Asian Journal of Chemistry Vol. 22, No. 8 (2010), 6321-6324

Antifungal Activity of Seeds of *Hyoscyamus niger* L. (Henbane) Against Some Clinically Relevant Fungal Pathogens

BASARAN DULGER*, NURCIHAN HACIOGLU, BEYZA S. GONCU† and FAHRETTIN GUCIN†
Department of Biology, Faculty of Science and Arts,
Canakkale Onsekiz Mart University, 17100 Canakkale, Turkey
E-mail: basarandulger@yahoo.com

The methanolic extracts obtained from the seeds of *Hyoscyamus niger* L. (Solanaceae) used as traditional medicine in Turkey were investigated for their ability to inhibit clinically relevant fungal pathogens as six *Candida* species (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13808, *C. guilliermondii* ATCC 6260, *C. krusei* ATCC 20298, *C. glabrata* ATCC 2001 and *C. parapsilosis* ATCC 22019) and two *Cryptococcus species* (*C. neoformans* ATCC 90112 and *C. laurentii* ATCC 34142) by microbroth dilution method. The plant displayed activity against all fungal cultures tested. The extracts possessed a strong antifungal potency. Greater activity was observed against both *Cryptococcus* species, with MIC values of 15 µg/mL.

Key Words: *Hyoscyamus niger* L., Antifungal activity, Medicinal plants.

INTRODUCTION

Medicinal plants have been known for their healing or disease-curing qualities for centuries. In particular, extracts and oils of these plants have formed the basis of many applications, including raw and processed food preservation, pharmaceutical, alternative medicine and natural therapies.

Hyoscyamus niger L. (Solanaceae), commonly known as Henbane, is widely distributed in Europe and Asia. The plant is said to possess anti-spasmodic, sedative and analgesic properties[1]. The narcotic alkaloids hyoscyamine, scopolamine and atropine are derived from this foul smelling weed. Its name is derived from the Anglo-Saxon Henn (chicken) and Bana (murderer) because when fowls eat the seeds of this plant, they become paralyzed[2-4]. In our field trip, it is determined that the aqueous extracts obtained from the seeds of *Hyoscyamus niger* has been applied for spilling over the larvae from eye, so the name of plant is locally 'shed-helmint'.

A bibliographical survey showed that there were no reports on antimicrobial activity of this plant. So, the aim of this work is to evaluate the antifungal activity of *H. niger* against clinically relevant the yeast cultures such as *Candida* and *Cryptococcus* species as wild-growing in Turkey.

†Department of Biology, Faculty of Science and Arts, Fatih Univesity, Istanbul, Turkey.

EXPERIMENTAL

The plant materials were collected from Kayseri, Turkey in September, 2009. Voucher specimens of the plant were deposited in the Biology Department at Canakkale Onsekiz Mart University.

Preparation of the extracts: The seeds of plant were extracted with aqueous 60 % methanol. Ten gram amounts of the seed materials were extracted in flasks placed in an ultrasonic bath first with 50 mL solvent for 1 h, then with 30 mL solvent for 45 min and finally with 20 mL of more solvent for 15 min, the overall extraction taking 2 h. The 3 extracts were combined, brought to a final volume of 100 mL with aqueous 60 % methanol. The methanol was removed vacuum rotary at 40 ºC until dryness. The resulting dried extract was stored in labeled sterile screw-capped bottles at -20 °C. The extract (in the form of sticky black substances) was dissolved in 0.1 mL of DMSO (5 mg/g) before testing.

Microbial test strains: *Candida* species (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13808, *C. guilliermondii* ATCC 6260, *C. krusei* ATCC 20298, *C. glabrata* ATCC 2001 and *C. parapsilosis* ATCC 22019) and *Cryptococcus* species (*C. neoformans* ATCC 90112 and *C. laurentii* ATCC 34142) were used for an antifungal evaluation. All fungal strains maintained on Sabouraud Dextrose Agar (SDA, Oxoid, Basingstoke, UK) at 4 °C.

Antifungal assays: Synthetic RPMI (Sigma, St. Louis, MO, USA) medium with L-glutamine buffered to pH 7.0 with 0.165 morpholine propane sulfonic acid (MOPS, Sigma) was prepared according to the CLSI M27-A2 Document[5] and used for minimal inhibitory concentration (MIC) determination. Fungal cultures, freshly grown at 35 °C and inoculums suspensions were prepared by the spectrophotometric method with a final inoculums of $1.5 \pm 1.0 \times 10^3$ cfu/mL used for suspectibility testing. Broth microdilution testing was performed in accordance with the guidelines of the CLSI M27-A2 document[5]. Susceptibility was determined by the microbroth dilution method performed in sterile flat-bottom 96-well microplates. Extracts and fractions were dissolved in dimethyl sulfoxide (DMSO, Merck, Darmstadt, Germany) after the addition of RPMI. Serial dilutions were then performed, using RPMI as diluents, maintaining a constant volume of 1000 µL per tube. The extracts were tested at eight concentrations that varied from 1000 to 7.8 µg/mL. From each dilution, 100 µL volumes were distributed in microplates. As a control for growth and sterility, RPMI alone was used without extracts or solvents. Amphotericin B was included at concentrations of 25 to 0.03 µg/mL, as positive antifungal controls. After inoculation of fungal strains, plates were incubated at 35 °C for 48 h for *Candida* species and 72 h for *Cryptococcus* species. All test were performed in triplicate. The endpoints were determined visually by comparison with the drug-free growth control well. MICs were defined as the lowest extract concentration for which the well was optically clear and were expressed in µg/mL.

RESULTS AND DISCUSSION

The MICs values concerning *in vitro* antifungal activities of the extracts are presented in Table-1. Greater activity was observed for the extracts of *H. niger* against both *Cryptococcus* species, with values of 15 μg/mL. The extracts have a strong effect against *Candida albicans* and *C. guilliermondii* as the same MIC values (60 μg/mL), fallowed yeast cultures, *C. tropicalis, C. krusei* and *C. parapsilosis* have susceptible to the extract at a MIC of 12.5 μg/mL. The highest MICs of the extract were 250 μg/mL against *C. glabrata*. In generally, the extracts have weaker antifungal effect than those of the standard antifungal antibiotic Amphothericin B.

TABLE-1
MINIMUM INHIBITORY CONCENTRATION VALUES OF
THE METHANOLIC EXTRACTS OF *H. niger*

Microorganisms	Minimum inhibitory concentration (mg/mL)	
	Plant extract (μg/mL)	Standard (Amphotericin B)
Candida albicans	60	1.0
Candida tropicalis	125	0.25
Candida guilliermondii	60	1.0
Candida krusei	125	0.5
Candida glabrata	250	1.0
Candida parapsilosis	125	0.5
Cryptococcus neoformans	15	1.0
Cryptococcus laurentii	15	2.0

Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than the other solvents[6]. According to present results, methanol extract has stronger and broader spectrum of antimicrobial activity. This information confirmed that the methanol has higher effective solvent for extraction of antimicrobial substances in *H. niger*.

There are no reports on the antimicrobial activity studies on *H. niger*. However, phytochemical analyses of *H. niger* have confirmed the presence of alkaloids[7], tyramine derivative[8], withanolides[8], lignanamides[9] and flavonoids[10]. The result indicated that *H. niger* possessed significant activity against bacteria. This activity may be indicative of the presence of metabolic toxins or the mentioned plant compounds. So, this plant extracts should be analyzed further, as it might provide a new compound effective against pathogens.

Fungi used in this study were chosen primary on the basis of their importance as opportunistic pathogens of humans. Accordance of the findings from National Nosocomial Infection Surveillance System (NNIS), 61 % of reported nosocomial fungal infections were due to *Candida albicans*, followed by other *Candida* spp. and *Cryptococcus* spp.[11]. *Candida albicans*, while naturally occurring in the intestinal flora, can cause oral trush and systematic infections. *Cryptococcus neoformans*

causes cryptococcosis, an opportunistic infection of the lungs especially in AIDS patients.

This study provides data on the antifungal properties of the methanolic extract obtained from *H. niger* against some clinically relevant fungi such as *Candida* and *Cryptococcus* species that could be able to studies for therapeutically useful. These extracts may be applied clinically for fungal infections, especially against candidiasis and cryptococcosis.

REFERENCES

1. B. Sajeli, M. Shai, R. Suessmuth, T. Asai, N. Hara and Y. Fujimoto, *Chem. Pharm. Bull.*, **54**, 538 (2006).
2. A.J. Carter, *J. Royal Soc. Med.*, **96**, 144 (2003).
3. A. Liberman and J.L. Mitchell, An Analytic Dictionary of English Etymology: An Introduction, University of Minnesota Press, pp. 108-111 (2008).
4. R.E. Schulles and E.W. Smith, A Golden Guide to Hallucinogenic Plants, Golden Press, New York, p. 22 (1976).
5. NCCLS, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Aproved Standard M27-A2, National Committee for Clinical Laboratory Standards, Wayne, PA, edn. 2 (2002).
6. C.I. Febles, A. Arias, M.C. Gil-Rodriguez, A. Hardisson and A.S. Lopez, *Anu. Est. Can.*, **34**, 181 (1995).
7. J.A. Duke, Handbook of Medicinal Herbs, CRC Press, Boca Raton FL, pp. 240-243 (1985).
8. C.Y. Ma, I.D. Williams and C.T. Che, *J. Nat. Prod.*, **62**, 1445 (1999).
9. C.Y. Ma, W.K. Liu and C.T. Che, *J. Nat. Prod.*, **65**, 206 (2002).
10. E. Steinegger and D. Sonanini, *Pharmazie*, **15**, 643 (1960).
11. J.W. Warren, in eds.: G.L. Mandell, J.E. Bennett and R. Dolin, Nosocomial Urinary Tract Infections, Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, Philadelphia: Churchill Livingstone, edn. 6, pp. 3370-3381 (2005).

(*Received*: 15 December 2009; *Accepted*: 12 May 2010) AJC-8695