Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

Antimicrobial Activity and Phytochemical Studies on Turkish Samples of *Papaver macrostomum*.

Ç. Ünsal, G. Sarıyar, B. Gürbüz Akarsu & A. Çevikbaş

To cite this article: Ç. Ünsal, G. Sarıyar, B. Gürbüz Akarsu & A. Çevikbaş (2007) Antimicrobial Activity and Phytochemical Studies on Turkish Samples of *Papaver macrostomum*., Pharmaceutical Biology, 45:8, 626-630, DOI: 10.1080/13880200701538948

To link to this article: https://doi.org/10.1080/13880200701538948

Published online: 07 Oct 2008.

Pharmaceutical Biology
2007, Vol. 45, No. 8, pp. 626–630

informa
healthcare

Antimicrobial Activity and Phytochemical Studies on Turkish Samples of *Papaver macrostomum*

Ç. Ünsal[1], G. Sarıyar[1], B. Gürbüz Akarsu[2], and A. Çevikbaş[2]

[1]Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkey; [2]Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Istanbul, Turkey

Abstract

Three alkaloids, cheilantifoline, mecambrine, and laudanosine, and two flavonoids, luteoline and tricine, have been isolated from two samples of *Papaver macrostomum* Boiss. & Huet ex Boiss. (Papaveraceae) of Turkish origin. Antimicrobial tests have been performed on the extracts obtained from these species. It has been found that diethyl ether and acetone extracts of two samples obtained from the aerial parts of the plant have antimicrobial activity against almost all bacteria tested. The existence of flavonoids and the antimicrobial activity of this species are reported for the first time.

Keywords: Alkaloids, antimicrobial activity, cheilantifoline, flavonoids, laudanosine, luteoline, mecambrine, *Papaver macrostomum*, tricine.

Introduction

In the *Flora of Turkey*, annual *Papaver* L. (Papaveraceae) species are grouped into four sections; Mecones Bernh., Carinatae Fedde, *Papaver* L., and Argemonorhoeades Fedde (Cullen, 1965; Güner et al., 2001). Of these sections, Carinatae is represented by only one species, *Papaver macrostomum* Boiss. & Huet ex Boiss, which is widely distributed in Turkey. This species is used as an antitussive and sedative together with *Papaver rhoeas* L. in the folk medicine of Turkey (Baytop, 1984). Previous investigations on the alkaloids of this species revealed the existence of the benzylisoquinoline (macrostomine, dehydromacrostomine, sevanine), aporphine (isocorydine), protopine (protopine), and rhoeadine (rhoeadine, papaverrubine A, B, D, E), types (Preininger, 1986). Recently, we reported the isolation of isopavine (amurensine, amurensinine) and protoberberine (cheilantifoline) types from *Papaver macrostomum* collected from Northwestern Turkey (Sarıyar, 2002).

In this work, we aimed to determine the alkaloid and flavonoid contents of two samples of *P. macrostomum* of Turkish origin collected from two different regions of eastern Turkey (M1 and M2) to determine the existence of chemotypes and to investigate the antimicrobial activities of the extracts prepared from the aerial parts of the plant using different solvents.

Materials and Methods

General

UV spectra were taken with a Jasco 530V spectrophotometer (UK). ^{1}H NMR spectra were measured on a Varian Unity Inova (500 MHz) instrument (USA) at ITL, University of Istanbul. ^{13}C NMR and Heteronuclear Multiple Quantam Correlation (HMQC) spectra were taken with a Varian Mercury-VX (400 MHz) spectrometer at Boğaziçi University. Mass spectra were obtained on a JEOL GC Mate II instrument (USA) at Research Resources Center, University of Illinois. IR spectra were run on a Perkin-Elmer 1600 Series FTIR instrument (USA).

Plant material

The aerial parts of *P. macrostomum* were collected from the eastern part of Turkey in Malatya in June 2001 (M1) and in Van in June 2002 (M2). Voucher specimens are deposited in the herbarium of the Faculty of Pharmacy,

Accepted: May 4, 2007.

Address correspondence to: Dr. Çağlayan Ünsal, Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, 34116 Beyazıt, Istanbul, Turkey. E-mail: caglayanu@gmail.com

DOI: 10.1080/13880200701538948

Istanbul University (M1 ISTE 79426 and M2 ISTE 81463). The plant identification was verified by Prof. G. Sarıyar.

Extraction and isolation

Extraction and isolation of alkaloids

The dried and powdered material (M1, 6 kg; M2, 8.796 kg) was percolated with ethanol at room temperature. The ethanol extract was concentrated under vacuum, and the residue was taken up in 5% hydrochloric acid. The acid extract was first washed with light petroleum and then with diethyl ether. The aqueous layer was made alkaline with NH_4OH to pH 7–8 and extracted successively with $CHCl_3$. The combined $CHCl_3$ extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness under vacuum to yield the tertiary alkaloid extracts of M1 (2.9392 g) and M2 (6.5328 g).

The tertiary alkaloid extracts were each separated on a column of silica gel eluting with $CHCl_3$, $CHCl_3$:MeOH (95:5, 90:10, 80:20). Fractions were evaporated and purified by preparative thin-layer chromatography on silica gel to afford the pure alkaloids.

The M1 column fractions 67–73 eluted with $CHCl_3$:MeOH (90:10) were purified by an aluminum oxide column and gave mecambrine (11 mg). The M2 column fractions 5–9 eluted with $CHCl_3$ and fractions 26–30 eluted with $CHCl_3$:MeOH (95:5) were separated by preparative TLC on silica gel with toluene: Me_2CO: MeOH:NH_4OH (45:45:7:3) and yielded cheilanthifoline (90 mg) and laudanosine (9 mg), respectively.

Extraction and isolation of flavonoids

The dried aerial parts of *P. macrostomum* (300 g) were extracted with petroleum ether in a Soxhlet apparatus. The petroleum ether extract (A) was concentrated and extracted with 60% EtOH, which was treated with $CHCl_3$ (B). The petroleum ether exhausted material was extracted with EtOH. The extract was concentrated, diluted with H_2O, and extracted with toluene (C) and $CHCl_3$ (D). Extract (D) was investigated for flavonoids.

Extract (D) of M1 and M2 were applied to a polyamide column (H_2O and increasing concentrations of MeOH as eluent). From M1 column, fractions 63–69 eluted with H_2O:MeOH (20:80) and, from M2 column, fractions 100–106 eluted with H_2O:MeOH (30:70), tricine (3 mg), and luteoline (4 mg) were obtained after a Sephadex LH20 purification with MeOH.

Antimicrobial activity tests

The antimicrobial activity tests were performed on the extracts obtained from the aerial parts of *P. macrostomum* (25 g) using solvents petroleum ether, diethyl ether, chloroform, acetone, and ethanol in a Soxhlet apparatus. The antibacterial and antifungal tests were carried out using the agar diffusion method followed by the dilution method for extracts that presented a bioactivity.

Extracts were tested against six strains of bacteria; *Staphylococcus aureus* (ATCC 6538), *S. epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 1539), *Proteus mirabilis* (ATCC 14153) and *Klebsiella pneumoniae* (ATCC 4352). Muelleor-Hinton agar (MHA) and Mueller-Hinton broth (MHB) were used for the bacteria.

Extracts were tested against six fungal strains; *Candida albicans* (ATCC 10231), *C. glabrata* (ATCC 90030), *C. guilliermondii* KUEN 998, *C. tropicalis* KUEN 1021, *C. pseudotropicalis (kefyr.)* KUEN 1012, and *C. krusei* (ATCC 6258). Sabouraud dextrose broth (SDB) and Sabouraud dextrose agar (SDA) were used for yeast. Minimum inhibitory concentration (MIC) was determined by the dilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (1997, 1999) (May et al., 1997). Results are shown in Tables 1 and 2.

Microorganisms were obtained from the Department of Microbiology, Faculty of Pharmacy, Marmara University, Istanbul.

Meropenem (512 μg/mL) served as the positive control for the tested bacteria, whereas fluconazole (5120 μg/mL) served as the positive control yeast. Petroleum ether, diethyl ether, chloroform, acetone, and ethanol were tested as solvent controls.

Results and Discussion

The structures of known alkaloids and flavonoids were elucidated through spectrocopic analysis and TLC by direct comparison with authentic samples. Mecambrine was isolated from the sample (M1) collected at Malatya, whereas a sample collected at Van (M2) yielded cheilantifoline and laudanosine. The presence of mecambrine and laudanosine has been shown in *P. macrostomum* for the first time. However, the amount of alkaloids in two samples has been found in very low quantity. The two samples also differ in their flavonoid contents, yielding tricine (M1) and luteoline (M2), which is the first report on the presence of these flavonoids in a *Papaver* species. This study revealed that these two samples of *P. macrostomum* were two new chemotypes having proaporphine and benzylisoquinoline-protoberberine types of alkaloids when compared with the findings of previous work that reported the existence of three chemotypes containing benzylisoquinoline, rhoeadine-protopine, and isopavine-protoberberine-aporphine types.

Although they differ chemically, the diethyl ether and acetone extracts prepared from the aerial parts of the two samples have similarities in their antimicrobial activity. It has been found that diethyl ether and acetone extracts

Table 1. Antimicrobial activity of first sample of *P. macrostomum*, which was collected from Malatya.

	Petroleum ether extract		Diethyl ether extract		Chloroform extract		Acetone extract		Ethanol extract		Meropenem	
	Zone diameter (mm)	MIC (μg/mL)	Zone diameter (mm)	MIC (μg/mL)	Zone diameter (mm)	MIC (μg/mL)	Zone diameter (mm)	MIC (μg/mL)	Zone diameter (mm)	MIC (μg/mL)	Zone diameter (mm)	MIC (μg/mL)
Bacteria												
Staphylococcus aureus ATCC 6538	14	3164.1	12	8055.56	3	6468.75	8	8666.67	1	4062.5	27	<0.125
Staphylococcus epidermidis ATCC 12228	32	6328.13	26	4027.78	–	–	5	4333.3	28	4062.5	31	0.25
Escherichia coli ATCC 11229	–		1	2013.89	–		7	4333.3	–		24	0.25
Pseudomonas aeruginosa ATCC 1539	–		9	8055.56	–		2	8666.67	3	4062.5	24	0.25
Proteus mirabilis ATCC 14153	–		16	12.65	5	25875	1	>1733.3	–		24	0.125
Klebsiella pneumoniae ATCC 4352	–		6	8055.56	–		1	8666.67	–		24	0.125
Yeasts											Fluconazol	
Candida albicans ATCC 10231	3	6328.13	–		–		6	4333.3	4	2031.25	34	<0.3125
Candida glabrata ATCC 90030	–		5	8055.56	–		–		–		29	<0.3125
Candida guilliermondii KUEN 998	–		–		–		–		6	4062.5	14	<0.3125
Candida tropicalis KUEN 1021	–		4	4027.78	–		2	8666.67	–		24	<0.3125
Candida pseudotropicalis (C. kefyr) KUEN 1012	–		–		–		–		5	8125	24	<0.3125
Candida krusei ATCC 6258	4	1582.03	1	4027.78	–		2	8666.67	–		6	<0.3125

MIC, minimal inhibitory concentration; –, no inhibition.

Table 2. Antimicrobial activity of second sample of *P. macrostomum*, which was collected from Van.

	Petroleum ether extract		Diethyl ether extract		Chloroform extract		Acetone extract		Ethanol extract		Meropenem	
	Zone diameter (mm)	MIC (μg/mL)	Zone diameter (mm)	MIC (μg/mL)	Zone diameter (mm)	MIC (μg/mL)	Zone diameter (mm)	MIC (μg/mL)	Zone diameter (mm)	MIC (μg/mL)	Zone diameter (mm)	MIC (μg/mL)
Bacteria												
Staphylococcus aureus ATCC 6538	4	32125	5	3180.56	5	26750	6	2500	1	6975	27	<0.125
Staphylococcus epidermidis ATCC 12228	30	64.25	26	3180.56	–		36	5000	25	6975	31	0.25
Escherichia coli ATCC 11229	–		–		–		2	5000	–		24	0.25
Pseudomonas aeruginosa ATCC 1539	–		8	1590.28	–			5000	3	3487.5	24	0.25
Proteus mirabilis ATCC 14153	–		3	3180.56	–		–		–		24	0.125
Klebsiella pneumoniae ATCC 4352	–		2	3180.56	–		1	5000	–		24	0.125
Yeasts											Fluconazol	
Candida albicans ATCC 10231	–		–		–		4	5000	4	6975	34	<0.3125
Candida glabrata ATCC 90030	–		–		–		–		–		29	<0.3125
Candida guilliermondii KUEN 998	–		1	1590.28	–		–		–		14	<0.3125
Candida tropicalis KUEN 1021	–		1	1590.28	–		–		–		24	<0.3125
*Candida pseudotropicalis (C. kefyr)*KUEN 1012	–		3	795.14	–		–		3	6975	24	<0.3125
Candida krusei ATCC 6258	–		2	795.14	–		1	5000	–		6	<0.3125

MIC, minimal inhibitory concentration; –, no inhibition.

of *P. macrostomum* have antimicrobial activity against almost all microorganisms tested.

As a result of our investigations on the alkaloids of samples of *P. macrostomum* growing in different regions of Turkey, the presence of three chemical races containing proaporphine, benzylisoquinoline-protoberberine, and isopavine-protoberberine-aporphine types has been found so far.

Acknowledgments

This work was supported by The Scientific Research Projects of Istanbul University (project no. T-1256/01112001). We express our sincere thanks to Prof. Dr. A. Douglas Kinghorn (College of Pharmacy, University of Illinois at Chicago) for mass measurements.

References

Baytop T (1984): *Therapy with Medicinal Plants in Turkey*. Istanbul, University of Istanbul, Publication No. 3295, p. 208.

Cullen J (1965): Papaveraceae. In: Davis PH, ed., *Flora of Turkey and the East Aegean Islands*, Vol. 1. Edinburgh, University Press, pp. 219–236.

Güner A, Özhatay N, Ekim T, Başer KHC (2000): *Flora of Turkey and the East Aegean Islands*, Vol. 11 (Supplement 2). Edinburgh, University Press, pp. 16–29.

May JL, King A, Warren CA (1997): Fluconazole disc diffusion testing for the routine laboratory. *J Antimicrob Chemother 40*: 511–516.

NCCLS (National Committee for Clinical Laboratory Standards) (1997): Performance Standards for Antimicrobial Disk Susceptibility Tests, 6th edition. Approved Standard M2-A6, Wayne, PA, NCCLS.

NCCLS (National Committee for Clinical Laboratory Standards) (1999): Performance standards for antimicrobial susceptibility testing. 9th International Supplement M100-S9, Wayne, PA: NCCLS.

Preininger V (1986): Chemotaxonomy of Papaveraceae and Fumariaceae. In: Brossi A, ed., *The Alkaloids*, Vol. 29. New York, Academic Press, pp. 1–98.

Sarıyar G (2002): Biodiversity in the alkaloids of annual *Papaver* species of Turkish origin. *Acta Pharm Turcica 44*: 159–168.