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Development of Anti-bacterial Ointment from Two Extracts of *Curcuma longa* L. and *Aloe vera* L.

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Authors' contributions

This work was carried out in collaboration between all authors. Author AKA designed the study, performed the statistical analysis wrote the protocol and the first draft of the manuscript. Authors NMN, LAA, AAQ, AAS, Adnan Al-Serry and SAZ managed the analyses of the study, the literature searches and sample collection and analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim of this Study: This study aimed to extract, formulate and evaluate an antibacterial ointment from extracts of two Yemeni plants.
Methods: The extraction of both *Curcuma longa* and *Aloe vera* was performed using alcoholic and hydro-alcoholic solvents, respectively. The in-vitro antibacterial activity of *C. longa* and *A. vera* extracts was investigated using well agar diffusion and disc diffusion method against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. Finally, the ointment was prepared from a combination of these two extracts then stability study testing was performed and also the in-vitro activity antibacterial of this ointment against the above bacteria was done.
Results: The extracts inhibited the growth of both bacteria with zone of inhibition between 6 mm-14mm at 0.52 mg-20 mg/100 µl. The MIC of *C. longa* and *A. vera* extract were 0.25 mg/100 µl and

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0.4 mg/100 µl respectively in *P. aeruginosa* and 0.25 mg/100 µl in *S. aureus* for both extracts. The prepared ointment showed satisfactory physicochemical results and the antibacterial activity was 20 mm and 7 mm for *P. aeruginosa* and *S. aureus,* respectively. The pH was in a proper range (pH 5.2-5.5). The formulation was found to be stable during stability study according to ICH guideline (25 ± 2℃/65 ± 5% RH and 40 ± 2℃/75 ± 5% RH) for o ne month. The assay test of active ingredient was done using UV -Visible spectrophotometry. Curcumin content was 59.70% to 59.78% in ointment. Aloin assay was 81 to 81.21% in the prepared ointment.

Conclusion: These finding showed that the formulated new antibacterial ointment is stable and economic formula and consider as database for further clinical studies in spite of both plants used safely in large doses in traditional medicine.

Keywords: Curcuma longa; Aloe vera; antibacterial; formulation; stability testing.

1. INTRODUCTION

Curcuma longa rhizome and *Aloe vera* leaf and juice extract are known to have significant antibacterial properties [1]. This is because of the presence of active compounds, mainly Curcumin [2] and Aloin [3].

Curcuma longa (Turmeric) is the dried rhizome of *Curcuma longa* (*Zingiberaceae*) [4]. Curcumin, the principle curcuminoid found in *Curcuma longa*, is well known for its multiple pharmacological and biological properties [5,6]. Curcumin also possesses potent antibacterial activity against a widely range of bacterial [7-9]. Alcohol extract and the essential oil of *C. longa* were shown in one study to inhibit the growth of most microorganisms including bacteria and fungi [10]. Curcuma showed improvement of the antibacterial activities for antibiotics, markedly Gentamicin, Amikacin, Ciprofloxacin, Cefixime, Cephotaxime, Vancomycin and Tetracycline [11,12].

Aloe vera is a perennial plant with yellow flowers and though, freshly triangular or spear-like leaves arising in a rosette configuration. Studies of the–vitro antimicrobial properties of the ethanolic extract of *A. vera* leaf gel revealed that it was active against most of the studied pathogenic bacteria and fungi, even at very low doses [13,14].

Both plants used widely, in traditional medicine, topically to treat the skin diseases and orally as spices and these plants are affordable and easily accessible and there is no evidence of bacterial resistance to whole-plant extracts.

The concept of poly-herbalism to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity [15,16].

Aloe vera and *Curcuma longa* chloroform and methanolic extracts combinations showed a synergistic effect against multi-drug resistant *E. coli* [17].

The present study was conducted to develop antibacterial ointment of poly-herbal extract of two Yemeni plants to present an economic, stable and effective formula with less or no bacterial resistance.

2. MATERIALS AND METHODS

2.1 Materials

Aloin and Curcumin obtained as a gift from Central Laboratory for Drugs Quality in Yemen.

2.2 Study Area

This study was performed in Al-Nasser University, Department of Pharmacy. The drying process was done in Sana'a University, Faculty of Pharmacy while the accelerated stability study was carried out in Global Pharmaceutical MFG. CO., Sana'a, Yemen.

2.3 Collection of Plant Samples

Curcuma longa rhizomes were bought from the local Yemeni market. *Curcuma longa* rhizomes were washed then dried under shade for 5 days. *Aloe vera* leaves were collected from Al-Ghaeel, Hamdan, Yemen and washed with water then sterilization by ethanol.

2.4 Extraction of *Curcuma longa* Rhizomes and *Aloe vera* leaves

2.4.1 Extraction of *Curcuma longa*

The extract of *C. longa* was prepared by maceration method [18]. This was by immersing 1 kg of dried powder of *C. longa* in 5 liters 96% ethanol and mixed by a shaker with 200 rotates per minute at room temperature for 2 days. The extract was filtered through Whitman filter paper No.1 then concentrated to semisolid brown colored mass by evaporating ethanol using a rotary evaporator (BUCHI rotavapor R-200, Germany), then dried by freeze dryer (CRYODOS-45, Spain).

2.4.2 Extraction of *Aloe vera*

The extract of *Aloe vera* was produced by maceration method as follow: 6 kg of *A. vera* leaves was immersed with 10 liters of 90% ethanol and 10% H_2O at room temperature for three days with shaking. The extract was filtered then concentrated to solid dark brown colored mass by evaporating the solvent using a rotary evaporator, then dried by freeze dryer.

2.5 Qualitative Analysis

2.5.1 Phytochemical evaluation of crude extract

2.5.1.1 A. Phytochemical evaluation of Curcuma longa and Aloe vera extracts

The phytochemical tests of *Curcuma longa* employed for alkaloids, tannins, saponins, flavonoids, terpenoids and phenol were applied as stated in [4,19]. Phytochemical screening of *Aloe vera* for alkaloids, terpenoids, flavonoids, tannins, anthraquinones and saponins were carried out as described below [20,21].

2.5.1.1.1 Test for Alkaloids

About 20 mg of the extract was dissolved in 2 ml of ethanol then few drops of 1% HCl was added. The mixture was heated, kept in steam and after cooling, the mixture was treated with few drops of Wagner's reagent.

2.5.1.1.2 Test for Tannins

About 20 mg of the extract was dissolved in 1 ml of distilled water in a test tube and 1-3 drops of 1% $FeCl_3$ were added.

2.5.1.1.3 Test for Saponins

40 mg of the extract was dissolved with 5 ml of distilled water and shaken vigorously till a stable persistent froth was obtained, the froth mixed with three drops of olive oil and shaken vigorously.

2.5.1.1.4 Test of Flavonoids

20 mg of the extract was dissolved in 1ml of distilled water 0.5 ml of dilute ammonia solution was added, after that concentrated H_2SO_4 was added.

2.5.1.1.5 Test for Terpenoids

20 mg of the extract was dissolved in 1ml of chloroform and 1ml of concentrated H_2SO_4 was added.

2.5.1.1.6 Test for phenol

To the extract 2 ml of 2% $FeCl_3$ was added.

2.5.1.1.7 Test for Steroids

0.5 g of the extract was dissolved in 3 ml of chloroform and filtered. Concentrated H_2SO_4 was carefully added to the filtrate. A reddish brown colour at interphase indicates a steroid ring.

2.5.1.1.8 Test for *Anthraquinones*

3 ml of the aqueous extract was boiled with 3 ml of H_2SO_4 and filtered while hot. 3 ml of benzene was added to filtered and shaken. The benzene layer was separated and 3 ml of 10% NH_3 added.

2.5.2 Thin layer chromatography (TLC) test for *Curcuma longa extract*

Separation of curcuminoids by TLC was done using TLC plate coated with silica gel, and chloroform and methanol (95:5) as mobile phase, after development, the plates were removed and dried and spots were visualized in ultraviolet light and retardation factor values (R_f) were calculated [22].

2.5.3 Thin layer chromatography (TLC) test for *Aloe vera extract*

Test solution was prepared by dissolving 0.25 g of dried extract in 20 ml of methanol R and heat to boiling in a water bath. Shake for a few minutes and decant the solution. Mobile phase

was a mixture of water R, methanol R, and ethyl acetate R (13:17:100 (V/V/V)). After development, the plates were removed and dried, then sprayed with a 100 g/l solution of potassium hydroxide R in methanol R and examine in ultraviolet light at 365 nm [23]. Finally, (R_f) values was calculated.

2.5.4 Antimicrobial activity testing for the extracts

Pseudomonas aeruginosa and *Staphylococcus aureus* used for the present investigation were obtained from MEDLAB laboratory, Sana'a, Yemen, the two bacteria were sub-cultured individually on nutrient agar plates and blood agar plates; then incubated for 24 hr.

2.5.4.1 Preparation of media

The required amount of Mueller Hinton agar was measured and prepared according to manufacturer's instructions and poured into conical flasks and covered by wrapping with aluminum foil then sterilized by autoclaving at 121℃ for 15 minutes. The media was allowed to solidify in petri-dishes. A sterile swab was used to pick the bacteria into the appropriate solidified media.

2.5.4.2 Preparation of Curcuma longa extract dilutions

A stock solution of *C. longa* extract was prepared by dissolving 5 g in 50 ml volumetric flask with 96% ethanol to get 1 mg/1 ml working stock solution. Whitman's filter paper was punched into 5mm disc form then sterilized. A sterile discs was incorporated individually with different concentration of *C. longa* extract (0.1 mg/100 µl, 0.250 mg/100 µl, 0.500 mg/100 µl, 0750 mg/ 100 µl, 1 mg/100 µl, 2 mg/100 µl and 4 mg/100 µl) using a micropipette. After sometime another doses of extracts were applied on disc. The disc were allowed to saturate and dry in air then used after drying then the in-vitro antibacterial test was performed.

2.5.4.3 Preparation of Aloe vera extract discs

A stock solution of *A. vera* extract was prepare by dissolving 10 g in 50 ml volumetric flask with 96% ethanol then filtered to get a stock working solution of 1 mg/1 ml. Whitman's filter paper was punched into 5 mm disc form then sterilized. A sterile discs was incorporated individually with different concentration of *Aloe vera* extract (0.1 mg/100 µl, 0.250 mg/100 µl, 0.500 mg/100 µl, 0.750 mg/100 µl, 1mg/100 µl, 2 mg/100 µl, 4 mg/100 µl, 10 mg/100 µl, 20 mg/100 µl) using a micropipette. After sometime another doses of extracts were applied on disc. The disc were allowed to saturate and dry in air then used after drying then the in-vitro antibacterial test was performed.

2.5.4.4 Antimicrobial assay of Curcuma longa extract

Antibacterial activity of *Curcuma longa* was tested by agar well diffusion method. Wells of 6 mm in diameter were made using a sterile borer. The well were filled with 100 µl with the solutions with different concentrations as the following 0.1 mg, 0.25 mg, 0.50 mg, 0.75 mg, 1 mg, 2 mg and 4 mg, respectively. The plates were incubated at 37℃ for 24 hr. The results were compared with standard Ciprofloxacin discs 5 µg and Tobramycin discs 10µg as positive control. The negative control was set up in parallel using the solvent that was used to reconstitute the extract. Antimicrobial activity was determined by measuring the zone of inhibition around each well.

2.5.4.5 Antimicrobial assay of Aloe vera extract

The antibacterial studies were carried out by disc diffusion technique. Previously prepared sterile discs of *Aloe vera* extract with concentrations of 0.1 mg, 0.250 mg, 0.500 mg, 0.750 mg, 1 mg, 2 mg, 4 mg, 10 mg and 20 mg were placed on plates. The plates were incubated at 37℃ for 24 hr. The effects were compared with standard Ciprofloxacin discs 5µg and Tobramycin discs 10 µg as positive control. Also the negative control was used. Antimicrobial activity was determined by measuring the zone of inhibition around each disc. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was recorded.

2.6 Antimicrobial Assay of the Final Product

Muller Hinton agar was prepared by dispersing 19 gm in 500 ml of D.W. then boiled and sterilized by autoclave. Small amount of *A. vera* and *C. longa* extract's ointment, were used at MIC 0.25%. Each well was incorporated with 40µl of *A. vera* and *C. longa* extract's ointment for each bacteria. These plates were incubated for 24 hours at 37℃ for bacteria.

2.7 Quantitative Test

2.7.1 *Ultra-violet spectrophotometry test for curcuma extract

2.7.1.1 Preparation of standard stock solution and calibration curve for Curcuma longa

Standard stock solution was prepared by dissolving 10 mg of *curcumin* in 30 ml ethanol then filtrate into 100 ml volumetric flask. The final volume was made up to 100 ml with ethanol to get the working standard stock solution of each 100 µg/ml. From stock solution series of solutions with the following concentration 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml were prepared. The absorbance was measured by UV-Visible spectrophotometer at 421 nm [24]. The procedure was repeated three times for each concentration.

2.7.1.2 Preparation of standard stock solution and calibration curve for Aloe vera

Stock solution was prepared by dissolving 10 mg of *Aloe vera* extract in 30 ml ethanol then filtrated into 100 ml volumetric flask. Final volume was made up to 100 ml with ethanol to get working standard stock solution of each 100 µg/ml. From stock solution series of solutions with the following concentration 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml were prepared. The absorbance was measured by UV-Visible spectrophotometer at 262.5 nm [25]. The procedure was repeated three times for each concentration.

2.7.1.3 Preparation of ointment sample solution

Stock solution was prepared by dissolving 4 g of ointment formulation in 50 ml ethanol then filtered into 100 ml volumetric flask. The final volume was made up to 100 ml with ethanol to get working stock solution of each 100 µg/ml. From stock solution series of solutions with the following concentration 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml were prepared. Then absorbance of curcumin was measured by UV-Visible spectrophotometer at 421 nm [24] and the absorbance of aloin was measured by UV-Visible spectrophotometry at 262.5 nm [25]. The procedure was repeated three times for each concentration.

2.7.1.4 Curcuma longa and Aloe vera ointment formulation

Different ointment formulas were prepared using different ointment bases with different concentrations. The formula in Table (1) was the best formula by different physicochemical properties of ointment formulations.

Table 1. The ingredients and their quantities used in ointment formula

Ingredient	Quantity
Aloe vera extract	0.25%
Curcuma longa extract	0.25%
Ethanol (96%)	5%
Almond oil	5%
With soft paraffin	90%

2.8 Procedure of Ointment Preparation

The required quantity of soft paraffin 90 g was completely melt on water bath. 5 ml of Almond oil was added with mixing. Finally, 0.25 mg of *A. vera* extract and 0.25 mg of *C. long* extract (MIC) were dissolved in 5ml of ethanol then added and mixed well until a homogeneous ointment was obtained.

2.9 Accelerated Stability Study for Ointment

Stability studies were carried out for formulation (ointment) according to International Conference on Harmonization (ICH) guidelines. Samples were kept at different storage conditions i.e. at 25℃ and at 40℃ with 75% RH (Relative Humidity) with intensive light in stability chambers, and observed for a period of one month at definite time intervals. A sufficient quantity of formula in suitable containers was stored in oven under 40±2℃ and 75%±5% RH for one month and all physical and chemical tests were done for the samples weekly through one month [26].

2.10 Quality Control of the Final Product

2.10.1 Physical evaluation

The prepared formula was evaluated for physical properties by using usual methods including viscosity, homogeneity and grittiness tests were done by using small quantity of product was pressed between the thumb and the index finger and by physical observation. The consistency and the texture of ointment were noticed. A small quantity of the sample was rubbed on the skin of the back of the hand to determine the homogeneity and spreadability. The spreadability is very important as show the behavior of ointment come out from the tube [27].

2.10.2 Drug content assay

Assay tests was done to *C. longa* and *A. vera* ointment formula using UV-Visible spectrophotometer.

2.10.3 Microbial test

The formulated ointment was inoculated on the plates of Muller Hinton agar media (was prepared according to manufacturer's instruction) by dispersing 38 gm in1000 ml of distilled water and heat to boiling then sterilize by autoclaving by streak plate method and a control was prepared by omitting the preparations. The plates were placed into the incubator then incubated at 37℃ for 24 hours for bacteria, at 32℃ for 3 days for fungi and the result was recorded. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control [28].

2.10.4 Packaging of the final product

Ointment was packed in dark collapsible plastic tube because *Aloe vera* is sensitive to light.

3. RESULTS AND DISCUSSION

3.1 A. Qualitative Analysis

3.1.1 Results of phytochemical evaluation of *Curcuma longa* extract

The results of preliminary phytochemical evaluation of *A. vera* and *C. longa* extract are summarized in Table 2.

Table 2. The results of phytochemical evaluation of *A. vera* and *C. longa* extract

Constituents	Result	
	***Aloe vera* extract**	***Curcuma longa* extract**
Flavonoids	+	+
Anthraquinones	+	-
Terpenoids	_	+
Steroids	+	-
Alkaloids	+	+
Tannins	+	+
Saponins	_	+
Phenolic compounds	+	+

The previous Table (2) showed the results of applied phytochemical tests for different constituents of *C. longa* extract which were positive results and indicate the presence of the active constituents of curcuminoids. Also indicated the presence of alkaloids, tannins, phenolic compounds, terpenoids, saponins and flavonoids components [29]. Also about the results of applied phytochemical tests for constituents of *A. vera* extract which positive results except saponins and terpenoids, they were negative results. The absence of saponins was reported [30], while the presence of alkaloids, tannins, terpenoids and flavonoids were confirmed. Another study reported the presence of alkaloid, saponins, tannins, flavonoids, and steroids [20]. These variations in results may be due to the difference in the extraction method, solvent used, the season of collection, or even the geographic source of plant, which may lead to different results [31].

3.1.2 Result of TLC of *Curcuma longa* extract

The result of TLC is illustrated in Table 3. The R_f value intense spot obtained with test solution was matching with R_f value of the standard curcumin [32]. The results of TLC study confirmed that the isolated compound is curcumin.

Table 3. The results of TLC analysis of *Curcuma longa* extract

Sample	$R_f$value
Standard curcumin	Spot = 0.7
Test sample of *Curcuma longa*	Spot = 0.77

3.1.3 Result of TLC of *Aloe vera* extract

The result of TLC of *A. vera* extract is illustrated in Table 4. The chromatogram obtained with the test solution shows in the central part a yellow fluorescent zone [23].

Table 4. The results of TLC analysis of *Aloe vera* extract

Sample	R_f value
Test sample of *Aloe vera* extract	Spot 1 = 0.28 Spot 2 = 0.26 Spot 3 = 0.39 Spot 4 = 0.4

The R_f value intense spot obtained with test solution was compared with R_f value of the Aloin R_f which was reported in [21] with R_f value of 4.6, while the R_f value 0.28 and 0.26 in Table 4 are related to Aloinoside A and B.

3.1.4 Results of antimicrobial test

3.1.4.1 Results of antimicrobial test for Curcuma longa extract

The anti-bacterial activity of *C. longa* extract was evaluated against *S. aureus* and *P. aeruginosa* by measuring the diameters of the zones of inhibition. It was found that *C. longa* extract is effective against *S. aureus* and *P. aeruginosa*. The measurements of zone of inhibition to somehow varied in *S. aureus* and *P. aeruginosa*. Maximum zone of inhibition was seen is 14 mm with the concentration of 4 mg/100 µl in bacteria *S. aureus* and 12 mm with the concentration of 4 mg/100 µl in *P. aeruginosa* Table 5 and the minimum inhibition zone of inhibition was 7mm with concentration of 0.250 mg/100 µl in *P. aeruginosa* and 8mm with concentration of 0.250 mg /100 µl in *S. aureus* Table 5. This is because of many constituents including curcumin which possesses potent antibacterial activity against a wide range of bacteria [5,6]. The methanol extract of *C. longa* revealed MIC values of 16 µg/mL and 128 µg/ml against *B. subtilis* and *S. aureus*, respectively [33]. The study of hexane and ethanol *C. longa* extract and curcuminoids (from ethyl acetate extract of curcuminoids isolated from *C. longa* with 86.5% curcumin value) against 24 pathogenic bacteria isolated from the chicken and shrimp showed the highest antimicrobial activity for ethanol extract with the MIC value of 3.91 to 125 PPT [34], However, curcuminoids elicited inhibitory activities against 8 bacteria of *S. agalactiae*, *S. intermedius*, *S. epidermidis*, *S. aureus*, *A. hydrophila, B. subtilis, B. cereus,* and *E. tarda*. Curcumin also exhibited inhibitory activity on methicillin resistant *S. aureus* strain (MRSA) with MIC value of 125-250 µg/ml [35]. The in vitro investigation of 3 new compound of curcumin, namely, indium curcumin, indium diacetyl-curcumin, and diacetylcurcumin, against *S. aureus*, *S. epidermis, E. coli*, and *P. aeruginosa* revealed that indium curcumin had a better antibacterial effect compared to curcumin itself and if may be a good compound for further in vivo studied. However, diacetyl-curcumin did not exhibit any antibacterial effect against tested bacteria [36].

3.1.5 Results of antimicrobial test for *Aloe vera* extract

The anti-bacterial activity of *Aloe vera* leaf extract was evaluated against *S. aureus* and *P. aeruginosa* by measuring the diameters of the zones of inhibition. It was found that *A. vera* extract is effective against *S. aureus* and *P. aeruginosa*. The measurements of zone of inhibition varied in *S. aureus* and *P. aeruginosa*. Maximum zone of inhibition was seen is 12 mm with the concentration of 20 mg/100 µl in both bacteria *S. aureus* and *P. aeruginosa* Table 6 and the minimum inhibition zone of inhibition was 6mm with concentration of 4mg/100µl in *P. aeruginosa* and 6mm with concentration of 0.250 mg/100 µl in *S. aureus* Table 6. The leaf extract used contains almost all the active constituents such as anthraquinones, saponins etc. which have antibacterial property. Studies have proved that the leaf extracts includes whole leaf components such as anthraquinones [37], dihydroxy-anthraquinones, and saponins which have direct antibacterial properties. This fact supports our study. Maximum antibacterial activity of *Bacillus cereus* was seen in methanol, ethanol and acetone gel extracts of *Aloe barbadensis* [38] observed no antimicrobial activity in the aqueous extract of *A. vera* leaves. Most of the studies have been carried out in the *Aloe vera*juice extract with organic solvents. It was also found that the gel and *Aloe vera* juice were effective against Gram positive bacteria such as *S. aureus* [39], *M. smegmatis, S. aureus, E. faecalis, M. luteus* and *B. sphericus* [40], Gram negative bacterias such as *E. coli, S. flexneri* [41], *P. aeruginosa*, *K. pneumonniae, S. typhimurium* [41], *Proteus spp* [42]. Reports are done on the fact that the *S. aureus*isolated from the wound infections is susceptible to both *A. vera* gel and ethanolic leaf extracts while *P. aeruginosa* was susceptible to *A. vera* ethanolic leaf extracts only [43].

Aloe vera and *Curcuma longa* chloroform and methanolic extracts combinations showed a synergistic effect against multi-drug resistant *E. coli* [17].

Table 5. The antimicrobial activity of *Curcuma longa* extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Concentration of extract (mg/100µl)	Zone of inhibition (mm)	
	P. aeruginosa	*S. aureus*
0.10	-	-
0.25	-	08
0.50	07	12
1.00	09	12
2.00	11	13
4.00	12	14
Standard Ciprofloxacin	09	15
Standard Tobramycin	12	10

Table 6. The antimicrobial activity of *Aloe vera* extract and the minimum inhibitory concentration (MIC)

Concentration of extract (mg/100µl)	Zone of inhibition (mm)	
	P. aeruginosa	*S. aureus*
0.10	-	-
0.25	-	06
0.50	-	06
01	-	07
02	-	07
04	-	08
10	08	09
20	12	12
Standard Ciprofloxacin	27	28
Standard Tobramycin	20	21

3.1.6 Results of antibacterial assay of final ointment

The ointment formula were tested against both bacteria that used in this study by well diffusion method and the results showed in Table 7.

Table 7. shows the antibacterial assay of *A. vera* and *C. longa* extract's ointment

Bacteria	*S. aureus*	*P. euroginosa*
Zone of inhibition	7mm	20mm

The minimum inhibition concentration of both extracts individually is approximately 0.25%. At this concentration both extracts showed that *Staphylococcus aureus* (gram positive bacteria) is more sensitive than *Pseudomonas euroginosa* (gram negative bacteria), but the combination extract in the prepared ointment formula showed that *Pseudomonas euroginosa* is more sensitive than *Staphylococcus aureus* with zone of inhibition 20 mm and 7 mm, respectively.

3.1.7 Results of quantitative tests

3.1.7.1 Result of UV-Visible spectrophotometer of Curcuma longa

3.1.7.1.1 Calibration curve of Curcumin standard

From the result in Fig. 1 which illustrated the calibration of the UV-Visiblespectrophotometer with working standard Curcuminand $r^2 = 0.999$.

3.1.7.1.2 Curcumin in ointment product

Assay test of Curcumin content in ointment formula:

Assay of Curcumin in ointment formula was calculated using the following formula:

Assay of curcumin = (abs. of sample/abs. of standard × conc. of standard /conc. of sample) x100 = (0.11/0.184×10/10) 100 = 59.78%

From the result obtained from Fig. 1, it was found that Curcumin obeys linearity within the concentration range of 10µg/ml to 50µg/ml and coefficient correlation was found to be 0.999. The regression of the curve was Y=0.008x+0.007. P-value is 0.001, correlation is significant at the 0.01 level. The assay of Curcumin in the ointment formula is 59.78%.

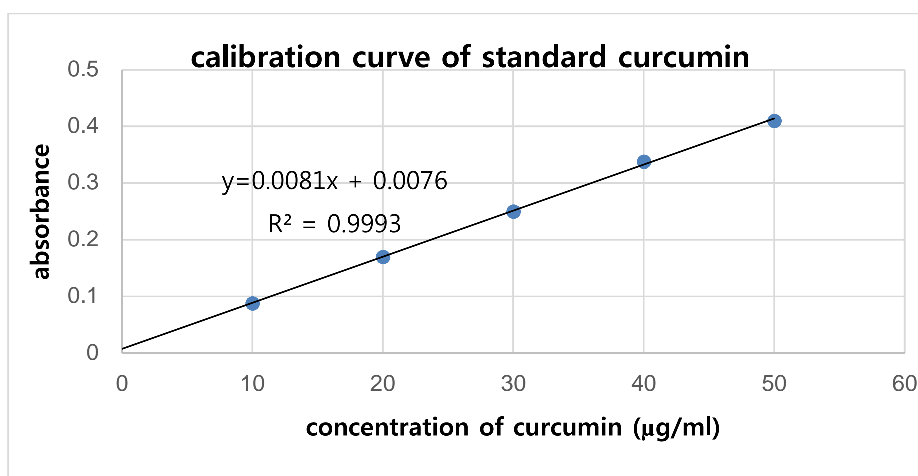

Fig. 1. Calibration curve of standard curucmin

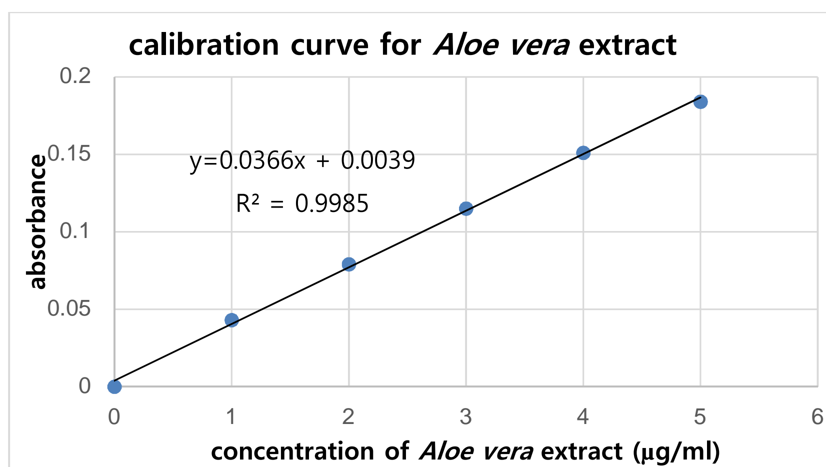

Fig. 2. Calibration curve of *Aloe vera* extract

3.1.7.2 Result of UV-Visible spectrophotometer of Aloe vera extract

3.1.7.2.1 Calibration curve of aloinstandard

From the result in Fig. 2 which illustrated the calibration of the UV-Visible spectrophotometer with working *Aloe vera* stock solution and r^2 = 0.998.

3.1.7.2.2 Aloin in *Aloe vera* ointment formula

Assay test of Aloin content in ointment formula:

Assay of Aloin in ointment formula was calculated using the following formula:

Assay of Aloin = (abs. of sample / abs. of standard × conc. of standard / conc. of sample) 100= (0.108/0.133 × 10/10) 100 = 81.21%

From the result obtained from Fig. 2, it was found that Aloin obeys linearity within the concentration range of 10µg/ml to 50 µg/ml and coefficient correlation was found to be 0.998. P- value is 0.000, correlation is significant at the 0.01 level. The regression of the curve was Y=0.036x+0.003. The assay of Aloin in the ointment formula is 81.21%.

3.1.7.3 Result of evaluation of sample dosage forms

3.1.7.3.1 Result of accelerated stability study

The formulated ointment was prepared and subjected to various parameters to be evaluated. The results have shown that the ointment maintained the same appearance and good feel on application after the accelerated

Table 8. The results of *C. longa* and *A. vera* ointment preparation under control and stressed conditions at different time points

Time period	Condi-tions	Curcumin Assay%	Aloin Assay%	Parameters					
				PH	Viscosity	Color	Spread-ability	Homog-eneity	Odor
0 day	RC	81%	59.71%	5.3	Good	Light orange	Good	Good	NC
	SC	81.08%	59.71%	5.2	Good	Light orange	Good	Good	NC
1st week	RC	81.12%	59.70%	5.5	Good	Light orange	Good	Good	NC
	SC	81.13%	59.73%	5.4	Good	Light orange	Good	Good	NC
2nd week	RC	81.13%	59.75%	5.3	Good	Light orange	Good	Good	NC
	SC	81.18%	59.78%	5.5	Good	Light orange	Good	Good	NC
3rd week	RC	81.17%	59.73%	5.4	Good	Light orange	Good	Good	NC
	SC	81.21%	59.74%	5.5	Good	Light orange	Good	Good	NC
4th week	RC	81.16%	59.73%	5.4	Good	Light orange	Good	Good	NC
	SC	81.20%	59.75%	5.5	Good	Light orange	Good	Good	NC

**NC.: No Change. *RC.: Room conditions (25℃, 65% relative humidity) *SC.: Stress conditions (40℃, 75±5% relative humidity)*

stability study performed. The initial viscosity of the prepared formula showed constant stability in comparison with after accelerated stability study performed. The pH of human skin typically ranges from 4.0 to 6.0. Therefore, the formula intended for skin application should have pH closer to this range (pH 5.2-5.5). In this study, there are no significant changes in formulated ointment. The active ingredient of *Curcuma longa* (Curcumin) and *A. vera* (Aloin) in ointment preparation showed good results. It was unchanged with time with range from 59.70% to 59.78% for Curucminin 0.25% *C. longa* extract and 80.21% to 81% for Aloin in 0.25% *A. vera* extract ointment.

3.1.8 Microbial test for the final product

The prepared ointment formula was conformed the pharmacopoeia requirements of microbial test (not more than 102 C.F.U. /ml for fungi and not more than 103 C.F.U./ml for bacteria) under suitable aerobic and anaerobic conditions.

4. CONCLUSION

The phytochemical analysis of ethanolic extract of *C. longa* and the hydroalcoholic extract of *A. vera* exhibited the presence of various phytochemicals including, tannins, phenolic compounds, terpenoids, saponins and flavonoids, but saponins and terpenoids were absent in *A. vera* extract. This study showed that the extract of *C. longa* and *A. vera* possess antibacterial activity. As a result it could be estimated that the plants chosen for antibacterial study has a good range of activity against *S. aureus* and *P. aeruginosa*. Thus, the formulations have been made. *A. vera* and *C. longa* antibacterial ointment. The prepared formulations showed physical and chemical stability during the study period. Stability parameters like visual appearance, texture, viscosity, spreadability and pH values of the formulations showed no significant variation during the study period. UV-Visible spectrophotometry of plants extracts and formulations confirm the presence of the active ingredients (Curcumin and Aloin) in high amounts. The formulation were found to be stable during stability study according to ICH guidelines (25 ±2℃/ 65 ±5% RH and 40±2℃/75 ±5% RH) for one month [26,44].

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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