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Potential to Value-Add Waste Orange Peels to Produce Natural Anti-Microbial Extracts

N. Muranda
Department of Chemical Engineering, Manicaland State University, Zimbabwe

M. M. Manyuchi
Department of Chemical Engineering, Manicaland State University, Zimbabwe
Department of Operations and Quality Management, Faculty of Engineering and the Built Environment, University of Johannesburg, South Africa

N. Sukdeo
Department of Operations and Quality Management, Faculty of Engineering and the Built Environment, University of Johannesburg, South Africa

Abstract

This study focused on assessment of the potential to use waste orange peels extracts as an anti-microbial substance. Waste orange peels from a local company were first collected and underwent size reduction in order for them to increase the surface area through powder formation. The powder then underwent solid-liquid extraction using water whereby the orange peels extract was formed. Afterwards, the orange peels extract was qualitatively analysed for the presence of tannins, saponins, flavonoids, terpenoids, cardiac glycosides, alkaloids, carbohydrates and phenols. Further, the potential for use of the orange peels extract as an anti-microbial agent was evaluated at extracts brix concentration of 3.6-21.6, shelf time of 7-35 days, pH of 2.1-3.4, temperature of 4-40 ^{o}C and orange peels extracts of 2-12 mL. The orange peels extracts tested positive for tannins, phenolics, carbohydrates, cardial glycoside, saponins and flavonoids, however, terpenoids were absent. The anti-microbial properties of the orange peels extracts were optimum at brix 3.6, shelf time of 7 days, pH 2.1, temperature 4 ^{o}C and orange peels extracts concentration of 12 mL. From this study findings, it is important to value-add waste orange peels extracts since they have natural antimicrobial properties.

Keywords
Anti-oxidants, anti- microbial, process conditions, value addition, waste management, waste orange peels

1. Introduction
Processed orange fruit waste is a very good example of a wasted resource which has a potential to be used as a raw material in the production of fuels, chemicals and other materials (Sharma et. al., 2017). The quantities of whole fruit oranges produced globally are around 68 million tons which accounts for about 8.5% of the total fruits which are currently been produced globally per annum (Aliyu et al.., 2016). Approximately 42-62% of these oranges are used as raw material by fruit juice processing industries. However, after processing 45-60% of these oranges end up as waste which usually is of no use for numerous Orange Juice and Oil Processors (Alothman et al., 2016).

Large quantities of processed orange fruit waste, roughly in the range 15-25 million tons per year are currently been produced globally by Orange Juice Processors (Barrales et aal., 2016). The processed orange fruit waste produced comprises of orange membrane materials, peels and seeds with peel materials been the major constituent accounting for approximately 44% of the waste weight. As a result of these large quantities of orange fruit waste been produced from orange juice and oil processing operations, numerous Orange Juice Processors in first world countries have invested in

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facilities that enable them to utilize processed orange fruit waste as a raw material in another profit-making side processes to produce anti-oxidants (De Melo et al., 2023).

In this study, a case study was undertaken at a local orange fruit juice processing company in Zimbabwe, in a bid to utilise its waste orange peels to produce anti-oxidants. The company, which is a strategic business unit specialises in the processing of whole fruit oranges to recover orange juice and oil. As one of the major orange juice processing facilities in Zimbabwe, the company currently has a capacity to process about 4200 tonnes of whole fruit oranges every year resulting in the production of antioxidants from processed orange fruit waste which amount to about 2200 tonnes.
Unlike the other Orange Juice Processors in developed countries, the company and other numerous local orange fruit juice processing companies have however not been able to invest in orange waste processing facilities which can enable value to be realised from the processed orange peels waste produced every season. One of the major limitations been that of the high capital investment costs required for the establishment of molasses and pectin pomace production plants which are expensive to erect. Figure 1 is showing an example of processed orange fruit waste dumped on a dumpsite after orange juice and oil have been recovered from whole fruit oranges.

Figure 1. Processed orange fruit waste dumped on an open ground dumpsite.

From the company's production records for 2017 it is evident that about 82% of the processed orange fruit waste which was produced by this company annually was just dumped at the company's dumpsite as useless waste. Whilst about 18% of the processed orange fruit waste was sold to a number of farmers who bought it as stock feed for their cattle. The selling price for processed orange fruit waste as stock feed is however very low (USD11 per ton), making the whole idea of selling processed orange fruit waste as stock feed financially unsound to the company. Figure 2 is showing quantities of processed orange peels waste which was produced in 2017 by the company from its orange processing operations.

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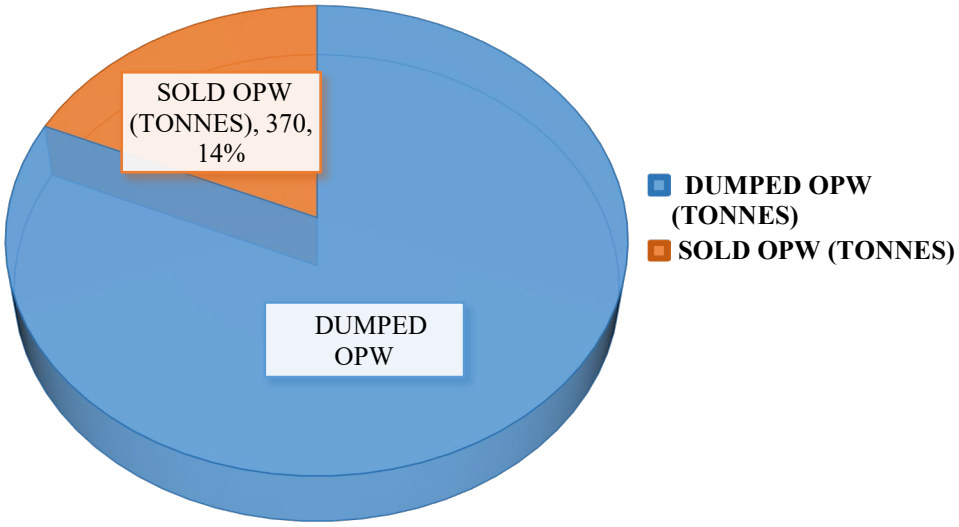

Figure 2. Orange fruit waste produced by the study company.

2. Problem Statement

Some of the large quantities of Processed Orange Fruit Waste produced globally each year (15-24 million tonnes) from orange juice and oil production operations have currently found use as raw materials in the production of pectin, flavonoid, fibre, and animal feed. However, a large amount of this waste is still dumped every year, posing both economic and environmental problems such as high cost of transportation, shortages of dumping site, and accumulation of high organic matter. Therefore, numerous alternative uses of orange peel are still desirable.

Some recent studies have indicated that orange peels contain polyphenolic and antioxidant compounds which give their extract the ability to be used as a preservative in some situations. Due to continuous expansion of the food and beverages industry, the demand for natural food additives and preservatives is increasing drastically. The general public have currently become more and more aware about the health effects of some of the synthetic preservatives which are currently been used in food. Henceforth researchers are currently developing numerous natural preservatives to meet this demand by consumers. Therefore, this research paper will focus on the possibility of waste orange peel extracts to be used as a natural preservative for the beverages industry since the demand of natural preservative is gradually increasing on the food industry market with a focus on the process conditions for the microbial activity.

2.1 Aim

The study aim is to investigate the possibility of using waste orange peel extracts as a natural preservative for the preservation of orange juice dilatants with a focus on the process parameters that affect microbial activity.

2.2 Objectives

The study objectives included to:

i. Conduct solid-liquid extraction of dried orange peel powder using hot water as a solvent.
ii. Conduct qualitative analysis of the orange peel extract.
iii. Investigate the effects of orange juice pH, brix value, time and storage temperature on the preserving ability of orange peel extract.

2.3 Research Questions

The study research questions were:

i. What are chemical constituents present in the orange peel powder extract for current study?
ii. What are the effects of pH, time, and temperature and brix value on orange peel extract preservative?
iii. What is the minimum concentration of orange peel power extract required for effective inhibition of microbial activity?

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2.4 Scope of The Study

This study focused on the investigation of the possibility of value to be added to the processed orange peel waste produced by orange juice producers. In case of this research, a local Zimbabwean company was used as a study case, where dried orange peel extract which will be prepared at the company and investigated for its ability to act as a natural antimicrobial against bacteria, moulds and fungi. Qualitative analyses will be carried out to determine, the composition of the orange peel power extract and any problems which might be encountered during the course of the experiment will be highlighted.

2.5 Study Significance

The study will enable the utilization of waste orange peel produced from orange juice processing operations there by reduce environmental pollution as well as transportation cost of the waste from the processing plant to dumpsites. It will also further enable the formulation and development of a natural preservative whose demand is increasing rapidly in the food processing industry.

3. Factors That Influence Use of Orange Peels Anti-Microbial Agent

3.1Effect of pH

Low pH values are known to be unfavorable for the growth of most microbial organisms, henceforth favor a longer shelve life. pH is defined the negative logarithm to base 10 of hydrogen ions which is a function used to quantify the acidity or alkalinity of any given food sample (Gómez-Urios et al., 2023). The interaction of a food sample's pH, storage temperature, redox potential, and preservative contribute towards the overall ability of a preservative to inhibit growth of pathogens and all other microbial organisms. In general, microbial organisms are known not to grow, or grow at a very slow rate, at low pH values usually below 4.6, but there are exceptions.

3.2 Effect of temperature

All microorganisms are known to be having defined temperature range in which they multiple and grow, with an optimum, minimum and maximum, growth temperature value. Deep knowledge on how the relationship between time, temperature, pH, sucrose percentage of a soft drink, and preservative type and quantity is a crucial factor when determining the proper storage conditions for a soft drink (Hasija et al., 2015). Temperature has an impact on both the replication time of microorganisms and their lag period. Usually over a specified temperature range, the rate at which organisms grow is defined as an Arrhenius relationship. This is in the range where the log of the microorganisms' growth rate constant will be proportional to the absolute temperature's reciprocal. The equation below only applies over the linear portion of the Arrhenius graph when determining microorganisms' growth rate is represented by Equation 1:

$G = -\mu / 2.303\ RT$ …… (1)

Where: G is the log growth rate constant, μ is the temperature characteristic (constant for a particular microbe), R is the Gas constant and T is the temperature (°K)

3.3 Impact of storage time

Storage time (shelf life) is also an important factor when considering the growth rate of microbial organisms. Therefore, determination of shelve life at various soft drink parameters is of paramount for all soft drinks and well food products in the food industry (Huang et al., 2014)0. Shelf life is defined as the time when a food product will be safe to consume in terms of its appearance, taste and microbial load from the time of its production. Factors like microbial safety and organoleptic qualities of a food product are used in determining a product's shelf life.

4. Materials and Methods

4.1Reagents and Chemicals

The chemicals that were used in this work included: orange serum agar, malt extract agar, citric acid mono-hydrate, iron (III) chloride, 1% NH_3, $CHCl_3$, concentrated H_2SO_4, CH_3CO_2H, Methanol, 1% HCI, Molisch reagent and Wagner reagent were obtained from the study company Quality Control Laboratory. In addition, 500 mL of olive oil were purchased from food world supermarket, orange peels and pasteurized 65 brix orange juice concentrate were collected from the study company processing plant.

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4.2 Instruments

The equipment's that were used included: the digital hand refractometer, colony counter, microwave, digital pH meter, conical flasks, incubator, microbiological petri dishes, electronic balance, pipette, mortar, pestle, screw cap test tubes, aluminium foil paper and No"1 filter paper.

4.3 Preparation of Orange Peel Extract

4.3.1 Pre-extraction preparation of the orange peel sample

Valencia orange (Citrus sinensis) peels were obtained from the study company processing plant peel bin during processing in Zimbabwe. The orange peels were thoroughly washed with running tap water for about 5 minutes so as to wash away orange pulp and seeds from the peels. The orange peels were afterwards dried in an incubator at 40 ^{o}C for a period of about two weeks.

4.3.2 Producing orange peel powder

Dried orange peels were then grinded to fine powder using a mortar and pestle so as to increase the surface area of extraction. The fine orange peel powder obtained from the grinding was stored under room temperature in airtight bottles until its use.

4.3.3 Solid-liquid extraction of orange peel powder

To obtain the orange peel extract, 45 g of orange peel powder were weighed using an electronic balance and they were transferred into a 500 mL beaker followed by the addition of 450 mL of boiling water which was heated to 100 ^{o}C. After this addition of hot water, the beaker was immediately covered with foil papers so as to avoid any possible evaporation of volatiles from sample. The sample was then placed in an accumulator at 50 ^{o}C where it left for 3 hrs. This sample was continuously swirled within the 3 hours so as to increase the possibility of extraction. After these hours the sample was then filtered using a No"1 filter paper to collect the orange peel extract which. This extract was kept in air tight plastic bottle and under refrigeration conditions (4 ^{o}C) till use.

4.4 Qualitative Analysis of Orange Peel Extract

4.4.1 Test for Tannins

A portion of the orange peel extract was taken and then diluted with distilled water in a ratio of 1:4. Four drops of 10% ferric chloride solution were afterwards added to the extract sample. Formation of a blue or green color indicated the presence of tannins (Ma et al., 2008).

4.4.2 Test for Saponins

A 2g sample of fine orange peel powder was boiled with 20 mL of distilled water in a water bath and filtered.10ml of the filtered sample was afterwards mixed with 5 mL of distilled water in a test tube and this mixture was shaken vigorously to obtain a stable persistent froth. The resulting froth was then mixed with about 3 drops of olive oil. For this test, the formation of emulsion indicated the presence of saponins (Ochuko et al., 2013).

4.4.3 Test for Flavonoids

A sample with 4 drops of 1% NH_3 solution were added to the orange peel extract sample in a test tube. The formation of a yellow coloration confirms the presence of flavonoid compounds (Lin et al., 2013).

4.4.4 Test for Terpenoids

A sample with 5 mL of the orange peel extract sample was mixed with 2 mL of $CHCl_3$ in a test tube. 3ml of concentrated H_2SO_4 was afterwards carefully added to the mixture to form a layer. The formation of an interface with a reddish-brown coloration indicated the presence of terpenois (Shharma et al., 2017).

4.4.5 Test for cardiac Glycosides

A sample with 5 mL of the orange peel extract solution was mixed with 1 mL of 1 % ferric sulphate solution in 2 mL of (5%) glacial acetic acid. One drop of concentrated sulphuric acid was afterwards added. Formation of a blue color indicated presence of a deoxy sugar (Soma and Genitha, 2014).

4.4.6 Test for Alkaloids

A sample with 3 drops of Wagner's reagent were added a 3 mL of sample of the orange peel extract sample. Formation of a brownish precipitate indicated the presence of alkaloids (Rizaldy et al., 2023).

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4.4.7 Test for carbohydrates

For the detection of the presence of carbohydrates, Molisch's test was used whereby one drop of concentrated sulphuric acid was added to l mL of the orange peel extract solution. Three drops of Molisch's reagent were added to the mixture, without mixing to form an upper phase. The presence of carbohydrates in the samples was indicated by the formation of a brown ring at the interphase (Umesh et al., 2018).

4.4.8 Test for Phenol

A sample with 3 drops few drops of 5% $FeCl_3$ solution were added to a 3 mL orange peel extract solution sample. The presence of phenols is indicated by the formation of deep blue-black colour (Aliyu et al., 2016).

5 Results and Discussion

5.1 Orange Peels Extract Constituents

The chemical constituents of the orange peels are shown in Table 1. The orange peels extracts tested positive for tannins, phenolics, carbohydrates, cardial glycoside, saponins and flavonoids, however, terpenoids were absent. These chemicals are critical components of anti-oxidants and anti-micro-biology chemicals. This gave a good indication that the orange peels extract from the study company can be used to produce anti microbiology chemicals.

Table 1. Chemical constituent of the waste orange peels

Chemical Constituent	Test Result	Interpretation
Tannins	+	Tannins present
Phenolics	+	Phenolics present
Terpenoids	-	Terpenoids absent
Alkaloids	+	Alkaloids present
Carbohydrates	+	Carbohydrates present
Cardial Glycosides	+	Cardial Glycosides present
Saponins	+	Saponins present
Flavanoids	+	Flavanoids present

5.2 Microbiological analysis

These analyses involved the examination of any Escherichia Coliforms in the orange juice sample over time. The microbiological plating of the orange juice samples was done so as to determine the levels of coliform bacteria, total acidophilic count, and heat resistant moulds in each of the samples with time. Orange Serum Agar (OSA) and Malt Extract Agar (MEA) was used as culturing media for these analyses. Orange Serum Agar was used for the cultivation and enumeration of microorganisms associated with the spoilage of orange juice cultivation of Lactobacilli, other aciduric organisms and pathogenic fungi whilst Malt Extract Agar was used for isolating, cultivating and enumerating of yeasts and molds. These media were prepared, and then mixed with the juice samples and media. The mixtures were then cultured in petri dishes using the pour plate method for the isolation of any bacteria, moulds and yeasts that might had formed in the juice samples. The petri dishes for each sample were then incubated for 48 hours at 30 ºC. After these hours the total number of colonies are counted using a colony counter.

5.3 Effect of process parameters on the orange peels extract anti-microbial activity

5.3.1 Effect of sucrose % (w/w)

Thee Pasteurized 65 brix orange juice concentrate from the study company processing plant was used to prepare samples with varying brix values (3.6, 7.9, 11.8, 15.2, and 21.6 brix) for the study of the effects of sucrose % on the orange peel extract preservative. A digital hand refractometer was used to attain these brix values whilst dilutions of the orange concentrate from 65 brix to the respective brix values were made with distilled water. 7.5 mL of a 43.38 mg/mL orange peel extract preservative was added to each of these orange juice samples each of 50 mL. These samples were kept room temperature ready for microbiological analysis which were done on each of the samples with time for 5 weeks. The anti-micro biology characteristics for the orange peels extract for bacteria, yeast and mould respectively were low at 3.6 brix a shelf time of 7 days (Figure 3). Increase in the brix value to 21.6 and the shelf life to 35 days resulted in reduced anti-microbiology activity by the orange peels extracts (Figure 3).

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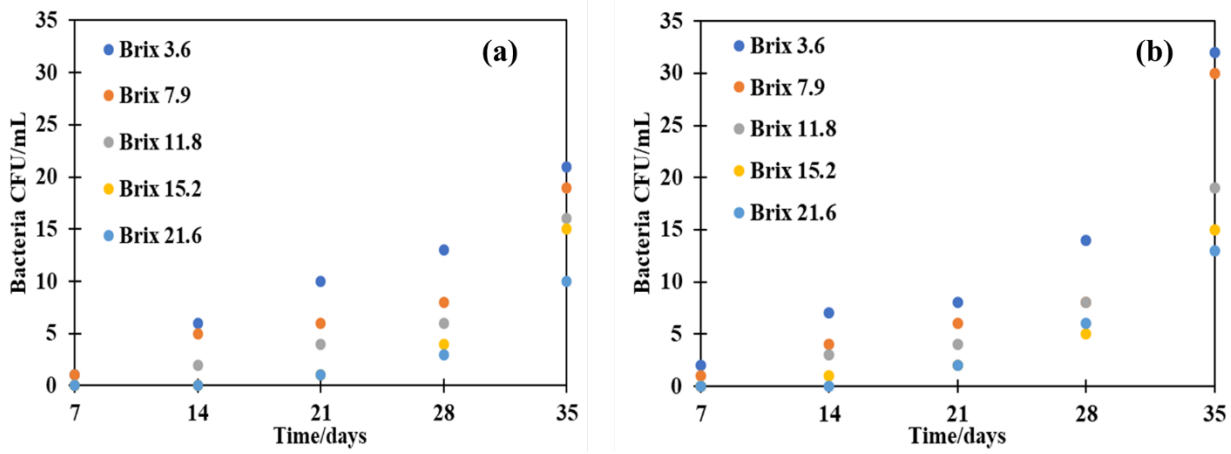

Figure 3. Effect of orange peels extract brix concentration and shelf time on bacteria growth

5.3.2 Effect of pH

To satisfy the pH requirements of the juice samples, citric acid mono-hydrate and a digital pH meter were used to adjust pH values of six 15 brix samples to 2.1, 2.3, 2.6, 2.9, 3.2, and 3.4 so as to enable the study of the effects of pH changes on the effectiveness of orange peel extract preservative at a fixed juice brix value. 7.5 mL of the 43.38 mg/mL orange peel extract preservative was added to these samples each sample with 50 mL of orange juice. These samples were kept in screw type test tubes at room temperature ready for microbiological analysis which were done on each of the samples with time for 5 weeks. The pH of the bacteria had an effect on the increase in their concentrations in CFU/mL. Acidic conditions o pH 2.1 and low shelf life of 7 days were ideal for conditions of the anti-microbiology properties of the orange juice extracts (Figure 4). Higher pH of 3.4 and shelf life of 35 days resulted in increased bacteria production hence low anti-microbiology activity.

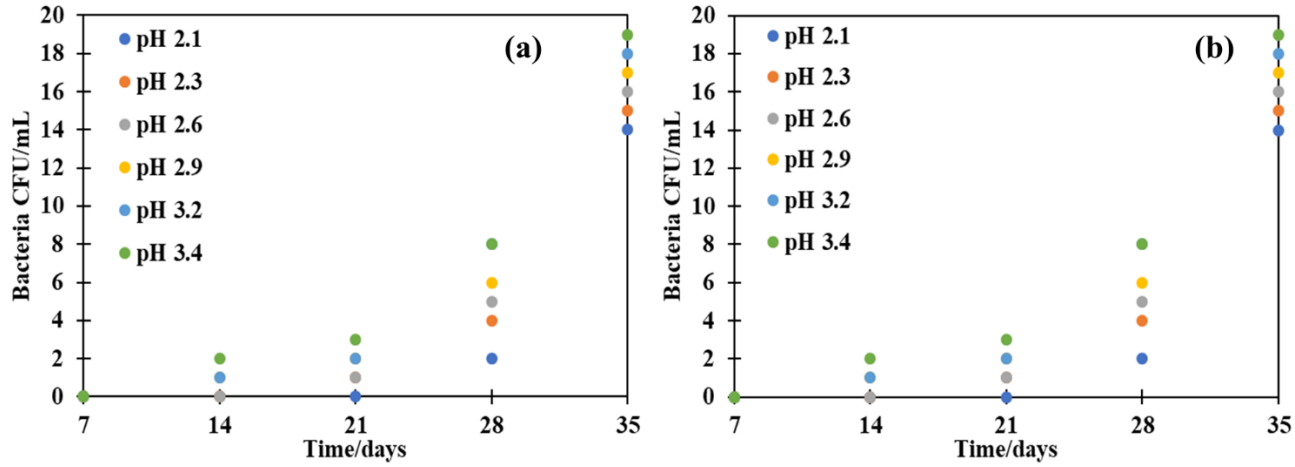

Figure 4. Effect of pH and time on growth in orange peels extracts

5.3.3 Effect of Time

Samples for the study of the effect of time on various 15 brix orange juices samples at varying volumes of the 43.38 mg/mL orange peel extract preservative of 2 mL, 4 mL, 6 mL, 8 mL, 10 mL and 12 mL were prepared. Each of these samples had 50 mL of orange juice. These samples were in screw type test tubes kept at room temperature ready for microbiological analysis which were done on each of the samples with time for 5 weeks. The concentration of the bacteria in CFU/mL increased over time for the various orange peels extracts with the highest being reached at 2 mL extract of waste orange peels and 35 days (Figure 5). This showed that the anti-microbiology activity of the orange peels extracts was higher at low shelf life of 7 days but with high orange peel extract of 12 mL (Figure 5).

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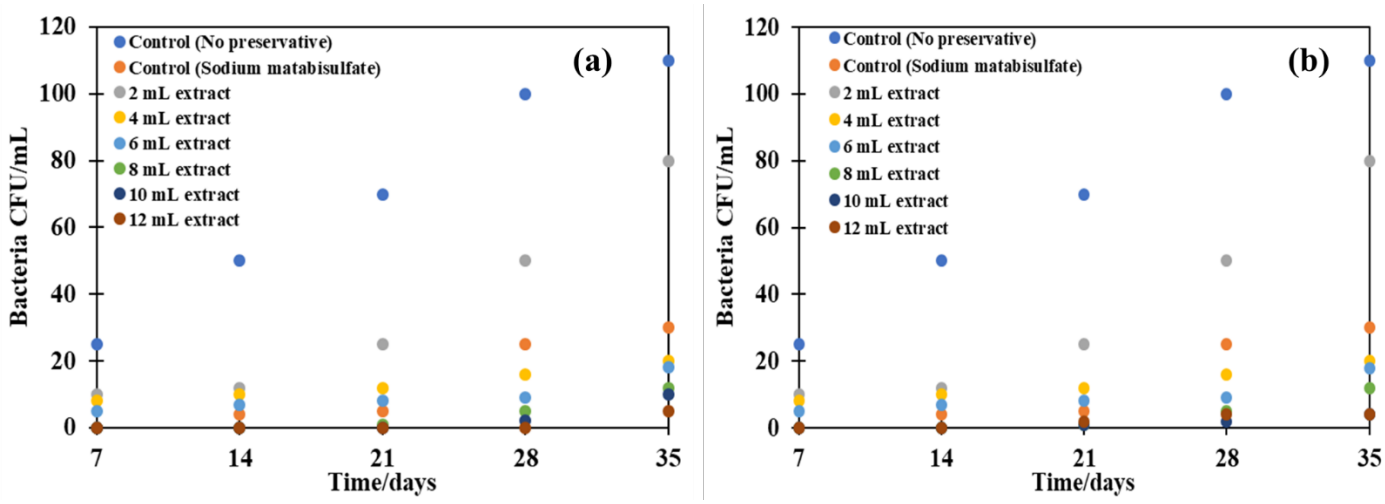

Figure 5. Effect of time and orange peels extracts concentration on bacteria growth

5.3.4 Effect of Temperature

For the study of the effects of temperature on the orange peel extract preservative, 15 brix orange juice samples, each with 7.5 mL of 43.38 mg/mL orange peel extract were prepared. Each of these sample constituted of 50 mL of orange juice. These samples were kept in screw type test tubes at varying temperature conditions of 4 ºC, 15 ºC, 15 ºC, 25 ºC, 32 ºC and 40 ºC. This temperature was attained through the use of a refrigerator and incubators. Microbiological analysis was done on each of the samples with time for 35 days. The concentration of the bacteria increased in CFU/mL over time for the various temperatures with the highest concentration being achieved at 40 ºC and 35 days (Figure 6). This gave an indication that the anti-microbiology properties of the orange juice extracts were highest at low temperature of 4 ºC and shelf life of 7 days.

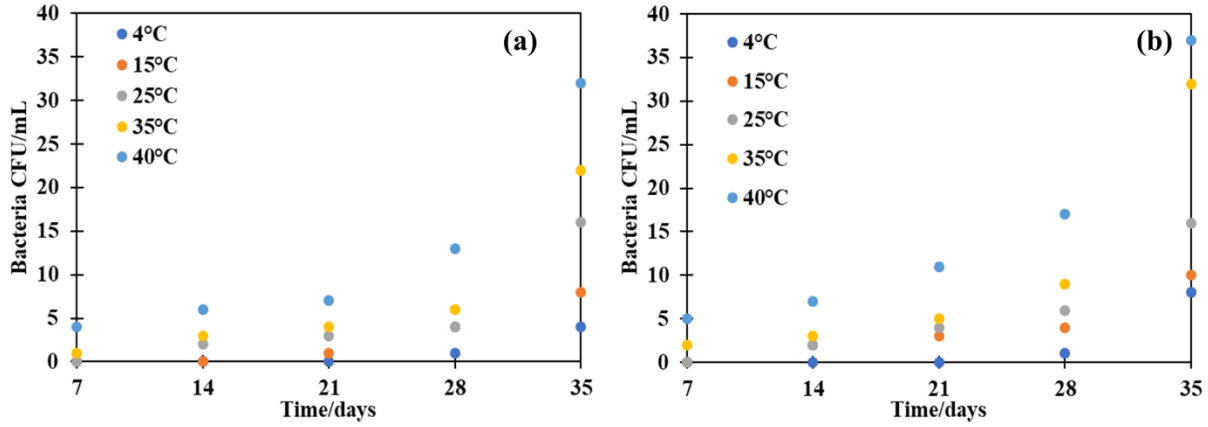

Figure 6. Effect of temperature and time on bacteria growth in orange peels extracts

6. Conclusion

Waste orange peels extracts are a good raw material source for the production of natural and organic anti-oxidants anti-microbiology substances. Waste orange peels are rich in tannins, phenolics, alkaloids, carbohydrates, cardial glycoside, saponins and flavonoids which are good constituencies of natural anti-oxidants and anti-microbiology substances. Orange peels extracts as a good source of anti-microbiology substances optimally inhibit bacteria growth at brix concentration of 3.6, pH of 2.1, temperature of 4 ºC, shelf life of 7 days a. Waste orange peels can be value added to orange peels extracts that can be used as an anti-microbiology substance under control conditions.

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