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LAMPIRAN

Lampiran 1. Skema Kerja Ekstraksi Isi Kapsul Kerang Darah *Anadara granosa* L. Difortifikasi Mikroalga *Spirulina platensis* dan Simplisia *Anadara granosa* L.

Isi kapsul dan simplisia *Anadara granosa* L.

- Isi kapsul ditimbang sebanyak 200 g, sedangkan simplisia *Anadara granosa* L. ditimbang sebanyak 100 g.
- Direndam menggunakan etanol 96% dengan perbandingan simplisia:pelarut yakni 1:3.
- Rendaman diaduk dan didiamkan selama 24 jam. Bejana maserasi ditutup dengan rapat menggunakan *wrap plastic*.
- Dilakukan penyaringan setelah 24 jam lalu diremaserasi hingga sebanyak 2 kali.
- Maserat pertama, kedua, dan ketiga dicampur dan dievaporasi pada suhu 37 °C menggunakan *rotary evaporator*.

Ekstrak kasar

Lampiran 2. Skema Kerja Uji Daya Hambat Ekstrak Etanol Isi Kapsul Kerang Darah *Anadara granosa* L. Difortifikasi Mikroalga *Spirulina platensis*

Ekstrak etanol isi kapsul

- Dibuat larutan dengan dua seri konsentrasi, yakni: a) 25, 50, 75, 100%, dan b) 10, 15, 20, 25%. Di samping itu, dibuat larutan ekstrak *Anadara granosa* L. konsentrasi 100% dan 25%.
- *Blank disk* steril direndam ke dalam ekstrak dan kontrol (ciprofloxacin 5 ppm dan etanol 96%) pada pelat tetes selama 15 menit. *Blank disk* lalu didiamkan hingga pelarut ekstrak penguap.
- *Blank disk* hasil rendaman diletakkan di atas permukaan media MHA padat yang telah dihomogenkan dengan suspensi bakteri uji.
- Kultur diinkubasi pada suhu 37 °C selama 48 jam.
- Zona bening diukur pada waktu inkubasi 1 × 24 jam dan 2 × 24 jam.

Rata-rata diameter zona bening

Lampiran 3. Skema Kerja Pemisahan Senyawa secara Kromatografi Lapis Tipis Preparatif (KLTP)

Pelat KLTP berlapis gel silika 60 GF_{254}

- Pelat KLTP (20 × 20 cm) berlapis gel silika 60 GF_{254} diaktifkan di dalam oven selama 10 menit pada suhu 105 °C.
- Sampel ditotolkan pada pelat menggunakan pipa kapiler.
- Pelat dimasukkan ke dalam *chamber* yang telah jenuh oleh eluen kloroform:etanol (90:10).
- Setelah mencapai batas atas, pelat KLT dikeluarkan dari *chamber* dan dikeringkan pada suhu kamar.
- Noda diamati di bawah sinar UV 254 nm dan 365 nm.
- Noda ditandai menggunakan spatula.

Kromatogram

Lampiran 4. Skema Kerja Kromatografi Lapis Tipis Bioautografi (KLT-Bioautografi)

Kromatogram

- Noda kromatogram dikeruk menggunakan spatula dan masing-masing dilarutkan ke dalam 2,5 mL etanol 96%.
- Larutan divorteks dan didiamkan selama 24 jam hingga fase diamnya mengendap.
- Larutan fraksi dan kontrol diteteskan pada *blank disk* steril.
- *Blank disk* hasil rendaman diletakkan di atas permukaan media MHA padat yang telah dihomogenkan dengan suspensi bakteri uji.
- Kultur diinkubasi pada suhu 37 °C selama 48 jam.
- Zona bening diukur pada waktu inkubasi 1 × 24 jam dan 2 × 24 jam.

Rata-rata diameter zona bening

Lampiran 5. Gambar Prosedur Ekstraksi Isi Kapsul Kerang Darah *Anadara granosa* L. Difortifikasi Mikroalga *Spirulina platensis* dan Simplisia *Anadara granosa* L.

(a)

(b)

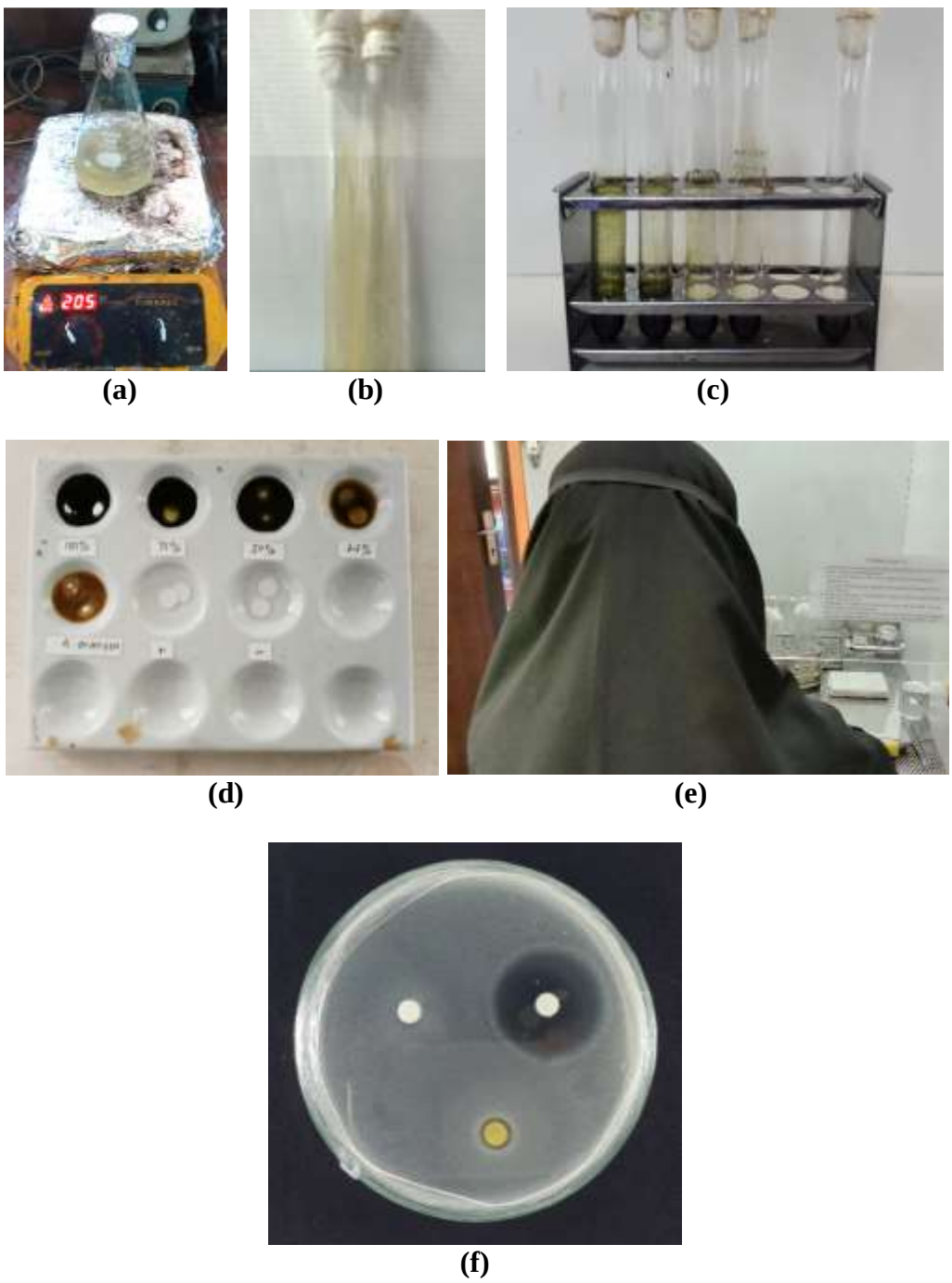
(c)

(d)

(e)

Gambar 1. (a) Penimbangan isi kapsul dan simplisia kerang darah *Anadara granosa* L., (b) Perendaman simplisia dalam etanol 96%, (c) Penyaringan filtrat dan residu, (c) Proses evaporasi ekstrak menggunakan *rotary evaporator* pada suhu 37 °C, dan (e) Ekstrak etanol isi kapsul dan *Anadara granosa* L.

Lampiran 6. Gambar Prosedur Uji Daya Hambat Ekstrak Etanol Isi Kapsul Kerang Darah *Anadara granosa* L. Difortifikasi Mikroalga *Spirulina platensis*



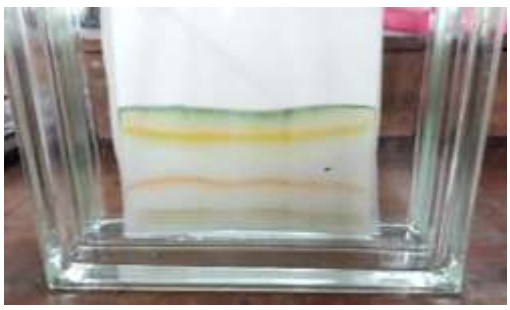
(a) **(b)** **(c)**

(d) **(e)**

(f)

Gambar 2. (a) Pembuatan media MHA, (b) Kultur bakteri *Staphylococcus aureus* dan *Pseudomonas aeruginosa*, (c) Larutan ekstrak, (d) Perendaman *blank disk* pada larutan uji, (e) Uji daya hambat, dan (f) Pengamatan dan pengukuran zona hambatan.

Lampiran 7. Gambar Prosedur Kromatografi Lapis Tipis Bioautografi (KLT-Bioautografi)



(a)

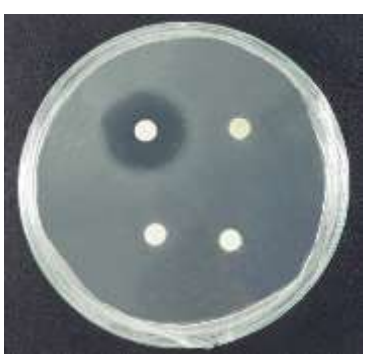
(b)

(c)

(d)

(e)

(f)



(g)

Gambar 3. (a) Proses elusi menggunakan fase diam pelat kaca (20 × 20 cm) berlapis gel silika 60 GF_{254} dan fase gerak kloroform:etanol (90:10), (b) Pengamatan kromatogram pada sinar UV 254 nm, (c) Pengamatan kromatogram pada sinar UV 365 nm, (d) Perendaman fraksi dalam etanol 96%, (e) Perendaman *blank disk* steril dalam larutan uji, (f) Uji daya hambat, dan (g) Pengamatan dan pengukuran zona hambatan.