

Comparative Efficacy of *Tagetes erecta* and *Centella asiatica* Extracts on Wound Healing in Albino Rats

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Abstract

The study was undertaken to evaluate the comparative efficacy of hydroalcoholic extracts of leaves of *Tagetes erecta* (*T. erecta*) and aerial parts of *Centella asiatica* (*C. asiatica*) on Excision, Incision and Dead space wound models in albino rats. Extract of *T. erecta* and *C. asiatica* ($P < 0.001$) showed significant increase in rate of wound contraction, epithelization and formation of scar faster in excision wound model compare to control group. *T. erecta* extract (250 and 500 mg/kg) showed significantly increased the wound breaking strength in incision wound model and wet and dry granulation tissue weights, breaking strength in a dead space wound model compare to control and *C. asiatica* treated group ($P < 0.001$). In this investigation the work is conclude that the comparison made between the above two plants, the *T. erecta* extract showed potent wound healing activity then the reported *C. asiatica* in different wound parameters.

Keywords: *Centella asiatica*, *Tagetes erecta*, Excision, Incision, Dead Space Wound Model

1. Introduction

Wound healing or wound repair, is the body's natural process. Have regenerating dermal and epidermal tissues. When an individual is wounded, a set of events takes place in a predictable fashion to repair the damages. Wounds were probably the first medical problem faced by human race. The field of pharmacologic modulation in wound healing is still in its infancy. Indians were acquainted with a far larger number of medicinal plants than the natives of other country. In fact ancient Indian surgeon sushruta has enumerated large number of herbal drug in the management of wounds singly or in combination. Some Ayurvedic medicinal plants, namely, *Centella asiatica*, *Curcuma longa*, *Aloe vera*, and *Tagetes erecta* Linn, were found to be effective on wound healing in experimental models.

Tagetes erecta (*T. erecta*) is commonly known as Marigold. The plant is widely distributed throughout India, Asia, Central Europe, USA and Africa. The leaves are reported to be effective against piles, kidney troubles, muscular pain, ulcers, wounds and earache. The pounded leaves are used as an external application to boils and

carbuncles. The steam distillation of fresh leaves offer 0.3% of essential oil with a strong, sweet lasting odor and contains d-limonene, ocimene, l-linalyl acetate, l-linalool tagetone, n-nonyl aldehyde, lutein [1].

Centella asiatica (*C. asiatica*) is commonly known as Indian pennywort. The plant is widely distributed in Asia, Africa, and North and South America [2]. This plant is reportedly useful in wound healing and protecting against ulcer formation and is reported to have antimicrobial properties. Extracts of the plant have been shown to accelerate wound healing through topical and systemic routes [3]. The extracts of *C. asiatica* plant are reported to have sedative, antidepressant, analgesic, and anticonvulsive effects [4]. It is also reputed within the Indian system of medicine for its use in treating chronic and obstinate eczema, psoriasis, syphilis and leprosy [5]. The titrated extract of *C. asiatica* contains three principal ingredients like asiaticoside, asiatic acid, and madecassic acid. All these ingredients are known to be clinically effective in the treatment of systemic scleroderma, abnormal scar formation [6] and wound-healing activity [7, 8]. The present study was planned to evaluate the comparative efficacy of the hydroalcoholic extract of aerial

parts of leaves of *T. erecta* and *C. asiatica* on different wound models.

2. Materials and Methods

2.1. Plant Materials

Leaves of *T. erecta* and aerial part of *C. asiatica* was collected during the month of November from the local areas of Burdwan District, West Bengal, India and both the plants were authenticated by Professor Udayan Sarkar, HOD, Dept of Botany, Sonamukhi College, Bankura, India. A voucher specimen (Reference no-SC341/06) of plants has been deposited in the department.

2.2. Preparation of Hydro Alcoholic Extracts

The plants material was cleaned in water and air dried, powder was made using an electric mixer. The powder (75 g) was packed into a Soxhlet apparatus and was extracted with 500 ml of hydro alcoholic solvent (350 ml alcohol-95% and 150 ml water) at 65°C to 70°C for 24 h. The extracts were dried under vacuum and weights of the dried mass were recorded (Yield: *C. asiatica*-10% and *T. erecta*-15%).

2.3. Animals

Healthy Wistar albino rats of either sex weighing between 180 - 250 g were used for the wound healing study and albino mice weighing between 25 - 30 g were used for the oral acute toxicity studies. The animals were housed individually in Poly propylene cages and maintained at 24°C ± 2°C, a 12 h light/dark cycle fed with standard pellet rat chow (Pranav agro industries Ltd. Sangli, India) and water *ad libitum*. The experimental protocol and procedures used in this study were approved by the Institutional Animal Ethics Committee

(Protocol no: IAEC /PP/06/07).

2.4. Toxicity Studies

The female albino mice weighing 20 - 25 g were used for the study. The acute oral toxicity studies of hydro alcoholic extracts of *T. erecta* and *C. asiatica* was carried out by using fixed-dose method according to OECD [9] guideline No. 401. Animals were kept fasting overnight prior to the experimentation.

2.5. Surgical Procedures and Treatment

2.5.1. Excision Wound Model

In the present experimental model two different types of creams were formulated of each extract (5% and 10% w/w of *T. erecta* and the other having 5% and 10% w/w of *C. asiatica*) and the wounds were medicated with one local application (5 - 10 mg) of each cream for every day until the day of epithelization and their excision wound healing effect on rats was observed. The animals were randomly allocated into five groups of six animals each. Group 1: Wounds untreated served as control; Group 2: Wounds treated with cream having *T. erecta* (5% w/w); Group 3: Wounds treated with cream having *C. asiatica* (5% w/w); Group 4: Wounds treated with cream having *T. erecta* (10% w/w); and Group 5: Wounds treated with cream having *C. asiatica* (10% w/w).

This surgical procedure was performed according to Panchatcharam. The wounding procedures were carried out under anesthetized condition by thiopentone sodium (40 mg/kg, ip), the backs of animals were shaved and sterilized with 70% ethyl alcohol. Excision wounds sized 2.5 cm diameter in average were made after leaving at least 5 mm space from the ears and treated topically every day until the day of epithelization (**Figure 1**). The physical attributes of wound healing viz wound closure

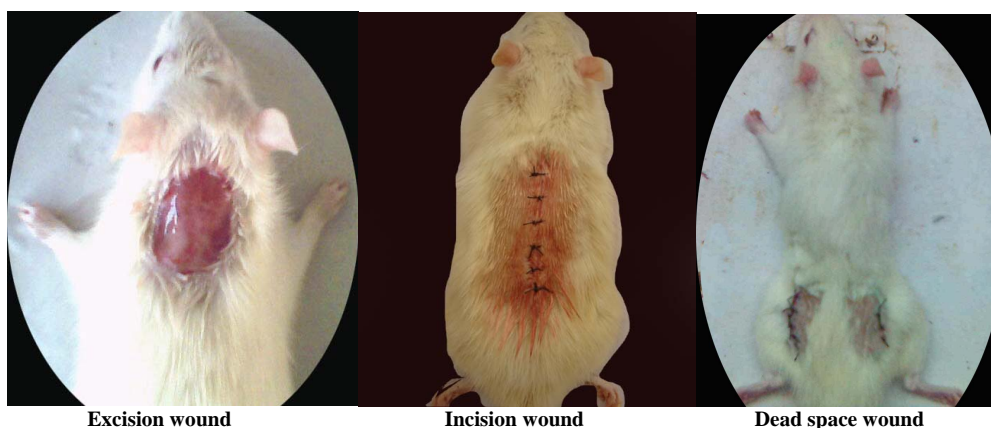


Figure 1. Different surgical wound models on rats.

(contraction), epithelization and scar features were recorded. The wound contraction was studied by traced 1 mm² graph paper on the day of wounding and subsequently on the alternate days, until healing was complete and calculated as percentage reduction of initial wound area [10].

2.5.2. Incision Wound Model

The animals were randomly allocated into five groups of six animals each. The extracts were dissolved in water and administered orally, daily for 10 days. Group 1: was assigned as vehicle control group and received no treatment; Group 2: received extract of *T. erecta* 250 mg/kg; Group 3: received extract of *C. asiatica* 250 mg/kg; Group 4: received extract of *T. erecta* 500 mg/kg; Group 5: received extract of *C. asiatica* 500 mg/kg, orally.

Under light ether anesthesia, the animals were secured to operation table in its natural position. Two Para vertebral straight incision of 6 cm each was made through the entire thickness of the skin, on either side of the vertebral column of the rat with help of sharp blade [11]. Wounds were closed with interrupted sutures, 1 cm apart and showed in **Figure 1**. The sutures were removed on the 8th day. The tensile strength of wound was determined on the 10th post wounding day by continuous constant water flow technique [12].

2.5.3. Dead Space Wound Model

In the case of dead space wound model the animals were randomly divided into five groups of six animals each. The extracts were dissolved in water and administered orally, daily for 10 days. Group 1: was assigned as control group and received no treatment; Group 2: received extract of *T. erecta* 250 mg/kg; Group 3: received extract of *C. asiatica* 250 mg/kg; Group 4: received extract of *T. erecta* 500 mg/kg; Group 5: received extract of *C. asiatica* 500 mg/kg, orally.

Physical and mechanical changes in the granulation changes in the granuloma tissues were studied in this model. These wounds were created by implanting two polypropylene tubes (0.5 × 2.5 cm each), one on either side in the lumber region on the dorsal surface of each rat and it showed in **Figure 1**. The wounds were sutured and mopped with cotton swabs in 70% alcohol. Animals were placed independently in cages after recovery from anesthesia. On the 10th post wounding day, the granulation tissue formed on the implanted tubes was carefully dissected out under anesthetic condition. The wet weight of granulation tissue was noted. The breaking strength of granulation tissue was measured by the method of Lee. Later, these granulation tissues were collected, dried at 60°C for 24 h. The weight of the dried granulation tissue was noted and expressed as mg/100g body weight.

2.6. Statistical Analysis

The data are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed by using one way ANOVA followed by Tukey multiple comparison test using Graph Pad Instate software (GPIS) (Version 1.13). $P < 0.05$ was considered statistically significant.

3. Results

The acute toxicity studies of the hydroalcoholic extract of leaves of *T. erecta* and aerial part of *C. asiatica* were found to be non-lethal up to dose of 2 g/kg body weight. Hence 1/8th and 1/4th of 2 g/kg dose (*i.e.* 250 and 500 mg/kg, orally) was selected for incision and dead space wound model. In the excision wound model, the *T. erecta* extract treated animals showed a significant increased percentage of wound contraction, decreased in the epithelization period and formation of scar, compare to *C. asiatica* extract treated animals. Data are presented in **Table 1** and **Figure 2**. In the incision wound model, a significant difference ($P < 0.001$) was observed with the treatment of *C. asiatica* and *T. erecta* in wound breaking strength group has compared to control group. Results are represented in **Table 2**. The effect of *C. asiatica* (500 mg/kg) and *T. erecta* extract (250 & 500 mg/kg) significantly increased the granulation tissue weight (wet and dry) and granulation breaking strength ($P < 0.001$) in the dead space wound model. By comparison, a significant decrease was observed in the *T. erecta* 250 mg/kg ($P < 0.05$) and non significant in *C. asiatica* 250 mg/kg treated group, data are presented in **Table 2**.

4. Discussion

The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort,

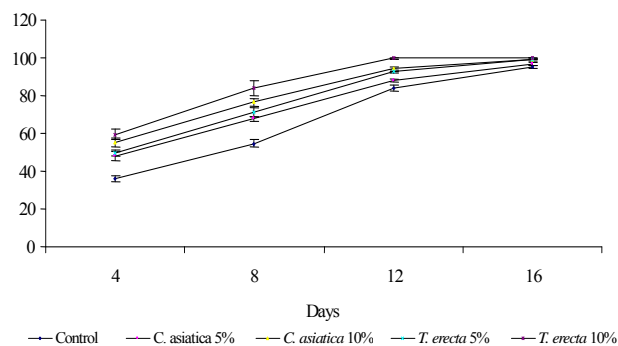


Figure 2. Effect of hydroalcoholic extracts of *Centella asiatica* and *Tagetes erecta* for percentage of wound contraction (in days), in excision wound model.

Table 1. Effect of hydroalcoholic extracts of *Centella asiatica* and *Tagetes erecta* for percentage of wound contraction (in days), epithelization (in days) and formation of scar in excision wound model.

Treatment	% Wound contraction in days				Period of epithelization (in days)	Mean size of scar area (in mm ²)
	4	8	12	16		
Control	36.29 ± 1.52	54.59 ± 1.90	83.94 ± 1.40	90.41 ± 0.80	21.80 ± 0.65	55.50 ± 1.78
<i>C. asiatica</i> 5% (w/w)	47.95 ± 2.06**	67.80 ± 1.40**	88.28 ± 0.70*	96.77 ± 0.64***	19.33 ± 0.76	48.33 ± 2.20
<i>T. erecta</i> 5% (w/w)	49.74 ± 1.49**	71.45 ± 2.30**	92.79 ± 0.74***	98.82 ± 0.39***	18.00 ± 0.51*	44.66 ± 2.50*
<i>C. asiatica</i> 10% (w/w)	55.11 ± 2.30***	76.50 ± 2.22***	94.12 ± 1.16***	99.48 ± 0.21***	17.66 ± 0.71*	43.33 ± 2.54*
<i>T. erecta</i> 10% (w/w)	59.59 ± 3.08***	83.73 ± 4.01***	99.72 ± 0.12***	100.00 ± 0.0***	14.16 ± 0.75***	29.00 ± 2.80***

The value are expressed as mean ± SEM, (n = 6). If * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control.

Table 2. Effect of hydroalcoholic extract of *Centella asiatica* and *Tagetes erecta* in incision and dead space wound models.

Treatment	Incision wound		Dead space wound	
	Breaking Strength, (g)	Wet tissue weight mg/100 g rat	Dry tissue weight mg/100 g rat	Braking Strength, (g)
Vehicle control	259.20 ± 7.45	152.30 ± 4.20	14.20 ± 1.04	256.10 ± 9.14
<i>C. asiatica</i> 250 mg/kg	281.20 ± 9.20*	175.60 ± 5.00	21.20 ± 0.99	280.60 ± 9.50
<i>T. erecta</i> 250 mg/kg	300.70 ± 13.60**	185.30 ± 4.20	22.28 ± 2.20*	297.10 ± 12.90
<i>C. asiatica</i> 500 mg/kg	324.50 ± 11.30***	193.50 ± 5.30**	25.27 ± 2.24**	331.70 ± 14.70***
<i>T. erecta</i> 500 mg/kg	351.40 ± 11.07***	214.50 ± 8.30***	27.21 ± 2.30***	353.60 ± 5.90***

The value are expressed as mean ± SEM, (n = 6). If * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Vehicle control.

and scarring to the patient. At the site of wound closure a flexible and fine scar with high tensile strength is desired [13,14]. The healing process begins with tissue reposition by cell proliferation presented in connective tissue. The main cells that trigger the wound healing process are the macrophage cells that remove foreign bodies and direct granular tissue development. Sequentially fibroblast and endothelial cells migrate towards the injured area increasing tissue permeability and collagen fiber production and it decreases vascularization by a contraction process by the scar process [15,16].

The ability of the wound is to heal is a biological process, which follows a definite pattern of cellular and molecular events, ultimately leading to complete repair of the injured tissues. High order animal's possess very limited regenerative capabilities except for those of some organs e.g. liver, parenchyma and outer surface of the skin. The healing rate differs among species and tissues and even between sites of the same tissue. However, in general the pattern of wound healing in domestic ruminants appears to be similar. The initial events of wound healing mechanism occurred smoothly under favorable condition. The normal process of wound healing may be disrupted. During this condition before using drugs,

many herbal preparations are extensively used in the rural area [17].

The result suggest that treatment with hydroalcoholic extract of *C. asiatica* and *T. erecta* have beneficial influence various phase of wound healing fibroplasias, collagen synthesis and wound contraction result in faster healing.

In the present study the significant increase in the breaking strength was observed in the animals treated with extract of *T. erecta* when compared to extract of *C. asiatica* on 10th post wound healing day.

In addition to excision and incision wound model, dead space wound model also revealed a significant increase in the dry granulation tissue weight indicating higher protein content and significant in breaking strength of granulation tissue both resutured incision wound was observed in the hydroalcoholic extract of *T. erecta* treated group compare to *C. asiatica* treated group.

The observation and result obtained in this study indicated that crude extract of *T. erecta* significantly stimulated wound contraction, breaking strength of the incision wound and increased in the dry granulation weight in the treated group compared with extract of *C. asiatica*. In this study one more attempt has been made to inclu-

sion *T. erecta* plant in the management of wound healing in folk medicine is justifiable.

5. Conclusions

In the present work it can be concluded that the comparison made between the above plants of hydroalcoholic extract of *T. erecta* showed promising wound healing activity then the reported *C. asiatica* in different wound parameters.

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7. References

- [1] T. Ghosh, A. Bose, G. K. Dash and T. K. Maity, "Wound Healing Activity of *Tagetes erecta* Linn Leaves," 2004. URL [http://www. Pharmainfo.net/exclusive/reviews](http://www.Pharmainfo.net/exclusive/reviews)
- [2] B. Brinkhaus, "*Centella asiatica* in Traditional and Modern Phytomedicine—a Pharmacological and Clinical Profile, Part I: Botany Chemistry Preparations," *Perfusion*, Vol. 11, 1998, pp. 466-474.
- [3] B. Brinkhaus, "*Centella asiatica* in Traditional and Modern Phytomedicine—a Pharmacological and Clinical Profile, Part II: Botany Chemistry Preparations," *Perfusion*, Vol. 11, 1998, pp. 508-520.
- [4] R. P. Lubadie, "An Ehnopharmacognostic Approach to the Search for Immunomodulators of Plant Origin," *Planta Medica*, Vol. 55, 1989, pp. 339-348. [doi:10.1055/s-2006-962024](https://doi.org/10.1055/s-2006-962024)
- [5] S. Chaudhary, S. Poddar, S. Sarkar and P. K. Das, "New Multidrug Regimen with Indigenous Drugs and Dapsone in the Treatment of Lepromatous Leprosy," *Indian Journal of Dermatology*, Vol. 32, No. 3, 1987, pp. 63-67.
- [6] S. S. Hong, J. K. Kim, H. Li and C. K. Shim, "Advanced Formulation and Pharmacological Activity of Hydrogel of the Titrated Extract of *Centella asiatica*," *Archives of Pharmacal Research*, Vol. 28, No. 4, 2005, pp. 502-508. [doi:10.1007/BF02977683](https://doi.org/10.1007/BF02977683)
- [7] A. Shukla, A. M. Rasik, G. K. Jain, R. Shankar, D. K. Kulashrestha and B. N. Dhawan, "In vitro Wound Healing Activity of Asiaticoside Isolated from *Centella asiatica*," *Journal of Ethnopharmacology*, Vol. 65, No. 1, 1999, pp. 1-11. [doi:10.1016/S0378-8741\(98\)00141-X](https://doi.org/10.1016/S0378-8741(98)00141-X)
- [8] S. Somashekar, S. L. Udupa and A. L. Udupa, "Effect of *Centella asiatica* Linn (Umbelliferae) on Normal and Dexametason Suppressed Wound Healing in Wistar Albino Rats," *The International Journal of Lower Extremity Wounds*, Vol. 5, 2006, p. 136.
- [9] Organization for Economic cooperation and development (OECD), "OECD Guidelines for Testing of Chemicals Acute Oral Toxicity," OECD, Paris, 1993. p. 401.
- [10] J. J. P. Morton and M. H. Malone, "Evaluation of Vulnary Activity by an Open Wound Procedure in Rats," *Archives Internationales de Pharmacodynamie*, Vol. 196, 1997, pp. 117-126.
- [11] H. P. Ehrlich and T. K. Hunt, "Effect of Cortisone and Anabolic Steroids on the Tensilestrength of Healing Wounds," *British Journal of Surgery*, Vol. 170, 1969, pp. 203-206.
- [12] K. H. Lee, "Studies on the Mechanism of Action of Salicylates II. Retardation of Wound Healing by Aspirin," *Journal of Pharmaceutical Sciences*, Vol. 57, No. 6, 1968, pp. 1042-1043. [doi:10.1002/jps.2600570633](https://doi.org/10.1002/jps.2600570633)
- [13] B. S. Reddy, R. K. K. Reddy, V. G. M. Naidu, K. Madhusudhana, S. B. Agwane, S. Ramakrishna and P. V. Diwan, "Evaluation of Antimicrobial, Antioxidant and Wound Healing Potentials of *Holoptelea integrifolia*," *Journal of Ethnopharmacology*, Vol. 115, No. 2, 2008, pp. 249-256. [doi:10.1016/j.jep.2007.09.031](https://doi.org/10.1016/j.jep.2007.09.031)
- [14] I. Süntar, U. Koca, H. Keleş and E. K. Akko, "Wound Healing Activity of *Rubus sanctus* Schreber (Rosaceae): Preclinical Study in Animal Models," *Evidence-Based Complementary and Alternative Medicine*, Vol. 2011, Article ID: 816156.
- [15] P. J. Michelle, M. Cristiana, T. G. R. Ana Lu'cia, T. F. Alik, A. F. R. Rodney, M. O. S. Ilza, A. F. Mary and E. C. Jo'ao, "Evaluation of Wound Healing Properties of *Arabidoea chica* Verlot Extract," *Journal of Ethnopharmacology*, Vol. 118, No. 3, 2008, pp. 361-366. [doi:10.1016/j.jep.2008.04.024](https://doi.org/10.1016/j.jep.2008.04.024)
- [16] S. K. Prasad, R. Kumar, D. K. Patel and S. Hemalatha, "Wound Healing Activity of Withania Coagulans in Streptozotocin-Induced Diabetic Rats," *Pharmaceutical Biology*, Vol. 48, No. 12, 2010, pp. 1397-1404. [doi:10.3109/13880209.2010.486837](https://doi.org/10.3109/13880209.2010.486837)
- [17] M. M. Alam, S. A. Islam, Y. Mohammed, N. S. Juyena and M. A. Hashim, "Comparative Efficacy of Two Medicinal Plant Extracts and an Antibiotic on Wound Healing," *Pakistan Journal of Biological Sciences*, Vol. 8, No. 5, 2005, pp. 740-743. [doi:10.3923/pjbs.2005.740.743](https://doi.org/10.3923/pjbs.2005.740.743)