

EXTRACTION OF *LANTANA CAMARA* FOR WOUND HEALING
APPLICATION

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Thesis submitted to the Faculty of Chemical and Natural Resources Engineering in
Partial Fulfillment of the Requirement for the
Degree of Bachelor of Engineering in Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

FEBRUARY, 2013

SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Engineering in Chemical Engineering.

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I hereby declare that the work in this project is my own except for quotations and summaries which have been duly acknowledged. The project has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Dedicated to my parents

ACKNOWLEDGEMENT

In preparing this thesis, I was in contact with many people, technicians, academicians. They have contributed towards my understanding and thoughts. First and foremost, all praise and gratitude to Allah SWT for giving me strength went through loads of difficulties to successfully finishing up my task. In particular, I wish to express my sincere appreciation to my supervisor, Profesor Madya Nordin Bin Endut for valuable encouragement, guidance and critics.

Not to be forgotten, all the lecturers, tutors and teaching engineers of Faculty of Chemical & Natural Resources Engineering (FKKSA) for their support and motivation during this project development, a deep thankfulness for everything and may God bless all of us.

Last but not least, entire family especially my beloved father and mother, Encik Abdul Pattah Bin Abdul Rahman and Puan. Robiah Binti Siraj and family members for their continuous supports for this project. All my fellow friends should also be recognized for their support. Their tips and views are indeed very useful.

ABSTRACT

Lantana camara L. is one of the plants that are central to the lives of traditional societies in India. It has been reported as a traditional folkloric medicine for the variety of diseases. The plant leaves are also used in some parts of India for wound healing, but there are no scientific reports on any wound healing activity of the plant. The aim of this experiment is to investigate the extraction of Lantana camara L. for wound healing application. Phytochemical Screening is used to determine the composition in the extract oil. The wound healing activity of Lantana camara L. Was studied by incorporating the hydro extraction in pure vaselline in a concentration of 5 % and 10% (w/w) and observe the percentage of wound closure in the control and extract treated groups. As a result, the percentage of wound closure will increase due to time on the treated group with 10 % extract compared with 5 % extract while control group takes more time for wound healing activity. This proves the significance studied that Lantana Camara L. can be applied as a wound healing agent.

ABSTRAK

Lantana camara L. adalah salah satu tumbuhan yang penting kepada kehidupan masyarakat tradisional di India. Ia telah dilaporkan sebagai ubat tradisional yang turun temurun digunakan untuk pelbagai penyakit. Daun tumbuhan ini juga digunakan di beberapa bahagian di India untuk penyembuhan luka, tetapi tiada laporan saintifik mengenai apa-apa aktiviti penyembuhan luka dikaitkan dengan tumbuhan ini. Tujuan eksperimen ini adalah untuk mengkaji process mengekstrak *Lantana camara* L. dan kesan terhadap aktiviti penyembuhan luka. Pemeriksaan fitokimia digunakan untuk menentukan komposisi dalam pati minyak. Aktiviti penyembuhan luka oleh *Lantana camara* L. telah dikaji dengan menggabungkan ekstrak dengan vaselline tulen dalam kepekatan 5% dan 10% (w / w) dan pemerhatian terhadap tahap penyembuhan luka dilakukan dengan melihat kadar penutupan luka dalam kumpulan yang dirawat. Hasilnya, peratusan penutupan luka akan meningkat dari semasa ke semasa ke atas kumpulan yang dirawat dengan 10% ekstrak berbanding dengan ekstrak 5% manakala kumpulan kawalan mengambil lebih banyak masa untuk aktiviti penyembuhan luka. Ini membuktikan bahawa *Lantana Camara* L. boleh digunakan sebagai agen penyembuhan luka.

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CHAPTER 1

INTRODUCTION

1.1 Overview of *Lantana camara*

There are several common names of *Lantana camara* L. around the world. According Global Invasive Species Database, different countries used different name such as ach man (Cambodia), angel lips, ayam (Malaysia), big sage, black sage, bunga Tayi (Malaysia), cambara de espinto (Brazil), cuasquito (Nicaragua), flowered sage (Jamaica), lantana, lantana wildtype, largeleaf Lantana (USA), latora Mao (Tahiti), Pha-ka-krong (Thailand), prickly lantana, shrub verbean, supirrosa (Spanish-Galapagos Islands), Wandelroeschen (German), white sage (Trinidad).



Figure 1.1 Flower of *Lantana camara* L. (Naeem *et al.*, 2009)

According to Sonibare and Effiong (2008) there are about 650 species of *Lantana camara* L. spread over 60 countries in the world. This species is a noxious weed belonging to Verbenaceae family that grows in tropical and warm regions worldwide. They are mostly cultivated for their ornamental purpose because of the variety color of flowers. The process helps this plant to spread faster than other herbal plants.

1.1.1 Physical properties of *Lantana camara*

Lantana camara L. from Verbenaceae family is a hardy, evergreen, straggling shrub with characteristic odor, it grows up to 3 m height, with or without minute prickles on the branches. It is among top ten invasive weeds on the earth. It is a perennial shrub found growing up to 2000 m altitude in tropical, subtropical and temperate parts of the world. The plant is spread widely over Himachal Pradesh, Uttarakhand, Uttar Pradesh and north-eastern States of India (Patel *et al.*; 2011).

1.2 Application of *Lantana camara*

Lantana camara L. known as herbal plant that use as treatment for various kinds of human disease. Research by Raina and Parwez (2008) shown that *Lantana camara* L. has abortifacient, antimalarial, anti-inflammatory and wound healing properties. *Lantana camara* is used in herbal medicine for the treatment of skin itches, as an antiseptic for wounds, and externally for leprosy and scabies (Patel *et al.*;

2011). This previous study shown that the plant contains antiseptic for the wound healing component. It is also contributing to one of the new alternative sources to replace other herbal plant.

1.3 Method of extraction

Lantana camara L. Can be extracted using many types of extraction method such as steam, solvent and hydro extraction. Refer to study by Sousa and Almeida (2011), this plant can extract using a hydro extraction process that well- known as hydro extraction. A different method is purpose by Qaisar and Chaunday (2009) that used dichloromethane and methanolic extraction. This solvent extraction contributes to different composition with the previous method.

1.3.1 Hydro Extraction

According from Medical and Aromatic Hand Book, water or hydro distillation is one of the oldest and easiest methods being used for the extraction of essential oils. In this method the plant material is fully dipped in the water. This process suffers from serious drawbacks such as the plant material near the bottom walls of the still comes in direct contact with the fire from the furnace, there is a likelihood of it getting charred and thus imparting an objectionable odor like burning note of the essential oil. Other than that, prolong the action of hot water can cause hydrolysis of some constituents of the essential oils such as esters. The process is

slow and the distillation time is much longer thereby consuming more firewood or fuel.

1.4 Problem Statement

Lantana species are widely spread weeds in warm climates but poisonous to stocks and human. The plant must continuously be suppressed and destroy in certain areas like Australia because it contain toxic in high percentage (Invasive Pest Fact Sheet). The study of *Lantana camara* L. will help to commercialize this plant from being destroyed without any contribution for the human being.

Study by Sagar and Sehgal (2005) claimed that this plant has been reported to be toxic to grazing animals such as cow, buffaloes, sheep and goats. The application of *Lantana camara* L. would help the farmers in order to reduce the possibility of animals died that cause by eating this plant that contains high toxicity.

Studies by Nayak and Raju (2008) shown that the extract sometimes used for the treatment of skin itches, as an antiseptic for wounds and externally for leprosy and scabies. However, there are only data shown that the *Lantana camara* L. has wound healing activity in the traditional way.

1.5 Research Objective

- 1.5.1 To extract the *Lantana camara* L. By using a hydro extraction process.
- 1.5.2 To study the effectiveness of wound healing activity by *Lantana camara* L. extract.

1.6 Research Questions/Hypothesis

There are two research questions that will help to achieve the research objective:

- 1.6.1 What is the method used for the extraction of *Lantana camara* L.?
- 1.6.2 How to measure the effectiveness of extracts for the application of wound healing?

1.7 Scopes of Proposed Study

In order to achieve the objective, the following scopes have been identified and to be applied:

- 1.7.1 Apply a hydro extraction to extract the *Lantana camara*.
- 1.7.2 Use phytochemical method analyzes the compositions in *Lantana camara* oil.
- 1.7.3 Apply the *Lantana camara* oil for wound healing activity.

1.8 Expected Outcome

An expected outcome of the research, there is a new data of wound healing process using extract from *Lantana camara* L.. Other than that, this research contributes to the wound healing application in modern way. In vivo wound healing activity of the extract will be concluded which given the optimum result same as in other herb plants.

1.9 Significance of Proposed Study

Lantana camara L. in wound healing application is contributing to a modern way in this world. There are research that has shown of application of this extract plant in burn healing but not wound healing even it is commonly used in the traditional way. Other than that, extraction of *Lantana camara* L. will give such the new data of chemical compositions according to geographical origin of plant that already proved by Sonibare and Effiong (2008).

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

Lantana camara L. became one of the new plants that use as medicine in the biomedical industry for the successful research year by year. There are four sub-chapters to look through in this chapter. The sub-chapter throughout the synthesis of journals and authentic articles of *Lantana camara* L. in bio-medical product.

2.1 Background of *Lantana camara*

Research by Naeem et al, (2009) shows that *Lantana camara* L. belongs to family *Verbenaceae*. There are 100 genera and nearly 2600 species of this family around the world. The plant consists two common types which are subtropical and tropical according to the condition of plant environment location. The plant is

cultivated as a decorative plant because of various attracting colors of the flower even though it is odor strongly compare as other flowers.

A previous study from Sonibare and Effiong (2008) recorded that about 650 species of *Lantana camara* L. consist around 60 countries in tropical and warm regions worldwide. This comparison of the year shown that this species of flower is spread very fast.

2.1.1 Physical Properties of *Lantana camara*

Refer to Veterinary Medicine Library, *Lantana camara* L. is a species that are grown up to 12 to 18 inches tall. It is different with the South which from Florida to California, it grows as a perennial shrub of 3 to 6 feet tall but in the tropical area, it may grow even taller. The leaves part are opposite, ovate, 1 to 5 inches long and 1 to 2 inches wide with very small rounded teeth, somewhat rough and hairy. Leaves are aromatic when crushed. Then, the flowers part are borne in dense cluster 1 to 2 inches across on the axis near the top of the stem. Each flower is tubular with 4 lobes flaring to about ¼ inch, initially yellow or pink that gradually changing to orange and deep red. Often, the different colored flowers are present in the same cluster. The last part which is the fruit is fleshy, greenish-blue to black and berrylike with each containing one seed.

Other research by Ghibalberti (2000) shown that this species is difficult to classify taxonomically since this species are not stable. It also has a unique characteristic that have widespread hybridization, the shape of inflorescence changes

with age and flower colors varies with age and maturity. This plant also can spread and growing luxuriantly at elevations up to 2000 m in tropical, sub-tropical and temperate regions.

2.1.2 Chemical Properties of *Lantana camara*

A previous study from Sousa and Almeida (2011) shown the result that *Lantana camara* L. consist of many active components that can use for various applications in the medical industry. The active component that contains in leaves consist of sesquiterpenes, a special β -caryophyllene, isocaryophyllene, germacrene D and bicycophyllene. This component use as antiseptic, inflammatory and antibacterial and also practically recovered cut, ulcers and swellings.

Oleanolic acid is a chemical component consist of the root of this plant used as oral drugs that can cure human liver disorders such as anti hyperlipidemias and anti tumor promoting agent. The highest percentage of insecticide component in flower help the flower essential oil promotes the *Aedes* mosquito oviposition.

2.2 *Lantana camara* as Plant-Based Medicine

Eventhough *Lantana camara* L. is highly toxic but this plant contains medicinally valuable. According to Patel and Kumar (2011) shown *Lantana camara* L. having considerable toxicity to human beings that posing a serious threat to human health. This plant also known as a troublesome prickly weed that cause the

death of animal that eat the plant. The toxicity that contains in the root part of the plant make the researcher only focusing experimental for excision studied.

This statement was supported by another study conducted by Bevilacqua (2010) which states that the gender *Lantana camara* L. is composed of 150 pantropical species, used as traditional medicines or as ornamental worldwide. This plant is popular in many applications of sudorific, carminative, antiseptic, antispasmodic and antiemetic.

Study by Sousa and Almeida (2011) shown that *Lantana camara* L. has been claimed to present activities antiprotozoal, antibacterial, antifungal, antioxidant, insecticidal, antiviral and allelopathic properties.

Another study by Patel and Kumar (2011) proved that *Lantana camara* L. used in herbal medicine for the treatment of skin itches, as an antiseptic for wounds, and externally for leprosy and scabies. Major natural products investigated in *Lantana camara* L. belong to the group of triterpinoides, flavonoides and other compounds.

2.3 Wound Healing

Wound healing involves a complex series of interactions between different cell types, cytokine mediators, and the extracellular matrix that occur in different stages. Refer the study by Mackay and Miller (2003), the phases of normal wound

healing include hemostasis, inflammation, proliferation, and remodeling. Each phase of wound healing is distinct according to the type of wounds such as burns or wounds caused by the cuts.

Study by Sasidharan (2010) shown that nearly 6 million people suffer from chronic wounds worldwide. The poor hygienic condition in some third world countries is the main cause of this problem. People from developing countries also suffered from this infected wounds because cannot afford to purchase an expensive modern drugs that might have side effects.

This problem became an inspiring to researchers in order to solve the wound infection by using plant products as an alternative solution. This product is potential agents for wound healing and most preferred because of widespread availability and effectiveness.

2.3.1 Wound Healing Application from *Lantana camara*

Lantana camara L. already use for traditional purposes to cure minor injuries such as cut. They only pounded leaves that contain an antiseptic agent that to be applied to cuts, ulcers and swelling.

According to Raina and Parwez (2008), *Lantana camara* L. is a shrub native of tropical America has completely been naturalized in many parts of India as an ornamental plant. The plant has abortifacient, antimalarial, anti-inflammatory and wound healing properties. The hydro-alcoholic extract and fresh juice of the leaves

have favored wound contraction. This is shown that the plant contains wound healing properties that can be used in medical application.

Research from Ghisalberti (2000) from Western Australia University shown that this plant used in folk medicine for the preparation of bitter tonics, sedatives, febrifuges, cough medicines, remedies for wounds and hypotensives.

Extraction of Lantana oil is sometimes used for the treatment of skin itches, as an antiseptic for wounds and externally for leprosy and scabies. This is because of sesquiterpenes and triterpenes inside the plant that come from a combination of isoprene. This chemical composition use as an antiseptic, antibacterial and anti-inflammatory.

2.4 Conclusion

In this chapter, we were discussing about those *Lantana camara* L. as plant-based medicine, *Lantana camara* L. in wound healing application and chemical component of wound healing essential oil. In the next chapter, we will discuss in detail about the method to produce essential oil and apply as wound healing from *Lantana camara* L.

CHAPTER 3

METHODOLOGY

3.0 Introduction

In this chapter, we are discussing more detail about the method to conduct a research on extraction of wound healing extract oil from *Lantana camara* L. by hydro extraction process. There are four elements that we are focusing on this study which are research design of experiment, materials and methods, wounds healing activity and expected result from this study.

3.1 Research Design

In this study, experiment is set-up through a combination of four stages as shown in Figure 2.1.

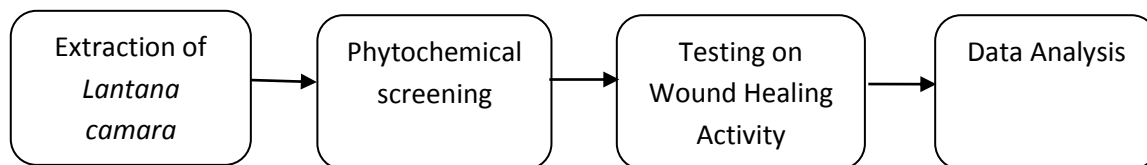


Figure 2.1 Research Design

First part of the experiment includes a hydro extraction process of *Lantana camara* L. by soxhlet apparatus. There is another method of extraction that used chemical solvent known as solvent extraction like methanolic and ethanolic extraction. Hydro extraction is used for this experiment because it is environmentally friendly. There is no chemical involve in the extraction in order to produce essential oil. Other than that, this method is through the production in lower cost and a simple extraction method that commonly use in production of essential oil. Then, extraction part will continue with the separation process to separate oil from water by using a rotary evaporator at a temperature of 115° C. Other separation technique that used in this experiment is by adding anhydrous sodium sulfate, Na₂SO₄ in 6 to 8 hours at room temperature.

After that, extract of *Lantana camara* L. is analyze through phytochemical screening to determine the composition of certain component such as terpernoid, tannins, flavonoid and saponins. Based on the screening result, experiment is proceeding with *in vivo* wound healing activity by using rats that followed a standard procedure from Sasidharan and Nilawaty (2010) that shown the procedure for testing of wound healing activity on rat in different plant species.

Final step has involved data analysis of wound healing activity. All data are recorded based on observation of wound healing activity using rat skin. The 15 rats

became specimens for this experiment will divide into three groups which receive treatment with modified vaselline in different percentage of extract and without it.

3.2 Materials and Methods

3.2.1 Plant Material

Fresh leaves of *Lantana camara* L. will collected from Kg. Chabang, Terengganu for the extraction. The species of *Lantana camara* L. that had been collected is from the Asian Verbenaceae family.

3.2.2 Extraction Process

Samples from fresh leaves that had been estimated around 350 grams will shade dried around 10 days and made into a coarse powder with the mechanical grinder for further use. The powder from *Lantana camara* L. leaves (320 g) will through a hydro extraction process in soxhlet type apparatus for 6 hours according to British Pharmacopoeia (1980) method. The essential oil collected will subsequently dry with anhydrous sodium sulfate (Na_2SO_4) in 6 to 8 hours according to Hand Book On Medical and Aromatic Plants and filtered by using Whatman no. 1 filter paper. Then, it kept refrigerated at below 4°C to be analyzed.

3.2.3 Phytochemical Screening

Chemical tests were carried out on the hydro extract using standardized procedures to identify the constituents as described by Rani (2012) and Mamta (2012).

3.2.3.1 Saponins

The extract (300 mg) was boiled in 5 ml water for 2 minutes. Then the mixture was cooled and mixed vigorously, and left to stand for 3 min. The formation of froth indicates the presence of saponins.

3.2.3.3 Terpenoids

The extract (300 mg) was mixed with 5 ml chloroform and warmed at 80 °C for 30 min. A few drops of concentrated sulfuric acid were added and mixed well into the mixture. The appearance of a red color indicates the presence of terpenoids.

3.2.3.4 Phenolics

Diluted NaOH, followed by diluting HCl, was added to the methanolic extract of the sample residue. The solubility and color change of the mixture were noted. A yellow solution with NaOH, which turns colorless with the addition of diluted HCl, confirms the presence of flavonoids.

3.3 Wound Healing Activity

3.3.1 Crude extract formulation

The 5% (w/w) and 10% (w/w) crude extract of Lantana leaf will be prepared by mixing the extract (1½ g) in pure vaseline (30 g) while another one using (3 g) in pure vaseline (30 g) obtained from a pharmacy.

3.3.2. Animal

Fifteen rats weighing between 150 and 200 g obtained from the Islamic International University Malaysia (IIUM) animal house will be used. The rats will be placed in a room with controlled cycles of 12 h of light and 12 h of darkness where the light went on at 7:00 am. Water and food will be provided to the animals. All the rats will be divided into three groups, namely, the treatment group with 5% extract, 10% extract and the control group. Experiments will be conducted in accordance with the internationally accepted principles of laboratory animal use and care (EEC Directive of 1986; 86/609/EEC).

3.3.3. *In vivo* Wound Healing Activity

The rats will be anesthetized using 45 mg/kg of diethyl ether given by the intraperitoneal route. A full-thickness wound (1.5 × 1.5 cm) will be made on a shaved dorsal area. After 24 h, the wounds will be treated topically with 10% and 5 %

formulated crude extract while the control rats will not treat with any extract for 12 days. The decrease in wound diameters during the healing process will be observed.

3.4 Conclusion

As a conclusion, methodology is the important part in the production of wound healing extracts from *Lantana camara* L. Important to identify whether the chosen method in the experiment is correct or not to make the whole process of production successful.

CHAPTER 4

RESULT AND DISCUSSION

4.0 Results

4.1 Phytochemical Analysis

The phytochemical screening of the hydro extracts of *Lantana camara* L. is depicted in Table 4.1. The phytochemical results reveal the presence of saponins, terpenoid and flavonoids in the extract

Table 4.1. Phytochemical Screening of Secondary Metabolites of *Lantana camara* L.

Secondary metabolites	Hydro extract
Saponin	+
Flavonoid	+
Terpenoid	+

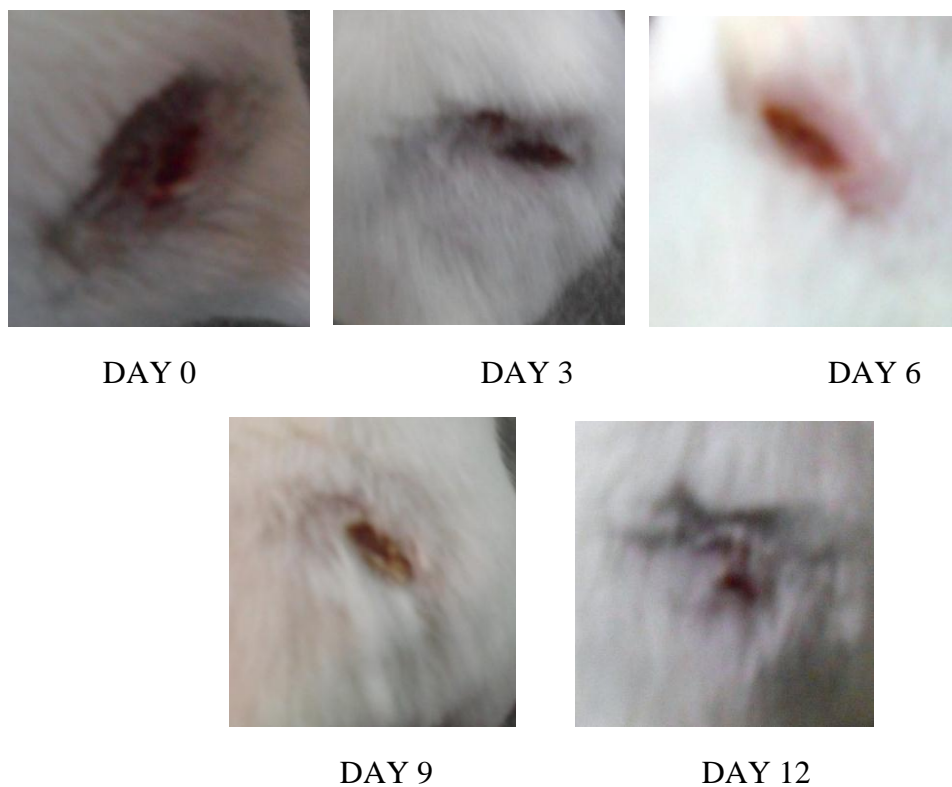
***Note:** present (+) , absent (-)

4.2 Wound Closure Rate

A significant difference in wound closure was observed between the three groups from day 3 onwards. The result based on the observation in later days, the rate of wound closure in the treated group for 10% extract was much faster than that in the treated group for 5% and control group. The wound closure was observed in the group treated with the *Lantana camara* L. leaf extract and control group for 12 days.

Figure 4 (a) shown the wound healing activity on the control group without using *Lantana camara* L. for the treatment. From the observation, it took around 16 days for the fully recovered from the wound.

Figure 4 (a) Photographic representation of the contraction rate on different days in the control group.



On the observation in two treated groups, wound closure rate in the treated group in Figure 4 (c) with 10% extract is faster with 12 days recovery compare with two other groups. The treated group in Figure 4 (b) with 5 % extract recover in 14 days.

Figure 4 (b) Photographic representation of contraction rate on different days in treatment group with 5 % extract.

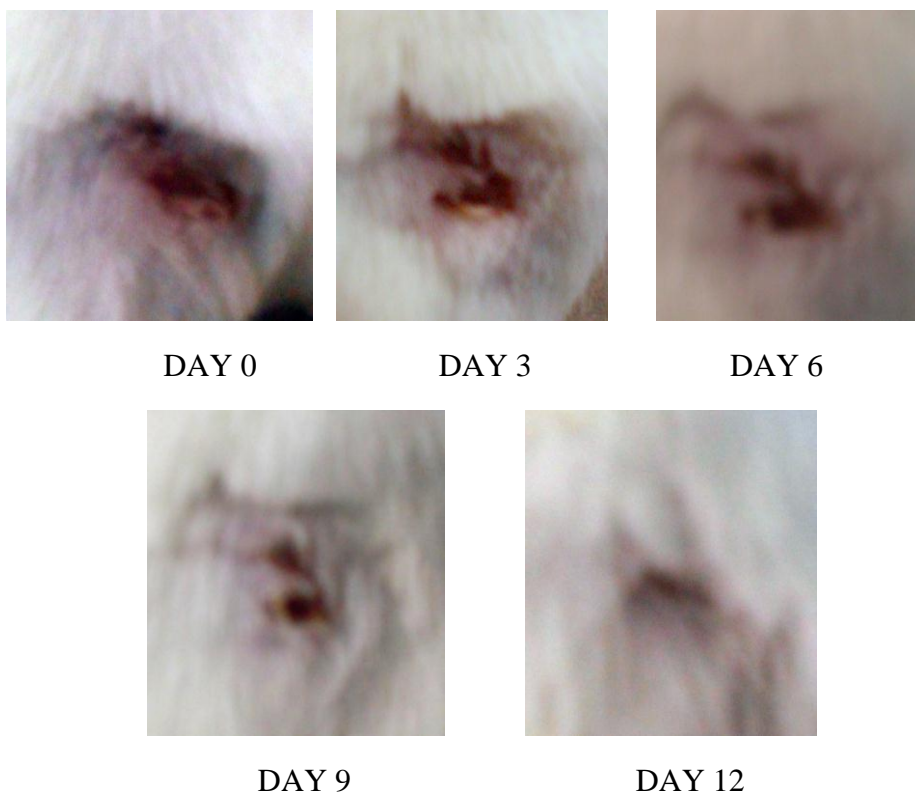
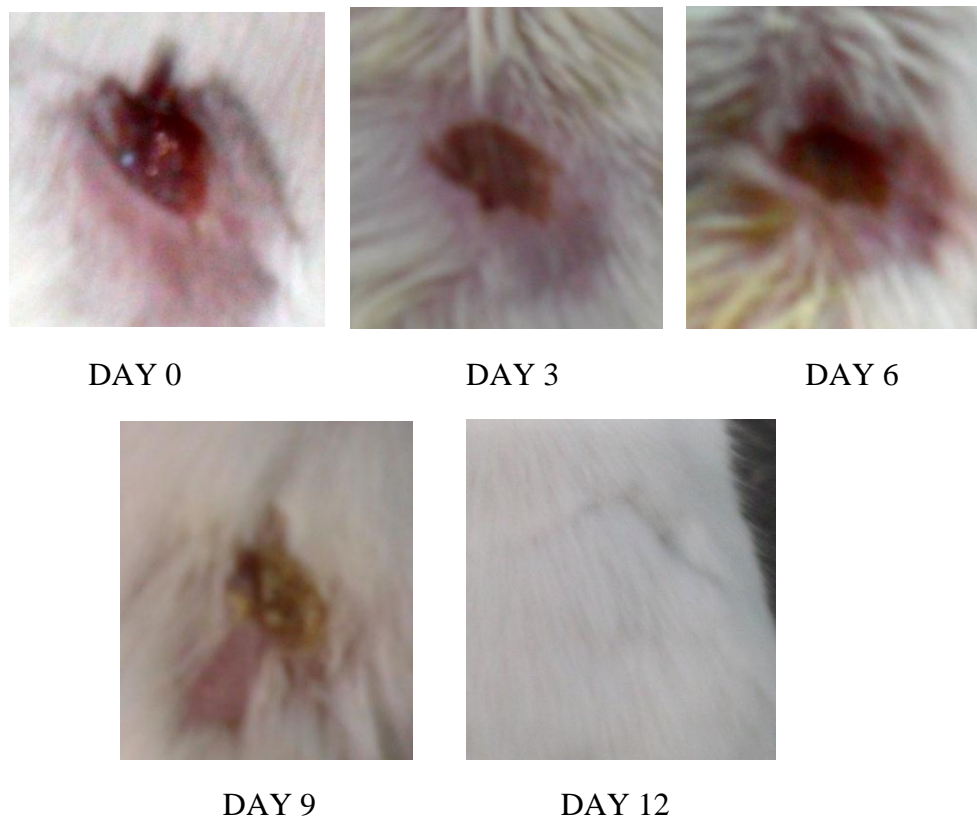


Figure 4 (c) Photographic representation of contraction rate on different days in treatment group with 10% extract.



4.3 Discussion

The present study describes some unique features of the leaf extract of the tropical plant *Lantana camara* L. with respect to its potential wound healing capacity in rats. Plant products are potential wound healing agents, and largely preferred because of their widespread availability. The leaf also absence of unwanted side effects, and effectiveness as crude preparations.

4.3.1 Phytochemical Analysis on *Lantana camara* L. Extract

Earlier it was reported by Sasidharan (2010) that *E. guineensis* is effective in wound healing in rats. Various activities were conducted in this study to evaluate the potential of *Lantana camara* L. as a wound healing agent. One such activity is the phytochemical screening test. The phytochemical results reveal the presence of saponins, terpenoid, and flavonoids in the hydro extraction.

The constituents of the oil leaf extract, such as terpenoids may play a major role in the wound healing process observed in this study. However, further phytochemical studies are needed to isolate the active compounds responsible for these pharmacological activities. This is because of the presence of terpenoid that known to promote the wound healing process, mainly due to their astringent and antimicrobial properties, which seem to be responsible for wound contraction and an increased rate of epithelialization (Scortichini, 1991). Studies with other plant materials also demonstrated the presence of similar phytochemical constituents, which were responsible for promoting wound healing activity in rats (Nayak, 2006).

Other phytochemical activity shown the presence of saponins that help to promote the wound healing by increase the wound closure. Saponins are the glycoside of triterpenes or steroids. It also includes the group of cardiac glycosides and steroidal alkaloids, therefore saponins may be used in traditional medicine as anti-infecting agents (Saxena Mamta *at el.*, 2012).

The flavonoids also present in the testing recognize as antioxidant activity. These phenolic compounds are a class of antioxidant agent which acts as free radical terminator (Rani and Saxena Mamta, 2012). These phytochemical compounds are important in the medicinal value of the plants.

4.3.2 Wound Healing Activity

A significant increase in collagen content due to enhanced migration of fibroblasts and epithelial cells to the wound site was observed during the wound healing process in the treated group (Figure 4 (b) and (c)). Due to the percentage of extract take by the treated group, the higher percentage with 10% extract make the process of wound closure more effective which is 50% recovery in 6 days rather than use in small amount of it (Figure 4 (a)). Clumps of degenerating neutrophils, necrotic changes, and the persistence of inflammatory exudates in the upper dermis with loss of the epidermis were also observed up to day 12. The treated rats with 10% extract showed marked epithelialization better than 5% extract with moderate amount of extracellular matrix synthesis, and new blood vessel formation.

The slow rate of wound closure in the control group might be attributed to the presence of microorganisms and their metabolites, which inhibit wound contraction and deteriorates the wound healing activity (Figures 4 (a)). This condition makes the period of wound closure became long which is 16 days compared with the treated group. The decreased collagen content in the control group might be due to a prolonged inflammatory phase where the degradation of collagen will be greater than its synthesis.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The result shown that the *Lantana camara* L. The extract is applied to a wound by improving the healing activities by the observation in 12 days. The application of hydro extraction of *Lantana camara* L. is expected to improve the phases of wound repair including wound contraction. As the *Lantana camara* L. possesses a wound healing property and its traditionally used in several African and Indian countries, our findings may provide the scientific rationale for the use of *Lantana camara* L. oil to promote healing of wounds .

5.2 Recommendation

The result of the study can be improved by applying a few of recommendation. The portion of extracting oil can be produced in higher quality by

increase the extraction time. In this study, there is only about 2 ml extract oil produce from 250 ml extract during 6 hours of the extraction process. Other than that, the effectiveness of *Lantana camara* L. can be improved by using the commercial product as a comparison. For future recommendation, GC-FID can be applied to the analysis of composition to get a concrete proves of the amount of bioactive molecule present in the extract.

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