SENSIVITY OF *BACILLUS SUBTILIS* TO WATER SOLUBLE ALKALOID EXTRACTS OF *CHELIDONIUM MAJUS* L. (PAPAVERACEA) ROOTS FROM AZORES

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ARQUIPÉLAGO

PAVÃO, M. LEONOR & RUY E. PINTO 1995. Sensivity of *Bacillus subtilis* to water soluble alkaloid extracts from Azores *Chelidonium majus* L. (Papaveracea) roots. *Arquipélago*. Life and Marine Sciences 13A: 93-97. Angra do Heroísmo. ISSN 0870-6581.

Water soluble alkaloid (WSA) extracts from *Chelidonium majus* L. (great celandine) roots, growing on uncultivated ground in the Azores, were prepared. The WSA showed antibacterial properties towards *Bacillus subtilis*. The effect of WSA appeared to be 1/10 of tetracycline. For concentrations lower than 100 µg/disc, no reproducible sensivity was observed. Chelidonine, protopine and allocryptopine had no action against *Bacillus subtilis*. Coptisine, which is reported as not exhibiting antibacterial activity, showed activity against *Bacillus subtilis* in a similar way as sanguinarine and berberine. Chelerythrine was the most active alkaloid (about 40-50% higher than sanguinarine). Sanguinarine and chelerythrine are generally accepted as the alkaloids responsible for the antibacterial properties of *Chelidonium* latex. Results suggest that, at least for the species existing in the Azores, berberine and coptisine also contribute to that biological activity .

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Prepararam-se extractos de alcalóides solúveis em água de raízes de *Chelidonium majus* L. (celidónia), colhida em terrenos incultos nos Açores. Estes extractos revelaram possuir propriedades antibacterianas em relação a *Bacillus subtilis*. O efeito dos extractos pareceu ser dez vezes menor do que o da tetraciclina. Para concentrações dos extractos inferiores a 100 µg/disco não foi detectada uma clara sensibilidade da cultura. A quelidonina, a protopina e a alocriptopina não são activos contra *Bacillus subtilis*. A coptisina, que não é citada na bibliografia como possuindo actividade antibacteriana, revelou actuar sobre *Bacillus subtilis* de um modo semelhante ao apresentado pela sanguinarina e pela berberina. A queleritrina foi o alcalóide que revelou maior efeito sobre *Bacillus subtilis*, cerca de 40 a 50 % superior ao da sanguinarina. A sanguinarina e a queleritrina são os alcalóides geralmente considerados como os principais responsáveis pela actividade antibacteriana do látex da quelidónia. Sugere-se que esta responsabilidade seja também atribuída à berberina e à coptisina, pelo menos no que diz respeito à espécie da planta existente nos Açores.

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INTRODUCTION

Chelidonium majus L. is a Papaveracea known for the pharmacological properties of its latex (BODALSKY & RZADKOWSKA 1957; KIM et al. 1969; BAUMMAN et al. 1971; KERY et al. 1987). These properties are mainly due to the alkaloid content of the plant (for a revision see SEQUEIRA DE MEDEIROS 1984). The total alkaloid extracts have antibacterial properties against pathogenic strains of *Staphylococcus*, *Streptococcus*, *Klebsiella* and *Bordetella* (GHEORGHIU et al. 1970). It is generally assumed that the quaternary benzophenanthridine alkaloids chelerythrine and sanguinarine are responsible, to a great extent, for this biological activity of the *Chelidonium* extracts (BODALSKI et al. 1957; LENFELD et al. 1981; GOMEZ & BAUM 1986; POPOVICI et al. 1986).

The purpose of the present study was to test the sensivity of *Bacillus subtilis* to water soluble alkaloid extracts from *Chelidonium* roots growing on uncultivated ground in the Azores. Results were obtained monthly for a one year growing cycle of the plant.

We also evaluated the antibacterial activity of the five major alkaloids shown previously to be present in the extracts: chelidonine, chelerythrine, sanguinarine, berberine, coptisine, protopine and allocryptopine (PAVÃO & PINTO 1995). For comparison, tetracycline, cloramphenicol and penicillin were used under the same conditions.

MATERIALS AND METHODS

Plant

Plants were harvested every 4 weeks, during a one year cycle, on uncultivated ground on the island of S. Miguel. Roots (3-4 kg) were separated from the aerial parts and dried at 50-60°C for 3 days. The dried materials from all the collected plants were combined and coarsely ground to a powder.

Chemicals and culture

All chemicals were of analytical grade. Berberine chloride ($2H_2O$), chelidonine and sanguinarine were from Extrasynthèse®, 69000 Geenay, France. Chelerythrine, protopine, allocryptopine and coptisine were generous gifts of Professor Jira Slavik (Purkine University, Brno, Czech Rep.). The purity of the standard alkaloids was verified by TLC, using 3 solvent systems (PAVÃO & PINTO 1995, this journal).

Triptone (Oxoid®), Plate Count Agar (Oxoid®) and *Bacillus subtilis* from Bactisubtil (Lepetit®) were used in microbiological tests. According to the laboratory description, each capsule of Bactisubtil contains a minimum of 10^9 cells of a pure, centrifuged and dried culture of *Bacillus subtilis*, strain IP 5832.

Susceptibility Test Discs (Oxoid®), containing 10 μg of tetracycline (TE_{10}), 10 μg of chloramphenicol (C_{30}) and 2 UI of penicillin G (P_2) were used.

Water soluble alkaloid (WSA) extracts

Determination of total alkaloids from roots was carried out according to KUSTRAK et al. (1982), as described by PAVÃO & PINTO (1995). TLC using 3 solvent systems, followed by fluorescence densitometry were carried out to separate, identify and quantify the five major alkaloids present in WSA: chelidonine, chelerythrine, sanguinarine, berberine and coptisine. Protopine and allocryptopine were also identified in all the extracts, but they were not evaluated. TLC revealed the presence of other alkaloid fractions, which were not identified (PAVÃO & PINTO 1995).

Testing material

WSA, prepared as previously described, were immediately used or, alternatively, dissolved in distilled water in suitable concentration and stored in plastic bags at –20°C. When necessary,

water was removed by evaporation. Methanolic solutions were prepared from each WSA (about 1 mg WSA ml^{-1}) and from each standard alkaloid (5 $mg \cdot ml^{-1}$).

Paper discs

Paper discs with a diameter of 6 mm were cut out from Whatman no.3 filter paper and sterilized at 180°C, during 30 min, in a Petri dish, where they were kept until required.

On each disc, successive portions of 50 µl of methanolic solutions of the testing material were applied, until the desired concentration was obtained (1 mg of standard alkaloid/disc and 0.5 mg WSA/disc). After each application, the solvent was evaporated using a cold air blow.

Antibacterial testing

The content of one capsule of Bactisubtil was poured into 10 ml of a 15 gl^{-1} triptone water solution and incubated at 37°C during 24h. For disc testing, 10 ml of the agar were used in a 100 mm diameter glass Petri dish. The surface was seeded with 0.5 ml of the broth culture and the discs were immediately applied. In all cases, paper discs, having pure solvent applied and evaporated as described above, were used as control. The activity of the testing materials was compared to the effect of TE_{10}, C_{30} and P_2. The antibacterial effect was estimated on the basis of the inhibition zone-diameter, after incubation at 37°C, during 24 h.

Also, we determined the minimum concentration of one of the considered extracts (containing 43 µg of chelertythrine, 67 µg of sanguinarine, 24 µg of berberine and 22 µg of coptisine, per milligram of WSA) at which no development of culture around the paper disc was observed. For this, methanolic solutions of the extract with suitable concentrations, were prepared and applied as previously described, so to obtain discs containing amounts of 50 to 550 µg.

RESULTS AND DISCUSSION

Chelidonine, protopine and allocryptopine did not show any effect upon *Bacillus subtilis* (Table 1). Coptisine was active against *Bacillus subtilis* in a similar way as sanguinarine and berberine (Table 1). The effect was equivalent to the activity of 30 µg of choramphenicol. Coptisne had not been previously reported as an antibacterial product, but has other pharmacological properties. Namely, it is one of the acitve anti-inflammatory constituents of *Coptis japonica* Makino (OTSUKA et al. 1981) and it was identified as one of the active principles of an aqueous extract of *Coptidis* rhizoma having an inhibitory effect on the bacterial colagenase from *Clostridium histolyticum* (TANAKA et al. 1991). Also, HATTORI et al. (1992) observed the antinephritic effect of berberine and coptisine in rats with original-type Anti-GBM nephritis.

Chelerythrine was the most active alkaloid against *Bacillus subtilis* and its activity was about 40-50% higher than that of sanguinarine.

Table 1

Antibacterial activity test of some alkaloids of *Chelidonium majus* L. against *Bacillus subtilis*. Values of active alkaloids represent mean±s.d. (5 samples)

Alkaloid	Inhibition zone diameter (mm)
Allocryptopine	0
Protopine	0
Berberine	9±0.5
Coptisine	8±0.6
Chelidonine	0
Chelerythrine	12±0.6
Sanguinarine	9±0.5

The minimum active concentration of WSA is approximately 80 µg, since a concentration of 100 µg still originated a clear inhibition zone,

which was not observed for 50 µg. Also, the effect of 100 µg of WSA appeared to be similar to that of 10 µg of tetracycline.

All the WSA showed a positive effect against *Bacillus subtilis* (Table 2), which reveals the presence of antibacterial alkaloids in the latex of the plant, throughout the growing season. However, maximum values were observed for winter extracts (December and January), when chelerythrine and sanguinarine concentrations (about 65µg/mg WSA) were higher than in the other months (PAVÃO & PINTO 1995).

Table 2

Antibacterial activity of water soluble alkaloid extracts from *Chelidonium majus* L. roots, along one year cycle.

WSA Alkaloid concentration (µg/mg WSA)					Inhibition zone diameter (mm)			
Month	Chr	S	B	C	WSA	P2	C30	TE10
Feb.	35.5	38.6	25.5	21.2	10	36	8	7
Mar.	39.9	25.6	16.4	17.7	8	38	8	8
Apr.	22.1	28.9	16.3	18.9	7	36	8	7
May	29.2	37.7	24.8	31.9	11	36	7	>6
June	19.3	12.5	11.6	16.0	>6	36	8	>6
July	27.4	31.4	31.2	35.5	8	36	8	>6
Aug.	25.2	35.0	26.4	27.8	8	36	9	8
Sep.	21.6	40.5	17.4	15.0	7	36	8	7
Oct.	30.4	54.5	21.8	15.2	8	36	8	7
Nov.	25.9	61.9	20.4	15.6	8	32	8	>6
Dec.	60.0	69.1	24.5	13.3	14	33	8	>7
Jan.	42.9	67.0	23.9	22.1	12	33	8	>6

Month=month of roots harvesting for preparation of extracts. Chr=Chelerythrine; S=Sanguinarine; B=Berberine; C= Coptisine; WSA= Water soluble alkaloid extracts; P2=Penicillin- 2UI; C30=Chloramphenicol-30µg; TE10= Tetracycline-10µg; Values of Alkaloid conc. (see PAVÃO & PINTO 1995); Values of WSA activity represent mean of 3 samples (10<error<15%); Values of P2, C30 and TE10 represent mean of 3 samples (error² 5%).

Results also suggest that coptisine may account for the observed activity of WSA. Namely, in the extract obtained from the roots harvested in May (Table 2), coptisine concentration was about twice as high as in December (13 µg/mg WSA), chelerythrine was about 50% (29 µg/mg WSA) and sanguinarine was 45% lower (38 µg/mg WSA); berberine was aproximately at the same concentration in both cases (25 µg/mg WSA).

A simple additive effect of the individual alkaloid actions can not be considered, but a synergistic effect could partially explain the results. However, WSA have a relatively large number of unquantified alkaloids (about 1/2 of total weight of WSA), whose action on *Bacillus subtilis* is also unknown, except for protopine and allocryptopine (Table 1). Also, the presence of a great amount of compounds on a disc may contribute to enhance the diffusion area of the material on culture. Thus, it is not possible to assess a simple relationship between the activity of an isolated alkaloid and its contribution, as a component of the extract, to the total effect of WSA.

Sanguinarine and chelerythrine are generally accepted as the main alkaloids responsible for the antibacterial properties of *Chelidonium* latex. The present study suggests that, at least for the *Chelidonium* species existing in the Azores, berberine and coptisine also contribute to that biological activity.

As *Bacillus subtilis* exists in mammalian intestinal flora, its sensivity to *Chelidonium majus* alkaloid extracts and individual components, when used in oral treatments, should be considered in further studies.

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