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Formulation of Polyherbal cream and evaluation of their antimicrobial potential

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Abstract

In recent years, there has been a progressive gradual development in the use of medicinal plants in developing nations, owing to the fact those herbal medicines are safe and have few side effects, especially when compared to synthetic pharmaceuticals. As the occurrence of unwanted side effects appears to be less common with herbal medications compared to allopathic treatments, the usage of herbal drugs is increasing, resulting in a rapid increase in the number of herbal drug makers. Since they are typically less expensive than synthetic medications People have been increasingly using herbal medicines during the last few decades. The present study was commenced to evaluate the quantitative profiling and antimicrobial activity of cream prepared from extracts of traditional plants like Kanthkari, Rasana, and Nirgundi. In the present study, a cream was formulated which comprised of an extract of plant materials. The dried plant materials of Kanthkari, Rasana, and Nirgundi were individually extracted using the maceration method.

These three different types of extracts were used for the preparation of cream and evaluated for the HPTLC profiling and antimicrobial activity. The HPTLC profiling of the extract and formulation was performed on pre-coated silica gel Aluminum Plate 60F-254 using Toluene: Ethylacetate: FormicAcid (8:1.5:0.5v/v/v) as mobile phase and a further quantity of Quercetin was evaluated in extract and formulation. The antimicrobial activity of prepared cream was evaluated against Escherichia coli and Staphylococcus aureus by disk diffusion method using reference disk of antibiotics. The result of antimicrobial the activity was determined by comparing the results of the zone of inhibition of formulation and solvent extract. The formulation showed significant activity against the tested bacterial pathogens. The disk diffusion method was used to assess the antibacterial activity of methanolic extract using a reference disk of antibiotics. The Rf value and spectral scanning of standard Quercetin (Rf 0.22) match with the values of extract and formulation indicate the presence of Quercetin in extract and formulation. Considering the ability of the golden treasure present in Kanthkari, Rasna, and Nirgundi. The Quality Control profiling of formulation and extract was performed by HPTLC, and antimicrobial activity was established by disk diffusion method. Formulated cream can use as a potential antibacterial against various bacterial infections.

Key-words: Polyherbal cream, Antimicrobial activity, Formulation

Introduction

Topical skin infections commonly occur and often present therapeutic challenges to practitioners, despite the numerous existing antibacterial agents available today. The necessity for developing new antibacterial means has increased significantly due to growing concerns regarding multidrug-resistant bacterial, viral, and fungal strains[1-4].Common examples for topical skin infections include diaper rash, cold sores, and tinea (also called pityriasis) versicolor. Topical delivery is the application of a drugcontaining formulation to the skin to treat cutaneous disorders (e.g. acne) or cutaneous symptoms of a general disease (e.g. psoriasis) with the goal of limiting the drug's pharmacological or other action to the skin's surface or within the skin.

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Its development is multifactorial, including skin wetness, friction, skin irritants, and pH change, which favors the growth of microorganisms including *Candida, Staphylococcus*, and *Streptococcus*[5].

India has a long history of medicinal herbs, as well as a variety of ancient medical systems. Traditional medicine, which is widely practiced in India, includes a vast variety of plants with varied therapeutic and pharmacological significance, and so provides a priceless reservoir of new bioactive compounds. In recent years, there has been a greater emphasis on plant research around the world, and data has accumulated to illustrate the enormous potential of medicinal plants employed in diverse traditional systems.From a drug development standpoint, plant chemical compounds will hit the drug target at certain places and rule over the synthetic substance[6]. As a result, plant chemical components are one of the greatest sources for most significant new medication discoveries. Traditional medicines are still used by more than 60% of the world's population to cure various diseases rather than allopathic medicine systems[7].Traditional medicinal plants including Kanthkari, Rasana, and Nirgundi are well-known for their therapeutic properties,Different parts of them have been used by Indian traditional healers to treat a range of diseases, such as Rasana, the plant is used for the inflammation and bronchitis, psoriasis, cough and piles. It is also used as antipyretic, analgesic, dyspepsia, rheumatoid arthritis, bitter, laxative and nervetonic.Thedecoctionoftheplantisusedtoprevent theswellingofjointsinarthritis, rheumatism and neurologicaldisorders [8,9]and Nirgundipossessesanalgesic,anti-parasitic, antibacterial,antipyretic,antihistaminic, analgesic, insecticidal, CNS depressant activity, and anti-fertility[10]. Kanthkari, Itiscommonlyusedinbronchialasthma, cough, worms, etc several othermedicinaluseslikeanthelmintic,antipyretic,anti-inflammatory,antitumor, cytotoxic activities, anti-asthmatic, antispasmodic, and hypotensive[11,12].The aim of the present study was to formulate topical creams containing extract of Rasna, Kanthkari and Nirgundi and to evaluate the *in vitro* antibacterial activity in the formulations against *Escherichia coli* and *Staphylococcus aureus*.

Experimental

Collection of plantsample

The fresh leaves and roots of Kanthkari, Rasana, and Nirgundi were collected from Minor Forest Produce Processing and Research Centre, Bhopal. The plantwas identifiedandauthenticatedby Ms.MahimaofVedantaTestingandResearchlaboratory,Bhopal.

Pharmacognostic study: The sample was evaluated on different parameters like Macroscopic, Microscopic, Physiochemical and Phytochemical to ensure the quality, authenticity of the plant material.

Table 1: Pharmacognostic investigation

Pharmacognostic investigation of Rasna	
BotanicalName	*Pluchea lanceolata*
Family	Asteraceae
CommonName:	Rasna
Macroscopic Examination	
Condition	Dried
Colour	Darkgreen
Odour	Characteristic
Taste	SlightlyastringentandBitter

Leaves	Leaves simple 2-5 cm long, 0.5 to 2cm broad, brittle, sessile obtuselanceolate, reticulate, midrib more distinct at the lower side, lateral veins somewhatrunningstraight parallel to the midrib; entire toothed near the apex, acute base symmetrical bothsurface pubescent.
Microscopic Examination	 **Figure 1.1:**
	TS passing through the mid rib shows one or occasionally two centrally located meritless encircled by thick walled, lignified, dorsiventrally well-developed sclerenchyma sheath, radially arranged xylem vessel and well developed phloem tissue; collenchyma tissue, almost reaching up to the meristele lies underneath both the epidermis.
Pharmacognostic investigation of Nirgundi	
BotanicalName	*Vitexnegundo*
Family	Verbenaceae
CommonName	Nirgundi leaves
Macroscopic Examination	
Condition	Dried
Colour	Darkgreen
Odour	Aromatic
Taste	Bitter
Leaves	Fresh leaf, leathery, palmately trifoliate to Penta foliate, the middle leaflet largest in size 6 to 12cm long 1.5 to 2cm wide with 1 to 2cm long petiole at base. Rachis long, leaflets, lanceolate, acute, entire or rarely crenate, pubescent and dark green on upper surface, tomentose and whitish on lower surface.

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Microscopic Examination	**Figure 1.2:** 
	TS of leaflet passing through the midrib is strongly convex on its lower side and somewhatflattened on its upper side, with an arc of meristele in the centre and narrow is bilaterallamina on its either side; the entire lower surface of the leaf pubescent. Mesophyll regionof the lamina shows 2-5 compact rows of the palisade cells discontinuous over the midribregionbutgettingslightlynarrow.
Pharmacognostic investigation of Kantakari	
BotanicalName	*Solanumvirginiatum*Linn.
Family	Solanaceae
MacroscopicExamination	
Condition	**Dried**
Colour	Yellowishgreen
Odour	Characteristic
Taste	Characteristic
Leaves	Brittle ovate oblong or rarely elliptic in shape 2 -12 cm in length, 2.5-5 cm inwidth, sinuate or sub pinnatified, sub acute, pubescent; midrib and lateral veins showsprominent sharp prickles; petiole cylindrical, 1 -2 cm in length, pubescent with spines andlongitudinallyrunningfurrowedupperside.
Microscopic Examination	  **Figure 1.3:**
	TS of leaf passing through midrib is dorsiventrally convex shows a narrow collenchymatous band underneath both the epidermis, a centrally located arc of biocollateral vascular bundle and two lateral narrow dorsiventral laminar extensions

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Physicochemical analysis:
Physicochemical analysis suchas Percentage ofashvaluesandextractivevalueswereredeterminedaccordingtothe Ayurvedic Pharmacopoeia of India[13].

Table 2: Physicochemical analysis

Experimental Results				
S.No	**Physicochemical parameters (% w/w)**	**Rasna Patti**	**Nirgundi**	**Kanthkari**
1	**Total ash**	12%	2.6 %	6.2%
2	**Acid-insoluble ash**	1.5%	0.62%	1.6%
3	**Alcohol-soluble extractive**	28.6 %	12.1%	12.2 %
4	**Water-soluble extractive**	26.2%	8.2%	11%

Preparation of Extract:
The two fraction of plant sample was prepared usingmethanol and Triple distilled water.
Methanolic Extract- Coarsely powdered 100g of drug was macerated and extracted with250 ml methanol at room temperature for 7 days and the extract was concentrated, frozen and lyophilized by lyophilizer.
Aqueous Extract-Coarsely powdered 100g of powdered drug was macerated and extracted with at room temperature for 7 days and the extract was concentrated by placed in oven at not more than 40°C for about 24 hours[14].

Preliminary PhytochemicalInvestigation.
The plant extracts were assessed for the existence of thephytochemical classes as described by Trease and Evans(2002), and Harborne (1973). Investigations on the preliminary phytochemical screening of Kanthkari, Rasana, and Nirgundiextracts revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids and carbohydrates in methanolic and aqueous extracts respectively[15,16]**.**

Table 3: Preliminary Phytochemical Investigation.

Chemical Constituents	**Methanolic extract**			**Water Extract**		
	Rasna Patti	**Nirgundi**	**Kanthkari**	**Rasna Patti**	**Nirgundi**	**Kanthkari**
Phenols	-	+	+	-	+	+
Glycosides	+	+	+	+	+	+
Flavonoids	+	-	+	+	-	+
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Alkaloids	-	+	+	-	+	+
Carbohydrates	+	-	+	+	-	+
Proteins and amino acids	+	-	+	+	-	+

Formulation
The ingredients were weighed as per the details given in Table below.The oily phase (Part A) that consisted of the emulsifier (stearic acid) and other oil soluble components was heated to 75±1° C on a water bath shaker (with constant stirring. The water soluble components were added to water

(Part B) and heated to the same temperature followed by addition of Rasna extract, Nirgundi extract, Kanthkari extract, methyl and propyl paraben with continuous stirring. To the heated aqueous mixture, oily phase was incorporated with continuous stirring on magnetic stirrer (Jindal Scientific Industries Pvt. Ltd) until the emulsion cooled down[17].

Table 4: Formulation of Herbal Cream

PART A (Oily Phase)		PART B (Aqueous Phase)	
Ingredient	**%w/w**	**Ingredient**	**%w/w**
Light liquid paraffin	27.85	Triethanolamine	1.71
Stearic acid	8.57	Glycerin	10.71
Glyceryl monostearate	7.50	Methyl paraben	0.21
Cetostearyl alcohol	4.28	Propyl paraben	0.21
Microcrystalline wax	0.21	Rasna extract	2.10
Hard paraffin wax	1.00	Nirgundi extract	2.10
		Kanthkari Extract	2.10
		Distilled water	q.s.100%

Evaluation of cream

The formulation properties of the cream were studied visually and characteristics like physical state, colour, odour and overall appearance was recorded[18].

Table 5: Evaluation Parameter of cream

Parameter	Result
Color	Dark Green
Odour	Sweet
Consistency	Smooth
State	Stable Semi Solid
pH	6.52±0.20
Spreadability	28.89 ± 1.11g.cm/sec
Viscosity	64000 cps (at least rpm of 10)

Estimation of Quercetin by HPTLC

Standard stocksolution:

Standard Quercetin was procured from Natural Remedies, Bangalore, India. 10mg/10ml Quercetinas standard solution was prepared in methanol.

Mobile phase: Mobile phase consists of a mixture of Toluene: Ethylacetate: Formic Acid (8:1.5:0.5v/v/v).

Preparation of test solution for Kanthkari*:*

10 grams of coarsely powdered drug were taken in 250 ml stoppered conical flask and extract with 100 ml alcohol for 24 hours by maceration with occasional shaking. The extract was decanted and made up to 100 ml in volumetric flask. The solution is filtered through Whatman filter paper no.41, 25 ml of the extract was taken from stock solution and dried on a water bath. Extract the dried bark with n-hexane (2x 5ml). Concentrate the pooled n- hexane extract to 5ml.

Preparation of test solution for Rasna:

10 grams of coarsely powdered drug were takenin 250 ml stoppered conical flask and extract with 100 ml alcohol for 24 hours by maceration with occasional shaking. The extract was decanted and made up to 100 ml in volumetric flask. The solution is filtered through Whatman filter paper no.41, 25 ml of the extract was taken from stock solution and driedon a water bath. The dried mark

was extracted with Petroleum ether 60-80°C (2x5ml). Concentrate the pooled Petroleum ether 60-80 °C extract to5ml.

Preparation of test solution for Nirgundi:

Take 10 grams of coarsely powdered drug were taken in 250 ml stoppered conical flask and extract with 100 ml alcohol for 24 hours by maceration with occasional shaking. The extract was decanted and made up to 100 ml in volumetric flask. The solution was filtered through Whatman filter paper no.41, 25 ml of the extract was taken from stock solution and concentrate on water bath to 5ml.

Instrumentation and chromatographicconditions

Total12bandswerespottedwithwidth of8mm byaCamag 100 microliter syringe on pre-coated silica gel aluminum Plate 60F-252 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat 5 (Switzerland). A constant applicationrateof150nl/swas employedandspacebetweentwo bandswas8 mm.Theslit dimension was kept 6 mm × 0.20 mm micro; 100nm/s scanning speed was employed. The mobile phase consisted of Toluene: Ethylacetate: Formic Acid (8:1.5:0.5v/v/v). Linear ascending development was carried out in a twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 20 min at room temperature. The length of the chromatogram run was approximately 70 mm. Subsequent to the development; the TLC plate was dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner in the absorbance mode at 252 nm. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between200 and 400 nm. The content of Quercetin was estimated in the extract and dosage forms by spotting 10µl solution of Kanthkari and 2 µlsolution of the Rasna, Nirgundi, and formulation sample on a TLC plate. The TLC plate was developed to a distance of 6 cm from the point of application. TLC plate was air dried and scanned on a Camag TLC Scanner in its absorbance mode at 252 nm.

Figure 2:Scanning of TLC plate via Camag TLC Visualizer

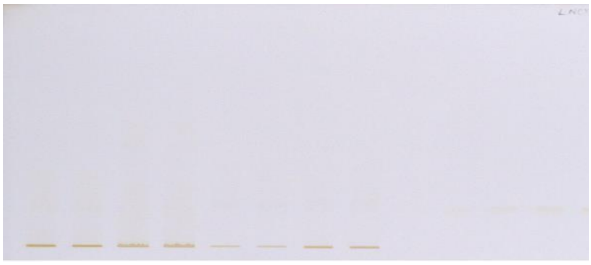
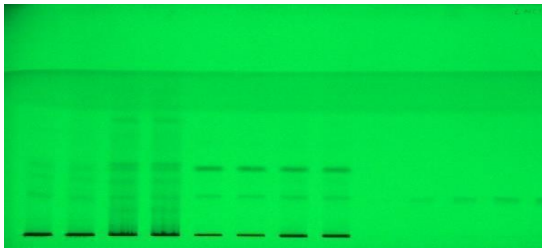
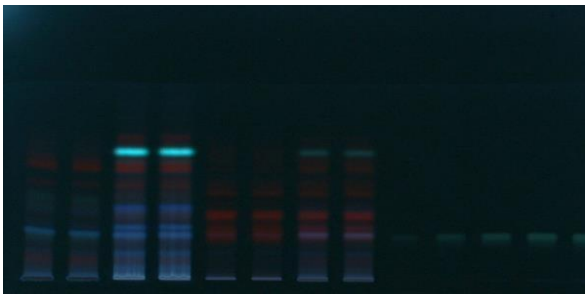
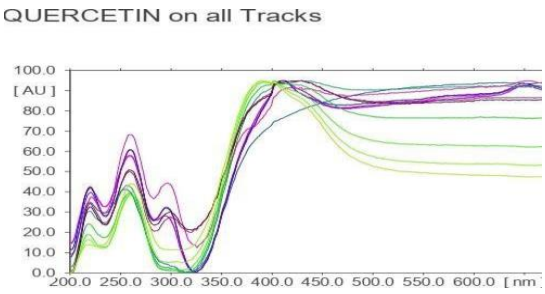
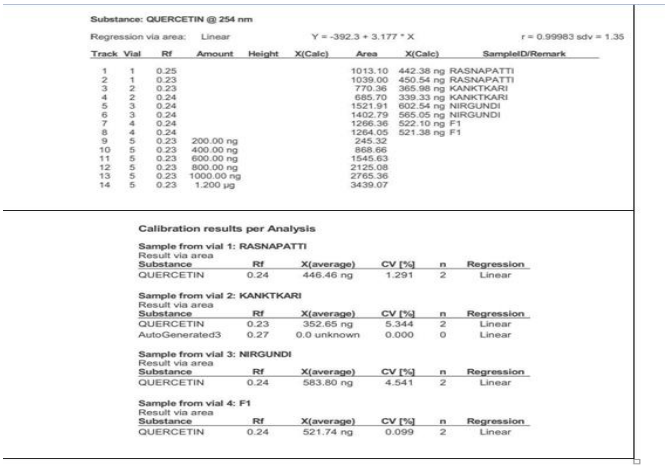
TLC plate under white light	**TLC plate under 252 nm**
	
TLC plate under 266 nm	**Spectral Scanning of Quercetin on all tracks**
	

Table 6: Rf value of extract and formulation indicate the presence of Quercetin

Tracks Number	Name	Rf value	Peak area
1.	Rasnapatti extract	0.25	1012.1
2.	Rasnapatti extract	0.22	1122.9
3.	Kantkari extract	0.22	681.9
4.	Kantkari extract	0.22	685.6
5.	Nirgundi extract	0.22	1521.9
6.	Nirgundi extract	0.22	1226.2
7.	Formulation	0.22	1266.2
8.	Formulation	0.22	1209.2
9.	Standard 1	0.22	228.2
10.	Standard 2	0.22	822.2
11.	Standard 2	0.22	1512.6
12.	Standard 2	0.22	2100.0
13.	Standard 5	0.22	2622.8
14.	Standard 6	0.22	2265.6

Estimation of Quercetin

The content of Quercetin was estimated in the extract and dosage forms by spotting 10µl solutionofKanthkariand2µlsolutionoftheRasna,Nirgundiandformulationsampleon a TLC plate. The TLC plate was developed to a distance of 6 cm from the point of application. TLC plate was air dried and scanned on a Camag TLC Scanner in its absorbance mode at 252 nm. The content of Quercetin was calculated by linear regression, and mean percentages were calculated from six replicate experiments.



Substance: QUERCETIN @ 254 nm

Regression via area: Linear Y = -392.3 + 3.177 * X r = 0.99983 sdv = 1.35

Track	Vial	Rf	Amount	Height	X(Calc)	Area	X(Calc)	SampleID/Remark
1	1	0.25				1013.10	442.38 ng	RASNAPATTI
2	1	0.23				1039.00	450.54 ng	RASNAPATTI
3	2	0.23				770.36	365.98 ng	KANKTKARI
4	2	0.24				685.70	339.33 ng	KANKTKARI
5	3	0.24				1521.91	602.54 ng	NIRGUNDI
6	3	0.24				1402.79	565.05 ng	NIRGUNDI
7	4	0.24				1266.36	522.10 ng	F1
8	4	0.24				1264.05	521.38 ng	F1
9	5	0.23	200.00 ng			245.32		
10	5	0.23	400.00 ng			868.66		
11	5	0.23	600.00 ng			1545.63		
12	5	0.23	800.00 ng			2125.08		
13	5	0.23	1000.00 ng			2765.36		
14	5	0.23	1.200 µg			3439.07		

Calibration results per Analysis

Sample from vial 1: RASNAPATTI
Result via area

Substance	Rf	X(average)	CV [%]	n	Regression
QUERCETIN	0.24	446.46 ng	1.291	2	Linear

Sample from vial 2: KANKTKARI
Result via area

Substance	Rf	X(average)	CV [%]	n	Regression
QUERCETIN	0.23	352.65 ng	5.344	2	Linear
AutoGenerated3	0.27	0.0 unknown	0.000	0	Linear

Sample from vial 3: NIRGUNDI
Result via area

Substance	Rf	X(average)	CV [%]	n	Regression
QUERCETIN	0.24	583.80 ng	4.541	2	Linear

Sample from vial 4: F1
Result via area

Substance	Rf	X(average)	CV [%]	n	Regression
QUERCETIN	0.24	521.74 ng	0.099	2	Linear

Antibacterial activity of plant extracts andformulation:

Anti-bacterialanalysis

Theanti-bacteriaanalysiswascarriedoutusingthe methanolicextractofplantstoassessthe antibacterial activity. Antibacterial activity was analyzed against *Escherichia Coli* and *Staphylococcus aureus*. The extract was prepared as in the standard procedure. 1mg of driedextractwasweighedandsuspendedin1mlofdi-methylsulphoxide(DMSO)tomake astocksolutionof1mg/mldose.Antibacterialactivity wasdeterminedbythediscdiffusion method to test the inhibitory zone formed by leaf extract. The sterile discs were taken and impregnated with the different concentrations of leaf extract then placed on nutrient agar media plates containing bacterial cultures and incubated at 26°C for 22 hrs. After incubation, the antibacterial activity of methanolic leaf extracts against the bacterial strains was assessed by measuring the diameter of the inhibition zoneformed[19].

Disk DiffusionMethod

Different concentrations of herbal drug are compared with standard antibiotics, as recommended in the CLSI (Clinical and Laboratory Standards Institute) guidelines. The disk of antibiotics contains:

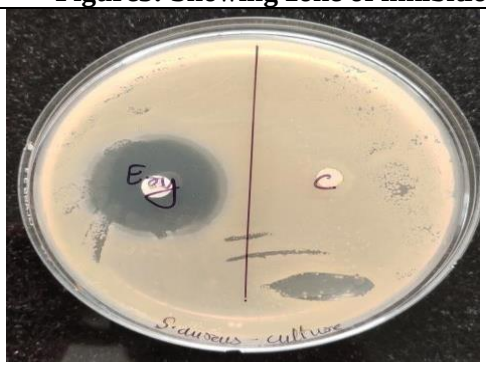
Standard Erythromycin–15mcg.

Herbal Drug concentrations usedare:

a) Stock Solution – 500mg/1000µl
b) 100mg/500µl
c) 65mg/500µl
d) 50mg/500µl
e) 25mg/500µl

The relative effectiveness of a compound was determined by comparing the diameter of the zone of inhibition of bacterial growth around the discs.The Antibacterial activity of plant extracts and formulation with respect to Erythromycin antibiotic disc is mentioned in the table 7:

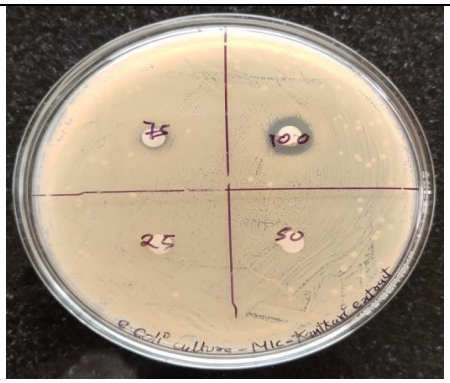
Figure3: Showing zone of inhibition on different concentration



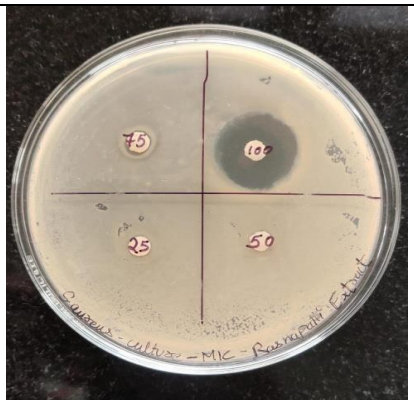
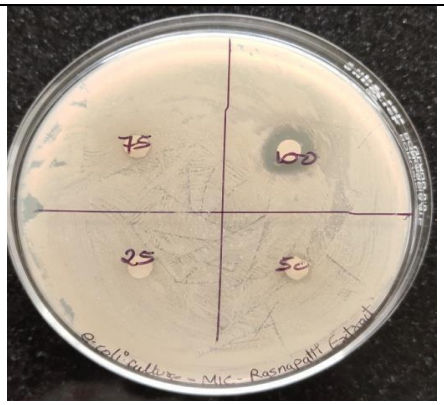
S.Aureus Culture

E.Coli Culture

S.Aureus Culture MIC-Kanthkari Extract



E.Coli Culture MIC-Kanthkari Extract

	
S.Aureus Culture MIC-RasnapattiExtrac	E.Coli Culture MIC-Rasnapatti Extract

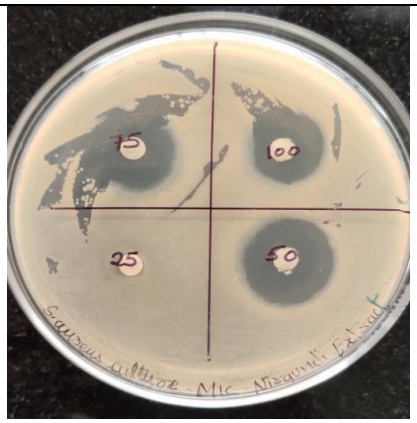
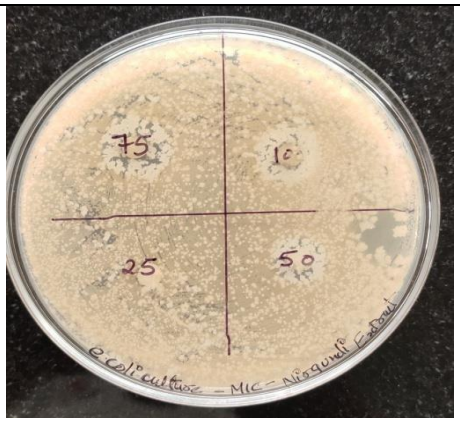
	
S.Aureus Culture MIC-Nirgundi Extract	E.Coli Culture MIC-Nirgundi Extract
	
S.Aureus Culture MIC-Formulation	E.Coli Culture MIC-Formulation

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Table 7: Effect of extract of rasnapatti and Erythromycin on Zone of inhibition ondifferent concentration

Name of extract	Name of micro-organisms	Zone of inhibition on different concentration					
Rasnapatti extract	*Staphylococcus aureus*	Eryth romycin	Control	25mg/ml	50mg/ml	65mg/ml	100m g/ml
		12 mm	0	0	0	1 mm	8 mm
	E. coli	2 mm	0	0	0	0	2 mm

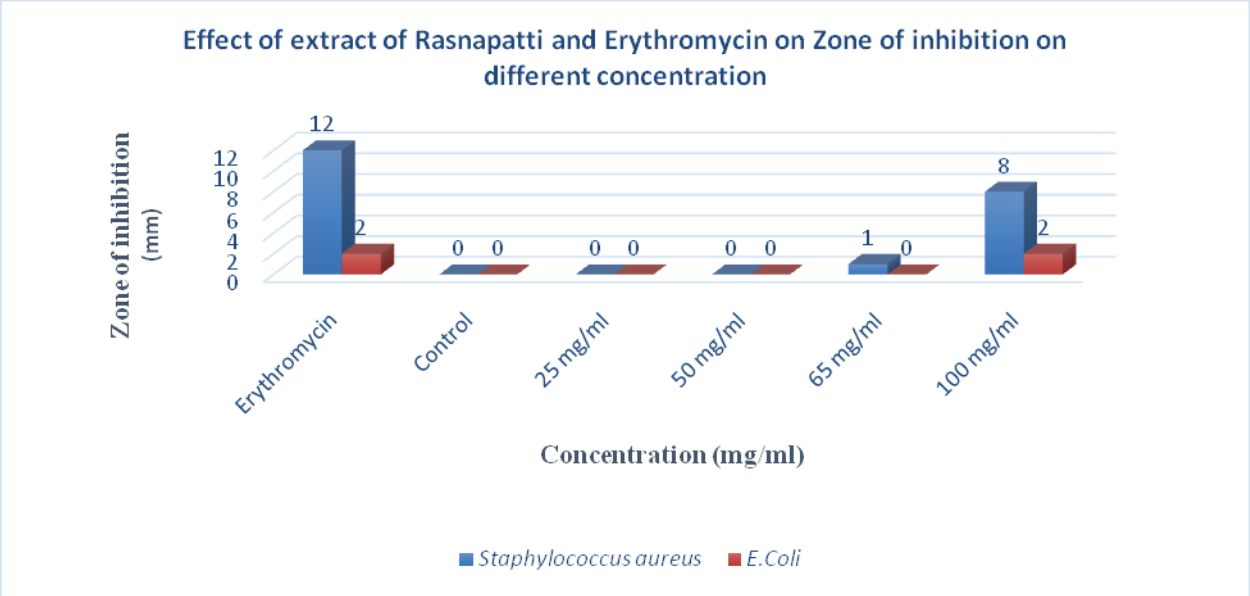

Figure 4: Effect of extract of rasnapatti and Erythromycin on Zone of inhibition on different concentration

Table 8: Effect of extract of Kantkari and Erythromycin on Zone of inhibition on different concentration

Name of extract	Name of micro-organisms	Zone of inhibition on different concentration					
Kantkari extract	*Staphylococcus aureus*	Eryth romycin	Control	25mg/ml	50mg/ml	65mg/ml	100m g/ml
		12 mm	0	0	0	6 mm	2 mm
	E. coli	2 mm	0	0	0	1 mm	2.2 mm

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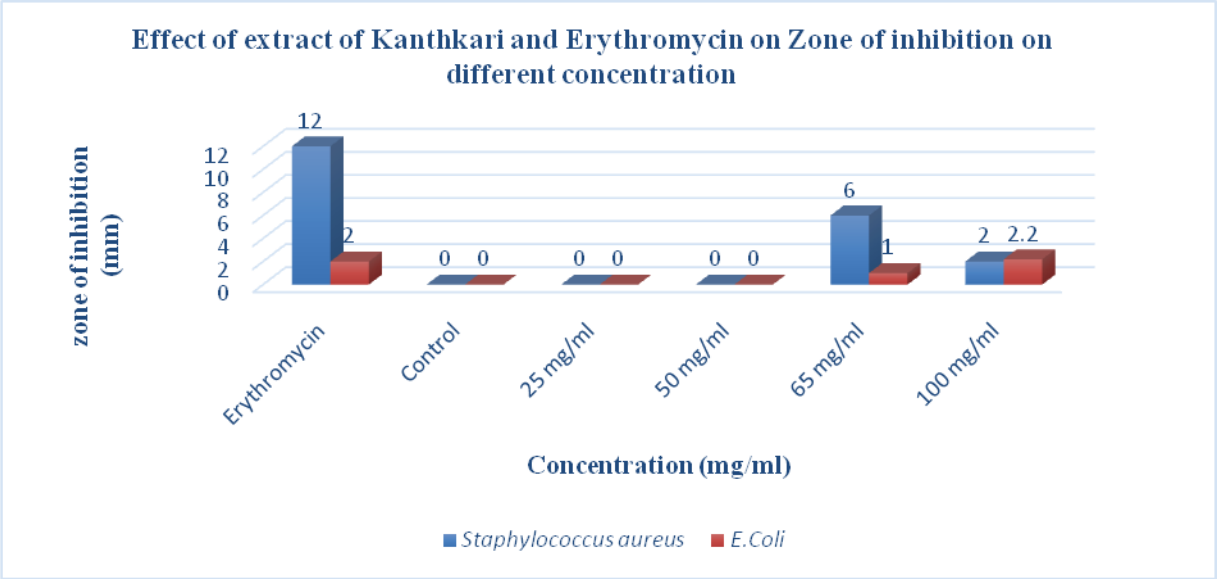

Figure 5: Effect of extract of Kantkari and Erythromycin on Zone of inhibition on different concentration

Table 9: Effect of extract of Nirgundi and Erythromycin on Zone of inhibition on different concentration

Name of extract	Name of micro-organisms	Zone of inhibition on different concentration					
Nirgundi extract	*Staphylococcus aureus*	Erythromycin	Control	25mg/ml	50mg/ml	65mg/ml	100mg/ml
		12 mm	0	0	6.5 mm	9 mm	8 mm
	E. coli	2 mm	0	0	2 mm	5.5 mm	5 mm

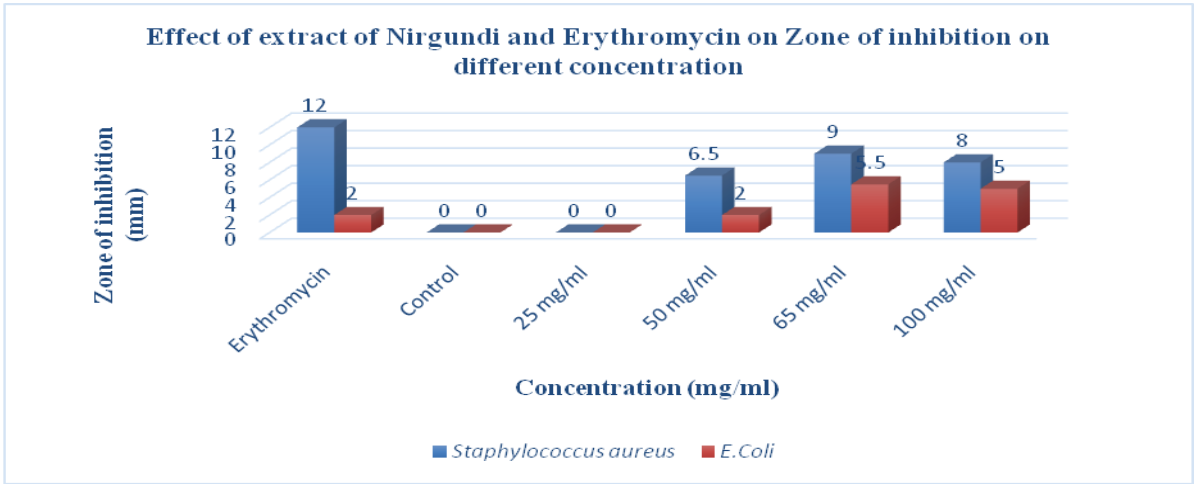

Fig6: Effect of extract of Nirgundi and Erythromycin on Zone of inhibition on different concentration

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Table 10: Effect of extract of Formulation and Erythromycin on Zone of inhibition on different concentration

Name of extract	Name of micro-organisms	Zone of inhibition on different concentration					
Formulation	***Staphylococcus aureus***	**Eryth romycin**	**Control**	**25mg/ml**	**50mg/ml**	**65mg/ml**	**100m g/ml**
		12 mm	0	5 mm	5.5 mm	6 mm	6 mm
	E. coli	2 mm	0	1 mm	2 mm	2 mm	2.5 mm

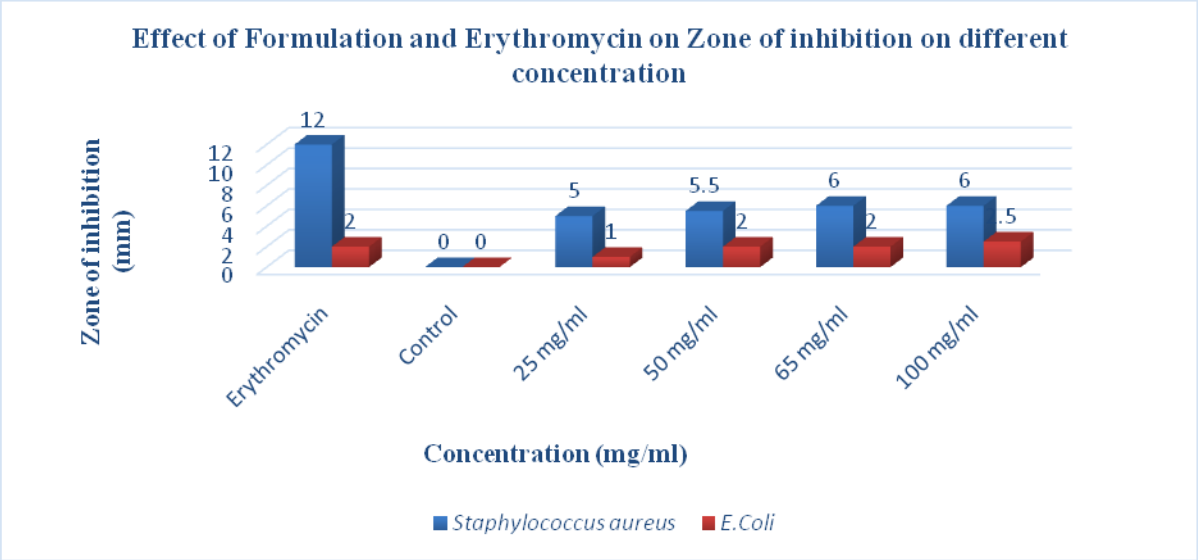

Fig 7: Effect of extract of Formulation and Erythromycin on Zone of inhibition on different concentration

Result and Discussion

The present study features a novel herbal skin care formulation with potential antibacterial properties. Prepared herbal cream contains combinations of herbal ingredients that are formulated to combat bacterial infection and reduce oxidative stress. This work deals with description, review of literature, pharmacognostical evaluation of plant material, preliminary phytochemical studies, thin layer chromatography, preparation of herbal cream comprising three plant extracts and in vitro antibacterial activity of extracts and formulation and pharmaceutical evaluation offormulations.

Analytical study of plant material

In recent years there has been an emphasis on standardization of medicinal plants of therapeutic potential. Identification and evaluation of plant drugs by pharmacognostic studies are very reliable, accurate and inexpensive means. According to World Health Organization (WHO) these studies are the first step towards establishing its identity and purity. This study was focused on the physical and chemical characters of roughly ground plant of Rasna, Kanthkari and Nirgundi.

Study started with the analysis of physical parameters of above-mentioned plants; in this, we studied Loss on drying, Total ash value, Acid insoluble ash value, Water soluble ash value,

Alcohol extractive value and Water extractive value. Preliminary physical characters of different extracts, qualitative chemical tests and TLC was performed to get an idea of presence of different classes of secondary metabolites, which are useful indicators of both efficacy and potential toxicity. The results of total ash value, acid insoluble ash value, water soluble ash values help in detected the extent of adulteration as well as establish the quality and purity of the drug. The solvent used for the extraction dissolves appreciable quantities of desired constituents. The selectivity of the solvent is important not only for the yield of one or more principal substances, but also for the qualitative and quantitative composition of the accompanying substances. Moreover, extractive values are primarily useful for the determination of exhausted or adulterated drugs. From the present study, it can be concluded that aqueous extractive values of selected medicinal plants were higher thantheethanol solvents. The result suggests that large quantity of polar soluble phytoconstituents were present in crudeextracts.

TLC profiling of ethanol extracts gives an impressive result that directing towards the presence standard Quercetin. The TLC method is the best choice for the identification of the secondary metabolite present in plants. The qualitative as well as quantitative HPTLC analysis under standard conditions provides chromatograms, which are very useful for controlling the quality of the phyto-pharmaceuticals. These studies provided referential information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbaldrugs.

In vitro antibacterialactivity

Antibacterial activity was done using ethanolic extract by disc diffusion method at different concentration against two bacterial strains showed varied level of zone of inhibition ranged from 1mm-9mm. Formulated cream shows significant result against *S.aureus and E.coli* thereby establishing promising dosage form .

Due to Restrictions of CPCSEA for the use of animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available. Hence, in the present study the assessment of the antimicrobial property of the herbal extract of Kanthkari, Rasana, and Nirgundi and formulation done by disc diffusion method at different concentration against two bacterial strains showed varied level of zone of inhibition.

Conclusion

The plant products over the synthetic compound in the treatment of diseases are needed because it does not have a deleterious effect in higher plants and animals including man. The urge to research new drugs from natural sources is now moving out of the herbalist's shop,awayfromthecoretextsintothedrugresearchlaboratories.Indiais hometoavariety of traditional medicine systems that rely largely on native plant species for their raw drug materials. Therefore,nowthereisaneedtolookbacktowardstraditionalmedicines,which can serve as a novel therapeutic agent. After getting information from various Vaidhyas, Ayurvedic doctors, and from a literature survey, it was found that *Kanthkari, Rasana,* and *Nirgundi* was used since ancient times to treat various diseases and prone to have antimicrobial properties. Hence, O/W emulsion-based cream formulation composed of *Kanthkari, Rasana, and Nirgundi*is prepared further HPTLC profiling and Antibacterial properties was evaluated. HPTLC profiling confirms the presence of Quercetin in formulation and extract with an Rf of 0.22. Antibacterial activity established by disk diffusion method shows 6mm and 2.5 mm the zone of inhibition by O/W emulsion-based cream formulation against *S.aureus and E.coli* respectively. It is concluded that the cream formulation exhibits significant antibacterial activity. The results authenticate the fact that prepared herbal skin care formulations could be used safely and effectively and are good alternatives for bacterial infection.

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