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PREPARATION, ANTIMICROBIAL EVALUATION AND STANDARDIZATION OF POLYHERBAL FORMULATIONS

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ABSTRACT

Objective: The present study was aimed to prepare various formulations, evaluate the antimicrobial efficacy, safety and stability of the prepared formulations and to standardize the formulations by various methods. **Methods:** The leaf and bark of the plants *Cassia fistula, Milletia pinnata, Ficus religiosa* and *Wendlandia thyrsoidea* were extracted and screened for antimicrobial activity. The active extracts were used to prepare eight formulations. They were Cream, gel, hand wash, sanitizer, soap, tooth paste, tooth powder and mouth wash. The formulations were subjected to antimicrobial and phytochemical screening. The total phenolic and condensed tannin contents were evaluated. The HPLC and HPTLC fingerprinting was done using tannic acid and gallic acid as standards. The formulations were further subjected to skin toxicity studies on albino rats in order to evaluate the safety. Accelerated stability studies were done by exposing the formulations to variations in temperatures and evaluating the physico-chemical parameters for 120 days. **Results:** The methanolic bark extracts exhibited good antimicrobial properties. Phytochemical screening of the formulations revealed the presence of tannins and polyphenols. The HPLC and HPTLC fingerprinting exhibited peaks corresponding to the retention times and Rf values of the standards. Results of animal studies revealed that they were safe and did not produce any inflammation and oedema after 7 days. Accelerated stability studies confirmed that the formulations were found to be stable. **Conclusion:** The formulations were safe with good antimicrobial effects and they were found to be stable after 120 days of stability studies.

KEYWORDS

Antimicrobial, Standardization, Fingerprinting and Animal studies.

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INTRODUCTION

Plants are known to produce a variety of secondary metabolites which are proven to poses potential antimicrobial effects, thus making them a rich source of different types of medicines[1]. Herbal medicinal products are of global importance both medicinally and economically. Although usage of these herbal medicines has increased, their quality,

safety and efficiency are serious concerns in industrialized and developing countries[2].

Oral hygiene is very important for the health. Bacterial infections that begin in the mouth can escalate to systemic infections and harm the organs if not controlled. Many herbal dentifrices are available in the market containing neem, aloe and turmeric as common ingredients however there are many more plants with proven antimicrobial and dentifrice properties which need to be formulated into various formulations and evaluate its efficacy[3].

The herbal formulations may be more appealing as they do not require alcohol and artificial preservatives. However there is a necessity to develop new methods to standardize them in order to maintain the quality, safety and efficacy of the formulations[4].

Cassia fistula Linn, belonging to the family *Leguminosae,* is adeciduous medium sized tree. Literature survey reveals the plant possess good antibacterial and antifungal properties[5,6]. *Ficusreligiosa* belonging to the family Moraceaeis is a very common tree in India. The methanolic bark extract of *Ficusreligiosa* is reported to poses good antimicrobial properties[7]. *Milletiapinnata* is a species of tree belonging to family *Fabaceae*. The fruits and sprouts of *Milletiapinnata* are used in folk remedies to treat cold, coughs, gonorrhoea and leprosy. The roots are used for cleaning gums and teeth, the oil is used as antiseptic[8]. Different parts of the plant *Milletiapinnata* are also reported to poses antibacterial properties[9]. *Wendlandia thyrsoidea* belonging to the family Rubiaceae is a small tree or large shrub, different parts of the plant are used in treatment of skin cuts and infections in traditional systems[10]. Different parts of *Wendlandia thyrsoidea* have also been reported to poses antimicrobial properties[11].

MATERIAL AND METHODS

Extraction of plants and preliminary antimicrobial screening of extracts

The leaves and bark of plants *Cassia fistula, Ficusreligiosa, Milletiapinnata* and *Wendlandia thyrsoidea* were collected from Mysore and Coorg districts, the specimens were authenticated at RRL, Bangalore. They were extracted by refluxing for 8 hours using methanol and aqueous methanol as solvents. The extracts were screened for antimicrobial activity by agar diffusion method against the microorganisms *Aspergillus Niger* (MTCC: 4325), *Candida albicans* (MTCC: 3958), *E coli* (MTCC: 521), *Staphylococcus aureus* (MTCC: 1144) and *Lacto bacillus* (MTCC: 903) and zones of inhibition were recorded.

Formulation of polyherbal preparations

The methanolic bark extracts of *Cassia fistula, Ficus religiosa* and *Milletia pinnata* that exhibited maximum antimicrobial effects were included in the formulations. Each extract was incorporated in the concentration of 0.15grams to produce a total concentration of 0.45grams per 10 grams of formulation. The formulations were prepared as per the following formulas.

Preparation of soap (10g)

Solidified basic glycerine soap was broken down to small pieces and melted on a water bath. 0.45grams of the extract combinations were added to the melted soap along with 5ml of ethanol, 0.033grams of stearic acid, 1ml of cinnamon oil and citronella oils each. The contents along with the melted soap were mixed uniformly for 30 minutes and moulded in circular moulds. The soap was allowed to solidify at room temperature until set and kept under physical observation for any characteristic changes.

Antimicrobial screening of formulations

The formulated cream and gel was tested against acne producing microorganisms, they were *P acnes* (MTCC: 1951) and *S epidermidis* (MTCC: 2639). The hand wash, soap and sanitizer were screened against organisms causing nosocomial infections, they were *C albicans* (MTCC: 3958), *E coli* (MTCC: 521), *S aureus* (MTCC: 1144) and *Paeruginosa* (MTCC: 1036). The tooth paste, mouth wash and tooth powder were screened against organisms causing dental plaque, they were *S mutants* (MTCC: 890), *S oralis* (MTCC: 2696) and *L bacillus* (MTCC: 903). All the 8 formulations

were evaluated at three different concentrations i.e. 0.25, 0.5 and 1.0g. The screening was done by agar diffusion method and zones of inhibition were recorded.

Phytochemical screening of formulations

The phytochemical screening of all the formulations was carried out by various chemical tests which are as follows.

- Tests for carbohydrates.
- Test for sterols.
- Test for saponins.
- Test for Tannins and polyphenols.
- Test for alkaloids.
- Test for aglycones:

a) Borntragers test for anthraquinone aglycone.
b) Test for cardiac aglycones.

Standardization of formulations

Total phenolic content determination[12]

Total phenolic content was estimated by using "Folin-Ciocalteu" assay method.

Sample preparation

To 1g of the sample was extracted with 10ml of water filtered and was added in a 25ml volumetric flask. Add 1ml of "Folin- Ciocalteu phenol" reagent, shake well and set aside for 5 minutes. After 5 minutes add 10ml of 7% sodium carbonate solution and make up the volume to 25ml with distilled water.

Standard preparation

A set of standard solution of gallic acid in the concentrations of 20, 40, 60, 80 and 100ug/ml were prepared in the same manner as sample preparation and incubated at room temperature for 90 minutes. The absorbance for the standard and sample was recorded against the reagent blank at 550nm, graph was plotted and the total phenols were expressed as mg of Gallic Acid Equivalent/ gram of the extract.

Total condensed tannin determination[12]

100mg of sample was dissolved in 10ml distilled water, 2ml of 5M HCL and 2ml of 37% formaldehyde were added and the mixture was heated for one hour. The reacted mixture was then filtered while hot through vacuum suction and the supernatant precipitate was washed with 10ml of hot water five times. The precipitate was dried and weighed. The yield of total condensed tannins was expressed as % of the weight of starting material.

HPLC fingerprinting of the formulations

The prepared eight formulations containing the active extract combinations were subjected to analysis by HPLC method. As the phytochemical analysis of them revealed the presence of phenols and tannins, Gallic acid and Tannic acid were used as standards.

The HPLC was carried out using an ODS, C-18 column (4.6mm x 250mm) with a particle size of 5um. HPLC pump used was Waters^{TM}510 US; detector used was Waters^{TM} 486 UV detector at a wave length of 320nm. Software used was Data Ace software. Elution was done by isocratic elution with solvent systems of Methanol: water (5:2) and acetonitrile: water (2:20). Flow rate was adjusted to 1ml/min, injection volume was 20ul and the run time was 15 min.

Preparation of standard solutions

Standard stock solutions of gallic acid and tannic acid were prepared by transferring 10mg of the standards in 10ml volumetric flasks and the volume made up to the mark with methanol to give a concentration of 1000µg/ml. These two stock solutions of gallic acid and tannic acid were prepared and stored in the refrigerator at 5^{o}C until use.

Preparation of sample solutions

0.25g of the formulations were added in 10ml of methanol, mixed well, filtered and the filtrate was used for analysis.

HPTLC fingerprinting of the formulations

The formulations were also subjected to HPTLC fingerprinting by using tannic acid and gallic acid as standards. The sample was prepared by dissolving 0.25g of the formulation in methanol. Solvent system used was pet-ether: methanol: formaldehyde (2.5:5:2.5). Plates used were silica gel 60 GF254 pre coated aluminium plates. About 5ul of the sample was applied on the TLC plates in the form of 6mm bands using linomat 5 applicator. HPTLC system used was CAMAG which was integrated with WIN CATS software programming. Densitometric scanning was done at 300nm.

Skin toxicity studies of formulations

The skin toxicity of formulations was carried on albino rats. Ethical clearance was obtained by the animal ethics committee before starting the study. 24 healthy albino rats were selected and divided into six groups with four rats in each group. Each group was assigned different formulations including a negative control. The back of the animals were shaved to make an area of about 40mm x 30mm and the fur removed 24 hours before application of the samples. Formulations were applies topically on the surface of the shaved skin and they were held in contact with the skin using a bandage dressing. Observation of sites was done at 24 hrs after application and repeated at 48 hrs, 72 hrs and 7 days. Scores corresponding to the skin reactions were allotted as per the Draize scoring system.

Accelerated stability studies of formulations

All the prepared formulations were evaluated for their stability by subjecting them to variations in temperatures. Three sets of formulations were used for the study, the first set of formulations were kept at 0-4ºC in a refrigerator, the second set of formulations were kept at room temperature and the third set were kept at 40ºC in incubator for 20, 40, 80 and 120 days, after which the physicochemical parameters of the formulations were evaluated. Parameters such as clarity, colour, odour, pH, spread ability, grittiness were evaluated.

RESULTS AND DISCUSSION

Antimicrobial screening of the extracts

The extracts of all the plants were subjected to antimicrobial screening against the selected microorganisms by agar diffusion method and the zones of inhibition were recorded. Results showed that the methanolic bark extracts of *C fistula, M pinnata* and *F religiosa* exhibited maximum activity with zones of inhibition ranging from 12 to 20mm, results are tabulated in Table No.1.

Antimicrobial evaluation of the formulations against specific microorganisms

All the 8 formulations were evaluated for antimicrobial activity at three different concentrations i.e. 0.25, 0.5 and 1.0g against specific microorganisms by agar well diffusion method. All the formulations exhibited antimicrobial effect with zones of inhibition ranging from 6 to 26mm. The tooth paste and sanitizer exhibited maximum zones of inhibition of 26mm at a concentration of 1gram, which was followed by the soap, gel and hand wash which exhibited zones of inhibition ranging from 16 to 24mm. The lowest zones of inhibition were observed in the tooth powder with zones of inhibition ranging from 8 to 12 at the highest concentration. The results are tabulated in Tables No.2 to Table No.4 and Figure No.1 to Figure No.10.

Standardization of the formulations

Determination of total phenol and tannin contents

All the eight formulations were evaluated for total phenol and condensed tannin contents. The total phenolic contents in the formulations were in the range from 41 to 73mg of gallic acid equivalent per gram. Among which maximum was present in soap and the lowest was observed in tooth powder. The total condensed tannins ranged from 44 to 87% W/W. The maximum tannin contents were found in soap which was followed by sanitizer and mouth wash, the tooth powder and gel was found to have the lowest tannin contents. The results are tabulated in Table No.5.

HPLC fingerprinting of the formulations using tannic acid and gallic acid as markers

The HPLC of a blank chromatogram was recorded with the solvent system and stabilized. This was followed by the two standards tannic and gallic acids using the solvent systems Methanol: water (5:2). Tannic acid at a concentration of 50ug/ml exhibited a peak at 4.9 minutes with a height and percentage area of 12.27 and 5.97% respectively. Gallic acid at a concentration of 50ug/ml was eluted at 6.90 minutes exhibiting a peak height and a percentage area of 17.40 and 11.55% respectively. The formulations exhibited various peaks with different retention times, among which they also exhibited peaks with retention times and peaks similar to the standards thus confirming the

presence of tannic acid and gallic acid in the formulations.

The cream formulation exhibited four peaks among which two peaks retention times lie in the range of the standard peaks. The gel formulation exhibited seven peaks with retention times ranging from 3.0 to 12.5 minutes, the gel also showed the presence of the standard compounds. The sanitizer and hand wash formulations exhibited six peaks with retention times ranging from 1.5 to 10.0 minutes. The tooth paste, soap, tooth powder and mouth wash also exhibited various peaks with retention times ranging from 1.5 it 12.5 minutes. The results are tabulated in Table No.6 and Figures No.11 and Figure No.12.

HPTLC fingerprinting of formulations using standards

The HPTLC fingerprinting of the formulations was done by using tannic acid and gallic acid as standards. Mobile phase used was pet-ether: methanol: formaldehyde (2.5:5:2.5). The chromatograms of tannic acid and gallic acid were recorded and their Rf values calculated, this was compared with the Rf values of the formulations which exhibited similar peaks at the same Rf values along with other peaks. Results are tabulated in Figures No.13 to Figure No.18.

Skin toxicity testing of formulations on albino rats

The cream, gel, hand wash, sanitizer and soap formulations were evaluated for skin irritant effects on albino rats. 24 animals were divided into 6 groups with 4 rats present in each group. A placebo gel was used as a negative control. Results showed that the formulations did not produce erythema or oedema/inflammation on the skin (scoring 0). However the group-6 on which soap was applied showed slight redness on days 2, 3 and 4 which disappeared from the fifth day (scoring1) which is negligible and may be due to other factors. The results are tabulated in Table No.7 and Figure No.19.

Accelerated stability studies of formulations

Accelerated stability studies were performed for the formulations by exposing them at temperatures ranging from 4^{o}C to 40^{o}C for a period of 20 days. After the specified period they were evaluated for their various physicochemical parameters such as color, odor, appearance, pH, spread ability and grittiness were evaluated. The formulations exhibited good appearance characteristics as well as the pH was found in the range of 6.5 to 7.5. The formulations didn't show any considerable changes in their parameters after the exposure for the specified time. This concluded that the prepared formulations were stable at different temperatures. The results are tabulated in Table No.8 and Figures No.20 to Figure No.27.

Formula for cream

S.No	Ingredient	Quantity taken (10g)
1	Extract combination	0.45g
2	White bees wax	2g
3	Liquid paraffin	6g
4	Borax	0.1g
5	Purified water	1.9ml
6	Rose oil	QS

Formula for gel

S.No	Ingredient	Quantity taken (10g)
1	Extract combination	0.45g
2	Sodium alginate	1.2g
3	Glycerine	0.2g
4	Methyl hydroxyl benzoate	0.02g
5	Calcium gluconate	0.005g
6	Purified water	QSP 10g

Formula for hand wash

S.No	Ingredients	Quantity taken (10ml)
1	Extract combinations	0.45g
2	Carbopol	0.075g
3	Triethanolamine	qs
4	Sodium lauryl sulfate	0.05g
5	Methyl paraben	0.025g
6	Distilled water	10ml

Formula for sanitizer

S.No	Ingredients	Quantity taken (10ml) HS1
1	Extract combination	0.45g
2	Citronella oil	1.0ml
3	Cinnamon oil	1.0ml
4	Carbopol	0.1g
5	Triethanolamine	0.1g
6	Glycerine	0.5ml
7	Polysorbate-20	0.1ml
8	Perfume	Qs
9	Methyl paraben	0.1mg
10	Alcohol	4.0ml
11	Water	2.0ml

Formula for tooth paste

S.No	Ingredients	Quantity taken (10g)
1	Extract combinations	0.45g
2	Calcium carbonate	1.5g
3	Peppermint oil	1.0ml
4	Saccharin	0.05g
5	Methyl paraben	0.25g
6	Sodium lauryl sulphate	2.0g
7	Acacia	2.0g
8	Glycerine	1.5ml
9	Water	qs

Formula for mouth wash

S.No	Ingredients	Quantity taken (10ml)
1	Extract combinations	0.45g
2	Sodium benzoate	0.1g
3	Saccharin	0.015g
4	Menthol	0.2g
5	Cinnamon oil	0.05ml
6	Sodium lauryl sulphate	0.2g
7	Sorbitol	0.5g
8	Glycerin	0.5ml
9	Water	qs

Formula for tooth powder

S.No	Ingredients	Quantity taken (10g)
1	Extract combinations	0.45g
2	Di calcium phosphate dihydrate	6.0g
3	Calcium carbonate	1.8g
4	Sodium lauryl sulphate	0.25g
5	Dextrose	0.9g
6	Menthol	0.05g
7	Sodium saccharin	0.01g
8	Clove oil	0.1ml

Table No.1: Zones of inhibition of various extracts by agar diffusion method

S.No	Extract	*Aspergillus niger*	*Candida albicans*	*E coli*	*Staphylococcus aureus*	*Lacto bacillus*
1	CB-MOH	14.0	18.0	14.0	12.0	20.0
2	CB-40MOH	10.0	12.0	12.0	8.0	6.0
3	CL-MOH	-	-	-	-	-
4	CL-40MOH	-	-	-	-	-
5	MB-MOH	16.0	14.0	16.0	12.0	18.0
6	MB-40MOH	12.0	6.0	12.0	10.0	16.0
7	ML-MOH	-	12.0	6.0	-	10.0
8	ML-40MOH	-	10.0	6.0	-	12.0
9	WB-MEOH	12.0	-	10.0	10.0	12.0
10	WB-40MOH	12.0	-	9.0	9.0	16.0
11	FB-MEOH	12.0	14.0	12.0	16.0	18.0
12	FB-40MOH	10.0	12.0	12.0	8.0	10.0
13	FL-MEOH	-	-	8.0	-	-
14	FL-40MOH	-	-	6.0	-	-

Note: CB= *Cassia fistula* bark, CL= *Cassia fistula* leaf, MB= *Milletia pinnata* bark, ML= *Milletia pinnata* leaf, WB= *W thyrsoidea* bark, WL= *W thyrsoidea* leaf, FB= *F religiosa* bark, FL= *F religiosa* leaf, MOH= Methanol, 40MOH= 40% Methanol. Indicates no activity.

Table No.2: Zones of inhibition of the cream and gel formulations

S.No	Formulation	Concentration(g)	Zones of inhibition (mm)	
			P acnes	***S epidermidis***
1	Cream	0.25	14.0	12.0
		0.5	16.0	14.0
		1.0	18.0	18.0
2	Gel	0.25	10.0	10.0
		0.5	16.0	16.0
		1.0	24.0	22.0

Table No.3: Zones of inhibition of the soap, sanitizer and hand wash formulations

S.No	Formulation	Conc. (g)	Zones of inhibition (mm)			
			C albicans	***S aureus***	***P aeruginosa***	***E coli***
1	Soap	0.25	10.0	6.0	16.0	8.0
		0.5	18.0	12.0	18.0	14.0
		1.0	22.0	18.0	24.0	24.0
2	Hand wash	0.25	12.0	12.0	14.0	12.0
		0.5	18.0	18.0	18.0	22.0
		1.0	22.0	24.0	20.0	24.0
3	Sanitizer	0.25	12.0	14.0	12.0	14.0
		0.5	20.0	22.0	16.0	18.0
		1.0	20.0	26.0	18.0	24.0

Table No.4: Zones of inhibition of Tooth paste, powder and mouth wash formulations

S.No	Formulation	Conc.(g)	Zones of inhibition (mm)			
			C albicans	***S mutans***	***S oralis***	***L bacillus***
1	Tooth paste	0.25	14.0	12.0	14.0	16.0
		0.5	22.0	22.0	24.0	22.0
		1.0	22.0	22.0	26.0	26.0
2	Mouth wash	0.25	16.0	18.0	20.0	18.0
		0.5	20.0	24.0	24.0	22.0
		1.0	24.0	20.0	20.0	16.0
3	Tooth powder	0.25	14.0	10.0	14.0	18.0
		0.5	18.0	18.0	18.0	20.0
		1.0	10.0	8.0	12.0	8.0

Table No.5: Total phenolic and tannin contents of the formulations

S.No	Formulation	Total Phenolics (mg of GAE/g)	Total tannins (%W/W)
1	Cream	58	64
2	Gel	47	59
3	Soap	73	87
4	Sanitizer	69	85
5	Hand wash	68	73
6	Tooth paste	57	62
7	Mouth wash	68	81
8	Tooth powder	41	44

Table No.6: HPLC fingerprinting of the formulations

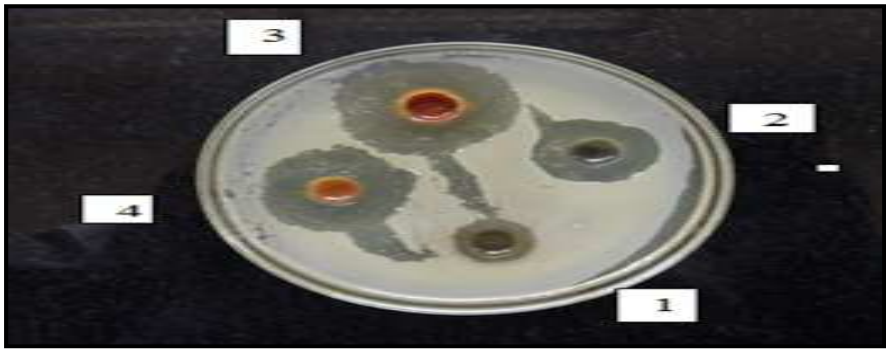
S.No	Formulations /Standards	No of peaks	Retention time (Min)	Peak area	% Area	Peak height
1	Tannic acid	01	4.97	2496906	5.97	12.27
2	Gallic acid	01	6.90	4554308	11.55	17.40
3	Cream	04	1.20, 2.12, 4.90, 6.87	2869305 3381704	6.86 8.57	14.10 12.92
4	Gel	07	2.86, 5.11, 6.91, 9.74, 10.98, 11.52, 12.87	1672743 4860545	3.99 12.32	8.22 18.57
5	Sanitizer	06	1.83, 1.85, 2.0, 4.31, 4.87, 7.12,	2055317 1633269	4.91 4.14	10.10 6.24
6	Hand wash	06	1.85, 2.52, 5.0, 6.91, 9.23, 10.10	1811121 4371088	4.33 11.08	8.90 16.70
7	Tooth paste	04	2.61, 4.98, 6.91, 8.45	2686158 3248216	6.42 8.23	13.20 12.41
8	Soap	06	2.51, 5.24, 6.87, 9.1, 9.88, 10.96,	2423647 4405115	5.79 11.17	11.91 16.83
9	Tooth powder	05	2.63, 3.21, 5.0, 7.23, 8.27	2543710 3088553	6.08 7.83	12.50 11.80
10	Mouth wash	05	5.1, 6.0, 6.98, 10.34, 11.1	2008513 4829136	4.80 12.24	9.87 18.45

Table No.7: Draize scorings for skin irritation test conducted on Albino rats (Average of 4)

S.No	Groups	Draize scorings					
		Day-1	Day-2	Day-3	Day-4	Day-5	Day-7
1	01 (Placebo)	00	00	00	00	00	00
		00	00	00	00	00	00
		00	00	00	00	00	00
		00	00	00	00	00	00
2	02 (Cream)	00	00	00	00	00	00
		00	00	00	00	00	00
		00	00	1.0	1.0	1.0	00
		00	00	00	00	00	00
3	03 (Gel)	00	00	00	00	00	00
		00	00	00	00	00	00
		00	00	00	00	00	00
		00	00	00	00	00	00
4	04 (Hand wash)	00	00	00	00	00	00
		00	00	00	00	00	00
		00	00	00	00	00	00
		00	00	00	00	00	00
5	05 (Sanitizer)	00	00	00	00	00	00
		00	00	00	00	00	00
		00	00	00	00	00	00
		00	00	00	00	00	00
6	06 (Soap)	1.0	1.0	1.0	1.0	00	00
		00	1.0	1.0	1.0	1.0	00
		00	1.0	1.0	1.0	00	00
		1.0	1.0	1.0	1.0	1.0	00

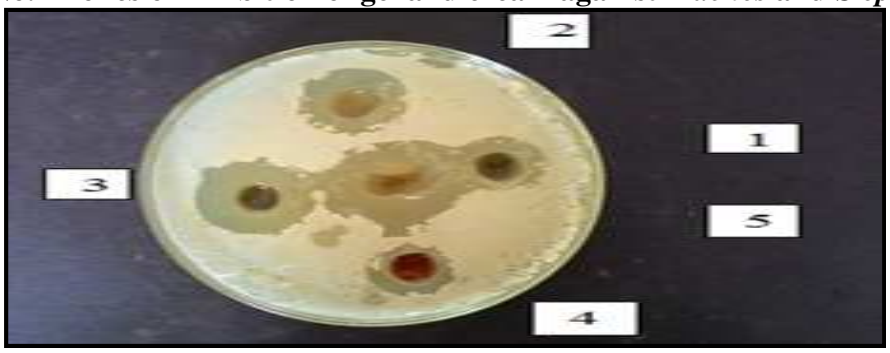
Table No.8: Results of stability testing of the formulations after exposing to accelerated temperatures

S.No	Formulations	Colour	Odour	Appearance	pH	Spreadability	Grittiness
1	Cream	No change	Fragrant	No change	6.5	Easily spreadable	Absent
2	Gel	No change	Agreeable	No change	6.8	Easily spreadable	Absent
3	Hand wash	No change	Fragrant	No change	7.1	-	Absent
4	Sanitizer	No change	Good	No change	6.2	-	-
5	Soap	No change	Fragrant	No change	7.0	-	-
6	Tooth paste	No change	Mint	No change	7.0	Easily spreadable	Absent
7	Tooth powder	No change	Mint	No change	6.2	-	Absent
8	Mouth wash	No change	Aromatic	No change	6.0	-	-



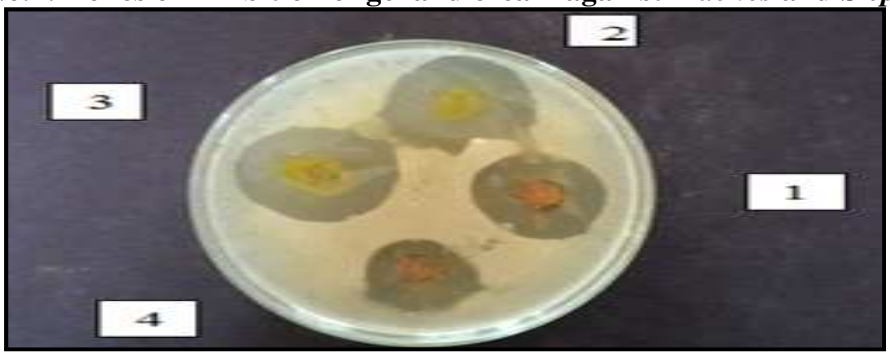
1=gel (0.25g). 2=gel (0.5g). 3=(1.0g). 4=cream (1.0g)

Figures No.1 Zones of inhibition of gel and cream against *P acnes* and *S epidermidis*



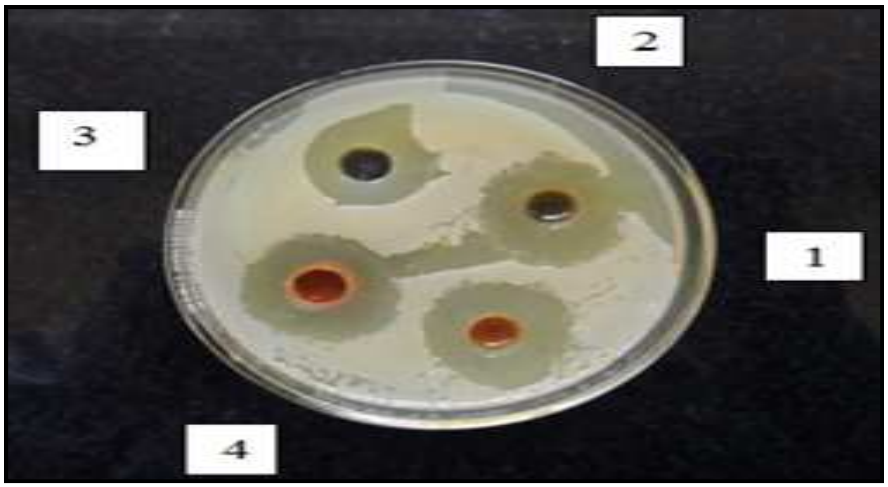
1=gel (0.25g). 2=cream (0.5g). 3=cream (1.0g). 4=ream (0.25g). 5=1=gel (1.0g)

Figures No.2: Zones of inhibition of gel and cream against *P acnes* and *S epidermidis*



1=soap (1g). 2=sanitizer (1g). 3=hand wash (1g). 4=hand wash (0.5g)

Figures No.3: Zones of inhibition of soap, hand wash and sanitizer against *C albicans* and *S aureus*

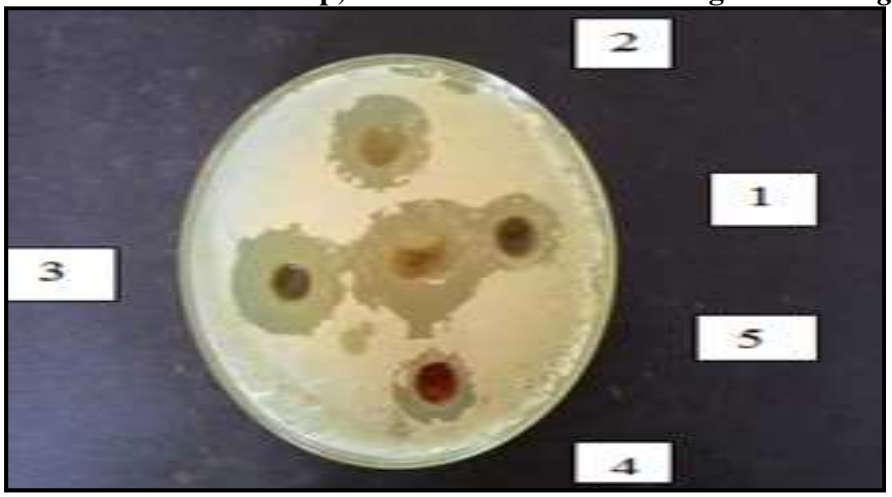


1=soap (1.0g). 2=sanitizer (0.5G). 3=hand wash (0.5g). 4=soap (1g)

Figures No.4: Zones of inhibition of soap, hand wash and sanitizer against *C albicans* and *S aureus*

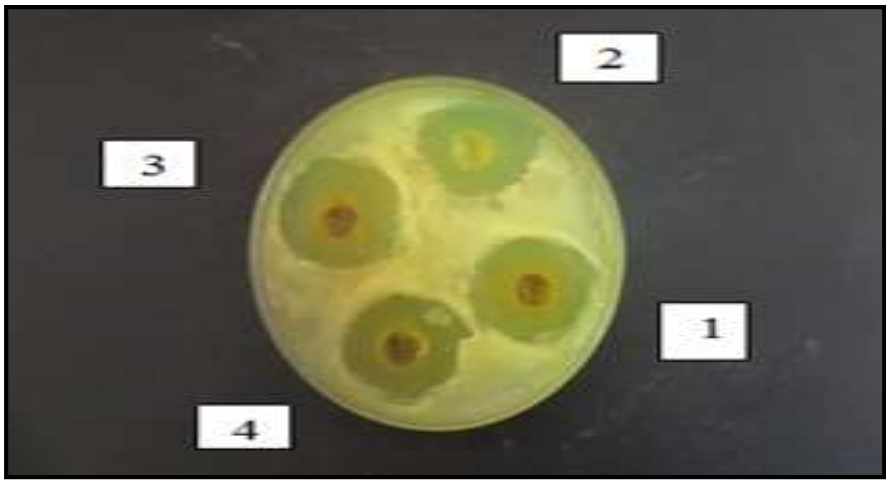
1=soap (0.25g). 2=sanitizer (0.5g). 3=hand wash (0.5g). 4=soap (0.5g)

Figures No.5: Zones of inhibition of soap, hand wash and sanitizer against *Paeruginosa* and *E coli*

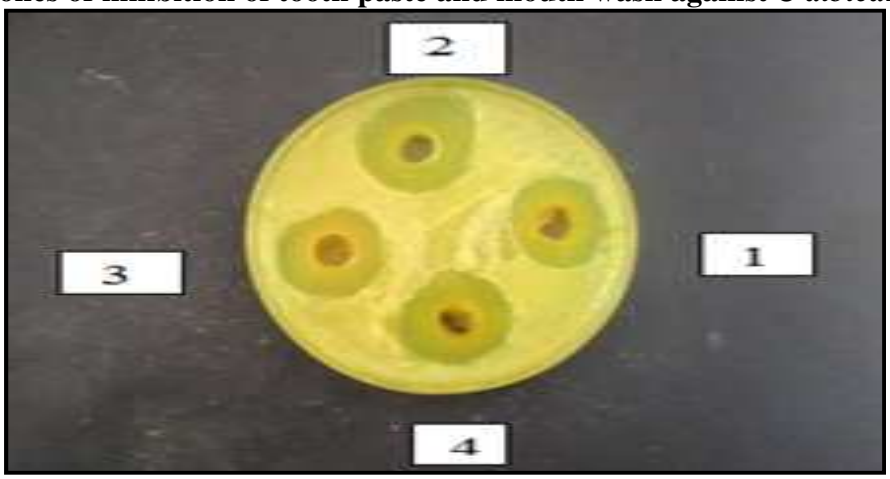


1=soap (0.25g). 2=hand wash (0.5g). 3=sanitizer (1.0g). 4=hand wash (0.25g). 5=soap (1g)

Figures No.6: Zones of inhibition of soap, hand wash and sanitizer against *Paeruginosa* and *E coli*

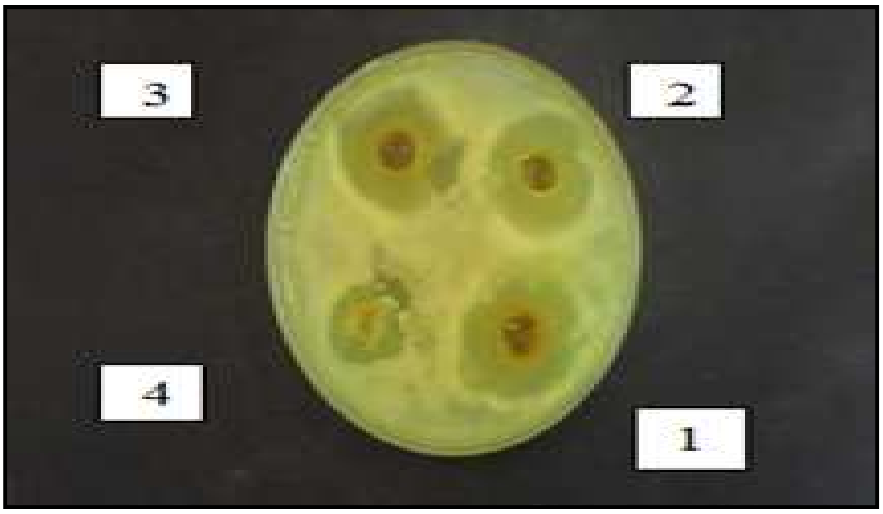


1=tooth paste (0.5g). 2=mouth wash (1g). 3=mouth wash (0.5g). 4=tooth paste (0.5g)
Figures No.7: Zones of inhibition of tooth paste and mouth wash against *C albicans* and *S mutans*



1=tooth paste (1g). 2=mouth wash (0.5g). 3=mouth wash (1g). 4=tooth powder (1g)
Figures No.8: Zones of inhibition of tooth paste and mouth wash against *C albicans* and *S mutans*

1=tooth paste (0.5g). 2=tooth paste (0.5g). 3=mouth wash (0.5g). 4=tooth powder (0.25g)
Figure No.9: Zones of inhibition of tooth paste and tooth powder against *S oralis* and *L acillus*



1=tooth paste (0.25g). 2=mouth wash (0.25g). 3=mouth wash (0.5g). 4= tooth powder (0.25g)
Figure No.10: Zones of inhibition of tooth paste and tooth powder against *S oralis* and *L acillus*

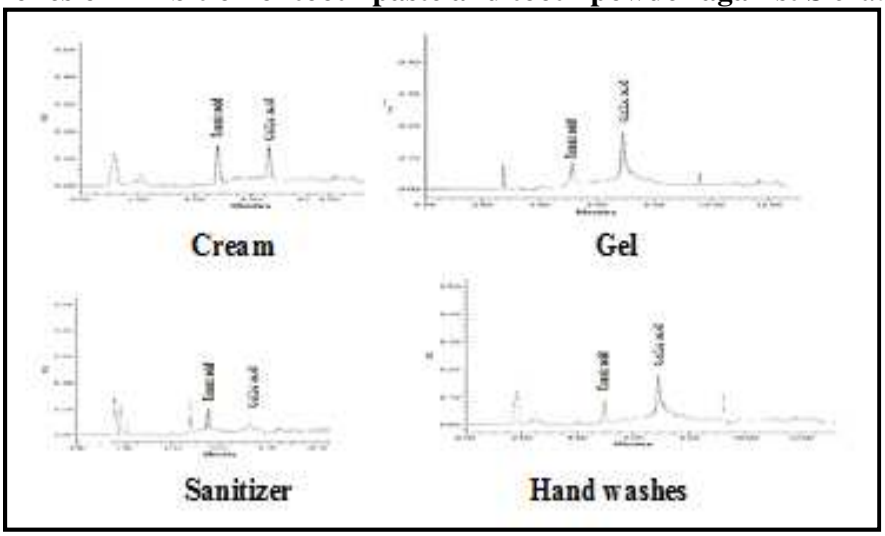


Figure No.11: HPLC profiles of Cream, Gel, Sanitizer and Hand wash

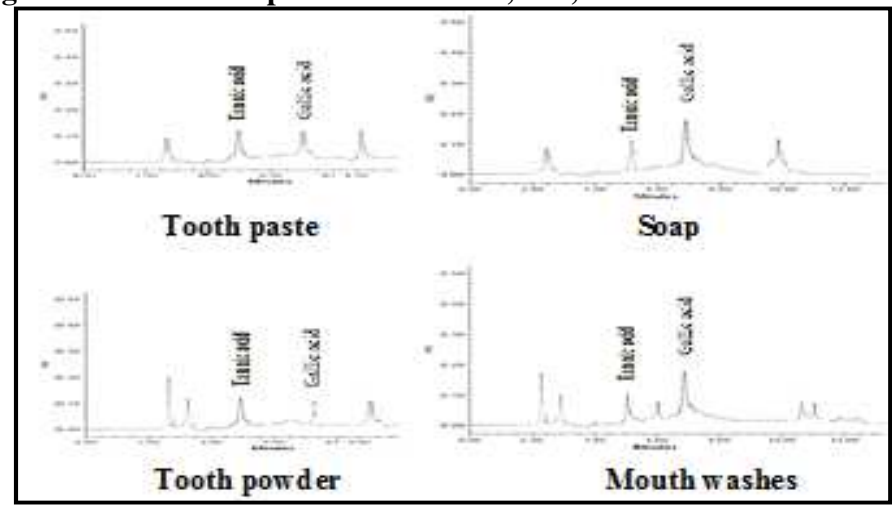
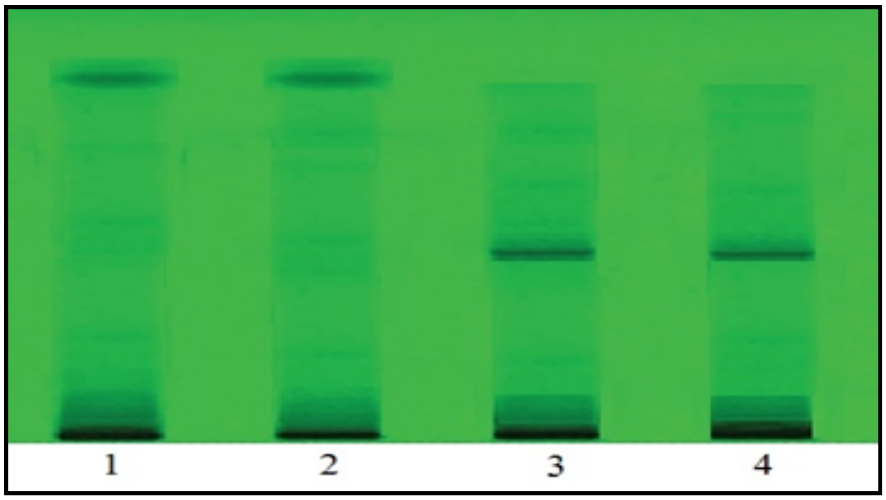


Figure No.12: HPLC profiles of Tooth paste, Soap, Tooth powder and Mouth wash



Track 1 and 2: Gallic acid. Track 3 and 4: Tannic acid
Figure No.13: TLC of Gallic acid and Tannic acid

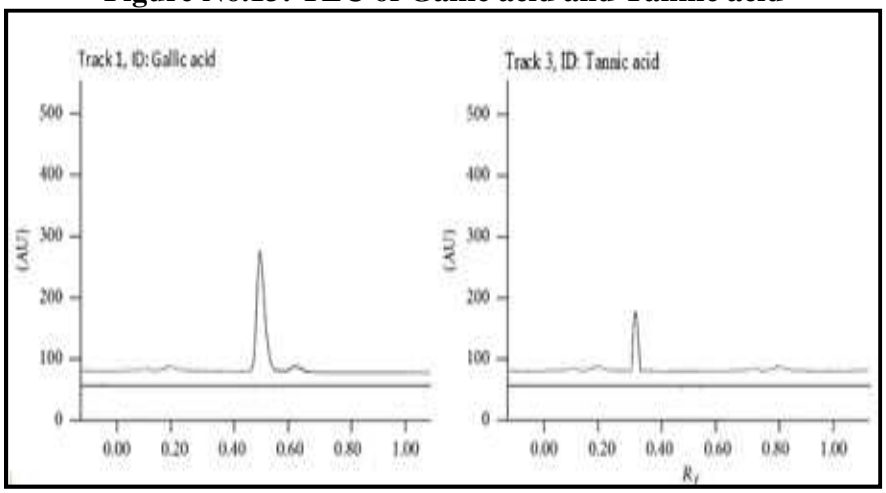
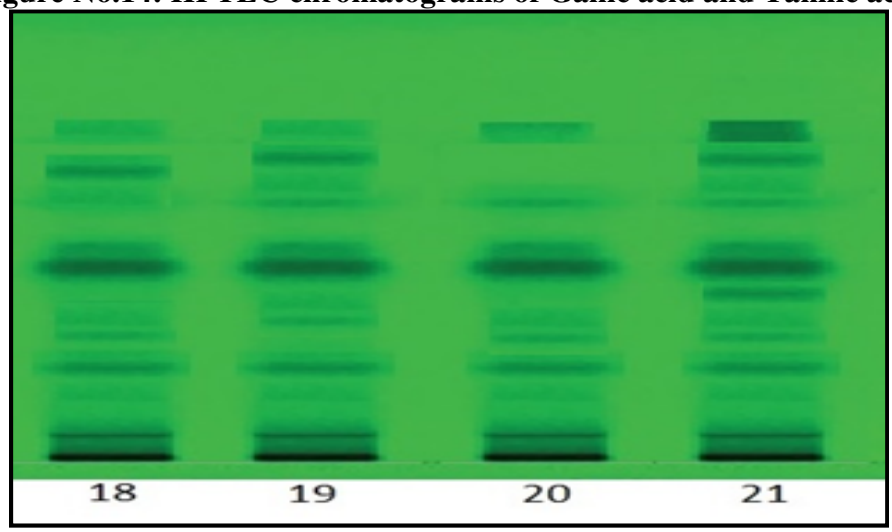


Figure No.14: HPTLC chromatograms of Gallic acid and Tannic acid



Track 18: Soap, Track 19: Sanitizer, Track 20: Hand wash, Track 21: Mouth wash
Figure No.15: TLC of soap, sanitizer, hand wash, mouth wash

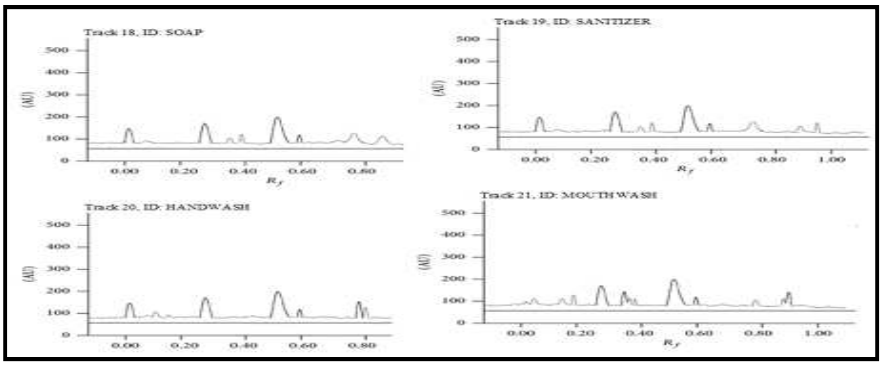
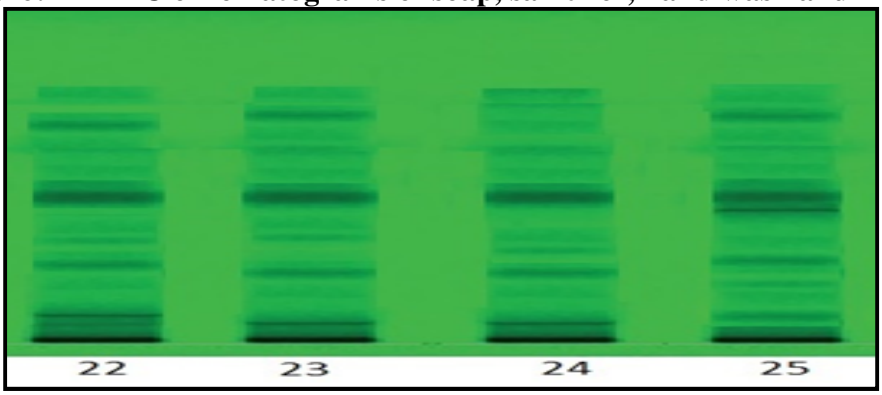


Figure No.16: HPTLC chromatograms of soap, sanitizer, hand wash and mouth wash



Track 22: Cream, Track 23: Gel, Track 24: Tooth paste, Track 25: Tooth powder

Figure No.17: TLC of cream, gel, tooth paste, tooth powder

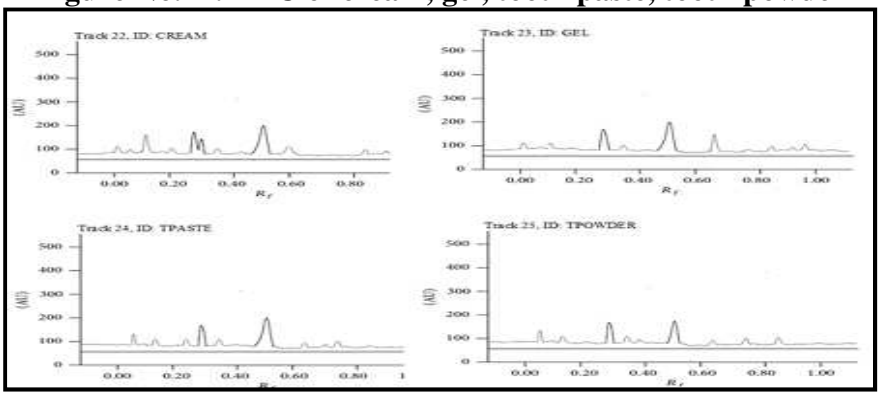
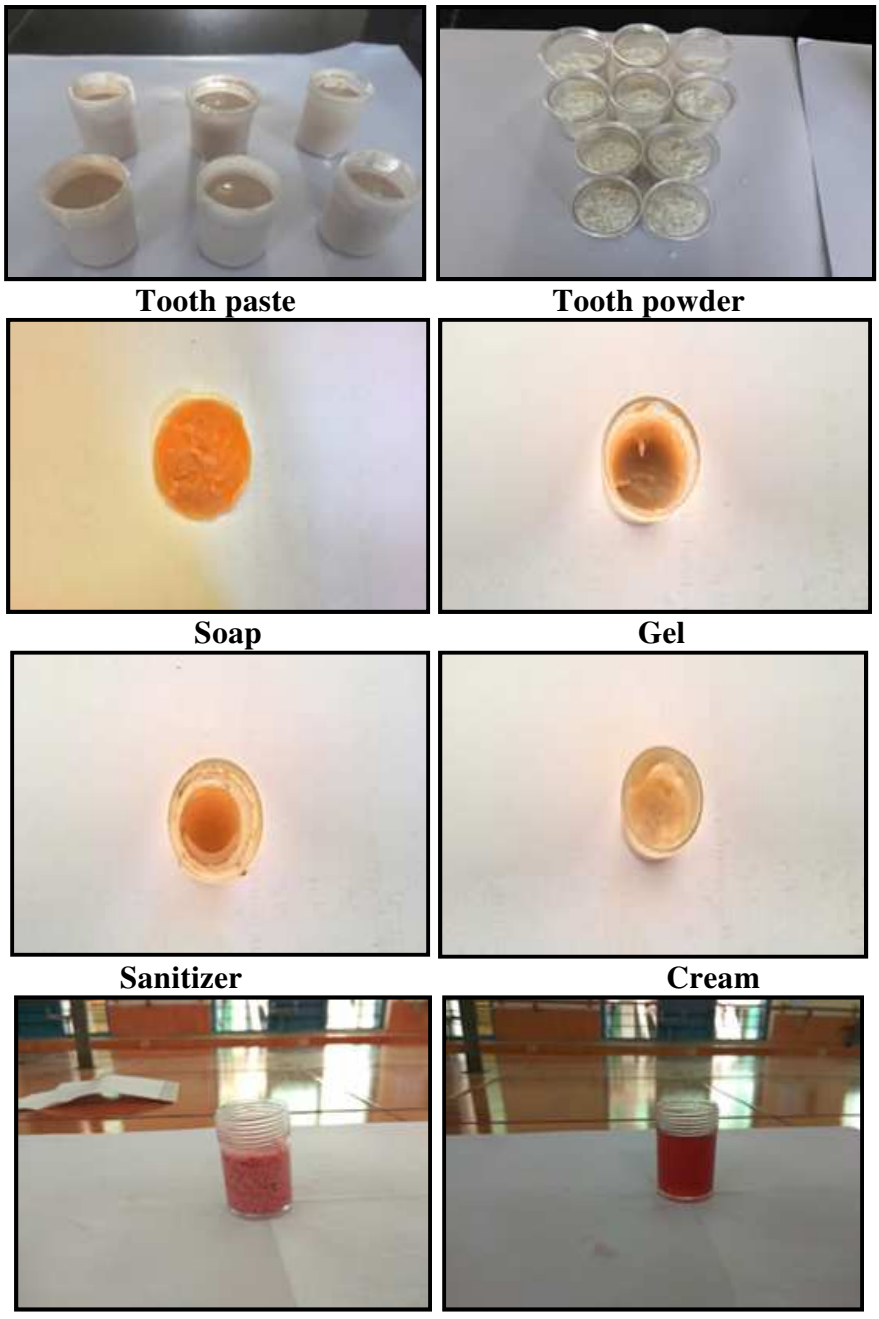


Figure No.18: HPTLC chromatograms of cream, gel, toothpaste and tooth powder

Figure No.19: Skin irritation test on the rats on day-1 and mday-7 of applying formulations



Figures No.20 to Figure No.27: Prepared formulations after accelerated stability studies

CONCLUSION

It was concluded that the plant extracts exhibited good antimicrobial activity against the selected microorganisms. The active extracts when combined together and included in formulations exhibited additive/synergistic effects. The formulations were stable with good physicochemical parameters even after 120 days of accelerated stability studies. The formulations can be further standardized and used commercially as good antiseptics, disinfectants and chemotherapeutic agents.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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