

Hot Water Extraction of Cinnamon for Formulation of Natural Wound Healing Gel

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Cinnamomum zeylanicum tree bark is rich in cinnamaldehyde, eugenol and camphor, contributing to its medicinal properties, including antimicrobial, anti-inflammation and antioxidant. This research looks into extraction of active compounds from cinnamon barks for formulation of natural wound healing gel. Current extraction processes are found to be time consuming with low productivity. Thus, we proposed a simple hot extraction process. Cinnamon barks were crushed into smaller pieces for Hot Water Extraction (HWE) under different temperatures, extraction times and solid-to-liquid ratio for the optimum yield of cinnamon extract. The extract was sent for Fourier Transform Infrared Spectroscopy (FTIR) to verify the presence of cinnamaldehyde and extract compositions. The gels formulated based on cinnamon extract and cinnamon oil were sent for rheology tests and Disk Diffusion Test with the latter benchmarking the flow properties and antimicrobial performances. When the extraction temperature increases from 60°C to 120°C, the total percentage of cinnamaldehyde increases from 3.01% to 3.08%. When the extraction time increases from 30 minutes to 60 minutes, the total percentage of cinnamaldehyde increases from 3.03% to 3.15%. However, only cinnamon oil gel samples show positive antimicrobial results of 10.3±0.5 mm, suggesting that high extraction temperature and long extraction time might degrades the cinnamaldehyde.

Keywords: Cinnamon Extract, cinnamaldehyde, wound healing gel, hot water extraction (HWE), Disk Diffusion Test

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Cinnamon is a spice commonly used by various cultures over the world for centuries. Cinnamon may be extracted from the inner bark of trees from the genus *Cinnamomum*, which is an evergreen plant containing two main varieties which are *Cinnamomum zeylanicum* (CZ) and *Cinnamomum cassia* (also known as CA). Besides being used in the culinary world, cinnamon is taken as a remedy for respiratory, digestive and gynaecological illnesses in the context of native Ayurvedic medicine [1]. Almost every part of the cinnamon tree ranging from the bark, leaves, flowers, fruit and up till the roots are all useful in terms of medicinal or culinary use. Different parts of the cinnamon tree may contain a significant variation in the chemical composition of the volatile oils extracted, suggesting that oils from different parts of the cinnamon tree might vary in their pharmacological effects [2]. Besides that, CNAD and Eugenol helps to protect the gut from injury from conditions of inflammation, infections and oxidative stress. Furthermore, CNAD has another added value of able to inhibit the growth of harmful fungus and also the mycotoxin contamination of the agricultural commodities. In the case of antimicrobial properties, both CNAD and Eugenol are among the active components against Gram-positive and Gram-negative

bacteria. CNAD can be derived into the form of essential oil, which possesses antifungal and antibacterial properties, or cinnamon extract that possesses antioxidant activity [3].

CZ can be used to extract in the form of the Cinnamon Essential Oil (CMO) or Cinnamaldehyde (CNAD). Previous cases shows that a direct contact to CMO for a long period of time leads to irritation and chemical second-degree burn [4]. From the incident, the involved patient broke a vial of CMO in his pocket without realizing it and left unwashed 2 days, ultimately leads to the chemical second-degree burn. Besides that, CMO has a very strong and pungent smell, which may cause discomfort to certain patients. The CMO can be extracted via many methods, including Steam Distillation (SD), Hydrodistillation and Ultrasonic Extraction (UE). However, SD requires a large amount of energy to generate the steam and it might take up a very long extraction time, from 1 to 5 hours [5]. Moreover, any thermosensitive components may be thermally degraded or hydrolyzed by the water throughout the process [6]. CMO is a hydrophobic volatile and mildly viscous oil [7], which means it can evaporate easily, which may affect its antimicrobial efficacy. Besides that, CMO also has a pungent smell,

which might not be inviting to every user. In addition, the direct contact of CMO to the human's skin may lead to irritation or even chemical burning in some cases. Thus, the use of CMO inside the topical gel might be a concern to the users. A study shows that CNAD, trans- Cinnamaldehyde specifically, is the main component of the CMO [8]. As the main constituent in CMO, CNAD contributes significantly to the antimicrobial effect of CMO [9]. Other studies also suggests that CNAD is the main component that possess antimicrobial effect [10]. Thus, CNAD should be used as an alternative replacement to eliminate the drawbacks of CMO but still retain its antimicrobial efficacy of the topical wound healing gel formulated.

EXPERIMENTAL

Chemicals and Materials

Cinnamon bark, Cinnamon Essential Oil (CEO) and Viscolam were purchased from the suppliers. Distilled water, filter paper discs, MH agar and bacterial cultures (*S. aureus* and *E. coli*) were prepared by UTM IBD Lab. Hot plate, beaker, grinder, thermometer, measuring cylinder, pipette, magnetic stirrer were used locally from UTM CLEAR Lab and UTM IBD Lab.

Preparation of Cinnamon Extract

The cinnamon bark was crushed into smaller pieces using grinder. 5g of the ground cinnamon bark were put into a beaker filled with 50ml of distilled water,

assuming 1g = 1ml distilled water. The amount of ground cinnamon and distilled water were kept constant throughout the extraction process for a constant solid-to-liquid ratio. The beaker was then placed on the hot plate to heat up the mixture of different temperatures. The extraction process was conducted of different temperature and different extraction time in accordance to Table 1. After the extraction process, the sample residue was filtered and left aside for cooling for 10 minutes to remove preliminarily any impurities. The cooled samples of Run 1, Run 4, Run 5 and Run 8 were sent for FTIR analysis at C19 Lab under Faculty of Science UTM. The remaining samples were stored for further formulation of the wound healing gel.

Preparation of Gel of Different Volumes of Viscolam

Another set of preparation was prepared to understand the effect of different volumes of Viscolam on the viscosity of the gel formed. Based on Table 2, different amounts of Viscolam, cinnamon essential oil (CEO) and distilled water were measured by using pipette and measuring cylinder for accurate measurements and then poured into a beaker. The beaker of mixture was homogenized by using a homogenizer at 300 rpm until a smooth and consistent gel was obtained. The mixture was then further agitated with a magnetic stirrer to ensure the uniform mixing of the components. The samples prepared were then sent for rheology tests to understand the shear stress and viscosity of the samples at different shear rates.

Table 1. Operating Parameters of Hot Water Extraction.

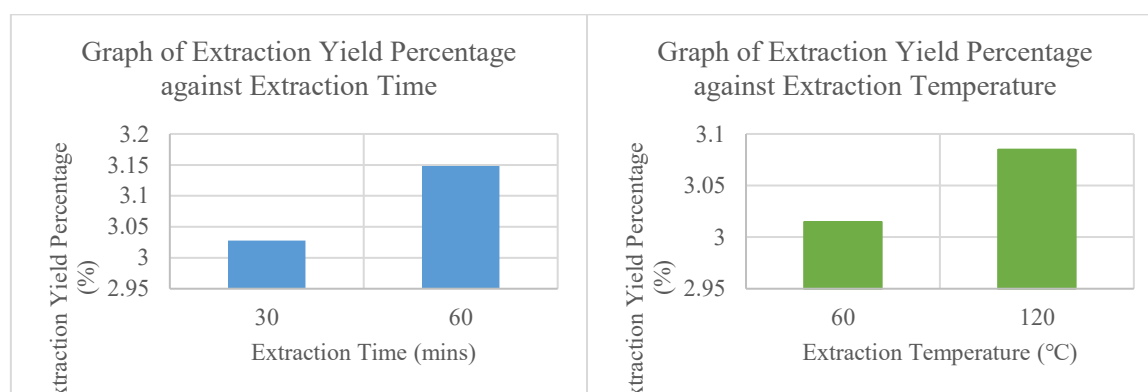
Parameters	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
Solid-to-Liquid Ratio	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10
Temperature (°C)	80	80	80	80	60	80	100	120
Time (mins)	30	40	50	60	45	45	45	45

Table 2. Gel Formulation of Different Viscolam Volumes.

Ingredients	S1	S2	S3	S4	S5	S6	S7	S8	S9
Viscolam (ml)	2	2	2	2	4	4	4	4	2
CEO (ml)	2	4	6	8	2	4	6	8	10
Distilled Water (ml)	96	94	92	90	94	92	90	88	88

Table 3. Detailed Composition of Wound Healing Gel Formulation.

Ingredients	A	B	C	D	E	F	G
Cinnamon Extract (%wt)	2	4	6	8	10	-	-
Cinnamon Essential Oil (%wt)	-	-	-	-	-	2	10
Viscolam (%wt)	2	2	2	2	2	2	2
Distilled Water (%wt)	96	94	92	90	88	96	88

**Figure 1.** Graph of Extraction Yield Percentage against Extraction Time (a, Left) and Extraction Temperature (b, Right).

Preparation of Wound Healing Gel

With reference of Table 3 and a basis of 100 ml, different amount of distilled water was poured into the beaker together with different amount of cinnamon extract and cinnamon oil. Then, the fixed amount of viscolam (approximately 2 ml) was added slowly into the beaker and stirred gently. The mixture was then homogenized with a homogenizer until a uniform mixture was obtained. The gel samples were then sent for disk diffusion test to understand the antimicrobial activity of the wound healing gel samples.

RESULTS AND DISCUSSION

Extraction Yield of Hot Water Extraction of Crushed Cinnamon Bark

Figure 1 shows the extraction yield percentage across different extraction time and extraction temperature of hot water extraction of ground cinnamon bark. Based on Figure 1 (a), it was observed that the extraction yield percentage increases with the extraction time, with 3.0277% at 30 minutes and 3.1483% at 60 minutes of extraction time. The same trend has been observed with the increment of extraction temperature, where the yield is 3.0146% at 60°C and 3.0848% at 120°C. Studies suggested that the

prolonged extraction time allows the progressive release of the cinnamaldehyde from the cinnamon bark matrix, leading to a higher yield since the diffusion of bioactive compounds were enhanced [11]. However, this does not mean that we can bluntly extend the extraction time due to throughput concern and a high temperature might degrades the cinnamaldehyde via hydrolytic degradation due to the long exposure to heat [12].

The same applies to the increase in extraction temperature. A higher temperature leads to a better extraction yield as the solubility and diffusion of the bioactive compounds has been enhanced [13]. Moreover, a higher temperature facilitates the breakdown of the cinnamon barks cell walls, enabling more bioactive compounds to be diffused out into the solvent. However, as mentioned above, a high temperature leads to the degradation of cinnamaldehyde. Thus, it is necessary to conduct a more detailed study to obtain the degradation temperature of the cinnamaldehyde. It can be verified based on the inhibitory area of the filter paper disks soaked with the wound healing gel. The antimicrobial activity of the wound healing gel should function as a preliminary filter whether the cinnamaldehyde was degraded.

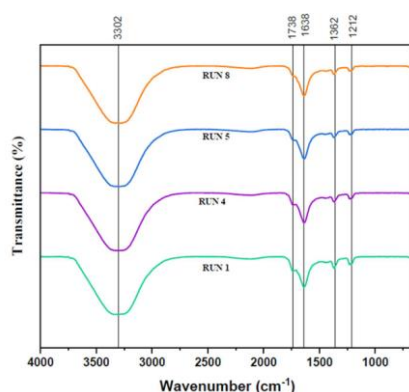


Figure 2. FTIR Results of Gel Samples.

Detailed Composition of Hot Water Extraction Yield Under FTIR

Based on Figure 2, it was observed that a broad absorption band between 3,200 to 3,600 cm^{-1} , indicating the presence of O-H bond stretching, commonly found across alcohols, phenols and carboxylic acids. [7, 11, 14] Next, the region around 3,000 to 3,100 cm^{-1} corresponds to the C-H bond stretching of the sp^2 hybridized carbons, indicating the presence of alkenes or aromatic structures in the sample. This resonates to the expected outcomes of the extract to be full with cinnamaldehyde, giving out a strong aromatic smell. Another peak was observed at the region around 1,700 cm^{-1} suggesting the C=O bond, to further narrow down the extract as ketones,

aldehydes or carboxylic acids. The next peak observed between 1,600 to 1,650 cm^{-1} suggested the presence of C=C bond, which is a common characteristic found in aromatic rings. For the region with a wavenumber of below 1,500 cm^{-1} , which is also known as the fingerprint region, enables the further analysis and confirmation of the extract composition. Two peaks observed between 1,000 and 1,300 cm^{-1} suggests the presence of C-O bond. With the combination of O-H, C=O and C-H bonds, it strongly proves that the composition of the extract contains multiple functional groups due to its structural complexity. Thus, it is deduced that the extract contains aldehydes and phenol, suggesting that it might contain cinnamaldehyde and eugenol, which resonates with the composition of cinnamon essential oil.

Table 4. Viscosity and Shear Stress of Cinnamon Gel.

Ingredients	S1	S2	S3	S4	S5	S6	S7	S8	S9
Viscolam (ml)	2	2	2	2	4	4	4	4	2
CEO (ml)	2	4	6	8	2	4	6	8	10
Distilled Water (ml)	96	94	92	90	94	92	90	88	88
Viscosity (mPa.s) at 50s ⁻¹ Shear Rate	18.6780	9.3639	4.2438	2.7095	39.0120	22.6970	15.5770	2.7120	-
Shear Stress (Pa) at 50s ⁻¹ Shear Rate	0.9339	0.4682	0.2122	0.1355	1.9506	1.1348	0.7788	0.1356	-

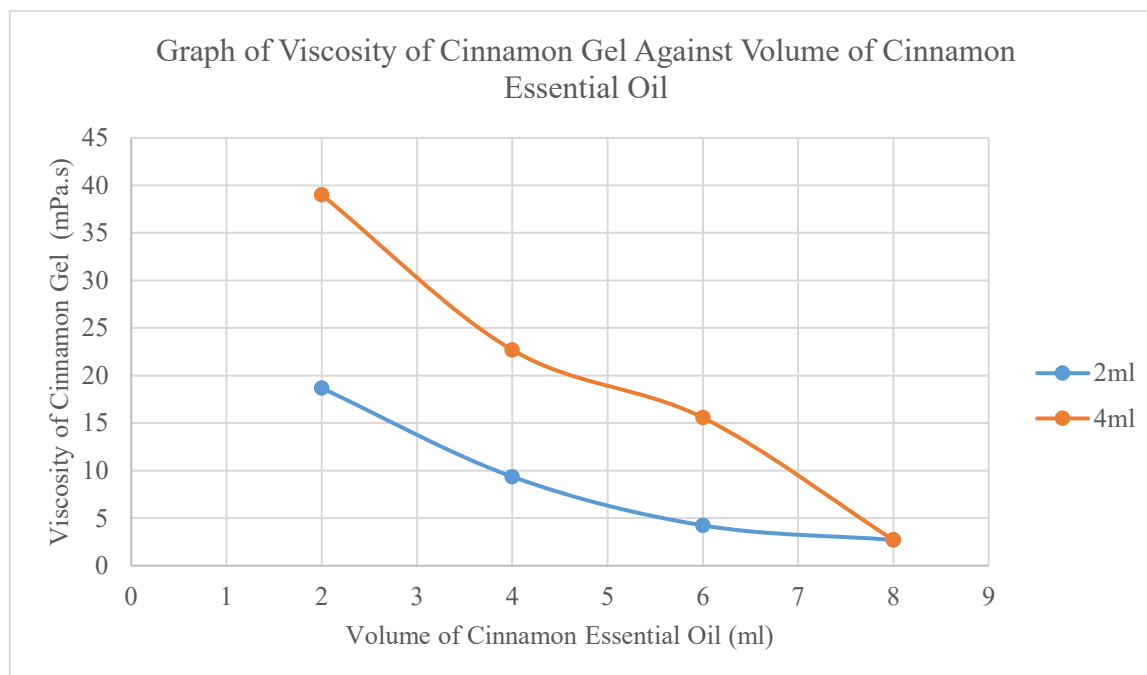


Figure 3. Graph of Viscosity of Cinnamon Gel against Volume of Cinnamon Essential Oil.

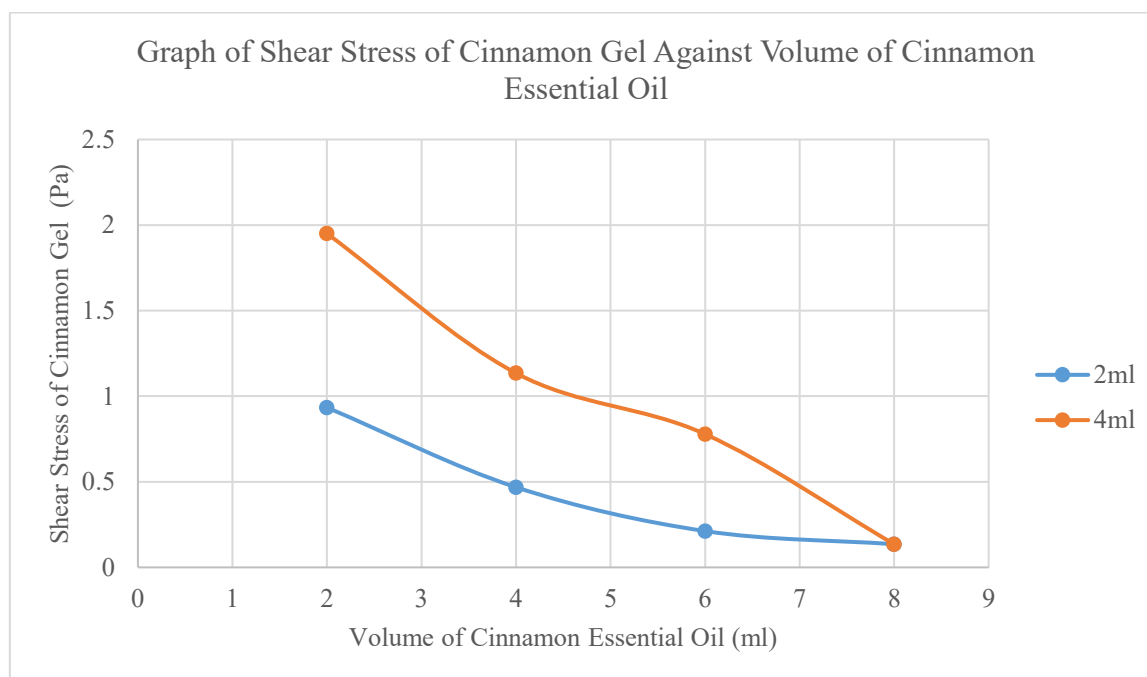


Figure 4. Graph of Shear Stress of Cinnamon Gel against Volume of Cinnamon Essential Oil.

Viscosity Study of Gels Made Up of Different Viscolam Amount

Table 4 and Figure 3 illustrates the relationship between the volume of cinnamon essential oil and viscosity of cinnamon gel at two different amount of viscolam. Table 4 and Figure 4 demonstrates the

relationship between the between the volume of cinnamon essential oil and shear stress of cinnamon gel at two different amount of viscolam. Based on the two graphs plotted, it was observed that both viscosity and shear stress decreases along with the increase of cinnamon essential oil with the amount of viscolam kept constant. With the amount of viscolam, which is

the emulsifier kept constant, the increase in cinnamon essential oil will only “dilute” the wound healing gel, making it easier to flow with a smaller amount of shear stress needed to be applied. Narrowing the scope to only 2ml of cinnamon essential oil, it was observed that with a higher amount of viscolam at 4ml made the gel to be more viscous and thus more shear stress needed to be applied for the gel to flow as a liquid. A conclusion can be drawn here is a higher amount of viscolam leads to higher viscosity,

whereas the higher amount of cinnamon essential needs to have the amount of viscolam to be increased simultaneously to maintain the desirable viscosity for a wound healing gel that can be stored as solid, but able to flow as a liquid when applied a certain amount of shear stress. To further developed a better gel, the gel samples should be sent for additional rheology tests such as amplitude sweep curve and frequency sweep curve tests, to further understand the flow properties.

Table 5. Inhibitory Area of Different Gel Formulation.

Ingredients	A	B	C	D	E	F	G
Cinnamon Extract (%wt)	2	4	6	8	10	-	-
Cinnamon Essential Oil (%wt)	-	-	-	-	-	2	10
Viscolam (%wt)	2	2	2	2	2	2	2
Distilled Water (%wt)	96	94	92	90	88	96	88
Inhibitory Area (mm) $\pm$ SD							
<i>S. aureus</i>	0.0	-	-	-	0.0	8.3 ± 0.5	10.3 ± 0.5
<i>E. coli</i>	0.0	-	-	-	0.0	8.6 ± 0.5	10.3 ± 0.5

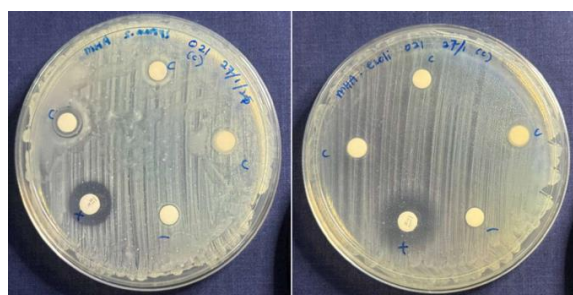


Figure 5. Disk Diffusion Test of Cinnamon Extract Gel against *S. aureus* (Left) and *E. coli* (Right).

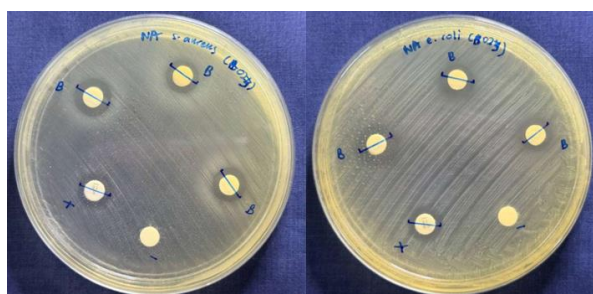


Figure 6. Disk Diffusion Test of Cinnamon Essential Oil Gel against *S. aureus* (Left) and *E. coli* (Right).

Antimicrobial Activity of Wound Healing Gel

Based on Figure 5 and Figure 6, it was observed that the gel made with cinnamon extract exhibits no antimicrobial activity against the two bacterial strains, while the gel made with cinnamon essential oil exhibits antimicrobial activity against the two bacterial strains as there are visible clear circular rings around the filter paper discs, also known as inhibitory zone. The + sign indicates a positive control, which is Streptomycin for *S. aureus* and Gentamicin for *E. coli*. The – sign indicates a negative control employed to ensure that the inhibitory area is not due to the filter paper discs. With the difference observed between Figure 5 and Figure 6, it suggests that cinnamon extract might not have the same antimicrobial activity as compared to cinnamon essential oil. This should be verified by repetitive experiments. If the situation persists, it can be deduced that the cinnamaldehyde and eugenol extracted have been degraded due to the high extraction temperature and long extraction time. Another deduction that can be made is that the high amount of distilled water dilutes the wound healing gel and indirectly reduces the antimicrobial activity of the wound healing gel from cinnamon extract. Lastly, another confirmation tests can be conducted with all the filter paper discs soaked with cinnamon extract gel and cinnamon essential oil gel to be placed together inside one petri dish of bacteria agar to rule out the factor of bacterial culture contamination.

CONCLUSION

This study managed to understand the relationship between different operating parameters and the extraction yield of the hot water extraction of the ground cinnamon bark. At the current stage of study, it is proven that a higher extraction temperature and longer extraction time increases the extraction yield by a tiny margin. The extract obtained is able to show the desired peaks under FTIR to identify the presence of aldehydes and phenols, which are cinnamaldehyde and eugenol respectively. The study also helps to understand that a higher amount of viscolam leads to the formation of a more viscous gel. For the antimicrobial tests, it proves that cinnamon in the form of cinnamon essential oil shows promising results as a wound healing bioactive compounds but the cinnamon extract might not be able to be used as the replacement of the cinnamon essential oil inside the wound healing gel. Thus, more research and studies should be done to further improve the antimicrobial activity and stability of the wound healing gel.

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