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Ramburrun, Shivani

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MPHARM DISSERTATION ABSTRACT

In vitro investigation of *Rosa rubiginosa* (rosehip) oil and essential oil combinations for the topical treatment of acne

Shivani Ramburrun[a]

[a] *Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa.*

Acne vulgaris is the eighth most prevalent disease worldwide. *Cutibacterium acnes* overgrowth and inflammation are responsible for acne pathogenesis. Essential oils are one of the leading natural products used for dermatological applications, including acne. Essential oils are combined with fixed oils to reduce irritation and toxicity, however, the effect of fixed oils on essential oil bio-activity requires further investigation. This study aimed to investigate the *in vitro* antimicrobial, toxicity, anti-inflammatory, and associated cytotoxicity, as well as the skin absorption and retention properties of *Rosa rubiginosa* (rosehip) fixed oil independently and in combination with essential oils to treat the pathophysiological mechanisms involved in acne.

For the antimicrobial activity, the minimum inhibitory concentration (MIC) was determined using the broth microdilution assay. The fractional inhibitory concentration index (ΣFIC) was calculated to evaluate the antimicrobial interactions of the oil combinations. *Rosa rubiginosa* fixed oil combined with *Cinnamomum zeylanicum* (true cinnamon) essential oil displayed antimicrobial synergy (mean MIC = 0.42 mg/mL, mean ΣFIC = 0.45) against three acne-inducing reference strains (*C. acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*). *Rosa rubiginosa* fixed oil combined with either *Eugenia caryophyllata* (clove), *Kunzea ericoides* (kanuka), *Melaleuca quinquenervia* (niaouli), *Melissa officinalis* (lemon balm), *Rosa damascena* (Damask rose), or *Styrax benzoin* (gum benjamin) essential oil displayed antimicrobial additive interactions. *Rosa rubiginosa* fixed oil combined with *Santalum austrocaledonicum* (New Caledonia sandalwood) essential oil displayed antimicrobial antagonism. These eight oil combinations were further analysed in various ratios. The ΣFIC for the various ratios were expressed graphically on isobolograms, which revealed that most of the antimicrobial effects were attributed to the essential oils rather than *R. rubiginosa* fixed oil.

When the toxicity was evaluated using the brine shrimp lethality assay, *R. rubiginosa* fixed oil was found to be non-toxic with a percentage mortality of 0.35-1.64% after 24 hrs and 0.74-2.00% after 48 hrs, across all concentrations in the range of 0.03-1.00 mg/mL. For the combinations, *R. rubiginosa* fixed oil combined with *K. ericoides* essential oil at 0.25 mg/mL produced the greatest reduction in toxicity, where the percentage mortality decreased significantly from 100% (*K. ericoides* essential oil independently) to less than 3% (1:1 oil combination) at 24 hrs (p-value = 0.04) and 48 hrs (p-value = 0.03).

For the anti-inflammatory and associated cytotoxicity studies, nitric oxide inhibition was investigated in lipopolysaccharide (LPS) induced RAW 264.7 murine macrophages treated with *R. rubiginosa* fixed oil and the eight essential oils. Simultaneous evaluation of cell viability (MTT assay) was used to confirm the absence of cytotoxicity of the test samples. *Rosa rubiginosa* fixed oil reduced LPS induced nitrite production by 18.75%, and the combination of *R. rubiginosa* fixed oil with *S. benzoin* essential oil reduced nitrite production by 33.80%, relative to the LPS control. Cytotoxicity in A549 human epithelial cells was ascertained by measuring cell viability, using a dual-staining method. The combination of *R. rubiginosa* fixed oil with *M. quinquenervia* essential oil showed the best non-cytotoxicity in A549 epithelial cells relative to the cell culture medium.

Skin absorption (via diffusion) and retention of topically applied *R. rubiginosa* fixed oil and the eight essential oils were studied using excised porcine skin. Fourier Transform Infrared spectroscopy (FTIR) was used to obtain the qualitative infrared profiles depicting the vibration transitions of *R. rubiginosa* fixed oil and the essential oils. Differences in the wavenumber positions and peak intensities (percentage transmittance) of the oils at various concentrations, were noted. The combinations of *R. rubiginosa* fixed oil with either *C. zeylanicum*, *E. caryophyllata*, *S. benzoin*, or *R. damascena* essential oil, were extracted from the skin after 24 hrs and qualitatively detected using FTIR. This suggested that FTIR could be a suitable method for detection and qualitative analysis of *R. rubiginosa* fixed oil and essential oils within the concentration range of 1.25-100.00% v/v.

This study revealed that *R. rubiginosa* fixed oil (containing 41.93% of linoleic, 35.73% of linolenic, and 15.45% of oleic acids) with *S. benzoin* essential oil (containing 72.20% of dimethyl phthalate, 7.20% of benzyl acetate, and 5.70% of ethyl cinnamate) is an effective inhibitor of LPS induced nitrite production (as a parameter of anti-inflammatory activity). *Rosa*

rubiginosa fixed oil with *S. benzoin* essential oil is a promising non-cytotoxic oil combination, able to absorb into and be retained within the skin when topically applied, which may be used to potentially treat the pathophysiological mechanisms involved in acne vulgaris. Future experimental analysis entails quantitative permeation studies of *R. rubiginosa* fixed oil and essential oils across an *in vitro* and/ or *ex vivo* skin model, to further support the use of these oils and combinations for treating acne pathogenesis.