FORMULATION OF ANTIBIOTIC OINTMENT FROM THE JATROPHA LATEX CURCAS

KHAFNIZA BINTI KHANAFIAH

Thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering

Faculty of Chemical and Natural Resources Engineering
UNIVERSITY MALAYSIA PAHANG

JANUARY 2012

ABSTRACT

Jatropha curcas is widely used in traditional folk medicine. Every part of the plant has its own advantages in treating diseases. The latex of this plant has strong antimicrobial activity. Secondary metabolites (tannins, saponins and flavonoids) that are present in the latex make it such a good antibacterial agent. It can be used as an antiseptic against cuts and wounds because of the healing effect. Even though the latex has been used traditionally as a plant medicament, scientific investigation including toxicological studies was very limited. Due to this condition, an antibiotic ointment is formulated using the dried latex of Jatropha as the active ingredient. The amounts of the active ingredient used in this experiment are 0.5g, 1.0g and 1.5g. In order to determine whether the cream is well formulated, the stability studies and the antimicrobial study are done. In vitro method is chosen to perform the antimicrobial test. It shows that as the active ingredient is increasing, the zone of inhibition is also increased. The potential of antimicrobial activities in the Jatropha latex is proven. Thus, antibiotic ointment can be used to kill microorganisms and prevent infections. The ointment is also shown good physical stability at room temperature and normal atmospheric condition after one month. Since the Jatropha latex has medicinal properties, it is potentially to be commercialized in industry.

ABSTRAK

Pokok Jarak telah digunakan secara meluas dalam perubatan tradisional. Setiap bahagian pada tumbuhan tersebut mempunyai kelebihan tersendiri dalam merawat pelbagai penyakit. Susu getah tumbuhan ini mempunyai aktiviti antimikrob yang kuat. Metabolit sekunder (tannin, saponin dan flavonoid) yang hadir dalam lateks membuatkan ia ejen antibakteria yang baik. Ia boleh digunakan sebagai antiseptic terhadap luka oleh disebabkan kesan penyambuhannya. Walaupun latexs ini telah digunakan secara tradisional sebagai tumbuhan ubatan, penyiasatan saintifik termasuk kajian toksikologi adalah sangat terhad. Disebabkan keadaan ini, krim antibiotic dirumuskan untuk menggunkan susu getah yg telah kering sebagai bahan aktif. Jumlah bahan aktif yang digunakan dalam eksperimen ini ialah 0.5g, 1.0g dan 1.5g. Untuk menentukan sama ada krim dirumuskan adalah bagus, ujian kestabilan dan ujian antimikrob telah dilakukan. Kaedah in vitro telah dipilih untuk melakukan ujian antimikrob. Ia menunjukkan bahawa semakin banyak bahan aktif yang digunakan, zon perancatan juga semakin meningkat. Potensi aktiviti antimikrob di dalam lateks pokok Jarak terbukti. Jadi, krim antibiotik bolrh digunakan untuk membunuh mikroorganisma dan mencegah jangkitan. Krim ini juga menunjukkan kestabilan fizikal yang baik pada suhu bilik dan pada keadaan atmosfera yang normal selepas satu bulan. Memandangkan lateks pokok Jarak mempunyai ciri-ciri perubatan, ia berpotensi untuk dikomersialkan dalam industri.

TABLE OF CONTENTS

	Page
SUPERVISOR'S DECLARATION	i
CANDIDATE'S DECLARATION	iii
DEDICATION	iv
ACKNOWLEDGEMENT	v
ABSTRACT	vi
ABSTRAK	vii
TABLE OF CONTENTS	viii
LIST OF TABLE	xi
LIST OF FIGURE	xii

CHAPTER 1 INTRODUCTION

1.1	Background of Study	1
1.2	Problem Statement	2
1.3	Objectives	2
1.4	Scope of Study	3
1.5	Rational and Significance	3

CHAPTER 2 LITERATURE REVIEW

2.1	*Jatropha curcas*	4
2.2	Jatropha Latex	7
2.3	Antimicrobial Activity	8
2.4	Wound Healing	10
2.5	Antibiotic Ointment	11

CHAPTER 3 METHODOLOGY

3.1 Apparatus and Equipments 12
3.1.1 Freeze Dryer 12
3.1.2 Analytical balance 13
3.1.3 Water bath 14
3.1.4 Hotplate stirrer 15
3.1.5 Homogenizer 16
3.1.6 Autoclave 17
3.1.7 Laminar air flow 18
3.1.8 Incubator 19
3.1.9 Microcentrifuge 20
3.2 Chemicals 21
3.3 Procedure 22
3.3.1 Collection of Jatropha Latex 22
3.3.2 Preparation of Dried Jatropha 23
3.3.3 Formulation of Antibiotic Ointment 24
3.4 Analysis Procedure 26
3.4.1 Stability Studies 26
3.4.1.1 Physical Stability 26
3.4.1.2 Centrifugation analysis 26
3.4.2 Antimicrobial Study 27
3.4.2.1 Preparation of Nutrient Agar Plates 27
3.4.2.2 Antimicrobial Test 28

CHAPTER 4 RESULTS AND DISCUSSIONS

4.1 Observations 29
4.1.1 Stability Studies 29
4.1.2 Antimicrobial Study 32
4.2 Results 34
4.3 Discussions 35

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions 37
5.2 Recommendations 38

REFERENCES		39
APPENDICES		43
A	Gantt Chart	43
B	Pictures in the Research	46

LIST OF TABLES

Table No.	Title	Page
3.1	The formulation ingredients of antibiotic ointment	21
4.1	The antimicrobial test data	34

LIST OF FIGURES

Figure No.	Title	Page
2.1	The chemical composition of *Jatropha curcas* various parts	5
2.2	The medicinal uses of *Jatropha curcas* various parts	6
3.1	Freeze dryer	12
3.2	Analytical balance	13
3.3	Water bath	14
3.4	Hotplate stirrer	15
3.5	Hand held homogenizer	16
3.6	Autoclave	17
3.7	Laminar air flow cabinet	18
3.8	Microbiological incubator	19
3.9	Microcentrifuge	20
3.10	Jatropha latex collection	22
3.11	Dried Jatropha latex in a Schoot bottle	23
3.12	Solution C at 45°C (ointment base)	25
3.13	Nutrient agar medium with 1.0g of Jatropha powder	28
4.1	The antibiotic ointment after one month	29
4.2	The ointment before the centrifugation analysis	31
4.3	The ointment after the centrifugation analysis	31
4.4	Ointment formulation with 0.5g of dried Jatropha latex	32
4.5	Ointment formulation with 1.0g of dried Jatropha latex	33
4.6	Ointment formulation with 1.5g of dried Jatropha latex	33
4.7	Graph of latex concentration against inhibition zone	35

CHAPTER 1

INTRODUCTION

1.1 Background of study

Jatropha curcas is one of the plants that have several of uses which bring beneficial to the medicinal development. The uses of any parts of the plant such as seeds, roots, leaves, bark, or stems for medicinal purposes has a long practiced outside of conventional medicine. Jatropha becoming one of the up-to-date analysis and research shows their value in the treatment and prevention of disease. The plant had been used for medicinal purposes long before recorded history.

The latex of Jatropha is used as an antiseptic against cuts and wounds. The previous study shows that latex of Jatropha has strong antimicrobial activity. For the medical purpose, the latex can be the active ingredient in formulating antibiotic ointment since it has the potential to kill microorganisms. Secondary metabolites that are present in the latex make it a good antibacterial agent. Antibacterial activity is the ability of a substance to inhibit or kill bacterial cells. Different types of antibiotics agents are being used in the treatment of one form of disease or the other (Reiner, 1984).

For the development, traditional medicine is an important source that has potentials. The antimicrobial researches are conducted to discover the antibacterial and antifungal agents. Medicated ointments contain a medicament dissolved, suspended or emulsified in the base. Ointments are used topically for several purposes, e.g. as protectants, antiseptics, emollients, antipruritic, keratolytics and astringents. Ointment bases are almost always anhydrous and generally contain one or more medicaments in suspension or solution or dispersion.

An antiseptic ointment is intended to destroy or inhibit the growth of bacteria. For the favorable in wound care, medicinal plants have been reported to be very beneficial. They are able to promote the rate of wound healing with minimal pain, discomfort, and scarring to the patient (Odimegwu et al., 2008).

1.2 Problem Statement

Jatropha curcas latex is one of the natural active ingredients that are being used for ointment formulation. There are also other plants and herbals used in ointment production such as aloe vera, sea cucumber, and neem but Jatropha latex has never been done. Moreover, even though the latex has been used traditionally as a plant medicament, scientific investigation including toxicological studies was very limited.

1.3 Objectives

1. To formulate an antibiotic ointment using the dried latex of *Jatropha curcas* as an active ingredient.
2. To study the stability of the antibiotic ointment formulation.
3. To study the antimicrobial activity of the antibiotic ointment.

1.4 Scope of Study

The raw material of this study is the latex of *Jatropha curcas*. A formulation of an antibiotic ointment will be formulated with various weight of Jatropha latex as the active ingredient which is 0.5g, 1.0g and 1.5g. A nicely formulated ointment is stored in an appropriate bottle to evaluate the stability of the ointment by keeping the ointment at different temperature conditions for two months. Centrifugation analysis is done at 10000rpm for 5 minutes. The antimicrobial activity of the latex is determined by using in vitro method which the diameter of the inhibition zone is evaluated.

1.5 Rational and significance

Jatropha curcas has much potential in term of economic significance such as medicinal uses, biodiesel, oil-soap production and plant cultivation. However, above all the products, the product that is being produced commercially is by petrochemical industry. In medicinal purposes, there is not much of studies are being done. The development of antibiotic ointment using Jatropha latex is a new research of study. Since Jatropha has the potential on economic significance, it can be commercialized in medical industry.

CHAPTER 2

LITERATURE REVIEW

2.1 *Jatropha curcas*

Jatropha curcas is widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and South East Asia (Cano-Asseleih, 1986; Cano-Asseleih et al., 1989). The genus of Jatropha belongs to tribe Joannesieae in the Euphorbiaceae family. It has approximately 170 known species. The first to name the physic nut Jatropha L. in "Species Plantarum" was Linnaeus (1753) and this is still valid until today.

The definition of the physic nut is a small tree or large shrub, which can reach a height of three to five meters, but under favorable conditions it can attain a height of 8 or 10m. Jatropha is derived from Greek words which are *jatr'os* (doctor) and *troph'e* (food) (Kumar and Sharma, 2008). It is implied for medicinal uses. Various parts of the plant have its own medicinal values. For examples, its bark contains tannin, and the flowers may attract bees, thus the plant has a honey production potential (Kumar and Sharma, 2008). The chemicals isolated from different parts of the plant are shown in the Figure 2.1.

Table 1 – Chemicals isolated from different parts of the plant

Various parts	Chemical composition	References
Aerial parts	Organic acids (*o* and *p*-coumaric acid, *p*-OH-benzoic acid, protocatechuic acid, resorsilic acid, saponins and tannins	Hemalatha and Radhakrishnaiah (1993)
Stembark	β-Amyrin, β-sitosterol and taraxerol	Mitra et al. (1970)
Leaves	Cyclic triterpenes stigmasterol, stigmast-5-en-3β, 7 β-diol. stigmast-5-en-3β,7α-diol, cholest-5-en-3β,7β-diol, cholest-5-en-3fl,7α-diol, campesterol, β-sitosterol, 7-keto-β-sitosterol as well as the β-D-glucoside of β-sitosterol. Flavonoids apigenin, vitexin, isovitexin	Mitra et al. (1970), Khafagy et al. (1977), Hufford and Oguntimein (1987)
	Leaves also contain the dimer of a triterpene alcohol ($C_{63}H_{117}O_9$) and two flavonoidal glycosides	Khafagy et al. (1977)
Latex	Curcacycline A, a cyclic octapeptide	Van den Berg et al. (1995)
	Curcain (a protease)	Nath and Dutta (1991)
Seeds	Curcin, a lectin	Stirpe et al. (1976)
	Phorbolesters	Adolf et al. (1984), Makkar et al. (1997)
	Esterases (JEA) and Lipase (JEB)	Staubmann et al. (1999)
Kernal and press cake	Phytates, saponins and a trypsine inhibitor	Aregheore et al. (1997), Makkar and Becker (1997), Wink et al. (1997)
Roots	β-Sitosterol and its β-D-glucoside, marmesin, propacin, the curculathyranes A and B and the curcusones A–D. diterpenoids jatrophol and jatropholone A and B, the coumarin tomentin, the coumarino-lignan jatrophin as well as taraxerol	Naengchomnong et al. (1986, 1994)

Figure 2.1: The chemical composition of *Jatropha curcas* various parts

Source: Kumar and Sharma, 2008

Jatropha curcas has thin, often greenish bark which exudes copious amounts of watery sap when cut with dark green , alternate, ovate to slightly lobed leaves with 3-5 indentations. It is a multipurpose shrub, widely cultivated as an ornamental and is considered to have originated in Latin Tropical America but presently grows throughout the arid, semi-arid, tropical and subtropical regions of the world (Hikwa, 1995; Henning, 1996; Makkar et al., 1997).

The roots, stems, leaves, seeds and fruits of *J. curcas* have been widely used in traditional folk medicine in many parts of West Africa. The seeds of *J. curcas* have been used as a purgative, antihelminthic, abortifacient as well as for treating ascites, gout, paralysis, and skin diseases. The seed oil of the plant has been used as an ingredient in the treatment of rheumatic conditions, itch and parasitic skin diseases, and in the treatment of fever, jaundice and gonorrhoea, as a diuretic agent, and a mouth-wash (Esimone CO, Nworu CS, Jackson CL, 2009).

The leaf has been used as a haemostatic agent. All parts of plant are very poisonous, especially the seed, and is known to contain a purgative oil, curcin, a phytotoxin known to cause dehydration, cardiovascular collapse as a result of hemorrhagic gastro-enteritis, and central nervous system depression (Adam, 1974; Ahmed and Adam, 1979; Abdu-Aguye et al., 1986). Uses of various parts of Jatropha in the treatment of disease are presented in Figure 2.2.

Table 3 – Uses of different parts of *J. curcas* in medicines (Heller, 1996; Kaushik and Kumar, 2004)

Plant part used	Diseases
Seeds	To treat arthritis, gout and jaundice
Tender twig/stem	Toothache, gum inflammation, gum bleeding, pyorrhoea
Plant sap	Dermatomucosal diseases
Plant extract	Allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies and small pox
Water extract of branches	HIV, tumor
Plant extract	Wound healing

Figure 2.2: The medicinal uses of *Jatropha curcas* various parts

Source: Kumar and Sharma, 2008

Medicinal plants like *J. curcas* have played a major role in the treatment of various diseases including bacterial and fungal infections. The extracts of many *Jatropha* species including *J. curcas* displayed potent cytotoxic, anti-tumor and anti-microbial activities in different assays. (Arekemase M.O., Kayode R.M.O., Ajiboye A.E., 2011)

It has been documented to have medicinal uses for human and veterinary purposes (Irvine, 1961). The latex combined with the powdered leaves is applied to sluggish wounds while when formulated as enema it is used for the treatment of gonorrhea (Irvine, 1961).

It is also used as an antiseptic against cuts and wounds. The healing effect of curcain a proteolytic enzyme from the latex on wound has been demonstrated (Nath and Dutta, 1992). However, one of the major problems of natural products is instability. Stability of an active substance is the capacity of that substance in a specific container/closure system to remain within its physical, chemical, microbiological and toxicological specification (Linter, 1975).

2.2 Jatropha Latex

Jatropha has traditionally been used for the treatment of various ailments such as skin infections, sexually transmitted diseases like gonorrhoea, as well as for jaundice and fever (Dalziel, 1937; Burkill, 1994; Chopra et al., 1956; Martinez, 1959). The latex is used to treat fungal infections in the mouth, bee and wasp stings and digestive problems of children in Mexico (Schmook and Serralta-Peraza, 1997).

From the research conducted by Oyi et al, 2007, the liquid latex lost all its activity within a week, while the dried latex maintained its activity for up to fourteen weeks period of study. The dried latex lost its activity gradually on exposure to light. Latex also did not possess steroid but it possessed saponins, tannins, alkaloids and glycosides (Arekemase M.O., Kayode R.M.O., Ajiboye A.E., 2011).

The latex is acrid and irritable to the skin (Watt and Breyer-Brandwijk, 1962). Curcain, a protease has been isolated from the latex of *Jatropha curcas* (Nath and Dutta, 1991). Scientific studies carried out have reported that extracts from *Jatropha* species have anti-bacterial, antitumor as well as anti-insect activities (Aiyelaagbe et al., 1998, 2000; Akinpelu et al., 2009).

One of the reported traditional uses of *Jatropha curcas l*eaves and latex is as a haemostatic or styptic: for example, when the latex or the crushed leaf of this plant is applied directly to cuts and bleeding wounds, the bleeding soon stops (Dalziel, 1955; Watt and Breyer-Brandwijk, 1962; Neuwinger, 1996). Furthermore, Villegas et al. (1997) have demonstrated a significant wound-healing activity in *Jatropha curcas* extracts.

The latex has a broad spectrum of antimicrobial activity, because it shows inhibitory activity against Gram positive, Gram negative bacteria and fungi but the activity is higher on bacteria compared to the fungi. The latex showed a very good activity against *Candida albicans* and *Tricophyton* sp. (Oyi et al, 2007). The latex of *J. curcas* also showed antibacterial activity against *Staphylococcus aureus* (Thomas, 1989)

2.3 Antimicrobial Activity

Antimicrobial agents are substances that kill microorganisms or inhibit their growth. They are widely employed to cure bacterial diseases. Antimicrobial agents that reversibly inhibit growth of bacteria are called bacteriostatic whereas those with irreversible lethal action on bacteria are known as bactericidal (Rajesh and Rattan, 2008). Ideally, antimicrobial agents disrupt microbial processes or structures that differ from those of the host. They may damage pathogens by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function, or blocking metabolic pathways through inhibition of key enzymes (Willey *et at.,* 2008).

Haslam *et al.* (1989) reported that plant extracts and their products are used in many parts of the world as the active principles in herb remedies. They are used locally in the treatment of infections, many centuries before scientific studies were discovered. Before an antimicrobial agent is accepted for use in human beings it must demonstrate most, if not all, of the following properties: selective toxicity (it should act on bacteria without damaging the host tissues); it should be bactericidal rather than bacteriostatic; it should be effective against a broad range of bacteria; it should not be allergic; it should remain active in plasma, body fluids etc.; it should be stable and preferably water soluble; desired levels should be reached rapidly and maintained for adequate period of time; it should not give rise to resistance in bacteria; it should have long shelf life; it should not be expensive (Rajesh and Rattan, 2008).

Previous works have shown that many *Jatrophas* species possess antimicrobial activities (Aiyelaagbe, 2001). The medicinal plants that are so used owe their efficacy to a direct action on the wound repair processes, or to the anti-inflammatory and antimicrobial properties (Esimone CO, Nworu CS, Jackson CL, 2009). The action of these plants on microorganisms have been found to be due to the presence of certain substances such as alkaloids, glycosides, volatile oils, gums, tannins, steroids, saponins, phlobatannins, flavonoids and a host of other chemical compounds referred to as secondary metabolites that are present in them (Kochlar, 1986; Sofowora, 1993; Oyagade *et al.*, 1999).

Previous research carried out by Igbinosa et al. (2009) investigated the antimicrobial properties of the crude extracts from the stem bark of *J. curcas* against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. Whereas, antimicrobial activity of *J. curcas* latex against *E. coli* and *S. aureus* have previously been reported (Thomas et al., 2008)

2.4 Wound Healing

Topical antimicrobial therapy is one of the most important methods of wound care (Rodeheaver et al.1980; Veerapur et al., 2004). Some medicinal plants have been employed in folk medicine for wound care (Udupa et al., 1995; Taranalli and Kuppast, 1996; Saha et al., 1997; Carson et al., 1998; Chitra et al., 1998; Veerapur et al., 2004; Rathi et al., 2004). Some of these plants either possess pro-wounding healing activities or exhibit antimicrobial and other related properties which are beneficial in overall wound care.

Wound is defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues (Ayello, 2005). Healing of wound is a biological process that is initiated by trauma and often terminated by scar formation (Rubin and Fabrex, 1996). The process of wound healing occurs in different phases such as coagulation, epithelization, granulation, collegenation and tissue remodeling (Fulzele et al., 2002).The process of wound healing has two components, first is formation of new tissue and other is protections from microbial invasion during the healing process.

Wound healing processes are well organized biochemical and cellular events leading to the growth and regeneration of wounded tissue in a special manner. Healing of wounds is an important biological process involving tissue repairs and regeneration. It involves the activity of an intricate network of blood cells, cytokines, and growth factors which ultimately leads to the restoration to normal condition of the injured skin or tissue (Clark, 1991). The aim of wound care is to promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration (Bowler et al., 2001).

In fokelore medicine, medicinal plants have been used widely in facilitating wound healing with high degree of successes. This has inspired many researches which are aimed at validating the claims and discovering mechanisms which possibly explains the potentials of these herbs on wound repair processes (Esimone CO, Nworu CS, Jackson CL, 2009).

2.5 Antibiotic Ointment

Natural products have been an important resource for the maintenance of life since ages; natural products are becoming increasingly important as alternative medicines. Wither directly as herbal drugs for the treatment of chronic diseases or as raw materials from which more or less complex chemical compounds with particular biological activity are isolated, the deeply held beliefs in the efficiency of natural products can and should be proven by scientific investigation bringing the tradition and experience in the ancient knowledge within the realm of science (Duke et al., 2002).

The treatment and control of diseases by the use of available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries. The intractable problems of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of noble compound to suppress or possibly eradicate the ever increasing problems of emergence of newer diseases though to be brought under control (Wurochekker et al., 2007).

Now at the start of a new millennium, it is estimated by the World Health Organization that, 80% of the world's inhabitants must rely on traditional medicines for health care; these traditional medicines are primarily plant-based (Akah et al, 1998). It is estimated that 25% of all prescriptions dispensed in USA contained a plant extract or active ingredients derived from plants (Goel,1986).

CHAPTER 3

METHODOLOGY

3.1 Apparatus and Equipments

3.1.1 Freeze Dryer

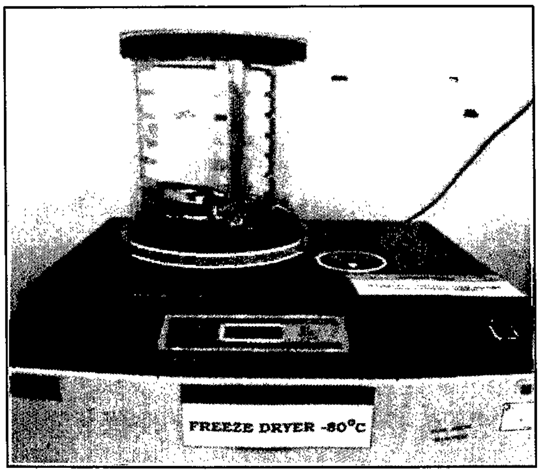

Figure 3.1: Freeze dryer.

Freeze-drying is used to preserve products containing water or solvents. Freeze dryer is used to change the liquid latex into dried latex (powder). The drying process is carried out in a vacuum chamber at pressures that are lower than the water vapour pressure of the frozen latex at their solidification temperature. It takes 1-2 days for all the water contained in the latex is gone.

3.1.2 Analytical Balance

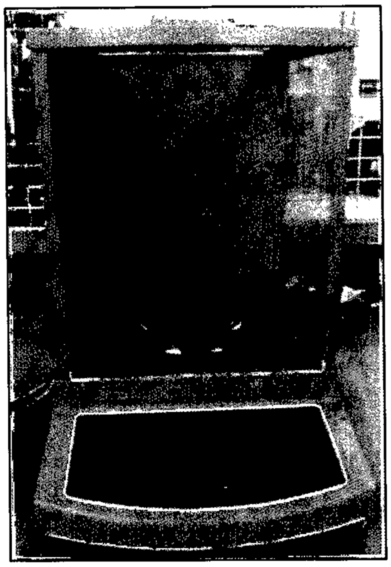

Figure 3.2: Analytical balance.

Analytical balance is used in order to weight all the chemicals involved in the experiment. The four decimal places analytical balance is used so that the measurement of all chemicals is accurate.

3.1.3 Water Bath

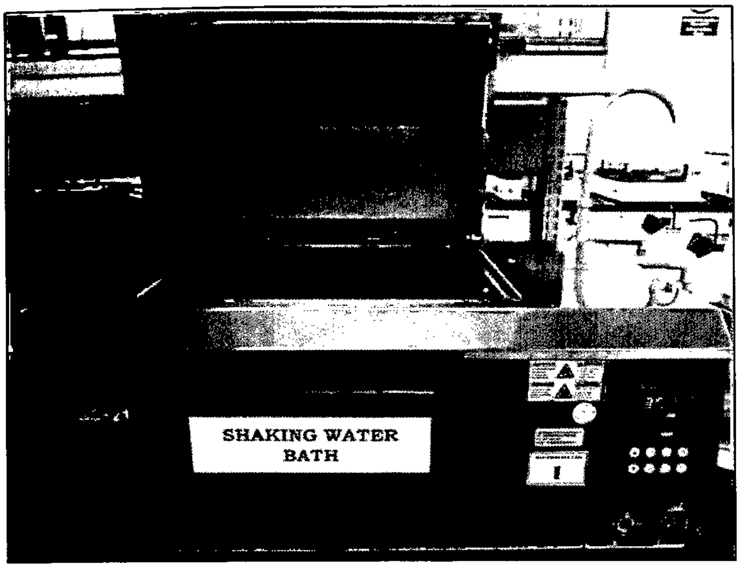

Figure 3.3: Water bath.

Water bath is used to mix all the oil phase ingredients. It must be mix at 70°C. It is also be used to mix the liquid and oil phases mixture at the same temperature. The water bath is used rather than hotplate because the heat distribution is more efficient.

3.1.4 Hotplate Stirrer

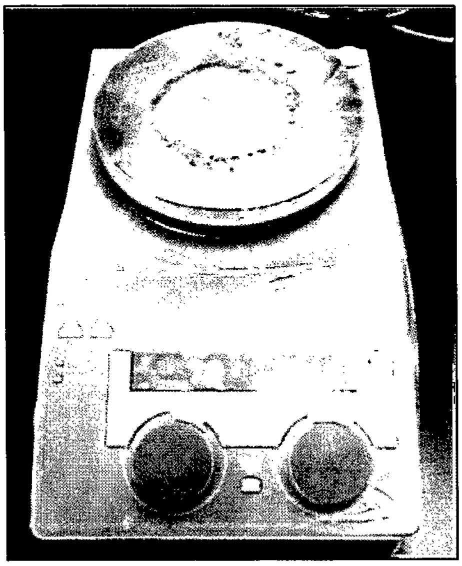

Figure 3.4: Hotplate stirrer.

Hotplate stirrer has two functions. One is to heat up something at a particular temperature and another one is to stir using the magnetic stirrer with a particular speed. In this experiment, the hotplate stirrer is used as to cool down the oil and liquid mixture using magnetic stirrer at certain speed until the temperature down to the range of 30°C-40°C.

3.1.5 Homogenizer

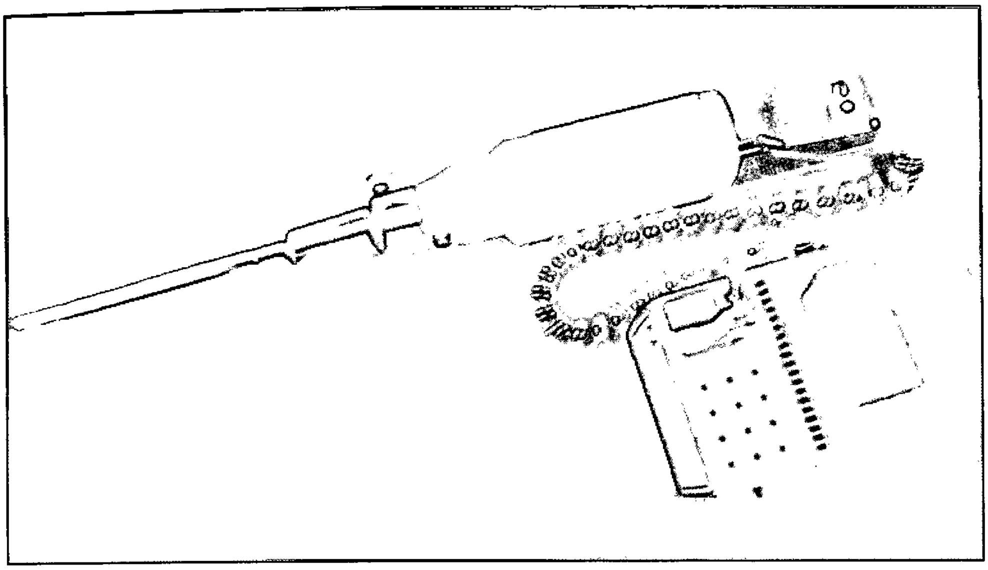

Figure 3.5: Hand held homogenizer.

Homogenizer is used to homogenize the ointment so that all the chemicals used in formulating the ointment is completely bind to each other. It can be said that the ointment is blended, mixed, emulsified, and stirred. When the ointment is homogenized, the ointment will have a better chemical structure.