

ABSTRACT

Six mushroom samples (*Ganoderma lucidum*, *Cordyceps militaris*, *Lignosus rhinocerotis*, *Pleurotus giganteus*, *Pleurotus floridanus* and *Auricularia polytricha*) were screened for their anticoagulant activity. Mushroom samples provided were freeze-dried and blended into powder form. Different concentrations of aqueous mushroom extracts were added to one ml of fresh bovine blood and well mixed. From the observations, among the six mushroom samples, only two samples namely *A. polytricha* and *L. rhinocerotis* showed anticoagulant activities in the preliminary screening.

Crude extracts of *A. polytricha* and *L. rhinocerotis* were tested for their *In-vitro* anti-platelet activity using fresh human blood. Platelet rich plasma 1.3×10^8 was obtained from the fresh human blood and the platelet aggregation activity was measured using spectrophotometer in percentage of transmittance values. Adenosine diphosphate was used to induce platelet aggregation in the study. Five concentrations (5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL and 25 mg/mL) of crude mushroom extracts were tested. Anti-platelet activity was shown in *L. rhinocerotis* crude extract. The crude sample was then dialysed using dialysis tubing 12 kDa in distilled water overnight. The proteins were then precipitated out using acetone and the *in-vitro* anti-platelet activity test was performed again. The protein precipitated also showed anti-platelet aggregation activity in the study.

Then, aqueous two phase system (ATPS) was done using 4 g of polyethylene glycol 50 % (PEG 8000) added with 2.9 g of phosphate 40 %. The anti-platelet aggregation activity was shown in the top phase of the aqueous two phase system (ATPS). The partially purified protein recovered in the top phase of the aqueous two phase system (ATPS) was then analysed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Two bands of approximately 50 kDa and 55 kDa

were observed in the gel. Non-denaturing PAGE (Native PAGE) was carried out and both of the bands were excised and tested. Anti-platelet aggregation activity was shown for both bands excised.

As the crude extract and the partial purified crude enzyme of *L. rhinocerotis* possessed anti-platelet aggregation capacity, further studies on the enzyme/s involved and the mechanism are needed. The enzyme/s can be further purified and sequenced to identify the protein.

ABSTRAK

Aktiviti antikoagulan bagi enam jenis cendawan (*Ganoderma lucidum*, *Cordyceps militaris*, *Lignosus rhinocerotis*, *Pleurotus giganteus*, *Pleurotus floridanus* and *Auricularia polytricha*) telah dikaji dalam kajian ini. Sampel cendawan yang diperuntuk dikeringkan secara sejuk beku dan dikisar sebelum digunakan. Kepekatan ekstrak cendawan yang berlainan ditambah dengan satu ml darah lembu yang segar dan dicampur sampai sehati. Dari pemerhatian, hanya dua sampel iaitu *A. polytricha* dan *L. rhinocerotis* di antara enam sampel cendawan yang dikaji menunjukkan aktiviti antikoagulan.

Kajian untuk menentukan anti-platelet aktiviti bagi *A. polytricha* dan *L. rhinocerotis* ekstrak dilakukan secara In-vitro dengan menggunakan darah segar manusia. Platelet-kaya plasma 1.3×10^8 diperoleh daripada darah manusia dan aktiviti pengagregatan platelet diukur dengan menggunakan spektrofotometer dalam peratusan nilai transmitan. Adenosine diphosphate (ADP) digunakan untuk mengaruh pengagregatan platelet dalam kajian ini. Lima kepekatan (5 mg / mL, 10 mg / mL, 15 mg / mL, 20 mg / mL dan 25 mg / mL) ekstrak cendawan telah diuji dan aktiviti anti-platelet telah ditunjukkan dalam ekstrak *L. rhinocerotis*. Kemudian, dialisis ekstrak sampel cendawan dilakukan dengan menggunakan tiub dialisis bersize 12 kDa. Selepas itu, protein dalam ekstrak yang telah melalui proses dialisis tersebut dimendakan dengan menggunakan aseton. Aktiviti anti-platelet yang telah dimendakan dikaji sekali lagi. Hasilnya, protein yang telah dimendakan juga menunjukkan aktiviti anti-pengagregatan platelet.

Kemudian, sistem dua fasa air telah dilakukan dengan menggunakan 4 g polietilena glikol 50% (PEG 8000 dengan 2.9 g fosfat 40%. Aktiviti anti-pengagregatan platelet didapati pada bahagian fasa atas sistem dua fasa air. Enzim yang diperolehi

daripada bahagian fasa atas sistem dua fasa air tersebut kemudiannya dianalisis dengan menggunakan gel electrophoresis poliakrilamide natrium dodesil sulfat. Hasilnya, dua jalur dalam lingkungan 50 kDa dan 55 kDa diperolehi dalam gel. Kedua-dua jalur tersebut kemudian dipotong dan diuji untuk menentukan aktiviti anti-pengagregatan platlet masing-masing. Aktiviti anti-pengagregatan platlet didapati daripada kedua-dua jalur yang diuji.

Kajian lanjut mengenai enzim dan mekanisme yang terlibat dalam aktiviti anti-pengagregatan platlet perlu dilakukan kerana ekstrak dan enzim separa tulen yang diperolehi daripada cendawan *L. rhinocerotis* ini telah menunjukkan aktiviti anti-pengagregatan platelet. Penulenan enzim boleh dilakukan lagi dan urutan enzim yang terlibat boleh dikaji pada masa akan datang.

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LIST OF SYMBOLS AND ABBREVIATIONS

APTT	Activated partial thromboplastin time
ADP	Adenosine-5'-diphosphate
AMP	Adenosine-5'-monophosphate
A	Alfa
ATPS	Aqueous two phase system
Ca	Calcium
Ca²⁺	Calcium ion
Cu	Copper
cAMP	Cyclic adenosine monophosphate
COX-1	Cyclooxygenase-1
Da	Dalton
°C	Degree celcius
DTT	Dithiothreitol
Γ	Gamma
GP IIb/IIIa	Glycoprotein IIb/IIIa
G	Gram
g/L	Gram per litre
rhG-CSF	Granulocyte-colony stimulating factor
GMP	Guanosine monophosphate
HPLC	High-performance liquid chromatography
HCl	Hydrochloric acid
pH	Hydrogen ion concentration
Fe	Iron
kDa	Kilo dalton
L	Litre
Log	Logarithm
Mn	Manganese
µg	Microgram
µg/mL	Microgram per microlitre
µL	Microlitre
mL	Microlitre
µM	Micromolar
mM	Milimolar
Mg	Milligram
mg/L	Milligram per litre
mg/mL	Milligram per microlitre
Min	Minute
M	Molar
MW	Molecular weight
Nm	Nanometer
%	Percent
P	Phosphorus
±	Plus minus
PAGE	Polyacrylamide gel electrophoresis
PEG	Polyethylene glycol
K	Potassium
PAR-1	Protease-activated receptors-1
PAR-4	Protease-activated receptors-4

PT	Prothrombin time
Rpm	Rotation per minute
Se	Selenium
Na	Sodium
NaCl	Sodium chloride
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TXA2	Thromboxane A 2
Tris-HCL	Tris hydrochloride
2D	Two-dimensional
V	Voltages