PERBANDINGAN KUANTITAS DAN KUALITAS ISOLAT DNA *Aspergillus niger* MENGGUNAKAN *FILTER BASED KIT*, *ALKALINE LYSIS* DAN *HEAT TREATMENT*

SKRIPSI

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Memperoleh Gelar Sarjana Kedokteran

Oleh

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RINGKASAN

Hizb Hamzah Al Kahfi Barqly. Fakultas Kedokteran, Universitas Islam Malang, 14 Oktober 2021. PERBANDINGAN KUANTITAS DAN KUALITAS ISOLAT DNA *Aspergillus niger* MENGGUNAKAN *FILTER BASED KIT*, *ALKALINE LYSIS* DAN *HEAT TREATMENT*. **Pembimbing 1**: dr. Hj. Noer Aini, M.Kes, **Pembimbing 2**: Rio Risandiansyah, S.Ked, MP, PhD

Pendahuluan: *Aspergillus niger* (*A. niger*) penyebab *Chronic Pulmonary Aspergillosis* (CPA) yang deteksinya dengan kultur membutuhkan waktu yang lama. Metode deteksi cepat dengan PCR memerlukan isolat DNA dengan kuantitas dan kualitas yang memadai dan umumnya diperoleh dengan *filter based kit* (FBK). Metode isolasi DNA yang sederhana, mudah dan murah perlu dikembangkan, namun analisa perbandingannya belum ada sehingga perlu dilakukan.

Metode: Penelitian eksperimental *in vitro* dengan *A. niger* yang dikuantifikasi dengan *haemocytometer*. Sampel jumlah selnya 10^9, 10^4 sel/ml dan kontrol normal saline (NS). Isolasi DNA dilakukan dengan metode *heat treatment* (HT), *alkaline lysis* (AL) dan FBK. Hasil isolat DNA dilakukan perhitungan kuantitas dan kualitas DNA dengan spektrofotometer nanodrop. Data dianalisis dengan *One Way ANOVA*, LSD, *Kruskal Wallis*, dan *Mann Whitney* ($p<0,05$).

Hasil: Pada 10^9 sel/ml, urutan peroleh kuantitas DNA dari terbaik ke terburuk ($p<0,05$) adalah HT>FBK>AL (194,8 – 10,6 µg/ml), sedangkan untuk kualitas $A_{260}/_{280}$ ($p<0,05$) adalah AL>FBK>HT dengan rentang (1,80 – 1,26), dan kualitas $A_{260}/_{230}$ ($p<0,05$) adalah AL>HT>FBK (0,85 – 0,32). Pada jumlah sel 10^4 sel/ml, urutan kuantitas DNA ($p<0,05$) adalah HT>FBK>AL (12,8 – 0,2 µg/ml), sedangkan untuk kualitas $A_{260}/_{280}$ ($p>0,05$) adalah FBK=HT=AL (1,90 – 1,50), dan kualitas $A_{260}/_{230}$ ($p<0,05$) adalah HT>FBK>AL (0,46 – 0,02). Pada NS, urutan perolehan kuantitas secara signifikan adalah HT>FBK=AL (4 – 0 µg/ml), dengan tidak ada perbedaan kualitas $A_{260}/_{280}$, namun untuk kualiats $A_{260}/_{230}$ ($p<0,05$) adalah HT>FBK=AL (0,59 – 0).

Kesimpulan: Kuantitas DNA terbaik pada konsentrasi 10^9 dan 10^4 sel/ml adalah metode HT, sedangkan kualitas DNA terbaik pada konsentrasi 10^9 sel/ml adalah pada metode AL dan pada konsentrasi 10^4 sel/ml adalah FBK.

Kata Kunci: *Aspergillus niger*; *Filter Based Kit*; *Alkaline Lysis*; *Heat Treatment*; *Kuantitas DNA*; *Kualitas DNA.*

SUMMARY

Hizb Hamzah Al Kahfi Barqly. Faculty of Medicine, Islamic University of Malang, Oktober 2021. QUANTITY AND QUALITY COMPARISON OF DNA ISOLAT *Aspergillus niger* USING FILTER BASED KIT, ALKALINE LYSIS AND HEAT TREATMENT METHODS. **Supervisor 1**: dr. Hj. Noer Aini, M.Kes, **Supervisor 2**: Rio Risandiansyah, S.Ked, MP, PhD

Introduction: *Aspergillus niger* (*A. niger*) causes Chronic Pulmonary Aspergillosis (CPA), in which its detection using culture methods requires a long time; therefore, PCR can be used for rapid detection. A successful PCR requires DNA isolates with adequate quantity and quality, which is commonly obtained using filter based kit (FBK). A simple, easy and inexpensive DNA isolation method needs to be developed, but comparative analysis needs to be done.

Methods: This is an *in vitro* experimental study with *A. niger* quantified by haemocytometer. The sample cell count used was 10^9, 10^4 cells/ml and the control was normal saline (NS). DNA isolation was carried out using heat treatment (HT), alkaline lysis (AL) and FBK methods. DNA isolates were calculated for quantity and quality of DNA using a nanodrop spectrophotometer. Data was analyzed by One Way ANOVA, LSD, Kruskal Wallis, and Mann Whitney ($p<0.05$).

Results: At 10^9 cells/ml, the order of DNA quantity from the best to worst ($p<0.05$) was HT>FBK>AL (194,8 – 10,6 µg/ml), while for quality $A_{260}/_{280}$ ($p<0,05$) was AL>FBK>HT with a range (1,80 – 1,26), and the quality $A_{260}/_{230}$ ($p<0,05$) was AL>HT>FBK (0,85 – 0,32). In the number of cells 10^4 cells/ml, the order of DNA quantity ($p<0.05$) was HT>FBK>AL (12,8 – 0,2 µg/ml), while for quality $A_{260}/_{280}$ ($p>0,05$) is FBK=HT=AL (1,90 – 1,50), and the quality $A_{260}/_{230}$ ($p<0,05$) is HT>FBK>AL (0,46 – 0,01). In NS, the order of significant quantity was HT>FBK=AL (4 – 0 µg/ml), with no difference in quality $A_{260}/_{280}$, but for quality $A_{260}/_{230}$ ($p<0,05$) it was HT>FBK= AL (0,59 – 0).

Conclusion: At both concentrations, the highest DNA quantity obtained was using HT, however, the highest quality at 10^9 cells/ml was using AL, and 10^4 cells/ml was using FBK.

Keywords: *Aspergillus niger*; *Filter Based Kit*; *Alkaline Lysis*; *Heat Treatment*; *DNA Quantity*; *DNA Quality*.

BAB I
PENDAHULUAN

1.1. Latar Belakang

Aspergillosis adalah penyebab *chronic pulmonary disease* yang biasa dikenali *chronic pulmonary aspergillosis* (CPA) yang sering menemani pasien dengan TB dan HIV (Rozaliyani *et al.*, 2020; Setianingrum *et al.*, 2020). Penyebab aspergillosis adalah *A. fumigatus*, *A. flavus*, *A. terreus*, dan *A. niger* (Wilopo *et al.*, 2019). Data epidemiologi global menunjukkan sekitar 3 juta orang menderita CPA (Brown *et al.*, 2012). Angka kematian pasien CPA adalah 50-85 % selama 5 tahun, terutama pasien CPA dengan penyakit pernyerta TB (Lowes *et al.*, 2017). Belum ada data pasti jumlah CPA di Indonesia, namun diestimasi berdasarkan perhitungan dan eksplorasi dari WHO di Indonesia, dari 274.397 pasien TB sebanyak 83.000 pasien menjadi CPA dalam 5 tahun (Wahyuningsih *et al.*, 2017). Sehingga upaya untuk menegakkan diagnosis aspergillosis perlu dilakukan.

Dalam menegakkan diagnosis aspergillosis perlu diketahui jenis *Aspergillus* sp. yang menginfeksi. *Gold standard* untuk pemeriksaan tersebut adalah dengan menggunakan pemeriksaan kultur dan histopatologis, namun kedua pemeriksaan tersebut tidak dapat menunjukkan genus dan spesies jamur dan memerlukan waktu yang lama. Hal ini menyebabkan penundaan diagnosis karena sensitivitas dan spesifisitas yang terbatas (Arvanitis *et al.*, 2014; Guarner dan Brandt, 2011; Van Burik *et al.*, 1998; Yu *et al.*, 2020). Metode PCR dapat digunakan untuk mengetahui jenis *Aspergillus* sp. dengan cepat, namun metode ini membutuhkan isolat DNA dengan kuantitas dan kualitas DNA yang baik (Wilopo *et al.*, 2019).

PCR selama ini pada jamur menggunakan isolat DNA dari metode *filter based kit* atau kit komersial (Tan dan Yiap, 2009), namun metode isolasi DNA ini memerlukan tambahan beberapa peralatan, dan bahan yang khusus yang tidak mudah diperoleh, dan memiliki harga yang mahal, sehingga sulit digunakan pada negara berkembang (Ali *et al.*, 2017; Shi *et al.*, 2018a, 2018b). Sehingga dibutuhkan metode isolasi DNA yang lebih sederhana yaitu metode *alkaline lysis* dan *heat treatment* yang memiliki keunggulan dengan proses yang cepat dan sederhana, biaya yang murah dan relatif mudah (Ali *et al.*, 2017; Dilhari *et al.*, 2017).

Penelitian mengenai kuantitas dan kualitas DNA *Aspergillus niger* (*A. niger*) yang diperoleh dengan metode *alkaline lysis*, *heat treatment* dan *filter based kit* belum dilakukan. Penelitian ini melakukan perbandingan kuantitas dan kualitas DNA *A. niger* dengan metode sederhana *alkaline lysis* dan *heat treatment* dibandingkan dengan metode *filter based kit*.

1.2. Rumusan Masalah

1.2.1. Bagaimana perbandingan kadar kuantitas DNA *Aspergillus niger* menggunakan metode isolasi DNA *alkaline lysis* dan *heat treatment* dibandingkan dengan metode *filter based kit* pada beberapa konsentrasi sampel?

1.2.2. Bagaimana perbandingan kadar kualitas DNA $A_{260/280}$ *Aspergillus niger* menggunakan metode isolasi DNA *alkaline lysis* dan *heat treatment* dibandingkan dengan metode *filter based kit* pada beberapa konsentrasi sampel?

1.2.3. Bagaimana perbandingan kadar kualitas DNA $A_{260/230}$ *Aspergillus niger* menggunakan metode isolasi DNA *alkaline lysis* dan *heat treatment* dibandingkan dengan metode *filter based kit* pada beberapa konsentrasi sampel?

1.3.Tujuan Penelitian

1.3.1. Penelitian ini bertujuan untuk mengetahui perbandingan kadar kuantitas DNA *Aspergillus niger* menggunakan metode isolasi DNA *alkaline lysis* dan *heat treatment* dibandingkan dengan metode *filter based kit* pada beberapa konsentrasi sampel.

1.3.2. Penelitian ini bertujuan untuk mengetahui perbandingan kadar kualitas DNA $A_{260/280}$ *Aspergillus niger* menggunakan metode isolasi DNA *alkaline lysis* dan *heat treatment* dibandingkan dengan metode *filter based kit* pada beberapa konsentrasi sampel.

1.3.3. Penelitian ini bertujuan untuk mengetahui perbandingan kadar kualitas DNA $A_{260/230}$ *Aspergillus niger* menggunakan metode isolasi DNA *alkaline lysis* dan *heat treatment* dibandingkan dengan metode *filter based kit* pada beberapa konsentrasi sampel.

1.4. Manfaat Penelitian

1.4.1. Manfaat Teori

Penelitian ini dapat digunakan sebagai landasan ilmiah perbandingan kuantitas dan kualitas DNA *A. niger* antara metode *filter based kit* dengan metode *alkaline lysis* dan *heat treatment*.

1.4.2. Manfaat Praktis

Manfaat praktis dari penelitian ini adalah pengembangan metode isolasi DNA menggunakan metode *alkaline lysis* dan *heat treatment* untuk isolasi DNA pada jamur.

BAB VII
PENUTUP

7.1. Kesimpulan

Berdasarkan hasil penelitian, analisis data serta pembahasan pada penelitian ini dapat disimpulkan bahwa:

1. Kuantitas isolat DNA *A. niger* pada konsentrasi 10^9 dan 10^4 sel/ml metode *heat treatment* lebih baik dibandingkan *filter based kit* dan *alkaline lysis*.
2. Kualitas ($A_{260}/_{280}$) isolat DNA *A. niger* pada konsentrasi 10^4 sel/ml didapatkan hasil paling baik dengan metode *filter based kit*, sedangkan pada 10^9 sel/ml hasil paling baik pada metode *alkaline lysis*.
3. Kualitas ($A_{260}/_{230}$) isolat DNA *A. niger* pada konsentrasi 10^4 dan 10^9 sel/ml pada ketiga metode tidak memenui kreteria kualitas ($A_{260}/_{230}$) baik.

7.2. Saran

Penelitian ini menyarankan beberapa hal berikut ini untuk penelitian selanjutnya sebagai bentuk pengembangan dan kemajuan suatu ilmu pengetahuan:

1. Untuk membuktikan secara aplikatif kuantitas dan kualitas dari isolasi DNA, penelitian ini dapat dilanjutkan ke uji-uji selanjutnya seperti elektroforesis, PCR, RT-PCR atau DNA *sequencing*.
2. Melakukan perbandingan kuantitas dan kualitas isolat DNA pada kurang dari konsentrasi 10^4 sel/ml dan lebih dari 10^9 sel/ml.
3. Pada penelitian selanjutnya dapat dilakukan modifikasi metode sederhana dengan tahapan proses isolasi DNA yang lengkap dengan cara menggabungkan beberapa tahap *alkaline lysis* dan *heat treatment* untuk mendapatkan kuantitas dan kualitas DNA yang lebih baik.

4. Pada penelitian selanjutnya metode *alkaline lysis* pada larutan alkali mengandung NaOH dapat melakukan eksplorasi peningkatan konsentrasi NaOH.

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