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Research Paper

Preformulation and formulation studies of the alcoholic extract of *Punica granatum* L., (Lythraceae) exocarp as antimicrobial ointment

Anna Muriel T. Jacinto*, Jose Rennel M. Talavera and Catherine B. Galvez

College of Pharmacy, University of Perpetual Help System DALTA, Las Piñas City, Metro Manila, Philippines

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* Corresponding author
College of Pharmacy, University of Perpetual Help System DALTA, Las Piñas City, Alabang-Zapote Road, Pamplona 3, Las Piñas City, Metro Manila, 1740 Philippines
E-mail: annamurieltalaverajacinto@gmail.com

ABSTRACT

Punica granatum L., (Lythraceae) is commonly known as pomegranate and contains various pharmacological property among which is its antimicrobial property. The objective of the study was to formulate an antimicrobial ointment. Methods used include physicochemical evaluation, microbial assay, compatibility of excipients, formulation, quality control and stability test of the ointment. Results revealed that the extract was a dark brown colored powder with like bark odor, disagreeable taste, acidic (pH 3), lighter than water (< 1.0 density), melts at 117.10ºC and was soluble in the following solvents: 95% ethanol, 0.1 N HCl, 0.1 N NaOH, 0.9% NaCl, phosphate buffer solutions (pH 4, 7 & 10) and petroleum ether. Furthermore, it was most sensitive to *Staphylococcus aureus* (28 mm) followed by *Trichophyton mentagrophytes* (23 mm), *Pseudomonas aeruginosa* and *Aspergillus niger* (22 mm) while least sensitive to *Candida albicans* (8 mm). The formulated ointment passed the quality control test but needed further reformulation to improve product stability. Hence, future studies recommend the exploration of other excipients, formulation of other dosage forms and employment of additional methods of preparation.

Key words: microbial assay, natural products, preformulation, pomegranate

1. Introduction

The *Punica granatum* L., (Lythraceae) fruit exocarp has an antimicrobial property. Its fruit peel extract exhibits antibacterial activity against several bacteria like *Streptococcus mutans*, *Streptococcus sanguis, Streptococcus mitis* (Vasconcelos et al., 2006), *Escherichia coli*, *Psuedomonas aeruginosa* and *Staphylococcus aureus* (Khan and Hanee, 2011). Likewise, it is also very effective against several fungi such as *Candida albicans* (Vasconcelos et al., 2006), *Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis* and *Microsporum gypseum* (Foss et al., 2014). In fact, its antimicrobial activity is attributed to several phytoconstituents present in the fruit exocarp such as punicalagin (Foss et al., 2014), ellagitannins and punicalagin (Machado et al., 2002) and phenolic punicalagins, gallic acid, fatty acids, catechin, quercitin, rutin, flavonols, flavones, flavonones and anthocyanidins (Jurenka, 2008). Considering the dearth of information on *Punica granatum*, this study bridged the lack of scientific studies on the preformulated and formulated antimicrobial ointment from the exocarp extract of this plant. Thus, the general objective of the study was to conduct a preformulation and formulation studies from the extracts of *Punica granatum* L. (Lythraceae) exocarp as an antimicrobial ointment.

Moreover, this scientific work is a response to the call of the Philippine Department of Science and Technology (DOST) for researches to reflect the National Unified Health Research Agenda for 2017–2022. One of the six (6) themes comprising these research priorities is global competitiveness in research and innovation in health. DOST promotes research as a tool for creating a novel solution to existing and emerging health problems through technology development and innovation in the fields of rapid advancement such as drug discovery and development (Philippine National Health Research System, 2017).

2. Material and methods

2.1. Materials

This study was conducted at the University of Perpetual Help System DALTA-College of Pharmacy, Las Piñas City, Metro Manila, Philippines. The microorganisms used in this study were *Staphylococcus aureus* ATCC 29213*, Pseudomonas aeruginosa* ATCC 27853*, Aspergillus niger* ATCC 16404*, Candida albicans* ATCC 10231 and

Tricophyton mentagrophytes ATTC 18748 which were purchased from the University of the Philippines-Department of Medical Microbiology. All chemicals and reagents used in the experiment were of analytical grade and were purchased from Merck (Darmstadt, Germany), Sigma (St. Louis, MO, USA) and HiMedia (India).

2.2. Methods

The design of the study was experimental and methods were derived from the United States Pharmacopeia 23/ National Formulary 18 (1995), Philippine Pharmacopeia (2004) and Modified Irritation Test in Rabbits, OECD, #404. Methods used include collection, extraction, physico-chemical evaluation, microbial assay, compatibility testing, formulation, quality control, sensitivity and stability test of antimicrobial ointment. All equipment used in this study were properly calibrated prior to use.

2.2.1. Collection of plant material

The whole plant was collected in Quezon Province, (Coordinates 13.3471° N, 122.5202° E) Philippines. It was air- dried and brought to the Research Center for Natural and Applied Sciences for authentication. About 10 kg of ripe pomegranate fruits that have no spoilage were hand-picked and washed with tap water. The exocarp was peeled off, chopped into pieces using a kitchen knife and dried in an oven until crispy at 50˚C. It was pulverized using the Wiley Mill Grinder.

2.2.2. Extraction and physicochemical evaluation of lyophilized extract

The powdered pomegranate peel was macerated in a 2 L Erlenmeyer flask for 24 hr using 80 percent ethyl alcohol. The extract was filtered using Whatmann filter paper no.1, concentrated in vacuo (Wilmad WG-EV311, Condenser H50-500 Lab-Tech, Rotavap RE-2000A) and lyophilized at Agcaoli Memorial Tissue Bank of the Department of the Orthopedics, University of the Philippines Manila-Philippine General Hospital. The lyophilized extract was weighed using Ohaus Analytical Balance (Serial no. 8329340171, USA), stored in a clean amber bottle and kept inside the refrigerator (4ºC) for succeeding tests. The following tests were performed in triplicate.

2.2.2.1. Physical characteristics were identified using the organoleptic evaluation method by describing the appearance, color, odor and taste.

2.2.2.2. The pH was measured by immersing the electrodes of a pH meter (TS-1, Suntex) at 25ºC in a 5 mL solution of the lyophilized extract obtained by diluting 1 g of lyophilized extract with 10 mL of purified water.

2.2.2.3. Bulk density using Method 1 (Measurement in a Graduated Cylinder). A quantity of material sufficient to complete the test was passed through a 1.00-mm (No.18) screen to break up agglomerates that may have formed during storage. Into a dry 250 mL cylinder, approximately 100 g of the test sample (M) was introduced without compacting. It was weighed with 0.1% accuracy. The powder was carefully leveled without compacting, if necessary and the unsettled apparent volume, VO was read to the nearest graduated unit. The bulk density, in g per mL, was calculated by the formula: (M) / (Vo).

2.2.2.4. Solubility was determined with the use of a mechanical shaker (MRC, USA) using different solvents such as water, 95% ethanol, 0.1 N HCl, 0.1 N NaOH, 0.9% NaCl, phosphate buffer solutions and petroleum ether. About 1 mg of lyophilized extract was placed in an Erlenmeyer flask and then 1 µL of solvent was added. The mixture was agitated at 100 rpm using an automatic shaker until the test sample was dissolved. If the lyophilized extract did not dissolve, a further 30 µL of solvent was added and its effect was noted. Successive amount of the solvent was added until the compound exhibited solubility. If the test sample immediately dissolved with the solvent, the amount of the solvent to be added was decreased.

2.2.2.5. Melting point was determined using differential scanning calorimetry (DSC-4000 Perkin Elmer, USA). About 5 mg of the sample was accurately weighed and placed inside the pan. The parameters used were the following: heat from 25˚C to 300˚C at 30˚C/min and nitrogen gas at 20 mL/min.

2.2.2.6. Screening test for tannins was determined qualitatively using the gelatin test and ferric chloride test as described by Guevara B. (2005). The formation of a jelly-precipitate indicates the presence of tannins. On the other hand, in the ferric chloride test, a blue-black color indicates the presence of hydrolysable tannins while a brownish-green color may indicate the presence of condensed tannins.

2.2.3. Microbial assay of lyophilized extract

Microbial assay was performed using the paper disk diffusion method by Kirby Bauer. The size of the disk was 6 mm. Nutrient agar (HiMedia, India) plates for bacterial strains and Sabouraud glucose agar (Titan Biotech Ltd, India) plates for fungi strains were prepared in triplicate. The media surface was inoculated with test microorganisms from broth culture that were previously standardized against 0.5 McFarland to obtain turbidity of approximately 1.5×10^8 CFU/mL of the test organism. The plant extracts were prepared at different concentrations: 1 g/mL, 0.75 g/mL, 0.50 g/mL and 0.25 g/mL. Nutrient broth and Sabouraud broth were used as solvents of the plant extract as well as negative control. Neomycin (Sigma-Aldrich) and Miconazole (Coloplast A/S DK-3050 Humlebaek, Denmark) were used

as positive controls. The inoculated petri dish were inverted and placed inside the incubator (Model No. 10-140) with a temperature of 35ºC for 24 hr for bacterial strain and fungi strain. Formations of a clear zone of inhibition were measured using a ruler and interpreted according to the following: < 10 mm expressed as inactive; 10–13 mm, partially active; 14–19 mm, active; > 19 mm, very active (Guevara, 2005). This test was performed inside the Biosafety Cabinet Level II (BC - Sanyo).

2.2.4. Compatibility test of excipients

The lyophilized extract and excipients were individually tested by differential scanning calorimetry (DSC-4000, Perkin Elmer, USA) as baseline data. Five (5) mg of lyophilized extract was mixed in a ratio of 1:1 with 5 mg excipient and was tested. The parameters used include heat from 25˚C to 300˚C at 30˚C/min and nitrogen gas at 20 mL/min. Another mixture that was previously exposed to different conditions (4˚C, 29˚C, 40˚C) was tested in DSC. The intended excipients used were the following: methyl paraben and propyl paraben (preservative); propylene glycol 4000 (water base ointment); stearic acid (emulsifying agent); NaOH solution (pH adjustment) and petrolatum (oil base ointment). The TA Universal Analysis software was utilized for the calculation and analysis of the melting peaks for each sample.

2.2.5. Formulation of ointment

Water-based and oil-based ointments were formulated through the mechanical incorporation method using mortar and pestle. Ten (10) formulations were done through trial and error before the proper formulations were achieved. After the proper combinations of excipients were determined, eight formulations were prepared as summarized in Table 1 were tested for quality control.

2.2.6. Quality control test of formulated ointment

The following quality control tests were conducted in triplicate in accordance to the standards of USP 23/NF 18 (1995).

2.2.6.1. Organoleptic test: About five (5) g of the sample was placed in the watch glass with white background and observed for their physical properties such as visual appearance, color, odor and smoothness.

2.2.6.2. Spreadability testing: It was carried out using two glass plates. About 1 g of the sample was placed at the center of the lower plate. A pre-weighed glass plate was placed above the lower plate. The diameter of the circle was measured after 1 minute. Afterwards, a 200 g flyweight of was placed at the center of the glass plate and the diameter of the circle was measured again after a minute. The procedure was repeated by placing another 200 g until a weight of 1000 g was achieved (Briedis and Sznitowska, 2011). The sets of weights were calibrated prior to testing.

2.2.6.3. pH: A 1% solution of ointment was prepared and its pH was determined by immersing the electrodes of a pH meter.

2.2.6.4. Viscosity: It was measured by Brookfield Viscometer (Model: VS-CRA-14S).

2.2.6.5. Microbial test: Because the active ingredient was of organic origin, a total microbial count test was performed to determine the microbial contamination of the samples. Tryptic soy agar was used as the media. The samples were compared to positive control utilizing *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aureginosa* ATCC 27853 as test microorganisms and negative control. Five mL of the sample was diluted with 45 mL of phosphate buffer 7.2 and was mixed with 1 mL of the mixture then was transferred into a plate containing tryptic soy agar. The mixture was gently mixed, then incubated at 35ºC for 24–48 hours and the microbial contamination was counted. Each sample was run in triplicate.

2.2.7. Sensitivity test of formulated ointment

This test utilized the Modified Dermal Irritation for Rabbits found in OECD guideline # 404. A certificate of

Table 1. Summary of Formulations of Antimicrobial Ointments (10 g).

	Water-based Formulations Concentrations in % (w/w)				**Oil-based Formulations Concentrations in % (w/w)**			
Ingredients	20%	40%	60%	80%	20%	40%	60%	80%
Powdered pomegranate extract (g)	2.00	4.00	6.00	8.00	2.00	4.00	6.00	8.00
Methyl paraben (g)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Propyl paraben (g)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Polyethylene glycol 4000 (g)	3.00	2.00	0.50	0.50	—	—	—	—
Stearic acid (g)	2.00	1.00	0.50	—	1.00	0.50	0.50	—
Sodium hydroxide solution (mL)	3.00	3.00	3.00	1.50	3.00	3.00	2.00	1.00
Petrolatum (g)	—	—	—	—	4.00	2.50	1.50	1.00

Animal Research Permit was obtained from the Bureau of Animal Industry prior to the conduct of the study. The formulated pomegranate ointment was tested on the intact skin of the rabbits. After 4 hours of continuous exposure, any effect on the application site was observed and noted. A period of 14 days was used for observing any change on the application site due to its exposure to the formulated ointments. Distilled water was used as negative control.

2.2.8. Stability test of formulated ointment

An accelerated stability study was conducted on the formulated antimicrobial ointment in accordance to the Philippine FDA ASEAN guidelines on the stability study of drug products version 6.0 May 2013. The formulated antimicrobial ointment in their packaging was placed inside an oven (40˚C ± 2/75% RH). The physico-chemical testing and microbiological stability were conducted at 0, 3 and 6 months.

3. Results and Discussion

3.1. Physicochemical evaluation of the lyophilized extract

The lyophilized extract was a dark brown colored powder with like bark odor, disagreeable taste, acidic (pH 3), lighter than water (< 1.0 density), melts at 117.10ºC and was soluble in the following solvents namely: 95% ethanol, 0.1 N HCl, 0.1 N NaOH, 0.9% NaCl, phosphate buffer solutions (pH 4, 7 & 10), and petroleum ether. Furthermore, there was formation of gelatin and blue-black colors in confirmatory tests which revealed the presence of hydrolysable tannins.

In fact, the disagreeable taste, like bark odor and brown color of the extract was attributed to active the component; (tannins and alkaloids) as described (Tyler, 1988) and found in confirmatory tests (Haque et al., 2015). Tannins are complex substances that usually occur as mixtures of polyphenols that are difficult to separate because they do not crystallize. They are customarily divided into two chemical classes, based on the identity of the phenolic nuclei involved and on the way they are joined. Members of the first class consist of gallic acid or related polyhydric compounds esterified with glucose. Because such esters are readily hydrolyzed to yield the phenolic acids and the sugar, they are referred to as hydrolysable tannins. Nonhydrolyzable or condensed tannins compose the second class. Basically, these tannins contain only phenolic nuclei but are frequently linked to carbohydrates or proteins. Most such tannins result from the condensation of 2 or more flavan-3,4-diols, such as leucocyanidin. When treated with hydrolytic agents, these tannins tend to polymerize, yielding red-colored products known as phlobaphenes. Tannins are noncrystallizable compounds that, with water, form colloidal solutions possessing an acid reaction and a sharp puckering taste.

3.2. Microbiological assay

The microbiological assay shows that the different concentrations of pomegranate extracts were susceptible to test microorganisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger* and *Trichophyton mentagrophytes.* However, it was resistant to *Candida albicans* (6–8 mm) as summarize in Table 2. The inactivity of *Candida albicans* confirm the results of the investigations conducted by a researcher from Jordan (Abdollahzadeh et al., 2011). Methanolic and hydroalcoholic extracts of pomegranate fruit peels were resistant to *Candida albicans.* However, it contradicts the results of the study of another investigator from Brazil (Anibal et al., 2013) wherein the macerated ethanolic pomegranate peel extract was found susceptible to *Candida albicans*. The differences in the

Table 2. Results of average zone of inhibition in different concentrations against test microorganisms using a 6 mm size disk.

Microorganisms	Zone of inhibition (mm)															
	1 g/mL conc.				0.75 g/mL conc.				0.50 g/mL conc.				0.25 g/mL conc.			
	Ave.	Std. Dev.	Positive control	Negative control	Ave.	Std. Dev.	Positive control	Negative control	Ave.	Std. Dev.	Positive control	Negative control	Ave.	Std. Dev.	Positive control	Negative control
Staphylococcus aureus	28	0.58	40	0	21	0.58	40	0	18	0.58	40	0	14	0.58	40	0
Pseudomonas aeruginosa	22	1.53	25	0	18	1.00	25	0	17	1.00	25	0	31	1.53	25	0
Candida albicans	8	2.52	30	0	6	0.58	30	0	8	0.47	30	0	9	0.82	30	0
Aspergillus niger	22	1.00	27	0	20	1.00	27	0	21	1.00	27	0	17	0.58	27	0
Trichophyton mentagrophytes	23	0.82	25	0	21	2.00	25	0	22	0.58	25	0	17	2.52	25	0

Legend: positive control drugs (Neomycin for antibacterial and Miconazole for antifungal) Reference: Guevara, Q.B. 2005
negative control (Nutrient broth for bacteria and Sabouraud broth for fungi)
< 10 mm, inactive
10–13 mm, partially active
14–19 mm, active
> 19 mm, very active

antifungal activities of pomegranate fruit peel extracts among studies could be partially explained by variations in extraction methods, freshness of fruits, variations in the season and region of growth, strains sensitivity and antimicrobial procedures adopted in tests (Al-Zoreky, 2009; Opara et al., 2009). *Pseudomonas aeruginosa* (31 mm) showed a very interesting result because it has the greatest zones of inhibition even at the lowest concentration (0.25 g/mL) of the plant extract and greater than Neomycin (25 mm), the positive control. In this case, it minimizes the possible toxic effects produced by the plant extract. The present study confirmed the findings of scholars (Khan and Hanee, 2011) which revealed that the ethanolic pomegranate peel extract (25.5 mm) is more sensitive than Tetracycline (21.5 mm) against *Pseudomonas aeruginosa. Staphylococcus aureus*, *Aspergillus niger* and *Tricophyton mentagrophytes* were active (14–19 mm) to very active (> 19 mm) at the different concentrations of the plant extract. This present study correlated with the studies done by several researchers (Abdollahzadeh et al., 2011; Foss et al., 2014; Khan and Hanee, 2011). Extracts of aqueous, methanolic and ethanolic pomegranate peel were sensitive to *Staphylococcus aureus* whereas the zones of inhibition range from 16 mm to 25 mm. Moreover, its sensitivity can be attributed to the phyto-constituents present, specifically tannins, as confirmed and identified by several academics (Chebaibi and Filali, 2013; Rahimi et al., 2012; Rajan et al., 2011). Tannins exhibited antimicrobial action by precipitating proteins from solutions and combined with other 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 the therapeutic applications of tannins. They are employed in medicine as astringents in the gastrointestinal tract and on skin abrasions. In the treatment of burns, the proteins of the exposed tissues are precipitated and form a mildly antiseptic, protective coat under which the regeneration of new tissues may take place (Singh et al., 2002; Tyler, 1988).

3.3. Compatibility of excipients

Using a differential scanning calorimeter, 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). A baseline data of melting points of the extract and excipients were established and compared to test samples exposed at different conditions. Any sudden increase or decrease of melting points among the test samples constitutes an incompatibility.

Table 3 summarizes the melting points of materials at various conditions based on the DSC thermograms obtained. It was shown that the newly lyophilized extract melted at 117.10˚C and when exposed to lower (4˚C) and higher temperatures (40˚C), its melting point decreased to 83.13˚C and 86.62˚C, respectively. At 29˚C, its melting point increased to 123.48˚C. Based on the melting points obtained, it can be concluded that pomegranate lyophilized powder was sensitive to temperature both at lower and higher conditions, an indication of instability. This can be explained by the nature of the powder itself, since it was not isolated, purified and identified. Furthermore, when this pomegranate powder was combined with the intended excipients like polyethylene glycol, it showed the highest upsurge (139.72˚C) in temperature at 40˚C but remains stable at lower temperatures (4˚C, 29˚C), but within the baseline temperature of 59.10˚C. Likewise, pomegranate powder in combination with methyl paraben showed an escalation in melting temperature (131.11˚C) at 40˚C. At a higher temperature of 40˚C, it can be concluded that incompatibilities happened in the pomegranate extract, polyethylene glycol and methyl paraben. However, propyl paraben combined with pomegranate powder showed almost the same melting temperatures (99.61˚C, 99.87˚C, 99.82˚C) when exposed at varying conditions. It is similar to stearic acid combined with pomegranate powder wherein the melting points (62.01˚C, 60.53˚C, 60.52˚C) were almost identical at varying conditions.

Table 3. Melting points of materials at various conditions.

Materials	Newly Lyophilized	4ºC	29ºC	40ºC
Pomegranate powder	117.10	83.13	123.48	86.62
Methyl paraben	134.84	—	—	—
Propyl paraben	107.11	—	—	—
Stearic acid	63.97	—	—	—
Polyethylene glycol	68.43	—	—	—
Combination of pomegranate and methyl paraben	112.54	127.86	126.99	131.11
Combination of pomegranate and propyl paraben	89.10	99.61	99.87	99.82
Combination of pomegranate and polyethylene glycol (4000)	59.10	58.83	57.52	139.72
Combination of pomegranate and stearic acid	61.78	62.01	60.53	60.52
Combinations of all	59.43	57.10	57.38	57.23

Table 4. Summary of results of quality control tests of antimicrobial pomegranate ointments.

Test	Concentrations of water-based				Concentrations of oil-based				Reference
	20% Extract	40% Extract	60% Extract	80% Extract	20% Extract	40% Extract	60% Extract	80% Extract	
Visual appearance	Semisolid, no insoluble particle, foreign matter and dirt	Semisolid, no insoluble particle, foreign matter and dirt	Semisolid, no insoluble particle, foreign matter and dirt	Semisolid, no insoluble particle, foreign matter and dirt	Semisolid, no insoluble particle, foreign matter and dirt	Semisolid, no insoluble particle, foreign matter and dirt	Semisolid, no insoluble particle, foreign matter and dirt	Semisolid, no insoluble particle, foreign matter and dirt	Absence of insoluble particles, foreign matter and dirt
Color	Light brown	Brown	Dark brown	Very dark brown	Light brown	Brown	Dark brown	Very dark brown	Color palette
Odor	No odor	No odor	No odor	No odor	No odor	No odor	No odor	No odor	Absence of odor
Smoothness	No grittiness felt	No grittiness felt	No grittiness felt	Grittiness felt	No grittiness felt	No grittiness felt	No grittiness felt	Grittiness felt	Absence of grittiness
Spreadability (cm)	Ave. 2.6	Ave. 3.0	Ave. 4.9	Ave. 1.5	Ave. 3.4	Ave. 3.5	Ave. 4.1	Ave. 1.6	The bigger the area, the more spreadable the sample
pH (Ave.)	6.20	6.37	6.50	6.55	6.48	6.17	6.60	6.65	5–9
Viscosity (Cp)	62 000	61 500	65 900	46 600	60 400	61 200	62 900	54 500	< 90 000
Microbial assay Ave. zone of inhibition (mm)									< 10 mm, expressed as inactive
S. aureus	20	25	32	30	23	28	37	33	10–13 mm, partially active
P. aeruginosa	10	10	25	25	11	15	26	23	14–19 mm, active
C. albicans	13	17	20	19	10	15	22	19	> 19 mm, very active
T. mentagrophytes	15	17	27	25	12	19	20	23	
A. niger	10	12	19	15	9	11	17	15	
Dermal Irritation	—	—	0	—	—	—	0	—	

Dermal Irritation Reference

Erythema and eschar formation:		Edema formation:	
No erythema	0	No edema	0
Very slight erythema	1	Very slight edema (barely perceptible)	1
Well-defined erythema	2	Slight edema (edges of area well-defined by definite raising)	2
Moderate erythema	3	Moderate edema (raised approximately 1 mm)	3
Severe erythema	4	Severe edema (raised more than 1 mm and extending beyond the area of exposure	4

It can be concluded that there were no incompatibilities manifested. Interestingly, when the active ingredient and all excipients were combined, the melting point of the mixture at different conditions was within the baseline melting point of 59.43˚C. Hence, it was concluded that no incompatibility occurred; therefore, researchers proceeded to formulation.

Lastly, petrolatum was not tested for compatibility testing as it liquefies at a lower temperature (38ºC melting point) before the actual testing in the DSC equipment, forming soot in the pan and causing damage to the equipment. It was a common excipient found in ointment, hence the researchers concluded to be safe and stable for formulation. Moreover, material safety data sheet (MSDS) stated that petrolatum was biologically inert, not a skin sensitizer in guinea pigs and non-allergenic to humans.

3.4. Formulation and quality control of antimicrobial ointment

Based on the quality control test done, amongst the water-based ointments formulated, the 60% concentration of extract passed the test. Its attributes were the following: semisolid, dark brown, no odor, smooth ointment, pH of 6.55, spreadability of 4.9 cm, viscosity of 65,900 Cp, sensitive to test microorganism and did not produce irritation when tested on animal skin. Among the oil-based ointments formulated, the 60% concentration of extract passed the quality control test. Its characteristics were the following: semisolid, dark brown, no odor, smooth ointment, pH of 6.60, spreadability of 4.1 cm, viscosity of 62,900 Cp, sensitive to test microorganism and does not produce irritation when tested on animal skin. These characteristics are summarized in Table 4. Finally, these two formulations of ointments were prepared to precede the stability study.

3.5. Stability study

After exposure to the accelerated condition of 40ºC for 3 and 6 months, both ointments obtained the following characteristics: solidified, black in color, burnt odor, rough in texture and decreased zone of inhibition to almost zero which are indications that the ointments have undergone degradation physically, chemically and therapeutically.

The ointment is a semisolid dosage form intended for topical application; if it solidified and hardened, then the active drug will not be released on the site of application therefore causing a therapeutic failure. As it solidifies, zero viscosity and spreadability are obtained. Viscosity and spreadability are important for ointment attributes so that the product is readily applicable on the required site and to make the product aesthetically appealing.

The only attribute that the ointments passed was the pH. It remained stable in pH throughout the testing period. Overall, the 60% water-based and 60% oil-based ointments were unstable based on the degradation manifested by the formulations.

4. Conclusions

This research established that the alcoholic extract of pomegranate peel has antimicrobial property and can be formulated into water-based and oil-based antimicrobial ointments. The formulated ointment passed the quality control tests but needed further reformulation to improve product stability. Hence, future studies recommend the exploration of other excipients, formulation of other dosage forms and employment of additional methods of preparation.

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