Investigating the synthesis of silver nanoparticles using multiple fruit extracts and their anticancer property

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1. Abstract

Since the last decade, nanotechnology has been advancing rapidly, and has been increasingly used in various fields such as medical, food, industrial purposes and many others, due to their unique physical and chemical properties. The use of silver nanoparticles as drug carriers has been explored and presents several advantages. They have the ability to eradicate cancer cells by flow and penetration to different regions of tumours through blood vessels and then to interstitial space in order to arrive at the target cells. However, silver nanoparticles have their limitations. Researchers have found that silver nanoparticles had a toxic effect on all cells including healthy ones, suppressing cellular growth and multiplication and causing cell death depending on concentrations and duration of exposure. Hence, this study investigates the synthesis of silver nanoparticles and its anticancer properties. This study utilises different fruit extracts to synthesise silver nanoparticles. Silver nanoparticles have been synthesised when the mixture turned brown. The absorption spectrum of silver nanoparticles peaked at about 420nm to 470nm. Afterwards, silver nanoparticles were added to *Saccharomyces cerevisiae* to determine their anticancer property. By measuring the absorbance spectrum, there was a lower absorption spectrum for yeast cells in nanoparticle solutions, signifying that there were less yeast cells alive. As *Saccharomyces cerevisiae* possess high homology to humans, by determining the number of yeast colonies; there were evidently less yeast colonies in *Saccharomyces cerevisiae* setups which contained silver nanoparticles. Therefore, the tests conducted proved that silver nanoparticles have cytotoxic effects on yeast cells. In conclusion, silver nanoparticles exhibit anticancer properties and may be a potential solution for cancer treatment.

2. Introduction

According to the National Cancer Institute (NCI), cancer is among the leading causes of death worldwide. In 2018 alone, there were 18.1 million new cases and 9.5 million cancer-related deaths worldwide. Therefore, the high mortality rate of cancer patients coerces the need for

technological breakthroughs in the medical field. Currently, there are a few technological treatments for cancer, such as chemopreventive and chemotherapeutic agents. However, these treatment methods may lead to a string of side effects such as increasing the incidence of endometrial cancer in women and long-term administration of tamoxifen and long-term administration of tamoxifen causes hepatic tumours in rats (Brown, 2002). Advancement of nanotechnology in the field of medicine has created a new hope in the therapeutic and pharmaceutical fields.

Nanomedicine is a developing field that could potentially make a huge positive impact on human health (Kumar et al., 2010). Nanoparticles possess unique chemical, physical and biological properties, which are useful in various fields like therapeutics, electronics, cosmetics, catalysis, among many others (Sriram et al., 2012). It offers a new aspect for tumour detection, prevention and treatment. Nanoparticles have the ability to eradicate cancer cells by flow and penetration to different regions of tumours through blood vessels and then to interstitial space in order to arrive at the target cells. The environmental and physiological characteristics may vary from one tumour tissue to another (Jannathul & Lalitha, 2015).

Among different nanoparticles like gold and copper, silver nanoparticles have an eye-catching role because silver has been shown *in vitro* to be more cytotoxic, this allows it to be more effective in killing or damaging cancer cells (Ip et al., 2006; Kathiraven & Ravi, 2014). Therefore, there is a possibility for silver nanoparticles to become anticancer agents (Raghunandan et al., 2011). The skin penetration ability of silver nanoparticles is much lower compared to that of other metal nanoparticles such as gold and copper (Wang et al., 2016). Silver nanoparticles are proven to have the ability to be anticancer therapeutic agents in the treatment of leukaemia, breast cancer, and lung cancer (Wei et al., 2015).

Yeast models have been employed to study numerous molecular aspects related to cancer development, as well as to determine the genetic contexts associated with anticancer drug sensitivity or resistance. This study uses the budding yeast *Saccharomyces cerevisiae* to present conserved cellular processes with high homology to humans, and it is a rapid, inexpensive and efficient compound screening tool. (Matuo et al., 2012)

Currently, there are numerous methods for the synthesis of silver nanoparticles, which can be categorised as chemical, physical and biological methods. Examples of physical and chemical methods include microemulsion techniques, UV-initiated photoreduction and photoinduced reduction (Wei et al., 2015). These methods are not cost-effective and may cause environmental damage. In contrast, using biological synthesis to synthesise silver nanoparticles is cost-effective and environmentally friendly. For example, research by Skiba and Vorobyova (2019) has shown that silver nanoparticles can be synthesised by using orange peel extracts and they tested for its dye degradation properties on methylene blue. Another research done by Ibrahim (2015) showed that silver nanoparticles can be synthesised using banana peel extract and he tested for their antimicrobial activity against representative microorganisms. Therefore, it is proven that silver nanoparticles can be biologically synthesised using fruit extracts.

Hence, it is of interest to compare the synthesis of silver nanoparticles using several different fruit extracts and compare their anticancer properties in the reduction of growth of yeast cells.

3. Objective

This study aims to compare the effectiveness of silver nanoparticles synthesised from various fruit peels in reducing the rate of growth and cell division in yeast cells.

4. Hypothesis

The hypothesis of the study is that silver nanoparticles synthesised from banana and orange peels are effective in reducing the rate of growth of yeast cells.

5. Materials and Methods

a. Materials

Saccharomyces cerevisiae was obtained from Carolina Biological Supply. Banana and orange were purchased from NTUC Fairprice CO. Silver nitrate, sodium chloride, potato dextrose (PD) broth, petri dishes, 0.45 mm microfilter were used.

b. Methods

i. Preparation of fruit peel extracts (Banana and Orange)

10 g of fruit peel was added to 100 ml of deionised water and heated to 80°C for 10 min. The mixture was filtered through a filter paper; the filtrate was collected and stored at 4°C until needed.

ii. Synthesis of silver nanoparticles using fruit peel extracts

1 mM silver nitrate solution was prepared in a bottle and wrapped with aluminium foil. 0.05 ml of each fruit peel extract was added to 10 ml of $AgNO_3$ solution. Silver nanoparticles are heated and stirred continuously. They would be synthesised when the mixture turned brown.

Silver nanoparticles were then centrifuged at high speed (9500 rpm for 10 min), washed three times with sterile water, and resuspended in sterile water.

iii. Analysis of silver nanoparticles

The absorption spectrum of silver nanoparticles was measured using a UV-vis spectrophotometer. The absorption spectrum of silver nanoparticles was within the range of 400nm - 470nm.

In the future, scanning electron microscopy can also be used to analyse the size and morphology of silver nanoparticles obtained.

iv. Growth of yeast cells

Yeast cells were inoculated into 10 ml PD broth and incubated with shaking at 30°C for 1 day. The absorbance at 600 nm was standardised at 0.8.

v. Effect of silver nanoparticles on growth of yeast cells

In the test setup, 0.125 ml of yeast culture was added to 1.25 ml of silver nanoparticle solution (synthesised from individual fruit peels) and 1.125 ml of PD broth was added. For control setup, 0.125 ml of yeast culture was added to 1.25 ml of sterile water and 1.125 ml of PD broth was added.

Three replicates of each setup were prepared. The mixtures were incubated at room temperature for 24h. Serial 10-fold dilutions are done with 0.85% sodium chloride solution. 0.1 ml of the diluted culture was spread on potato dextrose agar. Plates were incubated at 30°C overnight. The number of yeast colonies was determined.

6. Results and Discussion

a) Absorbance spectrum of synthesised silver nanoparticles

Silver nanoparticles synthesised from banana peel extract showed a peak in absorbance at 421.7nm.

Silver nanoparticles synthesised from orange peel extract showed a peak in absorbance at 459.8nm.

b) Effect of silver nanoparticles on growth of yeast cells

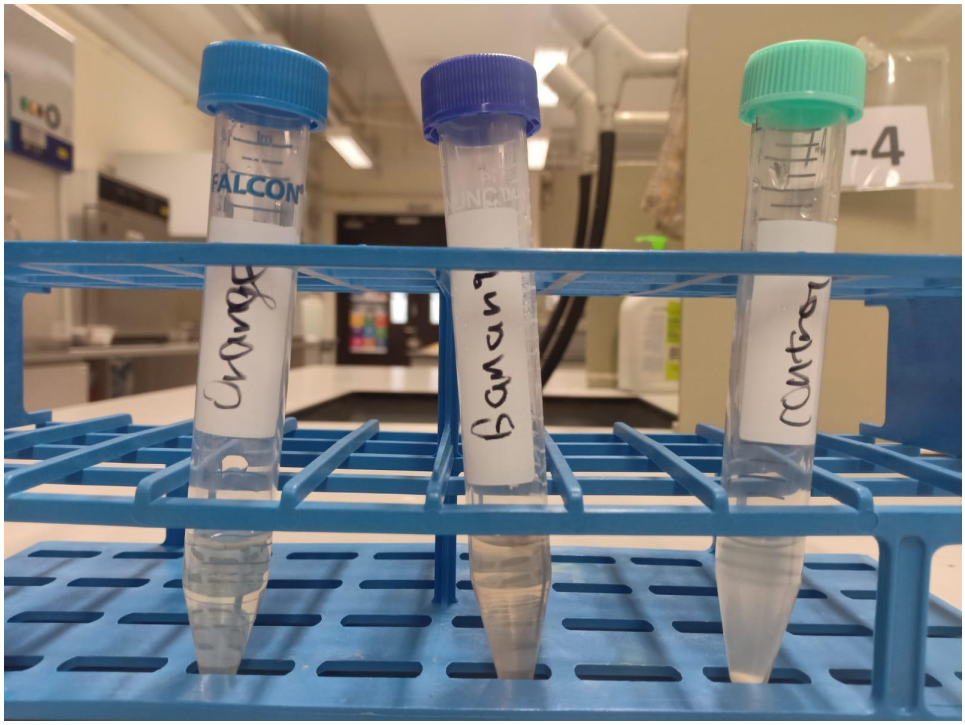

Fig 1.1: Experimental set-ups and control set-up

Sample no.	Absorbance at 600nm
Control	1.688
Control	2.128
Control	0.669
Banana	0.126
Banana	0.197
Banana	0.162
Orange	0.060
Orange	0.095
Orange	0.135

Absorbance spectrum of yeast cells in synthesised silver nanoparticles

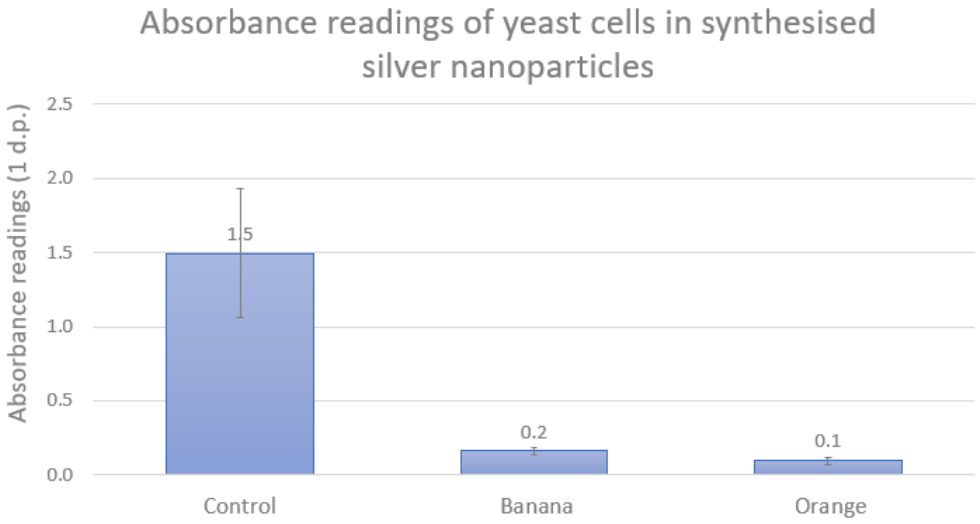

Fig 1.2: Effect of nanoparticles synthesised from fruit peels on the absorbance of yeast cultures

From Fig 1.2, the absorbance of yeast cells in the presence of silver nanoparticles synthesised from banana and orange peels was visibly lower than the absorbance of yeast cells in control set up, 0.2Au and 1.5Au respectively. which meant that there were less yeast cells alive in silver nanoparticle solution. The results signify that silver nanoparticles have a cytotoxic effect on yeast cells.

c) Number of yeast colonies

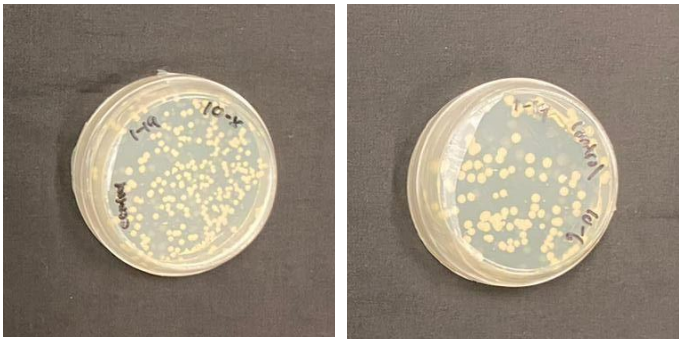

Fig. 2.1: Control set-ups. Dilution factor is 10-5 and 10-6 and the number of yeast colonies are 92 and 61 from left to right respectively.

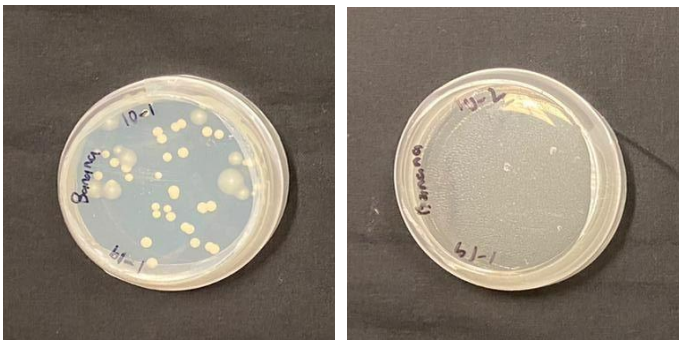

Fig. 2.2: Yeast colony in synthesised silver nanoparticles (Banana). Dilution factor is 10-1 and 10-2 and the number of yeast colonies are 21 and 1 from left to right respectively.

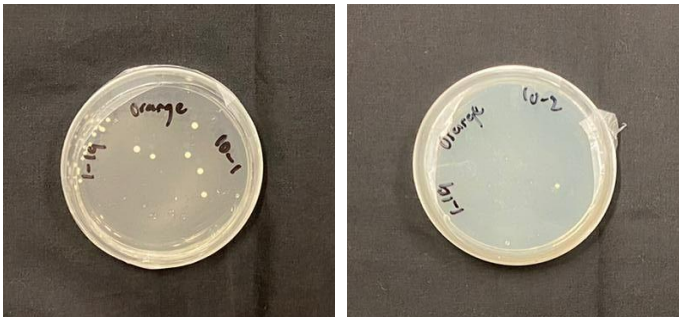

Fig. 2.3: Yeast colony in synthesised silver nanoparticles (Orange). Dilution factor is 10-1 and 10-2 and the number of yeast colonies are 28 and 0 from left to right respectively.

The mean colony count of *Saccharomyces cerevisiae* colonies in Fig. 2.1 was evidently higher than that in the synthesised silver nanoparticles set-ups (Fig. 2.2 and Fig. 2.3). The results signify that silver nanoparticles have anticancer properties, exhibiting cytotoxic effects on yeast cells. and were able to prevent the spread of yeast cells and even kill it in the process. The killing effect of silver nanoparticles have been proposed to be associated with the direct contact of nanoparticles to the yeast cell wall, followed by penetrating into cytoplasm. Direct contact of silver nanoparticles with large surface areas on a yeast cell wall could lead to membrane damage, resulting in the leakage of cellular contents and eventual cell death (Sondi, Salopek-Sondi, 2004; Liao et al., 2019). Besides, silver nanoparticles hinder yeast growth by reducing rates of transcription and cellular respiration, lastly, killing them (Galván Márquez et al., 2018).

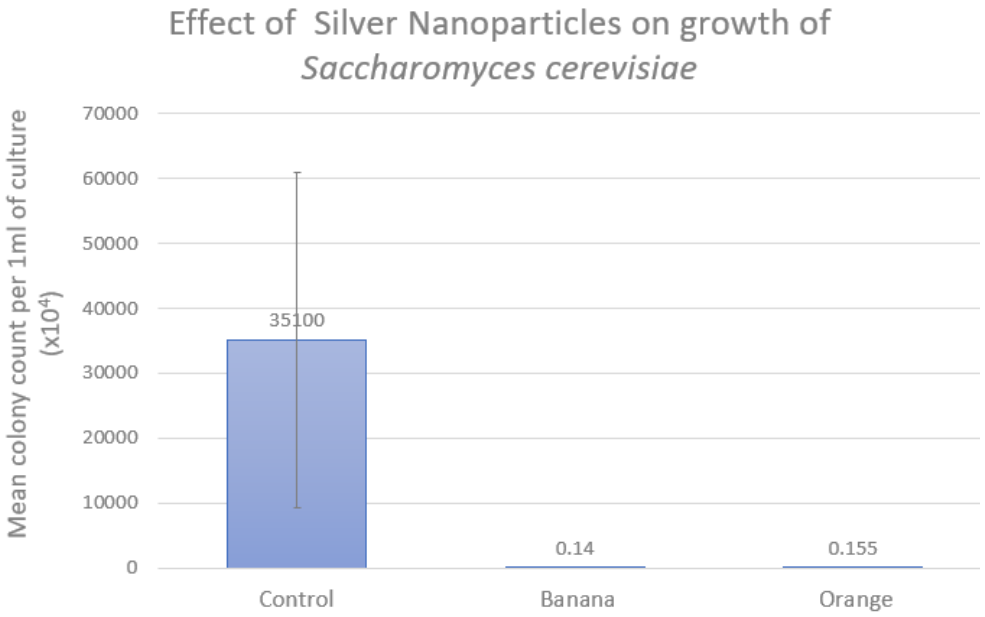

Fig. 2.4 : Effect of silver nanoparticles on growth of *Saccharomyces cerevisiae*

There is a significant effect of silver nanoparticles on growth of *Saccharomyces cerevisiae*. The mean colony count per ml of yeast culture for control set up is 250714 times the mean colony count per ml of yeast culture mixed with silver nanoparticles synthesised by banana peel. This shows the significant impact that silver nanoparticles have on the growth of yeast culture.

7. Limitations

There are some limitations of the project. Concentration of reducing agents found in different fruit peels are not fixed as we only manage to fix the mass of different fruit peels. Thus, this might affect the concentration of silver nanoparticles that are being synthesized, which affects the results when comparing the effectiveness of different fruit peels.

Polyvinylpyrrolidone (PVP), a capping agent which is used to control the growth of silver nanoparticles and standardise the size of the nanoparticles, was not used in our experiment. Thus, this might affect the size of silver nanoparticles that are being synthesized. As the effectiveness of silver nanoparticles varies with its size, our results may not be as accurate.

8. Conclusion

From all data collected, it can be concluded that silver nanoparticles synthesised by orange and banana peel extracts are able to reduce growth of *Saccharomyces cerevisiae* and they possess cytotoxic properties to kill *Saccharomyces cerevisiae*. Thus, silver nanoparticles have potential anticancer properties. Furthermore, using fruit peel extracts to synthesise silver nanoparticles can help to reduce and recycle waste as fruit peels are often discarded and wasted. Fruit peels can also replace toxic chemicals like sodium borohydride as reducing agents to synthesise nanoparticles. So, it is an environmentally friendly method compared to other methods of synthesising silver nanoparticles. It is also economically feasible to prepare fruit peel extracts to synthesise silver nanoparticles, allowing future hospitals to have a new way to treat cancer patients at a low cost and with increased effectiveness. For future work, more fruit peels should be tested to determine if they are able to synthesise silver nanoparticles as well to test for more effective results. Moreover, *Caenorhabditis elegans* can be used to further affirm the cytotoxicity of silver nanoparticles. Lastly, researchers can study more about silver nanoparticles synthesised by fruit peel extracts and test it on human cancer cells in clinical trials to affirm its anticancer properties.

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