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Pharmacological properties of *Anagallis arvensis* L. ("scarlet pimpernel") and *Anagallis foemina* Mill. ("blue pimpernel") traditionally used as wound healing remedies in Navarra (Spain)

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ABSTRACT

Ethnopharmacological relevance: : *Anagallis arvensis* and *Anagallis foemina* are traditionally used in Navarra (Spain) for dermatological purposes regarding wound healing properties. In some cases they are also used to threat internal infections although they are known to be toxic at high doses.
Aim of study: : Due to lack of studies, we decided to evaluate the potential of the plants as wound healing remedies measuring antimicrobial and anti-inflammatory properties using *in vitro* procedures.
Materials and methods: Antimicrobial effects were studied against four bacteria and one fungus. Anti-inflammatory properties were measured in terms of COX-1 and -2 inhibition as well as superoxide radical scavenging capacity.
Results: Both species exerted antimicrobial and anti-inflammatory effects. The methanolic extract obtained from *Anagallis arvensis* seemed to produce the highest inhibition in *Candida albicans* (MIC = 0.31 mg/ml). Inhibition of COX-1 and -2 was also stronger for methanolic extracts whereas aqueous were revealed as better free radical scavengers.
Conclusions: The study reveals that both species posses antimicrobial and anti-inflammatory activities related to their ethnomedicinal uses.

1. Plant material

Aerial parts of *Anagallis arvensis* L. and *Anagallis foemina* Mill. (Primulaceae) were collected in the province of Navarra (Spain) during spring time 2008. The material was botanically identified by S. Akerreta and R.Y. Cavero. Voucher samples were deposited at PAMP Herbarium of the University of Navarra: *Anagallis arvensis* (PAMP 18927) and *Anagallis foemina* (PAMP 18718).

2. Ethnobotanical importance and uses

Both species, popularly called "murajes" or "pasmobelarra" are used in the province of Navarra (Spain) regarding wound healing properties in human and veterinary ethnomedicine (Akerreta et al., 2007, 2010; Cavero et al., 2011). Aerial parts are used to prepare an ointment for the treatment of external infections such as wounds and infected pimples. In some cases, an infusion is prepared with the plants to threat internal or systemic infections, though, they are popularly known to be toxic during long-term consumption.

3. Previous phytochemical-pharmacological studies

Anagallis arvensis has been reported to contain saponins (Napoli et al., 1992; Shoji et al., 1994) and flavonoids (Kawashty et al., 1998); few works on biological effects have also been published (Shehata and Nassef, 1956; Rothwell and Marshall, 1986; Amoros et al., 1988; Ali-Shtayeh and Abu Ghdeib, 1999; Rivero et al., 2001; Apak et al., 2006). However, there are no pharmacognostic studies about *Anagallis foemina*.

4. Material and methods

4.1. Chemicals

2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), xanthine, xanthine oxidase from buttermilk, COX-1 from sheep seminal vesicles, human recombinant COX-2,

Abbreviations: COX, cyclooxygenase; PG, prostaglandin; ROS, reactive oxygen species; RSC, radical scavenging capacity; X, xanthine; XO, xanthine oxidase.

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Table 1
MIC (antimicrobial activity) and IC_{50} (superoxide radical scavenging activity) values.

Species	Extract	Antimicrobial activity—MIC (mg/ml)					Radical scavenging activity—IC_{50} (μg/ml)
		Bacillus subtillis	*Staphylococcus aureous*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	
Anagallis arvensis	MeOH	1.25	–	>2.5	–	0.3125	20.46 ± 2.89*
	Aq	–	–	–	–	–	10.73 ± 3.07*,#
Anagallis foemina	MeOH	1.25	–	2.5	–	0.625	27.82 ± 6.18
	Aq	–	–	–	–	–	18.67 ± 0.66#
Streptomycin		0.0125	0.0125	0.0125	0.0625		
Amphotericin B						0.0625	
Caffeic acid							1.38 ± 0.25

–, no activity.
* $p < 0.05$, compared with the same extract of *Anagallis foemina*.
# $p < 0.05$ compared with the methanolic extract of the same species.

nitroblue tetrazolium salt chloride (NBT), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and ^{14}C-arachidonic acid were purchased from Sigma-Aldrich. Caffeic acid was obtained from Extrasynthèse (Genay, France). The cytotoxicity detection kit was purchased from Roche (Indianapolis, USA). Culture media, penicillin-streptomycin were obtained from Gibco (Barcelona, Spain).

4.2. Preparation of extracts

Methanolic and aqueous extracts were prepared and used in the experiments according to a preliminary screening (López et al., 2008).

4.3. TLC analysis

Methanolic and aqueous extracts were evaluated in a flavonoid system (Wagner and Bladt, 1996). 100 μg of extracts was applied to Merck silica-gel 60_{F254} and eluted in ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:26). Three plates were prepared under same conditions. The first plate was tested for saponins using a blood reagent and saponin as reference. The second plate was sprayed with natural products – PEG (view under 366 nm) to detect flavonoids, using a mixture solution of rutin–chlorogenic acid–hyperoside as reference. The third plate was spayed with vanillin–sulphuric acid (observed at visible) as a general reagent to detect flavonoids and saponins using the same mixture of rutin–chlorogenic acid–hyperoside as reference.

4.4. Antimicrobial assays

The minimum inhibitory concentration (MIC) of each extract was determined for four bacteria and one fungus: *Bacillus subtillis* (ATCC 6051), *Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 9027) and *Candida albicans* (IMI 349010). The antibacterial activity was evaluated according to Eloff (1998) using streptomycin as positive control. The antifungal activity was performed according to Motsei et al. (2003). All experiments for the antimicrobial activity were done in three replicates.

4.5. COX-1 and COX-2 inhibitory activity

The COX assays were performed as described by Jäger et al. (1996) and Zschocke and van Staden (2000) using COX-1 from sheep seminal vesicles (Sigma) and human recombinant COX-2 (Sigma). Indomethacin was used as positive control for COX-1 activity and nimesulide as specific COX-2 inhibitor. Three experiments were performed. Percentages of prostaglandin production were calculated based on the conversion of ^{14}C-arachidonic acid by cyclooxygenases using liquid scintillation counting.

4.6. Free radical scavenging properties (X/XO assay)

Superoxide radicals were generated by the xanthine/xanthine oxidase (X/XO) system following the described procedure of Ribeiro et al. (2007). The radical scavenging capacity of the extracts is expressed as IC_{50} values, calculated by a non-linear regression with one phase exponential association equations using GraphPad Prism version 4.0. The effect of the extracts on xanthine oxidase was also evaluated in order to detect possible enzyme inhibition (Unno et al., 2004).

4.7. Statistical analysis

Data are expressed as means ± S.D. of three independent experiments. One-way ANOVA followed by Dunnett's multiple comparison test was used to compare control and treatments in the COX assay. Student *t*-test was used to analyze IC_{50} data from antioxidant activity. Statistics were performed with GraphPad Prism version 4.0. *p* values <0.05 were considered statistically significant.

5. Results and discussion

Anagallis arvensis and *Anagallis foemina* are of ethnobotanical importance in Navarra (Spain) regarding wound healing activities. However, few studies reveal its potential uses and neither ESCOP nor Comission E record official monographs of the plants. MIC activities are reported in Table 1. The methanolic extracts of both species were found to inhibit the growth of *Bacillus subtillis*, *Escherichia coli* and *Candida albicans*, being the methanolic extract obtained from *Anagallis arvensis* strongly active against *Candida albicans*.

Results on COX-1 and -2 activities are presented in Fig. 1 as percentage of prostaglandin (PG) production related to control (solvent blank). Extracts of *Anagallis arvensis* and *Anagallis foemina* inhibited cyclooxygenases at higher concentrations. In general, the methanolic extracts were more effective than the aqueous, as concentrations of 0.1 and 1 mg/ml reduced PG formation significantly ($p < 0.01$), whereas the aqueous were only active at 1 mg/ml. The positive controls indomethacin (5 μM) and nimesulide (200 μM) reduced PG formation until levels of 42% and 47% respectively.

Both *Anagallis arvensis* and *Anagallis foemina* exerted antioxidant properties in a dose dependent manner, but again *Anagallis arvensis* evidenced a higher activity. IC_{50} values (μg/ml) of methanolic extracts are 20.5 ± 2.9 (*Anagallis arvensis*) and 27.8 ± 6.2 (*Anagallis foemina*). IC_{50} values (μg/ml) of aqueous extracts are 10.7 ± 3.1 (*Anagallis arvensis*) and 18.7 ± 0.7 (*Anagallis foemina*). According to