



## Short Communication

Effect of “*Murcchana samskara*” on therapeutic efficacy of *Bhallatakadi Ghrita*

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## ABSTRACT

'*Bhallatakadi Ghrita*' (BG), comprising the plant extracts of *Semecarpus anacardium* L., *Argemone mexicana* L., *Cocculus hirsutus* L., and *Woodfordia fruticosa* K. '*Murcchana samskara*' of ghee before any '*ghrita-paka*' preparation evidenced the maximum acceptability for topical application. The current study dealt with the effect of the '*Murcchana*' process on the therapeutic efficacy of BG. In the first step, '*Murcchita*' ghee was prepared as per reference texts and then developed the '*Murcchita Bhallatakadi Ghrita*' (M-BG), which was further assessed for wound healing activity using incision and excision wound animal models. '*Murcchana samskara*' altered the wound healing ability of M-BG (100% wound contraction on 15th post wounding day with  $13.50 \pm 0.22$  days complete re-epithelization time and  $562.33 \pm 7.37$  g breaking strength). The presence of antioxidants, polyphenols, flavonoids, and fatty acids (known for their potential wound healing properties) in M-BG could accelerate the wound contraction rate ( $P < 0.001$ ). The present investigation has corroborated the Ayurvedic/traditional attribute of '*Murcchana samskara*' to augment the medicinal properties of the BG.

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## 1. Introduction

A polyherbal formulations (herbs in combination) demonstrate better therapeutic effects with reduced untoward effects and increased palatability [1]. The '*Ghrita*' is an Ayurvedic preparation

comprising cow ghee, treated with medicinal plants either as fresh or in the form of decoctions [2]. Cow ghee possesses regenerative properties, and therefore it is generally used to treat wounds in *Ayurveda*. Wound healing involves an orderly advancement of the events to resume the tensile strength by re-establishing the integrity of damaged tissues. In *Ayurveda*, many *ghrita* formulations are reported for wound management [2,3].

*Bhallatakadi ghrita* (BG), a polyherbal *Ghrita* formulation, is prepared using medicinal plant extracts viz. *Semecarpus anacardium* Linn. (Anacardiaceae), *Argemone mexicana* Linn. (Papaveraceae), *Cocculus hirsutus* Linn. (Menispermaceae), and *Woodfordia fruticosa* Kurz. (Lythraceae) [4].

Abbreviations: BG, *Bhallatakadi Ghrita*; M-BG, *Murcchita Bhallatakadi Ghrita*; WBS, Wound Breaking Strength; PI, Povidone Iodine; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; OECD, Organisation for Economic Co-operation and Development; ANOVA, Analysis of Variance; SEM, Standard Error of Mean.

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The 'Murcchana samskara,' i.e., the process of use of 'Murcchita' ghee (prepared by incorporating antioxidant herbs) before 'ghrita-paka kalpana' ensures better shelf life, improved acceptability, and therapeutic efficacy of ghrita formulation. The wound healing potential of BG [5] and the role of 'Murcchana samskara' on the shelf life and oxidative stability of ghrita preparations is reported [4–7]. Hence, the present study aimed to evaluate the effect of the 'Murcchana' process on the wound healing potential of BG using excision and incision animal models.

## 2. Materials and methods

### 2.1. Preparation of BG

The plant materials viz. fruits and leaves of *S. anacardium*, whole plant of *A. mexicana*, and *C. hirsutus*, and leaves and flowers of *W. fruticosa* were collected and authenticated under the supervision of Dr. Jayanthi J, Scientist 'C' and Dr. Benniamin A., Scientist 'C' at Botanical Survey of India (B.S.I.), Pune, Maharashtra, India for identification and authentication (Reference number BSI/WRC/Tech./2013/SND-1 Dated 06/12/2013; BSI/WRC/Tech./2013/JRB-01 Dated 27/11/2013; BSI/WRC/Tech./2013/GVG-01 Dated 31/12/2013; BSI/WRC/Tech./2013/GG-01 Dated 31/12/2013). The powdered materials of selected plant parts used for BG preparation were defatted for 2 h with petroleum ether (60–80 °C) using the Soxhlet apparatus followed by extraction (Soxhlet) with alcohol to obtain the alcoholic extract [4,8]. The extracts thus obtained were used to prepare BG formulation as per the Ayurvedic procedure (Supplementary File/Fig. 1).

### 2.2. Preparation of M-BG

The prepared BG was processed with 'Murcchana samskara' referring to the ancient Ayurvedic procedures and denoted by M-BG (Murcchita Bhallatakadi Ghrita) (Supplementary File/Fig. 2).

### 2.3. Pharmacological evaluation of M-BG

M-BG formulation was evaluated for wound healing potential following the guidelines of the CPCSEA (JSPM/CCOPR/IAEC/2017–18/02 dated 12.08.2017) [9]. Wistar rats (200–250 g) of either sex were procured and were kept under standard conditions at  $25 \pm 2$  °C with a 12/12 h light/dark cycle and fed with commercially available feed and water *ad libitum* regularly.

#### 2.3.1. Acute dermal toxicity test

The animals were separated into two groups,  $n = 6$ , where Group-I was treated as control and Group-II with M-BG topically (2000 mg/kg body wt.) The acute dermal toxicity test (OECD guidelines no. 402) of M-BG was carried by monitoring changes in fur, eyes, behaviour, and toxic dermal reactions for 14 days [10].

#### 2.3.2. Wound healing activity

Wistar rats (200–250 g) were alienated randomly into four groups,  $n = 6$ , viz. Group-I: Normal control treated with cow ghee, Group-II: reference standard group treated with 5% w/w Povidone-Iodine ointment USP (PI), Group-III: treated with BG, and Group-IV: treated with M-BG. The excision and incision wound animal models were used to assess the wound healing efficacy of M-BG [5].

The excision wounds were evaluated for wound closure (Planometric method) and epithelization time. The cow ghee-treated (control) and PI, BG, and M-BG-treated test groups were subjected to hydroxyproline estimation. The wound breaking strength (WBS) was determined by a continuous constant water technique for the incised skin of each animal in the incision wound model.

The results are reported as mean  $\pm$  S.E.M. The comparisons between groups were conducted using one-way ANOVA and Dunnett's t-test. Multiple comparisons with control were adopted to determine the statistical significance ( $p < 0.05$ ) between groups [7,11].

## 3. Results and discussion

Previous studies have reported the therapeutic efficacy, i.e., wound healing property of BG and the effect of the 'Murcchana' process on the stability of BG [4,12]. Furthermore, the presence of phenolic and flavonoidal phytoconstituents in BG and their antioxidant property encourage its use in wound healing [4]. In addition, reported bioactives from *Murcchita* herbs such as gallic acid, ellagic acid, chebulagic acid, phyllembin, belleric acid, rutin, quercetin, isoquercetin, vitamin C, luteolin, curcuminoids [13–15] may have corroborated the promising role in augmenting the therapeutic efficacy of M-BG.

The present research attempted to measure BG's enhanced therapeutic efficacy processed with 'Murcchana samskara'. The 'Murcchana' process ensures maximum acceptability and better shelf life of BG formulation [4,12]. The use of 'Murcchita ghee' is generally recommended in Ayurveda to enhance the therapeutic property of ghrita formulations. The herbs used in 'Murcchana samskara' (supplementary procedure) revealed profound antioxidant and anti-lipid peroxidation properties, thus protecting ghrita from oxidative damage.

### 3.1. Pharmacological evaluation of M-BG

The basic tissue repair processes in animal wound healing models, viz. excision and incision, were recorded to understand, develop, and validate the clinical treatment strategies. The M-BG was safe up to the 2000 mg/kg dose in the acute dermal toxicity test and devoid of any changes in fur, eyes, skin irritation, and behaviour in treated animals.

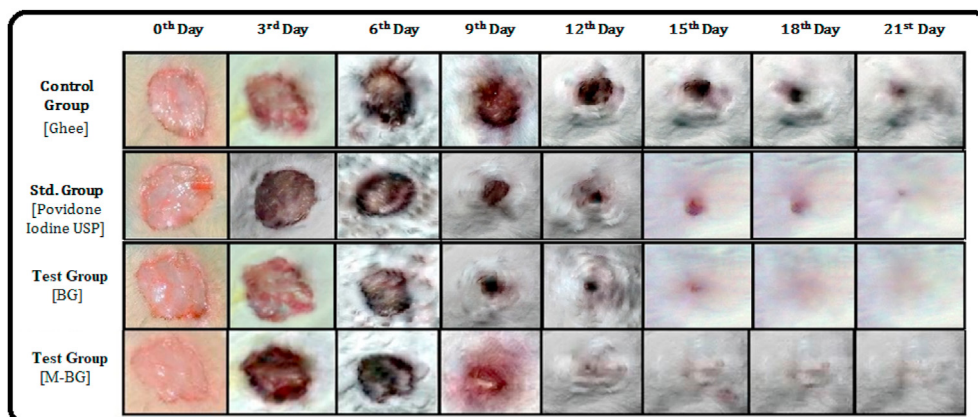
#### 3.1.1. Excision wound contraction

The wounded animals were assessed for about 21 days for contraction of excision wound area ( $\text{mm}^2$ ) and re-epithelialization time (days) (Fig. 1, Supplementary file/Fig. 3), and the percentage wound contraction was calculated (Supplementary file/Table 1).

The wound contraction was observed to be profoundly more from the 3rd day ( $p < 0.001$ ) in M-BG treated group compared to the control (cow ghee), and BG treated group. On 21st day post excision wounding, the control group showed  $97.32 \pm 1.09\%$  wound contraction, whereas 100% wound contractions was observed in BG treated group from the 18th day and in M-BG treated group from the 15th day, respectively. The re-epithelization time for all four groups was recorded (Supplementary file/Table 1). The ghee, PI, BG and M-BG treated groups showed complete re-epithelization on  $23.67 \pm 0.33$ ,  $17.67 \pm 0.49$ ,  $15.17 \pm 0.40$  and  $13.50 \pm 0.22$  days, respectively. The significantly ( $p < 0.05$ ) higher level of hydroxyproline in the M-BG treated group (Supplementary file/Table 1) proposes the ancillary role of M-BG in collagen formation in the wound healing proliferative stage.

#### 3.1.2. Wound breaking strength (WBS)

Breaking strength is an important factor in the wound healing process which reflects the sub-dermal organization of fibers in the newly deposited collagen [5]. The M-BG treated group shows significantly ( $p < 0.001$ ) higher WBS ( $562.33 \pm 7.37$  g) on the 10th day, whereas BG treated group and PI treated group shows  $531.50 \pm 5.89$  g and  $485.33 \pm 8.6$  g respectively as compared to control (Supplementary file/Table 1 and Supplementary file/Fig. 4).



**Fig. 1.** Wound contracting area on post-excision days of ghee (Control), PI ointment USP (Reference Standard), BG (Test) and M-BG (Test) treated groups where; BG- *Bhallatakadi Ghrita*; M-BG- *Murcchita Bhallatakadi Ghrita*.

### 3.1.3. Visual examination

The visual examination of the M-BG-treated group was carried out. Wounded animals were significantly clean and found to be free from swelling and redness (inflammatory reactions) compared with the cow ghee treated group.

## 4. Conclusion

The present study confirms the effect of the *Murcchana* process on therapeutic efficacy. The improved wound healing property of M-BG may be attributed to the antioxidants, flavonoids, and phenolics present in the *Murcchita* herbs used in the development of formulation and preparation of '*Murcchita ghee*'.

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None.

### Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Author contributions

**Shailendra Gurav and Manish Wanjari:** Conceptualization, Methodology, Writing - Review & Editing, Supervision, Project administration.

**Sandesh Wayal, Sonali Barke, Pradnya Jagtap and Ganesh Nigade:** Methodology, Animal Study, Software, Validation, Investigation.

**Nilambari Gurav, Muniappan Ayyanar and Satyendra prasad:** Writing - Original Draft, Visualization, Formal analysis, Resources.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaim.2022.100547>.

## References

- Sen S, Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: importance, challenges and future. *J Tradit Compl Med* 2016;7:234–44. <https://doi.org/10.1016/j.jtcm.2016.05.006>.
- Anonymous. *The ayurvedic pharmacopoeia of India*. 1st ed. New Delhi: Ministry of Health and Family Welfare Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy; 2007.
- Tonni S, Wali A. Dietary considerations of wound healing in Ayurveda. *J Nutr Food Sci* 2013;3:1–4. <https://doi.org/10.4172/2155-9600.1000227>.
- Wayal SR, Gurav SS. *Bhallatakadi ghrita*: development and evaluation with reference to *Murcchana* and *Shata-Dhauta* process. *J Ayurveda Integr Med* 2020;11:261–9. <https://doi.org/10.1016/j.jaim.2020.05.005>.
- Wayal SR, Gurav SS. Evaluation of wound healing potential of *Bhallatakadi Ghrita*—cow ghee based polyherbal formulation: in-vivo excision and incision wound model. *J Compl Integr Med* 2021. <https://doi.org/10.1515/jcim-2020-0179>.
- Gurav SS, Gurav NS, Patil AT, Duragkar NJ. Effect of explant source, culture media, and growth regulators on callogenesis and expression of secondary metabolites of *Curcuma longa*. *J Herbs, Spices, Med Plants* 2020;26(2): 172–90. <https://doi.org/10.1080/10496475.2019.1689542>.
- Gurav N, Gurav S, Wanjari M, Prasad S, Wayal S, Rarokar N. Development and evaluation of aphrodisiac potential of a classical ayurvedic formulation, '*Kaamdev ghrita*' in rat model. *J Ayurveda Integr Med* 2021;12(2): 294–301. <https://doi.org/10.1016/j.jaim.2020.09.007>.
- Amalraj S, Mariyammal V, Murugan R, Gurav SS, Krupa J, Ayyanar M. Comparative evaluation on chemical composition, in vitro antioxidant, anti-diabetic and antibacterial activities of various solvent extracts of *Dregea volubilis* leaves. *South Afr J Bot* 2021;138:115–23.
- CPCSEA. CPCSEA guidelines for laboratory animal facility. *Indian J Pharmacol* 2003;35:257–74.
- OECD. OECD guideline for testing of chemicals. 2015. [https://www.oecd.org/env/ehs/testing/2-TG\\_402\\_draft\\_26\\_Oct\\_2015.pdf](https://www.oecd.org/env/ehs/testing/2-TG_402_draft_26_Oct_2015.pdf). [Accessed 30 July 2016].
- Murade V, Waghmare A, Pakhare D, Dichayal S, Patil R, Wanjari M, et al. A plausible involvement of GABA/benzodiazepine receptor in the anxiolytic-like effect of ethyl acetate fraction and quercetin isolated from *Ricinus communis* Linn. leaves in mice. *Phytomedicine* 2021;1(3):100041. <https://doi.org/10.1016/j.phyplu.2021.100041>.
- Gurav NS, Gurav SS, Sakharwade SN. Studies on *Ashwagandha Ghrita* with reference to *murcchana* process and storage conditions. *J Ayurveda Integr Med* 2020;11(3):243–9. <https://doi.org/10.1016/j.jaim.2019.10.004>.
- Kumar N, Khurana SM. Phytochemistry and medicinal potential of the *Terminalia bellirica* Roxb.(Bahera). *Indian J Nat Prod Resour* 2018;9(2): 97–107.
- Variya BC, Bakrania AK, Patel SS. *Emblica officinalis* (Amla): a review for its phytochemistry, ethnomedicinal uses and medicinal potentials with respect to molecular mechanisms. *Pharmacol Res* 2016 Sep;111:180–200. <https://doi.org/10.1016/j.phrs.2016.06.013>.
- Nigam M, Mishra AP, Adhikari-Devkota A, Dirar AI, Hassan MM, Adhikari A, et al. Fruits of *Terminalia chebula* Retz.: a review on traditional uses, bioactive chemical constituents and pharmacological activities. *Phytother Res* 2020 Oct;34(10):2518–33. <https://doi.org/10.1002/ptr.6702>.