

Antimicrobial Activity of *Gonocytisus angulatus* (L.) Spach

Ufuk ABBASOĞLU*, Fatma TOSUN**, Ash AYDINLIOĞLU**

Summary: *In vitro* antimicrobial activity of ethanolic extracts and crude alkaloid mixtures obtained from roots, twigs, leaves, flowers and seeds of *Gonocytisus angulatus* (L.) Spach (Fabaceae) growing in Turkey was investigated by microdilution method. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* ATCC 19433 and *Staphylococcus aureus* ATCC 25923 were used as bacteria and *Candida albicans*, *Candida parapsilosis*, *Candida pseudotropicalis* and *Candida stellatoidea* as yeastlike fungi. It is observed that ethanolic extracts and crude alkaloid mixtures tested showed activity against all test bacteria and yeastlike fungi.

Keywords : *Gonocytisus angulatus*, Fabaceae, Antimicrobial activity

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Introduction

Gonocytisus angulatus (L.) Spach (Fabaceae) is an erect, slender shrub growing in East Mediterranean region¹. There is no report in the literature on *G. angulatus*. Our pharmacognosic researches are carried on *G. angulatus* growing in Turkey.

In this study, *in vitro* antimicrobial activity of ethanolic extracts and crude alkaloid mixtures obtained from different parts of *G. angulatus* was screened by the microdilution method^{2,3}.

Experimental

Plant Material

Gonocytisus angulatus used in this study was collected from Bornova (Izmir-Turkey). Herbarium

Gonocytisus angulatus (L.) bitkisinin Antimikrobiyal Aktivitesi

Özet: Türkiye'de yetişen *Gonocytisus angulatus* (L.) Spach (Fabaceae) bitkisinin kök, dal uçları, yaprak, çiçek ve tohumlarından elde edilen etanolü ekstraktların ve ham alkaloid karışımlarının *in vitro* antimikrobiyal aktivitesi mikrodilüsyon yöntemiyle araştırılmıştır. Bakteri olarak *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* ATCC 19433 ve *Staphylococcus aureus* ATCC 25923 maya benzeri fungus olarak *Candida albicans*, *Candida parapsilosis*, *Candida pseudotropicalis* ve *Candida stellatoidea* kullanılmıştır. Etanolü ekstrakt ve ham alkaloid karışımları, kullanılan tüm bakteri ve maya benzeri funguslara karşı aktivite göstermiştir.

Anahtar kelimeler : *Gonocytisus angulatus*, Fabaceae, Antimikrobiyal aktivite.

specimens are preserved in Ankara Üniversitesi Eczacılık Fakültesi Herbariumu (AEF No. 18473), Ankara, Turkey.

Media

The liquid growth medium used for bacteria was Müeller-Hinton Broth (Oxoid), and for yeastlike fungi was Sabouraud Liquid Medium (Oxoid).

Test Microorganisms

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* ATCC 19433 and *Staphylococcus aureus* ATCC 25923 were used as bacteria and *Candida albicans*, *Candida parapsilosis*, *Candida pseudotropicalis* and *Candida stellatoidea* as yeastlike fungi.

* Department of Microbiology, Faculty of Pharmacy, Gazi University, 06330 Hipodrom-Ankara, Türkiye.

** Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Hipodrom-Ankara, Türkiye.

All these microorganisms were obtained from Department of Microbiology, Faculty of Medicine, Hacettepe University.

Inoculation suspension

The microorganism suspensions used for inoculation were prepared at 10^6 CFU/mL concentration by diluting the fresh cultures at McFarland 0.5 density (10^8 CFU). It was known that there were 5×10^4 CFU/mL microorganism in each well after inoculation.

Equipment

Falcon^R microplates, which have 96 wells, were used for microdilution method. Brinkmann transferpette^R was used for two-fold dilution of the extracts in the wells.

Sample Preparation

Dried and powdered plant material (10 g) was extracted with ethanol in a Soxhlett apparatus. After concentration of extract under vacuum to 100 mL, ethanolic extract was obtained (from roots E_1 , twigs E_2 , leaves E_3 , flowers E_4 , seeds E_5).

Dried and powdered plant material (10 g) was extracted with methanol in a Soxhlett apparatus and the solvent was removed. The crude residue was taken up in 5 % aqueous H_2SO_4 and filtered. The aqueous acidic filtrate was extracted with chloroform to remove the non-basic substances and chloroform layers were discarded. The mother liquor was made basic with 35 % NaOH and extracted again with chloroform. The chloroform extract was dried with anhydrous Na_2SO_4 and evaporated under vacuum to give a crude alkaloidal mixture (from roots A_1 , twigs A_2 , leaves A_3 , flowers A_4 , seeds A_5). The crude alkaloidal mixture was dissolved in 100 mL ethanol.

Antimicrobial Activity Test

The screening of the antimicrobial activity of the samples was done using microdilution method. 0.1

mL of Mueller-Hinton Broth and Sabouraud Liquid Medium was placed into each well of microplates. 0.1 mL of 10 % extracts were added into the first row of microplates. The two-fold dilutions of the extracts (5%, 2.5%, 0.04%) were made by dispensing to the remaining wells. 0.1 ml of suspension at 10^6 CFU/mL microorganisms concentration was inoculated into the whole wells.

Ethanol-microorganism mixture, microorganism suspension and pure media were used as control wells.

The sealed microplates were incubated at $36^\circ C$ for 24-48 hr in a humid chamber. After this period of time, the turbidity in the wells were identified macroscopically by a concave mirror. The concentration of the extracts in the wells where no growth was seen, were assessed as the minimum inhibitory dilution (MID) of them.

Results and Discussion

There was no inhibitory activity in the wells containing ethanol. The microbial growth occurred and the media were not contaminated during tests. The MID values of extracts are given in Table 1. It is shown that the ethanolic extracts and crude alkaloid mixtures are more effective against yeastlike fungi than bacteria. Ethanolic extracts obtained from roots, twigs and seeds showed more antibacterial activity than crude alkaloid mixtures. But it is shown that, ethanolic extracts obtained from leaves and flowers, have equal antibacterial activity to that of crude alkaloid mixtures. Crude alkaloid mixtures obtained from roots, twigs and seeds showed more activity against Gram (+) bacteria than Gram (-) bacteria. Crude alkaloid mixtures obtained from leaves and flowers showed more activity against Gram (-) bacteria than the other crude alkaloid mixtures. Antifungal activity of crude alkaloid mixtures obtained from roots, twigs, leaves, flowers and seeds are similar to that of ethanolic extracts. The compounds responsible for the activity will be identified after our phytochemical studies are completed and the pure compounds are isolated.

Table 1. MID values of extracts (%)

Test Samples	Microorganisms							
	A	B	C	D	E	F	G	H
E ₁	0.16	0.16	0.16	0.16	0.04	0.04	0.04	0.04
E ₂	0.31	0.31	0.31	0.31	0.08	0.08	0.08	0.08
E ₃	0.31	0.31	0.31	0.31	0.04	0.04	0.04	0.04
E ₄	0.31	0.31	0.31	0.31	0.04	0.04	0.04	0.04
E ₅	0.16	0.16	0.16	0.16	0.04	0.04	0.04	0.04
A ₁	0.63	0.63	0.31	0.31	0.08	0.08	0.08	0.08
A ₂	0.63	0.63	0.31	0.08	0.08	0.08	0.08	0.08
A ₃	0.31	0.31	0.31	0.31	0.04	0.04	0.04	0.04
A ₄	0.31	0.31	0.31	0.31	0.04	0.04	0.04	0.04
A ₅	0.63	0.63	0.31	0.31	0.04	0.04	0.04	0.04

Microorganisms:

- A : *E. coli*
- B : *P. aeruginosa*
- C : *S. faecalis*
- D : *S. aureus*
- E : *C. albicans*
- F : *C. parapsilosis*
- G : *C. pseudotropicalis*
- H : *C. stellatoiden*

Ethanollic extracts:

- E₁ : roots
- E₂ : twigs
- E₃ : leaves
- E₄ : flowers
- E₅ : seeds

Crude alkaloid mixtures:

- A₁ : roots
- A₂ : twigs
- A₃ : leaves
- A₄ : flowers
- A₅ : seeds

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