



STUDIES ON PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL PROPERTIES OF *Citrullus lanatus* PEELS

(Kajian Jujukan Fitokimia dan Antimikrob Kulit *Citrullus lanatus*)

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Abstract

Citrullus lanatus is one of many species that are categorized under the genus *Citrullus*, which originates from Family Cucurbitaceae, the gourd family. It has been scientifically proven that many members of this family possessed pharmaceutical and medicinal values such as *Cucurbit pepo* (pumpkin), *Citrullus colocynthis* (bitter cucumber) and *Citrullus lanatus* (watermelon) is one of them. The aim of this study is to determine its phytochemical constituents and potential antimicrobial activity of *C. lanatus*, particularly in its peels. The peels were put under sequential extraction process involving two types of solvents, namely, methanol and hexane. *Citrullus lanatus* extracts were then evaluated in terms of its phytochemical constituents, by six qualitative phytochemical screening tests. In assessing *C. lanatus* potential antimicrobial activity, the peels extract were tested on two types of microorganisms, which are *Staphylococcus epidermidis* and *Trichophyton mentagrophytes* by using disk-diffusion method. For both solvents used, phytochemical screening tests verified the presence of alkaloid, flavonoid, saponin, tannin and terpenoid, except for phenolic compounds in the fruit peels. Result of antimicrobial activity revealed that *C. lanatus* peels extract impeded growth to both tested microorganisms. In conclusion, present study suggested that *C. lanatus* can be further developed to utilize its medicinal and pharmaceutical values.

Keywords: antimicrobial, *Citrullus lanatus*, disk-diffusion, phytochemical, sequential extraction

Abstrak

Citrullus lanatus merupakan salah satu daripada banyak spesies di bawah genus *Citrullus* yang berasal dari keluarga Cucurbitaceae, keluarga labu. Ia telah terbukti secara saintifik bahawa banyak ahli keluarga ini memiliki nilai farmaseutikal dan nilai perubatan dan tidak terkecuali *Cucurbit pepo* (labu), *Citrullus colocynthis* (timun pahit) dan *Citrullus lanatus* (tembikai). Tujuan kajian ini adalah untuk menentukan juzuk fitokimia dan potensi aktiviti antimikrob *C. lanatus*, terutamanya kulit. Kulit diekstrak secara berperingkat yang melibatkan dua jenis pelarut iaitu, metanol dan heksana. Ekstrak *C. lanatus* kemudiannya dinilai dari segi juzuk fitokimia, oleh enam ujian saringan fitokimia kualitatif. Dalam menilai *C. lanatus* aktiviti antimikrob yang berpotensi, ekstrak kulit yang telah diuji ke atas dua jenis mikroorganisma iaitu *Staphylococcus epidermidis* dan *Trichophyton mentagrophytes* menggunakan kaedah cakera resapan. Bagi kedua-dua pelarut yang digunakan, ujian saringan fitokimia mengesahkan kehadiran alkaloid, flavonoid, saponin, tanin dan terpenoid kecuali sebatian fenolik dalam kulit buah. Keputusan aktiviti antimikrob mendedahkan bahawa ekstrak kulit *C. lanatus* merencat pertumbuhan kedua-dua mikroorganisma yang diuji. Kesimpulannya, kajian ini mencadangkan bahawa *C. lanatus* mempunyai nilai-nilai perubatan dan farmaseutikal yang boleh dibangunkan.

Keywords: antimikrob, *Citrullus lanatus*, cakera resapan, fitokimia, pengekstrakan secara berperingkat.

Introduction

Medicinal plants or also known as natural remedies have been used since earliest times of mankind history. Although scientifically proven details about the chemical compounds in the plants were not yet discovered at those times, people had widely gained benefits from using the plants as medication. In fact, people from either developed or developing countries around the world are still consuming natural medications until today. In Malaysia, some of the serious ill-inflcted patients have turned to plant-based medications instead of common drugs in the hospital, such as using grape seed extracts to suppress cancer cells. The effectiveness of those extracts against cancer was stated by Sravanthi et al. [1].

Watermelon or scientifically known as *Citrullus lanatus* is among the favourite fruits for many people in the world. It is mainly composed of water, which is about 6% sugar and 92% water by weight. Watermelon belongs to Cucurbitaceae Family under Genus *Citrullus* [2]. Cucurbitaceae is a family that comprises of approximately 120 genera and over 900 species which is vastly distributed in tropical and subtropical regions of, Asia, Australia, Africa and America [3]. Genus *Citrullus* consists of basically four species; *C. colocynthis*, *C. ecirrhosus*, *C. rehnii* and *C. lanatus*. The fruits are usually oblong, ovoid or ellipsoid in shape with elliptical, flattened seeds that are commonly brown or black in colour, and uncommonly white in colour.

Phytochemical constituents or also known as secondary plant metabolites can be defined as non-nutritive chemicals naturally produced by plants. There are thousands of known and unknown phytochemical compounds such as alkaloids, anthocyanins, carotenoids, flavonoids, alkaloids, tannins, monoterpenes, phenolic acids, saponin and terpenoid. These chemicals are responsible in determining their characteristics; the aroma, colour and flavour. However, the main role of these chemicals is as protection and defence for the plants against diseases and damage. Phytochemical compound can be classify into its different bioactive and anti-diseases properties; antibacterial properties are demonstrate by terpenoids, alkaloids and phenolic while anticancer properties are exhibit by carotenoids and flavonoids [4].

Referring to past researches, several members from this family do possessed positive antimicrobial, antifungal and anti-parasite properties. *Cucumis melo* are capable in suppressing both bacteria and fungus [5]. Result from the study has shown that *C. melo* extracts exhibited resistance against several bacteria and fungus. In separate study by Gurudeeban et al. [6], *C. colocynthis* has shown broad-spectrum antimicrobial activity against 16 clinical microorganisms isolated from HIV positive patients. *Cucurbit pepo* seeds extracts, also known as pumpkin, are useful against intestinal parasites [7, 8]. The positive antimicrobial, antifungal and anti-parasite properties are shown in all three representatives sp chosen. Thus, *C. lanatus* which comes from the same family may have the potential to exhibit one of the stated properties.

Citrullus lanatus is one of the species originated from genus *Citrullus*. In Malaysia, it is locally known as "Tembikai Air". *Citrullus lanatus* is being widely studied in previous researches particularly on its fruits and leaves, but only a small scale of them explored into its peels. Thus, this study was aimed to determine the phytochemical constituents and potential antimicrobial activity of *C. lanatus* peels.

Materials and Methods

Plant sample collection

Citrullus lanatus fruits were bought from the nearby fruit stalls and the peels were collected and kept in sterilized container.

Plant sample extraction

The fruit peels were chopped into small pieces so that its surface area can be increased. The fruit peels were then dried in the oven for 4 days. Once dried, they were grounded into powder by using a grinder. The ground material were mixed thoroughly and left soaked in methanol. After that, the solution was filtered by using cloth strainer and paper filter. Later, the filtrate was evaporated using rotary evaporator. Evaporation from rotary evaporator yielded methanol crude extract. The crude extract was collected and stored in a beaker that had first been autoclaved and

was sealed using aluminium foil and parafilm at the cool room to prevent contamination of other microorganism [9]. The peel powder that previously soaked with methanol was then soaked in hexane, a process that is called as sequential extraction. Steps in collecting crude methanol extract were repeated to obtain crude hexane extract.

Qualitative phytochemical screening: Alkaloid determination

Alkaloids presence was determined through Meyer's and Wagner's test. 1 g of powdered sample was mixed with 5 ml methanol and 5 ml of 2M HCl. Then, the filtrate was treated with Meyer's and Wagner's reagent. The presence of alkaloid was denoted by the formation of precipitate [10].

Flavonoid determination

Methanol and hexane extract of *C. lanatus* peels were added with a small piece of magnesium ribbon. Concentrated hydrochloric acid was added to the solution. Changes of colour from orange to red, crimson or magenta indicated the presence of flavonoid [11].

Saponin determination

Saponin presence was determined through froth test. 2 g of powdered sample was boiled in 20 ml of distilled water for 30 minutes. The boiled solution was allowed to cool and then was filtered. 10 ml of the filtrate was added with 5 ml of distilled water. The solution was vigorously shaken for 15 minutes to detect any appearance of froth [12].

Phenolic compounds determination

Phenolic compounds were determined through ferric chloride test. 1.0 g of extract powder was added with 2 ml water and few drops of 10% ferric chloride. Blue and green coloration indicated the presence of phenolic compounds.

Tannin determination

Tannin presence was determined through ferric chloride test. An amount 0.5g of the crude powder extract of sample was boiled in 20 ml of water in a test tube. The solution was then filtered. The filtrate was then added with a few drops of 0.1% ferric chloride. Development of brownish-green and blue-black coloration indicated the presence of tannin.

Terpenoid determination

Terpenoid presence was determined through Salkowski test. An amount 2 ml of chloroform was mixed thoroughly with the sample extract solution. Next, 3 ml of concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids.

Antimicrobial assays: Disk-diffusion method

To test the antimicrobial activity, disk-diffusion method was applied. Firstly, nutrient agar and petri plates were sterilized through autoclaving. An amount 20 ml of agar medium was dispensed into petri plate in sterile conditions in a laminar flow to obtain plates of a uniform depth of 4 mm. The overnight inoculums containing *S. epidermidis* and *T. mentagrophytes* cells/ml were spread onto the surface of the solidified nutrient agar plates. Due to limited crude extract availability, crude extract was measured and used on mg of extract solute per ml of solvent basis.

For methanol crude, antimicrobial discs were impregnated into *C. lanatus* extracts of different concentrations namely 20, 40, 60, 80, 100 mg/ml. For hexane crude extract, only concentration of 20, 40, 60, 80 mg/ml were applied. By using sterile forceps, the discs containing the plant extracts were laid down on the surface of inoculated agar plate. The plates were incubated at 37 °C for 24 hours and the diameter of the zone of inhibition was measured in millimeter. Minimum inhibitory concentration (MIC) can be understood as the lowest concentration of antimicrobial agent at which a microorganism fails to exhibit visible growth on agar plate. Positive control for fungus and bacteria was nystatin and commercial ampicillin respectively. The negative control for fungus and bacteria was methanol and hexane themselves.

Results and Discussion

Qualitative phytochemical screening of *Citrullus lanatus*

Phytochemical test of *C. lanatus* peels, as illustrated in Table 1, shows methanol extract, *C. lanatus* peel yielded positive result to five out of six phytochemical namely alkaloid, flavonoid, saponin, phenolic compounds, tannin and terpenoid. The result also revealed that hexane extract of *C. lanatus*, in general, only yielded positive result from three out of six phytochemicals tests, where all remaining tests had shown negative result. Hexane extract contained alkaloid, saponin, terpenoid but flavonoid, phenolic compounds and tannins were not detected in it. For both methanol and hexane extracts of *C. lanatus*, it can be seen that phenolic compounds were absent in both type of extract.

Table 1. Qualitative phytochemical screening test of *C. lanatus* peel sample

Test	Methanol peel extract	Hexane peel extract
Alkaloid	+	+
Flavonoid	+	-
Saponin	+	+
Phenolic	-	-
Tannin	+	-
Terpenoid	+	+

Note: - = absent; + = present

According to Saxena et al. [4], three phytochemicals that were crucial in combating microorganisms including bacteria and fungus are terpenoids, phenols and alkaloids. Referring to Table 1, though phenols were absent, terpenoids and alkaloids were present in both type of extract. Thus, the presence of antimicrobial activity and positive test for phytochemical in the *C. lanatus* peels were responsible in the emergence of its antifungal and antibacterial property. Flavonoid was mainly functioned in antioxidants [13]. As flavonoid was present in *C. lanatus* peels, it can be expected then that these peels would make antioxidants remedy. Saponins were present in both of the peels extracts hold a pivotal role in killing insects particularly pests [14]. Thus, *C. lanatus* peels may have potential in serving as insecticide. Apart from that, the positive result of tannin as shown in the table may indicate that *C. lanatus* peels can be further explored and studied in terms of the tannin content as the phytochemical is beneficial in antidiarrheal, haemostatic and antihemorrhoidal [15].

Antimicrobial activity test

As depicted in the Table 2, it was generally revealed that both methanol and hexane extracts exhibited antibacterial and antifungal properties [16]. This was relevant as the anti-microorganisms phytochemicals (terpenoids, alkaloids) were present in either of the extract. However, the table has obviously shown that methanol extract of *C. lanatus* exhibited slightly stronger anti-microorganisms properties than hexane extract. The higher concentration of terpenoids and alkaloids in methanol as compared to hexane may possibly explain this situation.

Methanol extract displayed the strongest inhibition zone to *S. epidermidis* under 20 mg/ml of its concentration. The inhibition zone decreased to moderate level of inhibition and was maintained for the next three consecutive concentrations. At the highest concentration, zone of inhibition ceased. It can be therefore stated that the minimum inhibitory concentration (MIC) of methanol extract on *S. epidermidis* was at 20 mg/ml. Meanwhile, in *T. mentagrophytes*, the table exhibited that the zone of inhibition resulted by methanol extract was increasing through applying increasing concentrations of the extract. The MIC of methanol extract on *T. mentagrophytes* was also at 20 mg/ml.

Table 2. Antimicrobial activity of the *C. lanatus* peel extracts

Extract	Concentration (mg/ml)	Zone of Inhibition (mm)	
		SE	TM
Methanol	20	+++	++
	40	++	++
	60	++	++
	80	++	+++
	100	-	+++
Nystatin		-	++
Ampicillin		+++	-
Hexane	20	+	++
	40	+	++
	60	++	-
	80	-	-
Nystatin		-	+++
Ampicillin		++	-

SE: *Staphylococcus epidermidis*;
TM: *Trichophyton mentagrophytes*;
- = no inhibition zone;
+ = weak inhibition zone (≤ 4 mm);
++ = moderate inhibition zone (5-9 mm);
+++ = strong inhibition zone (≥ 10 mm)

Table 2 also portrayed that, for testing upon *S. epidermidis*, hexane extract generally resulted weak inhibition zone when using first two concentrations; 20 mg/ml and 40 mg/ml. At the concentration of 80 mg/ml, the zone of inhibition was absent. It can also be observed on the table that the MIC of hexane extract on the bacteria was 20 mg/ml. When tested on dermatophyte *T. mentagrophytes*, it can be seen from the Table 2 that the zone of inhibition had shown a decreasing trend through increasing of concentration used. The diameter of inhibition zone started at a moderate size and was consistent on the first two concentrations and then ceased for the last two concentrations. The MIC was 20 mg/ml. The antimicrobial properties of both extract suggested to concentration independent which is in others word time dependent. The percentage of time larger than MIC is associated with concentration-independent killing and short post-antifungal effect [17].

Previous study on antimicrobial activity of ethanol (polar solvent) and hexane (non-polar solvent) extracts of *C. lanatus* leaves, stems, fruits and seeds by Rahman et al. [18] can be used as reference point. Methanolic extracts exhibit strong antibacterial and antifungal property compare to hexane extracts. This explains that the phytochemical constituents in the fruits have similarities to that of peels.

Conclusion

Bactericidal and antifungal effects shown by two different extracts of *C. lanatus* peels on two types of microorganisms; a bacteria and a fungal, suggest that *C. lanatus* peels may provide opportunity for utilizing its potential as new herbal drug. Other phytochemicals that present in the peels beside the ones responsible for anti-microorganisms activities may open up a new ground for further studies to explore its other possible potential medicinal values and importance. Apart from stems, leaves, seeds and fruits of *C. lanatus* that displayed therapeutic actions which had been observed in previous studies, it can be concluded from this study that *C. lanatus* peels also possess the same capability.

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