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Anti-inflammatory and anti-ulcer activity of *Achillea alexandri-regis*

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Several plants from genus *Achillea* are used traditionally as aromatic bitters with astringent, chemostypic, choleretic and anti-inflammatory activity. Recent studies showed that some less known species of this genus posses significant pharmacological activity. A large number of different compounds including sesquiterpene lactones, flavonoids and polyacetylenes have been isolated from *Achillea* species. The anti-inflammatory activity of *Achillea* species is attributed to the presence of sesquiterpenes lactones (proazulenes), but the role of flavonoids is also very important [1–3].

Achillea alexandri-regis Bornmüller et Rudski is a stenoendemic species of Serbian Flora, found on mountain Osljak that presents northern branches of Sara mountain in Serbia [4]. In continuation of our research of wild growing species from Serbia, the methanol extract of herb of *Achillea alexandri-regis* has been screened for potential anti-inflammatory and gastro-protective action.

Using carrageenan-induced rat paw edema test it was found that the methanol extract given in doses of 50, 100 and 200 mg/kg (p.o.) showed significant dose dependent anti-inflammatory effects similar to those produced by diclofenac sodium (2.5, 5.0 and 7.5 mg/kg, p.o.), a strong non-steroidal anti-inflammatory drug (Table 1).

Also, pretreatment with the extract prevented experimental ulceration induced with ethanol (96%) in a dose dependent manner. There was a statistically significant difference compared to the control in doses of 400 and 600 mg/kg p.o. (Table 2).

The extract tested showed anti-inflammatory effects at lower doses than needed for the anti-ulcer effect. The results imply the potential use of *A. alexandri-regis* as herbal medicine with anti-inflammatory and anti-ulcer activity.

For HPLC analysis, the methanol extract was separated in ether, ethyl acetate and butanol fractions. Liquid chromatography analysis showed that polyphenolic compounds dominated in the extracts. The presence of apigenin-7-O-glycoside in the ethyl acetate and caffeic acid in the butanol extract was confirmed.

Table 1: Effect of methanol extract of *Achillea alexandri-regis* and diclofenac sodium on carrageenan-induced rat paw edema

Treatment	Number of rats	Doses (ml or mg/kg body wt)	Score (mean ± SE)	Effect (%)
Control	15	–	16.32 ± 12.27	0
Vehicle (75% methanol)	6	1	17.05 ± 6.83	–4.47
Methanol extract	7	50	17.18 ± 5.04	–5.3
Achillea alexandri-regis				
	7	100	10.63 ± 2.67*	34.87
	6	200	8.98 ± 3.59*	44.98
Diclofenac sodium	6	2.5	9.0 ± 5.84*	44.86
	8	5.0	7.21 ± 4.81**	55.83
	8	7.5	7.05 ± 5.46**	56.81

* $p < 0.05$, ** $p < 0.01$ (Mann-Whitney U-test)

Table 2: Effect of treatment with *Achillea alexandri-regis* in the prevention of induction of gastric ulcers

Treatment	Number of rats	Doses (ml or mg/kg body wt)	Score (mean ± SE)	Effect (%)
Control	8	–	2.9 ± 0.18	0
Vehicle (75% methanol)	6	1	2.7 ± 0.07	6.9
Methanol extract	5	200	2.95 ± 0.11	–1.72
Achillea alexandri-regis				
	6	400	1.91 ± 0.18*	34.14
	6	600	1.87 ± 0.51**	35.52

* $p < 0.01$, ** $p < 0.001$ (Mann-Whitney U-test)
Intensity score: 0-no lesions, 0.5-mild hyperemia, 1-less than 5 petechia or stronger hyperemia, 1.5-more than 5 petechia, 2-less than 10 petechia and erosions less than 4 mm in length, 2.5-more than 10 petechia and erosions less than 4 mm in length, 3-erosions more than 5 mm in length

It has been confirmed that free radicals were involved in the process of inflammation and pathogenesis of ethanol-induced stress ulceration. Antioxidants protect cellular damage by scavenging free radicals [5, 6]. Recent reports have suggested that *A. alexandri-regis* methanol, ethyl acetate and butanol extracts posses significant antioxidant properties attributed to the presence of flavonoids [7, 8].

Experimental

Whole plant *A. alexandri-regis* was collected on mountain Osljak (northern branches of Sara Mountain, Serbia) in July 1996. A voucher specimen is preserved in the Institute of Botany Herbarium (BeoU), Botanical Garden, University of Belgrade (No. 8402). Dried and grounded herb was macerated with light petroleum (1 : 10) for two days to remove fatty material followed by extraction with 75% methanol using the same procedure. The solvent was evaporated under low pressure. The final extract was dissolved in 75% methanol for testing the n-inflammatory and anti-ulcer activity. For HPLC analysis, the methanol extract was dissolved in boiling water and extracted with ether, ethyl acetate and butanol. The solvents were also evaporated under low pressure.

The carrageenan-induced rat paw edema test has been used as an experimental model of acute inflammation for screening anti-inflammatory activity according to the method described by Oyanagni et al [9]. The extract was administrated p.o. in doses of 50, 100 and 200 mg/kg body weight. Diclofenac sodium (ICN Yugoslavia) dissolved in water was used as a reference in doses of 2.5, 5.0, 7.5 mg/kg body wt p.o.

Ulceration was induced according to the method described by Szabo et al. by intragastric instillation of 96% ethanol (1 ml) to the rats [10]. The extract has been administrated p.o. 60 min before ethanol in doses of 200, 400 and 600 mg/kg body wt. The intensity of gastric lesions has been scored according to the modified scaling system by Szabo et al. Two independent observers estimated the intensity of gastric lesions. In case of difficulties to determine uniform values, the lesions were scored adding 0.25 to the lower value of the scale.

HPLC with a diode array detector (2150LKB pump, 2140 Rapid Spectral Detector, 2140 Rapid Spectral Detector Optical Unit, 2125 Rheodyne injector, sample 20 µl) has been used for analysis of ether, ethyl acetate and butanol extracts. Separation was performed on reversed phase material (LiChrosphere RP18, 5 µm, 250-4) with acetonitrile (40%)-acetic acid (1%) as mobile phase (flow rate 1 ml/min, room temperature). Identification was performed comparing retention times and UV spectra of unknown and reference compounds.

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Antimalarial constituents from *Guatteria amplifolia*

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In our search for natural substances with antimalarial activities from Colombian plants, we investigated the aerial parts of *Guatteria amplifolia* Tr. & Pl. (Annonaceae), a medium-sized tree distributed through the Occidental coast of Northern South America. In preliminary bioassays, we observed promising antimalarial activity with various extracts from this species.

Using chromatographic methods, we isolated four aporphine alkaloids identified as liriodenine, corydine, isocorytuberine and *O*-methylmoschatoline. Structural elucidation was performed using IR, UV, MS and ^{1}H and ^{13}C NMR mono- and bidimensional spectroscopy. The spectral data for the four alkaloids are in accordance with those reported. Liriodenine, corydine and *O*-methymoschatoline have been previously isolated from various genera of the Annonaceae, and particulary from the genus *Guatteria* [1–3]. Isocorytuberine has been isolated from *Trivalvaria macrophylla* (Annonaceae) [4], but, to our knowledge, it is the first time that its occurence in the genus *Guatteria* is reported.

The evaluation of the antimalarial activity of the purified alkaloids was performed *in vitro* against F32 (chloroquine sensitive) and D2 (chloroquine resistant) strains of *Plasmodium falciparum*. The results (Table) show good antimalarial activity for isocorytuberine and corydine, whereas liriodenine was notably less active and *O*-methymoschatoline inactive.

Experimental

1. Plant material

A sample of aerial parts was collected during march 1996 in Bajo Calima, Colombia. A voucher was deposited (BW 030) at the Herbarium of the Universidad del Valle (Cali).

2. Extraction and isolation

Dried ground aerial parts (250 g) were extracted following the usual work-up procedure [5] to give 1.8 g of an alkaloidal mixture. This mixture was fractionated by CC on silica gel using CH_2Cl_2-MeOH (9 : 1) as eluent. Further purification was achieved by preparative TLC on silica gel using the same eluent, affording 12 mg of liriodenine, Rf = 0.7, 15 mg of corydine, Rf = 0.6, 18 mg of isocorytuberine, Rf = 0.4, and 38 mg of *O*-methylmoschatoline, Rf = 0.8.

Table: Antimalarial activity (μM) against F32-Tanzania (chloroquine sensitive) and D2 (chloroquine resistant) strains of *Plasmodium falciparum*

	IC_{50} μM	
Compound	F32-Tanzania	D2
Isocorytuberine	3.05	4.88
Corydine	5.27	5.27
Liriodenine	54.5	36.3
O-Methylmoschatoline	>155	>155