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Formulation and Evaluation of Herbal Bandage Containing Curcuma Longa, Azadirachta Indica and Tridax Procumbens for Wound Healing

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Abstract
Bandages are commonly used to cover small wounds, providing protection against foreign elements. However, these conventional bandages lack pharmacological benefits. This study aims to design, develop, and assess a herbal bandage containing curcuma longa, azadirachta indica, and tridax procumbens linn for treating cuts, wounds, and burns. The selected herbal drugs are recognized for their safety and minimal side effects compared to allopathic medicines. The herbal drugs were processed into a powder and mixed to create an ointment base, resulting in a medicated herbal bandage. The ointment was transferred on a bandage with cavity. The ointment exhibited a homogeneous mixture, smooth texture, and a greenish color, without any grittiness. A diffusion study confirmed the release of phytochemical components from the ointment. The antibacterial effectiveness of the formulations against staphylococcus aureus and pseudomonas aeruginosa was evaluated using the rapid turbidimetric assay. Comparative results with the marketed formulation Fradiomycin® indicated turbidity values of 0.158 and 0.144, respectively. In vitro anti-inflammatory activity was assessed using the proteinase inhibitory method and protein denaturation assay, alongside the standard drug Diclofenac sodium®. The herbal bandage displayed a % proteinase inhibition of 40.81% and a protein denaturation of 48.02%, indicating potential anti-inflammatory properties. This research highlights the development of a herbal bandage with potential wound-healing benefits, which has potential antibacterial and anti-inflammatory activities.

Keywords: - Antibacterial, Herbal medicated bandage, Anti-inflammatory, Confirmatory test for Phytochemical constituents by diffusion, Wound healing

Introduction

Bandages are a standard of biomaterial applied to wounds for the purpose of preventing it from external environment. Currently more than 3000 types of dressings are available in the market for wound care. At wound site sometimes microorganisms enter in a wound as air exposure causes it

to be contaminated with bacteria and ultimately develop an infection. However, there is no bandage which have pharmacological activity. Hence there is need to of a bandage which not only offers covering but also will have activities like antimicrobial, anti-inflammatory which would result in quicker healing of wound. The present study aimed to design, develop, and evaluate the antimicrobial as well as anti-inflammatory activity of herbal bandage containing powdered herbal drugs [1]. Herbal drugs are beneficial for several skin related problems and also have wound healing effect. Herbal medications are always considered safer than allopathic medicines because it does not contain the side effects as like allopathic. In the present study we have selected curcuma longa, azadirachta indica and tridax procumbens linn for the formulation due to their anti-inflammatory, antimicrobial, wound healing activities, respectively [2-5]. In order to ensure that it is ready for use, the mixture of herbal drugs has been transferred into an ointment. The ointment was preferred over gel or cream because of topical use and the gels have slower and sustained activity which is not needed and cream may leak out. Additionally, water content of gels and cream can also make them more likely to be attacked by microbes or fungi. On other hand stability of ointment is more and it have capacity to hold its shape and content. The ointment was evaluated for its organoleptic properties, phytochemical constituents, antibacterial and anti-inflammatory activities. The formulated ointment was transferred onto bandage to make it ready to use, the marketed formulation Medigrip corn cap® concept was used [6-10].

Materials and methods: The part of plants of curcuma longa, azadirachta indica and tridax procumbens linn were freshly collected herbal garden of our college. The plants were identified and authenticated by, Department of Botany, KBP College Pandharpur, India. The Plant materials washed and dried under the shed and converted into powder by mechanical grinding. Then powders were passed through sieve no 16, and used for further study. Diclofenac sodium was obtained from J. B. chemicals and pharmaceuticals, Mumbai. The authenticated fresh bacterial cultures of staphyloccus aureous and Pseudomonas aeruginosa were obtained from department of biotechnology. Walchand College, Solapur. The Fridiomycin (Sofracare®) ointment, adhesive tape and liner were purchased from local surgical store.

Phytochemical Screening-

In order to determine the presence and absence of primary and secondary metabolites, phytochemical screening of plant extracts has been carried out using a standard procedure to verify the presence and purity of herbal medicinal products. Phytochemical tests for steroids, saponins, anthocyanins, coumarins, emodinants, alkaloids, proteins, amino acids, diterpenes, and phenol were carried out as per the earlier mentioned standard procedures [11-14].

Preparation of Bandage: -

Preparation of base for herbal drugs: - In a proportion of 4:1, bees wax and wool fat was transferred to a porcelain dish and melted. The powdered herbal drugs were mixed in a melted ointment base and cooled. The cooled ointment was further called as herbal ointment.

Table 1 Formulation chart of Herbal Bandage

Sr. No	Name of Ingredient	F1	F2	F3	F4	F5	F6
1.	Curcuma Longa	2%	2.5%	3%	10%	12.5%	15%
2.	Azadirachta Indica	1%	2%	3%	12.5%	15%	15%
3.	Tridax Procumbens linn	5%	6%	7%	10%	10%	10%
4.	Wool fat	73.6%	71.6%	69.6%	54%	50%	48%

5.	Bees wax	18.4%	17.9%	17.4%	13.5%	12.5%	12%

Preparation of bandage: -The 500mg ointment was applied in a cavity on an adhesive tape. The liner is used as a covering material. Initially adhesive tape was cut into a ($2cm \times 1cm$) and previously prepared cavity is kept at the center of the adhesive tape. The herbal ointment 500 mg was transferred into a cavity and finally covered with liner. The whole procedure of preparation of an herbal ointment and bandage was carried out under laminar air flow. This gives ready to use pharmacologically active herbal medicated bandage (Fig. 1 and Table 1).

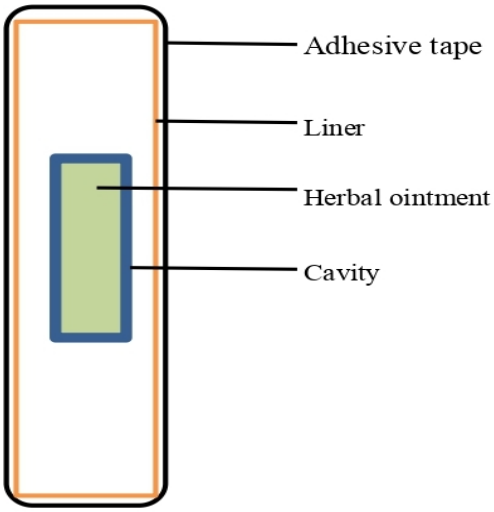

Fig. 1: Layout of Herbal medicated bandage

Evaluation

Phytochemical screening study: - Herbal drugs namely curcuma longa, azadirachta indica and tridax procumbens linn. were screened at a concentration range of 15%, 15% and 10%, respectively [15].

The general evaluation of ointment - The Prepared ointment was evaluated visually for colour, odour with the help of microscope beneath 10x magnification. The pH meter was used to determine pH of herbal ointment [16].

Confirmatory test for phytochemical constituent by diffusion study: -

A local diffusion cell, identical to a Franz diffusion cell, was created and medicinal bandage was placed over the donor and receptor compartments. The bandage cavity facing towards the donor compartment and adhesive tape towards the receiver compartment. In the receptor compartment, 32 ml of phosphate buffer was inserted at pH 6.8. In the receptor chamber, the temperature remained at 37℃ and was stimulated by magnetic beads. The 1 ml of sample was withdrawn and replaced it with an equal amount of buffer after each 15 minutes. For each chemical component, the diffusion study has been performed for a period of 2 hours. [17-18].

Rapid Turbidimetric assay: -

In order to determine antimicrobial activity, the Rapid Turbidimetric assay has been used. The bacterial species namely staphylococcus aureous and pseudomonas aeruginosa transferred into previously prepared broth individually and incubated for 24 hrs. at 37℃. After that herbal ointment was transferred into it. The Turbidity of ointment was calculated using a UV- Visible

spectrophotometer at 600 nm and compared with marketed formulation Fridomycin (Sofracare®) (0.3gm) [19-20].

In vitro anti-inflammatory activity: - The protein denaturation technique has been used to determine anti-inflammatory activity of the herbal ointment. Diclofenac sodium was used as a standard drug. The reaction mixture consisting of 2 mL of different concentrations of selected herbal drugs mixture or standard diclofenac sodium and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 2 mL of egg albumin and incubated at 27°C for 15 min. Denaturation was caused by incubation of the reaction mixture in water at 70 C for 10 minutes. After cooling, the absorbance was determined at 660 nm. All samples have been evaluated for the triplicate. (n=3). The percentage inhibition of protein denaturation was calculated by using the following formula:

$\%\ \text{inhibition} = \frac{Ac-At}{Ac} \times 100$

Where, At =absorbance of test sample; Ac=absorbance of control

In -Vitro anti-inflammatory activity by proteinase inhibitory method: -

The 1% bovine serum albumin solution was prepared and added to each test sample that contained a mixture of the herbal drugs. When the mixture had been stored at room temperature for 5 minutes, trypsin 250 was added. After centrifugation, an UV Visible spectrophotometer has been used to determine the absorbance of supernatant at 210 nm. All samples were assessed in the triplicate. (n=3). The percentage inhibition of proteinase activity was calculated by using the following formula:

$\%\ \text{inhibition} = \frac{Ac-At}{Ac} \times 100$

Where, At =absorbance of test sample; Ac=absorbance of control [21-22].

Result and Discussion: -

Evaluation of herbal drugs for purity-

The herbal drugs namely Curcuma Longa, Azadirachta Indica and Tridax Procumbens linn have been used in different proportions in an ointment to prepare herbal medicated bandage. As specified in the standards, the results of a phytochemical test for all herbal medicinal drugs show that certain essential phytochemical constituents are present (Table 2)

Table 2 Phytochemical screening

Sr. No	Test	Curcuma Longa	Azadirachta Indica	Tridax Procumbens linn
1.	Carbohydrate	(+)	(+)	(-)
2.	protein	(-)	(+)	(+)
3.	Glycoside	(+)	(-)	(-)
4.	Flavanoid	(-)	(+)	(-)
5.	Terpenoid	(-)	(+)	(-)
6.	Saponin	(+)	(+)	(+)
7.	Tannin	(-)	(+)	(+)
8.	Quinone	(+)	(-)	(-)
9.	Alkaloid	(-)	(-)	(+)
10	Coumarin	(-)	(-)	(+)

(+ presence of chemical constituents; - absence of chemical constituents)

General evaluation of ointment: -

In order to evaluate pharmaceutical ointment, the evaluation parameters are important tests. It has been established that the pH and thickness are satisfactory. The formulation has a pH of 5-6, which corresponds to normal skin pH and is not likely to irritate the skin [20]. It was found that herbal

preparations were free of gritiness, homogeneous mixture and with a smooth texture and the appearance of greenish colour. The thickness of the bandage has been found to be 1.078 mm, which is less than the marketed formulated bandage, namely the Medigrip corn cap, so that it can be applied to the skin easily and does not cause any problems (Table 3).

Table 3 General evaluation for ointment

Sr.no	Characters	F1	F2	F3	F4	F5	F6
1.	Colour	Green	Green	Green	Green	Green	Green
2.	Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
3.	Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
4.	Appearance	Glossy	Glossy	Glossy	Glossy	Glossy	Glossy
5.	pH	5-6	5-6	5-6	5-6	5-6	5-6
6.	Thickness	1.056	1.060	1.062	1.069	1.073	1.078

Confirmatory test for phytochemical constituent by diffusion study: -

To investigate the presence of herbal drugs and their stability, a diffusion study has been used. Curcuma Longa, Azadirachta Indica, and Tridax Procumbens linn have been shown to be successfully trapped and stable in the ointment by diffusion, phytochemical screening tests, Quinone, Flavonoid and Coumarin. The study also shows that the added herbal drugs have been stable, which would show their respective pharmacological effects (Table 4).

Table 4 Confirmatory test for phytochemical constituent by diffusion study

Sr.No	Drug	Chemical Constituents	Time (Min) 15	30	45	60	75	90	105	120
1	Turmeric	Quinone	- - - - - -	+ + + - + +	+ + + + + +	+ + + + + +	++ + + + +	+ + + + + +	- + - - + +	- - - - - -
2	Neem	Flavonoid	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + -	- - - - - -	- - - - - -
3	Tridax	Coumarin	+ - + + + +	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + +	+ + - - - -

(+ presence of chemical constituents; - absence of chemical constituents)

Rapid Turbidimetric assay: - Using a Rapid Turbidimetric assay, the developed formulations were investigated for their antimicrobial action against common bacteria such as Staphylococcus aureus and Pseudomonas aeruginosa. All formulations have been studied for the evaluation of antimicrobial activity. The optimized formulation showed highest turbidity of 0.158 on Staphylococcus aureous and 0.142 on Pseudomonas aeruginosa as compared to marketed formulation Fradiomycin® 0.109 and 0.143, respectively. Compared to other drugs, Curcuma Longa, has a higher influence on antibacterial activity. The activity increases gradually as concentration of Curcuma Longa, increases. The effect is mainly due to Curcuma Longa, richness in curcuminoid and phenolic compound, which might be contributed its higher antibacterial effect (Table 5 and Fig. 2). The results of antibacterial activity reveled ointments have potential in suppressing microbial growth and has comparable results with the standard drug fridomycin (Sofracare®). These results are in well accordance with earlier studies as Curcuma Longa has potential antibacterial activity [2].

Table 5 Antibacterial activity and Anti-inflammatory activity:

Sr.no	Sample	Antibacterial activity (Absorbance)		Anti-inflammatory activity (%)	
		Staphylococcus aureous	Pseudomonas aeruginosa	Proteinase inhibitory method	Protein denaturation assay
1	F1	0.196	0.181	23.25	27.61
2	F2	0.183	0.176	26.56	32.24
3	F3	0.178	0.164	29.21	36.10
4	F4	0.171	0.158	35.94	40.42
5	F5	0.166	0.146	38.98	42.24
6	F6	0.158	0.142	40.81	48.02
7.	Standard	0.109	0.143	49.87	53.49

In-vitro anti-inflammatory activity: - In-vitro anti-inflammatory activity in order to determine the anti-inflammatory activity of the formulation, the proteinase inhibitory method and the protein denaturation assay have been performed. The % Proteinase inhibition was found to be 40.81as compared to marketed formulation Diclofenac sodium® 49.87%. Protein denaturation was found to be 48.02% compared to marketed formulation Diclofenac sodium® 53.49%. (Table 5 and Fig. 2) [3] The results of anti-inflammatory and antibacterial activity are as per our hypothesis that Azadirachta Indica will have more effect on anti-inflammatory and antibacterial activity, respectively.

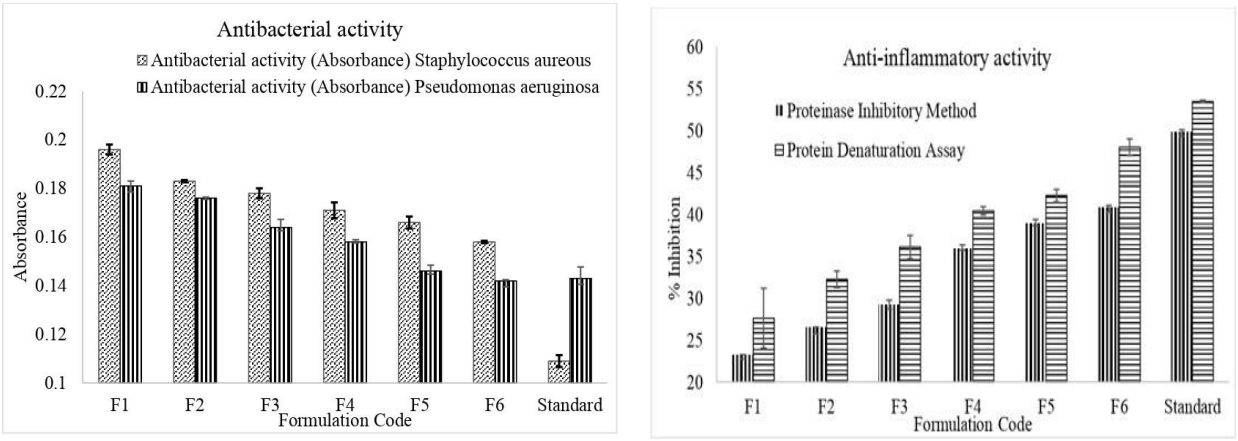

Fig. 2: Antibacterial activity and Anti-inflammatory activity

The results of anti-inflammatory and antibacterial activity are as per our hypothesis that aloe barbadensis miller and glycyrrhiza glabra will have more effect on anti-inflammatory and antibacterial activity, respectively.

Conclusion: -

The herbal bandage has potential to heal wounds and could replace the marketed bandages in terms of fast healing. As compared to the marketed formulations, the formulated herbal bandage has good antibacterial and anti-inflammatory activity. It's the first kind of bandage that's pharmacologically active. Diffusion study was utilized to confirm the presence and stability of herbal drugs. The phytochemical screening study of herbal medicinal products reveals the presence of essential chemical constituents in accordance with the standard. The pH of the formulation was found to be 5-6, which is acceptable and does not cause skin irritation. Anti-inflammatory activity of herbal medicated ointment was found to be 40.81% as compared to marketed formulation Diclofenac sodium® 49.87%.

Compliance with ethical standards -

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Conflict of interest-The authors declare that they have no conflict of interest.

Informed consent- Not Applicable

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Ethical approval-This article does not contain any studies with human participants or animals performed by any of the authors.

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