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*Keywords:* *hypericum mysorense, excision wound model, ointment.*

*GJMR-B Classification:* *NLMC Code: QV 55*




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# Evaluation of Methanolic Extract of Hypericum Mysorense Ointment for its Wound Healing Activity

Sankar C [α], Muthukumar S [σ], Arulkumaran G [ρ], Vinesha R [ω], Manimekalaim [¥] & Sandeep George Simson [§]

*Abstract-* The main objective of the present study is to formulate and evaluate semi-solid dosage forms of *Hypericum mysorense* for its wound healing activity. The stem of *Hypericummysorense* was extracted by continuous hot percolation/ soxhletationusing methanol as solvent and was evaluated for its phytochemical property, *in-vitro* antimicrobial and*in-vitro* antioxidant activity. Using this methanolic extract, semi-solid dosage form(ointment) was formulated. Ointment was prepared using 2% concentration of the extract by fusion method using emulsifying ointment. Hence, the ointment containing 2% methanolic extract of *Hypericummy-sorense* was evaluated for its wound healing activity using excision wound model. The effect produced by the extract ointment, in terms of wound contracting ability, wound closure time and histopathological characteristics were comparable to that of a standard drug povidone iodine ointment. Therefore, the present study concluded that the *Hypericum mysorense* at 2% concentration of the methanolic extract formulated as an ointment showed a better wound healing formulation for better patient care and pharmaco economical.

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## I. Introduction

*a) Overview of Traditional Herbal Medicine*

India is a birthplace of indigenous medicine such as Siddha, Ayurveda, and Unani where many herbs have been used for treatment of human ailments. About 65% of total global population remains dependent on traditional medicines for their primary healthcare. Herbs are occupying a comeback and an 'Herbal Renaissance' is blooming across the world. They have been evidently prized for their medicinal, flavoring and aromatic qualities for centuries, yet for a while they were over shadow by synthetic products of modern civilization. Folk medicine is generally defined as traditional medicine that is practiced by non-professional healers or embodied in local custom or lore, generally involving the use of natural and especially herbal remedies.

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Once having realized their sources and adverse effects, people are going back to nature with hopes of safety and security. The rich treasure of herbal drugs is forming a boon for our society. Plant derived compounds, apart from their nutritive values, could serve as important therapeutic weapons to fight various human and animal diseases, thereby making them indispensable in traditional medicine for treating a number of diseases. Plant drugs, popularly known as herbal medicines have since been unabatedly used to treat various diseases. The major challenge is to protect traditional knowledge and will prove to be a beneficial asset to our human surrounding. For all the ailments herbal formulations are proved to be effective without any side effects commonly seen with allopathic drugs.

## II. Ointment

An ointment is a homogeneous, viscous, semi-solid preparation, most commonly greasy, thick oil (oil 80% - water 20%) with a high viscosity which is intended for external application to the skin or mucous membranes. Ointments have a water number that defines the maximum amount of water that it can contain. They are used as emollients or for the application of active ingredients to the skin for protective, therapeutic, or prophylactic purposes and where a degree of occlusion is desired. Ointments are used topically on a variety of body surfaces. These include the skin and the mucous membranes of the eye (an eye ointment), vagina ,anus, and nose. An ointment may or may not be medicated. Ointments are usually very moisturizing, and good for dry skin. They have a low risk of sensitization due to having few ingredients beyond the base oil or fat, and low irritation risk. There is typically little variability between brands of generics and name brand drugs. They are often disliked by patients due to greasiness.

## III. Wound Healing Activity

Wounds are inescapable events in life. Wounds may arise due to physical, chemical or microbial agents. Wound healing involves a complex series of interactions between different cell types, cytokine mediators, and the extracellular matrix. The phases of normal wound



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healing include hemostasis, inflammation, proliferation and remodeling. Each phase of wound healing is distinct, although the wound healing process is continous, with each phase overlapping the next. Because successful wound healing requires adequate blood and nutrients to be supplied to the site of damaged tissue.

## IV. Materials and Methods

*a) Collection And Extraction*

The leaves of Hypericum mysorense was collected freshly during the month of October from Ooty, India, identified and authenticated .The collected plant, Hypericum mysorense was extracted by continuous hot percolation (soxhletation). 200g of powdered stem of Hypericum mysorense was defatted using petroleum ether. The marc obtained from the powdered plant part was successfully extracted with 250 ml of methanol by using soxhlet apparatus. The extraction was carried out for 48 hours. After extraction, the solvents were distilled out; the concentrated residues were analyzed by chemical tests.

*b) Preparation of Semi-Solid Formulation*

i. *Formulation of Ointment*

Preparation of Ointment Base(Emulsifying Ointment)

Emulsifying wax - 50g,White soft paraffin - 20g, Liquid paraffin - 30g

*Procedure*

Required quantities of emulsifying wax, liquid paraffin and white soft paraffin were weighed and melted. To this, adequate quantity of methanolic extract of plant was added and stirred well until a homogeneous mass were obtained. The composition of the prepared herbal ointment (F1) is listed in Table I.

*Table I:* Composition of Ointment

| Sl. No. | Ingredients | F1 |
|---|---|---|
| 1 | Methanolic extract of *Hypericum mysorense* stem | 2gm |
| 2 | Emulsifying Ointment | q.s to 100gm |

*c) Pharmacological Evaluation of the Formulated Ointment*

i. *Acute Skin Irritation Study*

The primary skin irritation test was performed on albino rats and weighing about 150-200gm. The animals were maintained on standard animal feed and had free access to water *ad libitum*. The animals were kept under standard laboratory condition. The total mass was divided into four batches, each batch containing six animals. Two batches of each were used for control and test. Dorsal hairs at the back of the rats were clipped off one day prior to the commencement of the study. Animals showing normal skin texture were housed individually in cages with meshes to avoid contact with the bedding. 50mg of the each formulation of different concentrations were applied over one square centimeter area of intact and abraded skin to different animals. Aqueous solution of 0.8% formalin was applied as standard irritant. The animals were observed for seven days for any signs of oedema and erythema.

ii. *Wound Healing Activity*

a. *Animals*

Wister rat of male sex, weighing 150-200g were used. All animals were obtained from KMCH College of Pharmacy. All animals were housed for at least one week in the laboratory animal room prior to testing. The selected animals were housed in polypropylene cages in standard environmental conditions (20-25°c). Fed with standard rodent diet and water as libitum. The experiments on animals were conducted in accordance with the international accepted principles for laboratory animal use and the experimental protocols duly approved by the institutional ethical committee ,KMCH college of pharmacy, Coimbatore, Reg No. 685/Po/02/a/CPCSEA.Dt:21st Aug 2002, IAEC No: KMCRET/M.Pharm/9/2013-14

## V. Results

The colour of the ointment was dark green with characteristic odour. The result clearly indicated that the ointment showed good spreadability and extrudability. The ointment showed a pH that lie in the normal pH range of human skin and iseasily diffusible.



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*Pharmacological evaluation of the formulated ointment*

*a) Wound healing activity*

*Table 7:* Percentage Wound closure

| Test Compounds | 0th day | 3rd day | 7th day | 13th day | 17th day | 19th day |
|---|---|---|---|---|---|---|
| | % WC | % WC | % WC | % WC | %WC | %WC |
| STD | 0 | 15.7 ±0.29 | 39.8±0.77 | 81.8±1.09 | 88.5±1.25 | 95.5±1.10 |
| Ointment Base | 0 | 13.5 ±0.49 | 27±0.83 | 52.5±1.24 | 66.8±1.24 | 88.2±1.88 |
| Control | 0 | 27.5±0.58 | 23.6±2.03 | 74.2±0.99 | 72±0.99 | 90.1±0.99 |
| Ointment Containing 2%Extract | 0 | 10±0.42 | 13.8±1.48 | 55.4±1.08 | 84.1±1.08 | 96.9±1.08 |

Results were expressed as mean (mm) ± SEM and were compared with the corresponding control group (simple ointment B.P.) by applying ANOVA test. P value was set <0.05 for all analyses.

*Table 8:* Figure showing percentage wound reduction

| Test compounds | TH 0 day | 3rd day | 7th day | 13th day | 17th day | 19th day |
|---|---|---|---|---|---|---|
| | %WR | %WR | %WR | %WR | %WR | %WR |
| STD | 100±0.00 | 84.3±0.29 | 60.2±0.77 | 18.2±1.09 | 11.5±1.25 | 4.42±1.10 |
| Ointment Base | 100±0.00 | 86.5±0.49 | 73±0.83 | 47.5±1.24 | 33.2±1.24 | 11.8±1.88 |
| Control | 100±0.00 | 72.5±0.58 | 76.4±2.03 | 25.8±0.99 | 28±0.99 | 9.81±0.99 |
| Ointment Containing 2% Extract | 100±0.00 | 90±0.42 | 86.1±1.48 | 44.5±1.08 | 15.8±1.08 | 3.02±1.08 |

Results were expressed as mean (mm) ± SEM and were compared with the corresponding control group (simple ointment B.P.) by applying ANOVA test. P value was set <0.05 for all analyses.

*Excision Wound On 0th Day*





Only Wound Wound + Ointment Base





Wound + Standard

Wound +Oint Containing 2% Meoh.Hms

*Figure:* Figure showing the wound healing activity from 0thday — 21th

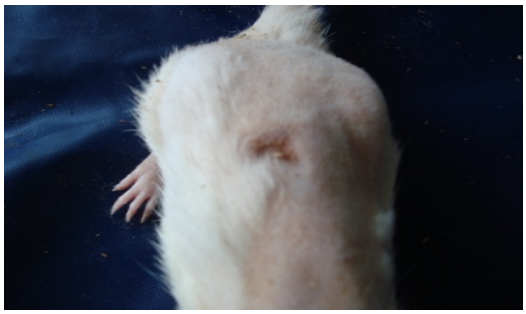


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*Excision Wound on- 21st Day*



Only Wound



Wound + Ointment Base



Wound + Oint Containing 2% Meoh.Hms



Wound+Standard

## VI. Discussion

Hypericum mysorense is an ornamental plant belonging to the family Hypericaceae, having antibacterial activity, against both gram-positive and gram-negative bacteria. Leaf and flower have strong antioxidant potential and used for liver disorders. Its stem is having strong antitumor, antipsychic and antiviral activities. Among the various indications where traditional herbal medicines are used, skin and skin related disorders are ranked top. Thus, the main objective of the present study is to formulate and evaluate semi-solid dosage forms of Hypericum mysorense for its wound healing activity. The stem of Hypericum mysorense was extracted by continuous hot percolation/ soxhletation using methanol as solvent,Using this methanolic extract, semi-solid dosage form (ointment) was formulated. Ointment was prepared with 2% methanolic extract using emulsifying ointment base.Hence, the ointment containing 2% Methanolic Extract of Hypericum mysorense stem was evaluated for its wound healing activity using excision wound model. The formulation did not produce any skin irritation for about a week when applied over the skin. Wister rats weighing around 150-200g were used for the study. They were divided into 4 groups consisting of 6 rats each. The 4 groups include standard(povidone iodine ointment), control( only wound), rats treated with ointment base and rats treated with 2% ointment. The effect produced by the extract ointment, in terms of wound contracting ability, wound closure time and histopathological characteristics were comparable to that of a standard drug, povidone iodine ointment. Thereby, the ointment formulated using 2% methanolic extract of Hypericum mysorense was found to possess wound healing activity.

## VII. Conclusion

The effect produced by the extract ointment, in terms of wound contracting ability, wound closure time and histopathological characteristics were comparable to that of a standard drug, povidone iodine ointment. Therefore, the present study concluded the wound healing activity of *Hypericum mysorense*stem at 2% concentration of the methanolic extract formulated as an ointment.

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*Conflict of Interests*

The authors declare that they have no conflicts of interest.

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