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FORMULATION AND COMPARATIVE EVALUATION OF POLYHERBAL PREPARATIONS FOR THEIR DISINFECTANT EFFECTS

Zeeshan Afsar[1*], Salma Khanam[2], Syed Aamir[3]

[1]Department of Pharmacognosy, Farooqia College of Pharmacy, Rajiv Gandhi University of Health Sciences, Mysore, Karnataka, India.
[2]Department of Pharmacognosy, Al-Ameen College of Pharmacy, Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka, India.
[3] Department of Pharmacology, Farooqia College of Pharmacy, Rajiv Gandhi University of Health Sciences, Mysore, Karnataka, India.

ABSTRACT

Background: The essential oils and extracts from several plant species can be used for their disinfectant properties for eliminating microorganisms related to skin, dental caries and food spoilage, including gram-negative and gram-positive bacteria, fungi and viruses.

Objective: To test different prepared formulations for the comparative disinfectant efficacy.

Methodology: The leaf and bark extracts of the plants *Cassia fistula*, *Ficus religiosa, Milletia pinnata* and *Wendlandia thyrsoidea* were subjected to antimicrobial screening. The extracts that exhibited maximum antimicrobial effect were used to prepare the cream, gel, hand wash, sanitizer and soap formulations. The prepared formulations were tested for their disinfectant efficacy by various methods such as Hand wash method, Degerming method, Ditch plate method and Rideal Walker coefficient methods.

Results: Results showed that all the formulations exhibited significant disinfectant properties among which the sanitizer and soap exhibited maximum disinfectant effect which was comparable with the commercially available market preparations.

Conclusions: The study concluded that plant extracts exhibits good antimicrobial activity and the prepared formulations exhibited good disinfectant efficacy, as well when evaluated by various disinfectant testing methods.

Keywords: Polyherbal preparations, disinfectant, comparative evaluation, Formulation

***Correspondence:** zeeshanafsar@gmail.com

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]. The essential oils and extracts from several plant species can control microorganisms related to skin, dental caries and food spoilage, including Gram-negative and Gram-positive bacteria, fungi and viruses. 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]. Plant extracts and products have been used for centuries in traditional medicine, functional food, natural dyes, cosmetics as detergents and in the treatment of diseases [3].

WHO report 80% of the world population relies on the drug from natural origin; hence there is a need to work in the field of development, evaluation and standardization of herbal formulations [4, 5].

Cassia fistula Linn, belonging to the family Leguminosae, is a deciduous medium sized tree. Literature survey reveals the plant possess good antibacterial and antifungal properties[6, 7].

Ficus religiosa belonging to the family Moraceae is a very common tree in India. The methanolic bark extract of Ficus religiosa is reported to poses good antimicrobial properties[8]. Milletia pinnata is a species of tree belonging to family Fabaceae. The fruits and sprouts of Milletia pinnata are used in folk remedies to treat cold, coughs, gonorrhea, and leprosy. The roots are used for cleaning gums and teeth, the oil is used as antiseptic[9]. Different parts of the plant Milletiapinnata are also reported to poses antibacterial properties[10]. 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[11]. Different parts of Wendlandia thyrsoidea have also been reported to poses antimicrobial properties[12].

MATERIALS AND METHODS

Plant material & extraction

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 using methanol and aqueous methanol by refluxation and the extracts were prepared for antimicrobial screening.

Antimicrobial screening of plant extracts

The leaf & bark extracts of Cassia fistula, Ficus religiosa, Milletia pinnata and Wendlandia thyrsoidea were subjected to antimicrobial screening by agar diffusion method against the microorganisms E coli (MTCC-1698), S aureus (MTCC-1143), P aeruginosa (MTCC-2453), P acnes (MTCC: 1951) and S epidermidis (MTCC: 2639).

Formulation of polyherbal preparations

The methanolic bark extracts of Cassia fistula, Ficus religiosa & Milletia pinnata that exhibited maximum antimicrobial effects were used in the formulations. Each extract was incorporated in the concentration of 0.15grams to produce a total extract concentration of 0.45 grams per 10 grams of formulation. The formulas used to prepare the formulations are as follows.

Afsar Z, Khanam S, Aamir S. Formulation and Comparative Evaluation of Polyherbal Preparations for Their Disinfectant Effects, The International Journal of Therapeutics 2018; 1(1):54-65.

Table 1: Formula for cream formulation

Ingredient	Quantity taken (10g)
Extract combination	0.45g
White bees wax	2g
Liquid paraffin	6g
Borax	0.1g
Purified water	1.9ml
Rose oil	QS

Table 2: Formula for gel formulation

Ingredient	Quantity taken(10g)
Extract combination	0.45g
Sodium alginate	1.2g
Glycerine	0.2g
Methylhydroxyl benzoate	0.02g
Calcium gluconate	0.005g
Purified water	QSP 10g

Table 3: Formula for hand wash preparation

Ingredients	Quantity taken(10g)
Extract combinations	0.45g
Carbopol	0.075g
Triethanolamine	qs
Sodium lauryl sulfate	0.05g
Methyl paraben	0.025g
Distilled water	10ml

Table 4: Formula for sanitizer preparation

Ingredients	Quantity taken(10ml) HS1
Extract combination	0.45 g
Citronella oil	1.0 ml
Cinnamon oil	1.0 ml
Carbopol	0.1 g
Triethanolamine	0.1 g
Glycerine	0.5 ml
Polysorbate-20	0.1 ml
Perfume	Qs
Methyl paraben	0.1 mg
Alcohol	4.0 ml
Water	2.0 ml

Procedure for preparation of soap

Solidified basic glycerine soap was broken down to small pieces and melted on a water bath. 0.45 grams of the extract combinations were added to the melted soap along with 5 ml 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.

Disinfectant efficacy evaluation by hand wash method(13)

The hand wash method was standardized after reviewing the literature. Using gloves the hands were washed for one minute with soft soap and dried. The dry fingers were immersed in the prepared suspension of microorganism for 5 seconds after which the fingers were withdrawn and dried in air for 3 minutes.

The fingertips were rubbed on petri dish containing the sterile medium and it was incubated. This acted as the positive control. Following the same way, the contaminated dry fingers were rubbed with the formulations for 5 minutes and the fingers were withdrawn and dried in air for 3 minutes and then rubbed on petridish containing the sterile medium and incubated for 24 hrs at 370C and observed for growth. The growth on the control media and the extract treated medium were compared.

Disinfectant efficacy evaluation by degerming method[14,15]

The degerming test method was carried out on hands using thumbs which were pressed on fresh sterile agar medium before and after exposure to sample and standard. After which the plates were incubated and observed for microbial growth. Each petri plate was divided into four parts (ie A, B, C, and D). Unwashed hands were used and the right thumb was pressed gently on surface A of the medium which acted as positive control. The same thumb was then immersed in savlon (standard) for one minute, removed and dried and pressed on surface B. Similarly the thumb were rubbed with the formulations and pressed on surface C & D. The test was performed in triplicate and all the plates were incubated at 37oC for 24 hrs, after incubation the plates were observed for the presence of growth which was compared with controls.

Disinfectant efficacy evaluation by ditch plate method[16-21]

Ditches were made in Mueller Hinton culture medium in petri dishes by using a punch. These ditches were filled with three concentrations of the formulations i.e. 0.25, 0.5 and 1.0g of the formulation was diluted to 10 ml and used. A total of four organisms' i.e. S aureus, P aeruginosa, E coli, and L bacillus were used. The organisms were streaked (length was measured) in one petri plate three on each side. The length of streak (LOS) was recorded and the plates incubated at 37oC for 24 hrs, after which the length of inhibition (LOI) and percentage inhibition was recorded.

Disinfectant efficacy evaluation by Rideal Walker coefficient method[22-24]

In this method of evaluation, the activity of the sample (formulation) was compared with the activity of the standard phenol and results are interpreted as Rideal Walker Coefficient. Results were calculated by dividing the dilution of test solution which shows growth at 2.5 and 5 minutes but no growth after 5 min by that dilution of phenol which shows growth at 2.5 and 5 minutes but no growth after 5 min.

Procedure

Successive dilutions of sample (formulations) to be evaluated along with a commercially available standard disinfectant savlon and the standard phenol are inoculated with a culture of test organism (Salmonella) at various time intervals and the inhibition of growth was evaluated.

Two sets of 20 test tubes, one for sample and another set for standard were taken and labelled. To both sets of test tubes, 5 ml of RW broth were added. To one set of test tubes (20) 5ml of prepared dilutions of the formulations were added and to another set of test tubes (20) 5 ml of prepared dilutions of phenol were added respectively as shown in the below table.

Table 5: Dilutions and Sub culturing time in minutes

Dilutions	**Sub culturing time in minutes**			
	10	**7.5**	**5.0**	**2.5**
1:10	A1	A2	A3	A4
1:20	B1	B2	B3	B4
1:30	C1	C2	C3	C4
1:40	D1	D2	D3	D4
1:50	E1	E2	E3	E4

The minimum dilution of sample which did not show growth at a specific time divided by the minimum dilution of standard which did not show growth at the specified time gives the Rideal Walker coefficient.

RESULTS

Antimicrobial screening of plant extracts

The various plants extracts were subjected to antimicrobial screening against the selected organisms by agar diffusion method. Results revealed that the methanolic bark extracts of Cassia fistula, Ficus religiosa & Milletia pinnata exhibited maximum zones of inhibition which are tabulated in the table:1 & figures:1-4.

Note: CL= Cassia fistula leaf, CB= Cassia fistula bark, ML= Milletia pinnata leaf, MB= Milletia pinnata bark WB= W thyrsoidea bark, WL= W thyrsoidea leaf. FL= Ficus religiosa leaf, FB= Ficus religiosa bark, MEOH=methanol extract, 40MOH= aqueous methanol extract.

Table 6: Zones of inhibition of various extracts of plants

Extracts	P acnes	S epidermidis	E coli	Staphylococcus aureus	P aeruginosa
CL-MOH	--	--	--	--	--
CL-40MOH	--	--	--	--	--
CB-MOH	14.0	18.0	14.0	16.0	20.0
CB-40MOH	10.0	--	12.0	8.0	6.0
FL-MEOH	8.0	10.0	--	--	--
FL-40MOH	15.0	--	8.0	--	--
FB-MEOH	10.0	16.0	18.0	20.0	22.0
FB-40MOH	--	8.0	14.0	12.0	12.0
ML-MOH	--	12.0	6.0	--	10.0
ML-40MOH	--	10.0	6.0	--	12.0
MB-MOH	16.0	14.0	16.0	22.0	18.0
MB-40MOH	--	6.0	12.0	10.0	16.0
WL-MEOH	--	9.0	8.0	8.0	10.0
WL-40MOH	--	--	--	--	--
WB-MEOH	--	14.0	10.0	--	12.0
WB-40MOH	--	9.0	9.0	--	--

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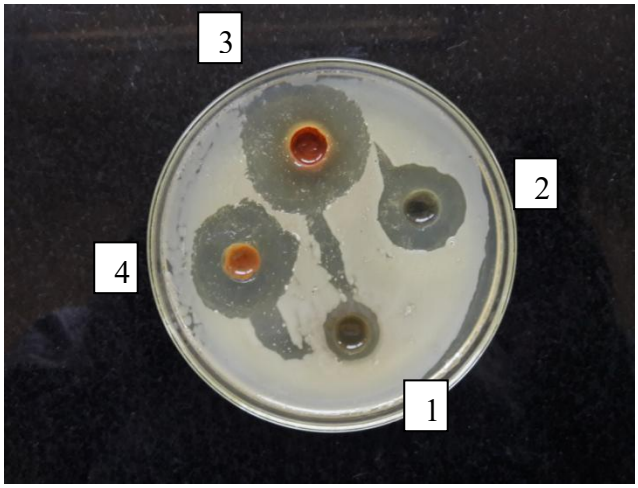


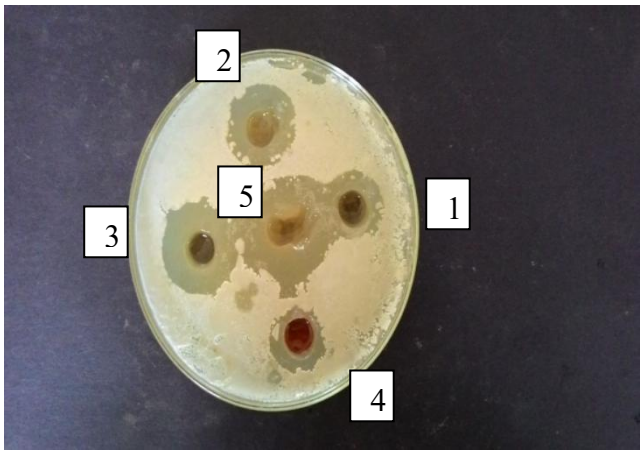
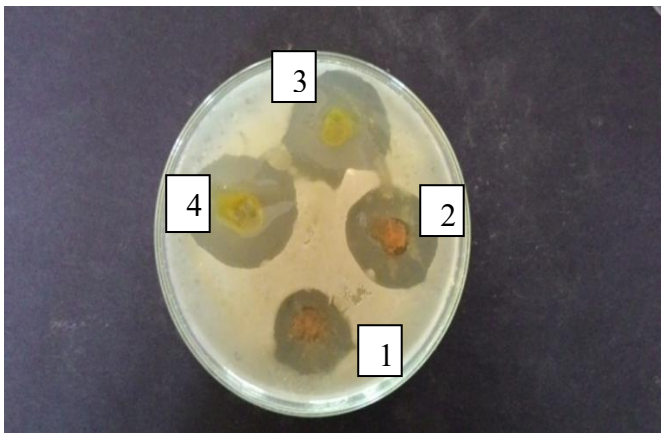
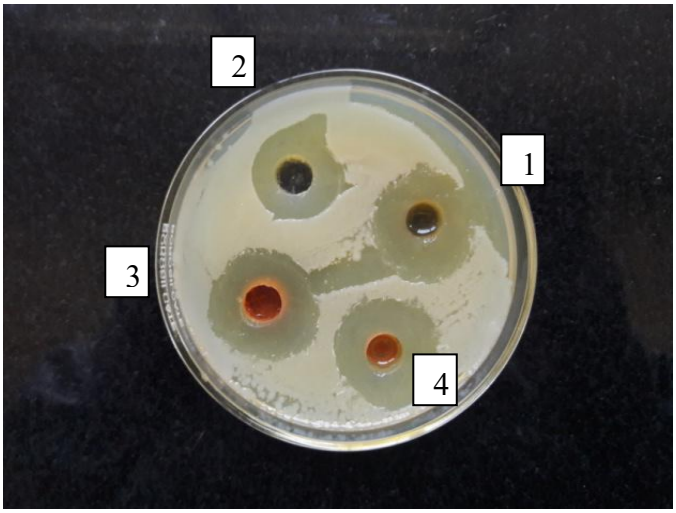


Fig 1 & 2: Zones of inhibition of the formulated gel and cream against *P acnes* & *S epidermidis*

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

Fig-2: 1=gel(0.25g). 2=cream(0.5g). 3=cream(1.0g). 4=cream(0.25g). 5=gel(1.0g).





Figures-3 & 4: Zones of inhibition of the formulated soap, handwash and sanitizer against E coli & S aureus

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

Fig-4: 1=soap(1.0g). 2=sanitizer(0.5g). 3=hand wash(0.5g). 4=soap(1g).

Evaluation of disinfectant activity by hand wash method

In the hand wash method, results showed that the extract treated hands exhibited significant difference in the growth pattern of microorganism when compared to the positive control. The standard savlon, sanitizer and soap inhibited growth completely but the hand wash, cream and gel formulations also reduced the growth of microorganisms significantly when compared to the positive control. The results are tabulated in the table: 2 & figures: 5-8.

Table 7: Results of disinfectant activity of formulations by hand wash method

Sl no	Combination no: 2/ Disinfectant	Time(Min)	Results
01	Positive Control	5.0	++++
02	Standard disinfectant	5.0	---
03	Cream	5.0	++
04	Gel	5.0	++
05	Hand wash	5.0	++
06	Sanitizer	5.0	---
07	Soap	5.0	---

Note: ++++ = more growth, + = less growth, --- = no growth.

Figures 5-8: Evaluations of microbial growth by hand wash method

Figure: 5. Positive control

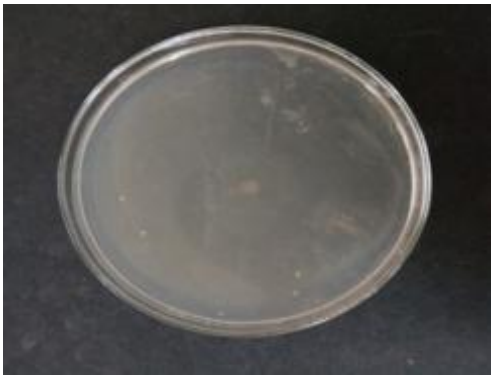


Figure 6: Standard (Savlon)

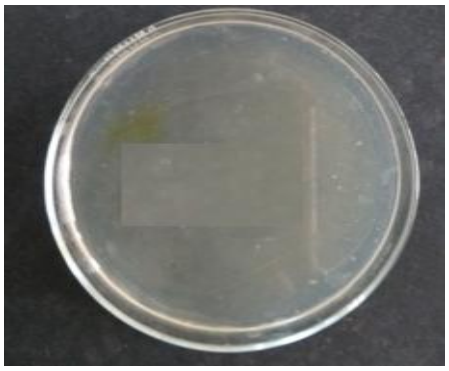


Figure: 7. Soap

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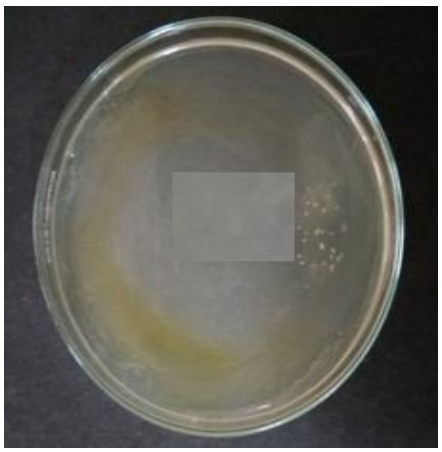


Figure: 8. Sanitizer

Disinfectant efficacy evaluation by degerming method

The Degerming test method was carried out on hands using thumbs which were pressed on fresh agar medium before and after exposure to sample and standard. Savlon was used as a standard disinfectant along with which the samples were compared. Without washing the hands all the plates showed growth which was taken as positive control. After treating the thumbs with the std and samples the plates were incubated. All the formulations exhibited growth inhibition with the standard, sanitizer and soap exhibiting complete inhibition whereas the plates with cream, gel and hand wash exhibited slight growth. Results are tabulated in the table: 3 and figures.

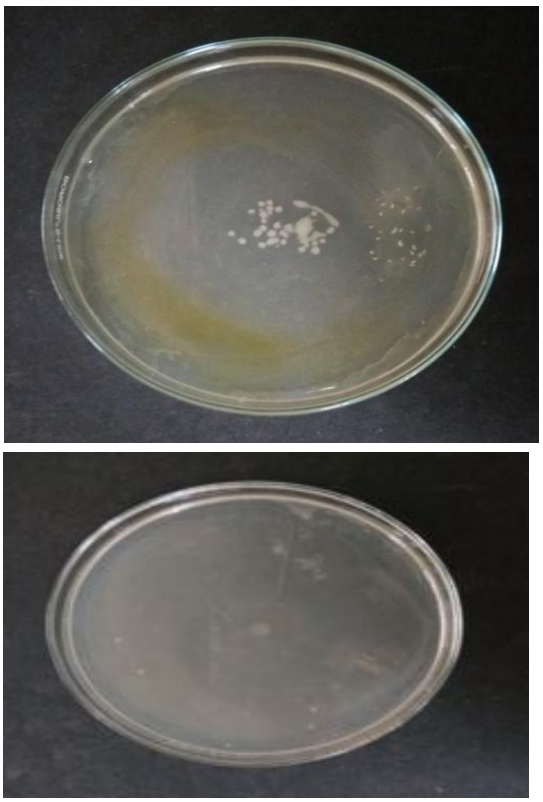
Afsar Z, Khanam S, Aamir S. Formulation and Comparative Evaluation of Polyherbal Preparations for Their Disinfectant Effects, The International Journal of Therapeutics 2018; 1(1):54-65.

Table 5: Results of Degerming test by formulations

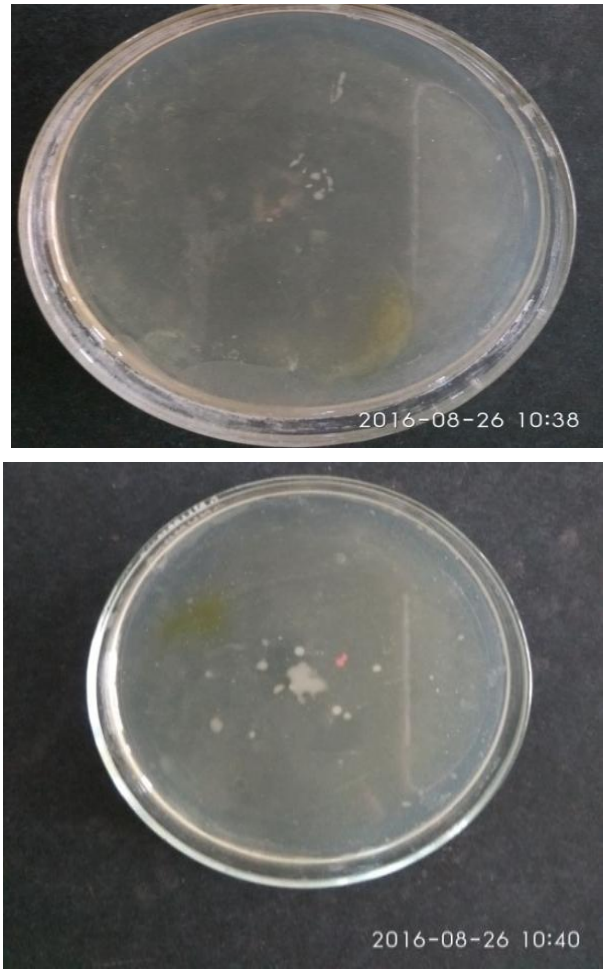
Formulation	Growth
Positive control	++++
Savlon (Std)	----
Cream	++
Gel	+
Soap	----
Sanitizer	----
Hand wash	+

Note: ++++ indicates microbial growth, ++ indicates lesser growth, ---- indicates complete growth inhibition.

Figures: 9-12. Results for microbial growth by degerming method



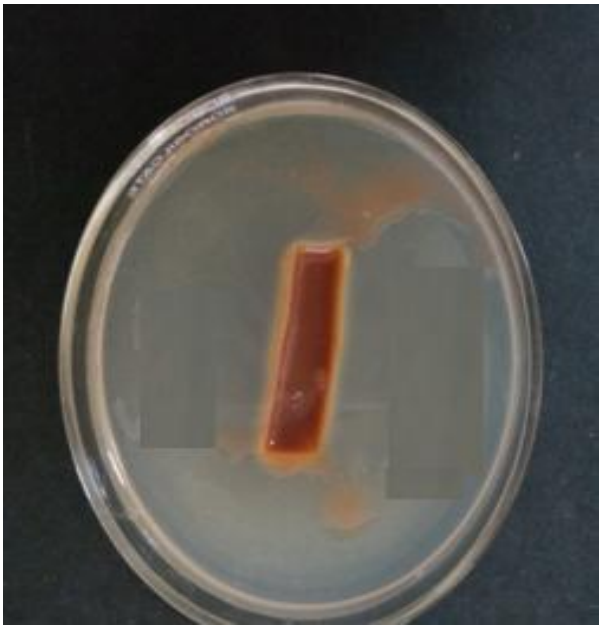
Figures: 9-10. Degerming test of the positive control and the standard savlon

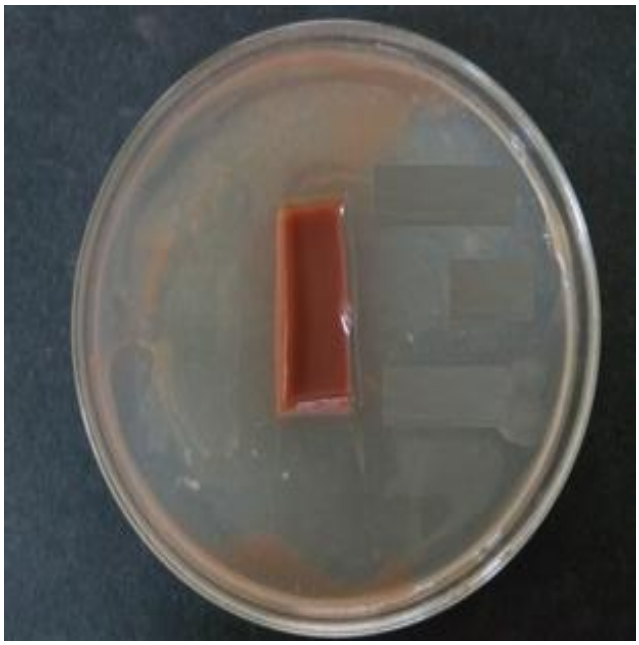


Figures:11-12. Degerming test of the Sanitizer and Soap

Disinfectant efficacy evaluation by ditch plate method

The prepared formulations were evaluated for their antimicrobial activity by ditch plate method against four microorganisms' i.e. *S aureus, P aeruginosa, E coli,* and *L bacillus*. Three concentrations of the formulations i.e. 0.25, 0.5 and 1.0g of the formulation was diluted to 10 ml and evaluated and their percentage inhibition were recorded. Results revealed that the soap and sanitizer exhibited maximum activity at a concentration of 1g with 100% growth inhibition against S aureus and E coli respectively; this was followed by gel which exhibited 96.5% inhibition at a concentration of 1g against S aureus and P aeruginosa. Results are tabulated in below table: 4 and figures 13, 14.





Figures: 13, 14. Disinfectant activity of sanitizer & soap to various microorganisms by ditch plate method

Disinfectant efficacy evaluation by Rideal Walker coefficient method

The disinfectant activity of the formulations was evaluated by Rideal Walker coefficient (RWC) determination. Among all the formulations, the sanitizer showed the maximum RWC of 0.8 which was comparable with that of the standard disinfectant "savlon", this was followed by soap which showed the RWC of 0.7. The hand wash exhibited the RWC of 0.6. Results are tabulated in table: 5.

Table 6. Results of "Redial Walker Coefficient" (RWC) of the formulations

Formulation	RWC
Savlon (STD)	0.95
Cream	0.4
Gel	0.5
Soap	0.7
Sanitizer	0.8
Hand wash	0.6

Table 7: Results of disinfectant activity of the formulations by Ditch plate method showing the percentage inhibition of microbial growth

Formulation	Conc (grams)	*S aureus*			*P aeruginosa*			*E coli*			*Lacto bacillus*		
	--	LOS (mm)	LOI (mm)	%INH	LOS (mm)	LOI (mm)	%INH	LOS (mm)	LOI (mm)	%INH	LOS (mm)	LOI (mm)	%INH
Gel	0.25	30	22	73.3	27	10	37.0	29	20	68.9	29	22	75.8
	0. 5	28	26	92.8	29	24	82.7	30	25	83.3	28	26	92.8
	1.0	29	28	96.5	28	27	96.4	28	27	96.4	31	30	96.7
Cream	0.25	31	20	64.5	32	15	46.8	30	12	40.0	28	12	42.8
	0. 5	29	25	86.2	29	20	68.9	29	18	62.0	31	25	80.6
	1.0	32	28	87.5	27	26	96.2	28	26	92.8	28	27	96.4
Soap	0.25	32	27	84.3	27	08	29.6	26	12	46.1	29	10	34.4
	0. 5	33	30	90.9	33	14	42.4	32	21	65.6	31	27	87.0
	1.0	32	32	100.0	26	22	84.6	27	27	100.0	27	27	100.0
Hand wash	0.25	28	24	85.7	29	08	27.5	28	11	39.2	26	04	15.3
	0. 5	30	27	90.0	32	16	50.0	30	18	60.0	28	12	42.8
	1.0	27	26	96.2	27	22	81.4	28	24	85.7	25	20	80.0
Sanitizer	0.25	27	24	88.8	28	26	92.8	28	24	85.7	26	22	84.6
	0. 5	29	26	89.6	30	28	93.3	32	30	93.7	29	26	89.6
	1.0	26	26	100.0	28	27	96.4	30	30	100.0	27	26	96.2

CONCLUSIONS

From this study, it was concluded that the plant extracts and prepared formulations exhibited good disinfectant properties along with good disinfectant efficacy when evaluated by various disinfectant testing methods. Among all the prepared formulations, the sanitizer and soap exhibited maximum antiseptic and disinfectant properties hence they need further standardization after which they can be manufactured for commercial purpose.

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