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DEVELOPMENT OF WOUND HEALING OINTMENT AND PRODUCT FORMULATION OF SOAP AND SANITIZER

Yuvasri.P*[1], Dr. N. Vanitha[2]

Department of microbiology, Hindusthan college of arts and science, Coimbatore, Tamilnadu, India – 641028[1,2]

Corresponding author*

Research highlights

- *Tridax procumbens, Curcuma aromatic* & *Aloe vera* has antibacterial activity, antifungal activity, antioxidant activity and anti-inflammatory properties.
- The bioactive compound from *Tridax procumbens, Curcuma aromatic* & *Aloe* vera are used in treatment of various alignments, such as burns, skin disease, diabetes, and foot ulcers.
- *Aloe vera* gels, Turmeric powder and Tridax plant are used in ointments, cosmetics, such as soap, shampoo, face wash, hair oil and moisturizer.
- *Aloe vera* is a natural moisturizer and its anti-microbial properties makes it perfect for use in hand sanitizers.

Abstract: The goals of using herbal plants as sources of therapeutic agents depends on many forms such as, bioactive compounds that can be isolated for direct use as drugs, or to produce bioactive compounds as pharmacological tools or to use the whole plant or part of it as a herbal remedy. The basic herbs have the answer with no side effects and effective remedies and the golden fact is use of herbal treatment is independent of any age group. When two or more herbs are used in the formulation they are known as polyherbal formulations. The traditional plant drugs are beneficial for several skin related problems and for wound healing. The present study has been undertaken to establish the antibacterial activity of Tridax procumbens, Curcuma aromatic, Aloe barbedensis. Herbal medicines are plant based medicines made from differing combinations of plant parts. E.g. leaves, flowers and roots. Each part can have different medicinal uses. Tridax procumbens, *C.aromatica and Aloe barbedensis* has been used for healing dermal wound. This is rich in alkaloids, steroids, carotenoids, flavonoids (such as catechins, centaurein and bergenins), fatty acids, phytosterols, tannins and minerals. It possesses anticoagulant, antioxidants, antiseptic, and antimicrobial.

Keywords: Tridax procumbens, Curcuma aromatica, Aloe barbadensis, tropical ointment, polyherbal ointment formulation, soap formulation, sanitizer formulation, antibacterial activity.

1.0 INTRODUCTION

Wound is a physical trauma where the skin is torn, cut or punctured. On exposure to air, microorganisms enter the wound which leads to wound contamination and finally development of infection.[1] Herbal medicines are plant based medicines made from differing combinations of plant parts. E.g. leaves, flowers and roots. Each part can have different medicinal uses. Tridax procumbens has been used for healing dermal wound. This is rich in alkaloids, steroids, carotenoids, flavonoids (such as catechins, centaurein and bergenins), fatty acids, phytosterols, tannins and minerals. It possesses anticoagulant, antioxidants, antiseptic, and antimicrobial.[2]

Tridax procumbens is a very promising species that produces secondary metabolites reported to have a variety of medicinal uses including among others, anti-anemic, anti-inflammatory, anti-diabetic and anesthetic properties. This species has a long history of traditional use by different communities. T. procumbens has diverse pharmacological properties including but not limited to: immunomodulatory, antioxidant, anti-hepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, and antimicrobial activities. The versatility of the species is most likely due to the

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plant's defense mechanisms, secondary metabolites such as flavonoids, alkaloids, tannins, carotenoids and saponins.[3]

Aloe has been used topically for cuts, burns, insects stings, bruises, acne and blemishes, poison ivy, welts, skin lesions, eczema and sunburns. Aloe vera gel can not only increase the amount of collagen in wounds but also change the composition of collagen, increase collagen cross-linking and thereby promote wound healing.[4]

Curcumin has been shown to possess significant anti-inflammatory, anti-oxidant, anti- carcinogenic, anti-mutagenic, anti-coagulant and anti-infective effects. Curcumin has also been shown to have significant wound healing properties. It acts on various stages of the natural wound healing process to hasten healing.[5]

The basic herbs have the answer with no side effects and effective remedies and the golden fact is use of herbal treatment is independent of any age group. When two or more herbs are used in the formulation they are known as polyherbal formulations. Numerous studies have been conducted with the extracts of and extract of turmeric rhizomes (Curcuma aromatica Family-Zingiberaceae) with the combination of many other herbal drugs.[6]

2.0 MATERIALS AND METHODS

2.1. COLLECTION OF PLANT MATERIAL:

Aerial parts of *Tridax procumbens* and *Aloe barbadensis* were collected from hindusthan college of arts and science, Coimbatore. The *Curcuma aromatica* were purchased in store, Coimbatore.[7]

2.2. PREPARATION OF PLANT EXTRACT:

The plants were washed in running tap water to remove the entrapped dirt and soil particles. Cleaned plants were shade dried at room temperature for 3 days. The dried plants was powdered with electrical blender and the powder were stored in glass bottles for further use. The *Aloe vera* were washed in tap water and the skin is wipped using 90% ethanol. The inner gel was collected and washed in tap water. The gel was blended and collecteDjuice. The juice were stored at 4-6°c.[8]

2.3. PHYTOCHEMICAL SCREENING

The phytochemical analysis for the extracts of *T.procumbens, C.aromatica and Aloe bbarbedensis* was carried out and the analytical color changes observed during the experiments.[9]

2.4. ANTIBACTERIAL ACTIVITY

The antibacterial activity of *Tridax procumbens, Curcuma aromatica & Aloe barbadensis* were done by agar well diffusion method.

2.4.1. COLLECTION OF MICROBES:

Bacterial strains such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococus pyogenes, Pseudomonas sp, Proteus sp,* were used for the study and were collected from PSGIMR college, Coimbatore. The collected microbes were maintained in Nutrient agar Broth and cultured in Nutrient Agar medium.

2.4.2. PREPARATION OF THE MEDIUM:

For Agar well diffusion method Muller Hinton Agar was prepared and sterilized in autoclave. The 20ml of Muller hinton agar medium was transferred into sterile petriplates and they were kept undisturbed for 30 minutes for solidification.

After solidification the bacterial strains were swabbed (Lawn culture) on the plates. Using a sterile well-boarer the well was cutted on the plates. The plant extracts were added on the wells using micropippete. The plates were incubated

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at 37°c for 24hrs.[10]

2.5. ANTIOXIDANT ACTIVITY:

The antioxidant activity of the *Tridax procumbens, Curcuma aromatica & Aloe baebadensis* samples was determined using the DPPH radical-scavenging activity.

2.5.1. DPPH Free Radical-Scavenging Activity:

The antioxidant activities of all turmeric extracts were evaluated according to the DPPH radical-scavenging activity. Briefly, 1 mL of the extract was mixed with 1.2 mL of 0.004% DPPH in methanol at varying concentrations (20-120μg/mL). The percentage of DPPH inhibition was calculated using the following equation. where is the absorbance of DPPH in the absence of a sample and is the absorbance of DPPH in the presence of either a sample or the standard.

100% Radical scavenging =(Abscontrol−Abssample)Abscontrol×100.[11]

2.6. FTIR ANALYSIS OF *Tridax procumbens, Curcuma aromatica* & *Aloe barbadensis*

FTIR spectroscopy is used to quickly and definitively identify compounds and their functional groups. Fourier transform infrared (FTIR) spectroscopy has been considered to be one of the most effective techniques to study and understand the chemical and surface chemistry in various types of membrane. In addition, the FTIR techniques can also be used to monitor the stability and durability of the specific membrane toward their performance.

The 1ml methanol extract of tridax, acetone extract of turmeric & ethanol extract of Aloe vera were used for FTIR analysis.[12]

2.7 THIN LAYER CHROMATOGRAPHY:

The precoated silica gel sheets are used to seperate the amino acids by thin layer chromatograpy. The chromatography chamber was saturated with chloroform, acetic acid and water in the ratio of 3:3.5:1.5. The samples of methanol extract of tridax, ethanol extract of aloe vera & acetone extract of turmeric are loaded on the TLC sheets. After the solvent reaches the top the plate were dried and then spraying reagent Aniline-diphenylamine- phosphoric acid was sprayed over the TLC sheets.

RF value =Distance moved by solute / Distance moved by the solvent. [13]

2.8. SYNERGISTIC ACTIVITY:

The interaction of two or more drugs when their combined effect is greater than the sum of the effects seen when each drug is given alone. Multidrug therapy is a useful method that focuses on inhibiting or destroying harmful agents (such as cancer cells or infections) as well as activating human body defense or healing mechanisms. It is the result of the progressive abandonment of the previously held dogma of monodrug therapy.

Synergistic effects are the combined effects of at least two drugs that have a greater influence than either of them could have had individually. It is what happens when chemical substances or biological structures interact, resulting in a larger overall effect than the sum of their separate effects.

The extracted samples were tested to identify the synergistic activity as A+B, B+C, C+A

A - Tridax (methanol extract)

B - Turmeric (acetone extract)

C - Aloe vera (ethanol extract)

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The technique in this experiment involves placing filter paper strips soaked in different solutions of antimicrobial agents onto seeded agar plates such that they overlap and after

incubation examining the nature of any areas of growth inhibition surrounding the intersections.[14]

2.9. OINTMENT FORMULATION :

The ointment base was used a carrier to deliver drug to the wound. A suitable base should be needed to formulate ointment. The base must be compatible with the extracts to be incorporated into it.[15]

2.9.1. OINMENT BASE COMPOSITION :

1. White paraffin wax - 12.5gm
2. White petroleum. - 12.5gm
3. PEG 200 - 4ml
4. Cetostearyl alcohol - 12.5gm
5. Methyl paraben - 0.025gm
6. Propyl paraben - 0.015gm

2.9.2. HERBAL COMPOSITION:

1. *Tridax procumbens* - 200mg
2. *Curcuma longa* - 200mg
3. *Aloe barbadensis* - 200mg

2.10. SOAP FORMULATION:

The aloe vera soap recipe contains plant-based skin-soothing herbs that help to repair and protect skin when hand washing. Aloe's PH level closely matches our skin.100ml aloe vera extract and 5grams of turmeric powder was mixed with 20ml liquid castile soap, 100 gm glycerine soap base melted by using double boiled method and mixed well with the above ingredients. For fragrance, 10 drops lime essential oil and lavender essential oil was added and poured in soap mould.[16]

2.11. SANITIZER FORMULATION:

The aloe vera extract can be used as an antimicrobial agent in the formulation of antimicrobial hand-sanitizer and other related products, rather than synthetic agents which are inherently harmful. The 250ml aloe vera hand sanitizer were Prepared by adding 150ml of Aloe vera extract. And 90ml isopropyl alcohol was added. Then add 5ml hydrogen peroxide for anti-infective and ripening agent. Add 5ml glycerine to protect the hand skin against dryness and dermatitis. Finally add few drops of lime essential oil for fragrence and mix well. And store the sanitizer in a proper container.[17]

2.12. ANTIBACTERIAL ACTIVITY:

The antibacterial activity of Polyherbal ointment, Bathing soap & Sanitizer were done by agar well diffusion method. They are active against Streptococcus sp, Staphylococcus sp, Pseudomonas sp, Bacillus sp, Klebsiella sp, E.coli, Proteus sp.,[18].

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2.13. SKIN IRRITANCY TEST:

The skin irritancy test determines whether the specific products result in skin irritation or an allergic reaction. Many different substances can cause a skin reaction, chemicals, preservatives and cosmetics. Skin irritancy test involves applying a small amount of a samples onto the skin by wrapping with gauze and leaving it for 24hours to see if a reaction develops. [19].

3.0 RESULT AND DISCUSSION

3.1 PHYTOCHEMICAL ANALYSIS

The phytochemical studies for the extracts (Methanol, Ethanol, Acetone, Chloroform and Ethyl acetate) of *Tridax procumbens,Curcuma aromatica* and *Aloe barbadebsis* the analytical color changes observed during the experiments.[20]

3.2. THIN LAYER CHROMATOGRAPHY:

1. The Rf value of *Tridax procumbens* is 0.50 indicates the presence of beta-sitosterol.[21]
2. The Rf value of *Curcuma aromatia* is 0.79 indicates the presence of curcuma.[22]
3. The Rf value of *Aloe barbadensis* is 0.91 indicates the presence of flavanoids.[23]

3.3. FTIR ANALYSIS:

3.3.1. FTIR ANALYSIS OF *Tridax procumbens*

For sample Tridax procumbens, the presence of multiple peaks3837, 2925, 2551, 1376, 1240, 1019 cm1 was confirmed. The broadened appearance of intense bands with O–H bond group was confirmed at 3837 cm1. Presence of OH peaks indicates the presence of residual moisture irrespective of heating and drying of samples.The peak bands at 2925, 2851 is confirmed as carboxyl group.

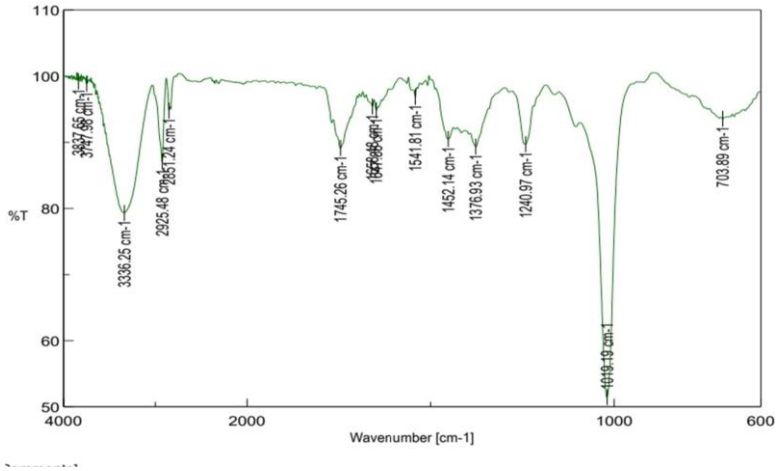

Fig 3.3.1 FTIR analysis of *Tridax procumbens*

3.3.2. FTIR ANALYSIS OF *Curcuma aromatica*

For *Curcuma aromatica*, the presence of multiple peaks at 1509, 1707, 1755, 2925, 2854 and 3664 cm1 was confirmed.

- The peak bands at 1509, 1707, 1755 cm1 represents C = O, 1055, 1092 represents alkyl amine, C-O

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stretching was confirmed at 1359 cm1.

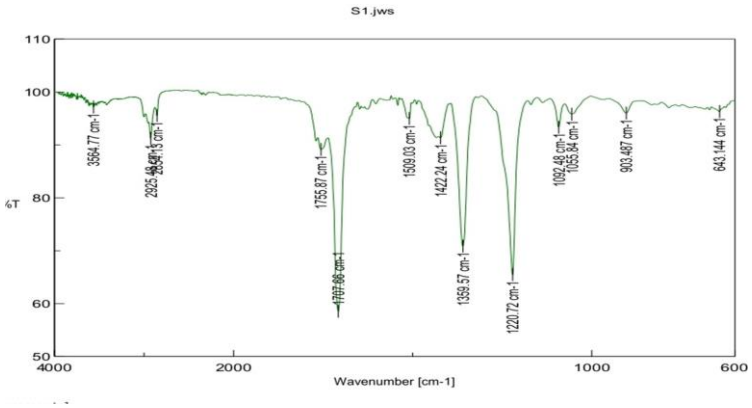

Fig 3.3.2 FTIR analysis of *Curcuma aromatic*

3.3.3. FTIR ANALYSIS OF *Aloe barbadensis*

For sample *Aloe barbadensis*, the presence of multiple peaks at 3747, 3078, 2974, 2880.

- The broadened appearance of intense bands with O–H bond group was confirmed at 3747 cm1.
- Presence of OH peaks indicates the presence of residual moisture irrespective of heating and drying of samples. The peak bands at 3078, 2974, 2880 cm1 represents Carboxyl acid and 2136 confirms C-N.
- Alkyl ketone & alkyl amine are represented in fingerprint region.

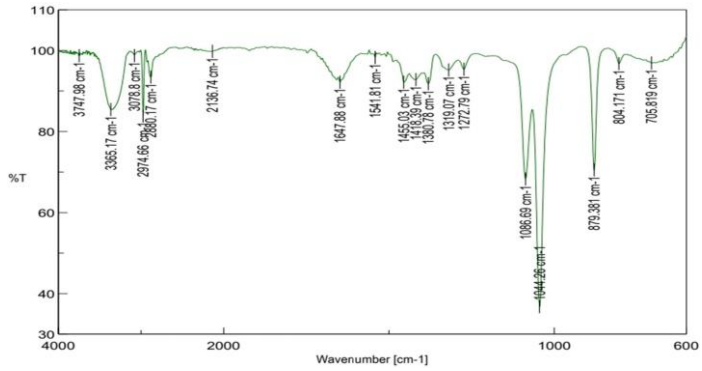

Fig 3.3.3. FTIR analysis of *Aloe barbadensis*

3.4. ANTIOXIDANT ACTIVITY:

3.4.1 ANTIOXIDANT ACTIVITY OF *Tridax procumbens*

By using DPPH method, the absorbance was calculated in uv spectroscopy, the percentage of antioxidant activity shows in Tridax extract is 91.2%

3.4.2 ANTIOXIDANT ACTIVITY OF *Curcuma longa*

By using DPPH method, the absorbance was calculated in uv spectroscopy, the percentage of antioxidant activity shows in turmeric extract is 65.2%

3.4.3 ANTIOXIDANT ACTIVITY OF *Aloe barbadensis*

By using DPPH method, the absorbance was calculated in uv spectroscopy, the percentage of antioxidant activity shows in *Aloe barbadensis* extract is 88.5%

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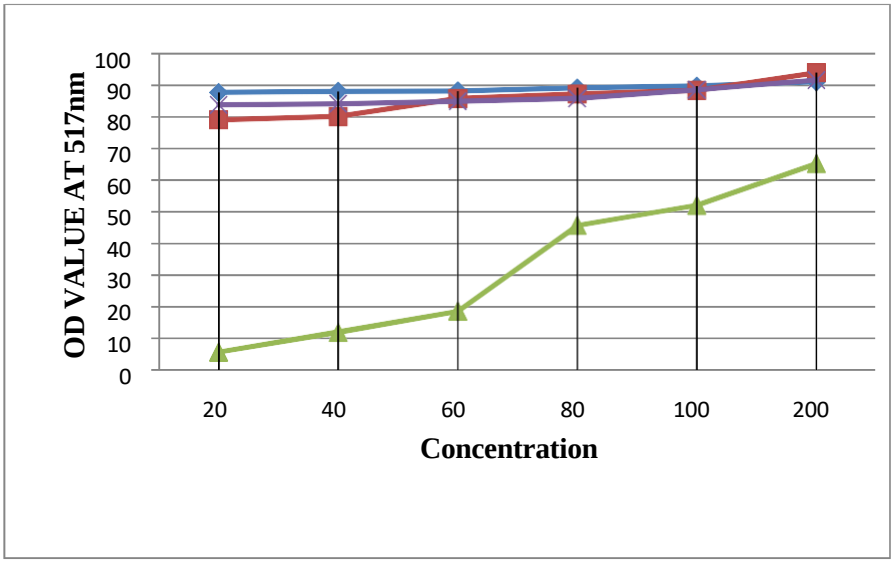
3.4.4.DPPH RADICAL SCAVENGING ACTIVITY:

S.NO	DILUTION	STANDARD (Ascorbic acid)	*Tridax procumbens*	*Curcuma longa*	*Aloe barbadensis*
1.	20µl	87.7%	79.1%	5.7%	83.8%
2.	40µl	88%	80.1%	11.9%	84.1%
3.	60µl	88.1%	85.8%	18.6%	84.9%
4.	80µl	89.1%	87.2%	45.7%	85.8%
5.	100µl	89.7%	88.3%	52%	88.5%
6.	200µl	91%	93.9%	65.2%	91.6%



3.5. SYNERGISTIC ACTIVITY OF POLY-HERBAL OINTMENT:

The synergistic activity of Polyherbal ointment shows higher antimicrobial activity compared to the individual. So the combination is said to be synergistic.

Combination of A+B (zone size) = A+B /2

3.5.1. TABLE (SYNERGISTIC ACTIVITY OF POLYHERBAL OINTMENT)

S.NO	ORGANISMS	SYNERGISTIC ACTIVITY(mm)
1.	*Staphylococcus sp*	40.5mm
2.	*Streptococcus sp*	37.5mm
3.	*Klebsiella sp*	34.5mm
4.	*Pseudomonas sp*	37.5mm

3.6. PREPARATION OF OINTMENT:

White paraffin wax was melted on hot plate, at temperature 70degree celcious, when the wax was completely melted, white petrolium was added allow the entire mixture remain the hot plate until liqufied. Following liquefication,

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removed from heat and allow them to congeal.

Fig 3.6 POLYHERBAL OINTMENT

3.7. ANTIBACTERIAL ACTIVITY OF POLYHERBAL OINTMENT:

The antibacterial activity of polyherbal ointment is done by agar well diffusion method. The antibacterial activity of ointment is tested against *E. coli, Streptococcus sp, Staphylococcus aureus, Pseudomonas sp, klebsiella sp, Proteus sp, Bacillus sp.,* The zone size of sample is measured in diameter.

3.7.1. TABLE (ANTIBACTERIAL ACTIVITY OF POLYHERBAL OINTMENT)

S NO	ORGANISM	20µL	60µL	100µL
1	*E. coli*	1.3cm	1.7cm	2.3cm
2	*Streptococcus sp*	1.5cm	1.7cm	2.1cm
3	*Staphylococcus sp*	1.5cm	1.6cm	1.8cm
4	*Proteus sp*	1.6cm	1.8cm	2cm
5	*Bacillus sp*	1.5cm	1.9cm	2.3cm
6	*Pseudomonas sp*	1.4cm	1.8cm	2cm
7	*Klebsiella sp*	15cm	1.7cm	2.3cm

3.8. PHYSIOCHEMICAL EVALUTION OF FORMULATED OINTMENT:

S.NO	PHYSIOCHEMICAL PARAMETERS	OBSERVATION
1.	Colour	Green
2.	Odour	Characteristic
3.	Consistency	Smooth
4.	pH	5.2
5.	Spreadability (seconds)	7sec
6.	Diffusion study (after 60min)	0.9cm

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7.	Loss on drying	20%
8.	Solubility	Soluble in boiling water, miscible with alcohol, ether, chloroform
9.	Washability	Good
10.	Non irritancy	Non irritant
11.	Stability study(2°C, 25°C, 37°C)	Stable

3.9. ANTIBACTERIAL ACTIVITY OF BATHING SOAP:

The antibacterial activity of bathing soap is active against gram positive *Staphylococcus sp.* the zone of inhibition is measured in diameter. The concentration of 100µl shows greater zone size of about (2.6cm)

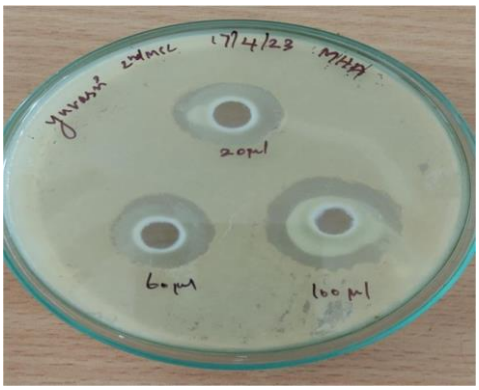

Fig 3.9.1. *Staphylococcus sp.*

Fig 3.9.2. Bathing soap

3.10. ANTIBACTERIAL ACTIVITY OF SANITIZER:

The antibacterial activity of Sanitizer is active against gram positive *Staphylococcus sp.*, and gram negative *E.coli*, the zone of inhibition is measured in diameter. The concentration of 100µl shows greater zone size of about (3.1cm)

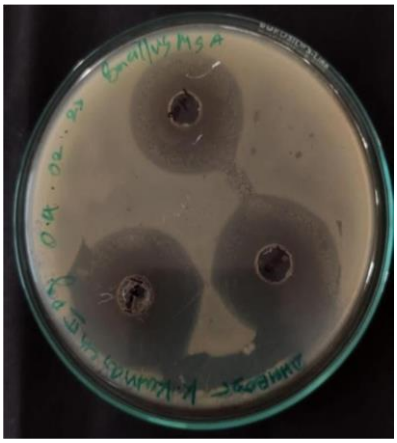

Fig 3.10.1 Staphylococcus sp

Fig 3.10.2.Hand sanitizer

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3.11. SKIN IRRITANCY TEST:

Skin irritancy test result suggest that are not allergic to most common allergens and allergen mixes. [24]

4.0 CONCLUSION

In Indian system of medicine majority of herbal products are made by using crude plant or portion of plant parts and their extracts. The leaves extracts of *Tridax procumbens, Curcuma aromatica & Aloe barbadensis* was taken for this present study and formulated for the tropical ointment and its properties. The ointment prepared using *Tridax procumbens, Curcuma longa & Aloe barbadensis* leaf extract was found to be good ointment, soap & sanitizer characteristics with respect to homogenicity, pH, viscosity, antibacterial activity. Ointment formulation containing leaf extract of using *Tridax procumbens, Curcuma longa & Aloe barbadensis* was successfully prepared with white paraffin wax and white petroleum as ointment base. The combination of Polyherbal ointment shows higher antimicrobial activity compared to the individual. The results of different chemical and physical test of ointment, bathing soap and sanitizer showed that the formation could be used tropically in order to protect skin against damage caused by *Staphylococcus sp.*, thus it can be concluded that there is a growing demand for herbal formulation in the world market and they are invaluable gift of nature. The skin irritancy test shows no allergens were formed by polyherbal ointment, bathing soap and sanitizer.

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