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FORMULATION AND EVALUATION OF TOPICAL POLYHERBAL GEL CONTAINING *COCCULUS HIRSUTUS* AND *TRIDAX PROCUMBENS*

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Keywords:

Cocculus hirsutus, *Tridax procumbens*, Herbal gel, Antimicrobial, Anti-inflammatory

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ABSTRACT: Topical medications are frequently used to treat skin conditions. Antibiotic-resistant bacteria have emerged. As a result, the man has looked to nature for healing. Traditional medicines work better because they are less likely to cause side effects and are more effective. The *Cocculu shirsutus* and *Tridax procumbens* leaves were authenticated, dried, and extracted. Phytochemical screening and physicochemical analysis of extracts were done. The antimicrobial studies and *in-vitro* anti-inflammatory activity of extracts were carried out. The polyherbal gel was formulated by varying the concentrations of the gelling agents. The formulation was evaluated for physical appearance, visual homogeneity, pH, viscosity, and accelerated stability studies. Six batches of polyherbal gel were formulated using different gelling agents and varying concentrations. The F4 formulation showed the best results. Six batches of polyherbal gel were formulated using different gelling agents and varying concentrations. The best results were shown by F4 formulation.

INTRODUCTION: Ayurveda, Siddha and Unani use herbal medicines to treat various diseases. Indian folk medicines can treat a variety of diseases such as inflammation, leprosy, scabies, skin infections, ulcers and wounds. Herbal medicines are commonly used to treat skin diseases [1]. For thousands of years, natural medicines have been used. The most common type of folk medicine is herbal remedies. Despite advances in synthetic drug chemistry, plants remain important sources of drugs for treating various diseases. An investigation is being conducted to determine the role of active principle in treating disease [2].

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Herbal medications have attracted the attention of developing countries due to their safety and lack of harmful effects. Because topical herbal preparations are widely used, they have gained much popularity and attention. Medicinal plants with antimicrobial properties are used to treat skin diseases. When gels are applied topically at the pathological site, the drug is released more quickly [3]. *Tridax procumbens* and *Cocculus hirsutus* have traditionally been used to treat a variety of skin conditions. *Cocculus hirsutus* belongs to the family of Menispermaceae.

It has a cooling effect. It heals impetigo, eczema, prurigo, blisters, cuts, and other skin problems. It has effective antibacterial and antifungal properties. *Tridax procumbens* has antimicrobial, anti-inflammatory and wound-healing properties. In folk medicine, the leaf extract is applied directly to wounds and used to treat skin infections. Local

healers use it to treat blisters, boils, and cuts. It has antifungal and antibacterial properties [4, 5]. Polyherbal formulations, according to Sarangdhar Samhita, have higher therapeutic efficacy. Polyherbal preparations contain multiple herbs in an appropriate ratio, which aids in achieving desired effects while lowering toxicity.

Single-plant active principles are sometimes incapable of producing the desired effects. Crude plant extract is more potent than individual isolated constituents. They produce a synergistic effect. Polyherbal preparations can be used to treat a wide range of diseases. Plant-based pharmacological agents have synergistic, potentiating, agonistic, and antagonistic effects. Because the dose required is small, the risk of side effects is reduced [6].

MATERIALS AND METHODS:

Collection and Authentication of Plant Materials: *Tridax procumbens* and *Cocculus hirsutus* leaves were collected from the Kolhapur region, Maharashtra. The leaves were authenticated in Central Research Facility, B.M.K Ayurveda Mahavidyalaya, Belagavi. (CRF/AUTH 70/2021 AND CRF/AUTH 71/2021 Respectively)

Processing of the Plant Material: After the leaves were authenticated, they were shade dried for fifteen days. The leaves were ground into a coarse powder. For future use, the powder was stored in an airtight glass container.

Extraction of Plants: *Tridax procumbens* dried leaf powder was macerated in methanol for 48 hours before being extracted on the Soxhlet apparatus with methanol as a solvent. Both extracts were combined and concentrated further. Similarly, *Cocculus hirsutus* leaf powder was macerated in methanol for 48 hours. Methanol was used to extract the marc. Both the maceration and Soxhlet extracts were combined and concentrated.

Formulation of Polyherbal Gel: Gelling agents such as Carbopol-934, Sodium alginate, and Sodium CMC were used to prepare six formulations. The gelling agent concentration was varied. The gelling agents were mixed in water and stirred for 30 minutes in a mechanical stirrer at 1200 rpm. Overnight, the mixture was soaked. The extract and preservatives were mixed into the gel. They were thoroughly mixed, and triethanolamine was added to adjust the pH to that of the skin [7].

TABLE 1: COMPOSITION OF DIFFERENT BATCHES (%W/W)

	F1	F2	F3	F4	F5	F6
Cocculus hirsutus	1	1	1	1	1	1
Tridax procumbens	1	1	1	1	1	1
Carbopol-934	1	1.5	-	-	-	-
Sodium CMC	-	-	2	3	-	-
Sodium alginate	-	-	-	-	2	3
Propylene glycol	4	4	4	4	4	4
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02
Triethanolamine	1.2	1.2	1.2	1.2	1.2	1.2
Water	qs	qs	qs	qs	qs	qs

Evaluation of Polyherbal Gel: The polyherbal gels were evaluated for the following parameters: colour, appearance, homogeneity, consistency, washability, greasiness, pH, spreadability and viscosity.

Physical Appearance:

Appearance: The appearance of the formulation was noted from visual observation.

Homogeneity: The homogenous nature of the formulation was checked by rubbing the sample between fingers.

Consistency: The consistency of gel was checked by applying it on the skin.

Colour: The sample was observed with naked eyes against a white background.

Appearance: Visual observation was used to determine the formulation's appearance.

Homogeneity: The homogeneity of the formulation was tested by rubbing the sample between fingers.

Consistency: The gel's consistency was determined by applying it to the skin [8].

Washability: The gel was applied to the skin, and its ability to remain on the skin or wash off was tested [8].

Greasiness: The gel was applied to the skin to check for greasy nature [9].

pH: The pH of the gel was measured using a digital pH metre. To test the pH, 0.5 g of the sample was dissolved in 10 mL of distilled water [9, 14].

Spreadability: The spread plate technique was used to determine sample spreadability. On a glass petri plate, 1 g of polyherbal gel was placed. Another plate covered it. For 1 minute, 50 grammes of weight were placed on the place. The weight was then removed, and the diameter of the formed circle was measured from three different angles. The average diameter was determined [9, 13].

Viscosity: The viscosity was measured using a Brookfield viscometer. Spindle 1 was used to check the sample's viscosity. The viscometer was set to 50 revolutions per minute. The measurements were taken in triplicate, and the average was calculated [9, 13].

Accelerated Stability Study: Stability testing was performed on the optimized batch. For one month, the gel formulations were tested for stability under accelerated conditions (40°C2°C, 75%5% RH) and room conditions (25°C2°C, 60%5% RH). The formulation was put in a container. Aluminum foil was used to cover the containers. At various time intervals, the gel formulation was evaluated for physical appearance, spreadability, pH and viscosity [10].

Antimicrobial Activity:

Agar well Diffusion Method: Antimicrobial property of plant extracts is accessed by the Agar well diffusion method. The Mueller Hinton agar medium was prepared and autoclaved at 20 psi for about 1 hour. The agar medium was removed from the autoclave. The agar medium was poured into petri plate and allowed to cool. The microbial cultures (*Staphylococcus aureus* and *Escheria coli*) were spread onto the agar medium using sterile cotton swab. Holes/wells of 6 mm were made using a sterile cork borer. The samples and standards were poured into the holes/wells. The plates were kept for incubation in an incubator at 37±2°C for 24 hours. Gentamycin was used as a standard. The zone of inhibition was measured. The antifungal activity was determined using the agar well diffusion method. *Candida albicans* were used to access antifungal activity. The plates were incubated in an incubator at 27±2°C for 48 hours. As a control, clotrimazole gel was used. The zone of inhibition was determined [11].

Anti-inflammatory Activity:

HRBC Membrane Stabilization Method: Human blood was collected from a healthy individual. HRBC suspension was made by transferring the blood into an isotonic buffer solution and centrifuging it for 10 minutes at 3000 rpm. The supernatant is separated and centrifuged with isotonic solution until clear supernatant is obtained. The HRBC suspension is mixed with sodium phosphate buffer and sample. The samples were collected at various concentrations. HRBC suspension and isotonic saline are used as controls. The mixture was incubated at 56°C for 30 minutes. It was then centrifuged again. At 560 nm, the absorbance of the supernatant was measured [12].

Protein Denaturation Assay: Protein denaturation results in the protein losing its original biological properties. Protein denaturation is linked to inflammation. This assay uses bovine serum albumin (BSA) or egg albumin as a protein to assess anti-inflammatory activity.

For 10 minutes, the reaction mixture is kept at 70°C in a water bath to induce protein denaturation. To prepare the reaction mixture, plant extract (1-500 µg/ml), 200 µL of egg albumin or 450 µL (5% aq. solution) BSA, 1400 µL of phosphate, and buffer saline were mixed together. The negative control is made by removing the extract from the above mixture. For 15 minutes, the mixture is incubated at 37°C. It is heated for 5 minutes at 70°C and then cooled. At 660 nm, absorbance is measured. As a positive control, diclofenac sodium is used. 12

$$\text{Percent inhibition of denaturation} = (1\text{-}D / C) \times 100$$

D: Absorbance of the test sample. C: Absorbance of negative control.

RESULT AND DISCUSSION: *Cocculus hirsutus* and *Tridax procumbens* leaves were collected and authenticated. For one week, the leaves were shade dried. A grinder was used to powder the leaves. Methanol was used to macerate *Cocculus hirsutus* leaf powder for 48 hours.

Methanol was used to extract the marc in a Soxhlet apparatus. The extracts were mixed and concentrated. Methanol was used as a solvent to macerate *Tridax procumbens* leaf powder for 48 hours. Methanol was used to extract the marc in a Soxhlet apparatus. The extracts were mixed and concentrated. Phytoconstituents present in the extract were found using phytochemical tests. *Cocculus hirsutus* extract contained alkaloids, steroids, terpenoids, carbohydrates, flavonoids, and tannins.

Tridax procumbens extract contained carbohydrates, alkaloids, tannins, terpenoids, and steroids. Flavonoids and phenolic compounds exhibit strong antimicrobial activity. *Cocculus hirsutus* is rich in flavonoids. The leaves of *Cocculus hirsutus* containsrutin, liquiritin and quercetin. *Tridax procumbens* contains flavonoids. So the extracts show good antimicrobial activity.

TABLE 2: PHYTOCHEMICAL SCREENING

Phytoconstituent	Test	*Cocculus hirsutus*	*Tridax procumbens*
Alkaloids	Dragendroff's test	+	+
	Mayer's test	+	+
	Hagers's test	+	+
	Wagner's test	+	+
Carbohydrates	Molisch's test	+	+
	Fehling's test	+	+
	Benedict's test	+	+
Flavonoids	Shinoda test	+	+
	Sulphuric acid test	+	+
Tannins	5 % Fecl3	+	+
	Lead acetate	+	+
Terpenoids	Salkowski's test	+	+
Saponins	Foam test	-	-
Steroids	Salkowski's test	+	+

Six batches of polyherbal gel were formulated using different gelling agents like Carbopol-934, Sodium alginate and Sodium CMC, varying their concentration. The gel was evaluated for pH, viscosity, appearance, spreadability, and consistency. F1, F2, F3 and F5 had good consistency. F4 and F6 had very good consistency. All the formulations were easily washable. The viscosity of the formulation were found in the range of 1267-2207. pH of formulations ranged from 6.37-6.49. The spreadability of formulations ranged from 5.4-6.2. Stability studies of the gel were performed by keeping the formulation at an accelerated temperature and then evaluating the gel. The formulation was found to be stable, as there was no change in its evaluation parameters. Batch F4 was stable. The antimicrobial activity of extract and optimum formulation was evaluated. The agar well diffusion method was used to access antimicrobial activity. *S. aureus* (gram positive) and *E. coli* (gram negative) were bacterial strains used. *C. albicans* were fungal strains used. The extracts and formulations showed good antimicrobial activity.

TABLE 3: EVALUATION OF POLYHERBAL GEL

	F1	F2	F3	F4	F5	F6
Color	**Green**	**Green**	**Green**	**Green**	**Green**	**Green**
Consistency	Good	Very good	Good	Very good	Good	Very good
Homogeneity	Good	Good	Good	Good	Good	Good
Appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Greasiness	Non-greasy	Non-greasy	Non-greasy	Non-greasy	Non-greasy	Non-greasy
Washability	Washable	Washable	Washable	Washable	Washable	Washable
pH	6.37	6.41	6.42	6.45	6.49	6.40
Spreadability	6.2	5.9	5.7	5.5	5.6	5.4
Viscosity	1267	1572	1819	2207	1807	2195

TABLE 4: *IN-VITRO* ANTIMICROBIAL ACTIVITY (EXTRACTS)

Sr. no.	Micro-organism	Zone of inhibition (mm)		
		Cocculus hirsutus extract	*Tridax procumbens* extract	Standard
1	*S.aureus*	20	23	27
2	*E.coli*	21	19	28
3	*C. albicans*	17	18	24

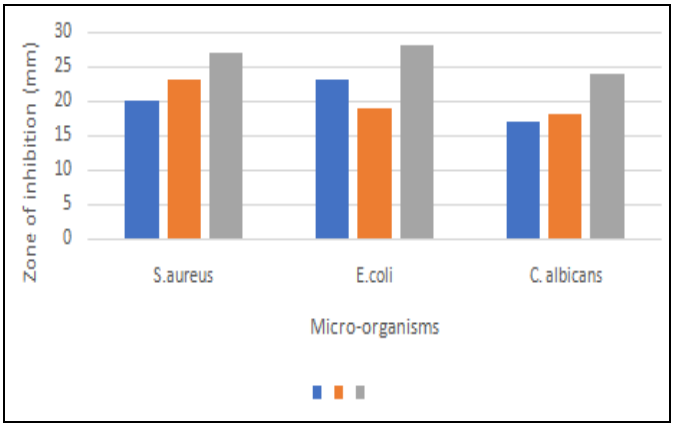

FIG. 1: ANTIMICROBIAL ACTIVITY OF EXTRACT

TABLE 5: *IN-VITRO* ANTIMICROBIAL ACTIVITY (FORMULATION)

Sr. no.	Micro-organism	Zone of inhibition (mm)	
		Polyherbal gel	Standard
1	*S. aureus*	23	26
2	*E. coli*	22	26
3	*C. albicans*	18	24

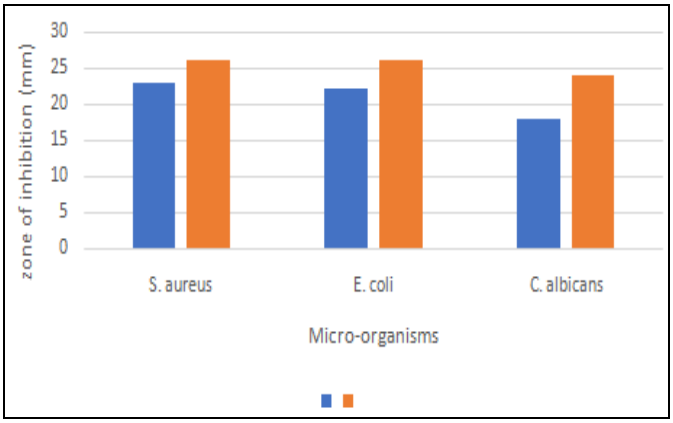

FIG. 2: ANTIMICROBIAL ACTIVITY OF GEL

In-vitro Anti-inflammatory Activity:

HRBC Membrane Stabilization Method: Extract of *Cocculus hirsutus*.

TABLE 6: *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY (HRBC MEMBRANE STABILIZATION METHOD) OF *COCCULUS HIRSUTUS* EXTRACT

Concentration (µg/ml)	Absorbance	Prevention of lysis.
300	0.169	32.93
200	0.182	27.77
100	0.193	23.41
Aspirin	0.157	37.69
Negative control	0.252	

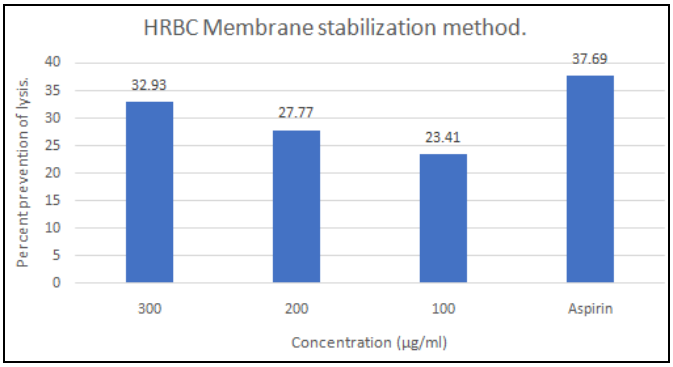

FIG. 3: *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY (HRBC MEMBRANE STABILIZATION METHOD) OF *COCCULUS HIRSUTUS* EXTRACT

TABLE 7: *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY (HRBC MEMBRANE STABILIZATION METHOD) OF *TRIDAX PROCUMBENS* EXTRACT

Concentration (µg/ml)	Absorbance	Prevention of lysis.
300	0.165	34.78
200	0.178	29.64
100	0.186	26.48
Aspirin	0.156	38.43
Negative control	0.253	

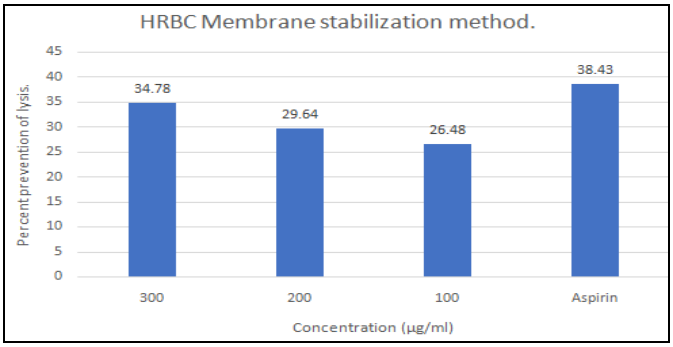

FIG. 4: *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY (HRBC MEMBRANE STABILIZATION METHOD) OF *TRIDAX PROCUMBENS* EXTRACT

Protein Denaturation Assay:

TABLE 8: *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY (PROTEIN DENATURATION METHOD) OF *COCCULUS HIRSUTUS* EXTRACT

Concentration (µg/ml)	Inhibition of protein denaturation (%)	
	Cocculushirsutus	Standard
100	12.14	15.92
200	23.39	28.00
300	31.98	37.03
400	43.23	49.32
500	58.72	61.57

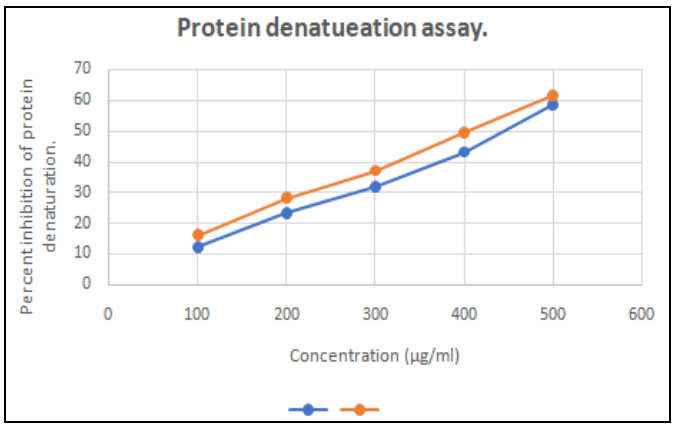

FIG. 5: *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY (PROTEIN DENATURATION METHOD) OF *COCCULUS HIRSUTUS* EXTRACT

TABLE 9: *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY (PROTEIN DENATURATION METHOD) OF *TRIDAX PROCUMBENS* EXTRACT

Concentration (µg/ml)	Inhibition of protein denaturation (%)	
	Tridax procumbens	Standard
100	11.54	15.92
200	24.45	28.00
300	30.75	37.03
400	43.55	49.32
500	56.12	61.57

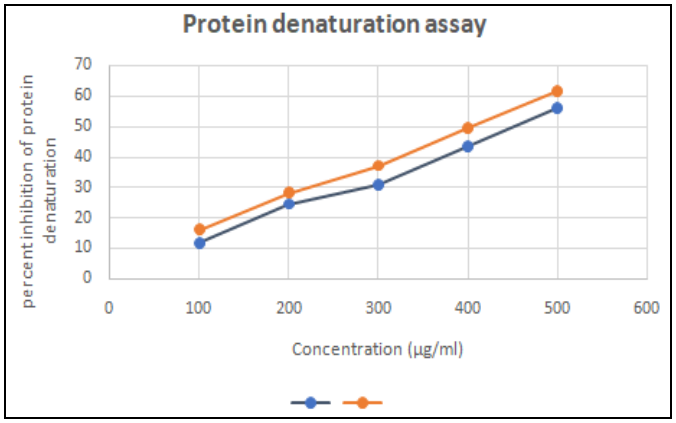

FIG. 6: *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY (PROTEIN DENATURATION METHOD) OF *TRIDAX PROCUMBENS* EXTRACT

TABLE 10: ACCELERATED STABILITY STUDIES OF OPTIMISED FORMULATION

Parameters	Initial	Room temperature			Accelerated temperature		
		25°C±2°C/RH 60±5%			40°C±2°C/RH 75±5%		
		7th day	15th day	30th day	7th day	15th day	30th day
pH	6.45	6.42	6.43	6.41	6.43	6.41	6.30
Viscosity	2207	2209	2206	2203	2205	2202	2200
Spreadability	5.5	5.52	5.54	5.51	5.5	5.53	5.57

CONCLUSION: Natural remedies are considered more acceptable than synthetic as they are safer. There is a growing demand for herbal formulations. The extract of *Cocculus hirsutus* showed presence of Alkaloids, steroids, terpenoids, carbohydrates, flavonoids and tannins. The extract of *Trida xprocumbens* showed presence of Alkaloids, steroids, terpenoids, carbohydrates, flavonoids and tannins. The extract of *Tridax procumbens* and *Cocculus hirsutus* showed good antibacterial, antifungal activity and anti-inflammatory activity. Formulation F4 was optimum in terms of viscosity, spreadability, consistency and pH. It can be concluded that poly-herbal gel showed good antimicrobial activity and anti-inflammatory activity.

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