Bacteriostatic activity of some Australian essential oils

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Australia possesses extraordinary vegetal resources which can offer extracts, essential oils, or other compounds for cosmetic preparations.

We shall describe work undertaken with Australian essential oils, supplied to us by the Museum of Applied Arts and Sciences in Sydney. This work was part of an exhaustive study on more than a hundred essential oils to determine which essential oils would show an interesting bacteriostatic activity. From these studies we have selected 10 essential oils as the subject of this article.

Melaleuca Alternifolia grows on the north coast of N.S.W. This is a leaf oil, commonly known as "Tea-Tree" oil. The yield from the leaves is between 2% and 3%. Its major constituents are 1-terpinen-4-ol and terpinene, 30% to 40% and 1,8-cineole and p-cymene, less than 5%.

Melaleuca Quinquenervia grows, for the most part, on the eastern coastline—stretching from N.S.W. as far as Queensland. It is also a leaf oil and yields from between 0.8% and 1.5%. Its major constituent is D-nerolidol, 80%. It is interesting to note that this oil, collected in N.S.W., shows up to 80% of the D-nerolidol. However, samples obtained in Queensland give results of about 40% D-nerolidol and linalool 40%. It would appear that the linalool takes over from the D-nerolidol in plants obtained in the warmer climate of Australia.

Melaleuca Viridiflora grows in northern Queensland and the Northern Territory. The yield is between 0.5% and 2%. Its major constituent is methyl cinnamate, 75%.

Eucalyptus Citriodora grows in southern Queensland and is cultivated in other parts of Australia. The oil is obtained from the leaves, yields between 0.5% and 2%. Its major constituent is citronellal, 60% to 80%.

Eucalyptus Dives Schauer grows mainly in central N.S.W. The leaves yield oil from between 3% and 5%. Its major constituent is 1-piperitone, 40% to 50%.

Eucalyptus Fruticetorum, more commonly known as Polybractea, grows in central N.S.W. (West Wyalong) and in Victoria (Bendigo). The yield of the oil is between 2.5% and 3.5%. Its major constituent is cineole, 80% to 85%.

Eucalyptus Radiata, more commonly known as Australiana, grows in central and eastern N.S.W. The yield is between 3% and 3.5%. Its major constituent is 1,8-cineole, 70% to 80%.

Australian sandalwood grows in western Australia. The oil is obtained from the wood in yields of 1.4% to 2.6%. Its major constituents are alpha- and beta-santalol and farnesol, 80%.

Callitris Hugelii more commonly known as Columellaris, grows in a belt from Victoria to Queensland, mainly in the center of the country. The yield from the wood is between 1% and 2%. Its major constituents are citronellic acid, 10% to 20%, and guaiol, 20%.

Eremophila Mitchellii grows in the central far west of N.S.W. and also in southern Queensland. It grows only in arid country. The yield from the wood is between 2.5% and 3.5%. Its major constituents are eremophilone, hydroxyeremophilone, hydroxydehydroeremophilone, and allied ketones, 90%.

Test procedure

Our problem was to find out the test method which could give us the best and most reproducible results. If one examines the studies carried out until 1954, one notices that most methods used can be classified into two groups: the methods using a solid medium and the methods using a liquid medium.

If one compares the results obtained by these different methods, one can see that for the same product the different methods give different results. For example, the activity of the essential oils of thyme on Escherichia coli is effective at the dilution of 1 in 800 or 1 in 3,000 according to the method used. For two different products the same method sometimes shows the same results, while another method achieves quite different antibacterial activities. In fact, the main difficulty encountered in the study of the essential oils comes from the fact that they are insoluble in aqueous media. It is with the aim of obtaining a reproducible method that the scientists from the University of Montpellier have tested the possibility of using emulsifiers to obtain a distribution of the oil in the medium which increases the contact between the bacterium and the oils to a maximum level. The dispersion of

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the oil in the culture medium is consequently at a maximum level but also has to be homogeneous and stable during the entire period of the experiment.

As the cationic emulsifiers present a slight bactericidal activity, the choice was oriented toward the nonionic emulsifiers. The most used nonionic emulsifiers having an HLB between 8 and 18 are the Span and the Tween range.

It is of primary importance that the emulsifier used meet three fundamental conditions. It must be inert and not possess any inhibiting or stimulating activity on the germ development; have no synergistic antiseptic activity for the oil; and be chemically stable and consequently have no chemical interaction with the constituents of the oil.

Only Tween 20 and Tween 80, when tested alone, have no antibacterial activity. The best antibacterial activities are obtained with mixtures of Tween 20 and oil in a proportion of 10 to 50 or 20 to 80. If greater quantities of Tween are used, the antibacterial power decreases. The chemical stability of the mixture emulsifier and oil is demonstrated by the fact that chromatograms of pure oil and oil mixed with emulsifier after a period of six months are absolutely the same.

The emulsion is prepared by mixing the compounds for 10 minutes; the final concentration is obtained by addition of sterile distilled water. These preparations are then ready to be used for the determination of the minimum inhibitory concentration in a solid medium. This method was used to test all the products studied.

Results and discussion

The results of these tests on the 10 oils are summarized in Table I.

It will be noticed that the essential oil of Australian sandalwood is, with the other variety of sandalwood used in perfumery, one of the most active essential oils against Staphylococcus aureus (gram positive bacteria). The minimum inhibitory concentration of the Australian sandalwood oil against this bacteria is similar to that of one of the most active preservatives: Bronopol (2,bromo-2nitropropane 1,3 diol).

These results tend to substantiate the long-held belief in the antiseptic properties of sandalwood oil. As this oil presents in vitro an excellent bacteriostatic activity against gram positive organisms, it is capable of having the same activity against the microflora of the skin.

Applications

The essential oil of sandalwood has been incorporated at two different concentrations (1% and 2%) in an aerosol formulation, and the activity of the finished formulation has been evaluated. The test procedure used for these tests simulates in a way the conditions realized in the use of the aerosol: Vaporize the test preparation for 3 seconds at a distance of 20 cm from a Petri dish containing Tryptic Soy Agar and inoculated on the surface with one drop of calibrated test suspension.

Controls constituted by an aerosol containing only the propellants, by an aerosol containing absolute alcohol, and by an aerosol preparation

Table I. Bacteriostatic Activity of the Tested Oils

The results are given by the range of the minimum inhibitory concentration expressed in P.P.M. (parties per million)

Oils Tested	Staphylococcus Aureus ATCC 6538	Escherichia Coli ATCC 10536	Pseudomonas Aeruginosa NCTC 1999	Candida Albicans	Aspergillus Niger
Melaleuca Alternifolia	5 000 2 500	2 500 1 250	40 000	400 200	156.25 78.125
Melaleuca Quinquenervia	5 000 2 500	>40 000	40 000	40 000	10 000 5 000
Melaleuca Viridiflora	5 000 2 500	>40 000	40 000	40 000	1 250 625
Eucalyptus Citriodora	2 500 1 250	5 000 2 500	40 000	625 312.5	625 312.5
Eucalyptus Dives	5 000 2 500	2 500 1 250	40 000	800 400	625 312.5
Eucalyptus Fruticetorum	10 000 5 000	5 000 2 500	40 000	1 250 625	10 000 5 000
Eucalyptus Radiata	10 000 5 000	5 000 2 500	10 000 5 000	625 312.5	5 000 2 500
Australian Sandalwood	200 100	>40 000	40 000	400 200	10 000 5 000
Callitris Hugelii	2 500 1 250	>20 000	20 000 10 000	2 500 1 250	10 000 5 000
Eremophila Mitchelii	625 312.5	> 20 000	40 000	800 400	10 000 5 000

without perfume have been incorporated in the series of tests. The results of the tests carried out with the aerosol preparation containing 1% or 2% of Australian sandalwood oil follow.

One notices a complete inhibition of the growth of the test organism Staphylococcus aureus. For that reason, one can assume that an aerosol formulation containing 1% or 2% of this essential oil would present in vivo an interesting deodorant activity. In the same way, one could assume that a perfume containing the same final concentration of sandalwood oil in an aerosol form would show in vivo interesting bacteriostatic and deodorant properties.

Conclusion

On the basis of the results obtained, one sees that sandalwood oil could be introduced into cosmetic formulations (creams, lotions, masks, deodorants, shampoos, bath oils) for its antiseptic activity. Perhaps it is even interesting enough to be the active ingredient in a complete cosmetic line. In fact, the purpose of this paper is to point out the interesting possibilities of natural oils and their contribution to cosmetics.

The indigenous Australian flora can no doubt provide many interesting isolated oils or extracts for this purpose. As mentioned previously, Australian sandalwood has given excellent results, but unfortunately it is now a protected species and therefore no longer commercially available. I believe this is only a temporary measure taken by the Western Australian Government and the time will come when we will be able to obtain it again. Australia has unlimited potential for essential oil production from its native flora, and I believe that this will be developed in years to come.