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SHORT COMMUNICATION

# Comparative Evaluation of Homoeopathic Medicines *Arnica montana, Calendula officinalis,* and *Hypericum perforatum* in Mother Tincture and 30C Potency on Cell Migration Assay

**Rupali Telang[1*], I. Manjula Kambi[2] and R. Deepa Mane[3]**

[1]Department of Homoeopathic Pharmacy, KLE Homoeopathic Medical College and Hospital, Yellur Road, Belagavi-590005. Affiliated to KLE Academy of Higher Education and Research, Belagavi - 590010, Karnataka, India; rupalitelang@yahoo.co.in

[2]Dr. Prabahkar Kore Basic Science Research Centre, Jawaharlal Nehru Medical College, Affiliated to KLE Academy of Higher Education and Research, Karnataka, Belagavi - 590010, Karnataka, India

[3]Department of Oral Pathology and Microbiology, KLE VK Institute of Dental College, Affiliated to KLE Academy of Higher Education and Research, Karnataka, Belagavi - 590010, Karnataka, India

## Abstract

**Background:** Wound healing is a compound process and is based on several components such as infection, tissue hypoxia, necrosis, exudate, and excess inflammatory cytokines. The fibroblast plays an important part. *Calendula officinalis* (CO MT and 30CH), *Hypericum perforatum* (HP MT and 30CH), and *Arnica montana* (AM MT and 30CH) are essential homeopathic remedies that are typically used for injuries. Studies on *in vitro* MTT assays have allowed us to screen a large number of plant products with antioxidant and cell mobilization properties that are essential for wound healing. **Aim:** The purpose of this study was to compare and examine the effects of mother tinctures and potencies on the Migration cell assay, as well as to evaluate the cellular toxicity and wound-healing properties of homeopathic medications using an *in vitro* scratch assay test. **Method:** MTT Assay and Migration Assay were conducted. Compounds of CO MT, CO 30CH, AM 30CH, AM MT, HP 30CH, and HP MT showed more than 85% of wound approximation at 24$^{th}$ hours compared to the negative control. Our results were Similar to standard care of neomycin for wound healing. **Results:** Compounds of CO MT, CO 30CH, AM 30CH, AM MT, HP 30CH, and HP MT showed more than 85% of wound approximation at 24$^{th}$ hour compared to the negative control. Our results were Similar to standard care of neomycin for wound healing. **Conclusion:** The study has evaluated the role of Homoeopathic medicines in healing wounds. *In vitro* cell line, it was discovered that Homoeopathic medicines improved the closure of wounds. Further, it was found that the medicines had no cytotoxic effects.

**Major Findings:** AMMT, AM30CH, COMT, CO30CH, HPMT, and HP30CH, lower concentrations like 7.8 µl/mL and 3.8 µl/mL had maintained the good viability of L929 (fibroblast) cell lines and showed more than 85% of wound approximation at 24$^{th}$ hour compared to the negative control.

**Keyboards:** *A. montana*, *C. officinalis*, Homoeopathic Remedies for Wounds, *H. perforatum*, MTT Assay, Wound Healing

## 1. Introduction

Injuries and physical trauma, including surgical cuts, bruises, contusions, open wounds, and nerve damage, are common in daily life and result from external forces. Wound healing is a complex biological process involving three main phases: inflammation, proliferation, and tissue regeneration. For optimal healing, each phase must occur in the correct sequence and at the appropriate time. Various factors,

*Author for correspondence

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such as infection, hypoxia, necrosis, excess exudate, and inflammatory cytokines, disrupt or delay this process[1-4]. Medications commonly used for wound management promote healthy granulation tissue, prevent suppuration, and reduce pus formation. This is usually achieved through both oral administration and topical application. Among the key cellular players, fibroblasts are crucial from the late inflammatory phase to complete epithelialization and extracellular matrix formation[5].

Homoeopathy is a system of medicine where treatment is based on individualization and symptom similarity. It aims not only to treat the injury but also the person as a whole, including underlying causes and contributing factors. Several homeopathic remedies are indicated for injuries, selected based on the nature of trauma, location, sensation, and modalities[6]. Three widely used and well-studied homeopathic remedies derived from the plant kingdom for injury and wound healing are *A. montana, C. officinalis, H. perforatum*[7].

*A. montana*, from the *Compositae* family and known as "Leopard's Bane," is used for trauma with bruising, soreness, and no bleeding[8-10]. *C. officinalis*, or "Marigold," also from the Compositae family, is indicated in clean, incised wounds with bleeding and post-surgical recovery due to its role in promoting granulation and preventing pus formation[10-12]. *H. perforatum*, or "St. John's Wort," from the *Hypericaceae* family, is used for injuries involving nerve-rich areas, especially fingertips, nails, and spinal trauma[13-14]. Although their efficacy has been demonstrated *in vivo*, this study aims to evaluate and compare these remedies *in vitro* using the scratch (migration) assay.

Mother Tinctures (MT) of these plants are prepared by combining 1-part fresh plant juice with 9 parts strong alcohol. Potencies like 30CH are derived through serial dilution and succussion (vigorous shaking). The following preparations were used: *A.montana* MT and 30CH, *C. officinalis* MT and 30CH, *H. perforatum* MT and 30CH. The *in vitro* scratch assay is a simple and cost-effective method to study cell migration. A "scratch" is created on a confluent monolayer of cells, and the migration of cells to fill the gap is observed and quantified. This model simulates *in vivo* wound closure processes and helps assess the wound-healing potential of various compounds[15-17]. The study aimed to evaluate and compare the effect of Mother tinctures and potencies on Migration cell Assay, and also to assess the wound healing property of Homoeopathy medicines by using an *in vitro* scratch assay test, as well as to evaluate their cellular toxicity.

## 2. Materials and Methods

### 2.1 MTT Assay

L929 cell lines were obtained from the National Centre for Cell Sciences (NCCS), Pune, and confirmed via STR profiling. Cells were cultured using complete media (DMEM + FBS + antibiotics) in a 5% $CO_2$ incubator under aseptic conditions. Sub-culturing was done at 85% confluence using trypsin as per standard protocols.

The MTT assay was performed on log-phase cells using a 96-well plate, seeded with $5 \times 10^3$ cells/well. Serial dilutions (500–3.9 µl/ml) of AM MT, AM 30CH, CO MT, CO 30CH, HP MT, and HP 30CH were tested in triplicate. After 24 hours of incubation, wells were treated with 20 µl MTT dye, wrapped in foil (due to photosensitivity), and incubated for 4 hours. Formazan crystals were dissolved using 100 µl DMSO and 25 µl glycine buffer. Absorbance was read at 570 nm, and the proliferation index was calculated using the formula below[18-20].

$$Surviving\ cells\ (\%) = \frac{Mean\ OD\ of\ the\ test\ compound}{Mean\ OD\ of\ control\ (untreated\ cells)} \times 100$$

## 3. Result

Figure 1 shows the Cytotoxicity assay of compounds named AMMT, AM30CH, COMT, CO30CH, HPMT and HP30CH, and HP30CH. We found that lower concentrations, like 7.8 µl/mL and 3.8 µl/mL, had maintained the good viability of L929 (fibroblast) cell lines.

Migration Assay Based on cytotoxicity results, 3.9 µl/mL was selected as the optimal concentration for the migration assay due to its minimal toxicity. L929 fibroblast cells in the log phase were trypsinized, and 70,000 cells per well were seeded into a 6-well plate and incubated for 24 hours. After forming a monolayer, a wound was created using a sterile 10 µl pipette tip. Test compounds diluted in media were added; neomycin

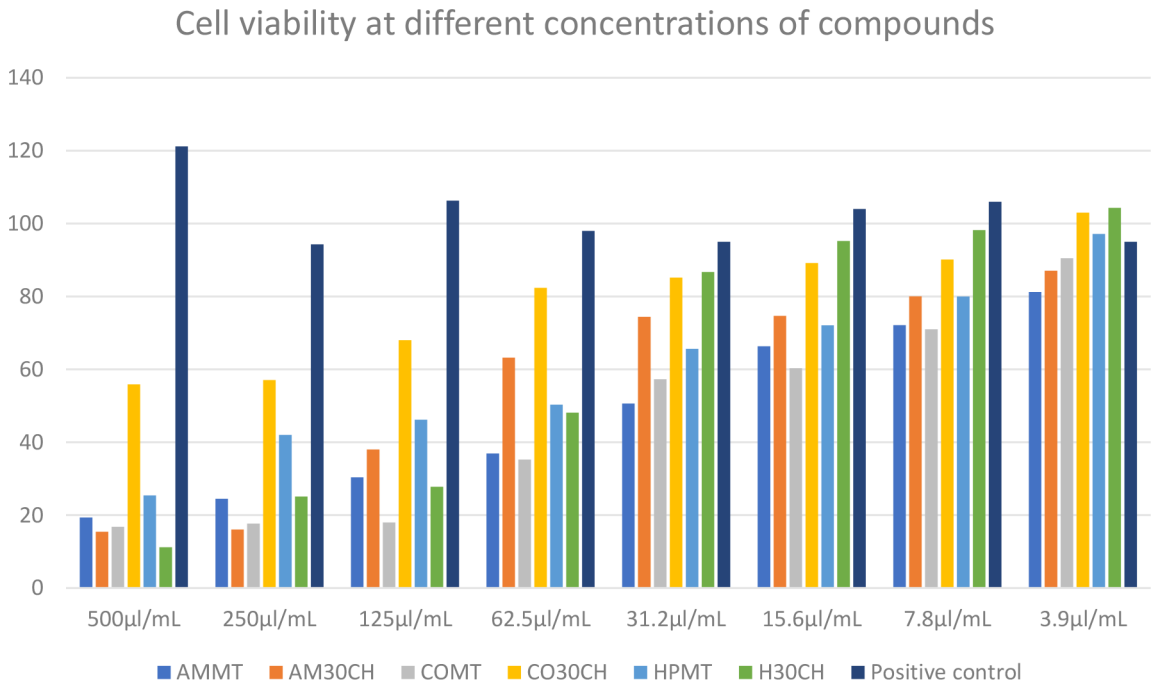



**Figure 1.** Graph showing the cell viability of compounds at different concentrations.

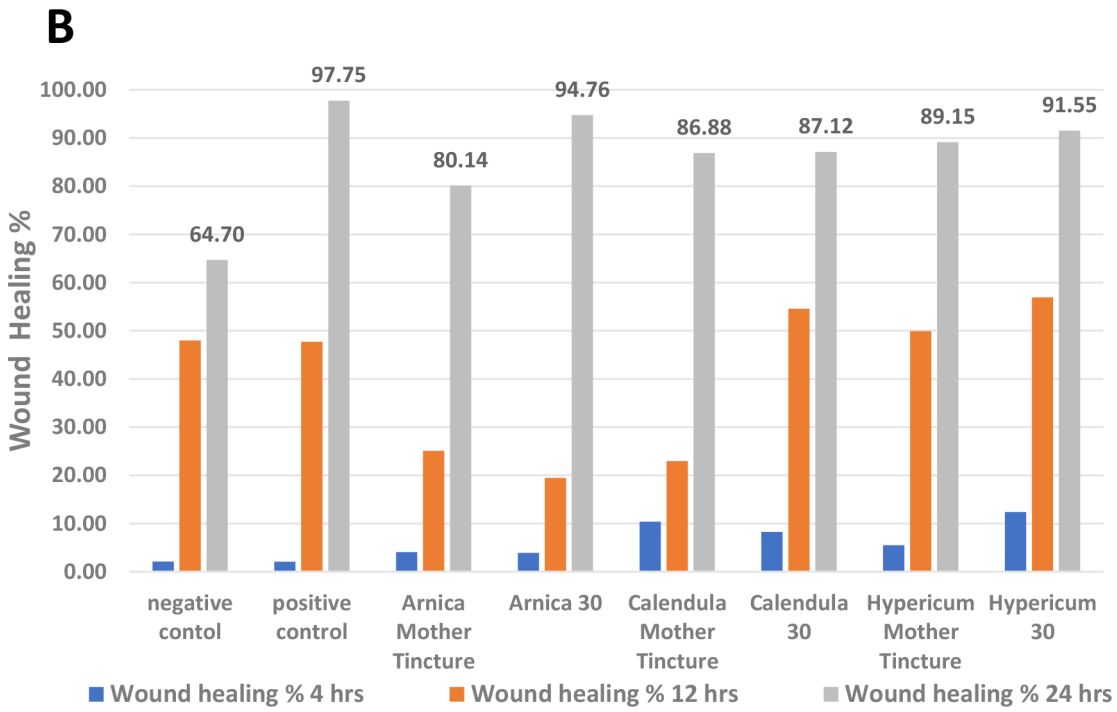



**Figure 2.** Graph showing wound healing percentages at 4, 12, and 24 hours for different compounds and controls.

served as the positive control, and untreated wells served as the negative control.

Images were captured at 0 and every 4 hours using an inverted microscope with a camera. Wound closure was analyzed using ImageJ software, and the percentage of wound healing was calculated using the formula[21-23].

$$Wound\ healing\ \% = \frac{Wound\ area\ at\ 0TH\ hour\ -\ wound\ area\ of\ 4TH\ hour}{Wound\ area\ at\ zero\ hour.}$$

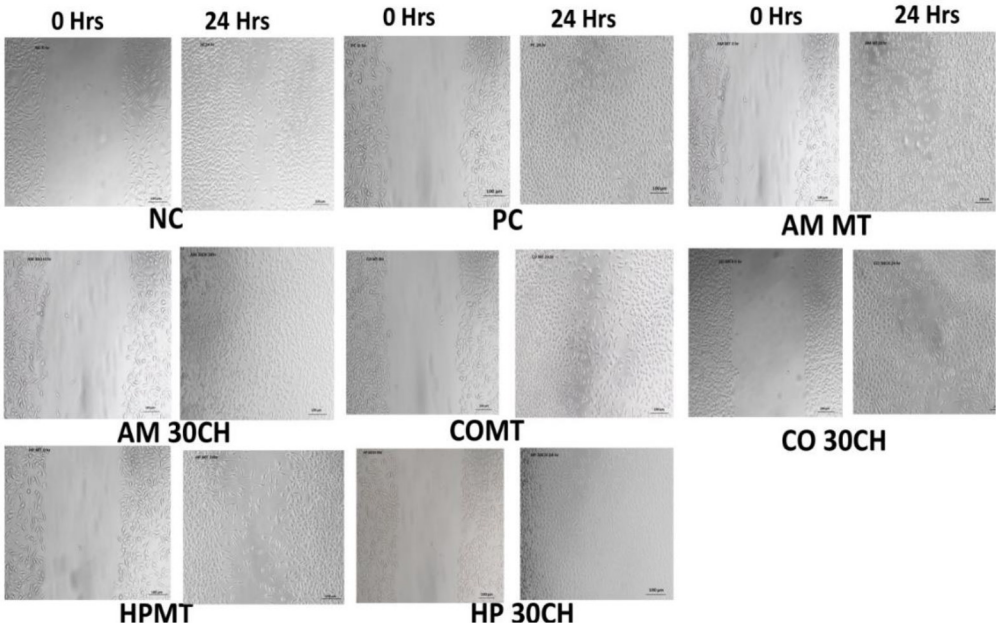



**Figure 3.** Images showing the wound healing percentage at 0 Hrs. and 24Hrs of all the compounds.

### 3.1 Migration

Based on MTT assay, we have considered all compounds with different concentrations for the migration assay. On calculating wound healing percentage of the test compounds, we observed that Compounds of COMT, CO30CH, AM30CH, AMMT, HP30CH, and HPMT showed more than 85% of wound approximation at 24$^{th}$ hour compared to the negative control. Our results were Similar to standard care of neomycin for wound healing, as shown in Figures 2 and 3.

## 4. Discussion

Wound healing varies between individuals and depends on factors such as infection, hypoxia, necrosis, exudate, and inflammatory cytokines. The process involves cell migration, collagen deposition, and mediator release[23-25]. Fibroblasts play a key role in inflammation to extracellular matrix formation, restoring the skin barrier[26-28].

Plant-based compounds are widely used for tissue repair due to their availability, minimal side effects, and effectiveness[29,30]. *A. montana* contains sesquiterpene lactones, flavonoids, and terpenoids with anti-inflammatory and antimicrobial properties, aiding in sprain and bruise treatment[31,32]. *C. officinalis* offers quercetin, triterpenoids, and carotenoids with anti-inflammatory and antioxidant effects[33,34]. *H. perforatum* provides hypericin, hyperforin, and flavonoids with antibacterial and neuroprotective benefits. Tannins in these plants support wound contraction and epithelialization. Tannic acid cross-linked collagen scaffolds have also shown improved wound closure and healing rates[35-37].

In the present study, cytotoxicity was shown by all the compounds in higher concentrations; in lower concentration cell viability of the fibroblast cell line was maintained by all the compounds. We observed that compounds of CO MT, CO 30CH, AM 30CH, AM MT, HP 30CH, and HP MT showed more than 85% of wound approximation at 24$^{th}$ hour compared to the negative control. Our results were similar to the standard care of neomycin for wound healing. Cell migration assay showed a significant (P value <0.0001) reduction in percent wound closure in the treatment group in comparison to the control. The p-value for the linear regression of wound healing % over time in the positive control (neomycin) group and the compound. This very low p-value indicates a statistically significant linear relationship between time and wound healing percentage in the positive control group and homeopathic medicines.

*In vitro* wound healing assays offer a non-invasive alternative to animal models for evaluating plant-based

compounds with antioxidant and regenerative properties. Homeopathic medicines, though highly diluted, showed ~87% healing, comparable to neomycin and significantly better than placebo (65%). This supports their biological activity, consistent with the Arndt-Schultz Law, which states that low doses can stimulate healing. These assays help validate the effectiveness of homeopathic remedies before proceeding to *in vivo* studies.

## 6. Conclusion

The study has evaluated the role of homeopathic medicines in healing wounds. *In vitro* cell line, it was discovered that homeopathic medicines improved the closure of wounds. It was additionally found that the medications did not have any cytotoxic effects. These results suggest that homeopathic medications can heal wounds and might be a good source of such properties. We are looking for alternatives as a result of the growing need for medications to treat bacterial resistance to antibiotics and to heal wounds sooner. One such option is homeopathy, which needs more thorough investigation and study to maximize the use of homeopathic medications in dermatology.

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## 9. Author Contribution

Dr Rupali Amar Telang- Idea, manuscript preparation, Review of Literature; Dr Manjula Kambi- Procedure, Analysis, Results, Verification; Dr Deepa Mane—Verification. All authors have read and agreed to the published version of the manuscript.

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