



Original Research Article

Phyllanthus Emblica and Zingiber Officinale on S. Cerevisiae

Anti-Aging Effect of *Phyllanthus Emblica* (Amla) and *Zingiber Officinale* (Ginger) on *Saccharomyces Cerevisiae*

Divya Darak, Devesh Kumar Joshi* , Princy Kumari Rajput, Ram Bhajan Sahu and Sonalika Singh

*Nims Institute of Allied Medical Science and Technology, Nims University (Rajasthan), Jaipur-303121.

Abstract: Aging is an age-related disease, and amla and ginger are best known for their anti-bacterial and anti-fungal activity. They also serve as anti-aging activity. Ginger's active ingredients, such as 6-gingerol and 6-shogaol, have anticancer properties against gastrointestinal cancer. Strong antioxidants such as ginger may reduce or even stop the production of free radicals. Amla is rich in vitamin C, contains phenolic compounds, is its main chemical constituent, and has strong antioxidant properties. Other active ingredients found in amla include ellagic acid, gallic acid, 1-O-galloyl-D glucose, chebulinic acid, quercetin, chebulagic acid, kaempferol, mucic acid, 1,4-lactone 3-Ogallate, isocorilagin, chebulanin, mallotusin, acylated apignin glucoside, and others. Moreover, it possesses anti-oxidative and anti-inflammatory qualities, that help slow down aging. *Astragalus membranaceus* which slows the ageing process was experimented on *in S. cerevisiae*, and the results were positive. Therefore, we have taken *S.cerevisiae* as a model organism to check the anti-aging effect. Various tests were conducted to assess the aging process. Amla outperformed ginger in the chronological lifespan experiment. Aqueous amla showed better result than aqueous ginger in the replicative longevity experiment. In the oxidative assay, amla 100 μ l and ginger 50 μ l concentration gave the best results. In verifying the antioxidative assay, the highest numbers of dead cells were present in Amla 50 μ l, Ginger 50 μ l, and *Astragalus* 75 μ l. According to the ANOVA test, the *p*-value in all the concentrations, i.e., 25 μ l, 50 μ l, 75 μ l, and 100 μ l was lesser than 0.05. In Effect of glucose, amla is better than ginger. Thus Amla and Ginger have anti-oxidative and anti-aging properties and are best to be used daily life.

Keywords: *Saccharomyces cerevisiae*, *Phyllanthus emblica*, *Zingiber officinale*, *Astragalus membranaceus*, ANOVA, ELISA

*Corresponding Author

Devesh Kumar Joshi, Nims Institute of Allied Medical Science and Technology, Nims University (Rajasthan), Jaipur-303121.

Date of Receiving 3 May 2024

Date of Revision 2 July 2024

Date of Acceptance 30 July 2024

Date of Publishing 3 October 2024

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Divya Darak, Devesh Kumar Joshi, Princy Kumari Rajput, Ram Bhajan Sahu, Sonalika Singh, Anti-Aging Effect of *Phyllanthus Emblica* (Amla) and *Zingiber Officinale* (Ginger) on *Saccharomyces Cerevisiae*. (2024). Int J Pharm Sci. 15(4), 1-17 <http://dx.doi.org/10.22376/ijpbs.2024.15.4.b1-17>

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Int J Pharma Bio Sci., Volume 15., No 4 (October) 2024, pp 1-17



I. INTRODUCTION

The aging process is marked by a decline in resilience and an increase in susceptibility, ultimately impairing an individual's capacity to cope with external stressors. Age diminishes the body's ability and increases its susceptibility to illness. Numerous external and internal factors, including environmental stress, diet, and exercise, can impact the rate of aging and cause cell damage and repair¹. Irregular DNA replication is one more cause of aging besides these. All chromosome ends are skipped over in a replication. Chromosomes end in telomeres, which are unimportant repeats of DNA called introns, to prevent critical DNA information from being removed. Telomeres shorten with each replication cycle until they are entirely eradicated. The cell is no longer dividing at this junction. Aging is a very serious problem for people. Due to the aging of cells, major health issues are listed above. Anti-aging compounds are present in some fruits, vegetables, or rhizomes to overcome this issue. Other compounds used for anti-aging include Nicotinamide mononucleotide, Nicotinamide adenine dinucleotide, Resveratrol, Nicotinamide riboside, Quercetin, and Sirtuin². Amla and ginger contain various phytochemicals that are used in anti-aging. Amla contains a potent antioxidant, vitamin C. It also has high levels of amino acids and proteins. Amla is a popular ingredient in hair and skin grooming products because of its high antioxidant content, which also helps prevent skin aging by increasing collagen production collagen^{3, 4}. With a lot of vitamin C, the human body produces the neurotransmitter norepinephrine, which helps dementia patients' brain function. It is also utilized in producing anti-aging cosmetics and is excellent for skin care⁵. Additional phytochemicals found in amla include isocorilagin, chebulanin, mallotusin, acylated apigenin glucoside, gallic acid, elagic acid, chebulinic acid, quercetin, chebulagic acid, kaempferol, mucic acid I, 4-lactone 3-O-gallate, and others⁶⁻⁸. The fruit can be used as a diuretic, laxative, liver tonic, stomachic, restorative, alterative, antipyretic, anti-inflammatory, hair tonic to prevent peptic ulcer and dyspepsia, and as a digestive compound. It can also be used alone or with other plants to treat various illnesses, including the common cold and fever⁹. The nutritional content of Amla per 100 grams includes 44 Kcal of energy, 10.18 grams of carbohydrates, 0.88 grams of protein, 0.58 grams of fat, and 4.3 grams of fiber. It also contains vitamins like folates (6 mcg), niacin (0.300 mg), acid (0.286 g), pyridoxine (0.080 mg), riboflavin (0.030 g), thiamin (0.040 g), vitamin A (290 IU) and vitamin C (27.7 mg). Additionally, it provides minerals such as potassium (198 mg), calcium (25 mg), copper (0.070 mg), iron (0.31 mg), magnesium (10 mg), manganese (0.144mg) phosphorus (27mg) and zinc(12mg)¹⁰. Numerous phytochemicals are also present in ginger, including [4]-gingerol, [6]-gingerol, [8]-gingerol, and [10]-gingerol. [14]-School, [6]-paradol, and 1-dihydroGingerenone A, 1, 7-bis-(4'-hydroxyl 3' ethoxyphenyl)-5-methoxyheptan-3-one, hexahydrocurcumin, tetrahydrocurcumin, - [10]-ginger-dione, and methoxy-[10]-gerol¹¹. Ginger also has medicinal properties: Stimulates digestion, lowers blood pressure, reduces nausea and inflammation, anti-bacterial properties, blood sugar regulator, reduces menstrual pains, positively affects cholesterol levels, lowers the risk of cancer, helps reduce weight, reduces muscle pain and soreness, protect against Alzheimer's disease, ease cold and flu¹². Because of its bioactive compounds, ginger has a wide range of pharmacological activities, making it a multi-target functional food and possible therapeutic for many aging-related

conditions. Application topically guards against UV-induced aging of the skin.¹³ Due to its potent antioxidant properties, ginger may reduce or even stop the production of free radicals¹⁴. One gm of fresh ginger contains the following nutritional values: 4.8 calories, 1.07 grams of carbohydrates, 0.12 grams of dietary fiber, 0.11 grams of protein, 0.05 grams of fat, and 0.1 grams of sugar. Fresh ginger contains trace levels of the following vitamins and minerals: iron, potassium, vitamin C, magnesium, phosphorus, zinc, folate, riboflavin, and niacin¹⁵.

1.1. Astragalus (*Astragalus membranaceus*)

By increasing the levels of antioxidant factors, *Astragalus membranaceus* can guard against oxidative stress. The *Astragalus radix* aqueous extract decreased the size of the myocardial infarction. In a rat model of myocardial ischemia, it enhanced cardiac function. This improvement is linked to antioxidant effects because it preserves the functioning of the superoxide dismutase (SOD), lowers levels of free radicals and malondialdehyde (MDA) production, and decreases cell apoptosis¹⁶. Acetone, polysaccharides, flavonoids, amino acids, and trace elements are the main components of *A. membranaceus*. The twelve triterpene-aminoglycosides that make saponins are astragalosides I–VIII, acetyl astragaloside I, astragaloside I and II, and soya-saponin¹⁰⁻¹². Catecosin-7-O-β-D-glucoside, calycosin-7-O-β-D-glucoside-6'-O-malonate, ononin, and (6aR,11aR) are the flavonoids found in *A. membranaceus*. (R)-7,2'-dihydroxy-3',4'-dimethoxyisoflavan-7-O-β-D-glucoside, (3R)-7,2'-dihydroxypterocarpan-3-O-β-D-glucoside, formononetin-7-O-β-D-glucoside-6'-O-malonate, and formononetin^{17, 18}. Initially, anti-aging studies were carried out on mice as a higher organism due to the short life span relative to humans. Still, recent studies have been carried out on smaller eukaryotic organisms like *S. cerevisiae*. Other models used for aging are fruit flies (*D. melanogaster*), nematodes, *C. elegans*, rhesus macaques (*Macaca mulatta*), fish, dogs and cats, birds, etc. Mice have the highest similarity of genes with humans, but their lifespan is longer than *S. cerevisiae*, and it is very difficult to handle them in the lab. After successful research on the anti-aging effect of Amla and ginger on *S. cerevisiae*, it can be further used in research on higher organisms. In our research, we have used *S. Cerevisiae* as a model system for anti-aging due to their fast growth, low cost, ease of storage, and ease of maintaining their cultures for longer. Compared to higher organisms, we can determine the aging mechanism in simple eukaryotic organisms like *S. cerevisiae*. *S. cerevisiae* is commonly used in metabolic studies and signaling pathways¹⁹. Baker's yeast is the most widely used strain in the baking industry. It acts as a leavening agent, causing the bread to rise or expand and become softer and lighter by turning the fermentable sugars in the dough into ethanol and CO₂^{20, 21}. After cells enter senescence, their survival time is known as their chronological life span, or CLS. Studies on *S. cerevisiae* have looked into aspects of aging. In order to regulate life span regulation from yeast to humans, broadly conserved signaling pathways have been identified. This technique quantifies the increasing lag time for outgrowth curves generated from the same aging culture on successive days. It is the aging culture's declining viable cell count that is the cause of this increase. Thus far, CLS assays utilizing an unconventional 96-well plate format has been employed in experimental setups to test the outgrowth method²². The term replicative lifespan (RLS) refers to the quantity of daughter cells a single mother cell in yeast produces before entering cellular senescence, which is a state in which cells

stop dividing²³. A compound's capacity to shield an organism from oxidative damage is known as the antioxidative assay. Antioxidant capacity could best be determined in a living cell system, but most assays used today are based on chemicals and conducted in vitro^{24, 25}. In verification of the anti-oxidative assay, dead cells were stained blue because due to oxidative stress given by H₂O₂ and ethanol treatment, the membrane of the cells was ruptured, and the stain penetrated inside easily. Live cells have intact membranes, and due to this, the stain can't penetrate inside the membrane. The effect of Amla and Ginger in combination assay was performed to check if there is any combined effect of amla and ginger on the growth of *S. cerevisiae* cells or if they can show their effect individually. To check the effect of high glucose on *S. cerevisiae*, cells were treated with a high concentration of glucose, i.e., 32 %, for a few hours and were spread on plates supplemented with Amla and Ginger extract to check the effectiveness²⁶.

2. MATERIALS AND METHODS

2.1. Materials

Agar as culture medium, Dextrose, Yeast Extract, Bacteriological Peptone, Sabouraud's agar, H₂O₂, *S. cerevisiae*, Amla, Ginger, *Astragalus*.

2.2. Methods

2.2.1. Yeast strain and Growth media

S. cerevisiae was isolated from baker's yeast. The isolated culture was identified and confirmed by PCR³⁴. *S. cerevisiae* was then cultured and isolated on Sabouraud's agar (0.1 g - peptone, 0.4 g - dextrose, 0.15 g - agar-agar, 10 ml - distilled water) and YPD agar (0.1 g - yeast, 0.4 g - peptone, 0.4 g - dextrose, 0.4 g - agar-agar, 10 ml - distilled water) by quadrant

method. Cultures were then preserved in YPD slants for further use in experiments.

2.2.2. Preparation of Powder and Extract (Aqueous, Ethanol, and DMSO)

Amla and ginger were grated and dried in a hot oven at 55° C. Both were grounded in a mixer, and fine powder was used in experiments. 4 g powder was mixed in 20 ml distilled water and 10 ml ethanol. It was kept in a water bath for 30 min at 100° C^{27, 28} for the DMSO extract. ethanol was evaporated from the ethanolic extract in an eppendorf tube by air drying, and pellets were dissolved in 5 % DMSO medium. 4 g *Astragalus* powder was mixed in 400 ml distilled water, and aluminum foil was wrapped to reduce the evaporation rate. The powder was taken in a water bath at 60° C for 1 h. Using a volumetric flask, the semi-solid solution was filtered through Whatman filter paper no.1. All the extracts were centrifuged at 10,000 rpm for 10 min. and then filtered by BioEra India membrane filter of 0.4 microns and preserved in sterilized Appendorf tubes²⁹.

2.2.3. Chronological Life Span Assay

7.7X10⁶ cells of *S. cerevisiae* were inoculated in 10 ml YPD broth and incubated for 24 h at room temperature. Amla and ginger extract, medium, and sample were added to a microtiter plate to make the total volume of 200 µl according to Table 1.1. All the controls (aqueous, ethanol, DMSO) were the same as in Table 1.1. Cultures were grown in a shaker incubator at 120 rpm at room temperature. Growth kinetics was recorded by measuring the OD value at 600 nm after 1st initial OD, which took OD every 2 h until the stationary phase reached^{22,23,31,32}

S.No.	Amla and Ginger sample	Medium	Culture
1	0 µl	180µl	20µl
2	25µl	155µl	20µl
3	50µl	130µl	20µl
4	75µl	105µl	20µl
5	100µl	80 µl	20µl

2.2.4. Replicative Lifespan Assay

The Replicative Lifespan Assay measures the number of times a cell can divide before it stops, which is an important factor in studying aging. This test helps researchers understand how long cells can continue to reproduce, which can provide insights into the aging process and the effects of different substances on cellular longevity. In our study, 7.7X10⁶ *S. cerevisiae* were inoculated in 10 ml YPD broth and incubated for 24 h at room temperature. After washing with phosphate-

buffered saline three times, *S. cerevisiae* cells were resuspended in 1.5 ml PBS buffer. 4 YPD plates containing 5.62 ml amla and 3.75 ml ginger extract each. Two negative controls were made with distilled water. Approximately 400 cells or 0.6 µl Culture were spread on YPD plates and incubated for 2 days at 28°C. After incubation, 10 micro colonies from each plate were suspended in 10 µl PBS and 1% methylene blue (1 micro colony in 1 Appendorf tube). Daughter colonies were counted on Neubauer's chamber under a 40x light microscope^{23,31,33}

S.No.	Volume of Amla and Ginger (ml)	Media (ml)
1	1.8 ml	15 ml
2	3.7 ml	15 ml
3	5.6 ml	15 ml
4	7.5 ml	15 ml

2.2.5. Anti-oxidative Assay

7.7×10^6 cells of *S. cerevisiae* were inoculated in 10 ml YPD broth and incubated for 24 h at room temperature. YPD agar plates were made for amla and ginger extract and control along with 9mM H_2O_2 (3 ml); the volume of extract was increased for 25 μ l, 50 μ l, 75 μ l, and 100 μ l as given in Table 2.1. 1.7 ml of 0.1 % fresh *Astragalus* extract was used as a positive control in 15 ml media. Controls were made the same as given in Table 1.2. 10 μ l (7680 cells) of 24 h-old yeast Culture was spread on agar plates. After being incubated at 28°C for three days, the growth of the yeast on the plate was seen and documented^{23,32}

2.2.6. Verification of Antioxidative Assay

7.7×10^6 cells of *S. cerevisiae* were inoculated in 10 ml YPD broth and incubated for 24 h at room temperature. 1 μ l of 24 h-old culture was added in a fresh sterile eppendorf tube. 50 μ l, 75 μ l, 100 μ l amla, and ginger extract were added and kept for incubation for 1 h. 200 μ l media was added to the Eppendorf tubes and incubated at 28°C for 24 h. 100 H_2O_2

was added and incubated for 3 h. 1 ml of PBS was added after incubation and centrifuged at 8000 rpm for 5 min. Pellets were washed 3 times with cold PBS, then treated with 15 % ethanol, and incubated for 20 min at room temperature. Again, centrifuged at 8000 rpm for 5 min. And resuspended the pellet in 200 μ l PBS. 5 μ l suspensions were taken in a fresh Eppendorf tube, and 5 μ l trypan blue was added and mixed thoroughly. Observed in Neubauer's chamber under 40 x, several *S. cerevisiae* dead cells were counted. Results were compared with control^{30,33}

2.2.7. Percentage Combination of Amla and Ginger

7.7×10^6 cells of *S. cerevisiae* were inoculated in 10 ml YPD broth and incubated for 24 h at room temperature. (25:75, 50:50, and 75:25) were percentage taken as a combination for Amla and Ginger for total volumes of 25 μ l, 50 μ l, 75 μ l and 100 μ l each as given in table 1.3. 20 μ l culture and media were added accordingly to make the final volume of 200 μ l in 96 well microtiter plates. Growth kinetics was recorded by measuring the OD value at 600 nm; after 1st initial OD, OD was taken every 2 h until the stationary phase was reached.

Table 1.3(a): Different percentage combinations of amla and ginger for total volumes of 25 μ l.

S.No.	Percentage Combination	Amla	Ginger
1	25: 75 %	6.25 μ l	18.75 μ l
2	50: 50 %	12.5 μ l	12.5 μ l
3	75: 25 %	18.75 μ l	6.25 μ l

Table 1.3(b): Different percentage combinations of amla and ginger for total volumes of 50 μ l

S.No.	Percentage Combination	Amla	Ginger
1	25: 75 %	12.5 μ l	37.5 μ l
2	50 : 50 %	25 μ l	25 μ l
3	75 : 25 %	37.5 μ l	12.5 μ l

Table 1.3(c): Different percentage combinations of amla and ginger for total volumes of 75 μ l

S.No.	Percentage Combination	Amla	Ginger
1	25: 75 %	18.75 μ l	56.25 μ l
2	50: 50 %	37.5 μ l	37.5 μ l
3	75: 25 %	56.25 μ l	18.75 μ l

Table 1.3(d): Different percentage combinations of amla and ginger for total volumes of 100 μ l

S.No.	Percentage Combination	Amla	Ginger
1.	25 : 75 %	25 μ l	75 μ l
2.	50 : 50 %	50 μ l	50 μ l
3.	75 : 25 %	75 μ l	25 μ l

2.2.8. Effect of Glucose on *S. cerevisiae*

Seven test tubes were taken, and serial dilutions were made of yeast. Culture from 10^{-1} – 10^{-7} . About 32 % glucose (3.2 g) was mixed in 10 ml distilled water in another test tube. 10 μ l (7680 cells) of 10^{-6} dilution were mixed in 32 % glucose solution and incubated for 3 h. 6 YPD plates were made accordingly: 2 for amla, 2 for ginger, and 2 for control as distilled water. After 3 h incubation, 10 μ l culture was taken from the glucose solution and spread on the YPD Plate containing amla, and the same was done for the ginger & control plate. Photograph of each plate was taken for 5 days, and changes in the number of colonies of *S. cerevisiae* were observed²⁶.

3. STATISTICAL ANALYSIS

All the data were analyzed using two-factor ANOVA.

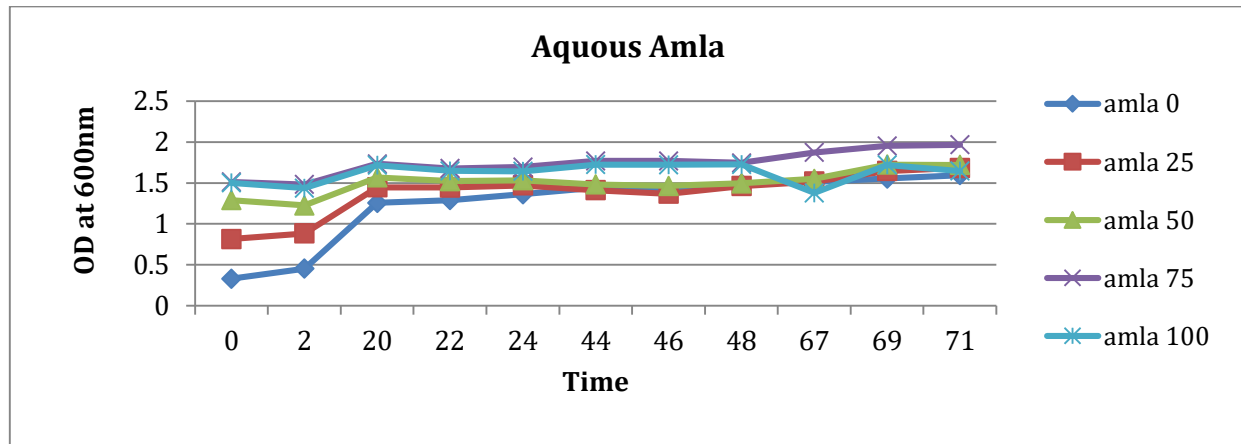
4. RESULTS AND DISCUSSION

4.1 Chronological Life Span Assay

According to Yanfei Lin *et al.*, the number of days that yeast viability equals 0 was considerably increased by CuB (Cucurbitacin B extract) from 15 ± 1 15 days to 17 ± 1 15²³. According to Paul P. Jung *et al.*, using CLS assay, a large heterogeneity of chronological life spans in a subset of 12 natural *S. cerevisiae* variants and even between closely related lab strains such as FY4 and W303²⁵. According to Postnikoff *et al.*, the results obtained through life-span assays vary greatly

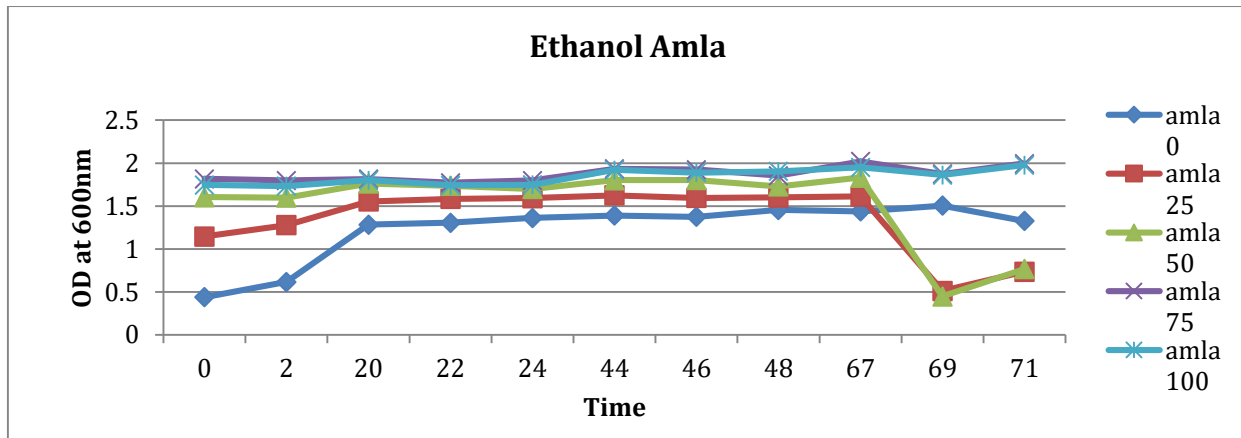
depending on the differences in environmental/nutrient conditions. Common manipulations include using complete media versus YPD and varying the type (such as raffinose, sucrose, galactose, or glycerol) and the concentration (0.05-4 %) of the carbon source³¹. According to Jinye Dang *et al.*, testing the chronological lifespan of the yeast strain BY4742 sourced from livestock in the presence of NLEs showed that NLE extracted from 50% or 75% ethanol significantly extended the cells' lifespans³². In this assay, we have used four different volumes, i.e., 25 µl, 50 µl, 75 µl, and 100 µl. *S. cerevisiae* culture was incubated with amla and ginger, and OD was taken after intervals of every 2 h. ANOVA test was performed to analyze

the results. In ANOVA, a Null hypothesis (H0) that the amla and ginger samples were the same, and an alternate hypothesis of both samples were different was made. In ANOVA, if the p-value is greater than 0.05, then H0 is accepted, and if the p-value is less than 0.05, then H0 is REJECTED, and based on this, we analyze whether the hypothesis will be accepted or rejected. Amla was better than ginger in aqueous, ethanol, and DMSO extracts. All the samples were compared with their respective concentrations of control. Here, the aqueous extract was considered the best compared to the other two, i.e., ethanol and DMSO. Here, the yeast survival rate was increased from 2 ± 1.5 days to 4 ± 1.5 days.



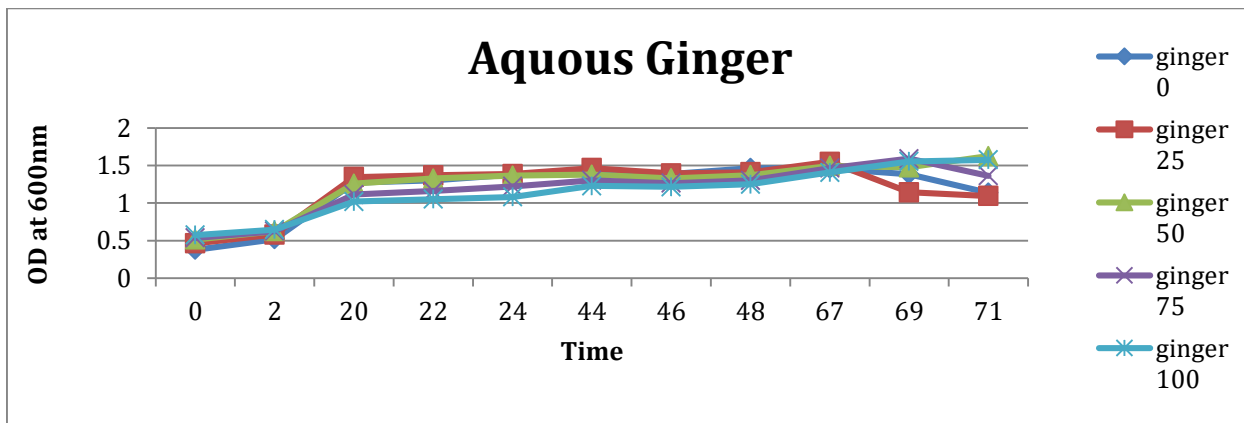
Graph 1: Graph representing OD v/s Time for Aqueous Amla. Here, 100 µl Aqueous amla extract gave the best result as compared to 0 µl, 25 µl, 50 µl, and 75 µl in the survival rate of yeast.

The constant growth has been seen from the 1st day (OD – 1.7555) to the 4th day (OD – 1.7687).



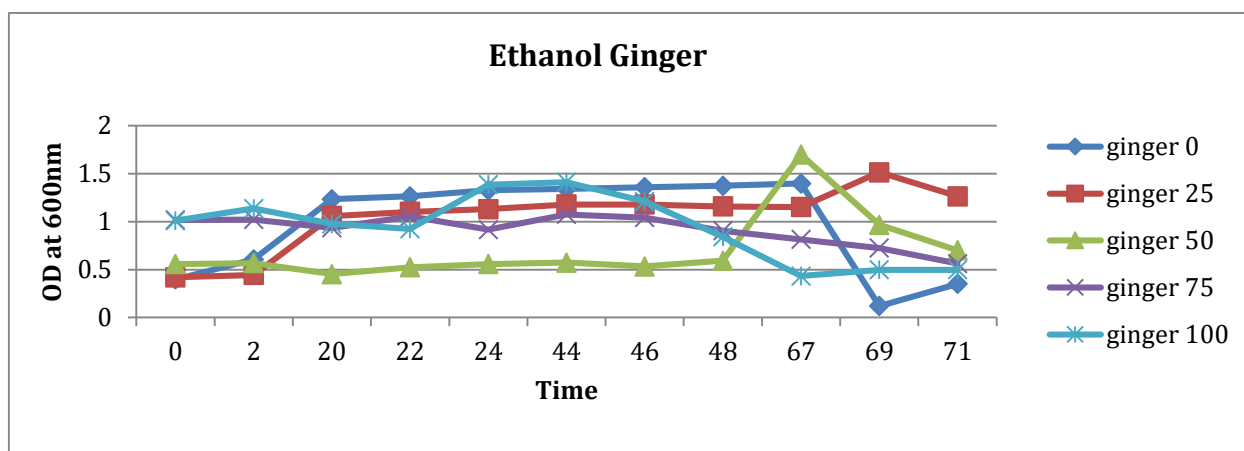
Graph 2: Graph representing OD v/s Time for Ethanol Amla. Here, 100 µl Ethanol amla extract gave the best result as compared to 0 µl, 25 µl, 50 µl, and 75 µl in the survival rate of yeast.

The constant growth has been seen from the 1st day (OD – 1.5467) to the 4th day (OD – 1.8799).



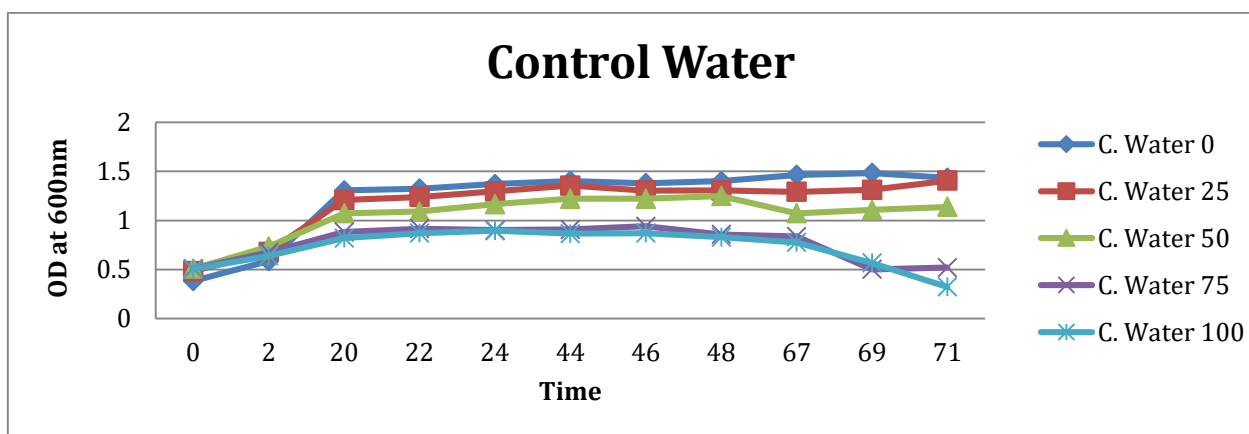
Graph 3: Graph representing OD v/s Time for Aqueous Ginger. Here, 100 μl Aqueous ginger extract gave the best result as compared to 0 μl, 25 μl, 50 μl, and 75 μl in the survival rate of yeast.

The growth increased from the first day (OD—0.567) to the fourth day (OD—1.7687).



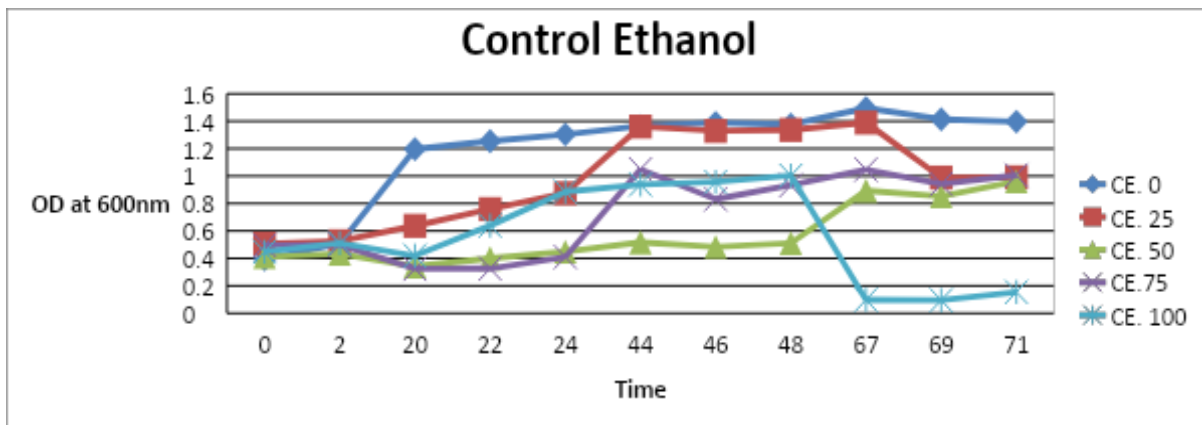
Graph 4: Graph representing OD v/s Time for Ethanol Ginger. Here, 100 μl Ethanol ginger extract gave the best result as compared to 0 μl, 25 μl, 50 μl, and 75 μl in the survival rate of yeast.

The growth increased on the first day (OD—1.067) but decreased on the fourth day (OD—0.645).

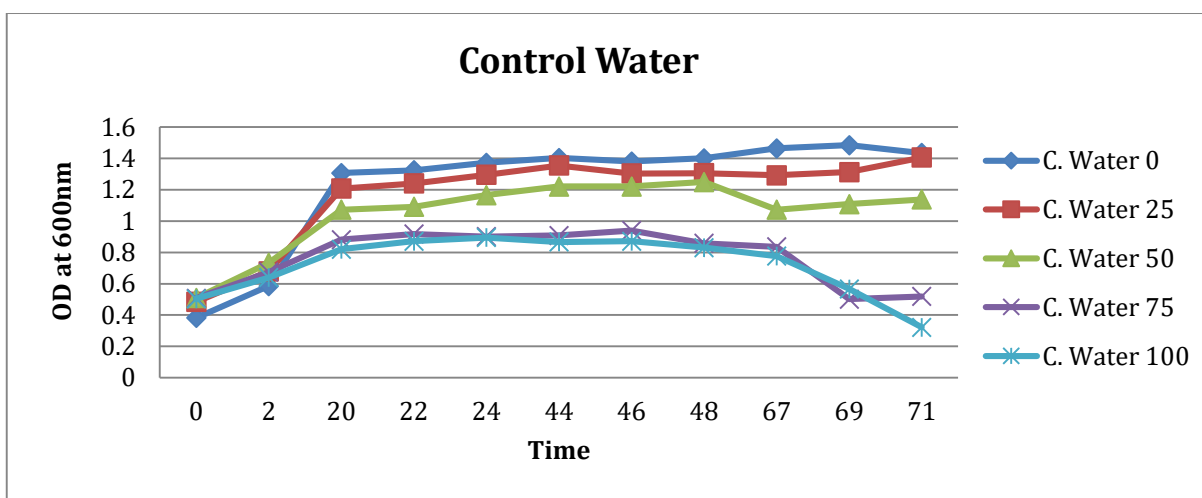


Graph 5: Graph representing OD v/s Time for Control Water. Here, 0 μl water gave the best result as compared to 0 μl, 25 μl, 50 μl, and 75 μl in the survival rate of yeast.

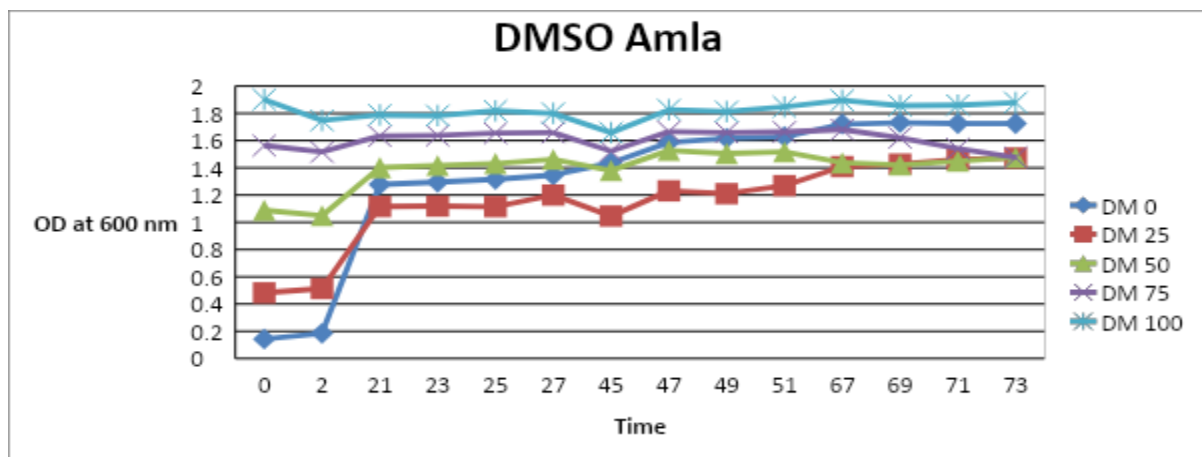
As we add water to the media, the growth rate declines due to dilution, and that's why, at 100 μl, the growth has decreased.



Graph 6: Graph representing OD v/s Time for Control Ethanol. Here, 0 μ l ethanol gave the best result as compared to 0 μ l, 25 μ l, 50 μ l, and 75 μ l in the survival rate of yeast.

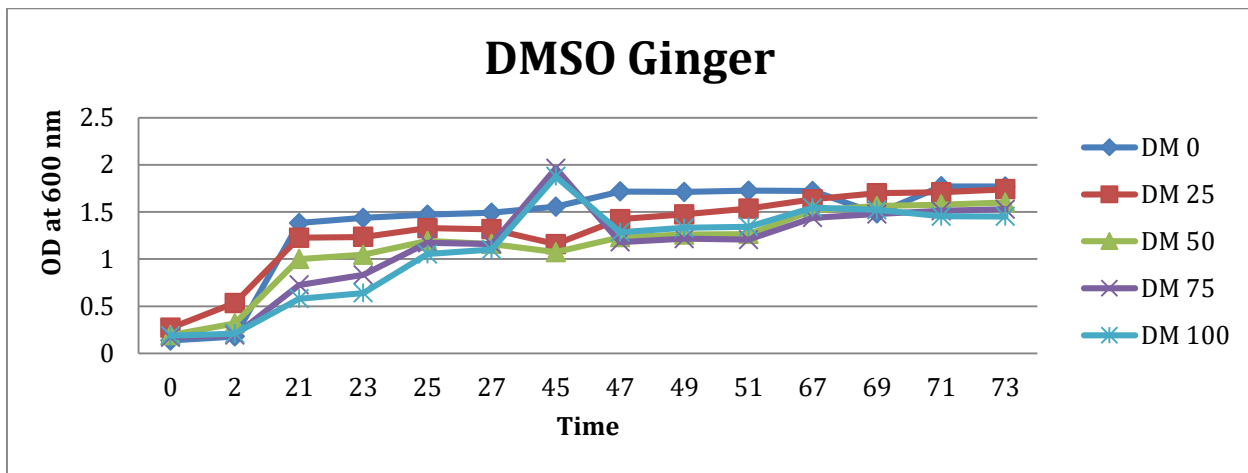


Graph 7: Graph representing OD v/s Time for Control Ethanol + Water. Here, 0 μ l Ethanol + Water gave the best result as compared to 0 μ l, 25 μ l, 50 μ l, and 75 μ l in the survival rate of yeast.

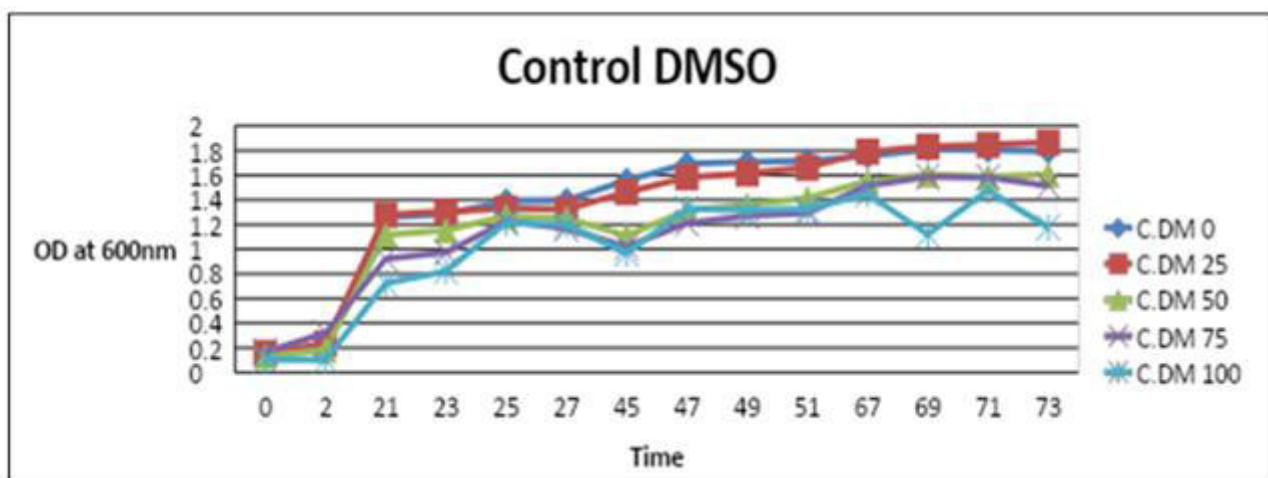


Graph 8: Graph representing OD v/s Time for DMSO Amla. Here, 100 μ l DMSO amla extract gave the best result as compared to 0 μ l, 25 μ l, 50 μ l, and 75 μ l in the survival rate of yeast.

The growth increased from the first day (OD—2.067) to the fourth day (OD—2.167).



Graph 9: Graph representing OD v/s Time for DMSO Ginger. Here, 0 μ l DMSO ginger extract gave the best result as compared to 0 μ l, 25 μ l, 50 μ l, and 75 μ l in the survival rate of yeast. The growth has been increased from the 1st day (OD – 0.367) to the 4th day (OD – 1.687).



Graph 10: Graph representing OD v/s Time for Control DMSO. Here, 25 μ l control DMSO gave the best result as compared to 0 μ l, 50 μ l, 75 μ l and 100 μ l in the survival rate of yeast.

4.2. Replicative Life Span Assay

According to Yanfei Lin *et al.*, the replication lifespan of the atg2 and atg32 mutants was unaffected by the CuB treatment. Atg2 and Atg32 did not significantly affect the yeast's ability to replicate²³. According to Postnikoff *et al.*, the results obtained through life-span assays vary greatly depending on the genetics of the yeast used.³¹ According to Stepień, K *et al.*, curcumin supplementation only impacted reproductive potential in the wild-type strain ($p < 0.05$). In the case of the *sod1Δ* and *rad52Δ* mutants, curcumin statistically significantly decreased the reproductive potential of the cells, with $p < 0.001$ for *sod1Δ* at 200 μ M curcumin concentration, and $p < 0.05$ for *rad52Δ* at 300 μ M curcumin concentration. In the case of the *SOD2* gene deletion, curcumin supplementation reduced the reproductive capacity of the cells; however, the changes were not statistically significant³³.

In our experiment, out of four volumes used in the above experiment, only 75 μ l in amla and 50 μ l in ginger gave the best results and were selected for further assay. In these experiments, cells treated with amla and ginger extract have more daughter colonies than their control. In the amla sample, 2441.6 average daughter cells are present in 10 μ l culture compared to the control, which has only 1260.8 average daughter cells in 10 μ l of culture. In the ginger sample, 2387.2 average daughter cells are present in 10 μ l culture compared to the control, which has 1062 average daughter cells in 10 μ l culture. It shows that after treatment with amla and ginger extracts, the cells do not move to the senescence phase early; rather, they multiply rapidly and form more daughter colonies. In the amla sample, daughter cells were approximately 2x times double that of control amla in 10 μ l of culture. In the ginger sample, daughter cells were approximately 2.3x times double that of control ginger in 10 μ l of culture.

Table 2.1: Comparison between average cells present in Amla Control and Amla Aqueous. In this table, in an Aqueous Amla, the daughter colonies double approximately in comparison with the control amla.

S. No.	Control Amla					Sample Amla				
	A	B	C	D	Total	A	B	C	D	Total
1.	13	19	12	14	14 (Avg) * 64 = 896 cells / 10 μ l	50	37	41	54	45 (Avg) * 64 = 2,912 cells / 10 μ l
2.	23	30	27	33	28 (Avg) * 64 = 1,792 calls / 10 μ l	42	38	40	45	41 (Avg) * 64 = 2,624 cells / 10 μ l

3.	12	16	15	21	16 (Avg) * 64 = 1,024 cells / 10µl	13	21	23	11	17 (Avg) * 64 = 1,088 cells / 10µl	
4.	12	09	07	09	09 (Avg) * 64 = 592 cells / 10µl	21	22	24	29	24 (Avg) * 64 = 1,536 cells / 10µl	
5.	05	07	09	08	07 (Avg) * 64 = 464 cells / 10µl	31	30	32	41	33 (Avg) * 64 = 2,112 cells / 10µl	
6.	07	14	08	08	09 (Avg) * 64 = 592 cells / 10µl	36	108	128	56	82 (Avg) * 64 = 5,248 cells / 10µl	
7.	27	22	15	21	36 (Avg) * 64 = 2,320 cells / 10µl	44	59	37	41	45 (Avg) * 64 = 2,880 cells / 10µl	
8.	43	38	31	33	36 (Avg) * 64 = 2,320 cells / 10µl	32	29	30	28	30 (Avg) * 64 = 1,920 cells / 10µl	
9.	15	12	24	17	17 (Avg) * 64 = 1,088 cells / 10µl	22	23	29	19	23 (Avg) * 64 = 1,472 cells / 10µl	
10.	28	21	25	21	23 (Avg) * 64 = 1,520 cells / 10µl	46	43	39	35	41 (Avg) * 64 = 2,624 cells / 10µl	
Avg					12,608 / 10 = 1,260.8	Avg					24,416 / 10 = 2,441.6
Large cell size											

Table 2.2: Comparison between average cells in Ginger Control and Ginger Aqueous. In this table, the daughter colonies doubled approximately in Aqueous ginger compared to control ginger.

S. No.	Control Ginger					Sample Ginger					
	A	B	C	D	Total	A	B	C	D	Total	
1.	37	23	20	21	25 (Avg) * 64 = 1,600 cells / 10µl	26	26	17	33	25 (Avg) * 64 = 1,600 cells / 10µl	
2.	18	19	28	29	23 (Avg) * 64 = 1,472 cells / 10µl	57	71	56	72	64 (Avg) * 64 = 4,096 cells / 10µl	
3.	17	10	17	18	15 (Avg) * 64 = 960 cells / 10µl	57	59	66	57	60 (Avg) * 64 = 3,840 cells / 10µl	
4.	16	19	25	19	20 (Avg) * 64 = 1,280 cells / 10µl	16	29	22	18	21 (Avg) * 64 = 1,344 cells / 10µl	
5.	31	10	05	11	14 (Avg) * 64 = 896 cells / 10µl	24	16	15	11	16 (Avg) * 64 = 1,024 cells / 10µl	
6.	07	09	14	12	10 (Avg) * 64 = 640 cells / 10µl	36	39	28	32	34 (Avg) * 64 = 2,176 cells / 10µl	
7.	07	07	04	08	06 (Avg) * 64 = 384 cells / 10µl	57	48	72	64	60 (Avg) * 64 = 3,840 cells / 10µl	
8.	06	12	08	15	10 (Avg) * 64 = 640 cells / 10µl	24	32	42	28	31 (Avg) * 64 = 1,984 cells / 10µl	
9.	17	14	10	18	15 (Avg) * 64 = 960 cells / 10µl	20	24	18	22	20 (Avg) * 64 = 1,280 cells / 10µl	
10.	31	27	26	28	28 (Avg) * 64 = 1,792 cells / 10µl	42	34	50	41	42 (Avg) * 64 = 2,688 cells / 10µl	
Avg.					10,624 / 10 = 1,062.4	Avg.					23,872 / 10 = 2,387.2
Small cell size											

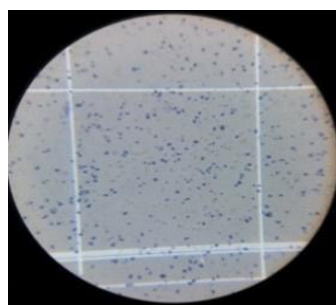


Fig. 9 - Aqueous Amla

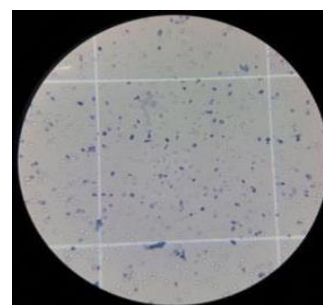


Fig.10 - Control Amla

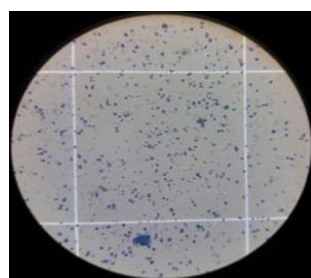


Fig. 11 - Aqueous Ginger

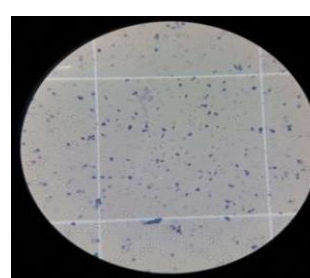


Fig. 12 - Control Ginger

4.3. Anti – Oxidative Assay

According to Yanfei Lin *et al.*, The survival rates were as follows: 38.09% ± 1.39 in the control group; 42.19% ± 0.87 in the RES group; 47.74% ± 2.03 in the 0.1 μM CuB (Cucurbitacin B) group ($p < 0.01$); 53.73% ± 0.73 in the 0.3 μM CuB group ($p < 0.001$); and 55.01% ± 1.35 in the 1 μM CuB group ($p < 0.01$). According to these findings, one of the main contributing factors to CuB's antiaging effects is the control of antioxidant activity²³. According to Jinye Dang *et al.*, when BY4742 cells were exposed to 50 mM H₂O₂ in a YPD medium for 60 or 120 min, a majority of the cells died. However, the pre-incubation of cells with NLE significantly promoted survival under H₂O₂-induced oxidative stress²³. In our experiment in the antioxidative assay, cells were treated with

9 mM H₂O₂ to give oxidative stress conditions and to check whether the cells would overcome the stress if given amla and ginger extract with varying volumes, i.e., 25 μl, 50 μl, 75 μl, 100 μl. Here in amla, 100 μl volume gave the best result. In ginger, 50 μl gave the best result for antioxidative assay compared with negative control as distilled water and positive control as *Astragalus*. Enhancing the antioxidant factors of *astragalus membranaceus* may inhibit oxidative stress. The results showed that when treated with 8mM ascorbate, yeast subjected to 4mM H₂O₂ continued to grow vigorously, suggesting that a relatively high amount of ascorbate is required to counteract the effects of 4mM H₂O₂. In contrast, gallic acid (found in Amla) demonstrated strong scavenging effects at a concentration of only 2 mM. This could be because gallic acid's three hydroxyl groups can donate hydrogen to reduce H₂O₂.



Fig. 13 – Amla 50 μl



Fig. 14 – Amla 75 μl

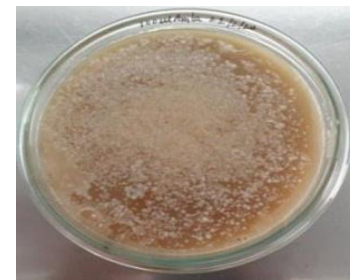


Fig. 15 – Amla 100 μl



Fig. 16 – Ginger 25 μl

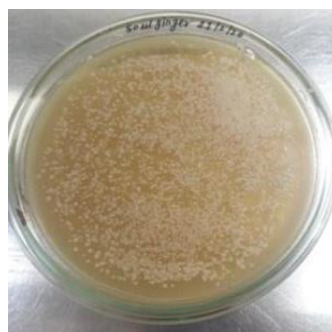


Fig. 17 – Ginger 50 μl



Fig. 18 – Ginger 75 μl



Fig. 19 – Control 25

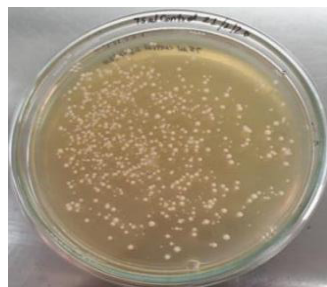


Fig. 20 – Control 50 μl

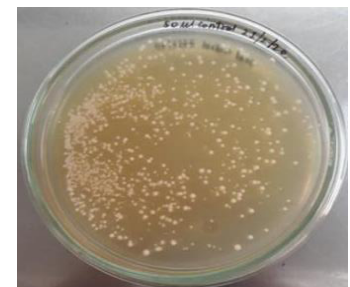


Fig. 21 – Control 75 μl

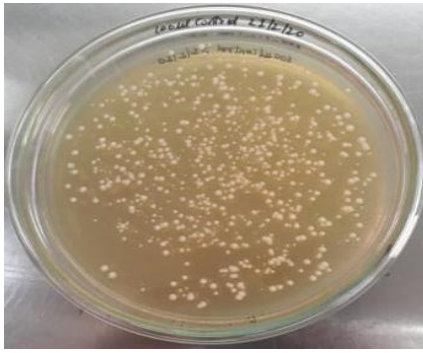


Fig. 22 – Control 100 µl

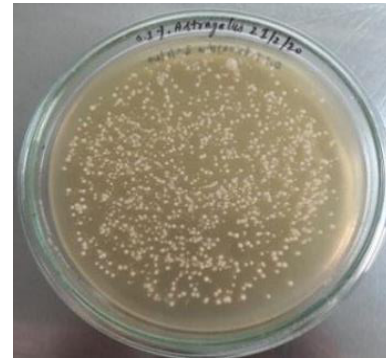


Fig. 23 – 0.1 % Astragalus

4.4. Verification of Anti-Oxidative Assay

According to Stepień, K *et al.*, curcumin supplementation caused a statistically significant increase in the level of ROS in the cell measured by DHE³³. We demonstrate that curcumin has an anti-oxidative effect in the yeast model. For verification of the antioxidative assay, cells were treated first with Amla and ginger and then with a high concentration of H₂O₂. 100 mM H₂O₂ concentration was given for 3 h and then washed with cold PBS and 15 % ethanol. The dead cells were stained with trypan blue dye, and counting was done on Neubauer's

chamber. Here, average cells present in Amla 50 µl are – 5072 dead cells, Amla 75 µl – 2112 dead cells and Amla 100 µl – 4256 dead cells, in Ginger 50 µl – 1360 dead cells, Ginger 75 µl – 944 dead cells and 100 µl – 192 dead cells, in Astragalus 50 µl – 2512 dead cells, 75 µl – 2640 dead cells and 100 µl – 1296 dead cells, as compared with control 50 µl – 3152 dead cells, control 75 µl - 4240 dead cells and control 100 µl – 4752 dead cells. In Amla 75 µl, the death rate of *S. cerevisiae* was lower and gave a better result than 50 µl and 75µl. In Ginger 100 µl, the death rate of *S. cerevisiae* was lower and gave a better result than 50 µ and 75 µl.

Table 4.1: Average dead cells present in different concentrations of Amla, Ginger, Astragalus, and Control. In 100 µl Ginger extract, yeast cells show less death than amla and Astragalus.

S.No.	Sample	Column A	Column B	Column C	Column D	Total
1.	Amla 50	86	81	61	89	317 / 4 * 64 = 5072 Dead cells
2.	Amla 75	38	16	54	24	132 / 4 * 64 = 2112 Dead Cells
3.	Amla 100	131	26	23	86	266 / 4 * 64 = 4256 Dead Cells
1.	Ginger 50	16	24	18	27	85 / 4 * 64 = 1360 Dead Cells
2.	Ginger 75	12	16	16	15	59 / 4 two-factored Cells
3.	Ginger 100	5	2	2	3	12 / 4 * 64 = 192 Dead Cells
1.	Astragalus 50	82	19	16	40	157 / 4 * 64 = 2512 Dead Cells
2.	Astragalus 75	52	38	42	33	165 / 4 * 64 = 2640 Dead Cells
3.	Astragalus 100	27	14	19	21	81 / 4 * 64 = 1296 Dead Cells
1.	Control 50	53	42	55	47	197 / 4 * 64 = 3152 Dead Cells
2.	Control 75	18	74	97	76	265 / 4 * 64 = 4240 Dead Cells
3.	Control 100	65	80	74	78	297 / 4 * 64 = 4752 Dead Cells

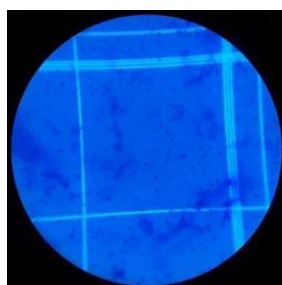


Fig. 24 – Amla 50 µl

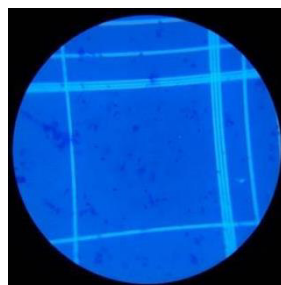


Fig. 25 – Amla 75 µl

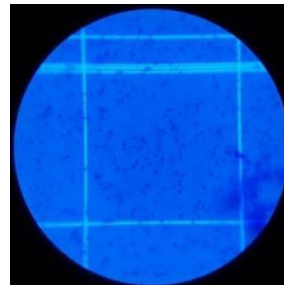


Fig.26 – Amla 100 µl

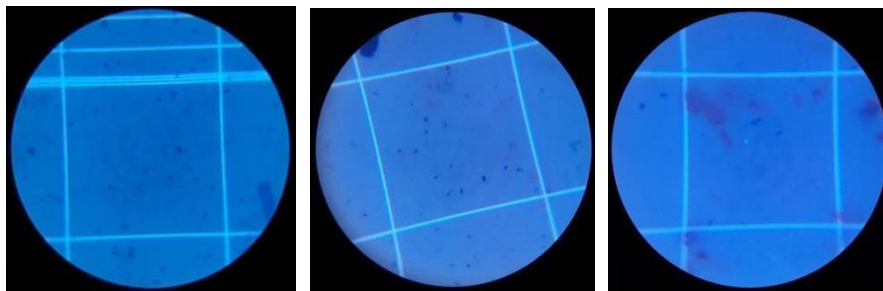


Fig. 27 – Ginger 50 µl Fig. 28 – Ginger 75 µl Fig. 29 – Ginger 100 µl

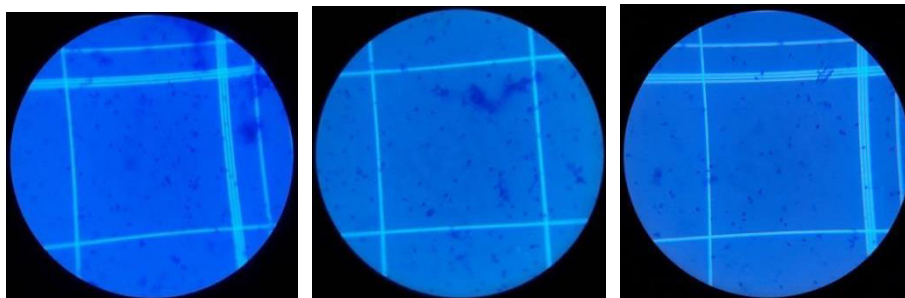


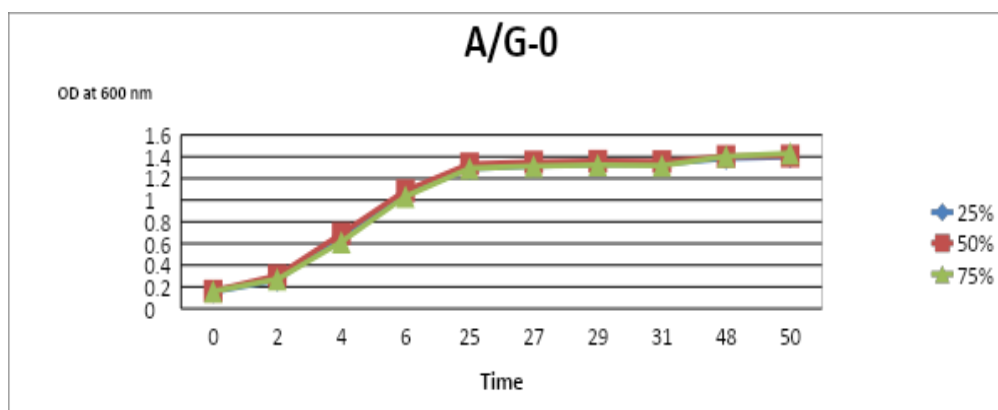
Fig. 30 – Astragalus 50 µl Fig. 31 – Astragalus 75 µl Fig. 32 – Astragalus 100 µl



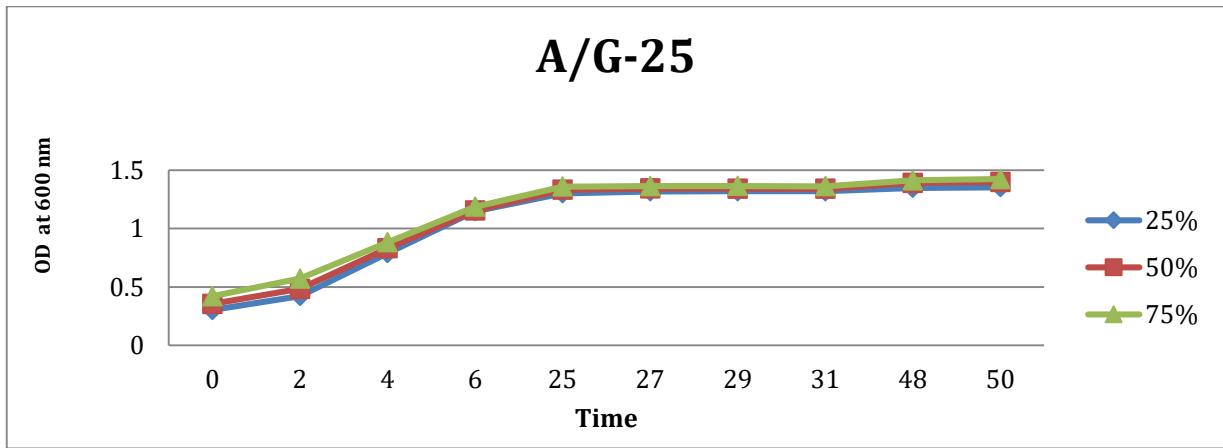
Fig. 33 – Control 50 µl Fig. 34 – Control 75 µl Fig. 35 – Control 100 µl

4.2 Effect of Amla and Ginger in Combination

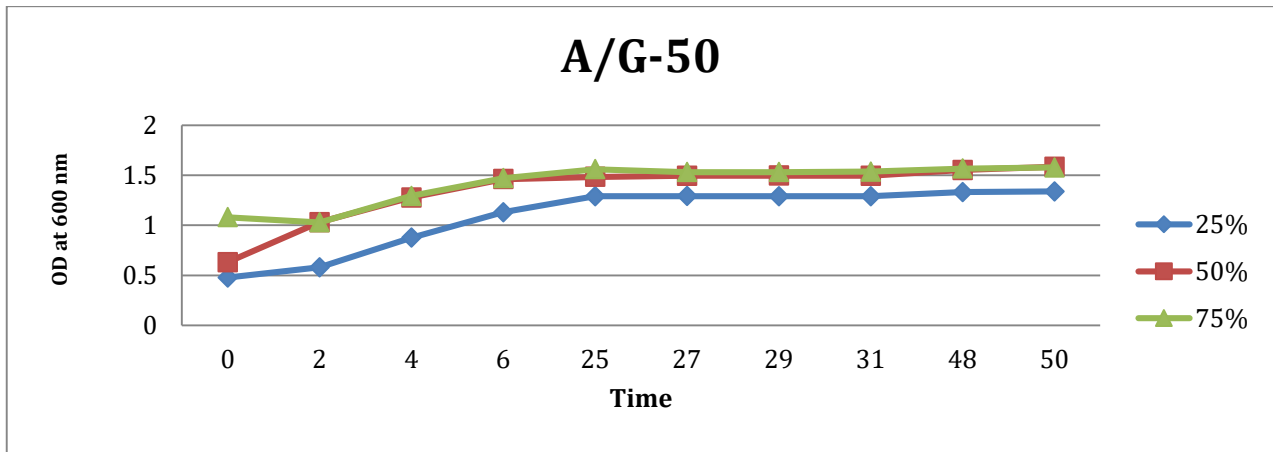
We have taken amla and ginger with different volumes in the chronological life span assay. Still, here we have mixed the two samples with different combinations such as 25:75 %, 50:50 %, and 75:25 % (Amla: Ginger) to clarify that if amla and ginger were mixed, whether they show the same effect as given independently or their effect was enhanced upon mixing. An ANOVA test was performed to analyze the results. The results were analyzed similarly to those in the chronological lifespan assay. Here, a 75:25 % (Amla: Ginger) combination in A/G 100 µl gave better results than all other volumes.



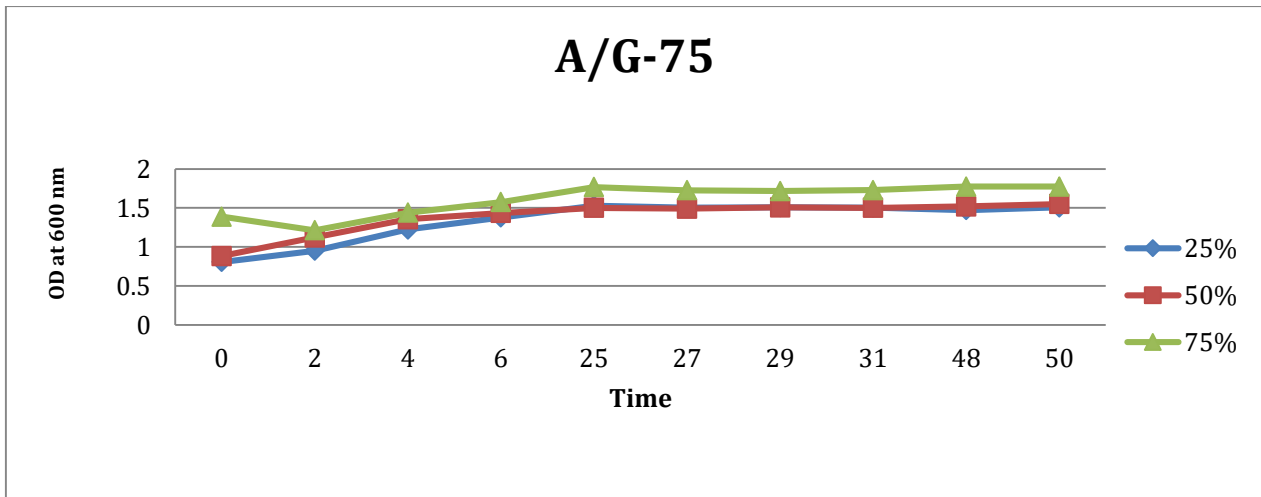
Graph II: Graph representing OD v/s Time for 0 µl concentration. Here, 75% (75:25) Amla: Ginger has shown increased growth from the 1st day (OD – 0.145) to the 3rd day (OD – 1.478) until constant growth has been reached.



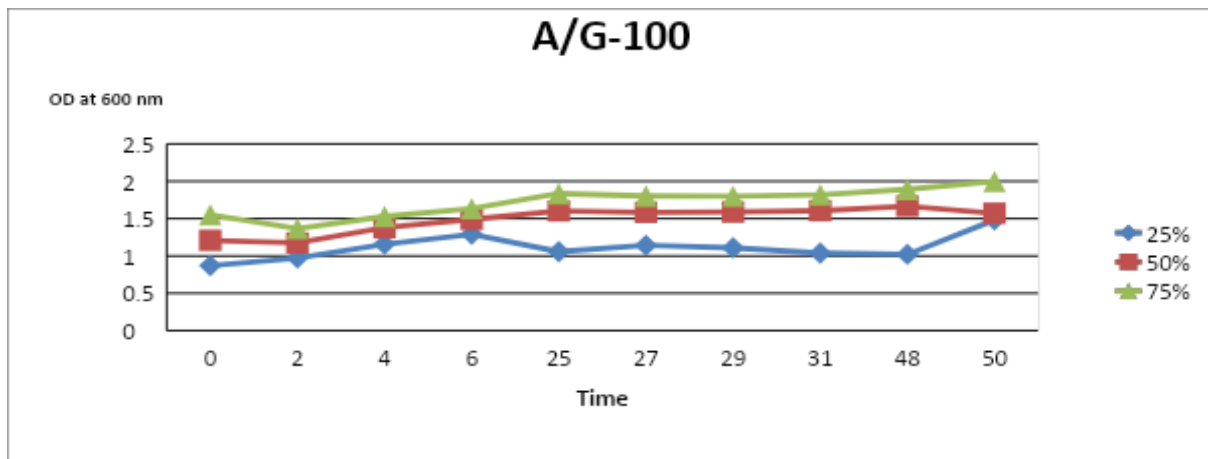
Graph 12: Graph representing OD v/s Time for 25 µl concentration. Here 75% (75:25) Amla: Ginger has shown increased growth from 1st day (OD – 0.476) to 3rd day (OD – 1.489) until constant growth has been reached



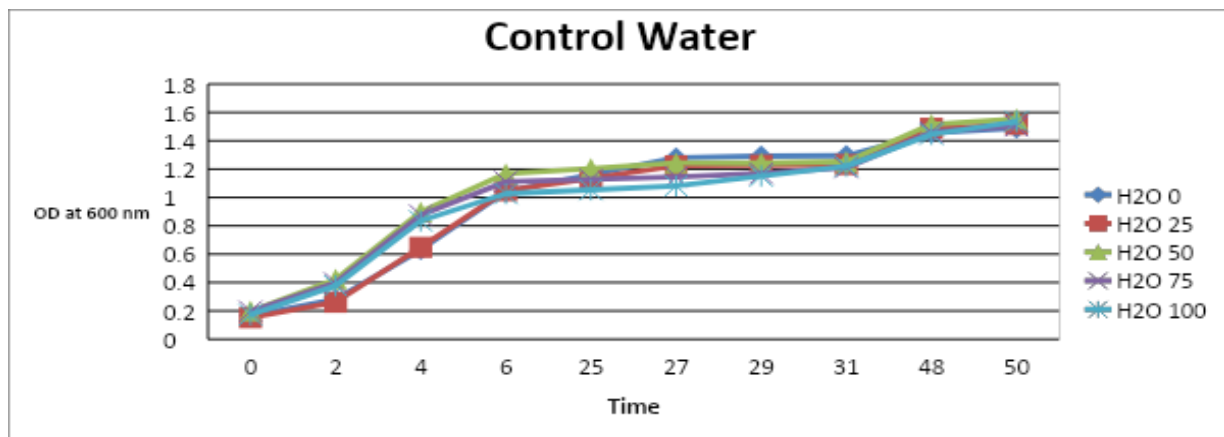
Graph 13: Graph representing OD v/s Time for 50 µl concentration. Here 75% (75:25) Amla: Ginger has shown increased growth from 1st day (OD – 1.148) to 3rd day (OD – 1.656) until constant growth has been reached



Graph 14: Graph representing OD v/s Time for 75 µl concentration. Here 75% (75:25) Amla: Ginger has shown increased growth from 1st day (OD – 1.497) to 3rd day (OD – 1.759) until constant growth has been reached



Graph 15: Graph representing OD v/s Time for 100 µl concentration. Here 75% (75:25) Amla: Ginger has shown increased growth from 1st day (OD – 1.569)to 3rd day (2.134) until constant growth has been reached



Graph 16: Graph representing OD v/s Time for Control Water. Here, 0 µl water gave the best result as compared to 0 µl, 25 µl, 50 µl, and 75 µl in the survival rate of yeast. As we add water to the media, the growth rate declines due to dilution, and that's why, at 100 µl, the growth has decreased.

4.5. Effect of Glucose on *S. cerevisiae*

According to Abigail Bryan et al., 2018 there was a significant drop in CO₂ production due to the saturation limit of *S. cerevisiae* after 3 days²⁶. In our experiment, *S. cerevisiae* cells were treated with 32 % glucose because the high sugar concentration affects the aging of cells. After 5 days, a few colonies on the plates showed that Amla and Ginger have an active compound that helps cells overcome the effect. Here, 129 visible colonies were observed in the medium supplied

with ginger extract compared to the control with 62 microcolonies. The 40% glucose concentration experiments showed the greatest variation in the carbon dioxide levels. The CO₂ production rate in the trials with an 80% glucose concentration was, on average, the highest. When yeast is combined with 100% glucose concentration, an unexpected transformation takes place. The yeast reached a saturation gradient, indicating that it could not produce any more CO₂, which caused a significant decrease in CO₂ production.

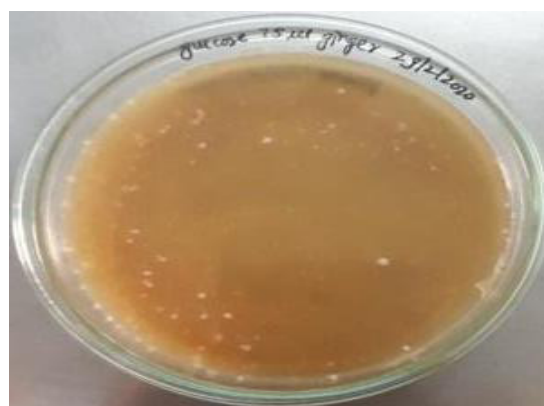


Fig. 29 - *S. cerevisiae* colonies on YPD plate with Ginger extract after treatment with glucose.

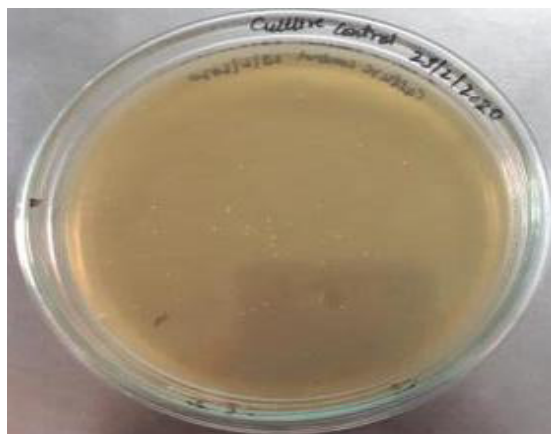


Fig. 30 - *S. cerevisiae* colonies on YPD plate as Culture control.

5. CONCLUSION

S. cerevisiae are eukaryotic and single-celled microorganisms. It was isolated on Sabouraud's agar and later on YPD agar using the quadrant plate technique. After the pure colony was isolated, it was preserved on a YPD slant for further use. Amla and ginger extract was extracted using three different methods, i.e., aqueous, ethanol, and DMSO, and was filtered by membrane filters. Equating all three methods it was confirmed that aqueous extract gave superior results on *S. cerevisiae* than Ethanol and DMSO. It was also confirmed that Amla was far better than ginger for its anti-aging effect on *S. cerevisiae*. The above results were established by performing different assays on *S. cerevisiae*. In the Chronological lifespan assay, we analyzed the results using statistical data, and for this, the ANOVA test was performed. In ANOVA, if p – the value is greater than 0.05, then H_0 is accepted, and if p – the value is less than 0.05, then H_0 is REJECTED. Here, in Aqueous Amla and Ethanol Amla p – the value was smaller than 0.05, so the Null hypothesis will be REJECTED. In Aqueous Ginger and Ethanol Ginger, p – the value was smaller than 0.05, so the hypothesis will be REJECTED. In aqueous ethanol and DMSO extract, Amla was better than ginger. All the samples were compared with their respective concentrations of control. Aqueous extract was considered the best of the other two, i.e., Ethanol and DMSO. In the replicative lifespan assay, according to the above results, out of 25 μ l, 50 μ l, 75 μ l, and 100 μ l, only 75 μ l in Amla and 50 μ l in ginger gave the best results and were used for this assay. In this assay, we analyze the result based on the number of daughter cells. *S. cerevisiae*, upon treatment with Amla and Ginger extract for 24 h, daughter cells were counted in Neubar's chamber. In Amla, there are 2441.6 daughter cells counted compared with the control, which has 1260.8 daughter cells. There are 2387.2 daughter cells in ginger compared with the control, which has only 1062 daughter cells. Here, Amla gave a better result than ginger. In the anti-oxidative assay, *S. cerevisiae* cells were treated with 9 mM H_2O_2 to provide oxidative stress conditions. In Amla, 100 μ l and in Ginger, 50 μ l volume gave the best results after comparing negative control as distilled water and positive control as *Astragalus*. In the Verification of Anti – Oxidative Assay, H_2O_2 concentration was increased to 100 mM, and cells were washed with cold PBS and then

treated with 15 % ethanol. After treatment, cells were mixed in trypan blue, and dead cells were counted in Neubar's chamber. Here, the highest number of dead cells was present in Amla 50 μ l, Ginger 50 μ l, *Astragalus* 75 μ l, and Control 100 μ l. In Amla 75 μ l, the aging rate of *S. cerevisiae* was lower and gave the best result than 50 μ l and 75 μ l. In Ginger 100 μ l, the death rate of *S. cerevisiae* was lower and gave a better result than 50 μ and 75 μ l. The ANOVA test was performed to measure the effect of Amla and Ginger in combination. According to the ANOVA test, p – the value in all the volumes, i.e., 25 μ l, 50 μ l, 75 μ l, and 100 μ l was smaller than the 0.05 value. So, the hypothesis for all the volumes was REJECTED. The hypothesis is REJECTED, i.e., it proves that Amla and Ginger's samples differed. Here, 75: 25 % combination in A/G 75 μ l and 100 μ l gave better results than all other volumes. In Effect on Glucose on *S. cerevisiae*, cells were treated with glucose because the high sugar concentration affects the aging of cells. After 2-3 days, a few colonies on the plates showed that Amla and Ginger have an active compound that helps cells overcome the effect. Here, 129 visible colonies were observed in the medium supplied with ginger extract compared to the control with 62 microcolonies. Hence, ginger gave a positive result.

6. ACKNOWLEDGMENTS

We thank Ms. Rama Bhadekar for her time and support at each stage of the dissertation process, and I would also like to thank Mr. Kashyap Pandya for his support.

7. AUTHOR'S CONTRIBUTION STATEMENT

Ms. Divya Darak conceptualized and gathered data for this work. Dr. Devesh Kumar Joshi and Dr. Sonalika Singh analyzed the data and provided the necessary input for the manuscript's design. Ms. Princy Kumari Rajput and Mr. Ram Bhajan Sahu discussed the methodology and results and contributed to completing the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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