Progress report : Ongoing research on the purification of ethanolic extracts with HFC R 134 a

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1. Theoretical reflection

While it can be shown that extraction of artemisia with ethanol is not more expensive than with hexane and produces a far higher yield of artemisinin (forthcoming report), the main problem that has be solved is finding an effective and cost efficient purification protocol.

Purification of crude extracts of artemisia has to take into account the following impurities:

- Green and yellow pigments.
- Only in the case of extraction with polar solvents such as hexane: Heavier fats: Waxes, oleoresins, other types of heavier oils
- Lighter oils
- Precursors of artemisinin such as artemisinic acid, arteanniun B, artemisitene
- Various other metabolites in small quantities such as camphor, limonene, pinene which will co-elute with the lighter oils
- Only in the case of alcoholic extraction: glucose and other sugars as well as other polar substances.

Pigments: The crude extracts may contain green and yellow pigments, which have to be removed by adsorption (usually with activated carbon) or by some partitioning process (for instance acetone in a polar solvent).

Waxes and heavier Oils: Ethanol as a polar solvent does not appear to co-extract waxes and heavier oils.

Lighter Oils: The lighter oils are usually removed in the last stage of purification by centrifugation after the artemisinin has crystallized or by washing with frozen lower grade alcohols (winterisation) followed by centrifugation.

Precursors of artemisinin: The precursors of artemisinin are apparently a big problem in the purification of hexane extracts and one of the reasons why partitioning between hexane and acetonitrile and chromatography become necessary. No mention is made of them in the literature in the case of ethanolic extraction and purification.

Sugars and other polar substances : The presence of sugars and some other polar substances is unique to alcoholic extracts. The are responsible for the fact that ethanolic crude extracts are heavier and more viscous than hexane extracts and that apparently evaporative crystallisation of the crude extract directly from the ethanol is not possible. The polar substances need to be removed either by liquid-liquid extraction involving a suitable partitioning process between a polar and a non polar solvent, or by drying the crude extract and extracting with a non-polar solvent thereafter .

2. HFC R134 a may be a suitable solvent for the purification of ethanolic extracts. Artemsinin is soluble in R134a and R 134 a has been used in the extraction of artemisia leaves. It has not been used for purification of ethanolic extracts before. It is predominantly polar and might thus be a suitable solvent to apply to the dried crude extract. While it may be argued that Supercritical or subcritical CO2 which is entirely polar might even be more suitable, using R134 a would have the advantage of allowing purification on much less expensive equipment.

2. Experimental

- 10 liters of ethanolic extract that had been pretreated with a small amount of activated carbon and filtered and contained 2.154 grams of artemisinin per litre (= 21.5 grams artemisinin). The extract was dark green.
- was evaporated to produce a concentrate of 485 ml weighing 372.82 grams with an estimated artemisinin content of 5.77%. The concentrate was dark green and very viscous.
- Of this concentrate 135,43 grams were taken and dried to produce 48.466 grams of dried crude extract that was dark green and sticky.
- The dried extract was filled into an extraction sheath and put into the high-pressure autoclave. It was extracted at 22° C and 7 bar three times and then evaporated and dried in an exsiccator producing the following yields : first extraction: 1 hour with 800 ml of R 134a, yielding 1.4682 g of a bright yellow powder, 16 hours with 970 ml of R 134 a, yielding 3.9032 g of a dark yellow powder, and another 12 hours with 970 ml of R 134 a, yielding 2.9 grams of dark yellow powder. In total 8.6317 grams of extract were harvested. The extract has a strong artemisia smell suggesting that the yellow color is due to artemisia oil. The artemisinin content (analytical work is still ongoing) is estimated to be between 60 and 80%.

The next steps in the trials that are planned:
Instead of evaporating the extract after extraction with R134 a , the solvent will be cooled (below 10°C) to allow for crystallization of the artemisinin and the oil removed by centrifugation. The loss of artemisinin that remains in the oily phase might have to be minimized by allowing it to crystallise in the oil, repeating centrifugation thereafter.

Alternatively the dried extract described above may be subjected to winterization or possibly be put back into the extractor to be extracted with R134 a at a much lower temperature at which maybe only the oil will be extracted while the artemisinin remains as a residue.

Once the major impurities have been removed, final purification may be performed by re-crystallisation from ethyl-acetate/hexane..

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