MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Microbiology and Biological Sciences (ISSN: 2408-7076) Vol. 6(9) pp. 108-117, November, 2018
Available online http://www.meritresearchjournals.org/mbs/index.htm

Original Research Article

Development of Antibacterial Cream from Acetone Extract of Matured Bark of Philippine *Tamarindus indica* L., (Leguminosae)

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Abstract

***Tamarindus indica* L., has various medicinal properties, among which is the antibacterial property. However scientific evidence that deals with preformulation and development into a suitable pharmaceutical dosage form is lacking. Therefore in order to fill this knowledge gap, this study aims to develop an antibacterial cream from the matured bark extract of Tamarind as an alternative medication to current synthetic brands. An experimental research design was used. Powdered bark was extracted using soxhlet method wherein 70% acetone was used as solvent. Physicochemical properties of extract were determined using the methodologies of USP and microbial assay were conducted using the paper disc diffusion method. Results revealed that plant extract had the following characteristics : dark brown crystalline powder with tamarind odor; bitter in taste; pH 8.38; density 0.09 g/mL; freely soluble in water, slightly soluble in (80 %v/v) ethanol, sparingly soluble in ether and very slightly soluble in chloroform; compatible with excipients such as methyl paraben (preservative), propyl paraben (preservative), sodium lauryl sulfate (emulsifying agent), stearyl alcohol (stiffening agent), white petrolatum (oleaginous base) and water (dissolving agent) ; not irritating when tested to rabbits and susceptible to *Staphylococcus aureus, Corynebacterium minutissimum* and *Streptococcus spp.* An antibacterial cream was manufactured using the mechanical incorporation and fusion method and passed the microbial, sensitivity and antibacterial tests. Based on the results presented, Tamarind bark can be developed into an antibacterial cream but a further study like long term stability testing is needed in order to determine its stability and shelf life.**

Keywords: Development, Antibacterial cream, Stem bark, *Tamarindus indica* L., (Leguminosae)

INTRODUCTION

Tamarindus indica L., belongs to the Family Leguminosae / Fabaceae (Kalia, 2005; Rummel, 2005; Garcia et al., 2003 ; Evans, 2002 ; Madulid, 2001 ; Tyler et al.,1988 ; Santos et al., 1981 and Quisumbing, 1978). It is commonly known in Myanmar as Beng-kong, Magyeng, Magyi, Mai-kyaing, Mak-k yeng, Manglon and Tamarind (Kress et al., 2003). In India it is known as Amlika, Amli, Imli, Ambli, Puliyan and Ambala (Anjaria et al., 2002). In Arabic it is known as Tamarindi (Quisumbing 1978). In the Philippines, the plant is populary called sampalok (Tagalog); asam (Sul., Tausug); kalamagi (Bisayan, Ibanag); kalamayi (Gaddang); onga kayo a masum (Maranaw); sala-magi (Ilocano, Pangasinan); salomagi (Ilocano); salumagi

(Hanunuo, Ilokano, Pangasinan); salunagi (Tinggian); sambag (Bisayan, Cebu; Bisayan, Panay; Butuanon, Maranaw); sambagi, amba (Bisayan); sambak, sambalagi (Bikol); sampag (Tausug); sibukan (Mansaka); tamarindo (Span.) (Rummel, 2005 ; Garcia et. al., 2003; Madulid, 2001 and Quisumbing, 1978).

Tamarind is a slow growing, long – lived, massive tree that reaches a height of 24 – 30 m, under favorable conditions, and may attain a spread of 12 m and a trunk circumference of 7.5 m. It is highly wind–resistant, with strong, supple branches, gracefully drooping at the ends, and has dark– gray, rough, fissured bark. The mass of bright–green, fine, feathery foliage is composed of pinnate leaves, 7.5 – 15 cm in length, each having 10 – 20 pairs of oblong leaflets 1.25 – 2.5 cm long and 5 – 6 mm wide, which fold at night. The leaves are normally evergreen but may be shed briefly in very dry areas during the hot season. Inconspicuous, 2.5 cm wide flowers, borne in small racemes, are 5 – petalled (2 reduced to bristles), yellow with orange or red streaks. The flower buds are distinctly pink due to the outer color of the 4 sepals, which are shed when the flower opens. The fruits, flattish, beanlike, irregularly curved and bulged pods, are borne in great abundance along the new branches and usually vary from 2 – 7 inches long and from 2 – 3.2 cm in diameter. One to twelve fully formed seeds are hard and glossy – brown squared, It is 1.1 – 1.25 cm in diameter and each is enclosed in a parchment like membranes. Native to tropical Africa, the tree grows wild throughout the Sudan and is so long ago introduced into and adopted in India that it has often been reported as indigenous. It is widely distributed from Africa to India and the Philippines and commonly cultivated. It flowers from April – October (Rummel, 2005 ; Evans, 2002 Tyler et al., 1988 ; Quisumbing 1978).

Tamarindus indica Linn., (Tamarind) is reported to have been used as refrigerants in fevers and as laxatives and carminatives, alone or in combination with lime juice, honey, milk, dates, spices or camphor (Quisumbing 1978). The parts of the plant, except for the roots are shown to have many medicinal uses. It is noteworthy that an antimicrobial property is manifested in almost all parts of the plant.

Tamarind plant extract is not only for medicinal use, it is also used in food industry. Several food preparations contain tamarind leaf extract such as canned beverages and soft drinks wherein it is used as a replacement for chemical acidulants and in preparations of tamarind wine. The active metabolites such as flavonoids, C- glycosides (vitexin, isovitexin, orientin and isoorientin), malic acid, tannins, tartaric acid and vitaxin are possibly responsible for such properties.

Tannins have been identified as the active metabolite from the bark of tamarind. Tannins are used in dermatological preparations for its astringent property. Evans (2002) described tannins as complex substances that usually occur as mixtures of polyphenols that are difficult to separate because they do not crystallize. They are generally subdivided into two groups, hydrolysable tannins and condensed tannins based on the identity of the nuclei involved and on the way they are joined. Members of the first group consist of gallic acid and hexahydroxydiphenic acid and their derivatives esterified with glucose. Since esters are readily hydrolyzed to yield phenolic acids and the sugar, they are referred to as hydrolysable tannins. The nonhydrolyzable or condensed tannins are also termed proanthocyanidins because on treatment with hot acid some of the carbon-carbon bonds were broken, yielding anthocyanidin monomers. Basically, these tannins contained only phenolic nuclei but frequently are linked to carbohydrates or proteins. Most such tannins result from the condensation of two or more flavan-3-ols, such as catechin, or flavan-3,4-diols, such as leucocyanidin. When treated with hydrolytic agents, these tannins tended to polymerize, yielding insoluble, usually red-colored products known as phlobaphenes. In many species, both types of tannins are present, although one type generally tends to predominate in a particular plant part. Cantoria (2003) further discussed the physical properties of tannins as yellowish white to light brown amorphous powder usually odorless; strong astringent taste; gradually darkens due to exposure to air and light. It is very soluble in water, glycerin, and alcohol; practically insoluble in chloroform or ether. It gives insoluble precipitates with albumin, starch, gelatin, most alkaloidal and metallic salts; produces a bluish-black color or precipitation with ferric salts. To identify tannins, Kar (2007) enumerated the following tests: (1) tannins form colloidal solutions possessing an acid reaction and a sharp tart taste, (2) they cause precipitation of solutions of proteins as well as of alkaloids, (3) they form bluish-black or greenish-black soluble compounds with ferric salts (4) they produce a deep red color with potassium ferrocyanide and ammonia; (5) they are precipitated by salts of copper, lead, and tin, and by strong aqueous potassium dichromate (or 1% chromic acid) solutions; and (6) in alkaline solutions, many of their derivatives absorb oxygen.In preparing drug formulations, it should be remembered that a solution of tannins gradually darkened when exposed to air and light through oxidation of phenolic groups to quinoid structures. It is incompatible with most enzymes, gums, salts of many metals, and many other substances. Tannins precipitate proteins from solutions and can combine with proteins, rendering them resistant to proteolytic enzymes. When applied to living tissues, this action is known as an “astringent action and forms the basis for therapeutic application of tannins. The matured barks of Tamarind are rich in tannin which is the active constituent that is responsible for the antibacterial property (Cantoria, 2003; Evans, 2002 and Quisumbing, 1978). Moreover, scientific evidence that deals with preformulation and development into a suitable pharmaceutical dosage form is lacking. Therefore in

order to fill this knowledge gap, this study aims to develop an antibacterial cream from the matured bark extract of Tamarind as an alternative medication to current synthetic brands.

METHODOLOGY

Collection of plant material

Tamarindus indica L., (Leguminosae) plant was collected from Rosario, Cavite in the Philippines. A prepared herbarium was submitted, identified and authenticated by Mr. Danilo Tandang of National Museum of Botany Division in Manila Philippines prior to experiment.

Preparation of crude drug and stock plant extract

All reagents used were of analytical grade. The bark from the trunk of the tree was taken at a height of at least 3 feet away from the base of the trunk. The matured barks were chopped into pieces, oven (Model No.: LAB 110) dried at 60˚C until crispy and grind until a desired particle size was achieved using Wiley Mill Grinder. The grind material was stored in a glass container for the duration of the experiment and a quality control tests such as organoleptic evaluation, foreign matter content and total ash were conducted based on methodologies of United States Pharmacopeia to determine its purity and quality.

The method of extraction employed in this study was Soxhlet Method. A 225 g of previously air-dried coarsely grounded tamarind bark was placed in the porous thimble. The partially filled thimble was placed in the Soxhlet inner tube. The flask was filled one-half full of 70% acetone, the volume of the solvent used was 500 mL. Then the unit was assembled and cooling water was turned on into the condenser. The unit was heated under the steam bath. A clear colorless extract at siphon tube indicates extraction was complete. The extract was evaporated to dryness to remove the solvent. The powdered acetone extract was placed in a tightly stoppered container and stored inside the laboratory refrigerator throughout the experiment. The container was properly labeled with the name of the plant, concentration of the plant extract in grams dried material / mL extract, and the date of extraction (Aguinaldo et. al., 2005 and Shugar and Dean, 1999, United States Pharmacopeia and Philippine Pharmacopeia 1). Physicochemical properties of acetone crude powdered extract were conducted in accordance to the methodologies of USP.

Purchase of microorganisms

Microorganisms namely: *Staphylococcus aureus* (ATCC 25923), Streptococcus spp and *Corynebacterium minutissimum* were purchased from College of Public Health - University of the Philippines Manila Campus and Department of Science and Technology. They were subcultured and used throughout the studies.

Microbial screening

Susceptibility testing was performed using the paper disc diffusion method by Kirby Bauer wherein nutrient agar plates were prepared in triplicate. The media surface were inoculated with microorganisms from a broth culture that were previously standardized against 0.5McFarland to obtained a turbidity approximately 1.5 x 10^8 CFU/mL of the test organism. Plant extract were prepared at different concentrations: 20 mg/mL, 40 mg/mL, and 80 mg/mL respectively. Sterile water was used as solvent and served as negative control while Fusidic acid (antibacterial drug) was used as positive control. Incubation was made at 35˚C for 24 h. The clear inhibition zones of diameters formed around the discs were measured using vernier caliper (Model No.: C/N 530-104) and interpreted according to Aguinaldo, et. al., 2005.

Purchase of rabbits

Prior to this test an animal research permit was obtained from Bureau of Animal Industry. Test animals were purchased from St. Luke's Medical Center Research and Biotech, Division. Animal test was conducted in accordance to IACUC Guidelines of the university and were acclimatized for 1 week prior to experiment proper.

Sensitivity test for local and topical applications

Patch Test. A group of eighteen adult rabbits was selected, 9 males and 9 females. The skin lateral to the spinal groove was shaved and cleaned. The left side of the groove in the animal was utilized as the negative control site and the right side as the test drug site. The sites were cleaned with 70% alcohol. The test drug was appropriately delivered and the negative drug control on the inoculation sites. Both sites were covered with sterilized gauze (1x1 cm in size). Surgical tapes were used to keep the gauze in place. All the test animals were rendered immobile and left undisturbed for 24 to 72 hours. The patches were removed after 24 hours of exposure and the reactions were evaluated according to the following listing.

Erythema and eschar formation	Score
No erythema……	0
Very slight erythema…… ……	1

Well-defined erythema……2
Moderate erythema……3
Severe erythema……4
Maximum possible erythema……...4

Edema formation	Score
No edema……	0
Very slight edema (barely perceptible)……	1
Slight edema (edges of area well-defined by definite rising)…… ……	2
Moderate edema (raised approximately 1 mm……	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure ……	4
Maximum possible edema score……..…..……	4

The average scores of 24 and 72 hours reading was computed. The averages of the scores for the patch and scratch tests were combined. This combined average was referred to as the primary irritation index.

Primary irritation index = ½ (average of the patch and scratch test).

In- Vivo Scratch Test. The procedure and the scoring for this test was the same as that in the patch test but with slight modifications. The skin was abraded, lateral to the spinal groove of the rabbit, by slightly scratching the skin five to seven times with a 20-gauge hypodermic needle containing Staphylococcus aureus. Immediately, the application of the test drug on the abraded skin patches was followed as the positive patch and the negative control patch. The results were observed and recorded as in the patch test (Aguinaldo, A.M. 2005).

Preparation and quality control of Tamarind cream

A 100 g cream was prepared using the mechanical incorporation and fusion method and packed in 5 gram aluminum tubes with closed mouth and epoxy-lined ends for crimping. The propylene screw caps were provided with a piercing device. Quality control tests of finished product were conducted in accordance to methodologies of USP.

RESULTS AND DISCUSSION

Quality control of crude drug

Crude powdered drug was dark to chocolate brown in color with light tamarind odor to earthly and bitter in taste. These characteristics can be attributed to active components of tannins and alkaloids as confirmed by Doughari 2006. Foreign matter content was 1.35% and did not exceed the USP Limit of >2 %. The lower the foreign matter content indicates that the crude drug was purer and contained fewer impurities. Foreign matter found included dirt, soil, spider web and ants corpse. Total ash found was 10.16 %. Total ash represented the inorganic salts which naturally occurs in the drug and adheres to it. This test provided a basis for judging the identity of the drug and cleanliness. Furthermore, when a standard for this has already been established, it gives information relative to its adulteration with inorganic matter (Knevel and DiGangi, 1977).

Physicochemical properties of acetone crude powdered extract

From 1,125.00 g of powdered trunk bark, 50.65 g of crude plant extract was obtained and a yield of 4.50%. Physicochemical tests revealed that crude plant extract was dark brown crystalline powder with tamarind odor, bitter in taste and had a pH of 8.38. Based on the result it was alkaline therefore, this should be adjusted to neutral pH prior to cream manufacture if necessary so that the cream will not be irritating in the presence of open skin. Care should be exercised however, that when adjusting the pH, the antibacterial quality of cream will not deteriorate. Density was found to be 0.09 g/mL and showed that it was lighter than water. This information was considered during dispensing of the extract. Since the extract was considered as a raw material, when one gram of it was dispensed, an overage occurred if the density was disregarded. The crude extract of Tamarind bark was freely soluble in water, slightly soluble in (80 %v/v) ethanol, sparingly soluble in ether and very slightly soluble in chloroform. Generally, it was soluble in non-organic or polar solvents and slightly soluble in organic / non-polar solvents. In the photoreaction test, the powdered crude acetone extract was placed in amber bottle and flint bottle and exposed to direct sunlight for 30 days. Samples were physically examined after one month. There were changes in odor (tamarind odor to absence of odor), consistency (viscous to solidified extract) and no changes in taste (acid) and color (dark brown). Tannin USP turned dark in color when exposed to light. However, since the extract contained an undetermined amount of tannin and alkaloid and was originally dark brown in color, no significant darkening was observed. Although the result may conclude that photo degradation took place on the basis of the physical attributes of the crude powdered acetone extract contained in the flint bottle, it would be practical to use amber bottle as storage container because of the storage statement in the USP. Hygroscopicity test used to determine how much water the test sample can lose or gain under a steady – state condition. Since the desiccator was a closed system the vapor provided by constant humidity solutions could not escape and remained within the desiccator, thus, ensuring steady state condition. Under this condition the test sample

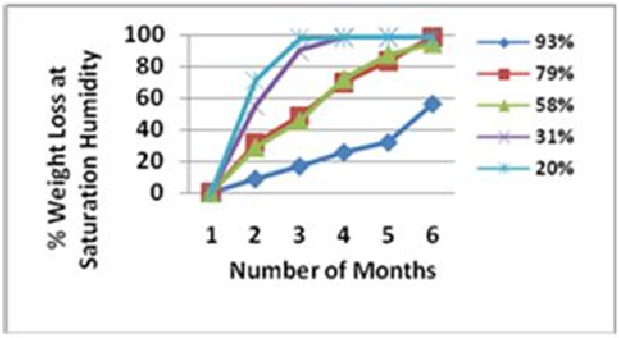


Figure 1. A Plot of Loss in Weight of Samples Exposed to Five Conditions of Humidity

contained "bound" water which was not easily removed by the evaporation. Using the traditional desiccator's method, the change in weight upon exposure to such conditions revealed that the accurate revealed weighed samples lost weight. Thus, it was found that the crude acetone extract had a high liquid content. This was demonstrated in a plot of percentage relative humidity against time expressed in months as shown in Figure 1. The Figure 1 showed the increasing % weight loss of samples that were stored in different environments of humidity such as 93, 79, 58, 31 and 20 % RH from 1st month to 6th months. The sample in 95% RH had the least number of % weight loss followed by samples stored in 79% and 58% RH while samples stored in 31% and 20% obtained the highest % weight loss. The test indicated that the crude acetone extracts had a high solvent content; therefore, the crude extract was lyophilized prior to formulation for complete removal of the solvent. Lyophilization was done at Agcaoli Memorial Tissue Bank of Department of Orthopedics, University of Philippines-Philippine General Hospital.

In the drug excipient compatibility test, a differential scanning calorimeter was used; a thermogram was obtained to reveal the characteristic melting point of any crystalline metabolite that may be present. Crystalline transitions, fusion, evaporation and sublimation were the obvious changes manifested in a thermogram (Wells, 2002). The following thermograms in Figure 2 (a) to (f) exhibit the melting curves of ; (a) powdered acetone crude extract of Tamarind at temperature of 187.20˚C using an energy of 66.87J/g, (b) methyl paraben at temperature of 127.60 ˚C using an energy of 192.0 J/g., (c) propyl paraben at temperature of 97.67˚C using an energy of 160.70J/g , (d) sodium lauryl sulfate at temperature of 102.58˚C using an energy of 20.79J/g , (e) stearyl alcohol at temperature of 60.56˚C using energy of 251.6J/g and (f) white petrolatum at temperature of 39.03˚C using an energy of 4.270J/g. Figure 2 (g) were thermograms of mixtures of excipients with the tamarind bark extract. The green curve represented the melting point of the powdered acetone crude extract, the excipient in blue color while the mixture of both the powdered crude acetone extract and the excipient was shown in black curves. It revealed that when tamarind (green line) combined with methyl paraben (blue line), the mixture (black line) melted at 127.60 ˚C at an energy of 72.50 J/g. It showed that there was a decrease in melting temperature when these two chemicals were added together; therefore they both melted and could be formulated into cream. The next thermogram in Figure 2 (h) revealed that when tamarind (green line) combined with propyl paraben (blue line), the mixture (black line) melted at 82.91 ˚C at an energy of 48.61 J/g. It showed that there was a decrease in melting temperature when these two chemicals were added together therefore they both melted and could be also formulated into cream. Another thermogram Figure 2 (i) revealed that when tamarind (green line) combined with sodium lauryl sulfate (blue line), the mixture (black line) melted at 102.98 ˚C at an energy of 48.61 J/g. It showed that there was a no change in melting temperature when these two chemicals were added together but sodium lauryl sulfate crystallizes at 200.08 ˚C at energy of 47.19 J/g. It showed that it is incompatible at temperature higher than 200 ˚C therefore the cream should not be manufacture beyond this temperature. Figure 2 (j) did not show any significant change in the thermogram of the mixture. Stearyl alcohol was therefore compatible with the extract. Lastly, Figure 2 (k) showed the compatibility of white petrolatum with the extract. No significant change was reflected in the thermogram of the combination.

An analysis of the thermograms revealed that the melting temperatures of the materials did not change significantly. However, sodium lauryl sulfate crystallized at a temperature above 200˚C, a sign of incompatibility. Manufacturing of the cream was by fusion. It was expected that the raw materials were intended to melt. Thus, the results of the test also indicated that the processing temperatures should not go beyond the maximum limit of 75˚C of the prescribed procedure. It validated the use of a steam bath. Wells (2002) explained

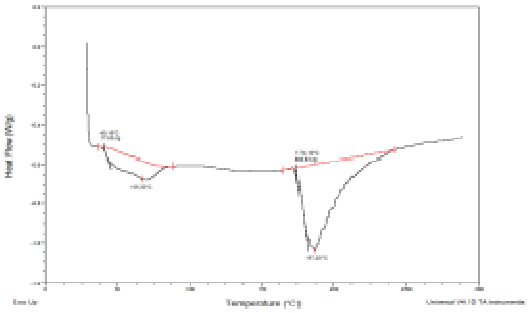


Figure 2 (a). Thermogram of Powdered Acetone Extract of Tamarind

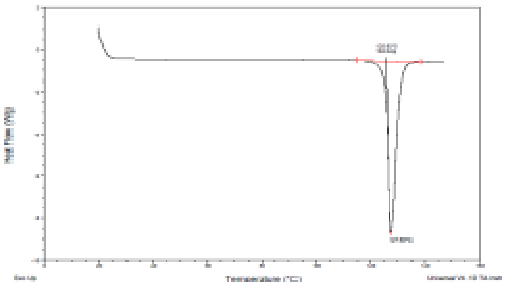


Figure 2 (b) Thermogram of Methyl Paraben

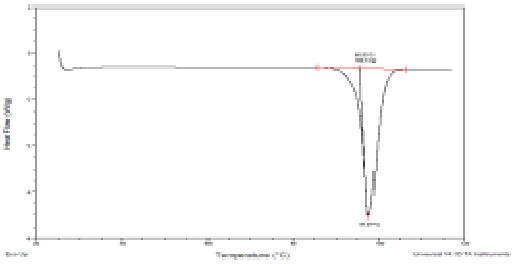


Figure 2 (c). Thermogram of Propyl Parabe Sulfate

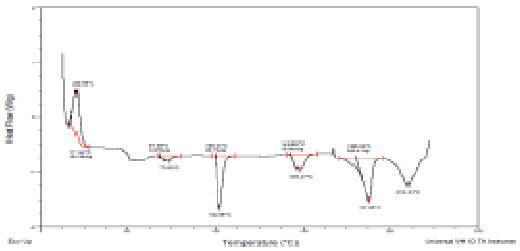


Figure 2 (d). Thermogram of Sodium Lauryl

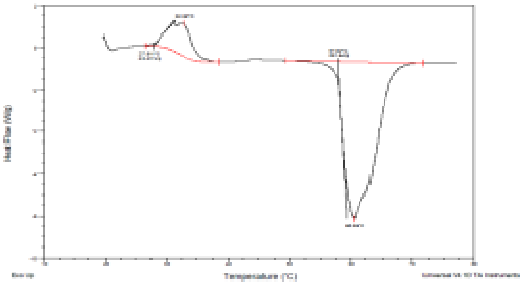


Figure 2 (e). Thermogram of Stearyl Alcohol

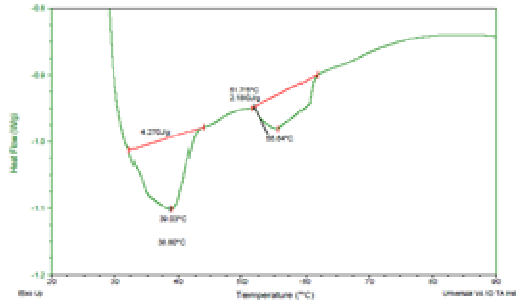


Figure 2 (f). Thermogram of White Petrolatum

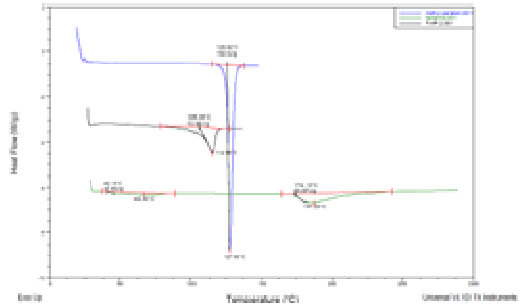


Figure 2 (g). Thermogram of a mixture of powdered acetone crude extract of Tamarind and Methyl Paraben

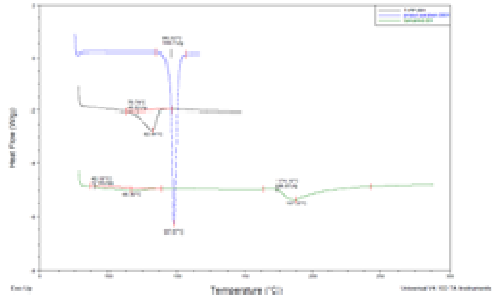


Figure 2 (h). Thermogram of a mixture of powdered acetone crude extract of Tamarind and Propyl Paraben

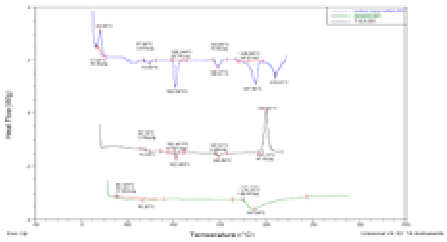


Figure 2 (i). Thermogram of a mixture of powdered acetone crude extract of Tamarind and Sodium Lauryl Sulfate

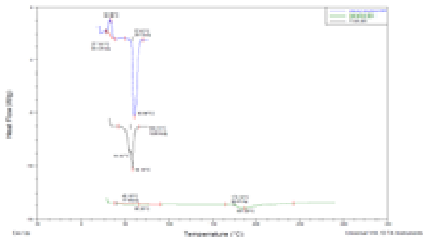


Figure 2 (j). Thermogram of a mixture of powdered acetone extract of Tamarind and Stearyl Alcohol

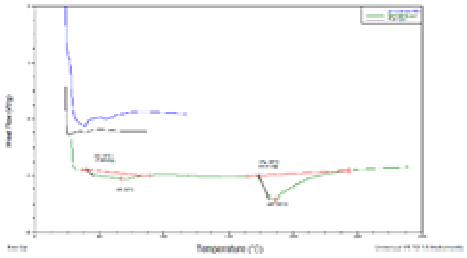


Figure 2 (k). Thermogram of a mixture of powdered acetone crude extract of Tamarind and White Petrolatum

Table 1. Average zones of inhibition (mm) of powdered acetone crude extract of Tamarind

Microorganisms	Concentrations (mg/mL)			Water	Fusidic acid
	20	40	80		
Staphylococcus aureus	11.7	19.7	20.8	0	29.7
Corynebacterium minutissimum	9.7	15.7	14.7	0	24.7
Streptococcus spp	8.7	14.7	14.7	0	24.8

Interpretation: < 10 mm, expressed as inactive; 10-13 mm, partially active; 14-19 mm, active; > 19 mm, very active (Reference: Aguinaldo, *et al.,* 2005)

the theoretical framework behind the use of Differential Scanning Calorimeter. “If no physical or chemical change occurred within the sample, then there was neither a temperature change nor input energy to maintain an isotherm. However, when phase changed occur, then, the latent heat suppressed a temperature change and the isothermal energy required registers a change as an electrical signal generated by thermocouples.”

Antibacterial property of plant extract

Tamarind bark crude extract exhibited an antibacterial effect against three organisms; namely, *Staphylococcus aureus, Corynebacterium minutissimum* and *Streptococcus spp* as shown in 1. *Staphylococcus aureus* was the most susceptible to all concentrations of extract followed by *Corynebacterium minutissimum* and *Streptococcus spp.* The higher the concentration the greater zone of inhibition formed. At 20 mg/mL concentration both *Corynebacterium minutissimum* (9.7) and Streptococcus spp. (8.7) were inactive while partially active for Staphylococcus aureus (11.7). On the other hand at 40 mg/mL and 80 mg/mL concentrations all the microorganisms were susceptible. However, such activity which is concentration dependent is weaker than the activity of the standard drug (fusidic acid).

Sensitivity test for local and topical applications

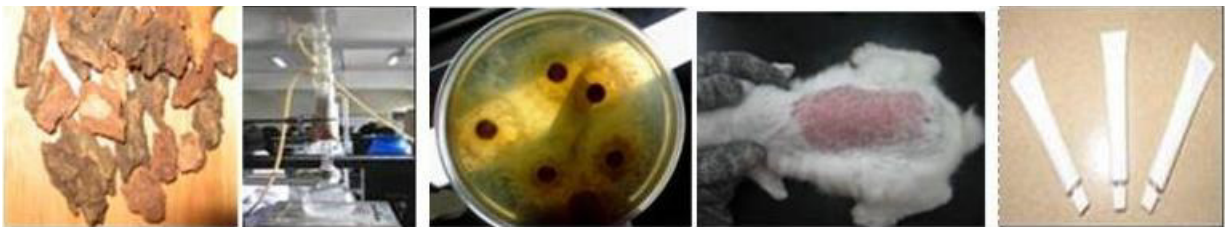
The safety of the powdered crude acetone extract of Tamarind bark was determined by a patch and scratch test using 18 rabbits (9 males, 9 females), and observed after 42 and 72 hours. The primary irritancy index was one. The test indicated that the crude acetone extracts had no irritating effect and therefore safe for topical formulation.

Table 2. Formulation of Tamarind Cream (40 % Trunk Bark)

Ingredients	***g/100 g***	***Phases***
Trunk Bark Extract of *T. indica*	40.0	Active ingredient
Stearyl Alcohol	3.0	Excipients - Phase 1:
White Petrolatum	44.0	Oleaginous base
Methyl Paraben	0.5	
Propyl Paraben	0.5	Excipients - Phase 2:
Sodium Lauryl Sulfate	3.0	Aqueous solution
Distilled Water, q.s.	100.0	

Table 3. Summary of Results of Quality Control Test of Cream

Parameters	**Results**	**Remarks**
1. Appearance	Semi-solid	Appearance of cream
2. Color	Dark brown	Natural color of cream
3. Odor	No characteristic odor	Absence of odor
4. Smoothness	No grittiness upon application on skin	Sensory test for texture of cream
5. pH	9.0	Alkaline
6. Microbial test		
6.1 *S. aureus*	0	Meets absence of microbial
6.2 *P. aureginosa*	0	growth to the test organisms
6.3 *E. coli*	0	
7. Sensitivity test	0	No irritation and inflammation effect
8. In-Vitro test	1	Very slight irritation



A **B** **C** **D** **E**

Figure 3 Experimental processes. Trunk bark **(A)** Soxhlet extraction of plant extract **(B)** Microbial assay of plant extract **(C)** Sensitivity test in rabbit **(D)** and Finished product the antibacterial ointment **(E)**

Preparation and quality control of Tamarind cream

A 100 g cream was prepared using mechanical incorporation and fusion method containing 40% trunk bark was presented in 2. The table showed the formulation of Tamarind cream. The ingredients were as follows: tamarind extract (40g) as active ingredient, stearyl alcohol (3g) as stiffening agent, white petrolatum (44g) as oleaginous base, methyl parabe (0.5g) as preservative, propyl paraben (0.5g) as preservative, sodium lauryl sulfate (3g) as emulsifying agent and water (q.s.) as solvent. The master formula was modified to produce a cream which was physically satisfactory and acceptable for topical application. The packaging materials purchased from Velfox Manufacturing consisted of 5 gram aluminum tubes with closed mouth and epoxy-lined ends for crimping. The propylene screw caps were provided with a piercing device.

The tamarind bark cream was subjected to quality control using the following parameters. The results were shown in Table 3. The table above showed that the developed product, antibacterial Tamarind cream was semisolid in appearance, has a dark brown color, no characteristic odor and not gritty. When tested it was alkaline (pH 9.0), absence of microorganisms such *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* and no irritation to rabbits.

As a summary of the development of antibacterial cream from matured bark of Tamarind, 3 was provided. It showed the experimental process started at harvesting and drying of trunk bark (Figure 3A), followed by Soxlet extraction of plant constituents using 80% acetone as solvent (Figure 3B), microbial assay of plant extract using paper disc diffusion method (Figure 3C), sensistivity test in rabbits (Figure 3D) and packaging in an aluminum tube of the antibacterial tamarind cream. The limitations of this

study were the accelerated stability testing, clinical trials of the formulated antibacterial cream and shelf life determination due to time constraints and limited availability of funds.

CONCLUSIONS

On the basis of results it is concluded that the crude drug, powdered Tamarind bark passed the quality control tests conducted therefore suitable for extraction process. The plant extract have the following physicochemical properties; dark brown crystalline powder with tamarind odor; bitter in taste; pH of 8.38; density of 0.09 g/mL; freely soluble in water, slightly soluble in (80 %v/v) ethanol, sparingly soluble in ether and very slightly soluble in chloroform; compatible with excipients such as methyl paraben (preservative), propyl paraben (preservative), sodium lauryl sulfate (emulsifying agent), stearyl alcohol (stiffening agent), white petrolatum (oleaginous base) and water (dissolving agent) ; not irritating when tested to rabbits and susceptible to Staphylococcus aureus, *Corynebacterium minutissimum* and *Streptococcus spp.* An antibacterial cream was developed however its stability and shelf life were not determined therefore it was recommended for future studies to purify, isolate, elucidate the bioactive compound and conduct a comprehensive stability testing for shelf-life determination.

ACKNOWLEDGMENT

We would like to acknowledge the following; First, the researcher would like to thank, JESUS, Almighty God as her guide and source of knowledge in doing this research : Philippine Council for Health Research and Development – Department of Science and Technology (PCHRD-DOST) for the generous research grant : College of Pharmacy Laboratory – University of Perpetual Help System DALTA Las Piñas City Campus and Graduate School – University of Santo Tomas for the use of their facilities : Dr. Gerardo Balaccua for treatment of data : Dr. Delia C. Ongtengco, Dr. Rosalinda Solevilla and Dr. Rebecca David who serve as panelists of this study : students, faculty members and Dr. Ma. Victoria E. Mendoza, Dean of College of Pharmacy – University of Perpetual Help System DALTA Las Piñas City Campus for moral support.

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