



Original Article

Formulation and Evaluation of Antibacterial Ointment Containing *Macrotyloma uniflorum* Seed Extract

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Abstract:

Skin infection occur commonly and often present therapeutic challenges to practitioners due to the growing concerns regarding multidrug resistance to bacterial, fungal strains^[1] The necessity for developing new antimicrobial means has increased. The aim of present study was to formulate topical ointment containing *Macrotyloma uniflorum* seed extract. The ointment was formulated by Fusion method. In formulation aqueous and alcoholic extract were used the extraction was carried by maceration technique. The ointment bases used in formulation are wool fat, cetostearyl alcohol, hard paraffin, white soft paraffin. The formulation was designed by Factorial method. The factors were designed in 3² for wool fat and hard paraffin. Formulation were evaluated for organoleptic parameters, general appearance, spreadability, viscosity, pH, irritancy, stability study. It was proved that this extract shows Antibacterial activity against human bacterial pathogens. The formulation show pale brown and pale green color for aqueous extract & alcoholic extract respectively with characteristic odour, smooth, soft semisolid consistency. The viscosity of aqueous and alcoholic extract containing formulation was in range of 2093.33 to 7473.33 cps. The pH of all formulation was found to be between 5.5± 0.152 and 6.8± 0.174 with better spreadability and poor washability due to presence of oleaginous ointment base. All the formulation were show non-irritancy and stable in nature.

Keywords: *Macrotyloma uniflorum*, Fusion method, Oleaginous, Spreadability.

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Introduction:

For the treatment of skin infection, diseases dermatological products such as ointments, creams, gels, patches, etc. The bacteria present over the skin has suitable environment for growth when infection, wound occur on skin. The Antibacterial products plays important role in healing of the infections. The ointment is an semisolid dosage form where the base was act as a drug carrier. Ointment base such as hard paraffin, soft paraffin, wool fat, cetosteryl alcohol. These ointment bases are mostly used in occlusive dressing because of its oleaginous nature. These bases result in retaining the drug for long period at infected part of the skin. The *Macrotyloma uniflorum* which was commonly known as Horse Gram belong to the family Leguminosae. The *M. uniflorum* seed comes under the category of millet which is highly nutritious pulse. These pulse are mostly cultivated in Tamilnadu, Andhra Pradesh, Karnataka. Along with high nutrient value it has been used for mainly health benefits such as Antibacterial activity, antifungal activity, antioxidant activity, cytotoxicity assessment, etc.

Materials And Methods:

Plant Material: The seeds of *Macrotyloma uniflorum*(Leguminosae) was collected from the village, mandrup south solapur, Maharashtra in octomber 2023. The botanical identification was made by Dr. S. D. Randive and Prof. Dr. M. N. Jagtap at Department of Botany D.B.F. Dayanand College, Solapur.



Fig no.1: Seeds of *Macrotyloma uniflorum*

Preparation of Aqueous extract: The aqueous extraction was carried out by maceration technique. In this 150 gm of seeds were weighed, washed and grind into coarse particle. Then it is transferred into 500 ml of RBF containing 250 ml of distilled water. For successive extraction it is kept for 5 days with continuous shaking. After it is filtered and solvent evaporation carried by electric water bath. The crude extract of about 13 gm was obtained.

Preparation of Alcoholic extract: The coarse powder (100 gm) was successively extracted with 80% ethanol (700 ml*48hr) by using Soxhlet Extraction technique. The extract was filtered and filtrate was evaporated by electric water bath to obtain crude semisolid ethanolic extract. The crude extract of about 9gm was obtained.



Fig No. 2: Extraction by soxhlet apparatus.

Phytochemical Test: The preliminary phytochemical test for tannins, phenols and amino acid was performed by standard protocol.

Microorganism: It was proved that the aqueous and alcoholic extract of *Macrotyloma uniflorum* shows antimicrobial activity for 9 human bacterial pathogen such as *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas*

argentinensis, *Pseudomonas* sp, *Bacillus subtilis*, *Vibrio harveyi*, *Salmonella paratyphi*, *Pseudomonas aeruginosa* and *Vibrio mimicus*.^[4]

Preparation of ointment formulation: The formulation of ointment is carried by Fusion method. The ointment bases such as wool fat, cetosteryl alcohol, hard paraffin, white soft paraffin are used. In this method the constituents placed together into melting pan and allowed to melt at 70°C for about 4 min and then cooled with continuous stirring. When the temperature reaches to 40°C the extract is added into the ointment base. The formulation was designed by Factorial method in which two factors such as wool fat and white soft paraffin are considered. These factors are designed by 3² ratio. The formulation was made separately using aqueous and alcoholic extract. The composition of all the formulation was mentioned in table no 1 & 2.



Fig No.3: Ointment by aqueous extract



Fig No.4: Ointment by alcoholic extract



Table 1. 1: Formulation table for aqueous extract containing formulation.

Formulation code (gm)	T ¹	T ²	T ³	T ⁴	T ⁵	T ⁶	T ⁷	T ⁸	T ⁹
Wool fat	0.75	0.75	0.75	0.5	0.5	0.5	0.25	0.25	0.25
Cetosteryl alcohol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Hard paraffin	0.75	0.5	0.25	0.75	0.5	0.25	0.75	0.5	0.25
White soft paraffin	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Aqueous extract	1	1	1	1	1	1	1	1	1
Methyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Table 2. Formulation table for alcoholic extract containing formulation.

Formulation code (gm)	P ¹	P ²	P ³	P ⁴	P ⁵	P ⁶	P ⁷	P ⁸	P ⁹
Wool fat	0.75	0.75	0.75	0.5	0.5	0.5	0.25	0.25	0.25
Cetosteryl alcohol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Hard paraffin	0.75	0.5	0.25	0.75	0.5	0.25	0.75	0.5	0.25
White soft paraffin	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Alcoholic extract	1	1	1	1	1	1	1	1	1
Methyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Evaluation Parameters:

- 1) **Organoleptic properties:** The formulated ointment batches are studied for its organoleptic properties such as color, odor & consistency.
- 2) **Viscosity:** The viscosity of the formulated batches was carried by Brookfield Viscometer using spindle no. 64 at 100 rpm. The readings of the viscosity was in cps.
- 3) **Spreadability:** The spreadability was carried by parallel plate method. The 1gm of sample prepared in 48hr before carrying the test. The test sample is placed between two glass plates 20X20 cm. then a weight of approximately 500 gm was placed on top for 1 min. After measuring the diameter spreadability was determined by formula.

Spreadability(S): $M \times L/T$

where, M: weight in gm

L: diameter

T: time

- 4) **Washability:** The ointment was subjected to washability by using tap water. The oleaginous base cause occlusive layer which result in poor washability that helpful to retain the active ingredient at infected body part/skin.
- 5) **pH:** The pH of an ointment should be same as that pH of the skin for better activity. The pH is determined by dissolving 0.5gm of ointment sample of each batch in separate 10ml of distilled water. The mixture is heated for uniform mixing. When temperature reaches at 30°C the pH was determined by Calibrated Systronics Digital pH meter.
- 6) **Irritancy:** The irritancy was determined by applying the ointment on human



skin. The presence of irritation, redness, itching to the subjected person was determined. Before application of ointment to personnel the patient consent form is taken in the presence of registered medical practitioner.

7) Antibacterial activity: The formulations were screened for invitro antibacterial activity by agar well

diffusion method. *S. aureus* (gram +ve) and *E. coli* (gram –ve) microorganism were used. Activity performed by using Muller-Hinton agar as a culture media. The test sample having concentration of 0.1gm/ml was prepared in DMSO solvent. The streptomycin (5mg/ml) was used as standard.

Results:

Table 3. Results of evaluation parameters for aqueous extract containing formulation batches.

Parameters	T ¹	T ²	T ³	T ⁴	T ⁵	T ⁶	T ⁷	T ⁸	T ⁹	Std
Viscosity (cps)	3423.33	3386.66	3102	7016	6470	3493.33	6903.33	4203.33	4723.33	3068
pH	6.66	6.25	5.82	7.2	5.58	4.78	6.12	5.22	7.1	5.90
Spreadability (gm.cm/sec)	35.41	35.83	41.66	21.66	23.33	34.33	23.75	34.16	33.33	40

Table 4. Result of evaluation parameter for alcoholic extract containing formulation batches.

Parameters	P ¹	P ²	P ³	P ⁴	P ⁵	P ⁶	P ⁷	P ⁸	P ⁹	Std
Viscosity(cps)	5266.67	4143	4153	2093.33	5093.33	5243.33	4236.66	5030	7473.33	3068
pH	6.54	6.11	7.24	5.85	6.10	5.82	6.18	6.21	6.33	5.90
Spreadability (gm.cm/sec)	32.5	35	32.91	37.5	29.16	31.83	34.16	35.83	20.83	40

Antibacterial Activity: The zone of inhibition of the formulation was compared with standard product Iodine povidone (betadine) ointment.

Table 5. Results of zone of inhibition where, A: Aqueous extract containing ointment B: Alcoholic extract containing ointment, std: Standard ointment sample.

Content	Zone of inhibition(mm)	
	<i>E. coli</i>	<i>S. aureus</i>
A	14	18
B	12	15
Std.	28	32

Figur No 3. Graph showing zone of inhibition for Aqueous and alcoholic extract containing ointment formulation.

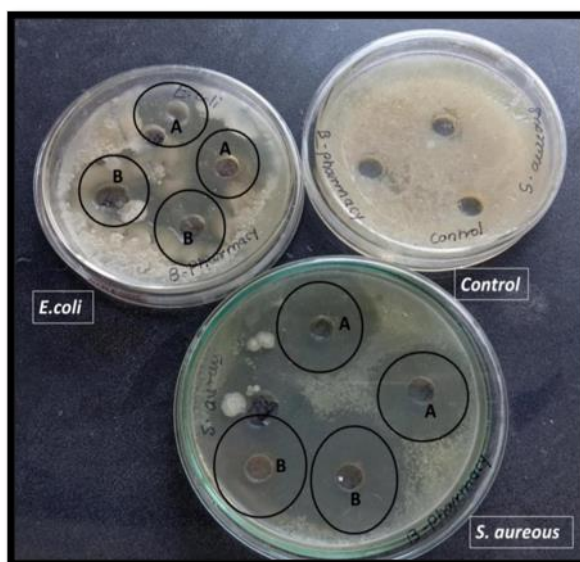
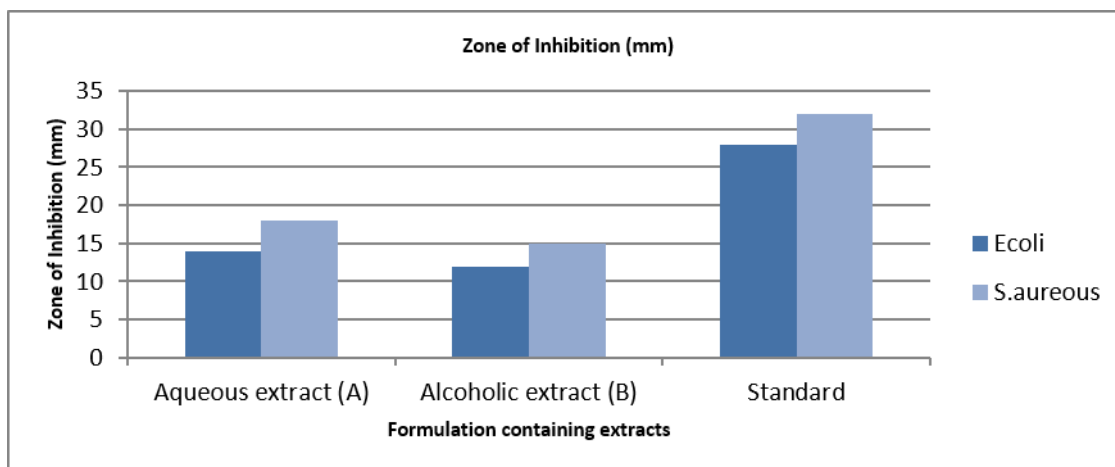


Fig No. 4: Formulation showing zone of inhibition

Discussion:

The ointment formulated by the extract of *Macrotyloma uniflorum* shows the antibacterial activity for both Gram positive and Gram negative bacterial species. The antibacterial activity may be showing due to the presence of tannins and phenolic compounds. The aqueous extract containing formulation shows a broad zone of inhibition compared to the alcoholic extract containing formulation on *E. coli* and *S. aureus* bacterial species. It may be because of

the solubility of tannins is more in an aqueous solvent. All the formulations show good consistency, smooth and semisolid appearance. The evaluation parameters of formulation code SB I were found to be most optimum. The evaluation parameter results were compared with the standard drug Iodine Povidone ointment (Betadine). The obtained formulation was able to prevent the spreading of infection and rapid healing activity. The poor washability of the formulation is



an advantage for retaining the active ingredient gradient for longer period.

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