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# ANTIBACTERIAL AND ANTIFUNGAL POTENTIAL OF HIMALAYAN MEDICINAL PLANTS FOR TREATING WOUND INFECTIONS

**UME HABIBA[1], MUSHTAQ AHMAD[1*], SHEHLA SHINWARI[1] SHAZIA SULTANA[1], ZABTA KHAN SHINWARI[2,3] AND MUHAMMAD ZAFAR[1]**

[1]*Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan*
[2]*Department of Biotechnology Quaid-i-Azam University, Islamabad, Pakistan*
[3]*Pakistan Academy of Sciences, Islamabad-Pakistan*
[*]*Corresponding author's email: mushtaqflora@hotmail.com, Tel: +0092-51-90643149.*

**Abstract**

Many bacterial and fungal strains are involved in wound infectious diseases as most of these strains become resistant to the most commonly used synthetic drugs in Himalayan region. Plant based natural products seem to be an alternative to this problem. The aim of this investigation was to evaluate the *In vitro* antibacterial and antifungal activities of 30 medicinal plants used in folk recipes by Himalayan people to treat wound infections against multi-drug resistant pathogens. In total of six medically important Myco-bacterial strains *Streptococcus pyogenes, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* were tested against methanolic plant extracts at 5 mg/ml concentration using agar disc well diffusion method to determined Minimum inhibitory concentrations (MICs).The plant extracts showed varied levels of MICs against test microorganisms. The strongest antibacterial activity was reported in methanolic extract of *Cynadon dactylon* (L.) Pers. against *Klebsiella pneumoniae* with 20.67±1.36 mm MICs, while *Candida albicans* was considered to be the most resistant pathogen with MICs9.6±0.57 mm. The findings were compared with results obtained using standard antibiotics, amoxicillin, ciprofloxacin, cefotaxime, fluconazole and itraconazole at conc.5mg/ ml. The results provide an evidence of folk medicinal uses of plants among the Himalayan communities to treat wounds. Further research needs to be carried out to identify the active molecules and evaluate the ***in vivo*** antibacterial and antifungal activities as well as toxicity level with clinical trials to use full potential of these plants for drug discovery development to control wounds globally.

**Key words:** Wounds, Antibacterial, Antifungal, Himalayan, Medicinal plants.

## Introduction

Wounds are physical injuries that result in opening or breaking of living tissues of skin. Wound healing is the natural process of body to regenerate and rebuild the disrupted skin tissues (Nguyen *et al*., 2010). Healing process initiates immediately after wounding. Though the healing process takes place by itself as an infection can seriously delay this healing process (Priya *et al*., 2002). Microbial organisms such bacteria and fungi most likely cause delayed wound healing and infections. Wound infections are most common in developing countries due to the poor hygienic conditions, while most of the people in developing countries who suffer an infected wound cannot afford to purchase expensive drugs, which might have side effects, secondly with the passage of time microorganism develop resistance against antibiotics. Consequently, after some time these antibiotics are not effective against the microbes (Walsh & Amyes, 2004). To compare microbial multi-drug resistant pathogens, scientists are looking-forward for the development of alternative and new medicines based on traditional herbal therapies. Natural sources such as plants, algae and animals provide a variety of natural medicinal compounds for the treatment of various infectious diseases (Ikram *et al*., 2015). Hence, plant products are seen as alternative solutions to the problem of wound treatment in developing countries. Plant products are potential agents for wound healing, and largely preferred because of their widespread availability and effectiveness as crude preparations (Sasidhran *et al*., 2010).

Both traditional and western systems of medicine for wound healing suffer from lack of resources and awareness. These need support and wider publication for relevant research to be pursued. Medicinal plants would be the best source to obtain a variety of drugs and therefore such plants should be investigated to understand better about their properties, safety and efficacy (Shinwari *et al*., 2015). Microbiologists all over the world are trying to formulate new antimicrobial agents and evaluation of the efficacy of natural plant products as the substitute for chemical antimicrobial agents. A lot of research has been investigated to develop the better healing agents and it has been a challenging task to discover healing agents and keep up pace with problems encountered (Kokane *et al*., 2009). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Adnan *et al*., 2014).

As in other parts of the world, diversity of medicinal plants in Himalayan range has traditionally played a great role in meeting significant part of the health requirements of indigenous communities, particularly for wound healing purposes (Abbasi *et al*., 2010). However with the revival interest of inhabitants and pharmaceutical industries on medicinal plants resources need to move their potential antibacterial and antifungal activities with particular emphasis on wound healing agents. In current age, the biological screening of medicinal plants have emerged as potential resource for addressing issues of rural health development among the communities of Himalayan range. In this region the medicinal plants are well documented as used among local communities by various workers in previous studies (Shinwari *et al.*, 2006). However the systematic investigation to prove the traditional claims of native communities regarding antibacterial and antifungal activities of Himalayan medicinal plants used for wound healing purpose are

lacking. The evolution and spread of antibiotic resistance, as well as the evolution of new strains of infection causing agents, is of great concern to the Himalayan as well as global health community. Our ability to effectively treat disease is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is traditional medicine. This study explores the comparative *In vitro* assessment of antibacterial and antifungal properties of plants used in folk medicine by Himalayan communities. We tested the hypothesis that methanolic plant extracts used to treat wounds infections often caused by bacteria and fungi would show antibacterial and antifungal properties in laboratory assays. This assessment would add to the existing knowledge about the potential of medicinal plant species for wound healing in this range for global circulation.

## Materials and Methods

**Ethnopharmacological information and plant material:** Medicinal plants studied in this investigation are frequently used by indigenous communities of different ethnic groups in Himalayan range for wound healing purpose since time immemorial .The prior rural appraisal (PRA) methods have been adopted during plant collection and ethnomedicinal documentation (Jamal *et al*., 2012). In total of 30 medicinal plant samples were collected from different geographical regions of Himalayan range including Azad Kashmir, Pakistan-China, Pakistan-Afghan and Pakistan-India borders. Plants were identified by a senior plant taxonomist Prof. Dr. Mir Ajab Khan and details of plants along with their voucher numbers are submitted in Herbarium (ISL) of Quaid-i-Azam (QAU) University Islamabad (Table 2).

**Microorganism used:** The detail of test organisms used in this study is presented in Table 1. For antibacterial and antifungal activities, Agar well diffusion method was used (Parekh & Chanda, 2007). Bacterial and fungus stains were obtained from Microbiology and Pathology Lab, Holy Family Hospital Rawalpindi, Pakistan. The bacterial cultures were maintained in Mueller-Hinton nutrient agar slants and fungal cultures were maintained in Potato dextrose agar slant at 28˚C.

**Table 1 Bacterial and fungal strains causing wound infections**

| S.No. | Lab No. | Sample isolation | Organism |
|---|---|---|---|
| 1. | HFHPL-4191 | Pus | *Streptococcus pyogenes* |
| 2. | HFHPL-3780 | Pus | *Staphylococcus aureus* |
| 3. | HFHPL-3848 | Urine | *Klebsiella pneumonia* |
| 4. | HFHPL-3811 | Pus | *Pseudomonas aeruginosa* |
| 5. | HFHPL-3737 | Hvs | *Candida albicans* |
| 6. | HFHPL-3900 | Swab | *Aspergillus niger* |

**Table 2. List of wound healing medicinal plants reported from Himalayan range.**

| S. No. | Taxon (Botanical name / local name / family/ voucher number) | Parts used |
|---|---|---|
| 1. | *Bergenia himalaica* Boiss. / Pather chat / Saxifragaceae / ISL-126911 | Root |
| 2. | *Calendula officinalis* Linn / Gainda / Asteraceae / ISL-126912 | Flower |
| 3. | *Calotropis procera* (Wild.) R. Br. /Ak / Asclepiadaceae/ ISL-126913 | Leaf, Latex |
| 4. | *Carissa opaca* Stapf-ex Haines / Garanda / Apocynaceae / ISL-126914 | Root |
| 5. | *Chenopodium botrys* L. /Sakhabooti / Chenopodiaceae / ISL-126915 | Leaf |
| 6. | *Cissampelos pareira* L. / Ghoresumi / Menispermaceae / ISL-126916 | Leaf, Root |
| 7. | *Clematis grata* Wall. / Dhand / Ranunculaceae / ISL-126917 | Leaves |
| 8. | *Cynadon dactylon* (L.) Pers. / Khabbalgrass / Poaceae/ ISL-126918 | Whole Plant |
| 9. | *Cyperus rotundus* L. / Motha / Cyperaceae / ISL-126919 | Root |
| 10. | *Dodonaea viscose* (L.) Jacq. / Sanatha / Sapindaceae/ ISL-126920 | Leaves |
| 11. | *Geranium pratense*L. / Kuratkachoo / Geraniaceae/ ISL-126921 | Whole Plant |
| 12. | *Mallotus philippensis* (Lam.) Muell.Arg. / Kamalia / Euphorbiaceae/ ISL-126922 | Leaves, Bark |
| 13. | *Malvestrum coromandelianum* (L.) Gareke. /Damhnibooti / Malvaceae / ISL-126923 | Leaves |
| 14. | *Mirabilis jalapa* L. / Gul-e-Abbasi / Nyctaginaceae / ISL-126924 | Leaves |
| 15. | *Otostegia limbata* L. / Koribooti / Lamiaceae / ISL-126925 | Leaves |
| 16. | *Oxalis corniculata*L. / Khatiboti / Oxiladaceae / ISL-126926 | Whole Plant |
| 17. | *Pistacia integerrima* Stew.ex Brand / Kanger / Anacardiaceae / ISL-126927 | Leaves |
| 18. | *Plantago lanceolata* Linn. / Ispagool / Plantaginaceae / ISL-126928 | Leaves |
| 19. | *Prunus persica* (L.) Bastch / Aru / Rosaceae / ISL-126929 | Leaves |
| 20. | *Ranunculus laetus* Wall.ex.H.and T. /Chambelbooti / Ranunculaceae / ISL-126930 | Leaves |
| 21. | *Ricinus communis* Linn. / Arind / Euphorbiaceae / ISL-126931 | Leaves |
| 22. | *Rumex dentatus* Spreng. / JanglipalakPolygonaceae / ISL-126932 | Whole Plant |
| 23. | *Saussurea heteromalla* (D.Don) Hand-Mazz./Kali ziri / Asteraceae / ISL-126933 | Leaves |
| 24. | *Solanum nigrum* Linn. / Kachmach / Solanaceae / ISL-126934 | Leaves |
| 25. | *Sonchus asper* L. / Mahtari / Asteraceae / ISL-126935 | Whole Plant |
| 26. | *Tegetes minuta* L./ Sat berga / Asteraceae / ISL-126936 | Leaves |
| 27. | *Trichodesma indicum* (Linn.) R.Br. /Juri / Boraginaceae / ISL-126937 | Leaves |
| 28. | *Verbascum thapsus* L. / Gidar Tambaku / Scrophulariaceae / ISL-126938 | Leaves |
| 29. | *Woodfordia fruticosa* (Linn.) S.Kurz. / Dhawi / Lytharaceae / ISL-126939 | Flower |
| 30. | *Ziziphus nummularia* (Burm.f.) Wight &Arn. /Janglibaer / Rhamnaceae / ISL-126940 | Leaves |

**Table 3. Bacterial and fungal growth inhibition zones with different plant extracts (mm).**

| S.No. | Taxon | Bacteria | | | | Fungi | |
|---|---|---|---|---|---|---|---|
| | | Gram positive | | Gram negative | | | |
| | | *S. aureus* | *S. pyogenes* | *K. pneumonia* | *P. aeruginosa* | *A. nigar* | *C. albicans* |
| 1. | *B. himalaica* | 17.67 ± 0.51 | 11.3 ± 1.3 | - | 17.67 ± 1.36 | 12.67 ± 1.86 | 14.7 ± 1.4 |
| 2. | *C. officinalis* | 12.6 ± 1.36 | 19.6 ± 1.36 | 14.6 ± 1.3 | 16 ± 0.89 | 17.67 ± 2.3 | 11.3 ± 1.4 |
| 3. | *C. procera* | 18 ± 1.78 | - | - | 18.3 ± 1.36 | - | 12.3 ± 1.52 |
| 4. | *C. opaca* | 17 ± 1.78 | - | 16.6 ± 1.36 | - | - | 12.3 ± 2.08 |
| 5. | *C. botrys* | 15.6 ± 1.86 | 15.3 ± 1.86 | - | - | 14.3 ± 2.08 | - |
| 6. | *C. pareira* | - | 17 ± 1.78 | 17 ± 0.89 | 18.67 ± 1.36 | 17.3 ± 1.52 | 12.3 ± 2.08 |
| 7. | *C. grata* | 17 ± 1.78 | 19 ± 0.89 | - | 15.7 ± 1.86 | 17 ± 2 | - |
| 8. | *C. dactylon* | 19.3 ± 1.86 | 15.3 ± 1.86 | 15.7 ± 2.25 | 14.6 ± 1.36 | 15.3 ± 3.05 | 13.67 ± 0.57 |
| 9. | *C. rotundus* | 16 ± 1.78 | 16.67 ± 1.36 | - | 16.3 ± 1.86 | 16.7 ± 1.52 | 14 ± 2 |
| 10. | *D. viscosa* | 17.3 ± 1.86 | - | 18 ± 0.89 | - | 15.6 ± 2.25 | 12.3 ± 2.08 |
| 11. | *G. pratense* | - | 16.7 ± 2.25 | 18.3 ± 1.9 | 18 ± 0.89 | 16.3 ± 2.08 | 12 ± 1 |
| 12. | *M. philippensis* | 16 ± 1.78 | 15.3 ± 0.51 | 20 ± 0.89 | 16 ± 1.78 | 19 ± 1 | 13.67 ± 1.52 |
| 13. | *M. coromandelianum* | 15.3 ± 2.25 | - | - | 17.3 ± 1.86 | - | - |
| 14. | *M. jalapa* | 17 ± 0.89 | 18.3 ± 1.36 | 17 ± 1.78 | 17.3 ± 1.86 | 18.3 ± 2.08 | 11.7 ± 2.08 |
| 15. | *O. limbata* | 18 ± 1.78 | - | - | 18.3 ± 1.36 | - | 12.3 ± 1.52 |
| 16. | *O. corniculata.* | 14.76 ± 0.60 | 17.67 ± 0.52 | 13.67 ± 0.52 | - | 18.67 ± 1.37 | 13 ± 0.90 |
| 17. | *P. integerrima* | 18.3 ± 1.9 | - | 20.67 ± 1.36 | 14.7 ± 1.03 | - | 11.3 ± 1.37 |
| 18. | *P. lanceolata* | 18.3 ± 1.4 | 18 ± 0.18 | 19 ± 0.90 | 19.16 ± 0.26 | 18 ± 0.90 | - |
| 19. | *P. persica* | - | 15.3 ± 2.74 | 16 ± 0.90 | 16.67 ± 2.25 | 15.3±0.52 | 11 ± 0.90 |
| 20. | *R. laetus* | 14.3 ± 1.37 | 15.67 ± 1.37 | 19.3 ± 1.03 | 14 ± 1.54 | 15 ± 0.90 | 12 ± 0.90 |
| 21. | *R. communis* | 12.6 ± 1.36 | 18.3 ± 0.51 | - | - | 16.3 ± 1.52 | 15.3 ± 1.51 |
| 22. | *R. dentatus* | 19.3 ± 1.86 | 18.3 ± 1.36 | 17 ± 0.89 | 16 ± 1.78 | - | 9.6 ± 0.57 |
| 23. | *S. heteromalla* | - | 17.6 ± 1.86 | 16.3 ± 2.25 | 12.3 ± 2.25 | 16.6 ± 1.52 | 15.3 ± 1.15 |
| 24. | *S. nigrum* | 19.3 ± 1.36 | 12.6 ± 1.36 | - | 15.6 ± 2.25 | 17 ± 1 | 14.8 ± 0.28 |
| 25. | *S. asper* | 14.6 ± 1.86 | - | - | 18.3 ± 1.03 | 16.6 ± 1 | 12.3 ± 1.07 |
| 26. | *T. minuta* | 15.67 ± 2.25 | 17.3 ± 1.36 | 18.3 ± 1.36 | 18.3 ± 1.86 | 16 ± 2 | - |
| 27. | *T. indicum* | 16 ± 0.89 | 18.3 ± 1.36 | 16.3 ± 1.03 | 18.3 ± 1.36 | 15.3 ± 2.08 | 14.3 ± 0.57 |
| 28. | *V. Thapsus* | 17.6 ± 1.36 | 16.3 ± 1.86 | 17.6 ± 1.36 | - | - | 11 ± 1 |
| 29. | *W. fruticosa* | 14.6 ± 2.73 | 17.3 ± 0.51 | 20.3 ± 1.37 | 19 ± 0.89 | - | 14 ± 1 |
| 30. | *Z. nummularia* | 15.7 ± 1.36 | 17.6 ± 1.36 | - | 16.3 ± 2.25 | 18.3 ± 1.52 | 10.3 ± 0.57 |

**Plant extracts preparation:** The fresh plant material was air-dried for one month at room temperature. It was pulverized by using mortar and pestle and electric grinder. The pulverized sample was kept in air-tight cellophane bags until use. Plant extracts were prepared by the new method (Bibi *et al*., 2011) with minor modifications. Powdered plant material (5 g) was macerated with 50 ml of methanol at room temperature for 5 days with frequent shaking. After five days the extract was filtered by using Whatmann No 1 filter paper. The combined filtrate was evaporated to dryness and stored. To prepare test solution, each plant extract (50 mg) was dissolved in 10 ml of 99.9% Dimethyl Sulphoxide (DMSO) to get 5 mg/ml concentration and stored. Pure DMSO (99.9%) was used as negative control. Commercial antibiotics and antifungal drugs, amoxicillin (5 mg/ml) and ciprofloxacin (5 mg/ml) and Cefotaxime (5 mg/ml), Fluconazole (5 mg/ml) and Itraconazole (5mg/ ml) were prepared in DMSO as positive control.

**Nutrient agar preparation:** The required amount of Mueller-Hinton (MH) agar and Potato Dextrose (PD) agar solutions were prepared as per manufacturer instructions (Oxoid Ltd, England). MH Agar was prepared by dissolving 3.9 g of MH agar in 100 ml of distill water, and PT agar was prepared by dissolving 3.8 g of PT agar in 100 ml of distil $H_2O$, then sterilized in autoclave, at cooling agar plates were prepared by pouring 15-20 ml of the medium into each sterilized Petri dishes and are allowed to set at room temperature.

**Preparation of turbidity standard and saline:** The turbidity standard was prepared by mixing 0.5 ml of $BaCl_2$ (0.048 M) in 99.5 ml of $H_2SO_4$ (1%). To prepare 0.9% saline solution, 9 g table salt (NaCl) was dissolved in 1000 ml of distill water, filtered, autoclaved and then stored (Bibi *et al*., 2011).

**Turbidity standard test:** Each bacterial and fungus culture was refreshed 24 hrs before experiment by streaking the culture on agar plates to obtain isolate colonies. 4-5 isolated colonies of these overnight cultures were then mixed with saline (0.9 % NaCl solution) in test tube and compared with McFarland solution for turbidity. Turbidity was corrected by adding sterile saline until McFarland turbidity standard $10^8$ Colony Forming Unit (CFU) per ml was achieved. These inocula were used for seeding of the nutrient agar (Parekh *et al*., 2011). Sterile

cotton swab was dipped into the turbid culture suspension and standardized inoculums were then inoculated aseptically on the dried surface of agar plates by streaking.

**Antimicrobial susceptibility tests:** Eight wells were made in seeded agar plates (six wells for extracts, one for positive and negative control each) with 8 mm sterile cork borer. With the help of micropipette, 50 µl of test solution was poured into respective well. One positive control (antifungal, antibiotic) and one negative control (DMSO) were applied to each petri plate and allowed to stand for 1 h at room temperature to diffuse the plants extracts in to medium before the plates were incubated at 37°C for 48 hours for bacterial and fungal cultures at 25˚C for 72 h. Negative control was included without adding the cultures to know the sterile conditions. After incubation period, antibacterial and antifungal activities were recorded by measuring the diameter of clear inhibition zones around each well. In this study triplicate plates were prepared to minimize the error. Zones were measured in mm for each organism. Negative control (DMSO) showed 0% antimicrobial activity while positive control (antifungal, antibiotics) showed 100% activity against pathogens. Extract showing zone of inhibition from 0-9 mm were considered ineffective 10-14 mm were effective and 15-20 mm were considered most effective. The entire microbial assay was carried out under aseptic conditions. Mean values of zone of inhibition were calculated with standard deviation procedure.

## Results and Discussion

In this investigation methanolic extract of 30 Himalayan medicinal plants were tested against six pathogenic strains of bacteria and fungi that cause infections to delay in wound healing. Data regarding zones of inhibition by plant extracts against bacterial and fungal strains is presented in Table 3. Botanical extracts were used in concentration of 5 mg/ ml. Parekh *et al*. (2006) reported that methanolic plant extracts exhibit more antibacterial activity as compared to aqueous extract. In this study it is found that plant extract showed maximum zone of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa* which indicate their highly sensitive nature while same extract showed minimum zones of inhibitions against *Candida albicans* which report this strain as highly resistant. In our findings, it is observed that *Cynadon dactylon* extract showed higher activities against all pathogens. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most common pathogens used previously against wound healing medicinal plants. Kumar *et al.* (2010) reported ethanolic leaf extract of *Calotropis procera* was not much effective against bacterial stains. While in our investigation, we observed methanolic leaf extract was highly effective against these pathogens (Table 3). Methanolic plant extract of *Sonchus asper* was found resistant against *Staphylococcus aureus,* and *Pseudomonas aeruginosa* as studied by Khan *et al.* (2010). Similarly Parekh & Chanda (2007) reported the antimicrobial potential of methanolic flower extract at conc. of 5 mg/ml against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa.* While (Kumar *et al*., 2010; Nair *et al*., 2005) studied the antibacterial potential of methanolic leaf extract of *Mirabilis jalapa* against *Staphylococcus aureus* and *Klebsiella pneumoniae.* Extract was highly effective against *S. aureus* and no activity against *K. pneumonia.* However during our investigation, we found that leaf extract of *Ricinus communis* was much active against *S. aureus* but not effective against *P. aeruginosa.* Previously Kensa & Yasmin (2011) studied ethanolic leaf extract at conc. 20 mg/ml and no activity was observed against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Similarly Jain *et al.* (2010) applied methanolic leaf extract of *Malvestrum coromandelianum* against *Staphylococcus aureus* but no activity was observed at conc. 100 mg/ml. While in this study *M. coromandelianum* leaf extract was found highly effective against *Staphylococcus aureus* with maximum zone of inhibition at conc. 5 mg/ml (Table 3). Kumar *et al.* (2010) checked the aqueous leaves extract against *Klebsiella sp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and found most effective against *P. aeruginosa,* less active against *Klebsiella sp.* and *S. aureus*. While we observed that leaf extract showed no effectiveness against *P. aeruginosa, K. pneumonia* and *S. aureus*. Parekh *et al*. (2006) studied aqueous and methanolic extract of *Cynadon dactylon* against *Staphylococcus aureus* and *Klebsiella pneumoniae.* Methanolic extracts showed no activity against these pathogens. In present studies plant showed high effectiveness against *S. aureus.*In this investigations leaf extract showed no effectiveness against *P. aeruginosa, K. pneumonia* and *S. aureus* while whole plant extract showed high activity against *S. aureus.* The variations in our findings in comparison with results of previous workers are might be due to difference in climatic conditions of plant habit, or due to variation in experimental conditions like chemicals and pathogens.

## Conclusions

This study reflected the use of 30 indigenous medicinal plants are promising source for wound healing among Himalayan communities. Present findings can contribute significant role towards Himalayan medicinal plants for rural health development in the region and projected as important resource for income generation through value addition. Further research needs to be carried out to identify the active molecules and evaluate the ***in vivo*** antibacterial and antifungal activities as well as toxicity level with clinical trials to use full potential of these plants for drug discovery development to control wounds globally.

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