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Research Article

Antibacterial acticivity of extracts from *Potentilla reptans* L.

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Abstract

Potentilla reptans is widely used in traditional medicine as an astringent, for treating diarrhoea, haemorrhoids and for bleeding gums. A recent ethnobotanical study has reported on the anti-mastitis effects of the aerial parts of *P. reptans* decoction. The aim of the present study is to evaluate the antibacterial potential of extracts and fractions, obtained from aerial parts of *P. reptans* against three strains of *Staphylococcus aureus.* The observed MICs were within the range of 0.325 – 2.5 mg/ml. Studied extracts and their fractions exerted mostly bacteriostatic effect, with the *n*-hexane fraction of hydroethanolic extract being the most active (MIC 0.313 mg/ml against *S. aureus* ATCC 6538 P). However, further investigations are necessary to reveal the precise mode of action of *P. reptans* against mastitis.

Keywords

Potentilla reptans, antibacterial activity, mastitis, traditional use

Introduction

Potentilla reptans L. is a stoloniferous perennial plant, belonging to the Rosaceae family, which is distributed in the Northern Hemisphere (Markova 1973). *P. reptans* is used traditionally for treatment of bleeding gums (Bulgaria, root decoction) (Stoyanov 1973), gastric ulcers (Turkey, aerial parts infusion) (Gürbüz et al. 2005), diarrhoea (Bulgaria, root infusion, Leporatti and Ivancheva 2003; Italy, aerial parts decoction, De Natale and Pollio 2007) and haemorrhoids (Bulgaria, root decoction, Stoyanov 1973; Spain and Italy, whole plant infusion De Natale and Pollio 2007, Calvo and Cavero 2014; Turkey, aerial parts infusion, Avcı et al. 2006). Recently, a study has documented a new ethnobotanical report for the anti-mastitis effect of *P. reptans* in the form of aerial parts decoction (Kozuharova et al. 2012).

Several compounds, mainly phenolics, have so far been isolated from the aerial parts from *P. reptans*. These are flavonoids and their glucosides (kaempferol, quercetin, rutin, quercetin-3-*O*-glucoside, kaempferol-3-O-gluco-

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side, apigenin-7-O-glucoside, luteolin-7-O-glucoside) as well as catechins and phenolic acids (chinc, caffeic, ferulic acid (Nedialkov 1994, Tomczyk and Latté 2009, Tomovic et al. 2015). Additionally, tannins have also been isolated from the aerial parts (Nedialkov 1994).

Mastitis in humans is relatively common, developing in 5–33% of women during lactation. It is a major cause of reduction of milk production and a reason for stopping breast-feeding. Clinical mastitis is a powerful risk factor for the vertical transmission of viral infections from mother to infant (Michie et al. 2003). Moreover, mastitis in humans has the potential for serious outcomes such as breast abscesses and septicaemia, in rare cases. Additionally, in dairy farms, mastitis is the costliest and thus, probably the most important disease. *Staphylococcus aureus* is the main etiological agent of acute mastitis and breast abscesses in humans, while in dairy cows, its prevalence worldwide varies between 5% and 18% (European Centre for Disease Prevention and Control 2017). Both human and dairy animal mastitis is treated with antibiotics, which contributes to the global antimicrobial resistance spread. It is estimated that infections caused by a subset of resistant bacteria are responsible for about 25000 deaths in Europe annually (Contreras and Rodríguez 2011). The increasing antibacterial resistance during the last decades has led to intense scientific efforts in the screening of plant extracts and fractions, based on the ethnobotanical database.

The present study aims to evaluate the antibacterial potential of extracts and fractions, obtained from aerial parts of *Potentilla reptans* against three strains of *Staphylococcus aureus*, including the methicillin-resistant strain, in order to verify the ethnobotanical use of the plant against mastitis.

Materials and Methods

Plant material and extracts preparation

Aerial parts of *Potentilla reptans* were collected in Sofia during the July 2015 flowering period. Four aqueous and one hydroethanolic extracts were obtained by different manners of extraction widely used in folk medicine (Table 1).

The extracts obtained were filtered and evaporated to dry weight. Extracts (except extract 4) were successively extracted with *n*-hexane, diethyl ether, ethyl acetate and *n*-butanol and submitted to antibacterial tests along with extracts 1-3 and 5.

Test microorganisms

Antimicrobial susceptibility testing was performed with the following bacterial strains: *Staphylococcus aureus* ATCC 6538 P (American Type Cell Culture, USA), *S. aureus* FDA 209(ATCC 6538, American Type Cell Culture, USA), methicillin-resistant *S. aureus* (MRSA) 1337 (Collection of the Stephan Angeloff, Institute of Microbiology, Bulgaria).

Culture medium and growth conditions

Muller Hinton agar (MHA) and Muller Hinton broth (MHB) were used for each bacterial strain in this study (CM0337B, resp. CM0405B, Thermo Scientific – Oxoid, UK). Microorganisms were grown at 37°C in aerobic conditions.

Minimal inhibitory (MIC) and bactericidal concentrations (MBC)

The antimicrobial activity was estimated by the broth micro-dilution method, according to Clinical and Laboratory Standards Institute procedures (CLSI) [15]. Minimal inhibitory concentrations (MICs) were determined visually as the lowest concentration without visible growth, expressed as milligram per millilitre. Minimal bactericidal concentrations (MBCs) were determined after overnight incubation in Muller Hinton agar of 100 µl of the untreated control and the samples incubated with ½ MIC, MIC and 2xMIC for 18 h at 37°C. MBC were read as concentrations where no visible growth occurred on the agar plates and were expressed as milligram per millilitre. The metabolic (respiratory) activity of treated microorganisms was estimated by spectrophotometric analysis and presented as the percentage of untreated control.

Biofilm formation assay

The biofilm formation of MRSA after exposure to extract 5.1 was determined following the protocol of Stepanović et al. (2007). Five concentrations of extract 5.1 in twofold serial dilutions (from 0.156 to 2.5 mg/ml) were applied. The biofilm formation was documented microscopically (40x) and quantified spectroscopically

Table 1. Preparation of *P. reptans* extracts.

Extract No	Plant material (g)	Solvent type	Solvent quantity (ml)	Extraction temperature (°C)	Extraction time	Extract weight (%)
1	10	Water	100	100	1 min	4
2	10	Water	100	100	10 min	4
3	10	Water	100	80	5 min	8
4	10	Water	400	100	40 min	1
5	5	70% Ethanol	100	Ambient	36 h	14

(550 nm). The minimum biofilm inhibition concentration (MBIC) was defined as the lowest concentration of the tested drugs that led to 50% inhibition on the biofilm formation. The GraphPad Prism software was used for calculation of the results.

Results and Discussion

Five extracts (1-5) were obtained from the aerial parts of *Potentilla reptans* following different modes of infusion used in the Bulgarian folk medicine (Table 1). The observed MBCs of extracts were within the range of 1.25 – 2.5 mg/ml. Comparison of their antibacterial activity with three *Staphylococcus aureus* strains, the etiological agent of mastitis, revealed that amongst the water extracts (1-4), those obtained after heating of the plant material at 100°C for 40 minutes, (4) were the most promising for direct consumption (Table 2). However, examining the antibacterial activity of the fractions, obtained from the aqueous extracts 1-3 by consecutive liquid-liquid extraction with *n*-hexane (1.1, 2.1 and 3.1), diethyl ether (2.2, and 3.2), ethyl acetate (1.3, 2.3 and 3.3), *n*-butanol (1.4, 2.4 and 3.4) and water residues (1.5, 2.5 and 3.5) showed that there are active principles concentrated in the ethyl acetate fractions 1.3 2.3 and 3.3, displaying MICs of 0.625 mg/ml against at least one of the tested bacterial strains (Table 2).

Meanwhile, the hydroethanolic extract 5 showed moderate antibacterial activity (Table 2). Its active compounds are distributed between the *n*-hexane (5.1) and diethyl ether (5.2) fractions, the first one being the most active fraction from all (MIC 0.313 mg/ml against *Staphylococcus aureus* ATCC 6538 P) and having the advantage of inhibiting the methicillin-resistant *S. aureus* MRSA 1337 strain (MIC 0.625 mg/ ml) as well. This result was confirmed by the determination of the respiratory activity of the bacteria and the evaluation of biofilm inhibition of MRSA (Fig. 1). The respiratory activity of penicillin-resistant *S. aureus* (Fig. 1A) was efficiently reduced by extract 5.1. It also strongly inhibited the biofilm formation of MRSA (Fig. 1B and 1C) on a dose-dependent manner. MBIC was calculated to be 0.19 mg/ml.

The observed results of antibacterial activities of the ethyl acetate fractions (1.3 2.3 and 3.3) could be attributed to flavonoids (rutin, quercetin-3-*O*-glucoside, kaempferol-3-O-glucoside, apigenin-7-O-glucoside, lute-

Table 2. Minimal inhibitory (MICs) and minimal bactericidal concentrations (MBCs) (mg/ml) of extracts and fractions from *Potentilla reptans* L. Legend: * For penicillin S ≤ 0.125 < R, according to EUCAST.

Test-bacteria	***S. aureus* ATCC 6538 P**		**MRSA 1337**		***S. aureus* 209**	
ExtractNo	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
1	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5
1.1	1.3	2.5	> 2.5	> 2.5	2.5	> 2.5
1.3	0.6	2.5	2.5	> 2.5	2.5	> 2.5
1.4	> 2.5	> 2.5	2.5	> 2.5	> 2.5	> 2.5
1.5	> 2.5	> 2.5	2.5	> 2.5	> 2.5	> 2.5
2	> 2.5	> 2.5	2.5	> 2.5	2.5	> 2.5
2.1	1.25	2.5	2.5	> 2.5	2.5	> 2.5
2.2	1.25	2.5	2.5	> 2.5	> 2.5	> 2.5
2.3	0.625	1.25	0.625	> 2.5	2.5	> 2.5
2.4	2.5	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5
2.5	> 2.5	> 2.5	2.5	> 2.5	> 2.5	> 2.5
3	> 2.5	> 2.5	2.5	> 2.5	2.5	> 2.5
3.1	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5
3.2	-	1.25	2.5	> 2.5	2.5	2.5
3.3	0.625	1.25	2.5	> 2.5	0.625	1.25
3.4	2.5	2.5	1.25	> 2.5	0.625	1.25
3.5	> 2.5	> 2.5	2.5	> 2.5	2.5	> 2.5
4	> 2.5	> 2.5	1.25	2.5	1.25	2.5
5	2.5	-	> 2.5	> 2.5	0.625	> 2.5
5.1	0.313	1.25	1.25	2.5	1.25	2.5
5.2	0.625	1.25	2.5	> 2.5	1.25	1.25
5.3	-	1.25	2.5	> 2.5	2.5	> 2.5
5.4	1.25	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5
5.5	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5
Penicillin referent antibiotic	0.00025*	0.00025	-	-	0.000008	0.000032
Gentamycinreferent antibiotic	0.00025	0.001	0.00025	0.001	-	-

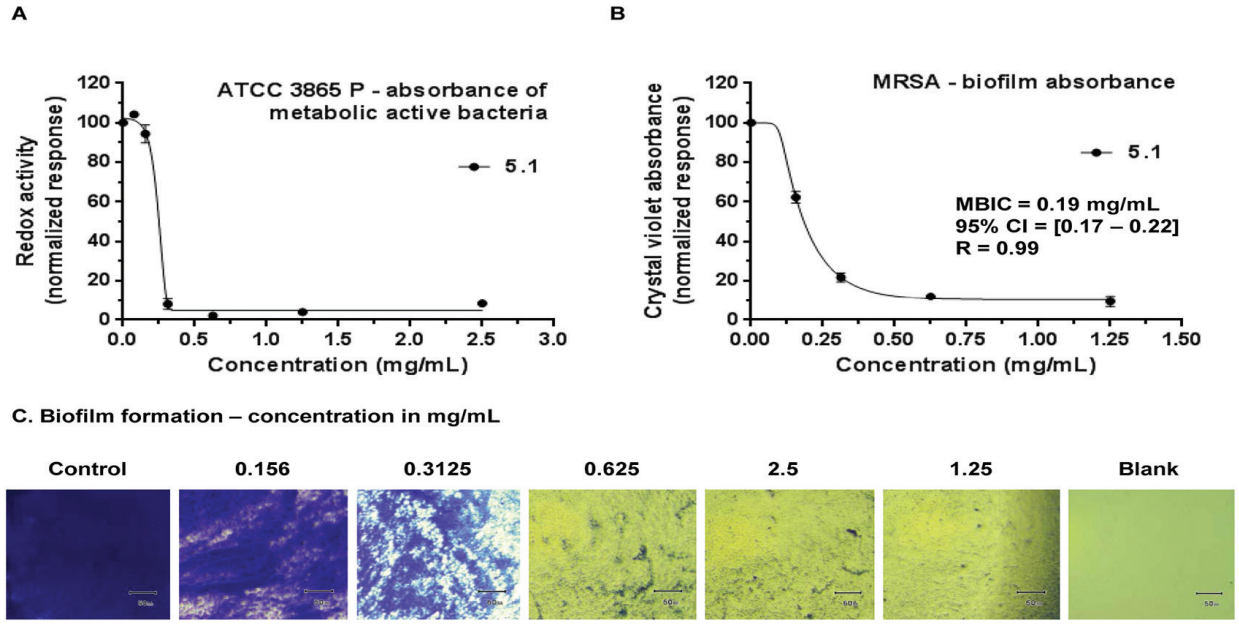

Figure 1. Metabolic activity of ATCC 3865 P and biofilm inhibition of MRSA after exposure to extract 5.1

olin-7-O-glucoside), presumably concentrated in these fractions (Nedialkov 1994). Antibacterial activities of quercetin-3-*O*-glucoside, luteolin-7-*O*-glucoside, rutin and kaempferol-3-*O*-glucoside against different pathogens, including *Staphylococcus aureus*, have been documented (Panizzi et al. 2000, Chiruvella et al. 2007, Akroum et al. 2009). At the same time, the antibacterial activity of hydroethanolic extract 5 and its *n*-hexane (5.1) and diethyl ether (5.2) fractions could be explained by the presence of flavonoid aglycones kaemferol and quercetin, presumably concentrated in these fractions (Nedialkov 1994). These compounds showed antibacterial activity against *S. aureus* (Hirai et al. 2010, Teffo et al. 2010). In addition, the displayed antibacterial effects could also be attributed to tannins – well-known as nature-derived antiseptics (Reddy et al. 2007, Yang and Liu 2014). Ellagitannins and flavonoids have been shown to possess synergistic antibacterial activity (Reed et al. 2005).

The active fractions deserve further attention in the context of the bioactivity-guided isolation of compounds with antibacterial activity. Such compounds might be sought amongst the major constituents of aerial parts of *Potentilla reptans*, namely quercetin, rutin, quercetin-3-O-glucoside, kaempferol-3-O-glucoside, apigenin-7-O-glucoside, caffeic and chinic acids (Tomczyk and Latté 2009 Tomovic et al. 2015, Coppo and Marchese 2014).

Conclusions

This study demonstrates that aerial parts of *Potentilla reptans* exert antibacterial activity and, hence, validates the rational basis for its traditional use. Meanwhile, the studied extracts and their fractions exerted mostly bacteriostatic effects, having the MBC/ MIC ratio generally above 4 (Mims et al. 1993). This moderate antimicrobial activity does not completely explain the reported beneficial effect of *P. reptans* against mastitis, which implies that other biological activities might additionally contribute to it. One possibility is an oxytocin-like action considering the mechanism of mastitis. The stretching of alveoles from retained milk leads to reduction of its production by the alveolar epithelium. In addition to reduced milk production, galactostasis (milk retention in milk ducts and alveoles) is a prerequisite for the development of lactation mastitis (Yu et al. 2018). Oxytocin plays an important role in the process of lactation and opening the milk ducts (Konar 2014) Our preliminary tests demonstrated agonistic activity of *P. reptans* extracts to the oxytocin and vasopressin receptors (Mincheva et al. unpubl. results). Moreover, the bioactivity-guided study for isolation of the active principles points to further analysis of the ethyl acetate fractions of the aqueous extracts and to the *n*-hexane and diethyl ether fractions of the hydroethanolic extract, which might lead to some of the compounds in the aerial parts of *P. reptans,* as well as to the discovery of novel potent compounds with no toxicity in this, so far, poorly studied plant (Mincheva et al. 1993). Further investigations are necessary to reveal the precise mode of action of *P. reptans* against mastitis.

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