phase, deceleration (decline) phase, stationary (stop) phase, and dead phase. However, the yeast cell growth pattern observed exhibited exponential phase only, declined at 40 minutes, and continued at different rates with the least (or higher) facilitating fractions continuing faster than the higher (or least) facilitating fractions including the standard drug. The growth pattern did not suggest dead of the yeast cells was observed by further exponential growth phase almost immediately after the decline. However, this could have been influence by time factor.

Additionally, extract fraction concentration of 31.25, 15.63, 7.81, and 3.91µg/mL (Figure 5- 8) demonstrated indications of a retarded yeast cell growth of – 20%, - 22%, - 24% and – 38% respectively. In general, it was observed from the result that the least enhanced extract fractions gradually transformed into higher facilitating extract fractions with a decrease in extract fraction concentration.

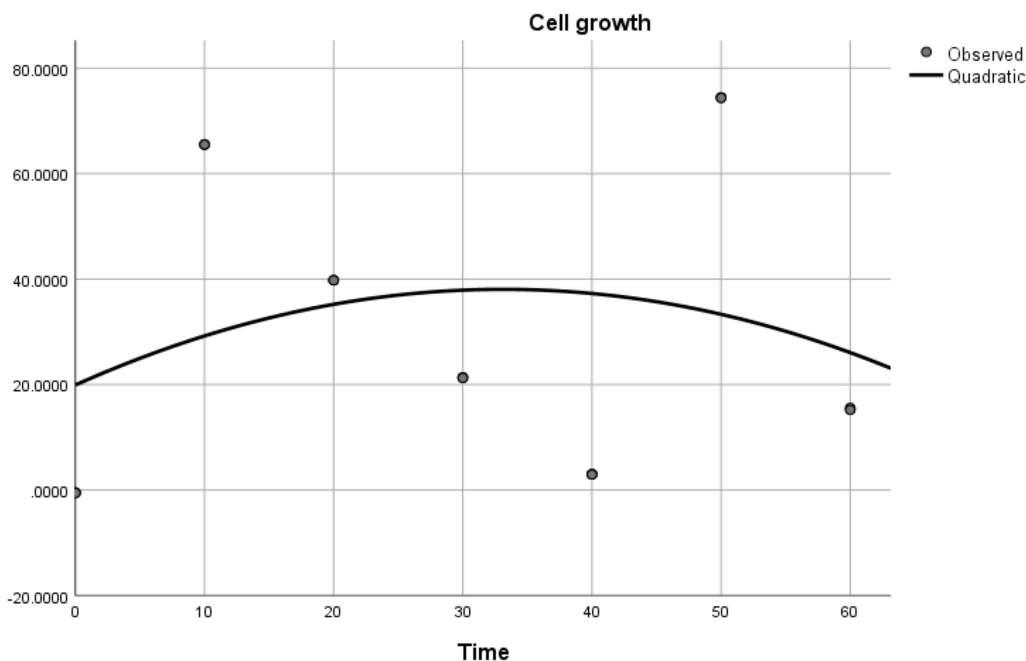


Figure 9: Showing quadratic plot of observed cell growth versus time (minutes) for BC1 kinetics at 3.91µg/mL with no cell growth occurring but only insulin release at the entire 60 minutes.

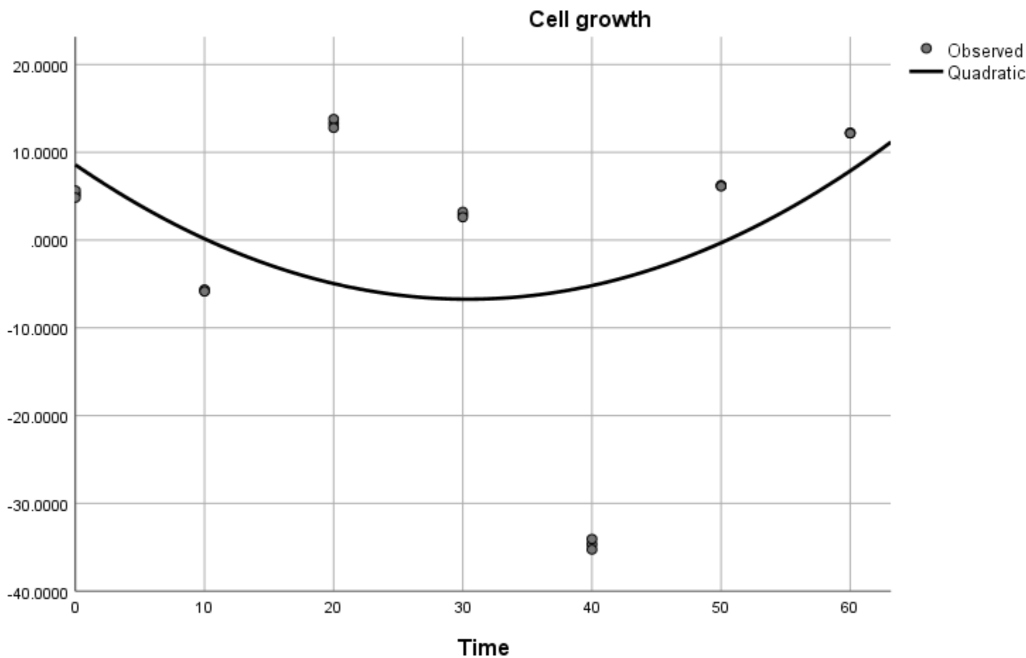


Figure 10: Showing quadratic plot of observed cell growth versus time (minutes) for BC2 kinetics at 3.91µg/mL with cell growth occurring at 0, 20, 30, and 40 minutes while insulin release took place at 10, 50, and 60 minures.

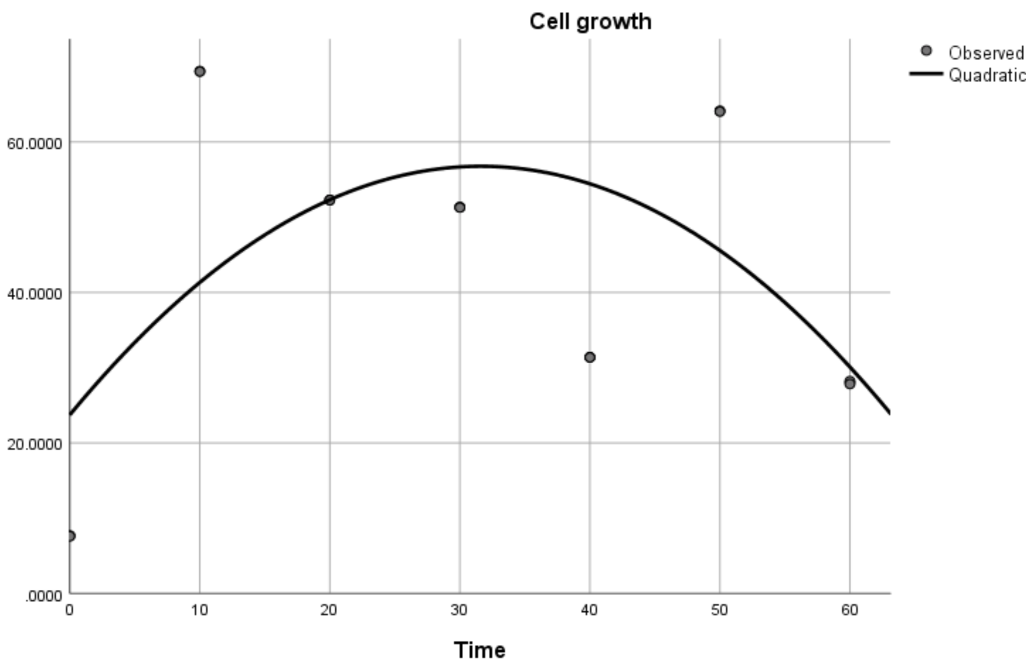


Figure 11: Showing quadratic plot of observed cell growth versus time (minutes) for BC1 kinetics at 31.25µg/mL with no cell growth occurring but only insulin release at the entire 60 minutes.

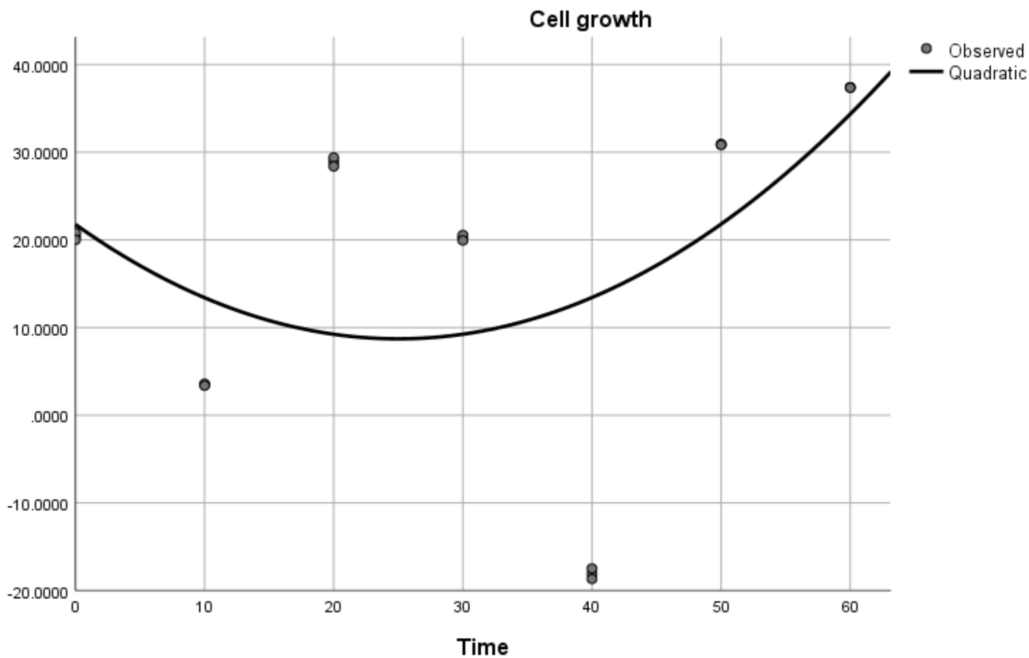


Figure 12: Showing quadratic plot of observed cell growth versus time (minutes) for BC2 kinetics at 31.25µg/mL with cell growth occurring at 0, 20, 30, and 40 minutes while insulin release took place at 10, 50, and 60 minutes respectively.

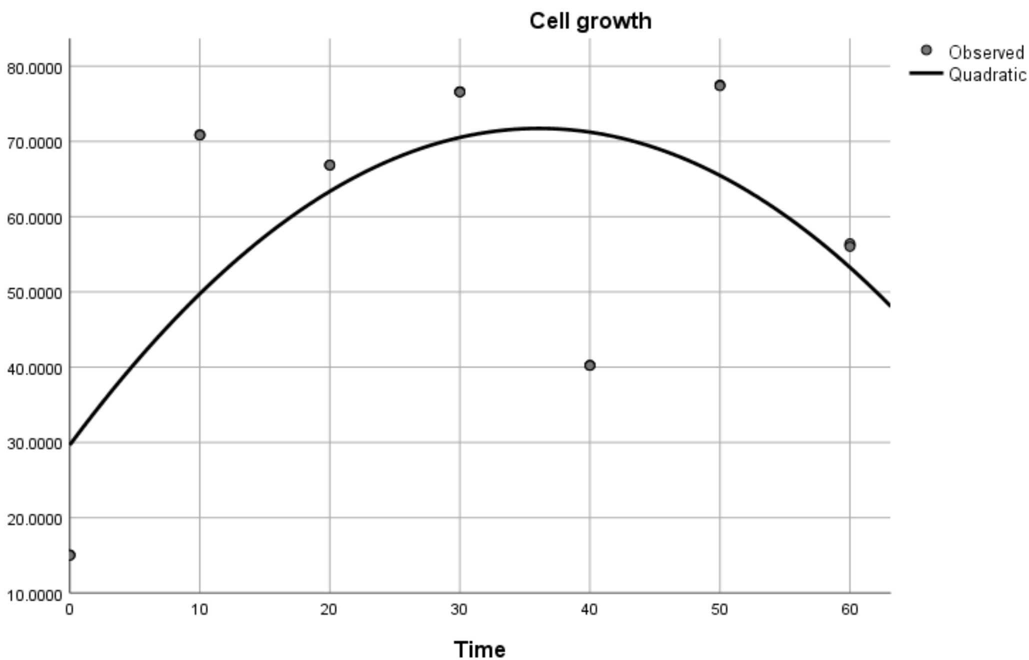


Figure 13: Showing quadratic plot of observed cell growth versus time (minutes) for BC1 kinetics at 125.0 µg/mL with no cell growth occurring but only insulin release at the entire 60 minutes.

International Journal of Dentistry, Diabetes, Endocrinology and Oral Hygiene, 6(1) pp,8-29, 2024
Print ISSN: ISSN 2631-567X
Online ISSN: ISSN 2631-5688
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development-UK

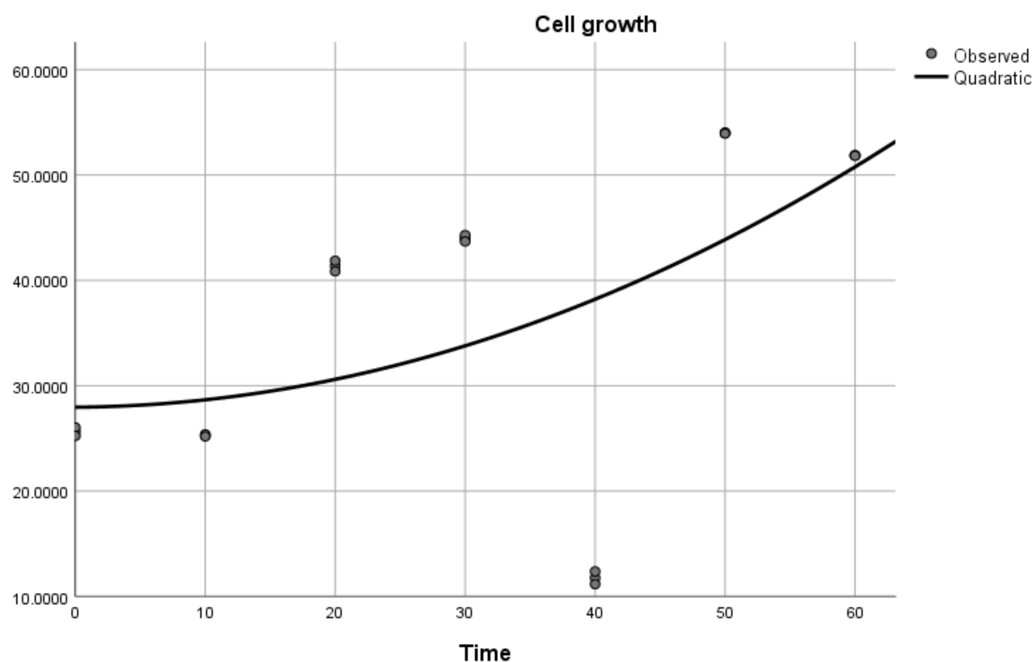


Figure 14: Showing quadratic plot of observed cell growth versus time (minutes) for BC2 kinetics at 125.0 µg/mL with cell growth occurring at 0, 20, and 30 minutes while insulin release took place at 10, 50, and 60 minutes respectively.

Statistical analysis of the yeast cell growth kinetics at extract fraction concentration of 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, and 500µg/mL (Figures 9-14) respectively showed observed cell growth as well as observed glucose uptake at the same time on the quadratic–kinetic plot.
The yeast cell growth was enhanced and facilitated by the extract fractions as nutrient energy for cell growth at specific–favourable time condition within the one-hour period of the experiment.

Table 2: Showing observed yeast cell growth kinetics and glucose uptake kinetics in situ.

Concentration of extract fractions (µg/mL)	Observed yeast cell growth	Time (Minutes)	Glucose uptake (µg/mL)	Time (Minutes)
BC1 3.91	NO	-	YES	0, 10, 20, 30, 40, 50, 60
BC2	YES	0, 20, 30, 40	YES	10, 50, 60
MET	YES	0, 10, 20	YES	30, 40, 50, 60
BC1 7.81	YES	60	YES	0, 10, 20, 30, 40, 50
BC2	YES	0, 10	YES	20, 30, 40, 50, 60
MET	YES	60	YES	0, 10, 20, 30, 40, 50

BC1 15.63	YES	0, 10, 20 40	YES	30, 50, 60
BC2	YES	50	YES	0, 10, 20, 30, 40, 60
MET	YES	10, 30, 40, 50	YES	0, 20, 60
BC1 31.25	NO	-	YES	0, 10, 20, 30, 40, 50, 60
BC2	YES	0, 20, 30, 40	YES	10, 50, 60
MET	YES	0, 10	YES	20, 30, 40, 50, 60
BC1 62.50	YES	10, 30, 50	YES	0, 20, 40, 60
BC2	YES	50	YES	0, 10, 20, 30, 40, 60
MET	YES	20, 40, 50, 60	YES	0, 10, 30
BC1 125.00	NO	-	YES	0, 10, 20, 30, 40, 50, 60
BC2	YES	0, 20, 30, 40	YES	10, 50, 60
MET	YES	0, 10	YES	20, 30, 40, 50, 60
BC1 250.00	YES	40, 50, 60	YES	0, 10, 20, 30
BC2	YES	0	YES	10, 20, 30, 40, 50, 60
MET	YES	50	YES	0, 10, 20, 30, 40, 60
BC1 500.00	YES	0, 20	YES	10, 30, 40, 50, 60
BC2	YES	10, 20, 40, 50, 60	YES	0, 30
MET	YES	0, 10	YES	20, 30, 40, 50, 60

Similarly, when conditions for cell growth was not favourable, glucose uptake was possible at the period cell growth was not observed. Detailed result of the quadratic-kinetic plot is shown in table 2 above.

Table 3: Showing rate of yeast cell growth with time at different extract fraction concentrations

Extract fraction	500µg/mL	250µg/mL	125µg/mL	62.5µug/mL	31.25µg/mL	15.63µg/mL	7.81µg/mL	3.91µg/mL
BC1	0.795529	0.468607	0.393205	0.272783	0.105914	0.00435	-0.03963	0.102463
BC2	0.232882	0.296891	0.380204	0.258626	0.209882	0.142438	0.068975	-0.01148
MET	-0.03167	0.256956	0.067693	0.00205	0.165379	0.105443	0.183761	0.206219

The kinetic rates (slopes) of the yeast cell growth with time at different extract fraction concentrations (Table 3) showed a decrease in the rate of cell growth and glucose uptake with a

corresponding decrease in extract fraction concentration. Kinetics of the extract fractions was higher and better than standard drug at all concentrations. The implication of this is that extract fractions exhibited potentials in facilitating the utilization of glucose nutrient for energy faster for cell growth compared to standard drug.

Table 4: Showing summary R^2 values from kinetic-quadratic modeling of extract fractions

µg/mL	BC1%	BC2%	MET%
3.91	4.9	14.2	21.6
7.81	10.8	13.4	16.9
15.63	23.6	21.1	6.8
31.25	32.7	22.5	5.6
62.50	38.0	27.8	0.0
125.0	41.8	29.6	1.1
250.0	55.0	18.0	7.8
500.0	90.6	11.4	35.1

Additionally, some of the extract fractions accomplished this process at a very low concentration of 3.91µg/mL; the cells utilized glucose as nutrient energy; facilitated by extract fractions for cell growth was a double process in situ; where it was possible for the extract fractions to sustain both cell growth and uptake of glucose from the system (Cirillo, 1962).

The extract fractions predicted function in the future to support cell growth and glucose uptake from the summary R^2 values from kinetic-quadratic modelling of extract fractions (Table 4) showed very high predictability to sustain facilitated utilization of nutrient (glucose) energy for yeast cell growth as observed by the high R^2 values (Song *et al*; 2014). The R^2 values of the extract fractions were higher than the standard drug at all concentrations. These predictions for yeast cell growth are at the same time predicting the function of the extract fractions to take up glucose from the system (Yang and Cao, 2017).

DISCUSSION

The kinetic modeling of yeast cell growth from the statistical point of view provided the opportunity to appreciate the outcome of the investigations such as the R^2, effect of the extracts on the cells or otherwise, and the quadratic plots of the observed cell growth for each of the best-selected extracts and their concentrations.

The rate of yeast cell growth at a concentration of 500µg/mL figures 1 showed growth which utilized between 12 to 85 percent of the extract fraction for enhanced process multiplication. The growth patterns were unstructured as such fifth and fourth-order polynomial equations for best fit were applied to model the structures. The growth was expected to follow microbial established growth phases (patterns) [lag (initial), exponential (growth), deceleration (decline) phase,

stationary (stop), and dead phase]. However, that was not seen as the cells exhibited exponential phases growth patterns only and declined at 40 minutes and, continued at different rates with the least facilitating fractions continuing faster BC2 (58 %).The growth pattern did not suggest the death of the yeast cells as seen by the further exponential phase growth almost immediately after the decline. But this was influenced by the time factor.

The rate of yeast cell growth at a concentration of 250µg/mL figure 2 showed growth which utilized 20 to 65 percent of the extract fraction for facilitating the process of utilizing available nutrient (glucose) energy for yeast cell multiplication. Extract fraction BC1 (80 %) was lower compared to the standard drug (82 %) facilitating the utilization of nutrient (glucose) for yeast cell growth only at this fraction concentration. The growth patterns were unstructured as such the sixth, fifth, and fourth-order polynomial equations for a best-fit model of the structures were adopted. Yeast cell growth did not follow established growth phases. However, in this study, the yeast cell exhibited exponential phases and declined at a time of 40 minutes and continued at different rates with the least facilitating fractions continuing faster BC2 (44 %). The growth pattern did not suggest the death of the cells as seen by the further exponential growth phase almost immediately after the decline. But the process, however, was influenced by time factors. This time factor is a phenomenon that exists within microorganisms. At a start of a batch culture when nutrient is supplied in the medium for the growth of the microorganisms (in this case, glucose facilitated by the extracts); the organisms require time to acclimatize with the new environment at such time, no obvious growth is seen (Lag phase). However, immediately after this stage; the cell continues to grow and multiply (Exponential phase). This continues until a stage is reached when there is sort of limiting nutrient; growth at this point seems to slow (Deceleration phase) and as this continue, the cell goes into the stationary phase. Cells usually can remain in this stationary phase for a long time waiting for growth conditions to be favourable (supply of more nutrients to support growth). However, in situations where there is no external supply of nutrient; microorganisms have an internal system where the stored energy is broken down to supply energy and sustain cell growth. But however, when the internal generated energy is exhausted and conditions remain unfavourable as result of no external supply; the cells are forced to go into death (Death phase). For this study, the cells reached a deceleration phase with limiting nutrient and declined, the cells used the time available to generate internal energy from the stored energy and continued with the growth; hence the exponential phase seen after the decline.

The rate of yeast cell growth at a concentration of 125µg/mL figure 3 showed growth which utilized 10 to 88 percent of the extract fraction for facilitating process utilization of available nutrient (glucose) energy for yeast cell proliferation. Extract fraction BC1 (79 %) was higher compared to the standard drug (75 5%) in facilitating the utilization of nutrients (glucose) for the yeast cell growth. The growth patterns were unstructured as such the fifth and fourth-order polynomial equations for a best-fit model of the structures were applied. The yeast growth showed established growth phases. However, the yeast cell growth exhibited exponential phases and declined at 40 minutes and continued at different rates with the least facilitating fractions continuing faster BC2 (41 %) and BC1 (79 %) including standard drug (75 %). The growth pattern

did not suggest the death of the yeast cells as seen by the further exponential growth phase almost immediately after the decline. But the process was influenced by the time factors.

The rate of yeast cell growth at a concentration of 62.5µg/mL figure 4 showed growth which utilized 5 to 80 percent of the extract fraction for facilitating process utilization of available nutrient (glucose) energy for yeast cell increase. The growth patterns were unstructured as such the fifth and fourth-order polynomial equations as best fit models for the structures were applied. Yeast cells showed established growth phases. However, cell growth exhibited exponential phases and declined at 40 minutes. But continued at different rates with the least enhancing fractions continuing faster BC2 (48 %) than the higher enhancing fractions BC1 (79%) including the standard drug (68 %). The growth pattern did not suggest the death of the cells as seen by the further exponential growth phase almost immediately after the decline. But the process was influenced by the time factors.

The rate of yeast growth at a concentration of 31.25µg/mL figure 5 showed growth which utilized 5 to 78 percent extract fraction for facilitating process utilization of available nutrient (glucose) energy for yeast cell proliferation. The growth patterns were unstructured as such the fifth-order polynomial equations as the best fit model for the structures was applied. Yeast cells showed established growth phases. However, cell growth exhibited exponential phases and declined at 40 minutes. But continued at different rates with the least enhancing fractions continuing faster BC2 (38 %) than the higher enhancing fractions BC1 (62 %) including the standard drug (58 %). The growth pattern did not suggest the death of the yeast cells as seen by the further exponential growth phase almost immediately after the decline. But the process was influenced by the time factors. Furthermore, there was an indication of retarded growth of – 20%.

The rate of yeast cell growth at a concentration of 15.63µg/mL figure 6 showed growth which utilized 5 to 78 percent extract fraction for facilitating process utilization of available nutrient (glucose) energy for yeast cell growth. The growth patterns were unstructured as such the fifth-order polynomial equations were generated as the best fit model for the structures. The yeast cells exhibited established growth phases. However, the growth presented was exponential phases which were stationed at 40 minutes and later continued at different rates. The least enhancing fractions continued faster BC2 (32 %) than the higher enhancing fractions BC1 (78 %) and standard drug (50 %)]. The growth pattern did not suggest the death of the yeast cells as seen by the further exponential growth phase almost immediately after the decline. But the process was influenced by the time factor. Furthermore, there was an indication of a possible retarded growth of – 22%.

The rate of yeast cell growth at a concentration of 7.81µg/mL figure 7 revealed that yeast growth utilized 80 percent of the extract fraction for facilitating process utilization of available nutrient (glucose) energy for yeast cell increase. Extract fraction BC1 (78 %) was higher compared to standard drug (50 %) promoting utilization of nutrient (glucose) for yeast proliferation. The growth patterns were unstructured as such the fifth and fourth-order polynomial equations were generated

as the best fit models for the structures. The yeast cells exhibited established growth phases. However, the growth presented exponential phases and declined at a time of 40 minutes. The least facilitating extract fraction continued at different rates faster BC2 (18 %) than the higher enhancing fractions BC1 (78 %) and standard drug (50 %). The growth pattern did not suggest the death of the yeast cells as seen by the continued exponential growth phase almost immediately after the decline. But the process was influenced by the time factors. Furthermore, there was an observed form of retarded growth of – 24%.

The rate of yeast cell growth at a concentration of 3.91µg/mL figure 8 revealed yeast growth which utilized 79 % of the extract fraction for promoting process utilization of available nutrient (glucose) energy for yeast cell growth. Extract fraction BC1 (78 %) was higher compared to standard drug (40 %) promoting utilization of nutrient (glucose) for yeast increase. The growth patterns were unstructured as such the fifth and fourth-order polynomial equations were generated as the best fit models for the structures. The yeast cells exhibited established growth phases. However, the growth presented exponential phases which declined at 40 minutes. But continued at different rates with the least promoting extract fractions continuing faster BC2 (11 %) than the higher promoting fractions BC1 (79 %) and the standard drug (40 %)]. The growth pattern did not suggest the death of yeast cells as seen by the continued exponential growth phase almost immediately after the decline. But the process was actually influenced by the time factor. Furthermore, there was an indication of possible growth retardation of – 38%.

At the concentration of 3.91µg/mL of BC1 kinetics, the value of R^2 was 4.9%, there was no effective difference in the extract fraction, and uptake of glucose by the cells was possible at the time of 0, 10, 20, 30, 40, 50, and 60 minutes. While there was no observed yeast cell growth. It is relevant to point out here that throughout the observed yeast cell growth plot; the point on the plots where there was no observed yeast cell growth occurring, uptake of glucose was possible there. For BC2 kinetics, the value of the R^2 was 14.2%, there was no effective difference in the extract fractions, and uptake of glucose by the cells was possible at the time of 10, 50, and 60 minutes. While the observed yeast cell growth occurred at the time of 0, 20, 30, and 40 minutes respectively. For standard drug (Metronidazole) kinetics, the value of R^2 was 21.6%, there was no effective difference in the extract fractions, and uptake of glucose by the cell was possible at a time of 30, 40, 50, and 60 minutes. While the observed yeast cell growth occurred at a time of 0, 10, and 20 minutes.

At the concentration of 31.25 µg/mL BC1 kinetics, the value of R^2 was 32.7%, there was an effective difference in the extract fractions, and uptake of glucose by the cells was possible at a time of 0, 10, 20, 30, 40, 50, and 60 minutes. While the observed cell growth did not occur. For BC2 kinetics, the value of R^2 was 22.5%, there was no effective difference in the extract fractions, and uptake of glucose by the cells was possible at a time of 10, 50, and 60 minutes. While the observed cell growth occurred at a time of 0, 20, 30, and 40 minutes respectively. For standard drug (Metronidazole) kinetics, the value of R^2 was 5.6%, there was no effective difference in the

extract fractions, and uptake of glucose by the cells was possible at a time of 20, 30, 40, 50, and 60 minutes. While the observed cell growth occurred at a time of 0, and 10 minutes.

At the concentration of 125µg/mL BC1 kinetics, the value of R^2 was 41.8%, there was an effective difference in the extract fractions, and uptake of glucose by the cells was possible at a time of 0, 10, 20, 30, 40, 50, and 60 minutes. While the observed yeast cell growth did not occur completely. For BC2 kinetics, the value of R^2 was 29.6%, there was an effective difference in the extract fractions, and uptake of glucose by the cells was possible at a time of 10, 50, and 60 minutes. While the observed cell growth occurred at a time of 0, 20, 30, and 40 minutes respectively. This suggests that where the yeast cells were able to grow; uptake of glucose was not possible. For standard drug (Metronidazole), the value of R^2 was 1.1%, there was no effective difference in the extract fractions, and uptake of glucose by the cells was possible at a time of 20, 30, 40, 50, and 60 minutes. While the observed cell growth occurred at a time of 0, and 10 minutes respectively.
At the concentration of 250µg/mL BC1 kinetics, the value of R^2 was 55%, there was an effective difference in the extract fractions, and uptake of glucose by cells was possible at a time of 0, 10, 20, and 30 minutes. While the observed yeast cell growth occurred at a time of 30, 50, and 60 minutes respectively. For BC2 kinetics, the value of R^2 was 18%, there was no effective difference in the extract fractions, and uptake of glucose by the cells was possible at a time of 10, 20, 30, 40, 50, and 60 minutes. While the observed cell growth occurred at the time 0 minutes only. For standard drug (Metronidazole) kinetics, the value of R^2 was 7.8%, there was no effective difference in the extract fractions, (tables 4.288, 4.289, 4.290) and uptake of glucose by the cells was possible at a time of 0, 10, 20, 30, 40, and 60 minutes. While the observed yeast cell growth occurred at a time of 50 minutes only.

At a concentration of 500µg/mL BC1 kinetics, the value of R^2 was 90.6%, there was an effective difference in the extract fractions, and uptake of glucose by the cells was possible at a time of 10, 30, 40, 50, and 60 minutes. While the observed yeast cell growth occurred at a time of 0, and 20 minutes respectively. For BC2 kinetics, the value of the R^2 was 11.4%, there was no effective difference in the extract fractions, and uptake of glucose by cells was possible at a time of 0, and 30 minutes. While the observed cell growth occurred at a time of 10, 20, 40, 50, and 60 minutes respectively. For standard drug (Metronidazole) kinetics, the value of R^2 was 35.1%, there was an effective difference in the extract fractions. It is important to note here that it was only at 500µg/mL that the standard drug (Metronidazole) was effectively different in the extract fractions; therefore, once again suggesting the comparative advantage of the extract over the standard drug. And uptake of glucose by cells was possible at a time of 20, 30, 40, 50, and 60 minutes. While the observed yeast cell growth occurred at a time of 0, and 10 minutes.

The summary of the R^2 values generated from the kinetic-quadratic modeling of the best extract fractions and the standard drug as contained in the discussion is shown in table 4 .

CONCLUSION

The results of the kinetics showed fraction with the highest R^2 value both at high and low concentrations was the most predictable extract fraction. The predictability of the extract fractions varied as some were able to increase their predictions with an increase in extract concentrations (BC1); while others decreased their predictions with an increase in extract fractions (BC2). However, the standard drug was only able to predict its future usefulness at the highest concentration of 500µg/mL; suggesting very low effectiveness of the drug compared with extract fractions. The R^2 value shows how useful is the prediction; with higher concentrations of the extract fractions, it is, therefore, easier to predict their performance even at low concentrations.
Extract fractions with good kinetics (rates) correspond with those of good glucose uptake. This also further suggests that the extract fractions are better compared to standard drugs. However, the extract fractions with the best kinetics (rates), do not correspond with those of best glucose uptake (low performance quite alright; but may only be a matter of time) for such fractions to also perform better as the other or even better than others. In other words, the extract fractions were found to be effective in different ways and useful. Those extract fractions with lower rates at low concentrations suggest that their rates are not like other extract fractions with high concentrations. This suggests that the low rates are also good however, with time, the extract fraction may not be dependable. Furthermore, the equations of reactions, shows that it can be predicted using the equations generated from the sixth, fifth and fourth-order equations of the kinetic models at the specific concentrations with time, i.e. at a given time X, the value of Y can be determined.

Kinetic modeling of the extract fractions with yeast cells has produced a "Time-Bound Insulin Releasing System" (TBIRS) in situ where uptake of glucose (mop up of excess sugar from the system) was possible at a specific given time. It is worth noting that on the same observed yeast growth plot, at such time when the growth of cells was occurring; uptake of glucose by the cells was inhibited. Also, it is interesting to note that at the lowest concentration of 3.91µg/mL, uptake of glucose by the cells was possible throughout the whole 60 minutes' period.

This study proposes further research in encapsulating the high bioactive extract fractions separately and coding them according to their different functions as a drug candidate for type 2 diabetics . And an animal trial of the drug will be necessary to monitor the in vivo performances of the drug and subsequent human volunteered trial.

Author contributions
Conceptualization, MA and EA.; methodology, MA and EA; validation, MA, KF,CP, EA; formal analysis, MA; investigation, MA.; resources, MA; data curation, MA and EA; writing original draft preparation, MA and EA.; writing review and editing, , MA, KF,CP, EA; visualization, MA; supervision, EA; project administration, EA. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement
The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of University of Nicosia; approval date is 15 January 2020.

Funding: "This research received no external funding".

Conflicts of Interest: state "The authors declare no conflict of interest."

REFERENCES

Bailey CJ, Day C (1996). Traditional plant medicines as treatments for diabetes. Diabetes Care 1989;12:553–64. 4.

Bailey CJ, Turner RC. Metformin. N Engl J Med;334:574–9.

Best CH, Scott MA(1923). Possible sources of insulin. J Metabol Res;3:177–9.

Best CH(1924). Recent work on insulin. Endocrynology;8:617–29.

Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA(1922). Pancreatic extracts in the treatment of diabetes mellitus. Can Med Assoc J;12:141–6.

Baggio LL, Drucker DJ(2007). Biology of incretins: GLP-1 and GIP. Gastroenterology;132:2131–57.

Collip JB(1923). Effect of plant extracts on blood sugar. Nature;111:571.

Cirillo VP(1962). Yeast Cell Membrane.;485–91.

Hubbard SR(2013). Structural biology: insulin meets its receptor. Nature;493:171–2.

Khanna P, Nag TN, Chandrajaia S, Mohan SV(1976). Process for isolation of insulin from plant source. U.S. Patent;3:945–88

Kabir OA, Olukayode O, Chidi EO, Christopher CI, Kehinde AF(2005). Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity.BMC Complement Altern.; 5:6

Li WL, Zheng HC, Bukuru J, De Kimpe N(2004). Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J Ethnopharmacol;92:1–21.

Menting JG, Whittaker J, Margetts MB, Whittaker LJ, Kong Gkw Smith BJ, Watson CJ, et al(2013). How insulin engages its primary binding site on the insulin receptor. Nature;493:241–5.

Mentreddy SR(2007). Medicinal plant species with potential antidiabetic properties. J Sci Food Agric;87:743–50.

Qaseem A, Humphrey LL, Sweet DE, Starkey M, Shekelle P(2012). Oral pharmacologic treatment of type 2 diabetes mellitus: a clinical practice guideline from the American College of Physicians. Ann Intern Med;156:218–31

. Rajeswari R, Sriidevi M(2014). Study of in vitro glucose uptake activity of isolated compounds from hydro alcoholic leaf extract of cardiospermum halicacabum linn. Int J Pharm Pharm Sci.;6(11):181–5.

Song Y, Wang J, Yau T(2014). Controlled Glucose Consumption in Yeast using a Transistor – Like Device', Scientific Reports; Metabolic Engineering; 4: 5429

Stud Nat Prod Chem 2000;21:457–96.

Wallis M(2015). Coevolution of insulin-like growth factors, insulin and their receptors and binding proteins in New World Monkeys. Growth Horm IGF Res;25:158–67.

Xavier-Filho J, Oliveira AEA, Silva LB, Azevedo CR, VenâncioTM, Machado OLT, et al(2003). Plant insulin or glucokinin: a conflicting issue. Braz J Plant Physiol;15:67–78.

Yabe D, Seino Y(2011). Two incretin hormones GLP-1 and GIP: comparison of their actions in insulin secretion and b cell preservation. Prog Biophys Mol Biol;107:248–56.

Yang J. and Cao Z(2017). Glucose-responsive insulin release: Analysis of mechanisms, formulations, and evaluation criteria, J Control Release.;10; 263: 231–239.