Research Article

Preparation of Herbal Antibacterial Ointment for the Treatment of Diabetic Foot Ulcer

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ABSTRACT

In contrast to chemotherapeutic medicines, which are primarily derived from plants, antibiotics are primarily obtained from microorganisms**.** Herbal medicine is the medical use of any plant's seeds, berries, roots, bark, leaves, fruits, or flowers. Herbal medications are also available in ointment form, in addition to conventional dosage forms. An ointment is a semisolid viscous fluid that is applied topically to various body surfaces. The primary purpose of this study was to develop and test an antibacterial diabetic foot ulcer ointment made from herbal plants. Herbal medications have grown in importance on a global scale, both medically and economically. This herbal ointment is more effective than manufactured drugs, which have some side effects. The alcoholic extracts of the selected plants and fruits were consumed in varied ratios at random, and antimicrobial tests were performed on the combinations. The most efficient combination was then established by comparing the zone of inhibition provided by the various extract ratios on *Staphylococcus aureus, Pseudomonas sp., Escherichia coli, and Enterococcus sp.* The powerful combination's minimum inhibitory concentration was then determined. The ointment base was made, and the ointment was formulated by combining the active components in the most effective ratio in the base. After the formulation was completed, was evaluated in terms of spreadability, stability, antimicrobial testing, etc.

Keywords: Isolation, Minimum Inhibitory Concentration, Herbal antimicrobial Ointment formulation, Spreadability, Antimicrobial testing.

INTRODUCTION

Diabetic foot ulcer (DFU) is an open sore or wound that occurs on the feet of people with diabetes. It is a serious complication of diabetes that can lead to infections and even amputations if left untreated[1].

Diabetic foot ulcers are caused by a combination of factors, including nerve damage (neuropathy), poor circulation, and high blood sugar levels. These factors can cause small cuts or blisters on the feet to become infected and not heal properly, leading to the development of a foot ulcer. People with diabetes should take steps to prevent foot ulcers by regularly checking their feet for any cuts or wounds, wearing comfortable shoes that fit well, and controlling their blood sugar levels. If a foot ulcer develops, it is important to seek prompt medical attention to prevent complications[2]. Diabetic foot ulcers are caused by a combination of factors that are related to diabetes. Here are some of the most common causes:

Neuropathy: People with diabetes can develop nerve damage (neuropathy) that can lead to loss of feeling in the feet.

Peripheral arterial disease: Diabetes can also cause poor blood flow to the feet due to damage to the blood vessels.

High blood sugar levels: High blood sugar levels can damage the blood vessels and nerves in the feet, making it more difficult for the body to heal wounds.

Foot deformities: Foot deformities, such as bunions or hammertoes, can increase the pressure on certain areas of the feet, increasing the risk of developing ulcers.

Poor foot care: Not taking proper care of the feet, such as not washing them regularly or wearing poorly fitting shoes, can also increase the risk of developing foot ulcers[1,3].

But researchers have introduced different herbal medicines and ointments that are too much effective for DFU. An herbal ointment is a topical preparation made from natural plant-based ingredients that are believed to have therapeutic properties. Herbal ointments typically contain a mixture of plant extracts, oils, and other natural ingredients that are known for their healing properties. Herbal ointments are often preferred by people who prefer natural remedies and are looking for alternative to synthetic or chemical-based products. Herbal medicine is the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medical reasons. Herbal medications are also available in ointment form, in addition to conventional dosage forms. An ointment is a viscous semisolid substance that is used topically on various bodily surfaces. The antibacterial tests of the combinations were performed using ethanolic extracts of the chosen plants in

various ratios[4]. Following that, the most effective combination was established by comparing the zone of inhibition provided by the 4 various extract ratios against *Staphylococcus aureus*, *Escherichia coli, Pseudomonas sp., and Enterococcus sp.*

MATERIALS AND METHODS

Isolation of pathogenic Microorganisms

Guru Nanak Institute of Dental Science and Research, Panihati, Sodepur, was used to segregate sewage water. Then the sewage water was streaked separately into different agar plates. Those plates contain EMB agar and Pseudomonas agar. *Staphylococcus aureus* was isolated from pure milk and it was streaked in MSA (Mannitol Salt Agar). Then plates were incubated for 24 hours at 37^0C. Two distinct golden colonies were identified and separated from the MSA plate (which had become yellow), and pure cultures of *Staphylococcus aureus* were produced in nutrient broth. Furthermore, two colonies with a green metallic sheen were recovered from EMB agar plates, and pure cultures of *Escherichia coli* were produced in nutrient broth. Similarly, pure cultures of *Pseudomonas sp.* were prepared. And the pure culture of *Enterococcus sp.* was given by Guru Nanak Institute of Pharmaceutical Science and Technology, Panihati, Sodepur.

Determination of Multidrug Resistance pattern among the isolated pathogens

It was observed that the pathogen was resistance to Amoxicillin, Cefixime, Azithromycin, and Tetracycline in isolated pathogens of *Escherichia coli, Enterococcus sp., Pseudomonas sp*., and *Staphylococcus aureus*. Muller Hinton agar plates were used for antibiotic susceptibility testing[5]. Antibiotic concentrations of 4mg/ml, 8mg/ml, 12mg/ml, 16mg/ml, and 20mg/ml were generated, with special care paid to the MIC_{90} values specified by the 2020 Clinical and Laboratory Standards Institute recommendations. Muller Hinton agar plates were utilized for antibiotic susceptibility testing. All the experiments were done in triplets.

Collection of plants and fruits samples

Carica papaya Linn*., Centella asiatica* Linn. were collected from Guru Nanak college campus, Panihati Sodepur, *Phyllanthus emblica,* and *Citrus sinensis* were bought from nearby market. Then *Citrus sinensis* peels were separated. Before extracting bioactive chemicals, all gathered leaves were air dried, while fruits and peels were sun-dried for 7-10 days.

Extraction, Isolation of plants and fruits samples –

The collected *C. papaya, C. asiatica* Linn*, P.emblica,* and *C. sinensis* peels were ground into a fine powder using the electronic blender. Then 50gm of each powder was soaked in different solvents. *C. sinensis* peel and *P. emblica* powders were soaked in methanol (100ml). *C. papaya* Linn. and *C. asiatica* Linn powder were soaked in acetone (100ml). Maceration is the method chosen for preparing plants and fruit extract. All the different mixtures were allowed to stand on a shaker at a speed of 180rpm for 3-4 hours for 5 days. After 5 days the mixtures were filtered through Whatman filter paper. After that, the different liquids were concentrated in a rotary vacuum evaporator and considered crude extracts. These extracts were weighed and kept in airtight vials at 4^0 C for future research[6].

Determination of the antimicrobial activity of the extracts

At first, all the crude extracts were prepared into the liquid for the MIC test. The agar well diffusion technique was used to test the effects of all extracts on bacteria. All four bacterial species including *Pseudomonas sp*., *Staphylococcus aureus*, *Escherichia coli,* and *Enterococcus sp.* were used for detecting the activity. *Citrus sinensis* peel extract against *Escherichia coli*., *Phyllanthus emblica* extracts against *Pseudomonas* sp., *Centella asiatica* Linn extract against *Enterococcus* sp. and *Carica papaya* linn extract against *S. aureus*. Muller Hinton agar plates were used for extract susceptibility testing. The plates were incubated at 37^0C for 24 hours. Plates were removed from the incubator after incubation, and the inhibition zone was noted. The experiments were carried out three times.

Quantitative Phytochemical Screening

Quantitative phytochemical screenings of all four extracts were carried out in accordance with conventional techniques to determine the total flavonoids and tannins each.

Determination of total Flavonoids content

The total flavonoid content was assessed using the minor modification approach. The preferred concentration of all extracts was measured. Then for this particular test, all solutions were added with determined concentration and uniformly mixed, all the incubation process was also done. Then the absorbance was measured at 510nm[7].

Determination of total Tannins content

Total tannin content was determined using tannic acid as a standard, and the method was slightly modified. The same concentrations of sample extracts were taken and with extracts were mixed the amount of freshly prepared 0.35% ferric ammonium citrate followed by 0.8% ammonia solution. The mixture's absorbance was then assessed at 525 nm[8].

Determination of concentration of the antimicrobial activity of extracts for Herbal ointment formulation -

Prepared different concentrations of each extract for comparing the results. The agar well diffusion method was used to test the effects of all extracts on bacteria, and all of the procedures have already been explained. There was used Dimethyl sulfoxide (DMSO) 2% as a control. The plates were incubated for 24 hours at 37°C. After incubation, plates were removed and the inhibition zone was measured. The experiments were carried out in triplicate.

Formulation of Ointment

Oil Phase Preparation:

Stearic acid and glyceryl monostearate were weighed using a weighing scale, then transferred to a 50 ml beaker. Liquid paraffin was then measured using a 10 ml graduated pipette, then poured into the beaker.

The beaker was then heated on a hot plate at 70-80° C to melt the solid material and ensure appropriate mixing. The oil phase is now ready.

Aqueous Phase Preparation –

- A graduated pipette was used to measure the desired amounts of glycerin and propylene glycol in a 50 ml beaker. Twenty ml of water was also added to the beaker.
- To ensure appropriate ingredient mixing, the beaker was then heated on a hot plate to a temperature of 70^0 to 80 °C.

Ointment Formation

The oil phase was then combined with the aqueous phase by gradually adding the oil phase to the aqueous phase while stirring continuously with a glass rod until the ointment was created. Then 1ml of each liquid extract added to that concentration, which was showed the maximum inhibition zone against responsible microorganisms. Then the ointment was then placed into an airtight container, and the ointment's assessment research was completed.

The physical appearance of ointment

I. **pH** – The pH of the prepared ointment was tested by dissolving 1gm ointment in 100ml of water and using a digital pH metre[10].

II. **Consistency** – The consistency of the prepared ointment was verified by hand. Take a pinch of ointment and rub it between fingers.

III. **Spreadability**–The spreadability of the prepared ointment was 300mg of ointment between two slides. A 50gm weight was placed on the upper slide. The weight was removed, and the excess formulation was scraped away. The bottom slides were secured to the apparatus's board, while the upper slide was secured with a nonflexible string to which a 20-gram load was applied. The time it took the slide to slip off was recorded[11].

Comparison of antimicrobial activity between Marketed ointment and Herbal ointment

This procedure involves melting the MSA, allowing it to cool at 45^0C, streaked *Pseudomonas* sp., *S. aureus*, *E .coli*, and *Enterococcus* sp. in individual sterile plates. In this method, after the agar plate has solidified, holes of about 90mm in diameter are made in the medium. Then the antimicrobial ointment is then placed in the well, and in another well, a commercial formulation is placed. The diameter of the zone of inhibition was then measured after inoculation at 37^0C for 2-3 days[12].

RESULTS

Table 1: Zone of inhibition of *E. coli* against different antibiotics

Concentrations of antibiotics (mg/ml)	Zone of inhibition of Azithromycin (mm)	Zone of inhibition of Amoxicillin (mm)	Zone of inhibition of Cefixime (mm)	Zone of inhibition of Tetracycline (mm)
4	13	0	12	15
8	15	0	25	16
12	19	0	37	24
16	29	0	43	37
20	30	15	61	46

Table 2: Zone of inhibition of *Pseudomonas sp.* against different antibiotics

Concentrations of antibiotics (mg/ml)	Zone of inhibition of Azithromycin (mm)	Zone of inhibition of Amoxicillin (mm)	Zone of inhibition of Cefixime (mm)	Zone of inhibition of Tetracycline (mm)
4	0	-	8	6
8	5	-	14	25
12	15	-	29	35
16	23	-	35	45
20	28	-	47	57

Table 3: Zone of inhibition of *enterococcus sp.* against different antibiotics

Concentrations of antibiotics (mg/ml)	Zone of inhibition of Azithromycin (mm)	Zone of inhibition of Amoxicillin (mm)	Zone of inhibition of Cefixime (mm)	Zone of inhibition of Tetracycline (mm)
4	0	0	-	11
8	0	9	-	17
12	0	12	-	23
16	11	16	-	29
20	14	21	-	34

Table 4: Zone of inhibition of *s. aureus* against different antibiotics

Concentrations of antibiotics (mg/ml)	Zone of inhibition of Azithromycin (mm)	Zone of inhibition of Amoxicillin (mm)	Zone of inhibition of Cefixime (mm)	Zone of inhibition of Tetracycline (mm)
4	-	-	29	7
8	-	-	39	14
12	-	-	48	24
16	-	-	59	32
20	-	-	68	40

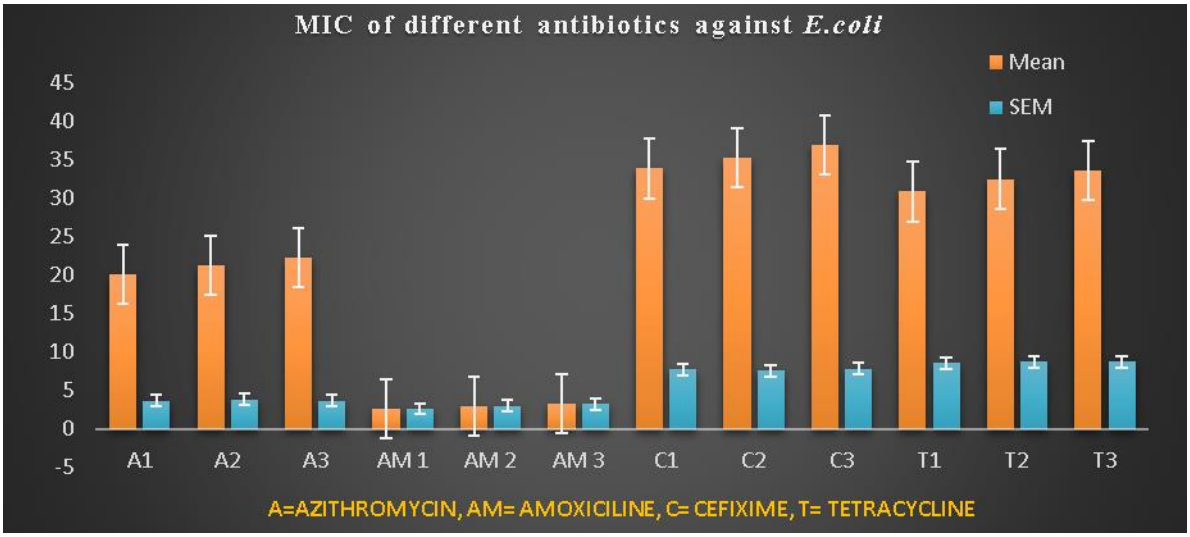

Figure 1: Comparison of the inhibition effect by MIC of different antibiotics against *E.coli* (For Azithromycin N =3, P value < 0.01), (For Amoxicillin N =3, P value < 0.01), (For Cefixime N =3, P value < 0.01), (For Teracycline N =3, P value < 0.01)

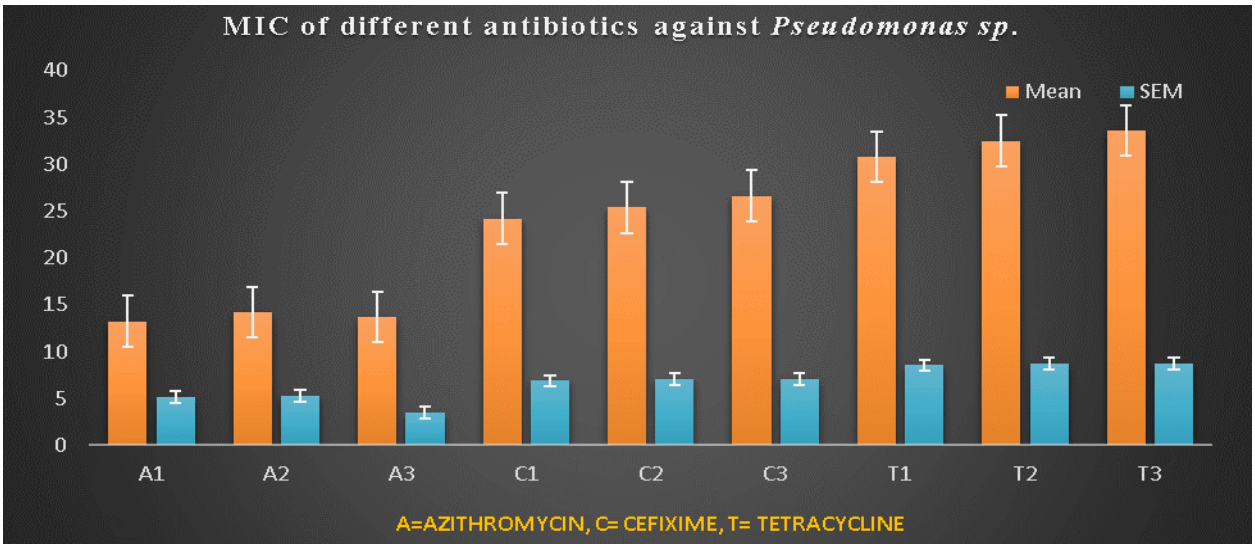

Figure 2: Comparison of the inhibition effect by MIC of different antibiotics against *Pseudomonas sp.* (For Azithromycin N =3, P value < 0.01), (For Cefixime N =3, P value < 0.01), (For Tetracycline N =3, P value < 0.01)

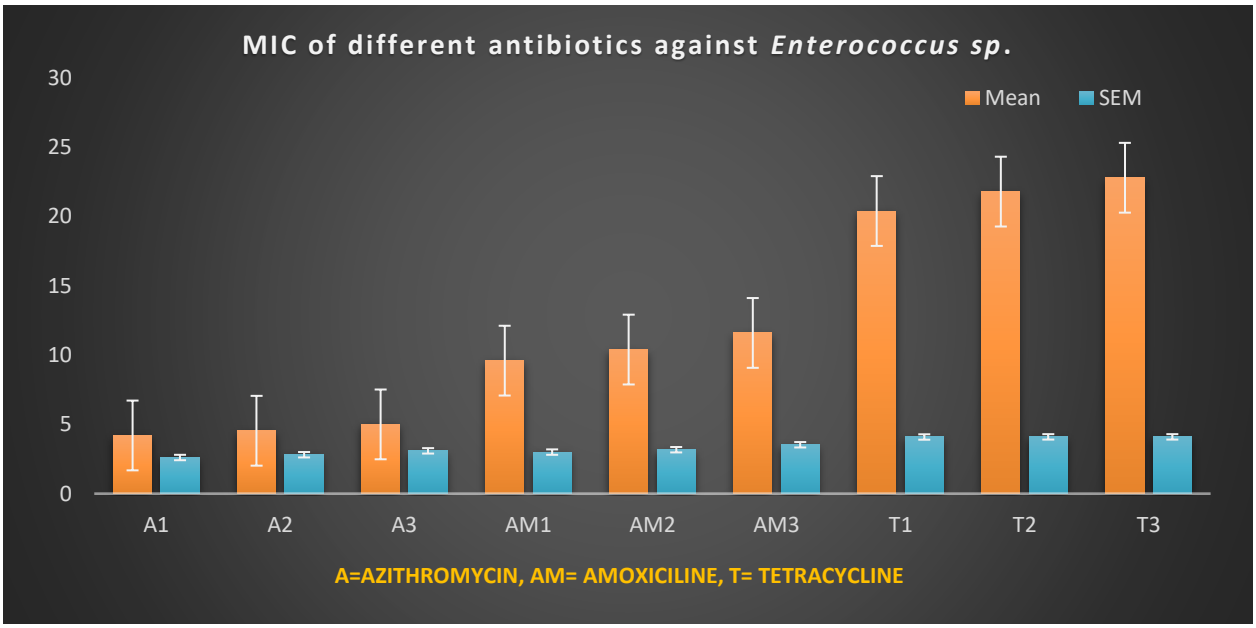

Figure 3: Comparison of the inhibition effect by MIC of different antibiotics against *Enterococcus sp.* (For Azithromycin N =3, P value < 0.05), (For Amoxicillin N =3, P value < 0.05), (For Tetracycline N =3, P value < 0.05)

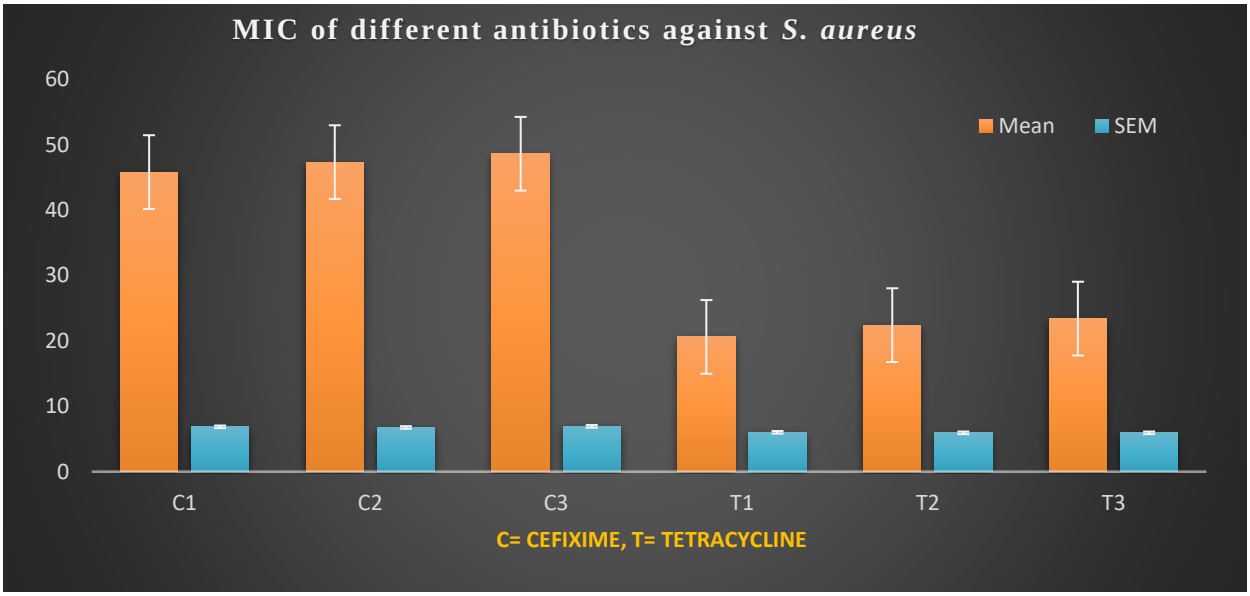

Figure 4: Comparison of the inhibition effect by MIC of different antibiotics against *Staphylococcus aureus* (For Cefixime N =3, P value <0.05), (For Tetracycline N =3, P value < 0.05)

Table 5: Screening of antimicrobial activity of plant extracts

Name of Extract	Concentrations of Extract (mg/ml)	Name of microorganisms	Zone of inhibition (mm)
P. emblica	200 250	*Pseudomonas sp*	210 260
C. Sinensis	200 250	*E. coli*	207 256
C. papaya	200 250	*S. aureus*	208 258
C. asiatica	200 250	*Enterococcus sp.*	206 257

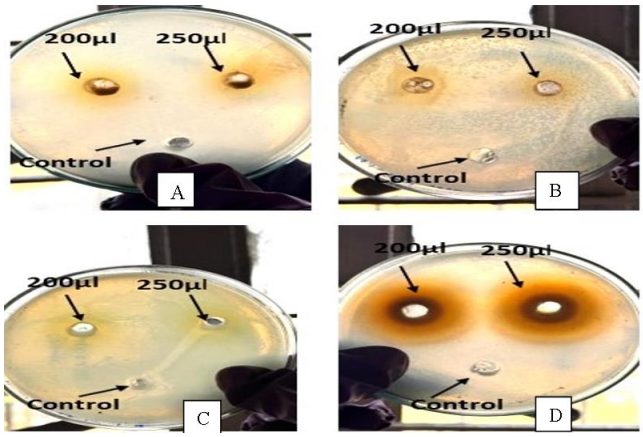

Figure 5: Screening of antimicrobial activity of plant extracts. A = *C. asiatica* against *Enterococcus sp*., B = *C. papaya* against *S. aureus*, C = *C. sinensis* against *E. coli*, D = *P. emblica* against *Pseudomonas sp.*

Phytochemical Analysis of Plant Extracts

Table 6: Total Flavonoids Content

Sample	Flavonoids Content (QE)/ gm dry extract
P. emblica 4000	0.112
P. emblica 8000	0.264
C. sinensis 4000	0.054
C. sinensis 8000	0.131
C. papaya 4000	0.043
C. papaya 8000	0.068
C. asiatica 4000	0.049
C. asiatica 8000	0.063

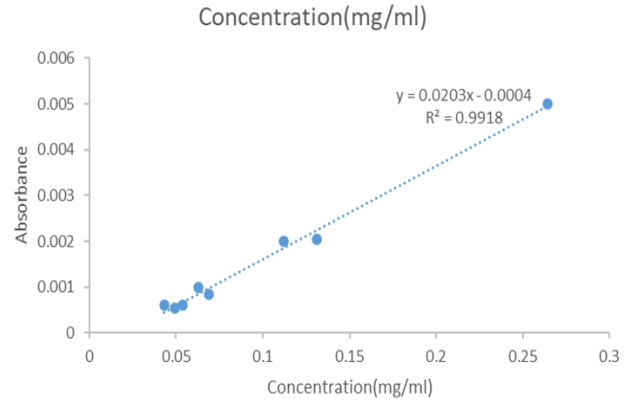

Figure 6: Determination of Flavonoids of different extracts

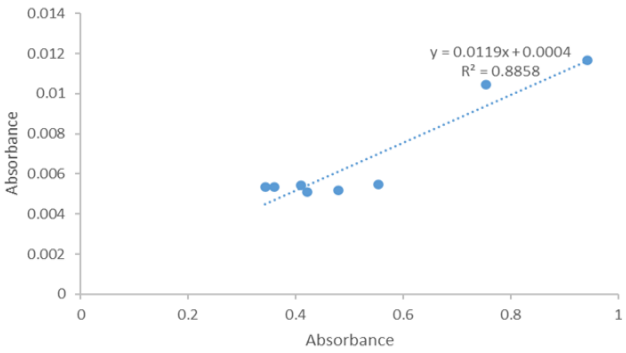

Figure 7: Determination of Tannins of different extracts

Table 7: Total Tannins Content

Sample	Tannin Content (TAE)/ gm dry extract
P. emblica 4000	0.754
P. emblica 8000	0.942
C. sinensis 4000	0.410
C. sinensis 8000	0.479
C. papaya 4000	0.421
C. papaya 8000	0.359
C. asiatica 4000	0.554
C. asiatica 8000	0.342

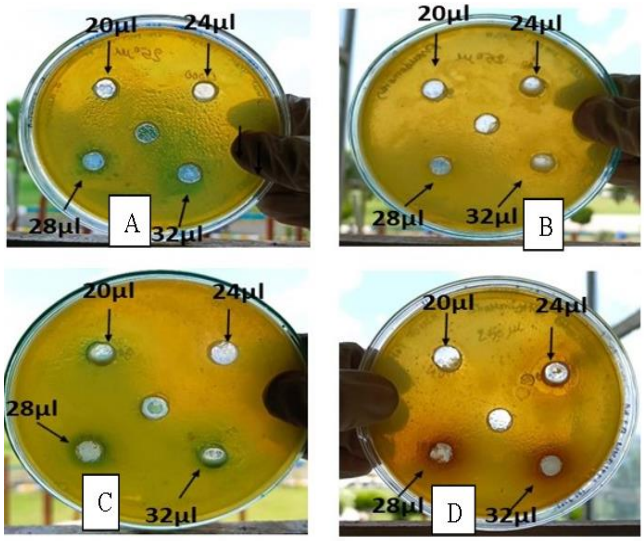

Figure 8: Determination of extracts concentration for ointment formulation. A = *C. papaya* against *S. aureus*, B = *C. asiatica* against *Enterococcus sp*., C = *C. sinensis* against *E. coli,* and D = *P. emblica* against *Pseudomonas sp.*

Figure 9: Preparation of Herbal ointment using *C. papaya* Linn, *C. sinensis* peel, *C. asiatica* Linn, and *P. emblica*

Table 8: Zone of inhibition of extracts for ointment formulation

Name of Extract	Concentrations of Extract (mg/ml)	Name of microorganisms	Zone of inhibition (mm)
P .emblica	20 24 28 32	*Pseudomonas sp*	25 30 36 42
C. sinensis	20 24 28 32	*E. coli*	24 29 35 44
C. papaya	20 24 28 32	*S. aureus*	26 31 37 43
C. asiatica	20 24 28 32	*Enterococcus sp.*	22 27 33 41

Evaluation Studies of Ointment

Evaluation Parameter	Observation
pH	6.23
Consistency	Smooth
Spreadability	Spreads easily

Table 9: Antimicrobial activity test of herbal ointment

Name of Microorganisms	Diameter of Zone of Inhibition	
	Standard (mm) (marketed ointment)	Test (mm) (Herbal ointments)
Pseudomonas sp.	15	11
Enterococcus sp.	14	12
S. aureus	17	13
E. coli	16	14

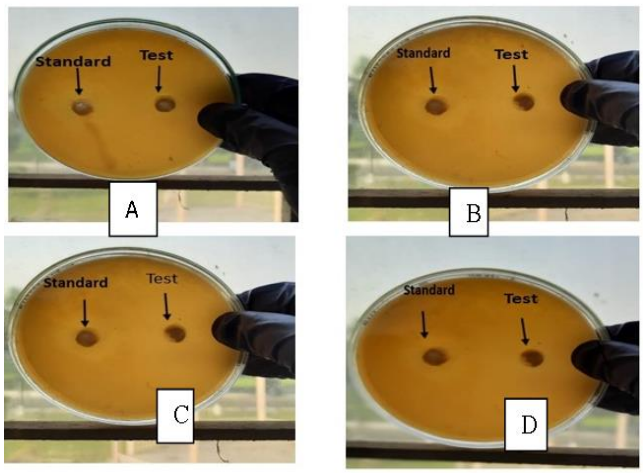

Figure 10: Antimicrobial activity test of Herbal Ointment. A = *S. aureus*, B = *E. coli*, C = *Enterococcus sp.*, D = *Pseudomonas sp.*

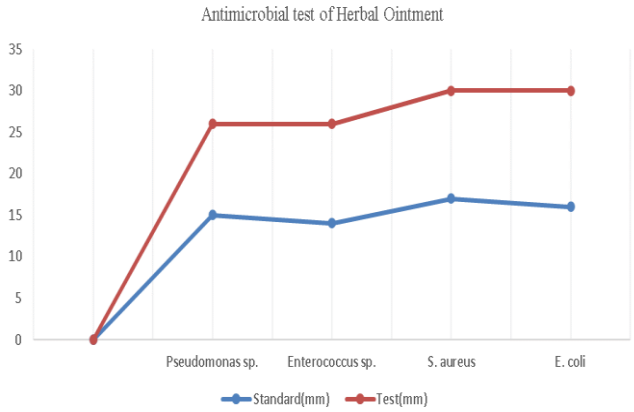

Figure 11: Representation of comparison between formulated Herbal Ointment and Marketed Ointment

DISCUSSION

As per **Table 1** cefixime shows highest anti-bacterial activity against *E.coli* with zone of inhibition 61mm against 20mg/ml concentration whereas **Table 2** reveals that Tetracycline elicit highest anti-bacterial activity against *P. aeruginosa* with ZOI 57mm at 20mg/ml. As per **Table 3** Tetracycline confer highest antibacterial property against *Enterococcus* with ZOI 34mm at 20 mg/ml and finally as per **Table 4** cefixime conferred highest antibacterial property against *S. aureus* with ZOI 68 mm at 20 mg/ml.

Comparing the antibiotic susceptibility of all the isolated pathogens against the selected antibiogram, as per **figure 1** MIC value for Azithromycin, Cefixime and Tetracycline is high with respect to standard MIC against *E.coli* as per CLSI guidelines, hence *E.coli* is resistant to Azithromycin, Cefixime and Tetracycline. As per **Figure 2** MIC of different antibiotics against *Pseudomonas* sp. shows resistance to Azithromycin, Cefixime and Tetracycline. As per figure 4 ***S aureus*** is resistant to Cefixime and Tetracycline. **Table 5** shows the antibacterial activity of 4 plant extracts against

the selected pathogens. Total flavonoid content and total tannin content was determined for the screened as per **Table 6** and **Table 7. Table 8** revealed the zone of inhibition of screened plant extract against the isolated pathogen and further **Table 9** revealed that herbal ointment is potentially effective with respect to marketed ointment and can be further developed for diabetic foot disease.

CONCLUSION

Herbal ointment is viscous semi-solid preparation, formed by the combination of different plant extracts. The herbal ointment has higher patient compliance because of its long-term healing capacity and lack of adverse effects. The demand for herbal preparations is rising right now on the global market. The primary goal of the herbal antimicrobial and antibacterial diabetic patient's ointment formulation was to cure or treat diabetic foot ulcers and injuries. It was concluded that the foot ulcer healing ointment which is prepared from natural sources has no side effects as compared to ointments which are prepared from synthetic compounds. This herbal ointment can kill particular microorganisms, which are responsible for diabetic foot ulcers. The wound healing ointment was examined using several parameters and determined to be suitable for application to the foot of diabetic patients where the ulcer occurred.

FUTURE ASPECTS

For diabetic foot ulcers continued research and clinical trials are essential to validate the efficacy and safety of herbal ointment. This includes evaluating the ointment's antimicrobial properties, wound healing effects, compatibility with conventional treatments, and long-term outcomes.

It is important to identify the active ingredients in herbs that contribute to the antibacterial properties of the ointment. Standardization of herbal extracts ensures consistency and quality control in the production of the ointment. Future research may explore innovative delivery systems or combination therapies that enhance the ointment's efficacy.

Formulation optimization can also help improve patient compliance by ensuring ease of application and a pleasant user experience. Future research could explore personalized medicine approaches, such as identifying biomarkers or genetic factors that impact treatment response. Increasing awareness among healthcare professionals, patients, and the general public about the potential benefits and appropriate use of herbal antibacterial ointments is important.

Compare the effectiveness of the herbal antibacterial ointment with conventional treatments such as antibiotics or standard wound care protocols. Comparative studies can help establish the superiority or non-inferiority of the herbal ointment in terms of wound healing, reduction in infection rates, and other relevant outcomes.

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