

Abstract for final submission

Antimicrobial and phytochemical validation of southern African plants used as soap substitutes.

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A major proportion of the population in southern Africa relies on medicinal plants commonly known as soapy plants for bathing and washing. There is limited scientific research that assess the effectiveness of southern African soap plants. Therefore, this study investigated the phytochemistry, antimicrobial activity, and toxicity of plants used in southern Africa as soap substitutes. Thereafter, an effective antimicrobial herbal soap was formulated and assessed for its antimicrobial efficacy.

A comprehensive literature review was conducted to gather information on plants used as soap substitutes in southern Africa. A total of 59 plant species were identified to be used for bathing and washing. A total of 26 plant species were collected based on availability at Walter Sisulu Botanical Garden, Random Harvest Indigenous Nursery and University of Johannesburg herbarium and University of the Witwatersrand storage. The organic and aqueous extracts were prepared and screened for the presence of alkaloids, terpenoids, and saponins. Methanol and acetone were the optimal solvents to extract alkaloids from 62.07% of plant extracts. Terpenoids were best extracted with ethanol (75.86% of plant extracts), followed by methanol (68.97% of plant extracts). Saponins were highly detectable using water (93.10% of plant extracts) and ethanol (82.76% of extracts). The qualitative evaluation of saponins using thin layer chromatography displayed a variety of saponins, including steroidal saponins that had R_f-values comparable to diosgenin (a steroidal aglycone used as a standard). *Sideroxylon inerme* subsp. *inerme* (16.3%) had a high percentage yield of saponins. *Hermannia cuneifolia* displayed the highest saponin content (262.41 ± 1.90 mg/g), followed by *Sideroxylon inerme* subsp. *inerme* (71.34 ± 1.01 mg/ml), *Acalypha glabrata* (70.48 ± 2.05 mg/g), and *Noltea africana* (68.53 ± 2.43 mg/g).

The organic and aqueous extracts of each of the selected soap plants were tested for their antimicrobial activity against skin-relevant pathogens. *Pelargonium peltatum* demonstrated the best antimicrobial activity against *Brevibacterium linens* and *Cutibacterium acnes* with an MIC value of 0.06 mg/ml. *Calodendrum capense* (leaves), *Noltea africana* (leaves), *Olea europaea* (leaves), *Pelargonium peltatum* (leaves), *Plectranthus ciliatus* (leaves), *Ptaeroxylon obliquum* (bark), and *S. inerme* subsp. *inerme* (leaves) organic extracts displayed noteworthy antimicrobial activity against the pathogen *C. acnes* with an MIC value of 0.06 mg/ml. The plants that demonstrated notable broad-spectrum activity against most of the tested pathogens were *Calodendrum capense* (leaves), *Pelargonium peltatum*, *Plectranthus ciliatus*, and *Ptaeroxylon obliquum* (bark).

The toxic profiles of the organic and aqueous extracts were evaluated to assess the safety of the plant species using brine-shrimp lethality assay (BSLA). Aqueous plant extracts were more toxic (65.52%) compared to organic plant extracts (62.07%). *Acalypha glabrata* (leaves), *Aloe maculata* (leaves), *Bauhinia bowkeri* (leaves), *Deinbollia oblongifolia* (leaves), *Ledebouria luteola* (bulb), *Pouzolzia mixta* (leaves), and *Sideroxylon inerme* subsp. *inerme* (leaves) organic and aqueous extracts demonstrated the lowest toxic effects at 24 and 48 h. *Aristaloe aristata* (leaves), *Calodendrum capense* (leaves) and *P. obliquum* (bark) organic extracts were non-toxic, and *Hermannia cuneifolia* (leaves), *Plectranthus ciliatus* (leaves), and *Ptaeroxylon obliquum* (leaves) aqueous extracts were non-toxic. *Crinum bulbispermum* (bulb), *Haemanthus albiflos* (bulb) and *Ilex mitis* (leaves) were highly toxic, with LC₅₀ values > 250 µg/ml after 48 h. *Pelargonium peltatum* displayed low toxicity at a concentration of 125 µg/ml.

The extracts of *Calodendrum capense*, *Pelargonium peltatum*, and *Ptaeroxylon obliquum* were then used for soap-making by the basic saponification reaction, and the physicochemical parameters and antimicrobial activity of the soaps were evaluated. The *Calodendrum capense* herbal soap had the lowest pH (10.79), moisture content (28%), and free caustic alkali (0.03%). *Pelargonium peltatum* and *Calodendrum capense* herbal soaps were categorized as first-grade soaps (84 and 80%, respectively). The antimicrobial efficacy of the soaps was determined by inoculating selected skin micro-organisms on agar containing soap formulations using the multipoint inoculator. Gram-positive micro-organisms were inhibited (MIC values of ≤ 1.57 mg/ml). All the tested micro-organisms except for *Enterobacter cloacae* were inhibited at a concentration of 12.5 mg/ml, which is comparable to the control, Protex®

commercial soap. The findings herein of the antimicrobial properties, phytochemistry, and toxicity contribute to the knowledge gaps that exist in the ethnobotanical literature of some southern African soap plants and provide evidence for their incorporation into soap formulation.