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CHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACTS OF CELANDINE (*CHELIDONIUM MAJUS* L.) PLANTS GROWING IN NATURE AND CULTURED *IN VITRO*.

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Celandine *(Chelidonium majus* L.) (Papaveraceae) is an important medical herb used in traditional and folk medicine throughout the world. In China it is used as a remedy for whooping cough, chronic bronchitis, asthma, jaundice, gallstones and gallbladder pains (Chang and Chang, 1986). In folk medicine of the Balkan countries, it is widely used for its choleric, spasmolytic, and sedative properties. Extracts from celandine are supposed to have antibacterial, antiviral, antifungal and anti-inflammatory effects. Fresh latex is used to remove warts, which are a visible manifestation of papilloma viruses (Colombo and Tome, 1995; Rogelj et al., 1998). The main active constituents of celandine are the alkaloids chelidonine, chelerythrine, sanguinarine, isochelidonine, and isoquinoline alkaloids with protopine (Franz and Fritz, 1979; De Rosa and Di Vincenzo, 1992). The alkaloid fraction is generally assumed to contribute to the antimicrobial activity of plant extracts (Hahn and Nahrstedt, 1991).

Celandine is a very common weed growing around human habitats, usually along walkways and footpaths, in crevices of walls and buildings, and in neglected spots and places. Containing the yellow latex rich with alkaloids, the aerial parts of celandine perish with the first frost, making the plant unavailable during winter until the early spring.

Celandine leaves, petioles, and seeds used for *in vitro* culturing were collected from plants growing in Kalemegdan Park, Belgrade, Serbia. Shoots and leaves of plants grown *in vitro* were collected from shoots produced by recurrent somatic embryogenesis on hormone-free medium as elaborated by Vinterhalter and Vinterhalter (2002).

In vitro plant cultures often produce secondary metabolites in quantities equal to those produced by plants growing in nature. Thus, Rosić et al. (2000) demonstrated high anthraquinone production by *in vitro* cultures of *Rhamnus fallax*, and Šavikin et al. (1999) described production of saponins in callus cultures of *Dioscorea balcanica*. In celandine, alkaloids produced by shoot cultures which leeched into the medium were visible as media discoloration (Vinterhalter and Vinterhalter, 2002).

The main purpose of this study was to examine total alkaloid content and antimicrobial activity of methanol extracts derivates of tissues from plants growing in nature and under conditions of *in vitro* culture.

HPLC analysis of total alkaloids was expressed as chelidonine (Table 1). Detection of chelidonine was done according to European Pharmacopoeia IV (Henning et al., 2003).

Antibacterial and antifungal tests were done with 96% methanol extract derivates (Soković et al., 2000) from leaves and petioles grown in nature and from shoots and somatic embryos cultured *in vitro.*

The following Gram (+) bacteria were used: *Bacillus subtilis* ATCC 10707, *Micrococcus luteus* ATCC 9341, *Sarcinia lutea* ATCC 9391, and *Staphylococcus aureus* ATCC 6538, in addition to the Gram (-) bacteria *Agrobacterium rhizogenes* A4M70GUS, *A. tumefacians* A281, *Escherichia coli* ATCC 35218, *Proteus mirabilis* (clinical isolates), and *Salmonella enteritidis* ATCC 13076; and the yeast *Candida albicans* (clinical isolates). The molds were from the collection of the IBISS Mycological Laboratory.

Test microorganisms were cultivated overnight at 37°C in TSB medium (tryptic soy broth - Biolife). Suspensions containing ~ 10^9 cells/ml were used.

Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in nutrient broth (TSB) with microorganism inocula. The plates containing bacteria were incubated for 24 h at 37°C, that of *C. albicans* for 48 h at 28°C. The following day, 50 µL of 0.2 mg/mL solution of INT (p-iodonitrotetrazolium violet, Sigma) was added to each row and the plate was returned to the incubator for at least half an hour to ensure adequate color development. Inhibition of growth was indicated by a clear solution or a definite decrease in the color reaction (Eloof, 1998). This value was taken as the minimum inhibitory concentration (MIC) of the compounds. The experiments for each sample were conducted in duplicate. Bifonazole was used as a positive control for *C. albicans* and streptomycin for bacterial species. This method was chosen because of its simplicity, sensitivity, and relatively low cost, being rapid and uncomplicated at the same time.

Table 1. Total alkaloids calculated as chelidonine contained in various explants of Celandine tissues. Values are means ± SE (n = 16)..

Total alkaloids (%)	collected from nature		*in vitro* cultured	
	leaves	petioles	embryos	shoots
chelidonine	1.40 ± 0.2	1.17 ± 0.2	1.58 ± 0.3	1.53 ± 0.2

Table 2. Antimicrobial activity (MIC - mg/ml) of methanol extracts from various Celandine explants as determined by the microdilution method..

Microorganisms	Plants grown in nature		Plants cultured *in vitro*		control
	leaves	petioles	embryos	shoots	
Agrobacterium rhizogenes	80	80	40	20	10[a]
Agrobacterium tumefacians	80	80	80	40	10[a]
Bacillus subtilis	80	80	80	40	10[a]
Candida albicans	-	-	-	80	80[b]
Escherichia coli	80	80	80	80	80[a]
Micrococcus luteus	40	80	80	80	10[a]
Proteus mirabilis	-	-	-	-	-
Salmonella enteritidis	80	-	80	80	80[a]
Sarcinia lutea	80	80	80	80	10[a]
Staphylococcus aureus	80	80	80	80	20[a]

–: no activity.
[a] Streptomycin; [b] bifonazole.

Chemical analysis (Table 1) showed that the total alkaloid content in shoots and embryos of *in vitro* cultured plants was higher than in leaves and petioles of plants growing in nature.

The results of testing antimicrobial activity are presented in Table 2. Methanol extracts of all samples showed significant activity against Gram (+) and Gram (-) bacteria and *C. albicans*. The extracts were not effective only against *P. mirabilis*. Extracts from various explants had higher antimicrobial activity against the tested microorganisms than that recorded for extracts from leaves and petioles of plants growing outdoors. When the results were compared with the antimicrobial activities of positive controls (streptomycin or bifonazole), some extracts showed equal antimicrobial activity against *E. coli*, *S. enteritidis*, and *C. albicans*.

The obtained results justify the use of *Chelidonium majus* in traditional folk medicine. Also, during the winter season, when we cannot collect *C. majus*, plants cultured *in vitro* can completely replace plants in nature.

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