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In Vitro Efficacy of *Crataegus oxycantha* L. (Hawthorn) and Its Major Components against ATCC and Clinical Strains of *Ureaplasma urealyticum*

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Abstract

Crataegus oxycantha L., commonly known as hawthorn, has traditionally been used for its beneficial effect on cardiovascular health, which is related to its flavonoid content. The aim of the present study was to evaluate the antibacterial properties of a fluid extract and a hydro-ethanolic macerate from buds of *Crataegus oxycantha* against clinical isolates of *Ureaplasma urealyticum*. The major purified flavonoids present in the extracts were also tested against ATCC strains and clinical isolates. Both the fluid extract and the hydro-ethanolic macerate were active against thirty-due clinical strains of *U. urealyticum*, with MIC ranges between 15.6 and 250 μg/ml and 15.6 and 62.5 μg/ml, respectively. All pure organic compounds, with the exception of rutin, showed activity against the strains tested, luteolin 3,7-diglucoside being the most active compound (MICs in the range of 0.48 and 1.95 μg/ml), followed by apigenin-7-*O*-glucoside (MICs in the range of 0.48 and 3.9 μg/ml). The activity of the pure flavonoids was greater against the clinical isolates compared to the ATCC strains. The data presented here demonstrate that flavonoids present in *Crataegus oxycantha* are effective against clinical isolates of *U. urealyticum* and could be used in combination with antibiotics in order to combat resistance.

Keywords

Crataegus oxycantha, Ureaplasma urealyticum, Flavonoids, Antimicrobial

1. Introduction

The genus *Ureaplasma*, considered part of the normal genital flora, is implicated in

several conditions like non-gonococcal urethritis, prostatitis, urinary and gynecological diseases, infertility, and lung disease in newborns. It belongs to the family *Mycoplasmataceae,* class *Mollicutes* order *Mycoplasmatales*. Several classes of antibiotics have proven not to be effective against *Ureaplasma* species, and a recent study has reported low susceptibilities to tetracycline [1]. Therefore, discovering novel bioactive compounds from plant products could help eradicate a number of infectious diseases. The growing concern over antibiotic resistance worldwide has posed an urgent need to develop novel antibacterial compounds. There is an increased effort by food industries in finding natural compounds with potential antimicrobial properties [2] [3].

Natural products have traditionally been regarded as a major source of important therapeutic agents, with their potential use against infectious diseases in folk medicine. Mixtures obtained by pure phytochemicals as well as plant extracts have successfully been studied for their antimicrobial properties [4] [5].

We have previously reported the antibacterial activity of polyphenols-rich natural products, including almonds [6] [7], pistachios [8], *Citrus* plants [9], *Vitis vinifera* L. [10], *Olea europaea* L. [11], *Citrus bergamia* essential oil [12], and juice [13]. In particular, the identification of novel compounds with bactericidal rather than bacteriostatic effect has attracted a lot of interests in recent years.

Crataegus oxycantha L., a tree belonging to the *Rosaceae* family, is commonly known as hawthorn, English hawthorn, May bush and whitehorn. It has traditionally been used for its positive effect on cardiovascular health, attributed to its flavonoid content, particularly high in oligomeric proanthocyanidins [14]. Animal studies have also indicated a protective effect of *Crataegus* extracts on plasma lipids, including total cholesterol, triglycerides and cholesterol fractions [15].

The flavonoid content in flowers, leafs and bud extracts from *Crataegus oxycantha* L. has been previously identified: the major identified compounds were luteolin, luteolin-3,7-diglucoside, apigenin, apigenin-7-O-glucoside and rutin [16]. The biological activity against arrhythmias exerted by this plant was due to the synergistic interaction amongst the several compounds with different chemical structures [16]. The study on the antibacterial activity of methanol and chloroform extracts from *Crataegus oxycantha* showed a bactericidal effect on pathogenic staphylococci when using flowers, with lower activity against *Bacillus* and *Pseudomonas* [17].

The aim of the present study was to evaluate the *in vitro* antimicrobial properties of a fluid extract and a hydro-ethanolic macerate from *Crataegus oxycantha* against clinical strains of *Ureaplasma urealyticum*, known to play an important role in vaginal infections and sexually transmitted diseases. In addition, the antimicrobial effect of the major flavonoid compounds present in the extracts was investigated against ATCC strains and clinical isolates.

2. Materials and Methods

2.1. *Crataegus oxycantha* Extracts

Two different preparations from buds of *Crataegus oxycantha* L. were used in the

study: 1 fluid extract and 1 hydro-ethanolic macerate. The extraction procedure has been previously reported [16]. Briefly, 1 ml of fluid extract (1 ml extract = 1 g of dried plant material) or 20 ml of the hydro-ethanolic macerate were extracted twice with ethyl acetate (20 ml). After evaporation of the solvent, residues were dissolved in de-ionised water (15 ml) and extracted eight times with 15 ml of ethyl acetate/acetone (2:1). The organic phases were combined and evaporated, whereas the residues were dissolved in methanol (5 ml) and analysed by HPLC. The composition of the extracts has been previously reported [16]. The major identified compounds were the following: luteolin (1.6 and 0.8 mg/ml), rutin (22.8 and 2.7 mg/ml), luteolin-3,7-diglucoside (1.8 and 0.1 mg/ml), apigenin-7-O-glucoside (0.3 and 0.2 mg/ml) in the fluid extract and the hydro-ethanolic macerate, respectively [16].

2.2. Microbial Strains and Culture Conditions

Three American Type Culture Collection strains (*Ureaplasma urealyticum* serotype I ATCC 27813, *Ureaplasma urealyticum* serotype II ATCC 27814 and *Ureaplasma urealyticum* serotype VII ATCC 27819) were used in the study. Thirty-due clinical strains of *U. urealyticum* were isolated from cervix, urethra, vagina and placenta during caesarean section at the Institute of Obstetric and Gynaecological pathology of the University of Catania [18]. The strains are kept within the *in-house* collection at the University of Messina, Microbiology sector (Messina, Italy). Cultures for antimicrobial activity tests were grown in 10-B Broth (pH 6.0, Sigma, Italy) [19]. Two antibiotics, azithromycin and erythromycin, used in the antimicrobial assays were obtained from Sigma (Milan, Italy).

2.3. Antimicrobial Testing

The minimum inhibitory concentrations (MICs) of the fluid extract and the hydro-ethanolic macerate of *Crataegus oxycantha* were determined against the thirty-due clinical isolates of *U. urealyticum*. The pure flavonoid compounds luteolin, rutin, luteolin 3,7-diglucoside and apigenin-7-*O*-glucoside were tested against the three ATCC strains of *U. urealyticum* and the clinical isolates. The broth microdilution method, according to CLSI [20], was used. The MIC was defined as the lowest concentration of compound which inhibited the color change in the broth at the time when the color of the control tube changed and the pH of the medium increased from pH 6.0 to pH 6.5 [21]. The MICs were read after an overnight incubation. All experiments were performed in triplicate. A positive control using antibiotics (azithromycin and erythromycin) and a negative control were included in each assay.

3. Results

The results of the *in vitro* susceptibility tests of the fluid extract and the hydro-ethanolic macerate of *Crataegus oxycantha* are presented in **Table 1**. The two extracts tested were active against *U. urealyticum* strains, the hydro-ethanolic macerate showing the lowest MIC range (15.6 - 62.5 μg/ml). As expected, azithromycin and erythromycin showed

comparable activities against the tested strains. These results showed that flavonoids present in *Crataegus oxycantha* were active against clinical isolates of *U. urealyticum*.

Table 2 reports the MICs of the four most abundant *Crataegus oxycantha* flavonoids against the *U. urealyticum* ATCC strains. All pure organic compounds, with the exception of rutin, showed activity against the strains tested, apigenin-7-*O*-glucoside being the most active compound (MICs in the range of 3.9 and 15.6 μg/ml), followed by luteolin 3,7-diglucoside (MICs in the range of 7.8 and 15.6 μg/ml). Table 3 reports the MICs of the pure isolated compounds against the thirty-due clinical strains of *U. urealyticum*. All compounds showed greater activity against clinical isolates compared to ATCC strains.

4. Discussion

The results of the present study demonstrated that flavonoids present in *Crataegus*

Table 1. MICs (μg/ml) of *Crataegus oxycantha* extracts against *U. urealyticum* clinical strains (32).

Compound	No. of occurrences at the indicated MIC											Range MIC
	0.48	0.97	1.95	3.9	7.8	15.6	31.25	62.5	125	250	500	
Azithromycin	12	19	1	0	0	0	0	0	0	0	0	0.48 - 1.95
Erythromycin	1	18	12	11	0	0	0	0	0	0	0	0.48 - 3.9
Fluid extract	0	0	0	0	0	2	1	22	1	9	0	15.6 - 250
Hydro-ethanolic macerate	0	0	0	0	5	16	10	6	0	0	0	15.6 - 62.5

Table 2. MICs (μg/ml) of pure flavonoids against *U. urealyticum* ATCC strains (3).

Compound	No. of occurrences at the indicated MIC											Range MIC
	0.48	0.97	1.95	3.9	7.8	15.6	31.25	62.5	125	250	500	
Azithromycin	2	1	0	0	0	0	0	0	0	0		0.48 - 0.97
Erythromycin	1	2	0	0	0	0	0	0	0	0	0	0.48 - 0.97
Luteolin 3, 7'-diglucoside	0	0	0	0	0	1	2	0	0	0	0	7.8 - 15.6
Apigenin 7-O-Glucoside	0	0	0	0	1	1	1	0	0	0	0	3.9 - 15.6
Luteolin	0	0	0	0	0	0	0	0	3	0	0	125
Rutin	0	0	0	0	0	0	0	0	0	0	0	>500

Table 3. MICs (μg/ml) of pure flavonoids against *U. urealyticum* clinical strains (32).

Compound	No. of occurrences at the indicated MIC											Range MIC
	0.48	0.97	1.95	3.9	7.8	15.6	31.25	62.5	125	250	500	
Luteolin 3, 7'-diglucoside	0	0	1	0	9	20	2	0	0	0	0	0.48 - 1.95
Apigenin 7-O-glucoside	0	0	0	4	6	3	15	4	0	0	0	0.48 - 3.9
Luteolin	0	0	0	0	0	0	0	2	2	26	2	15.6 - 250
Rutin	0	0	0	0	0	0	0	0	0	0	1	15.6 - 62.5

oxycantha are active against ATCC strains and clinical isolates of *U. urealyticum*. Therefore, those plant extracts could represent a potential source of natural antimicrobials, providing useful information for the development of novel antibiotics. Purified flavonoids from *Crataegus oxycantha* could also be used as natural antibacterial agents to combat infections caused by *Ureaplasma* strains. Together with genital tract infections and infertility, *Ureaplasma* genus is associated with adverse pregnancy outcomes and diseases in the newborn, as well as an increased frequency in HIV-infected patients.

Benmalek *et al.* [22] have quantified the flavonoids present in *Crataegus oxycantha* spp. *monogyna* leaves and berries and demonstrated an antimicrobial potential of these extracts against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Medicinal plants known to contain a range of phenolic compounds and flavonoids could be used to treat diseases originated by multidrug resistant bacteria. The activity of the plant extract may result from the synergistic interaction of the bioactives present. In this study we have shown that both the fluid extract and the hydro-ethanolic macerate from *Crataegus oxycantha*, as well as the major purified compounds, were active against the tested strains. Su *et al.* [23] have recently demonstrated the antibacterial activities of six polyphenols, including luteolin and quercetin, against clinical isolates of methicillin-resistant *S. aureus* (MRSA): synergistic and additive effects were observed when the combination of the two compounds was tested, without evidence of antagonistic effect. We have previously demonstrated that flavonoids from almond skins were active against food-borne pathogens and the interactions between compounds can alter the antimicrobial effectiveness [6]. We observed a synergism between naringenin and protocatechuic acid against *S. aureus* and *Salmonella enterica*, between naringenin and epicatechin against *S. enterica* and *S. aureus* and between protocatechuic acid and epicatechin against *S. aureus*. In this study we have shown that the flavonoid glycosides, including luteolin 3,7-diglucoside and apigenin-7-*O*-glucoside were the most active isolated compounds against *U. urealyticum* strains. However, when combined with the aglycones in the extract, their activity was reduced.

The activity of purified flavonoids could result in different susceptibility against species with various origins and background [24]. The low MIC ranges obtained with luteolin 3,7-diglucoside and apigenin-7-*O*-glucoside, could be used to develop therapeutic combinations between the purified flavonoid and macrolides, tetracyclines and fluoroquinolones, for which resistance has been reported. Recent results suggested that fruit flavonoids in their aglycone forms or as 3-O-glycosides were able to modulate norfloxacin-resistance in *S. aureus* strains over-expressing the *norA* gene [25]. A range of phenolic compounds identified in olive leaves, including luteolin7-O-glucoside, rutin, apigenin 7-O-glucoside and luteolin 4-O-glucoside, showed antibacterial and antifungal action [26]. The aglycone apigenin was active against five pathogens, including *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Proteus mirabilis*, the latter showing the maximum diameter of inhibition zone [27]. Luteolin and quercetin have been recently proposed in combination with ceftazidime for the treatment of infections caused by *Streptococcus pyogenes*: the

mechanisms of action of the synergistic effect observed related to an inhibition of peptidoglycan synthesis, increased cytoplasmic membrane permeability and decreased nucleic acid when the protein content of the bacterial cells was enhanced [28].

In the present study erythromycin was inhibitory to *U. urealyticum* clinical isolates, with MIC ranges between 0.48 and 1.95 μg/ml. However, in a previous investigation, the *U. urealyticum* isolates were inhibited by erythromycin at MICs ranging from 0.06 - ≥256 μg/ml and 16% of the tested strains were highly resistant to this antibiotic, with MIC values ≥256 μg/ml [21]. This trend demonstrated the isolates used in the present study had not developed antibiotic resistance to macrolides.

5. Conclusion

In summary the results of the present study showed that flavonoids from *Crataegus oxycantha* are effective against clinical isolates of *U. urealyticum*. However, further studies need to be performed to understand the precise mechanisms responsible for these interactions.

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