

## Resin-Salve from Norway Spruce - A Potential Method to Treat Infected Chronic Skin Ulcers?

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**Abstract:** The home-made resin salve from Norway spruce is traditionally and widely used in folk medicine to heal various skin infections and wounds in Northern Finland. We have performed laboratory studies to solve the mechanism of resin salve. The resin salve exhibited a bacteriostatic effect against all tested Gram-positive bacteria important in human medicine including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococcus (VRE), but was not effective against Gram-negative bacteria. An exception among the Gram-negative bacteria was *Proteus vulgaris* against which resin salve was effective. High amounts of lipophilic extractives, like resin acids were dissolved into water from the resin salve. Also, a large proportion of lignans and cinnamic acid were found in the water extract.

### INTRODUCTION

Some scientific reports have earlier described the use of resin-derived products to heal skin infections [1, 2]. Some investigations are published for the biological effects of the resins (rosin; colophon; resin acids). The studies indicate that resin may have antimicrobial and antifungal properties [3, 4, 5]. Therefore, it is not a surprise that the use of home-made resin salve from Norway spruce (*Picea abies* (L) Karsten) [6] is a widely used folk medicine to heal various skin infections and wounds in humans, at least in Northern Finland [7]. We have published case reports of healed pressure ulcers and surgical wounds treated with the traditional resin salve [8]. Due to encouraging empirical observations, we have become convinced for the value and possibilities of the spruce resin in the treatment of infected wounds and ulcers in clinical medicine.

### PREPARATION OF THE TRADITIONAL RESIN SALVE

The resin was originally collected in Kolari, Finnish Lapland, from Norway spruce trees (*Picea abies* [L.] Karst.). The traditional preparation procedure includes various steps and aims to prepare stiff raw-resin to grease-like lubricating salve. After the mechanical refinement of bark, wood tissue and other impurities, resin is mixed with salt-free butter in weight proportion of 1 to 3 (w/w) (Valio ltd, Finland) and liquefied and boiled at +100 °C. After intermixing of the ingredients, the mixture is cooled and stored in refrigerator until use.

The development of the resin products to more wide-spread or commercial use requires authorized laboratory conditions and standardized manufacturing protocol. As important is the separation of resin to its components, discover

the effective parts and carry out appropriate trials to prove the efficacy.

### BACTERIOLOGICAL STUDIES

Antimicrobial properties were studied against certain bacteria important in infected skin wounds. Muller-Hinton medium was used as Agar and FAB-medium as broth.

In the Agar diffusion test with Muller-Hinton agar plates, wells with a diameter of 8 mm were punched into the agar medium and filled with resin salve. After incubation of the plates for 18 hours at 35 °C, the zones of inhibition were measured. The zones of inhibition were clear but small. Therefore, we performed liquid media experiments as more sensitive method.

The liquid growth medium used in our experiments is Fastidious Anaerobe Broth (FAB) (Lab M Ltd, Bury, England). The medium is rich of nutrients and includes 0.75 gram of agar per liter. Due to bacterial growth, the broth becomes turbid or, depending of bacteria tested, visible colonies are formed in the medium. The experiments with the FAB medium were performed in two different ways. First, the bacteria tested were inoculated into FAB-medium with or without a drop of resin salve. Colonization of the bacteria was examined by turbidity of the growth medium and recorded as absent, mild, moderate or heavy (Fig. 1). Secondly, FAB medium pretreated with resin resin salve was prepared. In preparing the resin-pretreated FAB medium, a layer of resin salve was spread on the bottom of a Petri dish, after which 12 ml of FAB-solution was layered on this resin bed. After incubation the FAB medium was removed from the plate. The more detailed methods of these experiments is described in elsewhere [9].

Interestingly, our research on these antimicrobial experiments showed, that the resin-salve inhibits the growth of Gram-positive bacteria that are important causal agents in many skin infections in humans [9]. An exception among the Gram-negatives is *Proteus vulgaris* the growth of which is

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**Fig. (1).** Inhibition of growth of MRSA in FAB medium. Left tube FAB medium is inoculated with MRSA. Right tube FAB medium is inoculated with MRSA with drop of resin salve. Antibacterial activity of resin detected as an inhibition of the growth of MRSA in FAB medium.

also clearly inhibited by the resin-salve. The most interesting observation has been that the antimicrobial effect occurred against all staphylococci tested, including methicillin-resistant *Staphylococcus aureus* (MRSA). Antimicrobial activity is also found against vancomycin-resistant *enterococcus* (VRE). After liquid culture tests, viability of bacteria was also tested by cultivation of grafts on blood agar plates. Subcultures from the resin-FAB medium showed a regrowth of the bacteria, suggesting that the antimicrobial effect is more likely bacteriostatic than bacteriocidal [9]. The results of the antibacteriologic profile of the resin-salve are summarized in Table 1.

**Table 1. The Bacteria where the Growth Inhibitory Effect by Resin Salve has been Found in the Growth Medium**

<b>Gram positive cocci</b>
Staphylococcus aureus ATCC 25923 Staphylococcus aureus (MRSA) NEQAS 4937/98 Staphylococcus epidermidis ATCC 49461
Enterococcus faecalis ATCC 29212 Enterococcus faecalis (VRE) (vanB) EARSS UA605/01 Enterococcus faecium EARSS UA1527/01
Streptococcus pyogenes (A) ATCC 19615 Streptococcus agalactiae (B) NEQAS 6098/01
<b>Gram positive rods</b>
Arcanobacterium haemolyticum LABQ 237/95
<b>Gram negative rods</b>
Proteus vulgaris ATCC 8427

The Table is modified from Rautio et al., in press [9].

## ABIETIC ACID

After our findings about antibacteriological property of the resin salve, we have been interested in the protein composition of resin salve and the effective antimicrobiological molecule or molecules in resin. Conifer resin is a complex mixture of mono-, sesqui-, and diterpenoids. Resin accumulates at tree wound sites to kill invaders and both flush and seal the injury [10]. Diterpenoids, like resin acids are known to have antimicrobial effects, but they also show some toxic influences against aquatic organisms in high concentrations [4, 5, 11, 12, 13, 14, 15]. The main terpenoid component of spruce resin is dehydroabietic acid. Other resin acids present are levopimaric, pimaric, palustric, isopimaric, sandracopimaric, abietic and neoabietic acids. They can be easily detected by gas-liquid-chromatography (GLC) (Fig. 2).

The water extract of the resin-butter was obtained by sonication for 1 hour in a ultrasonic bath. It was further evaporated dry under nitrogen. Before GC/MS analysis, dried samples were silylated with 0,5 ml 20 % TMSI-pyridine mixture (TMSI=1-(trimethylsilyl)imidazole). The GC/MS analyses were performed using a HP 6890 GC-system equipped with mass selective detector 5973 and HP-5 capillary column (30m x 0,25 mm i.d., 0,25 um film thickness). Helium was used as carrier gas, flow 1,5 ml/min. The chromatographic conditions were as follows: initially temperature 180 °C; temperature rate, 5 °C/min; final temperature 300 °C for 5 min; injector temperature 280 °C and split ratio 1:20. MS-interface temperature was 300 °C and ion source temperature 230 °C. Mass spectra were obtained by electron impact (EI mode) ionization energy 70 eV.

A large proportion of diterpene resin acids (dehydroabietic acid as the major compound), lignans (laricresinol and matairesinol) and cinnamic acid were found in the water extract of the resin-butter (Fig. 2). The profile of extractives in water phase was similar for pure spruce resin than for resin-butter.

Our preliminary experiments (unpublished data) with abietic acid show striking similarities of antimicrobial (and antifungal) effects with the resin salve, indicating that the resin acids may be the therapeutically effective antimicrobial components of the home-made resin salve.

## CLINICAL RESEARCH

We are now running a randomized controlled trial to study the efficacy of the resin-salve with the standardized control treatment in patients with severe (stage II-IV) pressure sores in eleven health centers in Finland. The results of this pressure ulcer study will be ready during the year 2007. Preliminary results suggest that resin salve is more effective to heal wounds, than the control treatment. Our next clinical trial, which starts in the year 2007 is focused on healing of chronic surgical wounds and on prevention of the wound infections.

## CONCLUSIONS

The resin-salve seems to be effective against various Gram-positive bacteria, including MRSA and VRE. Therefore, resin-salve treatment may provide an effective option in therapy of patients with difficult infected ulcers. This is not a

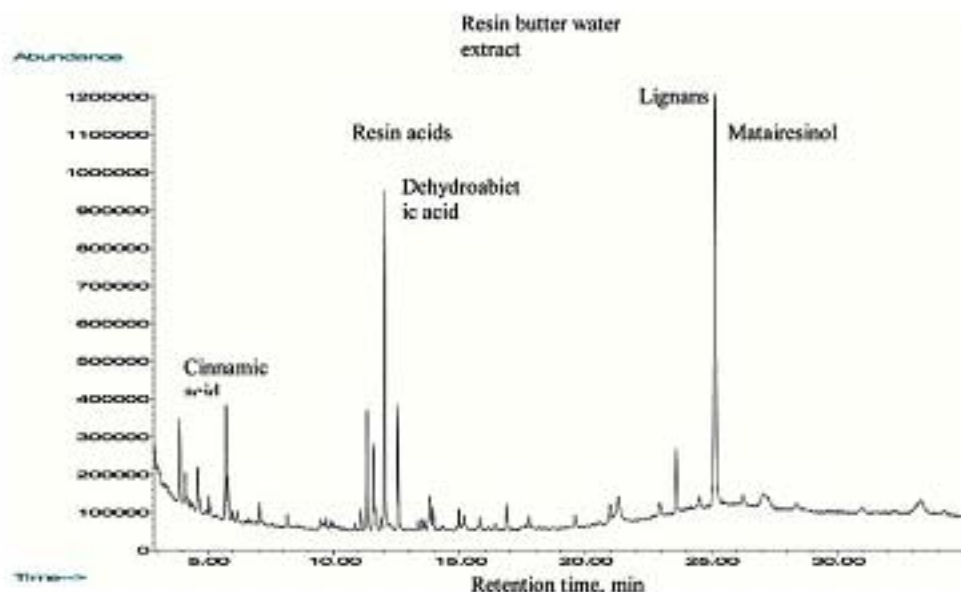


Fig. (2). Profile of extractives of spruce resin detected by gas-liquid-chromatography (GLC) and identified with mass spectra (MS). Resin acids and lignans are the major compounds detected in water extract of the resin-butter.

big surprise, because the plants are known to produce a variety of compounds that are experienced antimicrobial and potentially therapeutic tools [1, 2, 3, 16, 17, 18, 19, 20]. However, the results of clinical trials are warranted, before we can be sure about the clinical effects of resin salve or resin acids in treatment of the infected wounds. We also need much more experiments to resolve the possible wound healing mechanisms of the resin salve. We suppose that in addition to antimicrobial effect, the resin salve may have some other properties in healing of the wounds. It may for example promote the migration of endothelial, fibroblast and keratinocyte cells. To resolve these questions we will need more experimental and clinical studies, including investigations of effects of resin and its components on eukaryotic cells in culture conditions.

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