FORMULATION OF NATURAL NON-TOXIC MOUTHWASH LOTION FROM EXTRACTION OF JATROPHA CURCAS LATEX

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ABSTRACT

Latex from the Jatropha curcas (Euphorbiaceae) has been used traditionally in the medical region. It is can be used to cure many illness including the illness that related to tooth and anti-microbial activity. The Jatropha is actually originated from the central of America. The objective of this research is to formulate the non-toxics mouthwash lotion from the extraction of Jatropha curcas latex. The research is done in order as an improvement to dental health products. Since the Jatropha latex is commonly used in the medicinal region, this mouthwash is formulated as a new development to the uses of Jatropha latex. This formulation is regarding of uses of Jatropha Latex as the raw material, mixing with all the chemicals needed for mouthwash formulation. The latex will act as the active ingredient in this formulation. As for the method of analysis, In Vitro method will be used in order to test and observe the antimicrobial activities. Meanwhile to check the stability of this mouthwash, the stability test will be done. To formulate the mouthwash, various weight of Jatropha curcas latex will be added in the formulation. The various weight added give different result for the antimicrobial test that analyze via the zone of inhibition. The optimum value for the weight is determined by the zone of inhibition. The expected results from this formulation are the mouthwash is stabilizing in room temperature and normal atmospheric condition. This mouthwash is also expected to work for the antimicrobial action and prevent bad breath. As the conclusion, Jatropha curcas latex has very high potential in order to improve the formulation of the mouthwash and other dental health product, but further research need to be done to make sure the product produce is high quality.

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Jatropha curcas is a drought-resistant tree that belonging to the family Euphorbiacea (Suhaili et al., 2011). Jatropha curcas , (Euphorbiaceae) is a shrub , that are widely used in the tropical region. It is actually originated from the cetral of America and was distributed by the Portuguese seafarers via the Cape Verde Islands to the countries in Africa and Asia (Henning., 2000) Different parts of this plant such as leaves, roots, seed, latex, etc have been traditionally used for various purposes including the medicinal purpose. Some of the ethnomedical uses of the extract of Jatropha curcas leaves and roots include use as remedy for cancer, as an abortifacient, antiseptic, diuretic, purgative and haemostatic (Dalziel., 1995). According to Osoniyi and Onajobi (2003), the nut of the plant has also been used traditionally for the treatment of many ailments including burns, convulsions, fever and inflammation. Jatropha latex contains of alkaloids such as white latex of the Jatropha also use as disinfectant in mouth infections in children. The plant grows three and five meters in height and it can be attained a height of ten meters. Other than medicinal purposes, the Jatropha was also popular in the production of the biodiesel.

In this research, the Jatropha latex was mixed in the formulation of the mouthwash to the medicinal purposes. The Jatropha latex, as the other parts of Jatropha plant, also has medicinal effect. It contains tannins and saponin, wax and resin. The white latex serves as a disinfectant in mouth infections in children. It also has been used to cure toothache, as s mouthrinse to treat bleeding gums, treat gum inflammation and

many more. According to De Feo V,1989, the latex is used as a anti-inflammation by massaging the latex on the traumatic area. In his research paper about the plants that had used traditionally in oral care, Ganesan stated that young stem and latex of the Jatropha used as toothbrush, foetid smell and for cure mouth ulcer.

Mouthwash means that a liquid which is oral product that made to freshen the breath. It is also may kill the bacteria in the mouth or whiten the teeth. It was made by combining the appropriate raw materials based on the needs in the formulation. The mouth is home to hundreds of bacterial species that produce several fetid substances as a result of protein degradation (Krespi et al., 2006). The bad breath or a condition called halitosis made the mouthwash is needed to overcome the problem. The bad-breath or halitosis typically occurs upon first awakening or after a meal with garlic and onions.de In generally, the mouthwash comes with three types which are antibacterial products that are used to decrease the population of the bacteria, fluoride mouthwashes that used in help to improve the fluoride layer on tooth enamel. The mouthwash eliminated the bad breath in two ways; first way is they relieve it by killing the bacteria responsible for producing the foul odor and the second way is by masking the odor. The use of antimicrobial mouthwashes as chemotherapeutic adjuncts to mechanical oral hygiene regimens has become well established in dental practice (Mandel., 1988).

Formulation of mouthwash from extraction of Jatropha curcas latex was actually an improvement of the dental products. The mouthwash formulated is intended to function same to other mouthwash invented but with some additional, the medicinal purposes, the mouthwash can cure the illness due to toothache and mouth infections. The Jatropha latex was mixed in the composition of formulation as the active ingredient or can be classified as antibacterial agent. In the conventional mouthwash that has been invented, the example of the antibacterial agents used such as the quarternary ammonium compounds and the substantially saturated aliphatic acyl amides. In this formulation, various weights of Jatropha Latex were mixed with other components to find the maximum effect of inhibition zone in the anti-microbial test.

As for the toxicity, the research that has been carry on, it is shown that the jatropha latex is safe for the use in medicinal. The conjugating and synthetic ability were assessed by total and conjugating bilirubins, total protein and albumin, since all these parameters were not affected as seen from the results, it indicates that the latex is not hepatotoxic (T. Oduola et a.l, 2007).

1.2 Problem Statement

There are a lot of mouthwashes that available in the market nowadays. They are using active ingredient such as menthol, thymol, hexetidine, methyl salicylate, and many more. However, the mouthwash that highly contain of alcohol is found to be risk to the health of the consumer, the side effect that found such as risk of cancer. So some alternatives should be done as a solution and improvement to the product. One of the alternatives is to use the natural ingredient or formulation that involves the extraction of herbal from the medicinal plant. For the herbal mouthwashes, they are using sunflower oil and persica. The research regarding the formulation of mouthwash using the Jatropha latex has never been done. Scientific investigation including toxicological studies about the Jatropha latex was very limited eventhough it was used traditionally since a long time ago. Besides, this formulation also can improve the usage of medicinal plant to commercial product.

1.3 Objective

1) To formulate the mouthwash lotion using the jatropha latex
2) To determine the optimum formulation based on the concentration of latex powder.

1.4 Scope of Study

Raw material used in the study is the Jatropha curcas latex.One of the objectives in this research was to determine the optimum formulation based on the concentration of latex powder, so the latex will be added in various weights. The latex will be converted in the powder form before added in the formulation. Chemicals used in the

formulation are determined based on the basic properties of mouthwash formulation. As for the analysis, the antimicrobial test was done by using disc diffusion susceptibility method. The mouthwash was filled in the bottles and stored at the room temperature and normal atmospheric for two months to do the stability test. Then the antimicrobial test was done one again to check the antimicrobial activity.

1.5 Rational and Significance

Formulation of medicinal non-toxics mouthwash by extraction the Jatropha curacas latex is the new research study. As Jatropha has been use in medicinal field including the dental purposes, so it is high potential to produce a high quality mouthwash product that can fulfill the customers need. Several tests such a industrial and clinical test should be done before it can be marketed.

CHAPTER 2

LITERATURE REVIEW

2.1 Jatropha Curcas

The Euphorbiaceae family comprises approximately 8000 species, belonging to 321 gen-eras (Heller., 1996) which one of them is Jatropha .Jatropha is a multipurpose plant that originated in Central America but can now be found throughout the tropics, including Africa and Asia (Openshaw., 2000). It is also known as physic nut. The genus name Jatropha comes from the Greek iatrós (doctor) and trophé (food) which implies medicinal uses (Heller., 1996). According to Correll and Correll (1982), curcas is the common name for physic nut in Malabar, India.

All parts of Jatropha were used to many purposes, not only in the medicinal field. Fatty acid composition of Jatropha oil is similar to that of oil used for human consumption (Gubitz et al. ,1999). A total of 30–32% of crude protein can be obtained as a cake (Makkar et al., 1997). Apart from being a source of oil, the highly nutritious and economically extendable protein can be used as an animal feed (Aregheore et al., 2003).

Jatropha curcas can be used to prevent and/or control erosion, to reclaim land, grown as a live fence, especially to contain or exclude farm animals and also can be planted as a commercial crop (Openshaw., 2000). Aqueous extracts of physic nut leaves were effective in controlling *Sclerotium* sp., an *Azolla* fungal pathogen (Garcia and Lawas., 1990).

The plant can be raised by seeds or cuttings and reaches its maximum productivity by five years and can live up to 50 years. The plant is a drought resistant species which is widely cultivated in the tropics as a living fence, because it is not browsed by animal (Henning., 2000). The plant also has been used for many applications in around the world. The application and exploitation of the Jatropha plant is presented in the Figure 2.0. In the recent years, Jatropha becomes more popular primarily for the production of biodiesel.

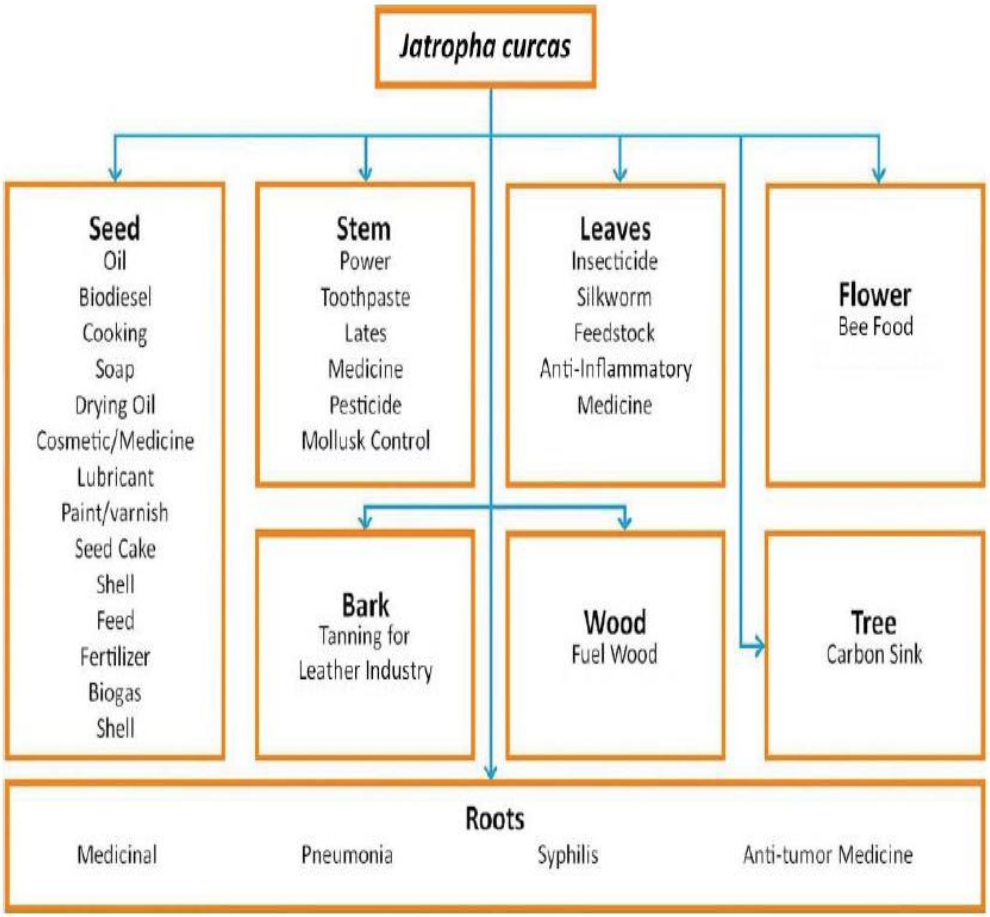

Figure 2.0: Uses of Jatropha curcas plant.
(Nahar and Ozores-Hampton., 2011)

All parts of Jatropha (seeds, leaves and bark) have been used in traditional medicine and for veterinary purposes for a long time (Dalziel, 1955; Duke, 1985b; Duke, 1988). Traditionally, the seeds have been harvested by women and used for medical treatments and local soap production (Duke 1983; Henning 2002).The uses of

various parts of Jatropha in medicinal field are shown in Figure 2.1. From the previous research, it was reported that some compounds (Curcacycline A) with antitumor activities were reportedly found in this plant (Van den Berg et al., 1995). The Curcacycline A was found in the latex. The chemical composition of various part of Jatropha curcas was reported in the paper and presented in the 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 2.1: uses of Jatropha curcas to treat disease

(Ashwani & Sharma.,2008)

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 2.2: Chemicals isolated and found in the various parts of the plant. (Ashwani & Sharma.,2008)

The root, stem, leaves, fruit, seed, bark and latex of the plant are largely used for the treatment of many diseases in different parts world (Rajore and Batra., 2003). One of the application is the oil, according to Heller in the book titled Physic Nut, Jatropha curcas L. , the oil has a strong purgative action and is also widely used for skin diseases and to soothe pain such as that caused by rheumatism. Because the seed is rich in nitrogen, in can be an excellent plant of nutrient source if detoxified (Makkar et al.,1998). The percentage of nitrogen in the seed compared to phosphorus, and potassium present in the Figure 2.3.

Besides that, according to Ochse (1980) " the young leaves may be safely eaten, steamed or stewed". They are also used as favored for cooking with goat meat that it was said to counteract the peculiar smell (Duke., 1983)

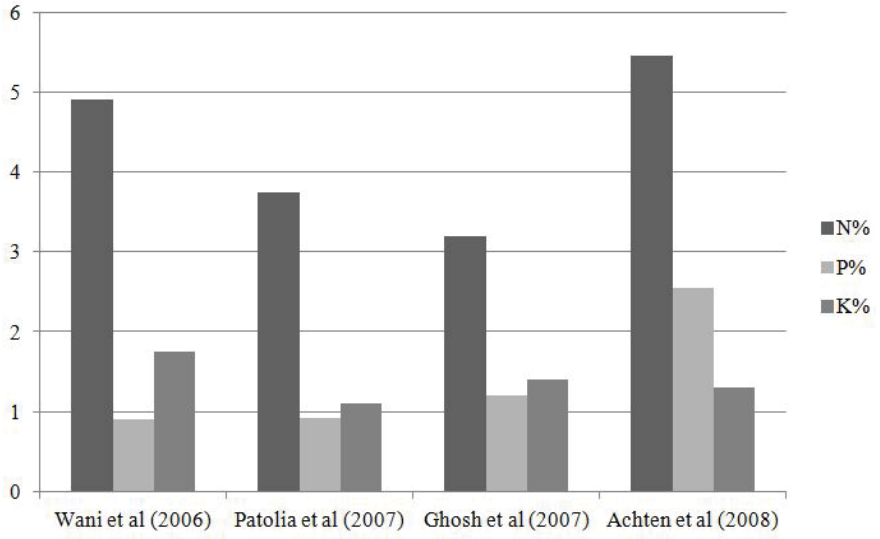

Figure 2.3: Percentage of nitrogen, phosphorus, and potassium in *Jatropha curcas* seed cake (Nahar and Ozores-Hampton., 2011)

Jatropha is being cultivated in 32 countries around the world, including India, Mali, Mexico, Sri Lanka, Nepal, Cambodia, South Africa, Tunisia, China, Bangladesh, Egypt, and the United States (Nahar and Ozores-Hampton., 2011).

As for the crop adaptability, the plant a highly adaptable species and is especially tolerant of severe heat, thrives in warmer weather and susceptible to freeze damage but can tolerate a light frost of relatively short duration (Nahar and Ozores-Hampton., 2011). As mentioned before, the Jatropha has been used centuries ago, the Figure 2.4 below shows about the traditional used and local processing that has been applied.

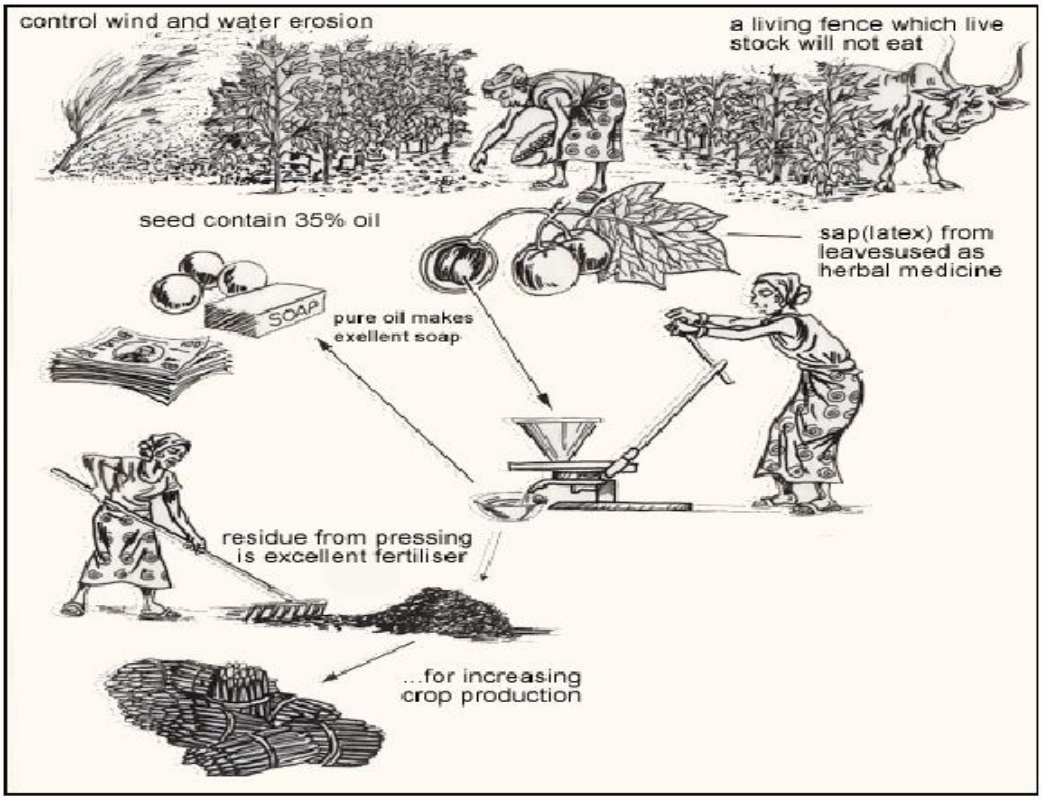

Figure 2.4: traditional used in and local processing of physic nut (J.curcas L.) (Jongschaap et al., 2007)

2.2 Jatropha Latex Poperties

In order to formulate the natural non-toxic mouthwash in this research, the Jatropha curcas latex has been used as the active ingredient. Jatropha latex has been used in the medicinal field traditionally. For the specific composition of the latex, it does contain tannins and saponin, wax and resins. Latex is applied typically to bee and wasp stings (Watt and Breyer-Brandwijk., 1962). In addition, according to Schmook and Serralta-Peraza 1997, the latex is used to treat fungal infections in the mouth, and digestive problems of children in Mexico.

In another research, it was reported that traditional uses of Jatropha curcas latex and leaves is a haemostatic or styptic: for example, when the latex or the crushed leaf of this plant was applied directly to cuts and bleeding wounds, the bleeding soon stops (Dalziel., 1955; Watt and Breyer-Brandwijk., 1962 ; Neuwinger., 1996).

The stem latex has been shown to possess coagulant activity and its mechanism of action as haemostatic agent found to be by precipitation of coagulant factors (Oduola et al., 2005). However, in order to make sure and prove the possible use of Jatropha latex for the formulation of mouthwash or in the dentistry field generally , many experiments should be carry on and the safety level of should be determine.

In the phytochemical screening carried out on the crude latex extract, it revealed the presence of saponins and tannins as had previously been reported on other parts of the plant (Igbinosa et al., 2009; Mishra et al., 2010; Oskoueian et al., 2011). Saponins and tannins, have been reported to possess antimicrobial activity (Diaz et al., 1988; Ogunleye and Ibitoye, 2003; Pretorius et al., 2003; Zakaria et al., 2010). From the present study carried out by Suhaili et al, the potential antimicrobial activity of the *J. curcas* latex extract, which could be attributed the the presence of saponins and tannis.

The plant is insecticidal, antibiotic and was used to cure the toothache and also as blood purifier. To be more focus to the important part of the plant that used in this study, the stem latex of jatropha is previously used by rular dweller, herbalist and peoples in the urban area to stop bleeding from nose, gum and skin. According to

Siregar and Kristiani, in tropical Africa and Southeast Asia the latex is used as a hemostatic and wound dressing and is said to be efficacious in treating scabies, eczema and ringworm, meanwhile in the Philiphine and Indonesia, a little latex on absorbent cotton is used to cure toothache.

In one of the studies about the latex, which is carried out by Osinoyi and Onajobi about the coagulant and anti-coagulant activity of Jatropha latex, they found that the Jatropha shows both the procoagulant and anticoagulant activity which the coagulant activity was being evident at high concentrations (lower dilutions) of the latex meanwhile the anticoagulant activity was evident at low concentrations (higher dilutions) of the latex. The research is very useful because it shows great of medical potential. The effect of Jatropha latex on clotting ime of blood shown in the Figure 2.5.

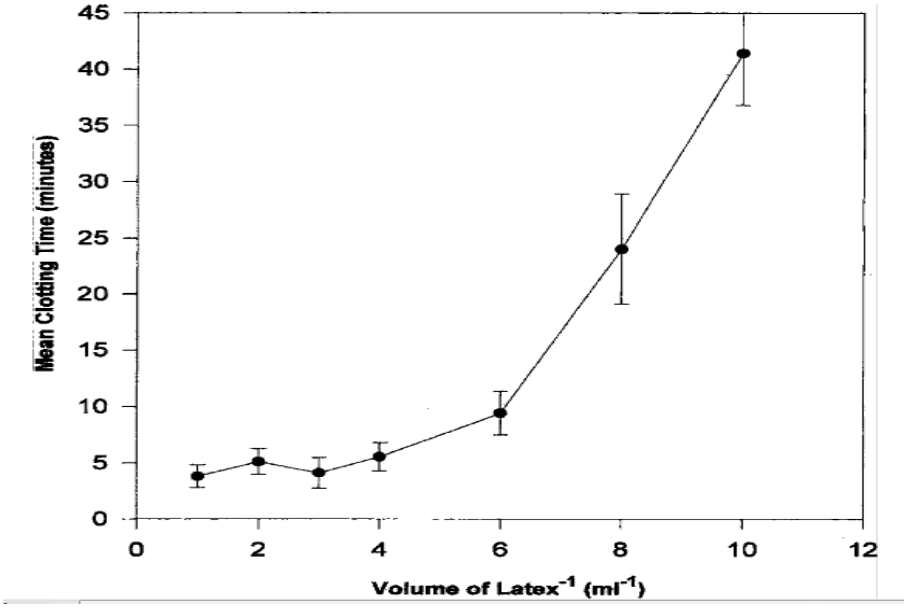

Figure 2.5: Effect of Jatropha curcas latex on the clotting time of blood (Osoniyi and Onajobi.,2003).

Eventhough the latex has been proved the performance and used in medicinal field and purposes, the scientific investigation including the toxicological study is very limited. But with the latest technology that available now, there are a lot of studies and test can be done.

2.2 Mouthwash Properties

Mouth bacteria have been linked to plaque, tooth decay and toothache. Plaque (a layer that forms on the surface of a tooth, principally at its neck; composed of bacteria in an organic matrix) has been linked to gingivitis, periodontal disease, or dental carries (Okpalugo et al,.2009). Neisseria, Staphylococcus, S.pneumoniae, Porphyromonas gingivalis, Diphtheriod, Fusobacteria and Haemophilus are the several types of mouth bacteria, some of useful but some are hurful. Inadequate care or management of the oral health can lead to pathogenic bacteria overgrowth.(Norman and Mosha., 1989). Early morning halitosis or bad breath is associated with oral cavity that is usually the principal source of physiologic malodor .Physiologic oral malodor can be controlled to varying degrees in most individuals by oral hygiene measures, such as tooth brushing, dental prophylaxis, tongue scraping and rinsing with antiseptic mouth washes (Tonzetich,.1977)

Mouthwash is one of the oral products that are available in the market nowadays. Mouthwashes are very useful in reduction of microbial plaques (Salehi and Momeni Danaie., 2006) and some peoples prefer to use it after brushed their teeth. Since years ago, it has experienced much improvement. There are three types of mouthwash that can be purchased: antiseptic, fluoride rinse or combination. In this study, Jatropha curcas latex was added as the new scientific study of the latex used in dental health product.

It is important to make sure the liquid mouthwashes formulated provide a comfortable feeling in the mouth during using, and it must have a pleasant flavor to obtain the customer acceptance. Base on the needs, there are some basic properties to formulate the mouthwash such as flavoring agent, solubilizer, preservatives, water, colorant and special ingredient that known as active ingredient. The active ingredient is commonly acts as the main ingredient called antibacterial agent.

Flavor in as important features that look by the customers or users, thus it is important to a mouthwash to have a very pleasant flavor. The examples of the flavors used in the formulation were, peppermint oil, menthol and methyl salicylate.

Another property is preservatives. The function of preservatives in the formulation is to prevent the growth of micro-organism in the mouthwash. The preservatives used must be safe to the users which are non-irritating, compatible with other components, and it should be used in combination. Examples of preservatives are sodium benzoate, methylparaben, and propylparaben.

All the properties was mixed base on the appropriate composition in the basic formulation method of mouthwash. All the components must be well mix in order to formulate a stable mouthwash, the stability and anti-microbial test was done to check the stability and effectiveness of the mouthwash. The very important property of the formulation was the active ingredients or anti-bacterial agents that played the main role as the medicine part in the mouthwash formulation, which to treat the mouth and teeth.

In the conventional mouthwashes that are available nowadays, the alcohol was most used as the active ingredient and antibacterial agent. But in the heavy consumed of alcohol will caused side effect to the health. For example, in a study conducted by Wynder and colleagues, they found a significant association between mouthwash use and oral cancer. According to Reilly and colleagues, the effect of varying alcohol content of mouthwashes has also been studied and significantly raised odds ratios for oral cancer, 1.4 in men and 1.6 in women, were reported in regular mouthwash users, after adjustment for tobacco, alcohol, education and other factors.

In this study, natural Jatropha latex will be used to make it more safe and environmental friendly. In the previous investigation it was reported the anti-parastic activity of the sap and crushed leaves of Jatropha. The latex has been proved to be effective to the antimicrobial activity, but there was insufficient information and study regarding the effectiveness of the mouthwash that includes the Jatropha curcas latex as the active ingredient and make the mouthwash formulated is a herbal and natural mouthwash that applying the extraction of latex from the medicinal plant. The sap also

showed the antimicrobial activity by inhibiting the growth of Candidia albicans and Staphylococcus. Recently, the use of the herbal mouthwash such as persica is increasing (Salehi and Momeni Danaie., 2006).

It is very important to the consumer to choose the right product of the oral care to avoid any affections and disease. A good mouthwash must be a combination of safe ingredients based on their properties. From the previous studies, it was advised that consumers to avoid all bacteria that make antibacterial claim unless there are herbs are being used in the formulation.

CHAPTER 3

METHODOLOGY

3.1 Raw Materials and ingredient

The raw materials in this experiment is the Jatropha Latex mixed in various weight, and the other ingredients in determine by the basic properties that must be added to formulate the mouthwash. The total 100ml of liquid mouthwash formulated with the combination all the components and it can divided into three phases A, B, and D. All the chemicals used in the formulation based on their phase presents in the Table 3.

Table 3.0: Mouthwash Formulation Ingredients

PHASE A	
Ingredients	Quantity used
Peppermint oil	0.125g
Tween 20	0.706g
Deionized water	1.5ml
PHASE B	
Propyl paraben	0.014g
Methyl paraben	0.1176g
water	50ml
sorbitol	12g
PHASE C	
Benzyl alkaniumchloride	0.01g
PHASE D	
Active ingredient	Various weight

3.2 The apparatus used:

3.2.1 Hot Plate

Figure 3.1: Hot Plate

Hot plate is used to heat up several ingredients in the formulation which are the ingredient in phase B. The temperature while heating should be 50°C. To get more homogenize mixture of the components, the magnetic stirrer was used during the process of heating.

3.2.2 Incubator

Figure 3.2: Incubator

When doing the in vitro method in anti-microbial analysis, nutrient agar was prepared using the nutrient broth and pours about 20ml into the petri dish. In this process, sterile petri dishes were used to inoculate the formulation on the mouthwash and the incubator will be use in order to control the temperature for the cultivation of bacteria. The temperature for the incubator was 37°C.