RESEARCH PAPER

Preformulation and Formulation Studies of the Alcoholic Extract of Bauhinia purpurea L. Leaves as Antimicrobial Ointment

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Abstract

Bauhinia purpurea is a medicinal plant known for its diverse pharmacological activities, including antimicrobial effects. This study aimed to develop an antimicrobial ointment using the alcoholic extract of *B. purpurea* leaves. The investigation involved physicochemical characterization, antimicrobial evaluation, excipient compatibility studies, formulation development, quality control testing, and stability assessment. The extract appeared as a dark brown powder with a bark-like odor, unpleasant taste, acidic pH (~3), density lower than water (<1.0), and a melting point of 117.10°C. It exhibited solubility in 95% ethanol, 0.1 N HCl, 0.1 N NaOH, 0.9% NaCl, phosphate buffer solutions (pH 4, 7, and 10), and petroleum ether. Antimicrobial testing showed the highest activity against *Staphylococcus aureus* (28±2.03 mm), followed by *Trichophyton mentagrophytes* (23±1.87 mm), *Pseudomonas aeruginosa* (22±1.44 nm), and *Aspergillus niger* (22±1.98 mm), while minimal activity was observed against *Candida albicans* (8±0.06 mm). Although the formulated ointments met quality control requirements, further optimization is required to enhance stability. Future work should focus on alternative excipients, different dosage forms, and improved formulation strategies.

Keywords: Stability study, Preformulation, antimicrobial, *Bauhinia purpurea*, natural products

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Introduction

The Bauhinia purpurea L., leave has an antimicrobial property. Its fruit peel extract exhibits antibacterial activity against several bacteria like Streptococcus mutans, Streptococcus sanguis, Streptococcus mitis [1], Escherichia coli, Psuedomonas aeruginosa and Staphylococcus aureus [2]. Likewise, it is also very effective against several fungi such as Candida albicans [3], Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis and Microsporum gypseum [4]. In fact, its antimicrobial activity is attributed to several phytoconstituents present in the fruit exocarp such as punicalagin, ellagitannins and punicalagin [5] and phenolic punicalagins, gallic acid, fatty acids, catechin, quercitin, rutin, flavonols, flavones, flavonones and anthocyanidins [6]. Considering the dearth of information on Bauhinia purpurea, 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 Bauhinia purpurea 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 research 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 [7].

2. Material and methods

2.1. Materials

The *Bauhinia purpurea* L. leaves were collected from Neelakanta Agrahara village regions of Malur Taluk, Kolar district of Karnataka, India. The plant specimens were authenticated by the Department of Pharmacognosy, East Point College of Pharmacy, Bangalore, India with the Reg. No: EPCP/M.Pharm/2020-2021. Hard paraffin, white soft paraffin, cetostearyl alcohol, and wool fat were procured from S D Fine Chem Ltd, Maharashtra, India. EDTA (disodium salt) was obtained from AVA Chemicals Pvt. Ltd. Maharashtra, India. Paraformaldehyde and xylene solution were purchased from Merck Life Science Pvt. Ltd. Maharashtra, India. Hematoxylin and eosin (H&E) staining kit was obtained from HiMedia Laboratories Pvt. Ltd. Maharashtra, India. Pure analytical grade chemicals and reagents were used for this research.

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, physicochemical 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.

2.3. Extraction of B. purpurea L.

Green formulative process is the sustainable and eco-friendly formulation of local ointments from the extracts of plants without the use of chemical additives or toxic chemicals. In the present work, B. purpurea L. leaves were powdered and passed through a sieve (10/40) and shade-dried and hygienically collected. Ethanolic extract of B. purpurea was prepared by Soxhlet extraction as per the previous literature [8]. 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. This eco-friendly formula ensures that the bioactive ingredients like flavonoids, tannins, and phenolic acids responsible for their antioxidant, anti-inflammatory, and healing properties are preserved and provide less environmental pollution and chemical residues.

2.4. Physicochemical evaluation

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

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 extract 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 extract immediately dissolved with the solvent, the amount of the solvent to be added was decreased.

Melting point was determined using differential scanning calorimetry (DSC-4000 Perkin Elmer, USA). About 5 mg of the extract 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 [8].

Screening test for tannins was determined qualitatively using the gelatin test and ferric chloride test as described by Guevara B. (2005) [9]. 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.

2.5. Anti-microbial assay

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 was 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 [10]. This test was performed inside the Biosafety Cabinet Level II (BC - Sanyo).

2.6. Compatibility test of excipients

The lyophilized extract and excipients were individually tested by differential scanning calorimetry (DSC-4000, Perkin Elmer, USA) as baseline data 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 (40+0.5 ºC , 29+0.5 ºC, and 4+0.5 ºC) 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 extract.

2.7. Formulation of ointment

Water-based and oil-based ointments were formulated through the mechanical incorporation method using mortar and pestle. 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 (2020).

Table 1. Various formulation of oil and water-based ointments

	Oil based concentration (%w/w)				**Water- based concentration (%w/w)**			
Percentage	**10 %**	**20 %**	**50 %**	**75 %**	**10 %**	**20 %**	**50 %**	**75 %**
Extract (g)	1.0	2.0	5.0	7.5	1.0	2.0	5.0	7.5
Propyl paraben (g)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Methyl paraben (g)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Stearic acid (g)	2.00	1.00	0.50	-	1.00	0.50	0.50	-
PEG (g)	-	-	-	-	3.00	2.00	0.50	0.50
NaoH (mL)	3.00	3.00	2.00	1.00	3.00	3.00	3.00	1.50
Petrolatum (g)	4.00	2.50	1.50	1.00	-	-	-	-

2.8. Quality control test

The following quality control tests were conducted in triplicate in accordance with the standards of USP 23/NF 18 (2020). Organoleptic test performed by 2 mg of the extract was placed in the watch glass with white background and observed for their physical properties such as visual appearance, color, odor and smoothness.

2.8.1. Spreadability test

It was carried out using two glass plates. About 500 mg of the extract 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 min. Afterwards, a 50 g flyweight of was placed at the center of the glass plate and the diameter of the circle was measured again after a min. The procedure was repeated by placing another 50 g until a weight of 1000 g was achieved (Briedis and Sznitowska, 2011). The sets of weights were calibrated prior to testing.

2.8.2. pH and Viscosity

A 1% solution of ointment was prepared, and its pH was determined by immersing the electrodes of a pH meter. Viscosity was measured by Brookfield Viscometer (Model: VS-CRA-14S).

2.9. Anti-microbial test

Because the active ingredient was of organic origin, a total microbial count test was performed to determine the microbial contamination of the extracts. Tryptic soy agar was used as the media. The extracts were compared to positive control utilizing Staphylococcus aureus ATCC 29213 and Pseudomonas aureginosa ATCC 27853 as test microorganisms and negative

control. 5 mL of the extract 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 to 48 hours and the microbial contamination was counted. Each extract was run in triplicate.

2.10. Sensitivity test of the ointment

This test utilized the Modified Dermal Irritation for Rabbits found in OECD guideline # 404. A certificate of Animal Research Permit was obtained from the Bureau of Animal Industry prior to the conduct of the study. The formulated Bauhinia purpurea 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.

2.11. Stability study

An accelerated stability study was conducted on the formulated antimicrobial ointment in accordance with 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. Preformulation outcome

The lyophilized extract was a dark brown colored powder with like bark odor, disagreeable taste, acidic (pH 5.7), heavier than water (> 1.0 density), melts at 118.6 ºC 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 and 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 and found in confirmatory tests [11]. 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. Bacterial sensibility

The microbiological assay shows that the different concentrations of Bauhinia purpurea extracts were susceptible to test microorganisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger and Trichophyton mentagrophytes*. However, it was resistant to *Candida albicans* (6 ±1.08–9±1.01 mm) as summarize in Table 2. The inactivity of *Candida albicans* confirm the results of the investigations conducted by a researcher from Jordan [12]. Methanolic and hydroalcoholic extracts of Bauhinia purpurea were resistant to Candida albicans. However, it contradicts the results of the study of another investigator from Brazil wherein the macerated ethanolic Bauhinia purpurea extract was found susceptible to Candida albicans. The differences in the antifungal activities of Bauhinia purpurea 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. *Pseudomonas aeruginosa* (22±1.44 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 slightly lesser than Neomycin (25 ±2.81 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 which revealed that the ethanolic Bauhinia purpurea extract (25.7 ±2.21 mm) is more sensitive than Tetracycline (18.8 ±1.88 mm) against *Pseudomonas aeruginosa*. Staphylococcus aureus, *Aspergillus niger* and *Tricophyton mentagrophytes* were active (17 ±1.78–23±1.87 mm) to very active (> 16 mm) at the different concentrations of the plant extract. This present study correlated with the studies done by several researchers [13]. Extracts of aqueous, methanolic and ethanolic Bauhinia purpurea were sensitive to *Staphylococcus aureus* whereas the zones of inhibition range from 14±1.53 mm to 28±2.03 mm. Moreover, its sensitivity can be attributed to the

phytoconstituents present, specifically tannins, as confirmed and identified by several academics [14,15]. 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 regenera tion of new tissues may take place [16,17].

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

Microorganisms	0.25 g/mL	0.5 g/mL	0.75 g/mL	1g/mL	Positive control	Negative control
Pseudomonas aeruginosa	17 ±1.21	17 ±1.43	18 ±1.81	22± 1.44	25 ±2.81	0
Staphylococcus aureus	14± 1.53	18± 1.53	21± 1.57	28± 2.03	40 ±2.41	0
Aspergillus niger	17 ±1.09	20 ±1.87	21 ±1.65	22± 1.98	27 ±1.19	0
Candida albicans	9±1.01	8 ±1.78	6 ±1.08	8±0.06	30 ±2.77	0
Trichophyton mentagrophytes	17 ±1.78	22± 1.76	21 ±1.99	23± 1.87	25 ±1.92	0

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 [18]. A baseline data of melting points of the extract and excipients were established and compared to test extracts exposed at different conditions. Any sudden increase or decrease of melting points among the test extracts 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 120.28 ± 3.67 ˚C and when exposed to lower (4 ±˚C) and higher temperatures (40 ±˚C), its melting point decreased to 85.74 ± 2.26 ˚C and 82.41 ± 2.32˚C, respectively. At 29˚C, its melting point increased to 120.01 ± 3.72 ˚C. Based on the melting points obtained, it can be concluded that Bauhinia purpurea 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 Bauhinia purpurea powder was combined with the intended excipients like polyethylene glycol, it showed the highest upsurge (60.62 ± 1.09˚C) in temperature at 40˚C but remains stable at lower temperatures (4˚C, 29˚C), but within the baseline temperature of 57.04 ± 0.51˚C. Likewise, Bauhinia purpurea powder in combination with methyl paraben showed an escalation in melting temperature (129.54 ± 1.32˚C) at 40˚C. At a higher temperature of 40˚C, it can be concluded that incompatibilities happened in the pome granite extract, polyethylene glycol and methyl paraben. However, propyl paraben combined with Bauhinia purpurea powder showed almost the same melting temperatures (100.03 ± 1.32˚C, 101.62 ± 2.32˚C, 101.76 ± 2.32˚C) when exposed at varying conditions. It is like stearic acid combined with Bauhinia purpurea powder wherein the melting points (59.75 ± 0.35˚C, 58.19 ± 0.67˚C, 59.91± 0.63˚C) were almost identical at varying conditions.

Table 3. Melting points of materials at various stress conditions

Materials	4+0.5 °C	29+0.5 °C	40+0.5 °C	Newly lyophilised
Extract	82.41 ± 2.32	120.01 ± 3.72	85.74 ± 2.26	120.28 ± 3.67
Propyl paraben	-	-	-	129.11 ± 0.32
Methyl paraben	-	-	-	128.46 ± 0.51
Stearic acid	-	-	-	64.15 ± 0.18
PEG 5000	-	-	-	69.11 ±0.11
Extrac + Propyl paraben	100.03 ± 1.32	101.62 ± 2.32	101.76 ± 2.32	90.28 ±0.02

Extrac + Methyl paraben	130.71 ± 2.32	124.17 ± 1.32	129.54 ± 1.32	109.87 ±1.11
Extrac + Stearic acid	59.75 ± 0.35	58.19 ± 0.67	59.91± 0.63	59.16 ± 1.31
Extrac + PEG	57.04 ± 0.51	56.26 ± 1.89	140.53 ± 0.76	60.62 ± 1.09
Combinations of all	55.28 ± 0.57	55.89 ± 0.39	54.83 ± 0.39	60.97 ± 1.03

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 60.97 ± 1.03˚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 (39 ± 0.23ºC 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. Quality control test for ointment

Based on the quality control test done, amongst the oil-based ointments formulated, the 50% concentration of extract passed the test [19]. Its attributes were the following: semisolid, dark brown, no odor, smooth ointment, pH of 5.39 ± 0.71, spread ability of 3.4 ± 0.21cm, viscosity of 60 400 ± 345.71Cp, sensitive to test microorganism and did not produce irritation when tested on animal skin. Among the water-based ointments formulated, the 50% concentration of extract passed the quality control test. Its characteristics were the following: semisolid, dark brown, no odor, smooth ointment, pH of 5.20 ± 0.18, spreadability of 4.89 ± 0.16cm, viscosity of 62 000 ± 432.12 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.

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

	Oil based concentration (%w/w)				**Water- based concentration (%w/w)**			
Test	**10 %**	**20 %**	**50 %**	**75 %**	**10 %**	**20 %**	**50 %**	**75 %**
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
color	Light brown	Brown	Dark brown	Dark brown	Light brown	Brown	Dark brown	Dark brown
Odor	No odor	No odor	No odor	No odor	No odor	No odor	No odor	No odor
Smoothness	No grittiness	No grittiness	No grittiness	Grittiness felt	No grittiness	No grittiness	No grittiness	Grittiness felt
pH	5.39 ± 0.71	5.30 ± 0.20	5.71 ± 0.19	5.75 ± 0.11	5.20 ± 0.18	5.38 ± 0.17	5.54 ± 0.14	5.72 ± 0.17
Spreadability	3.4 ± 0.21	3.42 ± 0.32	3.67 ± 0.13	4.24 ± 0.14	4.89 ± 0.16	3.02 ± 0.17	4.08 ± 0.11	1.50 ± 0.11

Viscosity	6040 ± 345.71	6120 ± 371.78	6290 ± 402.71	5450 ± 409.34	6200 ± 432.12	6150 ± 413.54	6590 ± 422.32	4660 ± 413.43
Microbial assay	ZOI							
Pseudomonas aeruginosa	23 ±2.81	28 ±1.81	36 ±1.44	33 ±2.81	20 ±1.21	25 ±1.43	32 ±1.81	30 ±1.44
Staphylococcus aureus	11 ±2.41	15 ±1.57	28 ±2.03	23 ±2.41	11 ±1.53	10 ±1.53	25 ±1.57	25 ±2.03
Aspergillus niger	10 ±1.19	16 ±1.65	22 ±1.98	19 ±1.19	14 ±1.09	17 ±1.87	27 ±1.65	19 ±1.98
Candida albicans	12 ±2.77	19 ±1.08	20 ±0.06	23 ±2.77	15 ±1.01	18 ±1.78	26 ±1.08	25 ±0.06
Trichophyton mentagrophytes	9 ±1.92	11 ±1.99	17 ±1.87	15 ±1.92	10 ±1.78	12 ±1.76	20 ±1.99	16 ±1.87

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 [20]. 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 Bauhinia purpurea 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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