A study of the production of essential oils in chamomile hairy root cultures

E. MÁDAY¹, É. SZÖKE¹, Zs. MUSKÁTH² and É. LEMBERKOVICS¹

Institutes of ¹Pharmacognosy and ²Pharmacy, Semmelweis University, Budapest, Hungary

Received for publication: 13 August 1999

Keywords: Matricaria recutita, chamomile (camomile), essential oil, hairy root, gas chromatography

SUMMARY

The active substances in chamomile (Matricaria recutita L.) belong to chemically different structural types. The largest group of medically important compounds forming the essential oils are primarily chamazulene, (−)-α-bisabolol, bisabololoxides, bisabolonoxide A, trans-β-farnesene, α-farnesene, spathulenol and the cis/trans-en-in-dicycloethers. Flavonoids, coumarins, mucilages, mono- and oligosaccharides also have pharmacological effects. We studied the production of essential oils in genetically transformed cultures. Sterile juvenile chamomile plants were infected with A4-Y strains of Agrobacterium rhizogenes. They are known plant pathogens and are capable of inducing so-called hairy roots. The transfer DNA segment of the Ri-virulence plasmid of A. rhizogenes becomes integrated in the genome of the plant cells. The isolated hairy roots grow rapidly on hormone-free media. In order to obtain bacteria-free media, we cultured the transformed roots on Murashige-Skoog (MS) medium supplemented with carbenicillin (800 mg/l). To study the production of essential oils, the clones were propagated on liquid and solid MS and Gamborg (B5) media, respectively. According to gas chromatography, the composition of the essential oil of hairy root cultures on different media was found to be similar, but differing in proportion. The main component of the essential oil which was identified by gas chromatography and mass spectrometry was trans-β-farnesene, as in the intact roots.

INTRODUCTION

The properties of chamomile (Matricaria recutita L.) are widely known, both to the medical profession and in folk medicine. The plant owes its properties to a number of groups of active substances which have a complex effect. The active substances belong to chemically different structural types. The largest group of medically important compounds forming the essential oils in the inflorescence (0.3–1.5%) are primarily chamazulene, (−)-α-bisabolol, bisabololoxides, bisabolonoxide A, the cis/trans-en-in-dicycloethers, trans-β-farnesene and α-farnesene, and spathulenol. Flavonoids, coumarins, mucilages, mono- and oligosaccharides also have pharmacological effects (1,2).

The antiphlogistic activity of chamazulene and (−)-α-bisabolol is demonstrated by stimulating histamine release at concentrations higher than 10−5 M, while the en-in-dicycloethers have a moderately stimulatory effect at concentrations lower than 10−4 M and a strong inhibitory effect on histamine release at concentrations above that (3). Although further studies are still required, according to some authors chamazulene, but not matricine, may contribute to the anti-inflammatory activity of chamomile...
extracts by inhibiting leukotriene synthesis and additional antioxidative effects (4). The anti-inflammatory activity of bisabololoxide A and bisabololoxide B is much lower than that of (-)-α-bisabolol (5). The effect of (-)-α-bisabolol is 2-fold higher than that of its isomers (6). The ulcer-protective function of (-)-α-bisabolol has been determined by animal tests (7). Strong spasmytic activity is attributed to crude bisabololoxides and cycloethers. The spasms caused by histamine on isolated cavy bowel are significantly reduced by the crude bisabololoxides (86%) and cycloethers (87%) (8). Regarding the flavonoid group, luteolin and apigenin strongly inhibit the infiltration of leucocytes (9,10). Moreover, recent studies have shown that apigenin also has an antiphlogistic activity if applied topically (11). Chamomile oil has an antibacterial effect at concentrations above 0.025% on Gram-positive bacteria, and an antifungal effect on Candida albicans (12). A water-alcohol extract of chamomile affects inhibition in Staphylococcus and Streptococcus sp. at 10 mg/ml concentrations while in Trichomonas sp., it affects activation at 2.5 mg/ml concentrations (13). According to microbiological investigations, (-)-α-bisabolol has a cid effect on C. albicans (12). Some studies have been made on the antivirulent effect of some flavonoids (14), which may open up new perspectives regarding chamomile in the field of medical treatment. Farnesene is known mostly for its juvenilis-hormone like activity (15), but its anti-inflammatory effect has also been determined (16).

In addition to the positive properties of chamomile, it also has certain negative effects (17,18), including the allergic effects characteristic of the Asteraceae family (19,20).

The increasing need for high quality drugs cannot be provided by plants growing in the wild. For several years the systematic and controlled cultivation of chamomile types of reproducible pharmaceutical quality has been undertaken on a world-wide basis. The claim that a drug has a high content of active substances has set new tasks for researchers in the field of medicinal plant improvement. The adoption of biotechnological methods has opened up a new perspective in this area.

**MATERIALS AND METHODS**

**Materials**

Seeds of Matricaria recuita L. (Degumil) were obtained from Kerepes (20 km from Budapest) at the end of May and during the first week of June 1997. *A. rhizogenes* strains A4-Y, 15834 and R-1601 were used for infection purposes.