

ABSTRACT

This study was carried out to evaluate the biological activities of *Penicillium* sp. (KUM60280). The methanol and dichloromethane crude extracts were evaluated for their antimicrobial, antioxidant, cytotoxic and anti-human papillomavirus (HPV) activities. The amounts of total phenolic compound in the extracts were also evaluated. The antibacterial capacity was screened against Gram positive bacteria (*Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Streptococcus mutans*, *S. mitis*, *S. sanguis*) as well as Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *P. vulgaris*). Antifungal potentials on the other hand, were evaluated against *Candida albicans*, *C. parasilopsis* and *Saccharomyces pombe* by the disc diffusion method. The results of antimicrobial assay indicated that extracts of *Penicillium* sp. were non-toxic against all the tested microorganisms. Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) assay and the reducing power assay. The subfraction methanol and dichloromethane extracts of *Penicillium* sp. was able to reduce the stable free radical DPPH[•] with an EC₅₀ values of 26.8 mg/ml ± 0.05 µg/ml and 50.5mg/ml ± 0.05 µg/ml, respectively. *Penicillium* sp. crude extracts are powerful radical scavengers based on the inhibition of scavenging component. The reducing powers of methanolic extract and dichloromethane extracts from mycelia were 1.161 and 0.431 mg/ml, respectively at 5 mg/ml. The total phenolics content which was found to be higher in methanol crude extract as compared to the dichloromethane crude extract were 107.87g GAE/100g and 58.43g in GAE/100g, respectively. The methanol and dichloromethane extracts of *Penicillium* sp. were evaluated for their cytotoxic

potentials against the human mouth epidermal carcinoma cell line (KB), human epidermal carcinoma of cervix cell line (CaSki), human colorectal cancer cell line (HT29 and HCT119), human breast cancer cell line (MCF7), human ovarian cancer cell line (SCOV3) and the normal human fibroblast cell (MRC5) using the *in vitro* neutral red cytotoxic assay. Generally, extracts of *Penicillium* sp. produced ED₅₀ values of more than 20 µg/ml and were therefore considered not cytotoxically active. Extracts were analyzed qualitatively at various concentrations for anti-HPV 16 E6 activities in HPV 16-containing cervical cancer-derived cell line (CaSki) using immunocytochemistry technique. Generally, the HPV 16 E6 oncoprotein was not suppressed in CaSki cells by the crude extracts of *Penicillium* sp. Therefore, the extracts could be suitable as antioxidative agents and have a potency to become one of antioxidant drugs.

ABSTRAK

Penyelidikan ini dijalankan untuk mengkaji aktiviti biologi *Penicillioptis* sp. (KUM60280). Ekstrak methanol dan ekstrak dikloromethane disaring untuk melihat aktiviti antimikrobial, antioksidan, sitotoksi dan anti *human* Papillomavirus. Kuantiti jumlah komponen phenolik juga dijalankan. Kapasiti antibakteria disaring menentang bakteria Gram positif (*Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Streptococcus mutans*, *S. mitis*, *S. sanguis*) disamping bakteria Gram negatif *Escherichia coli*, *Pseudomonas aeruginosa* and *P. vulgaris* dan kajian antikulat pula menggunakan *Candida albicans*, *C. parasilopsis* dan *Saccharomyces pombe* dijalankan menggunakan kaedah cakera penyerapan. Keputusan antimikrobial menunjukkan semua mikroorganisma yang terlibat dalam kajian ini resistan terhadap ekstrak-ekstrak *Penicillioptis* sp. Penyaringan antioksidan pula dijalankan menggunakan keupayaan ekstrak menyingkirkan radikal 2,2 diphenyl-1-picrylhydrazyl radicals (DPPH[•]) dan esei penurunan kuasa terhadap ekstrak-ekstrak. Subfraksi ekstrak methanol dan ekstrak dikloromethane *Penicillioptis* sp. berupaya menurunkan kestabilan radikal bebas DPPH[•] dengan EC₅₀ masing-masing 26.8 mg/ml ± 0.05 µg/ml dan 50.5mg/ml ± 0.05 µg/ml. Penurunan kuasa juga menunjukkan kesan yang signifikan terhadap ekstrak *Penicillioptis* sp. (KUM60280). Kajian menunjukkan kesan ekstrak methanol lebih baik berbanding dikloromethane iaitu 107.87g GAE/100g dan 58.43g in GAE/100g masing-masing. Kesan sitotoksik bagi ekstrak *Penicillioptis* sp. menentang *human mouth epidermal carcinoma cell line (KB)*, *human epidermal carcinoma of cervix cell line*

(CaSki), human colorectal cancer cell line (HT29), (HCT119), human breast cancer cell line (MCF7), human ovarian cancer cell line (SCOV3) dan normal sel human fibroblast cell (MRC5) yang dikenal pasti menggunakan *in vitro* esei *neutral red cytotoxicity*. Secara keseluruhannya ekstrak-ekstrak *Penicillioptis* sp. mempunyai nilai ED₅₀ kurang daripada 20ug/ml menunjukkan tiada aktiviti sitotoksik pada *Penicillioptis* sp. Ekstrak-ekstrak *Penicillioptis* sp. (KUM60280) telah dianalisa secara kualitatif pada kepekatan yang berbeza (25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml) untuk aktiviti anti HPV 16 E6 di dalam sel terbitan kanser servik, CaSki menggunakan kaedah immunohistokimia. Kedua-dua ekstrak menunjukkan kesan yang lemah terhadap aktiviti penyekatan ekspresi HPV 16 E6 onkoprotein dalam sel CaSki. Daripada kajian yang dijalankan mencadangkan *Penicillioptis* sp. berpotensi untuk dieksploitasi secara efektif untuk digunakan agen pengoksidaan dan berpotensi dalam industri pharmaseutikal.

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List of abbreviations

BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
cfu	colony forming unit
DCM	Dichloromethane
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
EC ₅₀	Effective concentration to give 50% antioxidant activity
FRAP	Ferric reducing ability of plasma
g	Gram
GAE	Gallic acid equivalents
GYMP	Glucose-Yeast-Maltose-Peptone
H ₂ O ₂	Hydrogen peroxide
IC ₅₀	Concentration that caused 50% inhibition
KUM	Kulat Universiti Malaya
MEA	Malt Extract agar
MHA	Muller-Hinton Agar
mg	Milligram
ml	Milliliter
nm	Nanometer
NR	Neutral Red
ORAC	Oxygen radical absorption capacity
PBS	Phosphate buffer saline
PDA	Potato dextrose Agar
SDA	Sabouraud dextrose agar
ROS	Reactive oxygen species
µg	Microgram
YPDA	Yeast Peptone Dextrose Agar