CINNAMON BARK EXTRACT FOR THE FORMULATION AND CHARACTERISATION OF ANTIMICROBIAL CREAM

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ABSTRACT

The main objective of this study was to formulate a stable W/O cream containing different strength of methanolic Cinnamomum cassia extract. It is known that the methanolic extract has an antibacterial activity and antioxidant, anticancer, antidiabetic and anti-cholesterol properties. Dried cinnamon bark was collected from Shub Alam, Malaysia during February 2014 and authenticated at Botany department at University Putra Malaysia. The dried barks were grinded to powder and used for extraction using maceration method. The extract was assessed for antibacterial properties. Then, a set of creams were prepared from the extract in different compositions to the formulations and stored at different accelerated conditions for a period of four weeks to predict the stability of these creams. Evaluation of creams was based on the physical stability, phase separation, centrifugation, pH of the formulations and test for microbial growth in formulated creams parameters. The result of the study showed significant changes in the pH of all creams in temperature above 25 °C and insignificant changes in temperature below 25°C with the passage of time. Great stability was observed in the formulations in term of phase separation, liquefaction, and color. The pH of all the formulations was 5.9-6.36, which is comparable to the 5.7-7 pH level of the skin. All these formulations showed no phase separation and color change during the test period, except for cream with 4%. From the present study, it can be concluded that it is possible to develop creams containing Cinnamomum cassia herbal extracts with a good antibacterial property.

Keywords: cinnamon bark extract, antimicrobial, phytochemical, maceration, methanolic extract

INTRODUCTION

Herbs have been called part of “Nature's pharmacy.” Although their action can in some ways be similar to modern drugs, herbal remedies are generally gentler and safer. Many of the drugs used in conventional medicine are derived from herbs. Herbalism uses the whole plant or whole parts of the plant, such as the leaves, the flowers, or the roots. Using the whole plant helps decreases the side effects that may occur when using isolated components. Herbs are plants actually grown fresh or purchased in dried form. They include the tropical aromatics, such as pepper, cinnamon and cloves etc. 1.

Spices are mainly used in small quantities for flavoring or coloring or as preservatives that kill pathogens or stop their growth. They also have certain medicinal properties and are used in pharmaceutical, perfumery, cosmetics and several other industries.

Cinnamon is a spice obtained from the inner bark of several trees from the genus Cinnamomum that is used in both sweet and savory foods. The word cinnamon comes from the Greek kinnamomon 2.

It is a small classic tree, with a 11-16 meters height which is (32.8-49.2 feet). It is belonging to the family Lauraceae, native to Sri Lanka and South India. The flowers have a greenish color and have a distinct odour and arranged in panicles. The fruit is a purple one-centimeter berry containing a single seed. Its flavor is due to an aromatic essential oil which makes up 0.5 to 1% of its composition. Cinnamon is high in antioxidant and antibacterial activity, and in medicine and traditions it is used to suppress cure common cold, toothache, to treat diarrhea and other problems of the digestive system and to fight bad breath and reported to have remarkable pharmacological effects in the treatment of type II diabetes. The oil extract of Cinnamon which aid in the preservation of certain foods through its antimicrobial properties. 3,5.

MATERIALS AND METHODS

The Study Area

Figure 1: Cinnamon bark 37
The research was carried out at research laboratory two of Management and Science University between the month of February and April 2014.

**Collection of Plant Materials**

Cinnamon bark (Figure 1) was collected in the local market in Shah Alam. The plant was sent to University Putra Malaysia, Selangor for plant species identification and authentication. The authentication number of the provided sample is 34b/0098/2015 which confirms the identity of the studied sample.

**Preparation of Plant Extracts**

Plant materials were finely grinded to powder by using a blender. Fifty grams of each plant material in powder form was weighed in an Erlenmeyer of 500 ml to which 300 ml of methanol is added for pre-extraction. The Erlenmeyer is placed in dark for three days in room temperature. The mixture was filtered using Whatman No. 1 filter paper then Bacterial filters. The filtrates were exposed to 60 °C in water bath for 30 min for methanol evaporation. The filtrates were kept at 4 °C until use.

**Preliminary Phytochemical Screening**

The extracts of cinnamon bark were subjected to phytochemical studies to find out the present of tannins. Extract was diluted with distilled water. Few drops of FeCl3 1% were added into 5 mL of diluted extract. Any changes in color were recorded.

The chemical constituents (Figure 2) were put in consideration while studying the extract.

![Figure 2: Chemical constituents extracted from Cinnamon bark](image)

**Measurement of Antimicrobial activity**

Disc diffusion method was applied to assess antimicrobial activities of the plant extracts against selected bacteria. The principle of this method was the different concentrations of plant extract would diffuse from the disc into agar containing the spread tested microorganisms. The presence of antimicrobial activity was identified by the appearance of inhibition zones which is the area where the microorganism could not grow due to the inhibitory effect of antimicrobial agent.

The different concentrations of crude extracts were dissolved in distilled water. 0.1g, 0.3g and 0.4g of crude extract were weighed and dissolve in 10ml of distilled water to produce 1%, 3% and 4% of extract.

Basically, three agar plates were used for the test. The first plate was placed with disc gentamycin as positive control, distilled water as negative control and 3% extract concentration. The third plate was placed for control, which include antibiotic gentamycin as positive control, distilled water as negative control and 4% extract concentration. Plates were incubated at 37°C for 24 h & zone of inhibition measure.

**Cream base formulation**

In this study, oil in water cream was prepared by the addition of oily phase to the aqueous phase with continuous stirring. To prepare the base, aqueous phase consisting of Tween 60 (10%), Citric Acid (0.7%), Sodium Borax (3%) and distilled water (up to 100%) was heated up to 70°C. At the same time, an oily phase that consisted of Paraffin Oil (10%), Beeswax (10%) and Stearic Acid (10%) was heated to the same temperature.

After heating, oily phase was added to the aqueous phase slowly. Stirring was continued until the mixture of oil and water phase homogenous and preservative, Methyl Paraben (0.5%) was added. The mixture of oil phase and water phase were stirred...
until it become semisolid. The finish cream was kept for further stability tests as well as bio-efficacy test.

Preparation of formulation

Aqueous phase consisting of Tween 60 (10%), Citric Acid (0.7%), Sodium Borax (3%) and distilled water (up to 100%) was heated up to 70°C. At the same time, an oily phase that consisted of Paraffin Oil (10%), Beeswax (10%) and Stearic Acid (10%) was heated to the same temperature and then the cinnamon extract (1%) was added to the water phase and being stirred until homogenous.

After both of the water and oily phase are homogenous, oily phase was added to the aqueous phase slowly. Stirring was continued until the mixture of oil and water phase homogenous and preservative, Methyl Paraben (0.5%) was added. The mixture of oil phase and water phase were stir until it become semisolid. Then, the same procedure was done to get cream of concentration of 3% and 4 %. The finish cream was kept for further stability tests as well as bio-efficacy test.

Bio-efficacy test

Agar well diffusion method was used to screen antibacterial activity of cream contain different concentration of *cinnamomum cassia* extract. Negative and positive controls were used. Fucidin cream was used as standard antibacterial drug and base cream was used as the negative control. After media were solidified, holes were made by using 5 mm cork borer. Plates were inoculated with test bacterial strains and creams were place in well, incubated at 37°C for 24 h & Zone of inhibition measured. Each experiment was carried out in triplicate. The zones of inhibition were then recorded in millimeters.

Cream evaluation test

Few tests were conducted in order to evaluate the stability of the cream. Several analysis were conducted such as pH determination, stability test and centrifugation test, and physical analysis. All these tests were conducted to guarantee that the formulation are with the desired properties. Stability tests were performed as well, under different conditions for creams to note the effect of these conditions on the storage of creams. These tests were performed on samples kept at 4°C (cold room), 25°C (in room temperature) and 40°C (in oven). Physical characteristic of cream will be analyze organoleptically (color, homogeneity, appearance and feel) and physically (liquefaction and phase separation) at various intervals for 28 days.

Determination pH of various formulated creams is performed by using digital pH meter. It was measured by direct immersion of the electrode of pH meter in formulated creams. Centrifugal tests are performed for cream immediately after preparation. The centrifugal tests are repeated for cream after 24 hours, 7 days, 14 days, 21 days, and 28 days of preparation. The centrifugal tests are performed at 5000 rpm for 10 minutes by placing 5g of sample in disposable Stoppard centrifugal tubes. The test conducted on the different range of temperature which are 4°C, 27°C and 40°C for specific period of time.

Statistical analyses

Statistical analysis was performed using t-test or one-way analysis of variance (ANOVA). P-values of less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical Tests

The results of the photochemical screening of cinnamon bark extracts (Figure 3), which revealed the presence of tannins compound. The present of tannins were observed when few drops of FeCl3 were added into 5 mL of diluted extract, blackish green color were produce. A change in the color of blackish green indicates that there is present of Tannins.

Phytochemical test Tannins (Figure 4: chemical structure of Tannin) Using FeCl3 1%. Phytochemical test is a qualitative test for the suspected presence of tannin in the extract of cinnamon barks. Phytochemical test conducted in this study that adding extracts with FeCl3 1% reagent indicated by the color change of green or blue-black ink.

In this study, the test for tannin is positive since the color of extract solution changed into blackish green. This result indicates there are present of tannin. Tannins can be used as an antibacterial because it has a phenol group, so if phytochemical with FeCl3 1% test gives a positive result it made possible the samples contained phenolic compounds and possible one of them is tannin. Because tannins are polyphenolic compounds. This was confirmed by Harbourne, classic way to detect simple phenol extract is added to a solution of FeCl3 1% in water, which cause the color green, red, purple, blue and black strong. Formation of green or blue-black ink on the extract after added with FeCl3 1% as tannins will form complexes with Fe16 ions.

Figure 3: Phytochemical screening test of tannins to the cinnamon bark extract

Figure 4: Chemical structure of Tannin
extract being extracted does contain antibacterial properties and can be used to formulate the antibacterial cream.

**Antibacterial Activity**

The extracts were subjected to the antibacterial activity using disc diffusion method. Mueller agar was used to culture the bacteria Staphylococcus aureus. The bacterial suspension was spread uniformly on the solid agar using the cotton swab. Sterile Whatman filter paper disc with diameter of 6mm was impregnated with extract of different concentration. the standard 6mm disc of Gentamycin were used as the positive control and distilled water as a negative control. Antibacterial activity of Methanolic extract of Cinnamon bark via disc diffusion method revealed that there was antibacterial effect of Cinnamon bark extracts against the tested bacteria, S. aureus. The means of inhibition zones was tabulated in table 1.

**Table 1: Means of inhibition zones (mm) of Methanolic extract of Cinnamomum cassia bark against tested bacteria**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Means of inhibition</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
<td>3%</td>
</tr>
<tr>
<td>S. aureus</td>
<td>1.0cm</td>
<td>1.3cm</td>
</tr>
</tbody>
</table>

In comparing the Fucidin cream as positive control to S. aureus, b Fucidin cream had a significantly (p < 0.05) stronger inhibition effect than extract solutions with 45mm zone of inhibition. This could be due to the fact that the Fucidin cream is a pure chemical while the cinnamon extracts were crude extracts. All cinnamon extract solutions were effective against inhibiting the growth of bacteria S. aureus. 4% of extract shows the most zone of inhibition which is 14mm followed by 3% and 1% of extracts with 13mm and 10mm respectively. This supports the reported use of *cinnamomum cassia* in many countries as a traditional herbal medicine.

**Table 2: Means of zones of inhibition of various Formulations against S. aureus**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Means of inhibition (mm)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cream 1%</td>
<td>Cream 3%</td>
</tr>
<tr>
<td>S. aureus</td>
<td>-</td>
<td>11</td>
</tr>
</tbody>
</table>

In comparing the Fucidin cream as positive control to S. aureus, Fucidin cream had a significantly (p < 0.05) stronger inhibition effect than cream base, cream 1%, cream 3% and cream 4%. This could be due to the fact that the Fucidin cream is a pure chemical while the cinnamon extracts were crude extracts. Not all the creams were effective against inhibiting the growth of bacteria S. aureus. Only cream containing 4% or cinnamon extract could inhibit bacterial growth. This show that cinnamon extract can be formulated into antibacterial cream and possess antibacterial activity even after being formulated into creams.

**Cream Bio-Efficacy**

Cream bio-efficacy test of antibacterial activity of cream containing cinnamon bark extract via well diffusion method revealed that there was antibacterial effect of the formulated cream against the tested bacteria S. aureus (see appendix 6). Means on inhibition zones of S. aureus are shown in the Table 2. Only cream 4% has minimum zones of inhibition.

**Table 3: Summary of the obtained result of the phase separation evaluation of the cream for the whole period of 28 days**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Homogeneity</th>
<th>Color</th>
<th>Appearance and feel</th>
</tr>
</thead>
<tbody>
<tr>
<td>cream base</td>
<td>Good</td>
<td>White</td>
<td>Smooth, wet</td>
</tr>
<tr>
<td>Cream 1%</td>
<td>Good</td>
<td>Very light brown</td>
<td>Smooth, wet</td>
</tr>
<tr>
<td>Cream 3%</td>
<td>Good</td>
<td>Brown</td>
<td>Smooth, wet</td>
</tr>
<tr>
<td>Cream 4%</td>
<td>Good</td>
<td>Brown</td>
<td>Smooth, wet</td>
</tr>
</tbody>
</table>

In this study, the base and the formulation were divided into three samples separately and these samples were kept at different storage conditions i.e. at 4°C in refrigerator, at 25°C and at 40°C. These samples under different storage conditions were observed for a period of 28 days at definite time intervals. The samples were observed with respect to change in homogeneity, appearance and feel, color and phase separation. There are no changes in homogeneity of all creams in temperature of 4°C, 25°C and 40°C during the whole observation period of 28 days. As for color, appearance and fell, no change for all creams were noticed in temperature of 4°C and 25°C during the whole observation period of 28 days. There is also no change color, appearance and fell in for cream base in temperature of 40°C during the whole observation period of 28 days. No change in the color of base and formulation at the end of observation periods may be attributed to different factors.
contributing to the cream stability, such as the components of oil phase, i.e. paraffin oil which is a colorless, transparent, tasteless, non-fluorescent liquid. There was changes in color, appearance and fell for the cream 1% which the cream change from very light brown into light brown in day 7 and the appearance and fell become very wet in day 28 at temperature of 40°C. For the cream 3%, the color started to change from brown to dark brown on day 14 and the appearance and fell become very wet and slightly rough at day 21. As for the cream 4%, the color changed from brown to dark brown on day 7 and become darker at day 21. The appearance and fell of cream 4% started to become very wet and slightly rough on day 7 until the end on the study. What we can say here, cream base did not change physically during period of 28 days. But cream 1%, cream 3% and cream 4% have change in color, appearance and fell under the storage of temperature 40°C.

No phase separation was observed in any of the samples kept at 4°C, 25°C and 40°C up to the observation period of 28 days. This indicated that all formulation was relatively stable considering phase separation as a parameter of stability.

**pH Stability in Different Temperature**

The pH of human skin typically ranges from 4.5 to 6.0 and 5.5 are considered to be average pH of the skin. Therefore, the formulations intended for application to skin should have pH close to this range. In this study, the pH of freshly prepared base and cream 1%, cream, 3% and cream 4% was 5.90, 5.98, 5.99 and 5.99, respectively, which is within the range of skin pH.

In the beginning of the study, pH values of the samples of cream base, cream 1%, 3% and 4% were 5.90, 5.98, 5.99 and 5.99, respectively. pH values of all of the formulation in temperature of 4°C, 25°C and 40°C was plotted in the graphs as shown in the Figure 4, Figure 5 and Figure 6 respectively.

![Figure 4: pH of different formulation over time (days) in temperature of 4°C](image)

![Figure 5: pH of different formulation over time (days) in temperature of 25°C](image)
After a week has passed, at temperature of 4°C, the pH value of cream base, cream 1%, cream 3% and cream 4% has increased to 6.15, 6.09, 6.10 and 6.14 respectively. As for temperature of 25°C, pH value has increased 5.96, 6.01, 6.03 and 6.04 respectively and at temperature of 40°C, the pH value has increased to 5.95, 6.07, 6.14 and 6.12.

Further increase in pH were noticed at the end of the study which were at temperature of 4°C, the pH value of cream base, cream 1%, cream 3% and cream 4% has further increased to 6.28, 6.29, 6.34 and 6.36 respectively. As for temperature of 25°C, pH value has increased to 6.00, 6.07, 6.10 and 6.11 respectively and at temperature of 40°C, the pH value has increased to 5.95, 6.07, 6.14 and 6.12. The pH values of all the samples kept under the mentioned storage conditions were found to be increasing gradually in the 1st week until 28th day. The pH values of all formulation are within the range of 5.9 to 6.36 which is within the range of skin pH.

By using two-way analysis of variance (ANOVA) technique at the 5% level of significance, it was found that the change in pH of all formulations were insignificant at different levels of time and temperature of 4°C and 25°C but there was a significant difference in change of pH all samples of formulation at temperature of 40°C. It was concluded that there was insignificant change in pH of all formulations under storage conditions of temperature below 25°C for 28 days. The creams were stable at lower temperature due to increased phase viscosity. It can be suggested that a stable W/O formulation using extract of cinnamon bark can be formulated into stable cream. The extract has antibacterial properties and all formulations were

CONCLUSION

The results of present investigation revealed that the methanolic extract of cinnamon bark has clearly indicated the characteristic of antibacterial properties. The result indicates there are present of tannins which is a phenol group and have properties like alcohol is an antiseptic that can be used as an antimicrobial component. As for microbial assay, all cinnamon extract solutions were effective against inhibiting the growth of bacteria *S. aureus*. Fucidin cream as positive control to *S. aureus*, had a significantly (*p* < 0.05) stronger inhibition effect than all the formulations. Only cream containing 4% or cinnamon extract could inhibit bacterial growth. This is maybe due to poor cream penetration of formulation that contains 1% and 3% of extracts. This show that cinnamon extract can be formulated into antibacterial cream and possess antibacterial activity even after being formulated into cream, pH values were found stable in temperature of 4°C and 25°C. Values of pH of all formulations were found not significantly increasing in the 1st week until 4th week in temperature of 4°C and 25°C with *p*-value >0.05. pH value was significantly increase over time in temperature of 40°C with *p*-value <0.05. pH values of all formulation are within the range of 5.9 to 6.36 which is within the range of skin pH. All formulation had no change in color in temperature of 4°C and 25°C and had change in color under the storage of temperature 40°C except for the cream base. Phase separation was seen for all creams in all temperature during period of 28 days. As for centrifugation test, all creams were stable at temperature of 4°C and 25°C for 28 days. Phase separation were seen in cream base after 28 days and formulation containing 1%, 3%, 4% extract at 21 days at temperature of 40°C. Creams may be more stable at lower temperature due to increased phase viscosity.
stable at temperature below 25°C. Only formulation that contain 4% of extract possess antibacterial properties.

REFERENCES

6. The authentication specimen number of the provided sample is 34h/0098/2015, University Putra Malaysia

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