KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
BADAN LAYANAN UMUM UNIVERSITAS LAMPUNG

SP2D

SURAT PERINTAH PENCAIRAN DANA (SP2D)

PM 00354/LS/LP2M/2019	Dari: Bendahara Pengeluaran
31 Juli 2019	Tanggal: 02 Agustus 2019
Lembaga Penelitian dan Pengabdian kepada Masyarakat UNILA	Nomor: 01877/UN26/SP2D/2019
	Tahun Anggaran: 2019

Belanja 5742.002.001.057.A.525119 Belanja Penyediaan Barang dan Jasa BLU Lainnya

Bank BNI 46 Cabang Pembantu UNILA

...h memindahbukukan dari rekening nomor 019.3922.784

Uang sebesar	Rp.	52.500.000,00
PPH	Rp.	0,00
PPN	Rp.	0,00
Jumlah diterima	Rp.	52.500.000,00

Lima Puluh Dua Juta Lima Ratus Ribu Rupiah

Drs. Suratman Umar, M.Sc., Griya GM Indah Blok C2/12A RT 004 RW 002 Gedung Meneng Ra... Bandar Lampung

14.590.651.7-323.000

0443717626 (Bpk Suratman)

BNI Cabang Tanjung Karang

Pembayaran Tahap I 70% untuk Kegiatan Penelitian Kerjasama Internasional Unila sesuai dengan SPK nomor. 3093/UN26.21/PN/2019 tgl 10 Juli 2019, BAST nomor. 3123/UN26.21/PN/2019 tgl 11 Juli 2019 BAP nomor. 3183/UN26.21/PN/2019 tgl 12 Juli 2019

...sa Pengguna Anggaran
...ektor Bidang Umum dan Keuangan,

...mad Kamal
...610101198503100...

Bandar Lampung, tanggal seperti di...
Bendahara Pengeluaran UNILA,

Muhammad Ismail
NIP. 198907062010121011

PT BANK NEGARA INDONESIA (PERSERO) Tbk. BNI KANTOR CABANG UNILA

1. ... Bendahara Pengeluaran 2. Biru untuk BPP 3. Hijau untuk Bank

TRANSFER DARI 1... 27704 REKTOR UNIVERSITAS LAMPUNG IDR 52.500.000,00-
TRANSFER KE 0443...626 Bpk SURATMAN IDR 52.500.000,00
TANPA BUKU
BERITA ...UN26/SP2D/19 02082019
12 - TANJUNG KARANG

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS LAMPUNG
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKA
GedungRektoratLantai 5, Jalan Prof. Dr. SumantriBrojonegoro No. 1 Bandar Lampung 3514
Telepon (0721) 705173, Fax. (0721) 773798, e-mail : lppm@kpa.unila.ac.id
www.lppm.unila.ac.id

SURAT PERJANJIAN (KONTRAK) PEKERJAAN PELAKSANAAN KEGIATAN PENELITIAN KERJASAMA INTERNASIONAL

NOMOR : 3093/UN26.21/PN/2019
TANGGAL : 10 Juli 2019

ANTARA

PEJABAT PEMBUAT KOMITMEN
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
UNIVERSITAS LAMPUNG

DAN

Drs. Suratman Umar, M.Sc (Ketua)
PENANGGUNGJAWAB KEGIATAN PENELITIAN DENGAN JUDUL
Investigation Of Antibacterial Bioactive Compounds From Jengkol Plant (Pithecellobium lobatum) Through Bioassay Guided Fractination Approach

FAKULTAS MIPA
UNIVERSITAS LAMPUNG
BANDAR LAMPUNG
2019

RINGKASAN KONTRAK

Kegiatan yang dananya berasal dari DIPA BLU Universitas Lampung

1.	No./Tgl.DIPA	:	DIPA-042.01.2.400954/2019, 05 Desember 2018
2.	Kode Keg./Sub.Keg/MAK	:	5742.002.001.057.A.525119, Tahun Anggaran 2019 (Penelitian)
3.	No. dan Tanggal SPK	:	3093/UN26.21/PN/2019, Tanggal 10 Juli 2019
4.	Nama Penanggungjawab	:	Drs. Suratman Umar, M.Sc / Penanggung Jawab Kegiatan Penelitian KERJASAMA INTERNASIONAL Unila
5.	Alamat Penanggungjawab	:	Jl.Prof. Sumantri Brojonegoro No.1 Bandar Lampung
6.	Nomor Pokok Wajib Pajak	:	14.590.651.7-323.000
7.	Nilai SPK/Surat Perjanjian	:	**Rp 75.000.000,-**
8.	Uraian dan volume Pekerjaan	:	Penelitian dengan Judul "**Investigation Of Antibacterial Bioactive Compounds From Jengkol Plant (Pithecellobium lobatum) Through Bioassay Guided Fractination Approach**
9.	Cara Pembayaran	:	1. Kegiatan penelitian pembayaran angsuran I (satu) sebesar 70% (dari nilai pekerjaan) atau 70% x Rp 75.000.000,- yakni sebesar Rp 52.500.000,- (*Lima puluh dua juta lima ratus ribu*), setelah surat perjanjian pelaksanaan pekerjaan ini ditandatangani oleh kedua belah pihak dan menyerahkan proposal-proposal kegiatan tersebut dari Pihak Kedua kepada Pihak Pertama. 2. Kegiatan penelitian pembayaran angsuran II (dua) sebesar 30% (dari nilai pekerjaan) atau 30% x Rp 75.000.000,- yakni sebesar Rp 22.500.000,- (*Dua puluh dua juta lima ratus ribu*), setelah pekerjaan selesai 100% dinyatakan dengan Berita Acara Serah Terima pekerjaan dan menyerahkan laporan hasil kegiatan dari Pihak Kedua kepada Pihak Pertama. 3. Pembayaran tersebut di atas dilakukan melalui kas Badan Layanan Umum (BLU) ke Rekening Pihak Kedua pada Bank : **BNI Tanjung Karang** dengan nomor rekening 0443717626, a.n. **Drs. Suratman Umar, M.Sc** sebagai penangung jawab kegiatan penelitian **KERJASAMA INTERNASIONAL** Universitas Lampung.
10.	Jangka waktu pelaksanaan	:	119 (Seratus Sembilan Belas Hari) kalender terhitung tanggal 10 Juli – 5 November 2019
11.	Tanggal Penyelesaian Pekerjaan	:	5 November 2019
12.	Jangka waktu Pemeliharaan	:	-

Ketentuan Sanksi :

1. Apabila terjadi ketelambatan pekerjaan tanpa adanya alasan yang diterima oleh pemberi pekerjaan dikenakan sanksi/denda sebesar 1/1000 (satu permil) untuk setiap hari keterlambtam denga denda makismal sebesar 5%, (lima persen) dari jumlah harga borongan.
2. Segala resiko yang timbul akibat keterlambatan pekerjaan tersebut ini sepenuhnya menjadi beban dan tanggung jawab pihak II. Maka kami sebgai pihak I dapat membatalkan SPK secara sepihak dan pihak II tidak berhak menuntut kerugian apapun dari instansi kami.

Bandar Lampung, 10 Juli 2019
Pejabat Pembuat Komitmen
LPPM Universitas Lampung,

Warsono, Ph. D.
NIP.196302161987031003

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS LAMPUNG
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKA
GedungRektoratLantai 5, Jalan Prof. Dr. SumantriBrojonegoro No. 1 Bandar Lampung 35145
Telepon (0721) 705173, Fax. (0721) 773798, e-mail : lppm@kpa.unila.ac.id
www.lppm.unila.ac.id

SURAT PERJANJIAN (KONTRAK) PEKERJAAN ELAKSANAAN KEGIATAN PENELITIAN KERJASAMA INTERNASIONAL UNIVERSITAS LAMPUNG

NOMOR : 3093/UN26.21/PN/2019
TANGGAL : 10 Juli 2019

da hari ini **Rabu** tanggal **Sepuluh** bulan **Juli** tahun **Dua Ribu Sembilan Belas**, kami ng bertanda tangan di bawah ini :

Nama : Warsono, Ph. D.
Jabatan : Pejabat Pembuat Komitmen LPPM Universitas Lampung
Alamat : Jl. Prof. Soemantri Brojonegoro No.1 Bandar Lampung

anjutnya dalam perjanjian ini disebut **PIHAK PERTAMA**

Nama : Drs. Suratman Umar, M.Sc
Jabatan : Penanggungjawab Pelaksanaan Kegiatan Penelitian KERJASAMA INTERNASIONAL dengan Judul "***Investigation Of Antibacterial Bioactive Compounds From Jengkol Plant (Pithecellobium lobatum) Through Bioassay Guided Fractination Approach***".
Alamat : Jl. Prof. Soemantri Brojonegoro No.1 Bandar Lampung

anjutnya dalam perjanjian ini disebut **PIHAK KEDUA**

IAK PERTAMA DAN KEDUA berdasarkan :

Peraturan Presiden nomor 54 tahun 2010; tentang pengadaan barang/jasa pemerintah
Undang-undang RI nomor 17 tahun 2003 tentang Keuangan Negara;
Undang-undang nomor 20 tahun 2003 tentang Sistem Pendidikan Nasional;
Undang-undang nomor 15 tahun 2004 tentang Pemeriksaan Pengelolaan dan Tanggung Jawab Keuangan Negara;
Keppres Nomor 42 tahun 2002 nomor 72 tahun 2004 tentang Pelaksanaan Anggaran Pendapatan dan Belanja Negara;
Peraturan Menteri Keuangan Nomor 606/KMK.66/2004 tentang Pedoman Pembayaran Pelaksanaan Anggaran;
DIPA Universitas Lampung Nomor DIPA-042.01.2.400954/2019, tanggal 05 Desember 2018

ngan ini menyatakan setuju dan sepakat untuk mengikat diri dalam suatu perjanjian aksanaan pekerjaan, dengan ketentuan dan syarat-syarat tercantum dalam pasal-pasal

PASAL 4
JANGKA WAKTU PELAKSANAAN

Jangka waktu pelaksanaan kegiatan Penelitian **KERJASAMA INTERNASIONAL** Universitas Lampung tersebut dalam pasal 1 adalah 119 (Seratus Sembilan Belas Hari) terhitung sejak ditandatanganinya perjanjian ini. Laporan ini harus diserahkan PIHAK KEDUA selambat-lambatnya tanggal 5 November 2019 sebanyak (3) Tiga Eksemplar.

Apabila laporan Penelitian tidak diselesaikan tepat pada waktunya, PIHAK KEDUA dapat mengajukan Adendum sebanyak 1 kali saja, dan apabila PIHAK KEDUA berhenti/diberhentikan dari jabatan atau dipindahkan ke instansi lain, PIHAK KEDUA wajib mempertanggungjawabkan penggunaan dana penelitian yang telah diterima dari PIHAK PERTAMA, selanjutnya PIHAK PERTAMA berhak menunjuk orang lain untuk melaksanakan pekerjaan tersebut.

PASAL 5
SANKSI

Jika **PIHAK KEDUA** tidak dapat melaksanakan pekerjaan sesuai dengan batas Waktu pelaksanaan yang tercantum dalam pasal 4 dalam perjanjian ini maka untuk tiap hari keterlambatan **PIHAK KEDUA** wajib membayar denda keterlambatan sebesar 1/1000 (satu permil) dari nilai kontrak.

PIHAK KEDUA bertanggung jawab penuh apabila dalam pelaksanaan pekerjaan ini tidak sesuai dengan ketentuan yang berlaku, atau terdapat hal – hal atau temuan pemeriksaan yang mengakibatkan kerugian negara.

PASAL 6
PENYELESAIAN PERSELISIHAN

Jika terjadi perselisihan antara kedua belah pihak, pada dasarnya akan diselesaikansecara musyawarah.

Jika perselisihan itu tidak dapat diselesaikan secara musyawarah, maka akan diselesaikan oleh "panitia pendamai" yang berfungsi sebagai juri/wasit yang dibentuk dan diangkat oleh kedua belah pihak yang terdiri dari:
- Seorang wakil dari PIHAK PERTAMA sebagai anggota
- Seorang wakil dari PIHAK KEDUA sebagai anggota
- Seorang pihak ketiga yang ahli sebagai Ketua, yang telah disetujui oleh PIHAK KEDUA

Keputusan panitia pendamai ini mengikat kedua belah pihak, dan biaya penyelesaian perselisihan yang dikeluarkan akan ditanggung secara bersama.

Jika keputusan ini sebagaimana dimaksud ayat 3 pasal ini tidak dapat diterima oleh salah satu pihak, maka penyelesaian perselisihan akan diteruskan melalui pengadilan Negeri.

PASAL 7
LAIN-LAIN

Segala sesuatu yang belum diatur dalam surat perjanjian ini yang dipandang perlu oleh kedua belah pihak akan diatur lebih lanjut dalam surat perjanjian tambahan (*Addendum*) dan merupakan perjanjian yang tidak dapat terpisahkan dari perjanjian ini.

Surat perjanjian ini dibuat rangkap 4 (empat) untuk Pihak Pertama dan Pihak Kedua, selebihnya diberikan kepada pihak-pihak yang berkepentingan dan ada hubungannya dengan pekerjaan.

PASAL 8
PENUTUP

Surat perjanjian ini dibuat dan ditandatangani oleh kedua belah pihak di atas materai Rp.6.000.,- (enam ribu rupiah) pada lembar ke satu dan lembar kedua yang mempunyai kekuatan hukum sama.

Perjanjian ini berlaku mulai tanggal ditandatangani oleh kedua belah pihak.

HAK KEDUA nanggungjawab Kegiatan,	PIHAK PERTAMA Pejabat Pembuat Komitmen LPPM Universitas Lampung,
s. Suratman Umar, M.Sc P 196406041990031002	Wársono, Ph. D. NIP 19630216 198703 1 003

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS LAMPUNG
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
GedungRektoratLantai 5, Jalan Prof. Dr. SumantriBrojonegoro No. 1 Bandar Lampung 35145
Telepon (0721) 705173, Fax. (0721) 773798, e-mail : lppm@kpa.unila.ac.id
www.lppm.unila.ac.id

==========

SURAT PERNYATAAN TANGGUNGJAWAB MUTLAK

ng bertanda tangan di bawah ini :

a m a : Drs. Suratman Umar, M.Sc
P : 196406041990031002
oatan : Penanggungjawab Kegiatan Penelitian **KERJASAMA INTERNASIONAL**

nyatakan dengan sesungguhnya :

1. Perhitungan tahap I sebesar 70% yang terdapat pada kegiatan Penelitian **KERJASAMA INTERNASIONAL** Unila sebesar Rp 52.500.000,- (Lima puluh dua juta lima ratus ribu) telah dihitung dengan benar. Sesuai kontrak Nomor : 3093/UN26.21/PN/2019, Tanggal 10 Juli 2019.
2. Apabila dikemudian hari terdapat kelebihan atas pembayaran kegiatan Penelitian **KERJASAMA INTERNASIONAL** Unila, kami bersedia menyetorkan kelebihan tersebut ke Kas Negara.
3. Segala akibat yang mungkin timbul dari perubahan di atas menjadi tanggungjawab kami sepenuhnya.

Demikian pernyataan ini kami buat dengan sebenar-benarnya.

Bandar Lampung, 10 Juli 2019
gungjawab Kegiatan,

METERAI TEMPEL
EEAFF803192897
6000
ENAM RIBU RUPIAH

Drs. Suratman Umar, M.Sc
NIP 196406041990031002

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS LAMPUNG
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
GedungRektoratLantai 5, Jalan Prof. Dr. SumantriBrojonegoro No. 1 Bandar Lampung 35145
Telepon (0721) 705173, Fax. (0721) 773798, e-mail : lppm@kpa.unila.ac.id
www.lppm.unila.ac.id

==========

SURAT PERNYATAAN TANGGUNGJAWAB MUTLAK

ıg bertanda tangan di bawah ini :

m a	: Warsono, Ph. D.
P	: 196302161987031003
atan	: Pejabat Pembuat Komitmen LPPM Universitas Lampung

ıyatakan dengan sesungguhnya :

1. Perhitungan tahap I sebesar 70% yang terdapat pada kegiatan Penelitian **KERJASAMA INTERNASIONAL** Unila sebesar Rp 52.500.000,- (Lima puluh dua juta lima ratus ribu) telah dihitung dengan benar. Sesuai kontrak Nomor : 3093/UN26.21/PN/2019, Tanggal 10 Juli 2019.
2. Apabila dikemudian hari terdapat kelebihan atas pembayaran kegiatan Penelitian **KERJASAMA INTERNASIONAL** Unila, kami bersedia menyetorkan kelebihan tersebut ke Kas Negara.
3. Segala akibat yang mungkin timbul dari perubahan di atas menjadi tanggungjawab kami sepenuhnya.

Demikian pernyataan ini kami buat dengan sebenar-benarnya.

Bandar Lampung, 10 Juli 2019
Pejabat Pembuat Komitmen,

Warsono, Ph. D.
NIP 196302161987031003

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS LAMPUNG
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKA
GedungRektoratLantai 5, Jalan Prof. Dr. SumantriBrojonegoro No. 1 Bandar Lampung 3514
Telepon (0721) 705173, Fax. (0721) 773798, e-mail : lppm@kpa.unila.ac.id
www.lppm.unila.ac.id

Nomor : 3093/UN26.21/PN/2019
Lampiran : 1 (satu) berkas
Perihal : Pengajuan SPP dan SPM

10 Juli 2019

Kepada Yth.
Wakil Rektor II
Universitas Lampung
Di Bandar Lampung

Dengan ini kami sampaikan permohonan penerbitan SPP dan SPM untuk keperluan pembayaran tahap I sebesar 70% Kegiatan Penelitian **KERJASAMA INTERNASIONAL** Universitas Lampung sebesar Rp 52.500.000,- (Lima puluh dua juta lima ratus ribu) yang dilaksanakan sesuai dengan Surat Perjanjian nomor : 3093/UN26.21/PN/2019, Tanggal 10 Juli 2019.

Atas perhatian dan kerjasamanya diucapkan terima kasih.

Penanggungjawab Kegiatan,

Drs. Suratman Umar, M.Sc
NIP 196406041990031002

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS LAMPUNG
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
GedungRektoratLantai 5, Jalan Prof. Dr. SumantriBrojonegoro No. 1 Bandar Lampung 35145
Telepon (0721) 705173, Fax. (0721) 773798, e-mail : lppm@kpa.unila.ac.id
www.lppm.unila.ac.id

BERITA ACARA SERAH TERIMA PEKERJAAN

Nomor : 3123/UN26.21/PN/2019

Pada hari **Kamis** tanggal **Sebelas** bulan **Juli** tahun **Dua Ribu Sembilan Belas**, kami sampaikan yang bertanda tangan di bawah ini :

1. Nama : Warsono, Ph. D.
   Jabatan : Pejabat Pembuat Komitmen Lembaga Penelitian Dan Pengabdian Kepada Masyarakat Universitas Lampung
   Alamat : Jl. Sumantri Brojonegoro No.1 Bandar Lampung 35145
   Selanjutnya disebut sebagai Pihak Pertama

2. Nama : Drs. Suratman Umar, M.Sc
   Jabatan : Penanggung Jawab Kegiatan Penelitian KERJASAMA INTERNASIONAL
   Alamat : Jl. Sumantri Brojonegoro No.1 Bandar Lampung 35145
   Selanjutnya disebut sebagai Pihak Kedua

Dengan ini menyatakan telah dilaksanakan serah terima pertama pekerjaan pelaksanaan kegiatan Penelitian **KERJASAMA INTERNASIONAL** Universitas Lampung setelah Surat Perjanjian Pelaksanaan Pekerjaan di tandatangani oleh kedua belah pihak dengan pembayaran dilaksanakan dalam dua angsuran yaitu pertama sebesar 70% dan kedua 30% dari nilai pekerjaan, sebagai berikut :

1. **Pihak Pertama** telah menerima dari **Pihak Kedua** proposal pelaksanaan kegiatan Penelitian **KERJASAMA INTERNASIONAL** Universitas Lampung sesuai dengan Surat Perjanjian Pelaksanaan Pekerjaan No : 3093/UN26.21/PN/2019,Tanggal 10 Juli 2019
2. **Pihak Kedua** telah menyerahkan kepada Pihak Pertama proposal pelaksanaan kegiatan Penelitian **KERJASAMA INTERNASIONAL** Universitas Lampung sesuai dengan Surat Perjanjian Pelaksanaan No : 3093/UN26.21/PN/2019, Tanggal 10 Juli 2019.

Demikian berita acara serah terima pekerjaan ini dibuat untuk dapat dipergunakan sebagaimana mestinya.

PIHAK PERTAMA	PIHAK KEDUA
PEJABAT PEMBUAT KOMITMEN	PENANGGUNG JAWAB KEGIATAN,
LPPM UNIVERSITAS LAMPUNG,	METERAI TEMPEL 6000 ENAM RIBU RUPIAH
Warsono, Ph. D.	Drs. Suratman Umar, M.Sc.
NIP 196302161987031003	NIP 196406041990031002

TAHUN ANGGARAN : 2019
DIPA NOMOR/TANGGAL : 042.01.2.400954/2019
: Tanggal 05 Desember 2018
MAK : 5742.002.001.057.A.525119

KWITANSI

SUDAH DITERIMA DARI : Pejabat Pembuat Komitmen LPPM Universitas Lampung

BANYAKNYA UANG : ***Lima puluh dua juta lima ratus ribu***

UNTUK PEMBAYARAN : Tahap I Pekerjaan Penelitian **KERJASAMA INTERNASIONAL** Universitas Lampung Tahun 2019 sesuai dengan Surat Perjanjian No. 3093/UN26.21/PN/2019, Tanggal 10 Juli 2019, dan BAP No. 3183/UN26.21/PN/2019, Tanggal 12 Juli 2019

JUMLAH : **Rp 52.500.000 -**

Bandar Lampung, 12 Juli 2019
Yang Menerima
Penanggungjawab
Kegiatan Penelitian,

METERAI TEMPEL
21AFF803192892
6000

Drs. Suratman Umar, M.Sc
NIP 196406041990031002

Setuju dibayar
Pejabat Pembuat Komitman
LPPM Unila,

Warsono, Ph. D.
NIP 19630216 198703 1 003

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS LAMPUNG
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
GedungRektoratLantai 5, Jalan Prof. Dr. SumantriBrojonegoro No. 1 Bandar Lampung 35145
Telepon (0721) 705173, Fax. (0721) 773798, e-mail : lppm@kpa.unila.ac.id
www.lppm.unila.ac.id

BERITA ACARA PEMBAYARAN
Nomor : 3183/UN26.21/PN/2019

Pada hari **Jum'at** tanggal **Dua belas** bulan **Juli** tahun **Dua Ribu Sembilan Belas**, kami yang bertanda tangan di bawah ini :

1. Nama : Warsono, Ph. D.
   Jabatan : Pejabat Pembuat KomitmenLPPM Unila
   Alamat : Jl. Sumantri Brojonegoro No.1 Bandar Lampung
   DISEBUT SEBAGAI PIHAK PERTAMA

2. Nama : Drs. Suratman Umar, M.Sc
   Jabatan : Penanggungjawab Kegiatan Penelitian **KERJASAMA INTERNASIONAL**
   Alamat : Jl. Sumantri Brojonegoro No.1 Bandar Lampung
   DISEBUT SEBAGAI PIHAK KEDUA

Dengan ini telah melaksanakan Kegiatan Penelitian KERJASAMA INTERNASIONAL Unila, sesuai dengan Surat Perjanjian Nomor : 3184/UN26.21/PN/2019, Tanggal 12 Juli 2019

PIHAK KEDUA berhak menerima pembayaran dari **PIHAK PERTAMA** sebesar 70% dari nilai kontrak atau 70% x Rp 75.000.000,- = Rp 52.500.000,- (Lima puluh dua juta lima ratus ribu) yang digunakan untuk 1 kegiatan penelitian, melalui kas Badan Layanan Umum Universitas Lampung.

PIHAK KEDUA sepakat atas jumlah pembayaran tersebut di atas dan dibayarkan melalui Nomor rekening : 0443717626 **BNI Tanjung Karang** atas nama : **Drs. Suratman Umar, M.Sc** penanggungjawab kegiatan penelitian **KERJASAMA INTERNASIONAL** Universitas Lampung.

Demikian berita acara pembayaran ini dibuat untuk dapat dipergunakan sebagaimana mestinya.

Bandar Lampung, 12 Juli 2019

II. PIHAK KEDUA
Penanggungjawab Kegiatan
Penelitian KERJASAMA INTERNASIONAL,

Drs. Suratman Umar, M.Sc
NIP 196406041990031002

I. PIHAK PERTAMA
Pejabat Pembuat Komitman
LPPM UNILA,

Warsono, Ph. D.
NIP 19630216 198703 1 003

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS LAMPUNG
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
GedungRektoratLantai 5, Jalan Prof. Dr. SumantriBrojonegoro No. 1 Bandar Lampung 35145
Telepon (0721) 705173, Fax. (0721) 773798, e-mail : lppm@kpa.unila.ac.id
www.lppm.unila.ac.id

SURAT PERNYATAN TANGGUNG JAWAB BELANJA

Yang bertanda tangan di bawah ini :

1. Nama : Drs. Suratman Umar, M.Sc
2. Alamat : Jl. Sumantri Brojonegoro No.1 Bandar Lampung

Berdasarkan Surat Keputusan Nomor : 1688 /UN26.21/PP/2019 tanggal 21 Juni 2019 dan perjanjian kontrak Nomor : 3093/UN26.21/PN/2019, tanggal 10 Juli 2019 mendapatkan Anggaran Penelitian dengan judul ***"Investigation Of Antibacterial Bioactive Compounds From Jengkol Plant (Pithecellobium lobatum) Through Bioassay Guided Fractination Approach"***

Dengan ini menyatakan bahwa :

1. Biaya kegiatan penelitian di bawah ini meliputi ;

No	Uraian	Jumlah
1	Tahap I Persiapan dan Pelaksanaan	Rp 52.500.000,-
	Jumlah	**Rp 52.500.000,-**

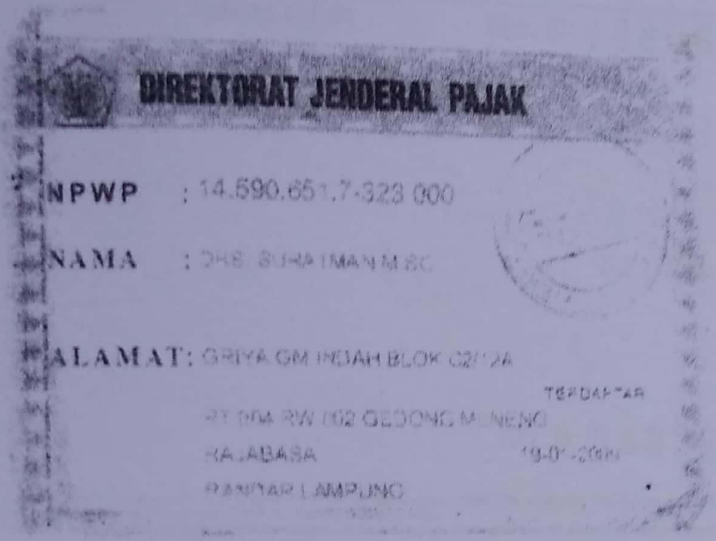
2. Jumlah uang tersebut pada angka 1, benar-benar dikeluarkan untuk pelaksanaan kegiatan penelitian dimaksud.

Demikian surat pernyataan ini dibuat dengan sebenarnya.

Bandar Lampung, 12 Juli 2019

METERAI TEMPEL 6000 ENAM RIBU RUPIAH

Drs. Suratman Umar, M.Sc
NIP 196406041990031002


DIREKTORAT JENDERAL PAJAK

NPWP : 14.590.651.7-323.000

NAMA : DRS. SURATMAN M.SC

ALAMAT: GRIYA GM INDAH BLOK C2/12A

TERDAFTAR

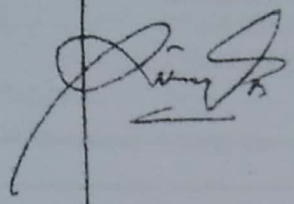
RT 004 RW 002 GEDONG MENENG

KAJABASA 19-07-2004

BANDAR LAMPUNG

Contoh Tanda Tangan

Pengesahan Petugas Bank



TANJUNG KARANG

No. D 5883641

Kantor Cabang

No. Rekening 0443717626 - IDR

Nama Bpk : SURATMAN

142 - 28691

TAPLUS (September'15) - GMU

NO. D 5883641

BNI

1. Penarikan BNI Taplus dapat dilakukan di semua Teller BNI di dalam negeri, di BNI ATM, ATM LINK, ATM Bersama dan ATM Prima di seluruh Indonesia serta ATM berlogo Cirrus atau Maestro di seluruh dunia.
2. BNI Taplus dilengkapi dengan fasilitas Kartu Debit BNI dan BNI e-Banking yang memudahkan Anda dalam melakukan transaksi perbankan.

PERHATIAN:

1. Periksa buku BNI Taplus Anda sebelum meninggalkan Bank.
2. Jika buku BNI Taplus/Kartu Debit BNI hilang, agar segera memberitahu Bank.
3. Penarikan tunai dengan surat kuasa hanya dapat dilakukan di Kantor Cabang pembuka rekening dengan membawa buku BNI Taplus dan menunjukkan asli identitas diri (KTP/SIM/Paspor) Pemberi dan Penerima Kuasa.
4. Penyalahgunaan buku BNI Taplus/Kartu Debit BNI oleh pihak ketiga yang bukan karena kesalahan Bank menjadi risiko penabung sepenuhnya.
5. Penarikan di Teller harus menyertakan buku BNI Taplus dan asli identitas diri yang berlaku. Penarikan dengan nominal tertentu di Teller yang rekeningnya memiliki fasilitas Kartu Debit BNI harus menyertakan Kartu Debit BNI.
6. Perubahan data nasabah agar dilaporkan kepada Bank.
7. Rekening yang tidak bertransaksi selama 6 bulan berturut-turut akan dinyatakan pasif (dormant). Rekening dormant dapat aktif kembali dengan transaksi pendebetan/pengkreditan melalui fasilitas BNI e-Banking, penarikan tunai, penyetoran tunai, pembelanjaan di merchant.
8. Rekening dormant yang tidak diaktifkan dan bersaldo nihil dalam jangka waktu tertentu akan otomatis ditutup oleh sistem.
9. Penggantian buku BNI Taplus yang habis karena mutasi/rusak dapat dilakukan di seluruh Kantor Cabang BNI di dalam negeri.
10. Penggantian buku BNI Taplus karena hilang yang memiliki fasilitas Kartu Debit dapat dilakukan di seluruh Kantor Cabang BNI di dalam negeri.
11. Penggantian Kartu Debit BNI karena hilang/masa berlaku telah tempo/rusak dapat dilakukan di seluruh Kantor Cabang BNI di dalam negeri.

Keterangan Kode Transaksi:

1. Penyetoran
2. Penarikan
3. Kliring
4. Pemindahbukuan
5. Pajak
6. Bunga
7. Pembentukan Kelebihan
8. Pemindahan Saldo
9. Biaya Administrasi
10. Akun Transaksi Db
11. Akun Transaksi Cr
13. Transaksi Debet Phone Banking
22. Penarikan ATM
23. Transaksi Kredit Phone Banking
24. Transaksi Maestro/POS
26. Transaksi Tarik Cirrus/Link/Bersama/Prima
26. Biaya Inquiry Cirrus/Link/Bersama/Prima
27. Pembayaran-Pembayaran
28. Biaya Tarik Cirrus/Link/Bersama/Prima
42. Pemindahan melalui ATM
83. Biaya Penolakan Transaksi Cirrus karena dana tidak cukup
84. Biaya Penolakan Transaksi Cirrus akibat hal-hal lain
92. Reversal/Pembatalan ATM
94. Reversal/Pembatalan Maestro/POS
95. Reversal/Pembatalan Tarik Cirrus/Link/Bersama/Prima
96. Reversal/Pembatalan Biaya Inquiry Cirrus/Link/Bersama/Prima
98. Reversal/Pembatalan Pembayaran
97. Reversal/Pembatalan Biaya Tarik Cirrus/Link/Bersama/Prima
90. Reversal/Pembatalan Biaya Tarik Cirrus/Link/Bersama/Prima

FINAL REPORT

PENELITIAN KERJASAMA INTERNASIONAL (PKSI)
UNIVERSITAS LAMPUNG

INVESTIGATION OF ANTIBACTERIAL BIOACTIVE COMPOUNDS FROM JENGKOL PLANT (*Archidendron jiringa* (Jack) I. C. Nielsen) THROUGH *BIOASSAY GUIDED FRACTINATION* APPROACH

RESEARCH TEAM:

Dr. Noviany, M.Si	**NIDN: 0019117301; SINTA ID: 259839**
Andi Setiawan, Ph.D	**NIDN: 0022095803; SINTA ID: 6670317**
Prof. Dr. Sutopo Hadi, M.Sc	**NIDN: 0030056902; SINTA ID: 37012**
Dr. Bambang Irawan, M.Sc	**NIDN: 0003036504; SINTA ID: 6047876**

COLLABORATOR:
Dr. Mohamad Nurul Azmi Mohamad Taib
UNIVERSITI SAINS MALAYSIA

Funded by:
DIPA BLU Universitas Lampung
No. 3093/UN26.21/PN/2019, Date: 10 July 2019

FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM
UNIVERSITAS LAMPUNG

NOVEMBER 2019

HALAMAN PENGESAHAN
PENELITIAN KERJASAMA INTERNASIONAL UNIVERSITAS LAMPUNG

Judul Penelitian	: Investigation of Antibacterial Bioactive Compounds from Jengkol Plant (*Archidendron jiringa* (Jack) I. C. Nielsen) Through *Bioassay Guided Fractination* Approach
Manfaat Sosial Ekonomi	: Membantu Meningkatkan Kesehatan Masyarakat
Jenis Penelitian	: Penelitian Terapan
Ketua Peneliti	
a. Nama Lengkap	: Dr. Noviany, M.Si
b. NIDN	: 0019117301
c. Sinta ID	: 259839
d. Jabatan Fungsional	: Lektor Kepala
e. Program Studi	: Kimia
f. Nomor HP	: 089628708515; 081377792816
g. Alamat surel (email)	: noviany@fmipa.unila.ac.id
Anggota Peneliti (1)	
a. Nama Lengkap	: Andi Setiawan, Ph.D
b. NIDN	: 0022095803
c. Sinta ID	: 6670317
d. Program Studi	: Kimia
Anggota Peneliti (2)	
a. Nama Lengkap	: Prof. Dr. Sutopo Hadi, M.Sc
b. NIDN	: 0030056902
c. Sinta ID	: 37012
d. Program Studi	: Kimia
Anggota Peneliti (3)	
a. Nama Lengkap	: Dr. Bambang Irawan, M.Sc
b. NIDN	: 0003036504
c. Sinta ID	: 6047876
d. Program Studi	: Biologi
Jumlah mahasiswa yang terlibat	: 2 orang
Jumlah alumni yang terlibat	: -
Jumlah staf yang terlibat	: 1 orang
Lokasi Kegiatan	: Laboratorium Kimia Organik dan LTSIT Unila
Lama Kegiatan	: 8 bulan
Biaya penelitian	: Rp. 75.000.000,-
Sumber Dana	: DIPA BLU Universitas Lampung

Bandar Lampung, 24 Oktober 2019

Menyetujui
Ketua LPPM

Prof. Dr. Ir. Hamim Sudarsono, M.Sc
NIP.196001191984031002

Pengusul
Dekan FMIPA Universitas Lampung

Drs. Suratman Umar, M.Sc
NIP.196406041990031002

SUMMARY

Infectious diseases caused by bacteria has become the global health issues especially antibacterial drug resistance. The most serious concern with antibacterial resistance is that some bacteria have become resistant to almost all antibacterial drugs and making them less effective. Overusing or misusing of these drugs can make resistance develop even faster. Various efforts have been made by many researchers to find antibacterial drugs including through chemical constituent studies from natural plants. Plants are the one of the most important natural resources because they are relatively safe from the side effects and cheap. One of the potential plants that has antibacterial properties is the jengkol plant (*Archidendron jiringa* (Jack) I. C. Nielsen). Jengkol plant is the one of Lampung's natural resources that has not been optimally used, and its utilization is in line with Unila's superior research focus in the field of technology development and drug health. In this research, a chemical constituents study of roots and stem barks of jengkol plants have been carried out through several stages including isolation, purification, identification, and biological activity evaluation. The stages of isolation of secondary metabolites were carried out by extraction technique followed by fractionation using chromatographic methods such as vacuum liquid chromatography (VLC), and column chromatography (CC). Meanwhile, characterization and identification of the active fraction was carried out by proton Nuclear Magnetic Resonance (^{1}H-NMR). The pure compounds will be tested for their biological activity against grams positive *Bacillus subtilis* and negative bacteria *Escherichia coli* using the agar disc diffusion and dilution method. The research outputs obtained has been prepared as a draft manuscript that ready to submit to international reputable scientific journals such as the Brazillian Journal of Pharmacognosy (International, Elsevier, IF: 1.754, Q2) or Pharmaceutical Biology (International, Q2 Scimago, Scopus indexed).

Keywords: Jengkol plant; *Archidendron jiringa*; antibacterial; bioassay guided fractionation; grams positive and negative bacteria

PRAKATA

Bismillah,

Alhamdulillah puji dan syukur penulis ucapkan ke hadirat Alloh Subhanahu wata'ala atas segala rahmat dan karunia-Nya laporan kemajuan penelitian ini dapat diselesaikan dengan baik sesuai dengan doa dan harapan. Laporan kemajuan dengan tema penelitian:" Investigation of Antibacterial Bioactive Compounds From Jengkol Plant (*Archidendron jiringa* (Jack) I. C. Nielsen) Through *Bioassay Guided Fractination* Approach" ini merupakan salah satu persyaratan yang harus dibuat dan menjadi bagian dari rangkaian kegiatan penelitian secara keseluruhan.

Dalam pelaksanaan kegiatan penelitian dan penulisan laporan kemajuan ini tidak lepas dari berbagai kesulitan dan rintangan, namun itu semua dapat penulis lalui berkat rahmat dan pertolongan-Nya serta bantuan dan dorongan semangat dari berbagai pihak yang membantu baik secara langsung maupun tidak langsung.

Pada kesempatan ini, penulis menyampaikan terimakasih sedalam-dalamnya kepada:

1. Direktorat Riset dan Pengabdian Kepada Masyarakat Kementerian Riset, Teknologi, dan Pendidikan Tinggi atas dana hibah penelitian yang diberikan melalui skim DIPA-BLU Penelitian Kerjasama Internasional (PKSI) Tahun Anggaran 2019.
2. Lembaga Penelitian dan Pengabdian Kepada Masyarakat Universitas Lampung yang telah memberikan kepercayaan, banyak dukungan dan fasilitas sehingga penelitian ini dapat terlaksana dengan baik.
3. Jurusan Kimia Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Lampung atas fasilitas sarana dan prasarana laboratorium demi terselenggaranya penelitian ini.
4. Prof. Dr. Sutopo Hadi, M.Sc, selaku anggota dalam tim penelitian, terimakasih atas bantuan, saran, dan kerjasamanya sehingga penelitian ini dapat terlaksana dengan baik.

5. Andi Setiawan, Ph.D, selaku anggota dalam tim penelitian, terimakasih atas bantuan dan kerjasamanya selama penelitian dilakukan.
6. Dr. Bambang Irawan, M.Sc selaku anggota dalam tim penelitian, terimakasih atas bantuan dan kerjasamanya selama penelitian dilakukan.
7. Dr. Mohamad Nurul Azmi Mohamad Taib dari Department of Chemical Sciences, Universiti Sains Malaysia atas bantuan, kerjasama, saran dan masukannya yang berharga dalam riset ini.
8. Bapak Dr. Eng. Suripto Dwi Yuwono, M.T., selaku ketua Jurusan Kimia Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Lampung atas dukungannya selama penelitian.
9. Wiwit Kasmawati, PLP Laboratorium Kimia Organik, yang telah memberikan bantuan dan kerjasama yang baik dalam pelaksanaan penelitian ini.
10. Dicky Sialdian dan semua anggota NRG atas segala bantuan, dukungan, kerjasama, dan kesabarannya yang luar biasa dalam menjalani penelitian ini.
11. Semua pihak yang tidak dapat dituliskan satu persatu yang telah membantu penulis sehingga penelitian ini dapat terselenggara dengan baik.

Semoga Alloh Subhanahu wata'ala membalas segala kebaikan bapak dan ibu serta semua pihak yang telah membantu dalam penelitian ini.

Penulis sangat menyadari bahwa laporan ini masih jauh dari kesempurnaan, namun semoga hasil penelitian ini dapat memberikan manfaat yang banyak kepada para pembaca khususnya baik penulis pribadi. Aamiin.

Bandar Lampung, 28 Oktober 2019
Peneliti

Dr. Noviany, M.Si

CONTENTS

PAGES

I. INTRODUCTION

1.1 Research Background

Infection disease is a serious global health problem and its caused the deaths of 13 million people worldwide every year, especially in developing countries like Indonesia. WHO data shows that viral, bacterial, fungal and parasitic infections are the biggest cause of death for the world's population. The use of antibiotics in the prevention of infectious diseases is the only solution[1]. While the continuous use of antibiotics raises the new problems for health especially the resistance of microorganisms that cause infection.

Bacteria are the most abundant organisms and widespread in living things. In terms of distribution and number, most bacteria may cause disease in humans and other living things[2]. *Bacillus subtilis* and *Escherichia coli* are the common pathogenic bacteria in humans, *E. coli* is a gram-negative bacterium found in the large intestine of humans, and it is a major cause of diarrheal diseases, especially in infants and children[3,4]. The mortality rate from diarrhea in Indonesia is still around 7.4%, while the mortality rate due to persistent diarrhea is higher at 45%. In 1000 inhabitants, 200-374 subjects experience diarrhea with 60-70% of whom are children under the age of 5 years[5].

Bacterial resistance to antibiotics is a problem that has not been resolved until now. Refdanita et al.[6] found that *E. coli* was resistant to chloramphenicol group antibiotics by 83.9% and amoxicillin by 86.2%. The greater the percentage of bacterial resistance to an antibiotic state that bacteria are no longer susceptible to these antibiotics. Various studies to overcome the bacterial resistance have been carried out, but there have been no effective reports. Thus, it is necessary to look for new antibacterial substances that are still active and selective. The search for antibiotic sources from natural ingredients is still the main trend for researchers. The ability of bioactive compounds of natural materials as a healing medium is estimated because of the content of secondary metabolites, including terpenoids, steroids, coumarin, flavonoids, and alkaloids.

One of the plants that has not been studied intensively in Indonesia is the family of Fabaceae. The Fabaceae family has quite interesting bioactivity such as antioxidants, antimalarial, anticancer, and antibacterial. The jengkol plant

(*Archidendron jiringa* (Jack) I. C. Nielsen) which is belongs to the family Fabaceae is commonly used by the Indonesian people as traditional medicine. Jengkol leaves are efficacious as medicine for eczema, scabies, sores, and ulcers, the skin of the fruit is used as ulcer medicine[1]. Several studies have been carried out on jengkol plants, both the leaves, fruit peel, and seeds. Nurussakinah (2010)[7] has carried out the phytochemical screening on jengkol fruit peel, seeds, bark and leaves extracts. Based on these screening its contain alkaloids, steroids, triterpenoids, glycosides, saponins, flavonoids, and tannins. While research on the roots and bark of the jengkol plant has never been done.

Based on above explanation, it is necessary to do chemical research on the stem bark of the jengkol plant (*A. jiringa*) by isolating and identifying secondary metabolites, followed by testing the antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aurogenosa*. Bioassay-guided approach through their antibacterial activity will be used to isolate the bioactive compounds.

1.2 Objectives of Research

This study will be conducted through 3 stages: extraction, isolation / fractionation / purification based on antibacterial bioassays-guided approach. Antibacterial testing shall be carried out using a dilution technique using grams positive and negative bacteria. The specific objective of the study is to obtain antibacterial active fractions/compounds from the roots and bark of *A. jiringa* which are described in detail in the research method.

1.3 Importance of Research

The utilization of Lampung's local natural resources is one of the leading research focuses by the University of Lampung (Unila) in the field of technology development and medicinal health. The antibiotic resistance is the major issues and its encourages researchers to study the antibacterial potential of available Lampung natural products which is not been studied and utilized optimally. For example, jengkol plants was chose to be studied because the chemical composition from this plant have potential to explored as a natural antibacterial substance. This finding can be used for preclinical screening *in vitro* / *in vivo* for the discovery of new antibiotic materials. It also reflexes the Unila's strategic plan in the prevention of infectious and tropical diseases caused by bacterial.

The theme proposed in this project is relevant to the 2016-2020 Unila research strategic plan, and in line with the superior field road map outlined in the theme research roadmap: Superior Material Based on Natural Resource Conservation, output/expected product is a superior biological-based material product and local natural resource-based organic active product[8].

Based on this strategic plan and road map of researcher (Fig.1), the jengkol plant which grows in Lampung will be used as a source of natural ingredients that is very potential to be developed. The results of the research obtained are expected to increase the economic value of jengkol plants and help improve the welfare and health of the community through research that is synergized with other fields such as medicine, pharmacy, and management in a sustainable manner. Thus, this research is very urgent to be carried out to support the strategic plan achievements and the 2016-2020 Unila research road map.

II. LITERATURE REVIEW

Indonesia's biodiversity, especially on high and low levels of plants, places Indonesia as the third 'megadiversity' country after Brazil and China. This wealth makes research and exploration of new sources of bioactive compounds from plants will be more practical and beneficial especially for researchers in Indonesia. Because until now, plants still play an important role in the discovery of new drugs to overcome various human diseases[9]. According to Tulp et al.[10], it is estimated that nearly 50% of the circulating drugs are now sourced from plants. Therefore, interest in the source of bioactive compounds can help make the new drug industry more efficient.

Exploration of new bioactive compounds from Indonesian plants, has become one of the prospective and profitable research focuses, especially for the pharmaceutical industries. Because until now, plants still play an important role in the discovery of new drugs to overcome various diseases. The Leguminosae or Fabaceae plant family, especially the species in the Papilionoideae subfamily has long attracted the attention of researchers not only due to variations in the structure of the compounds produced, but also because of their interesting biological activity. Several types of secondary metabolite compounds such as alkaloids, flavonoids, coumarins, phenylpropanoids,

anthraquinones, terpenoids and cyanogenate glycosides have been successfully isolated from this plant family[11]. Among the various groups of compounds, the type of isoflavonoids is the main component found in the subfamily Papilionoideae.

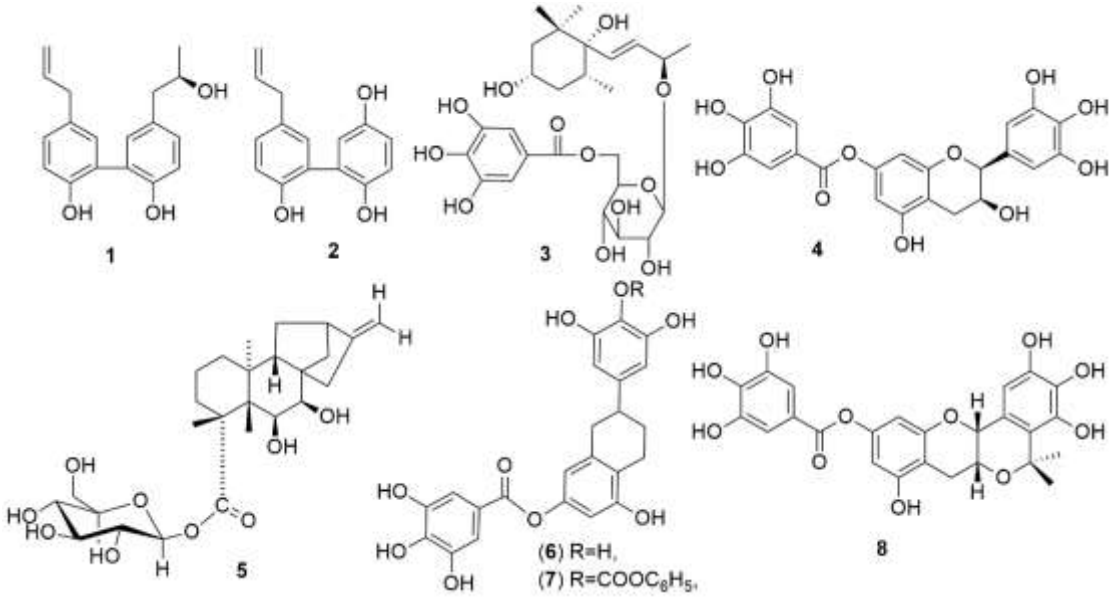
In 2007, Veitch[12] reported that more than 420 new compounds of isoflavonoid were successfully isolated from this plant. In line with these findings, it turns out that in nature, this group of compounds has a broad distribution of biological functions such as antimicrobials, insect repellents, allelopathic substances[13], inhibitors of pathogenic/disease attacks[14]. In addition, the results of clinical studies of isoflavonoid compounds showed a positive effect on human health and nutrition including in the prevention of heart disease, menopausal symptoms and osteoporosis[15-18].

Among 13,000 species in subfamily Papilionoideae, *A. jiringa* is one of the most potent medicinal plants to be developed as a new source of bioactive compounds. Several chemical studies have been reported from different species, including Lou et al.[19], who have isolated two new 3,3'neolignan compounds, namely clypearianine A (**1**) dan randaiol (**2**) from the twigs and leaves of *Pithecellobium clypearia* which exhibited antioxidant activity. Other researchers reported that a new clypearoside (**3**) along with a stereoisomer, (-)-(2S,3S)-epigallokatecin-7-gallate (**4**) has been obtained from the water leaves and stem bark extracts of the same plant[20].

In 2008, a new compound was identified as (-) 19-β-D-glucopyranosil-6,7-dihydroxikaurenoate (**5**) from *P. albicans* seeds[21]. In addition, Li et al[22] have successfully isolated two antiviral flavan derivatives that were identified as 7-O-galloyltricetifavan (**6**) and 7,4′-di-O-galloyltricetifavan (**7**) from the methanol extract of *P. clypearia* leaves. Furthermore, compound (**8**) has been obtained from the twigs and leaves of the same plant as well which showed to inhibit the expression of proinflammatory cytokines IL-6 or MCP-1 induced by influenza H1N1 virus in human A549 lung carcinoma cells[23]. According to the literature search, the most previous study that has been done were carried out on the seeds as well as the leaves of plants, while the root and stembark had not been done intensively. Therefore, in this study, the chemical investigation will be conducted on the root and stembark of *A. jiringa* extract and the antibacterial activity will be evaluated.

Based on the research roadmap of the researcher (Fig.1) which is in line with Unila's strategic plan, exploration of the secondary metabolites content from the

Leguminosae family plants through isolation and characterization techniques has been carried out since the last decade. From previous studies that have been done on the roots of turi plants (*Sesbania grandiflora*), obtained one terpenoid, betulin acid, seven compounds isoflavonoids, xenognosin B, liquiritigenin, 7,2',4'-trihydroxyisoflavone, demethylvestitol, vestitol, medicarpin, sativan, together with one new natural phenolic compound, 1,1'-bi-2-naphthol[24,25]. All isolated compounds were tested for their activity in vitro against strains of *M. tuberculosis* bacteria. Another recent study on the part of *S. grandiflora* bark has been isolated from two new phenolic compounds, sesbagrandiflorain A and B which also show anti-TB bioactivity in the medium category[26]. Based on the results of these studies, it can be stated that *S. grandiflora* is a valuable traditional medicinal plant especially in dealing with diseases caused by bacterial infections.



Jengkol plant (*A. jiringa*) have close kinship with turi plant, both of which are included in the Leguminosae family. Based on the chemotaxonomy of the plant, jengkol plant is predicted containing the same bioactive components as turi plant. This fact is also supported by the traditional knowledge of Indonesian in particular Lampungnese people who have used some parts of the jengkol plant as traditional medicine. Therefore, the study of jengkol plant is urgent to be done to find alternative sources of other bioactive compounds and clarify other potential of these plants as a

useful source for the discovery of new types of drugs, especially those that have the potential as antibiotics.

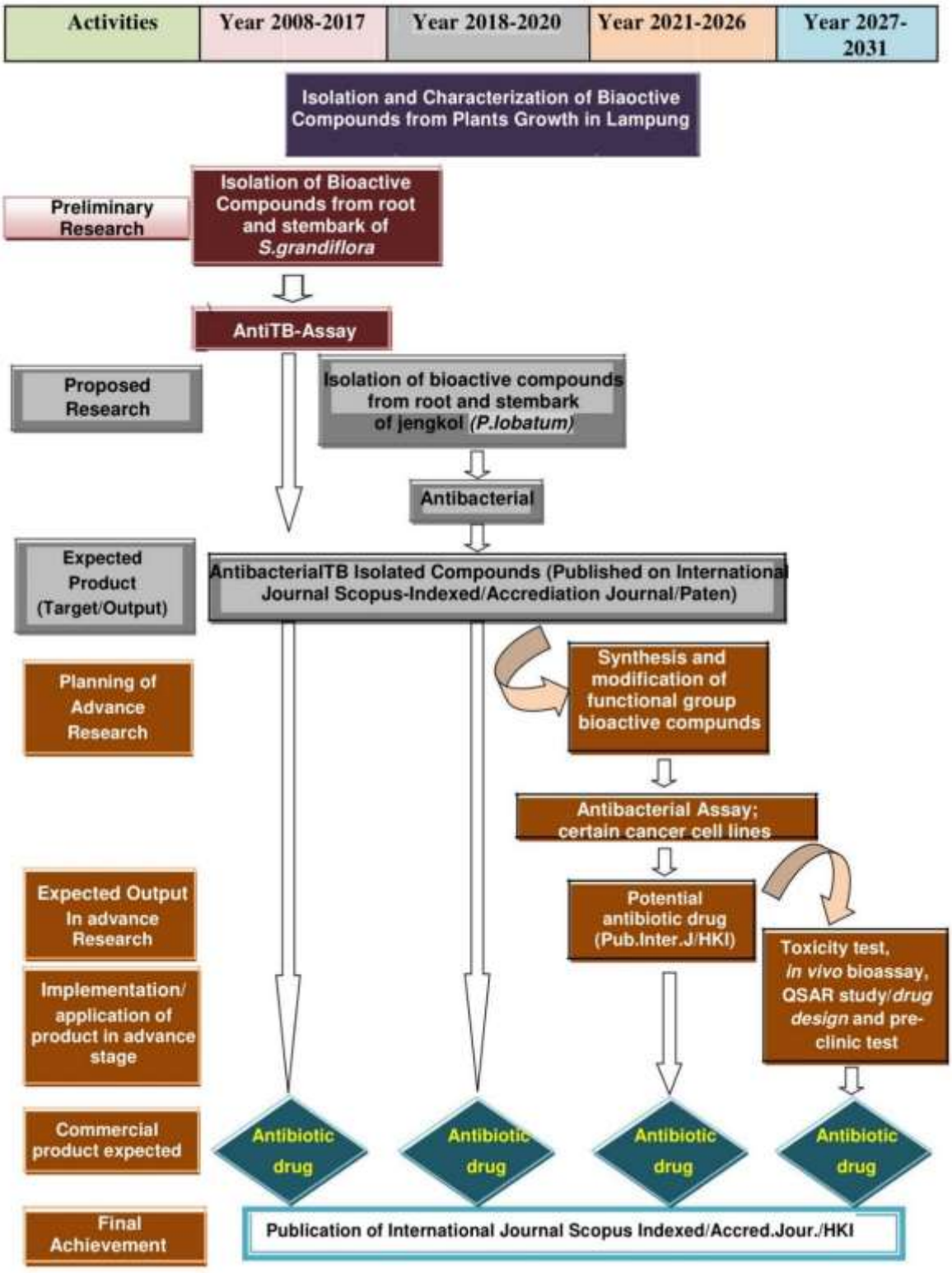


Figure 1. The Road Map of Research

In this study, isolation and identification of bioactive compounds from the root bark and stem bark of *A. jiringa* were carried out. Bioactive compounds were obtained through the bioassay guided fractination approach which relies on antibacterial tests in vitro against gram positive and negative bacteria. Pure compounds that show strong antibacterial activity will be used for the next stage of research such as in vivo and pre-clinical trials with collaborators from various other disciplines.

III. RESEARCH METHODOLOGY

This research will be carried out for approximately 7 months, starts from March to September 2019 in Organic and LTSIT Laboratories University of Lampung and NMR instrument facility at Universiti Sains Malaysia. The stem roots of *A. jiringa* were collected in March 2019 in Gedongmeneng, Bandar Lampung, Indonesia. The identity of the plant specimen was authenticated and deposited at the Bogoriense Herbarium, Bogor, Indonesia.

3.1 Instruments, Chemicals, and Solvents

The laboratory equipments and instruments will be used in this study, including laboratory glasswares, rotary evaporator, thin layer chromatography (TLC), vacuum liquid chromatography (VLC), analitical balance, Eppendrof tubes, melting point apparatus, UV lamp, NMR, and UV-Visible spectrophotometers.

The commercial chemicals and reagents used in the isolation and characterization of all the isolated compounds were as follows: acetone, acetonitrile, benzene, cerium (IV) sulphate tetrahydrat, chloroform, dichloromethane, diethyl ether, dimethyl sulfoxide, ethanol, ethyl acetate, *n*-hexane, hydrochloric acid, isopropylalcohol, methanol, sephadex LH-20 silica gel G 60, silica gel 60 GF_{254}, silica gel 60 PF_{254} containing gypsum, TLC aluminium sheets, and toluene.

3.2 Antibiotics, Bacterial Strains, and Consumables

Chloramphenicol, amoxicillin; gram positive bacteria: *Bacillus subtilis and* gram negative bacteria: *Escherichia coli*; nutrient agar broth, Resazurin; disposable sterile petri dishes, disposable syringe, micropipette tips, tissue culture plates 96-well.

3.3 Experimental Procedures

A. Preparation of extracts

Fresh chopped roots/stembarks (2.5 kg/each part of plant) were cleaned by rinsing under running tap water to remove soil and dirt. The roots was dried in an open space for three weeks and the air-dried roots are finally ground into powder form. The powdered air-dried roots (1.5 kg) were extracted with polar gradient solvent maceration technique. The solvent used at this stage starts from the solvent which has the lowest polarity, *n*-hexane, followed by ethylacetate and ends with a high polarity organic solvent, methanol. Each extraction was going carried out three times in each type of solvent. Before changing the type of solvent, the extract residue was first dried air for at least three days or until the residue is dry / free of solvent. The filtrates obtained from maceration obtained from polar gradient solvents were then separated from the residue by ordinary filtration. The filtrates were then concentrated under reduced pressure using a vacuum rotary evaporator. The process of isolation and purification have been carried out to concentrated methanol extract obtained.

B. Isolation and purification

Isolation and purification of methanol extract from roots/stembarks of jengkol plant was done in the usual way, through several stages of chromatographic separation which includes vacuum liquid chromatography and column chromatography using various gradient organic solvents polarity such as *n*-hexane, chloroform, dichloromethane, ethylacetate, acetone, isopropyl alcohol and methanol, either in the form of a mixture with a certain ratio or without a solvent mixture. The active extract was then purified by column chromatography using a suitable solvent. The biactive fraction was determined by determination of NMR spectroscopy and by thin layer chromatography (TLC) using eluent variations.

C. Structure Elucidation

Determination of the predictions of bioactive fraction has been determined spectroscopically using NMR spectroscopic analysis.

3.4 Screening of the Antibacterial activity

For the testing antibacterial activity, the microbial strains employed in the biological assay are Gram positive bacteria: *Bacillus subtilis and* gram negative bacteria: *Escherichia coli* (obtained from stock culture Hospital Abdul Muluk, Bandar Lampung, Indonesia). Original culture were further stored at low temperature in the

refrigerator to maintain stock culture. Fresh cultures were used for testing antibacterial activity using disc diffusion assay method.

A. Disc diffusion assay

The antibacterial activity of the stem roots extract (*n*-hexane, ethyl acetate and methanol) have been tested by disc diffusion method[27] against pathogenic bacteria gram negative (*Escherichia coli*) and gram positive (*Bacillus subtilis*). In this method, freshly prepared agar media was dispensed into the sterilized petri-dish. The agar was allowed to solidify and 100µl of bacterial suspension poured over the agar media and spread by a spreader or a rod. Ampicillin and chloramfenicol (30µg/dish) were used as positive control, while methanol was used as a negative control. In the each culture medium petri-dish four dish were used, one disk of antibiotics, two discs separately for (*n*-hexane, ethyl acetate and methanol) extracts, one disk used as a control (methanol). The plates were sealed and incubated overnight at 37ºC in the incubator. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The diameter of zone of inhibition (mean of three replicates SD) as indicated by clear area was measured to determine the antibacterial activity. The experiment was replicated three times to confirm the reproducible.

B. Determination of MIC via Resazurin assay

Resazurin assay has been performed in 96 well titration plates with some modification[28]. In complete nutrient broth two fold dilutions of plant extracts and antibiotics were prepared in the test wells. The final concentration were 20µ of each bacterial suspension was added to 180 µl of antibiotics and plant extracts (30-0.02mg/ml in sequence) contained in culture medium as well as the antibiotics concentration will be 0.06 mg/l amphicilin and chloramfenicol 0.12mg/l. For comparative study control plates were prepared only with culture medium and bacterial suspension. The plates were sealed and incubated for 12 hours at 37 C for additional 5 hour. At the intervals of 1 hour plates were observed for colour change blue to pink and pink to colorless in live bacterial strains containing wells. Preliminary micro titre plate assay revealed that the fast decolonization of resazurin extract didn't possessed antibacterial potential. The bioactivity of extracts were screened which showed that the extracts inhibit the dye reduction. The flow chart of the research can be seen in Fig.2.

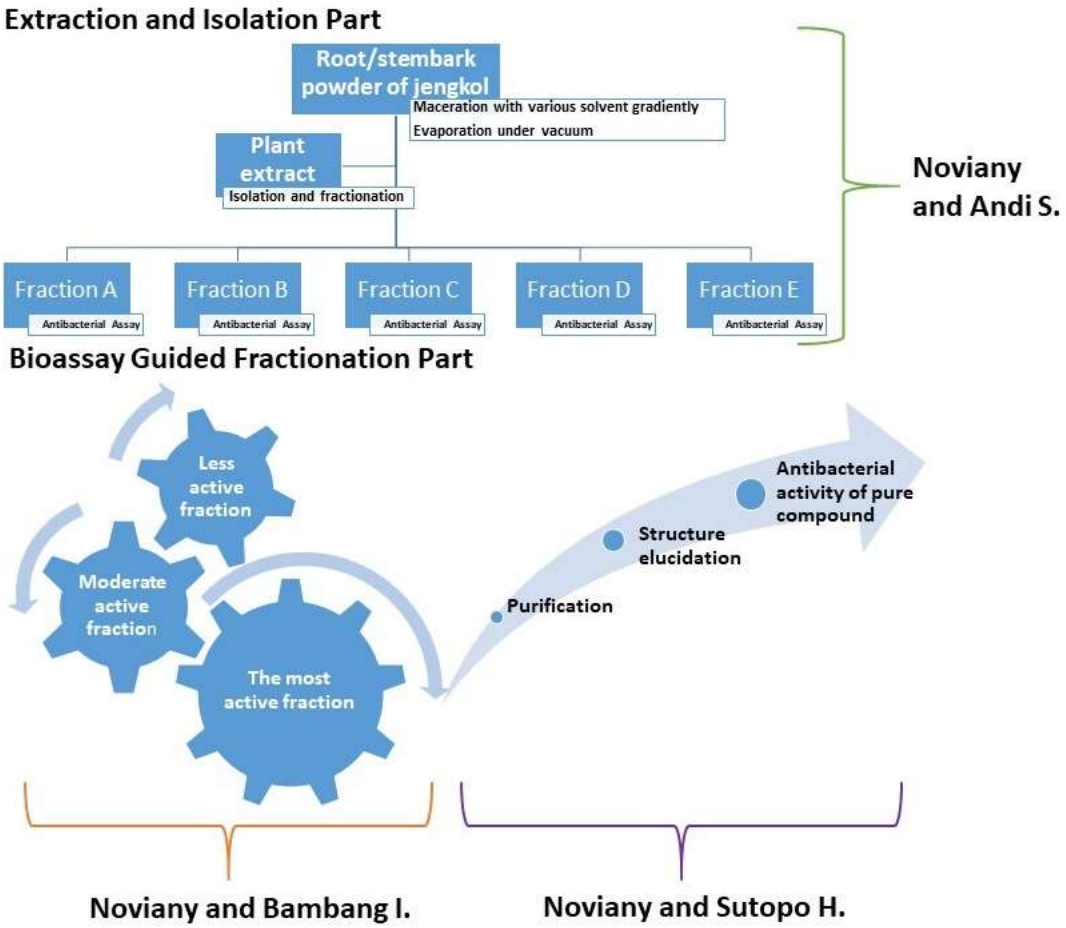
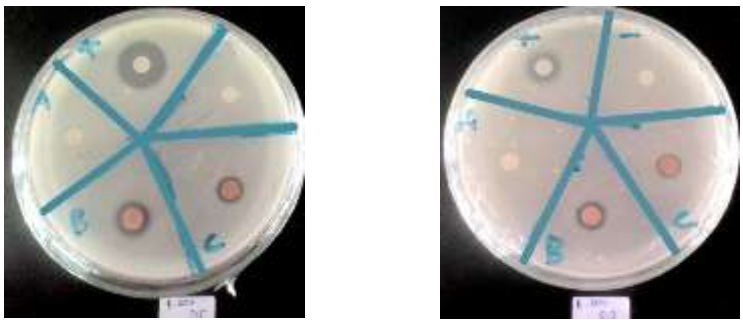


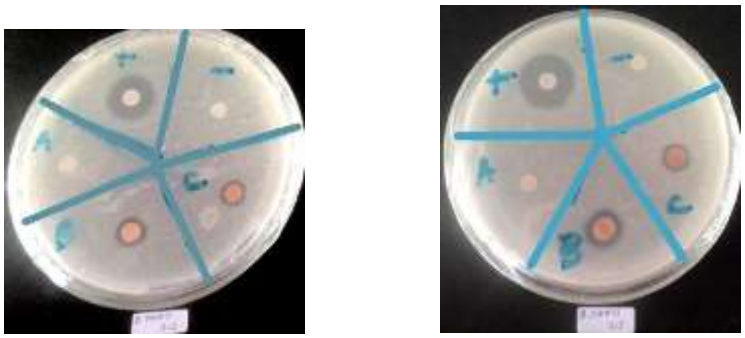
Figure 2. The Flow Chart of Research

IV. RESULTS AND DISCUSSIONS

The plant material was macerated using gradient polarity of solvent and afforded *n*-hexane (3.6 kg), ethyl acetate (55.8 kg), and methanol (67.2 kg) extracts. EtOAc extract performed the most bioactive extract against two bacterial strains, *Escherichia coli* and *Bacillus subtilis* using agar disc diffusion (Fig. 3; Table 1) and microdilution methods (Fig. 4; Table 2).



(a) (b)



(c) (d)

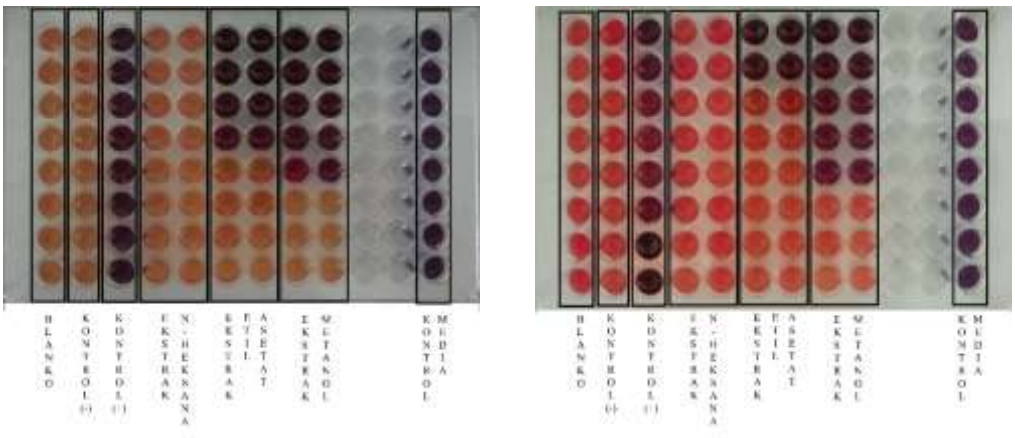
A: *n*-hexane; B: EtOAc; C: methanol; (+): chloramphenicol (*B. subtilis*) and amoxicillin (*E. coli*); (-): MeOH 12,5%

Figure 3. Antibacterial assay result by disc diffusion method (a) *E. coli* (0,5 g/disc); (b) *E. coli* (0,3 g/disc); (c) *B subtilis* (0,5 g/disc); (d) *B. subtilis* (0,3 g/disc)

Table 1. Zone diameter of inhibition of stem roots extracts (in mm) against *E. coli* and *B. subtilis*

Concentration	Zone diameter of inhibition against *E. coli*			Zone diameter of inhibition against *B. subtilis*		
	***n*–hexane**	**EtOAc**	**MeOH**	***n*–hexane**	**EtOAc**	**MeOH**
0,3 mg / disc	-	10	8	-	8	7
0,5 mg / disc	-	11	9	-	9	8

Among three extracts tested as tabulated on Table 1, EtOAc extract was found as the most active with the inhibition zone ranging of 8-11 mm against both bacterial strains.



(a) (b)

Figure 4. Antibacterial assay result by dillution method (a) *E. coli*; (b) *E. coli*

Table 2. MIC's values of *n*–hexane, EtOAc, and methanol against *E. coli* and *B. subtilis*

No	Extract types	OD mean of *E. coli*	OD mean of *B. subtilis*	MIC's value (µg/mL) of *E. coli*	MIC's value (µg/mL) of *B. subtilis*
1	*n*–hexane	0.76838	0.54231	-	-
2	EtOAc	1.88706	1.48556	12,5	50
3	methanol	1.68644	1.66288	6,25	6.25
4	positive	1.773	1.48025	0,78	0,78
5	negative	0.74013	0.57575	-	-

Antibacterial bioactivity testing using dilution method against *E. coli* and *B. subtilis* bacteria was carried out to find out extracts that had better antibacterial bioactivity. Each extract was dissolved in 12.5% methanol and made in a concentration of 2 mg/ mL, then the ability of the three extracts was tested as an antibacterial agent. Methanol is a good solvent in the extraction of bioactive compounds, because methanol is able to dissolve all types of compounds ranging from those that are polar, semi-polar and even non-polar in small concentrations[29]. The test results based on Table 5 showed that semi-polar ethyl acetate extract and polar methanol have a minimum inhibitory concentration better than n-hexane extract. In the dilution antibacterial test it is not enough to use visual observation only to observe the presence or absence of bacterial growth. But this tends to be subjective from each person's eyesight so the risk of error is relatively greater. This is because the color test makes it difficult to observe, so absorbance values before and after incubation are used to help determine the presence or absence of bacteria. The wavelength used to measure the number of microbes is 600 nm, because cells in the mitochondria and cytoplasm absorb at that wavelength[30].

After incubating for 18 hours and measuring Optical Density, the reaction color was added to rezazurin and re-incubated for 4 hours until the color changes. This color change occurs because rezazurin has a blue color that does not flourescent and can be reduced to a flourescent pink color. The change in color from blue to pink is an indicator of cell reduction. Color change in rezazurin is carried out by enzymes in cells in the mitochondria and cytoplasm[31]. Based on the results of the antibacterial tests that have been carried out, ethyl acetate extract (EA) was chosen for the fractionation and purification process because it has the best antibacterial activity in

inhibiting bacterial growth to a minimum inhibitory concentration of 12.5 and 50 µg/mL compared to extract *n*-hexane which has no inhibition against all bacterial strains. Furthermore, the EA extract was monitored using thin layer chromatography (TLC) to determine the separation pattern using a combination of eluents in order to obtain the separation pattern of the components of the compound. The eluent combination used is ethyl acetate/acetone 1: 1 as can be seen in Figure 5.

(a) (b)

Figure 5. TLC chromatogram of EtOAC with eluent EtOAc / acetone 1:1 (a) sprayed by staining reagent of cerium(IV)sulphate; (b) observed under UV lamp ƛ 254 nm

Based on the antibacterial activity results of extracts, the ethyl acetate extract (55.8 g) was selected to be isolated and fractionated further using VLC method on silica-gel (35-70 Mesh). The column was eluted with a stepwise gradient polarity of solvent systems including *n*-hexane–EtOAc (100%-0% of *n*-hexane); EtOAc-acetone (100%-0% of EtOAc); and acetone-MeOH (100%-0% of acetone), affording 23 fractions (200 mL each). Due to the similarity of TLC's profile of some fractions using EtOAc-acetone (1:1) as eluent (Fig.2), these fractions were analysed also by ^{1}H-NMR spectroscopy. According to their chemicals profile analysed by TLC and their proton NMR spectrum, these fractions were grouped and combined into seven primary fractions, E_21 (fr.1-10), E_22 (fr.11), E_23 (fr.12), E_24 (fr.13), E_25 (fr.14-15), E_26 (fr.16-19), E_27 (fr.20-23). All fractions E_21- E_27 were tested for their antibacterial property separately using disc diffusion and microdilution methods. The MIC's values and optical density (OD) means of bioactive fractions tested were tabulated on Table 3.

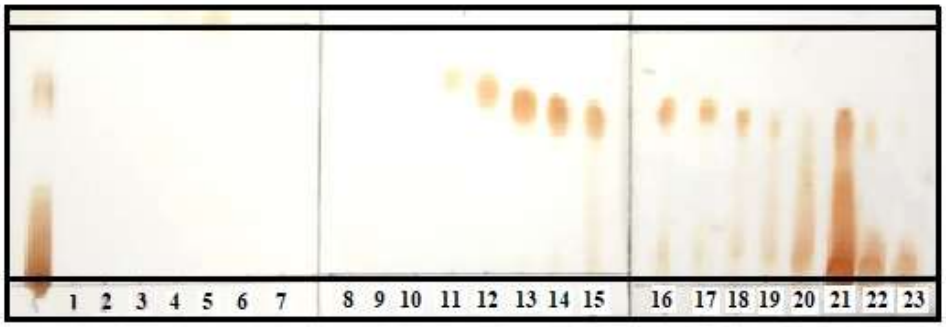


Figure 6. TLC chromatogram of EtOAc extract fractionation by VLC

Tabel 3. The MICS's values and optical density (OD) means of all fractions tested against *E. coli* and *B. subtilis*

No	Fraction code	OD mean against *E. coli*	OD mean against *B. subtilis*	MIC (µg/mL) against *E. coli*	MIC (µg/mL) against *B. subtilis*
1	$E_2$1	1.16363	1.02819	50	-
2	$E_2$2	1.49338	1.54488	25	25
3	$E_2$3	1.25269	1.74956	25	25
4	$E_2$7	1.39525	2.34931	25	6,25
5	Positive control	2.16563	2.28425	0,78	0,78
6	Negative control	0.86413	0.60838	-	-

Noted: (+) control: chloramphenicol (*B. subtilis*) and amoxicillin (*E. coli*); (-) control: MeOH 12.5%

Three fractions exhibited antibacterial activity against both bacterial strains with quite similar MIC's values, therefore fraction $E_2$2 (439.5 mg) was subjected to further fractionate due to the simplest chemical profile on its TLC (Fig.7). Fraction $E_2$2 was redissolved in acetone and then purified with CC on silica gel G-60 (35-70 Mesh) using *n* – hexane / isopropyle alcohol with the ratio volumes of 70/30, 60/40, 50/50, and 40/60, generating 6 major subfractions, $E_2$2a (132.0 mg); $E_2$2b (8.4 mg); $E_2$2c (9.0 mg); $E_2$2d (47.0 mg); $E_2$2e (2.0 mg); and $E_2$2f (50.3 mg). All subfractions were screened against both bacterial strains tested using microdilution methods. The MIC's values and optical density (OD) means of bioactive subfractions were described on Table 4. Among them, two subfractions ($E_2$2e and $E_2$2f) performed the most antibacterial activity against *B. subtilis* and *E. coli* with the MIC's values ranging of

12.5-25 µg/mL. Only subfraction $E_2$2f was selected to be analyzed further by ^{1}H-NMR spectroscopy as having sufficient quantity.

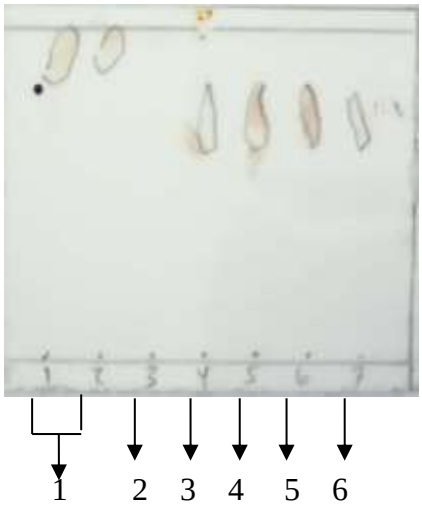


Figure 7. TLC chromatogram of CC of fraction $E_2$8$_1$ with eluent isopropyl alcohol / *n*–hexane (6/4)

Table 1. The MIC's values and optical density (OD) means of sub fractions tested against *E. coli* and *B. subtilis*

No	Subfractions code	OD mean against *E. coli*	OD mean against *B. subtilis*	MIC (µg/mL) against *E. coli*	MIC (µg/mL) against *B. subtilis*
1	$E_2$8$_1$1	1.49731	1.27825	50	100
2	$E_2$8$_1$2	1.44425	1.22788	50	50
3	$E_2$8$_1$3	1.29094	0.99919	25	50
4	$E_2$8$_1$4	1.259	0.84613	25	50
5	$E_2$8$_1$5	1.39463	1.18294	12,5	25
6	$E_2$8$_1$6	1.34638	1.141	25	25
7	Positive control	1.54525	1.45938	0,78	0,78
8	Negative control	0.85238	0.5695	0	0

Based on the results obtained it can be seen a decrease in the minimum inhibitory concentration compared to the antibacterial activity test on VLC results as observed on the purification results of fraction E_{13}. This fact occurs due to the possible

composition of active compounds contained in the sample. According to Priya et al.[32], when an increase or decrease in a component of an active compound there is a possibility of influencing the activeness of the compound itself. From the test results shown in Table 4, it was found that the E_28_16 subfraction gave a smaller value of the minimum inhibitory concentration compared to other subfractions both against *E. coli* and *B. subtilis* bacteria. Based on the activeness of the two subfractions, E_28_16 was then selected for ^{1}H-NMR analysis to predicted the structure of the E_28_16 subfraction.

The spectrum shown in Figure 8 indicates that the E_28_16 subfraction is not pure. However, interpretation of the ^{1}H-NMR data can still be done by referring to the results of the phytochemical test of the fraction which shows the content of phenolic compounds or flavonoids in the subfraction, therefore a comparison is sought in journals reporting the presence of phenolic or flavonoid compounds in the same plant type to estimate the types of bioactive compounds which is obtained. From the NMR spectrum of proton subfraction E_28_16 shows indications of aromatic protons in the chemical shift region δH 6 - δH 7 ppm. The signal for the methoxy group (-OCH_3) at δH 3.7 ppm, the proton signal at 8.01 ppm with the peak of the chemical shift of the hydroxyl group (-OH) attached to an aromatic ring. As well as the typical peaks for alkanes at 0.89 - δH 2.2 ppm.

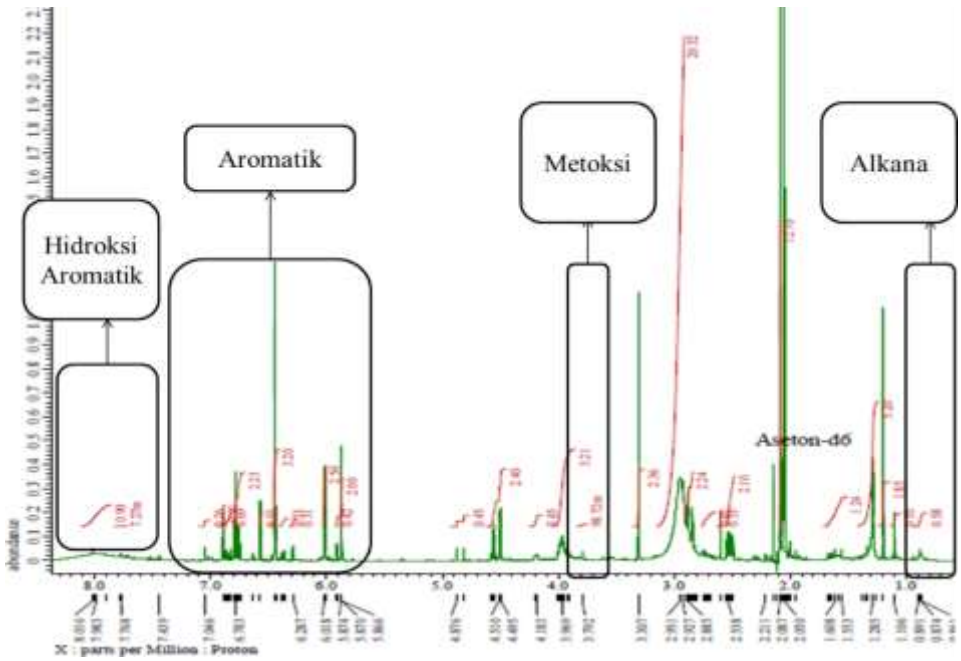


Figure 8. ^{1}H-NMR spectrum of E_28_16 subfraction

Sopian et al.[33] (2019) have succeeded in isolating phenolic compounds from the stem of the jengkol plant and were identified as 1-(2,6-dihydroxy-4-methoxyphenyl)-decan-1-one (Figure 19). Based on the comparison of the proton chemical shift values between the isolated subfaction and 1-(2,6-dihydroxy-4-methoxyphenyl)-decan-1-one, it is estimated that the main active compound contained in the $E_2 8_1 6$ subfaction is the phenolic compounds namely 1-(2,6-dihydroxy-4-methoxyphenyl) decan-1-one (Fig. 9). However, further purification in the E2816 subfaction still needs to be done to ensure the active compounds which are responsible for inhibiting the test bacteria.

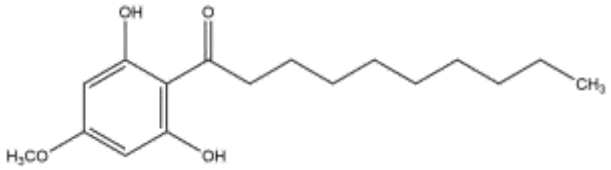


Figure 9. Proposed structure of $E_2 8_1 6$ subfaction: 1-(2,6-dihydroxy-4-methoxyphenyl)-decan-1-one

V. OUTPUTS

The targeted outputs of the research (both mandatory and additional) are mentioned as below:

1. Publishing in International reputable scientific journals (Targeted: submitted to Asian Pacific Journal of Tropical Medicine, Q2 Scimago, Scopus indexed or if possible the Journal of Asian Natural Products Research, Q2 Scimago, Taylor and Francis, IF: 1091). These journal targeted were proposed and mentioned in the proposal, but due to the insufficient data obtained from the research, the targeted journal was changed to Brazillian Journal of Pharmacognosy (International, Elsevier, IF: 1.754, Q2) or Pharmaceutical Biology (International, Q2 Scimago, Scopus indexed). Additionally, the status of paper in this research is still as a draft of manuscript (Appendix 1). The preparation of manuscript is still going in progress. However, we will update the status of the paper once it has been submitted and under review.
2. Attending to International Conference/Seminar as oral presenter (Targeted: registered). This output has been achieved and approved as can be seen in Appendix 2.

3. The Technology Readiness Level (TRL) has been achieved from this study is TRL 6.
4. Obtained the prototipe/lead componds: some bioactive fractions exhibited good antibacterial activity with the MIC's values ranging of 12.5-25 µg/mL (see Appendix 3).

The final output of the research is described on fishbone chart which can be seen on Fig.10.

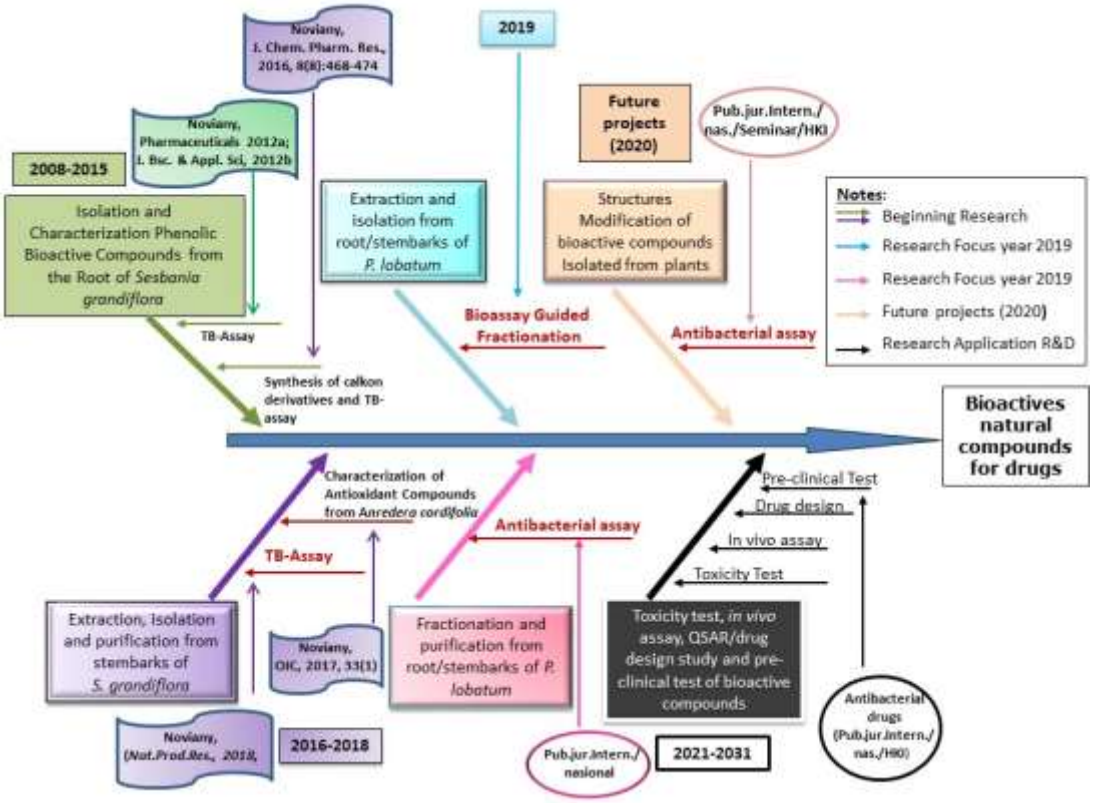


Figure 10. The Fishbone Chart of Research

VI. CONCLUSIONS

Based on the results of the research conducted it can be concluded that:

1. Ethyl acetate extract of jengkol (*Archidendron jiringa* (Jack) I. C. Nielsen) root bark has antibacterial activity against *E. coli* and *B. subtilis* bacteria which is better than *n*-hexane extract.
2. E13 fraction and E28 fraction have better antibacterial activity against *E. coli* and *B. subtilis* bacteria compared to other fractions from the same column.

3. E13 fraction and E28 fraction have better antibacterial activity against *E. coli* and *B. subtilis* compared to E13 subfaction and E28 fraction as a result of its purification.
4. Based on the ^{1}H-NMR result data the compounds detected in the E_28_16 subfraction is phenolic compound type.

VII. SCHEDULE OF RESEARCH

No	Aktivities	Month, year 2019							
		2	3	4	5	6	7	8	9
1	Sampling for roots and stembark of jengkol	■							
2	Maceration	■	■						
3	Extraction and fractionation		■	■	■				
4	Bioassay guided fractionation			■	■				
5	Isolation using chromatography techniques			■	■	■			
6	Purification				■	■	■		
7	Structure elucidation					■	■		
8	Antibacterial assay of pure compounds						■	■	
9	Final report and write up the manuscript							■	■

REFERENCE

1. Salni., H. Marisa, dan R.W. Mukti. 2011. Isolasi Senyawa Antibakteri dari Daun Jengkol (*Pithecolobium lobatum* Benth) dan Penentuan Nilai KHM-nya. *J. Pen. Sains*. 14: 1-2.

2. Siti Juariah, Shofriyanti Oktaviyani. The Activity Test Of Ethanol Extract Jengkol Skin (*Pithecellobium jiringa*) To Inhibit of Fungus Growth *Candida albicans*. *Prosiding*, 1-Sep 2016 1th Celscitech-UMRI 2016.

3. Susilowati, D.N., R.D. Hastuti, dan E. Yuniarti. 2007. Isolasi dan Karakterisasi Aktinomisetes Penghasil Antibakteri Enteropatogen *Escherichia coli* K1.1, *Pseudomonas pseudomallei* 02 05, dan *Listeria monocytogenes* 5407. *J. AgroBiogen*. 3 (1):15-23.

4. Maryati., R.S., Fauzia, dan T. Rahayu. 2007. Antibacteria Activity Test of *Ocimum basilicum* L. Toward *Staphylococcus aureus* and *Escherechia coli*. *J.Pen. Sains Tek.*. 8 (1): 30 – 38.

5. Rizal, M., Yusransyah, dan S.N., Stiani. 2016. Uji Aktivitas Antidiare Ekstrak Etanol 70% Kulit Buah Jengkol (*Archidendron paucifiorum* (Benth.) I.C.Nielsen) terhadap Mencit Jantan yang Diinduksi Oleum Ricini. *Jurnal Ilmiah Manuntung*. 2 (2): 131-136.

6. Refdenita., R. Maksum., A. Nurgani, dan P. Endang. 2004. Pola Kepekaan Kuman Terhadap Antibiotika di Ruang Rawat Intensif Rumah Sakit Fatmawati Jakarta Tahun 2001 – 2002. *MAKARA Kesehatan*. 8 (2). 41-48.

7. Nurussakinah. 2010. Skrining Fitokimia dan Uji Aktivitas Antibakteri Ekstrak Kulit Buah Tanaman Jengkol (*Pithecellobium jiringa* (Jack) Prain.) terhadap Bakteri *Streptococcus mutans, Staphylococcus aureus* dan *Escherichia coli. (Skripsi)*. Universitas Sumatra Utara. Sumatra Utara.

8. Lembaga Penelitian dan Pengabdian Kepada Masyarakat (LPPM) Universitas Lampung. 2017. Rencana Strategis Penelitian Universitas Lampung Tahun 2016-2020.

9. Balunas, M.J., and Kinghorn, A.D., 2005, Drug discovery from medicinal plants, *Life Sci*. 78: 431-441.

10. Tulp, M., Bruhn, J.G., dan Bohlin, L. 2006. Food for thought, *Drug Discov. Today,* 11 (23-24): 1115-1121.

11. Wink, M., and Mohamed, G. I. A. 2003. Evolution of chemical defense traits in the Leguminosae: mapping of distribution patterns of secondary metabolites on a molecular phylogeny inferred from nucleotide sequences of the *rbc*L gene. *Biochem. Syst. Ecol., 31*(8), 897-917.

12. Veitch, N. C. 2007. Isoflavonoids of the Leguminosae. *Nat.Prod.Rep. 24*, 417-464.

13. Dixon, R. A., and Sumner, L. W. 2003. Legume natural products: understanding and manipulating complex pathways for human and animal health. *Plant Physiol., 131*, 878-885.

14. Graham, T. L., and Graham, M. Y. 2000. Defence potential and competency, redox conditioning effects of salicylic acid genistein. *Plant Mic. Interac., 5*, 181-219.

15. Cogolludo, A., Frazziano, G., Briones, A. M., Cobeno, L., Moreno, L., Lodi, F., Salaices, M., Tamargo, J., and Perez-Vizcaino, F., (2007). The dietary flavonoid quercetin activates BKCa currents in coronary arteries via production of H_2O_2, role in vasodilatation. *Cardiovasc. Res., 73*, 424-431.

16. Cornwell, T., Cohick, W., and Raskin, I. 2004. Dietary phytoestrogens and health *Phytochemistry, 65*, 995-1016.

17. Di, X., Yu, L., Moore, A. B., Castro, L., Zheng, X., Hermon, T., and Dixon, D. 2008. A low concentration of genistein induces estrogen receptor-alpha and insulin-like growth factor-I receptor interactions and proliferation in uterine leiomyoma cells *Hum. Reprod., 23*, 1873-1883.

18. Kottra, G., and Daniel, H. 2007. Flavonoid glycosides are not transported by the human Na+/glucose transporter when expressed in *Xenopus laevis* oocytes, but effectively inhibit electrogenic glucose uptake. *J. Pharmacol. Exp. Ther. , 322*, 829-835.

19. Lou, L-L., Li, L-G., Liu, Q-B., Li, D-Q., Liu, Z-X., Huang, X-X., Song, S-J. 2016. 3,3'-Neolignans from *Pithecellobium clypearia* Benth and Their Anti-inflammatory Activity, *Fitoterapia*, 112, 16-21.

20. Li, L., Peng, Y., Hu, C., Liu, C., Liu, Q., Li, L., Wu, J., and Song, S. 2015. Antioxidant Activity of Chemical Constituents Isolated from *Pithecellobium clypearia, Rec.Nat.Prod*. 9:3, 284-291.

21. Mena-Rejon, G. J., Sansores-Peraza, P., Brito-Loeza, W. F., and Quijano, L. 2008. Chemical Constituents of *Pithecellobium albicans*, *Fitoterapia*, 79, 395-397.

22. Li, Y., Leung, K-T., Yao, F., Ooi, L.S.M., and Ooi, V.E.C. 2006. Antiviral Flavan form the Leaves of *Pithecellobium clypearia, J. Nat. Prod.* 69, 833-835.

23. Kang, J., Liu, C., Wang, H., Li, B., Li, C., Chen, R. and Liu, A. 2014. Studies on the Bioactive Flavonoids Isolated from *Pithecellobium clypearia* Benth, *Molecules*, 19, 4479-4490.

24. Noviany, Osman, H., Mohamad, S., Wong, K. C., Awang, K., and Zahariluddin, A. S. M. 2012a. The Chemical components of *Sesbania grandiflora* roots and their antituberculosis activity. *Pharmaceuticals, 5*, 882-889.

25. Noviany, Osman, H., Wong, C. K., Awang, K., and Manshoor, N. 2012b. Isolation and characterisation of l,l'-binaphthalene-2,2'-diol, A New Biaryl Natural Product from *Sesbania grandiflora* Root. *J. Bsc. & Appl. Sci., 8*, 253-256.

26. Noviany, Arief Nurhidayat, Sutopo Hadi, Tati Suhartati, Muhammad Aziz, Neny Purwitasari, and Iman Subasman. 2018. Sesbagrandiflorain A and B: isolation of two new 2-arylbenzofurans from the stem bark of *Sesbania grandiflora*. *Nat. Prod. Res.*, 32, 21, 2558–2564.

27. Bauer, A.W., Kirby, W.M.M, Sheriss. J.C et al., 1966. Antibiotic susceptibility testing by a standardized single disk method. *Ame. J. Clin. Pathol*.45: 493496.

28. Karuppusamy, S., and Rajasekaran, K.M. 2000. High Throughput Antibacterial Screening of Plant Extracts by Resazurin Redox with Special Reference to Medicinal Plants of Western Ghats Global. *J. Pharmacol*.3 (2): 63-68

29. Eloff, J. N. 1998. National Committee for Clinical Laboratory Standards (1990) Method for Dilution Antimicrobial Susceptibilty Test for Bacteria That Grow Aerobically - Second Edition; Approved Standard. *J. Ethnopharmacol*. 60. Hlm 1-8.

30. Clesceri, L. S., Greenberg, A. E., and Eaton, A. D. 1998. Standard Methods for The Examination Of Water and Wastewater. *American Public Health Association*. ISBN 0875532357. Hlm 1325.

31. Sarker, S. D., Nahar, L., and Kumarasamy, Y. 2007. Microtitre Plate - Based Antibacterial Assay Incorporating Rezazurin As An Indicator of Cell Growth, and Its Application In The *In Vitro* Antibacterial Screening of Phytochemicals. *Elsevier*. 42. Hlm 321-324.

32. Priya, V., Mallika, J., Surapaneni, K. M., Saraswathi, P., and Chandra, S. G. 2010. Antimicrobakterial Activity of Pericarp Exstract of *Garcinia mangostana* Linn. *International Journal of Pharma Sciences and Research*. 8. Hlm. 278-281

33. Sopian, A., Darmawan, A., dan Simanjuntak, P. 2019. Identifikasi Senyawa Kimia Fenolik Dalam Ekstrak Etil Asetat Kulit Buah Jengkol. *Ejournal Poltektegal*. 8(1). Hlm 51-55.

APPENDIX 1. DRAFT OF MANUSCRIPT

Bioassay-Guided Separation Approach for Characterization of New Antibacterial Fractions from the Stem Roots Extracts of *Archidendron jiringa*

Noviany Noviany[a,*], Dicky Sialdian[a], Andi Setiawan[a], Sutopo Hadi[a], Bambang Irawan[b], Mohamad Nurul Azmi[c]

[a]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung 35145, Lampung, Indonesia*

[b]*Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung 35145, Lampung, Indonesia*

[c]*School of Chemical Sciences, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia*

***Corresponding author at:** Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung 35145, Lampung, Indonesia

E-mail address: noviany@fmipa.unila.ac.id (N. Noviany).

ORCID ID

Noviany: 0000-0002-4046-6134

ABSTRACT

Infectious diseases caused by bacteria has become the global health issues especially antibacterial drug resistance. The most serious concern with antibacterial resistance is that some bacteria have become resistant to almost all antibacterial drugs and making them less effective. *Archidendron jiringa* is one of the most potent medicinal plants to be developed as a new source of antibacterial components. In current study, the separation of bioactive fractions of *A. jiringa* stem roots was carried out through several stages including isolation, fractionation, and characterization based on the antibacterial assay-guided approach. The stages of isolation of secondary metabolites were conducted by gradually extraction followed by fractionation using chromatographic methods. The antibacterial potential of extracts has been evaluated by the disc diffusion and microdilution methods employing the resazurin assay against one gram negative resistant bacteria, *Escherichia coli* and one positive bacteria, *Bacillus subtilis*. Among three extracts obtained, the ethyl acetate and methanol extracts performed the significant antibacterial effect, while no antibacterial activity was showed on the hexane extract. The fractionation of ethyl acetate extract led to the isolation of the most bioactive fractions ($E_2 8_1 5$ and $E_2 8_1 6$) with the MIC's values ranging of 12.5 - 25 µg/mL for both bacteria strains. Due to the less quantity, only the fraction $E_2 8_1 6$ was subjected to analyse by ^{1}H-NMR spectroscopy. The results exhibited that the bioactive fraction was obtained as a mixture of at least three major constituents. However, the purification of the bioactive fraction is required to further clarify the antibacterial compound that can be utilized as a new promising antibacterial agent. The bioassay-guided separation approach and the dye resazurin as an indicator of the growth of bacteria are applied for the first time for the phytopharmacological investigation from this plant. The present study represented the effective method for subsequent finding and isolation of potential novel antibacterial constituents from *A. jiringa* stem roots, in particular against the multi-drug resistant strains.

Keywords
Antibacterial assay, *Archidendron jiringa*, bioassay-guided separation, medicinal plant; resistant bacteria

ABBREVIATIONS

B. subtilis	*Bacillus subtilis*
CC	column chromatography
E. coli	*Escherichia coli*
EtOAc	ethyl acetate
IPA	isopropyl alcohol
IR	infrared
MeOH	methanol
M. tuberculosis	*Mycobacterium tuberculosis*

NMR	nuclear magnetic resonance
TLC	thin layer chromatography
UV	ultraviolet
VLC	vacuum liquid chromatography

Introduction

Infection disease is a serious global health problem and its caused the deaths of 13 million people worldwide every year, especially in developing countries like Indonesia. WHO data shows that viral, bacterial, fungal and parasitic infections are the biggest cause of death for the world's population. The use of antibiotics in the prevention of infectious diseases is the only solution[1]. While the continuous use of antibiotics raises the new problems for health especially the resistance of microorganisms that cause infection. Bacteria are the most abundant organisms and widespread in living things. In terms of distribution and number, most bacteria may cause disease in humans and other living things[2]. *Bacillus subtilis* and *Escherichia coli* are the common pathogenic bacteria in humans, *E. coli* is a gram-negative bacterium found in the large intestine of humans, and it is a major cause of diarrheal diseases, especially in infants and children[3,4]. The mortality rate from diarrhea in Indonesia is still around 7.4%, while the mortality rate due to persistent diarrhea is higher at 45%. In 1000 inhabitants, 200-374 subjects experience diarrhea with 60-70% of whom are children under the age of 5 years[5].

Bacterial resistance to antibiotics is a problem that has not been resolved until now. Refdanita et al.[6] found that *E. coli* was resistant to chloramphenicol group antibiotics by 83.9% and amoxicillin by 86.2%. The greater the percentage of bacterial resistance to an antibiotic state that bacteria are no longer susceptible to these antibiotics. Various studies to overcome the bacterial resistance have been carried out, but there have been no effective reports. Thus, it is necessary to look for new antibacterial substances that are still active and selective. The search for antibiotic sources from natural ingredients is still the main trend for researchers. The ability of bioactive compounds of natural materials as a healing medium is estimated because of the content of secondary metabolites, including terpenoids, steroids, coumarin, flavonoids, and alkaloids.

One of the plants that has not been studied intensively in Indonesia is the family of Fabaceae. The Fabaceae family has quite interesting bioactivity such as antioxidants, antimalarial, anticancer, and antibacterial. The jengkol plant (*Archidendron jiringa* (Jack) I. C. Nielsen) which is belongs to the family Fabaceae is commonly used by the Indonesian people as traditional medicine. Jengkol leaves are efficacious as medicine for eczema, scabies, sores, and ulcers, the skin of the fruit is used as ulcer medicine[1]. Several studies have been carried out on jengkol plants, both the leaves, fruit peel, and seeds. Nurussakinah (2010)[7] has carried out the phytochemical screening on jengkol fruit peel, seeds, bark and leaves extracts. Based on these screening its contain alkaloids, steroids, triterpenoids, glycosides, saponins, flavonoids, and tannins. While research on the stem roots of the jengkol plant has never been done.

In our ongoing investigation for new lead constituents from medicinal plants, we elaborated the bioactive secondary metabolites of *A. jiringa* stem roots and assayed their antibacterial activity. The aims of the study is to obtain antibacterial active fractions from the stem roots of *A. jiringa* based on bioassay-guided separation approach through their antibacterial property.

Materials and methods

Plant material

Samples of the stem roots of *A. jiringa* were assembled on 25 January 2018 from Unila's Housing area at Gedongmeneng District, Bandar Lampung, Lampung Province, Indonesia. The plant specimens (NV6/NRGD/2018) was identified at the Herbarium Bogoriense, LIPI Bogor, Indonesia.

General experimental procedures

TLC was done on silica gel 60 GF_{254} plates (Merck; 0.25 mm) and sprayed with staining reagen $Ce(SO_4)_2$. Column chromatography (CC) was made on silica gel (Kieselgel 60, 70-230 mesh ASTM; Merck) and sephadex LH-20. ^{1}H NMR spectrum was measured in acetone-d_6 (TMS as an internal standard), on an NMR Agilent 500 MHz spectrophotometer (Agilent Technologies, JNM-ECZ500R/S1) or Bruker 500 MHz spectrometer. Finally, *Microplate Reader* Hospitex-Italy was used

to measure the absorbance resulted on the Resazurin assay, while UV spectra were performed using an Eppendorf BioSpectrometer® kinetic instrument.

Bacteria strains and biochemicals

Ampicillin and chloramphenicol were purchased from Sigma Aldrich, resazurin sodium salt was purchased from Sigma-Aldrich, gram positive bacteria: *Bacillus subtilis* ITBCCB148 was obtained from the Microbiology and Fermentation Technology Laboratory, Bandung Institute of Technology, gram negative bacteria: *Escherichia coli* UNIATCC25922, nutrient agar broth, disposable sterile petri dishes (Idealcare), disposable syringe, micropipette tips, sterile tissue culture plates 96-well.

Preparation of extracts

Fresh chopped the stem roots of *A. jiringa* (2.5 kg) was cleaned by rinsing under running tap water to remove soil and dirt. The samples was dried in an open space for three weeks and the air-dried roots are finally ground into powder form. The powdered air-dried stem roots (1.5 kg) were extracted with polar gradient polarity of solvent using maceration technique. The solvent used at this stage starts from the solvent which has the lowest polarity, *n*-hexane, followed by ethyl acetate and finally with a high polarity organic solvent, methanol. Each extraction was conducted three times in each type of solvent. Before changing the type of solvent, the extract residue is first dried air for at least three days or until the residue is dry/free of solvent. The filtrates obtained from maceration obtained from polar gradient solvents are then separated from the residue by ordinary filtration. The filtrates are then concentrated under reduced pressure using a vacuum rotary evaporator to yield *n*-hexane (3.6 kg), ethyl acetate (55.8 kg), and methanol (67.2 kg) extracts, respectively. Each extract obtained was subjected to assay of its antibacterial activity using agar disc diffusion and microdilution methods. The general research flowchart can be seen in Fig.1.

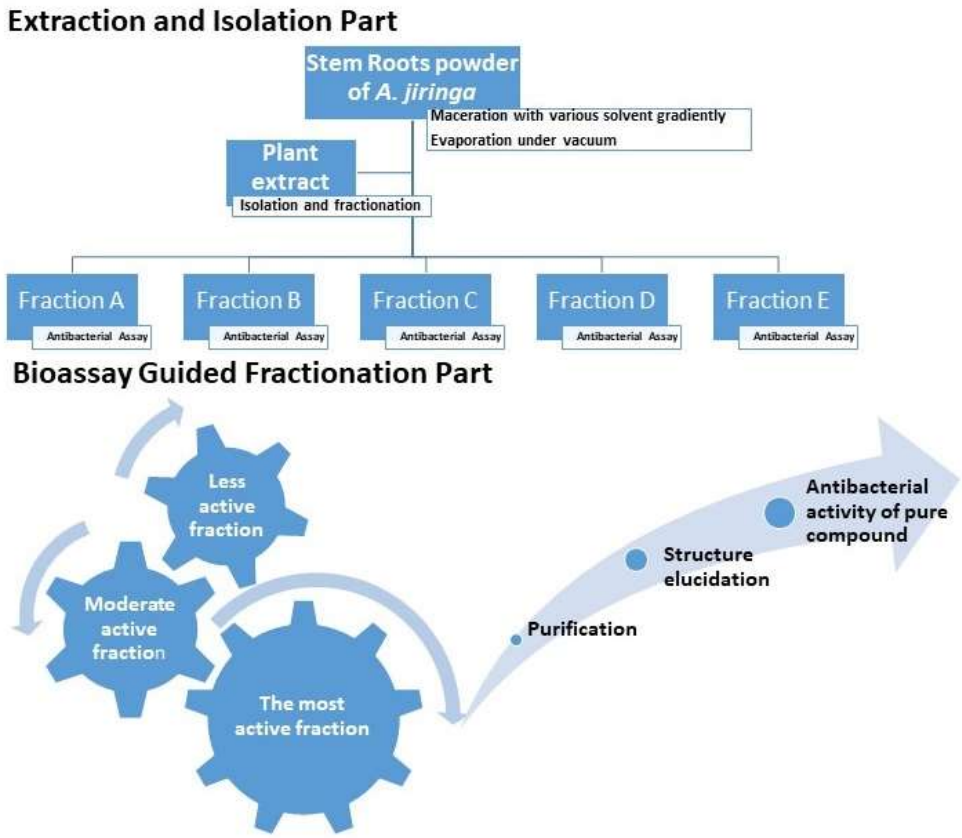


Figure 1. Research flow chart of bioassay guided separation

Bioassay guided separation of ethyl acetate extract

Based on the antibacterial activity results of extracts, the ethyl acetate extract (55.8 g) was selected to be isolated and fractionated further using VLC method on silica-gel (35-70 Mesh). The column was eluted with a stepwise gradient polarity of solvent systems including *n*-hexane–EtOAc (100%-0% of *n*-hexane); EtOAc-acetone (100%-0% of EtOAc); and acetone-MeOH (100%-0% of acetone), affording 23 fractions (200 mL each). Due to the similarity of TLC's profile of some fractions using EtOAc-acetone (1:1) as eluent (Fig.2), these fractions were analysed also by ^{1}H-NMR spectroscopy. According to their chemicals profile analysed by TLC and their proton NMR spectrum, these fractions were grouped and combined into seven primary fractions, E_21 (fr.1-10), E_22 (fr.11), E_23 (fr.12), E_24 (fr.13), E_25 (fr.14-15), E_26 (fr.16-19), E_27 (fr.20-23). All fractions E_21- E_27 were tested for their antibacterial property separately using disc diffusion and microdilution methods.

The MIC's values and optical density (OD) means of bioactive fractions tested were tabulated on Table 1.

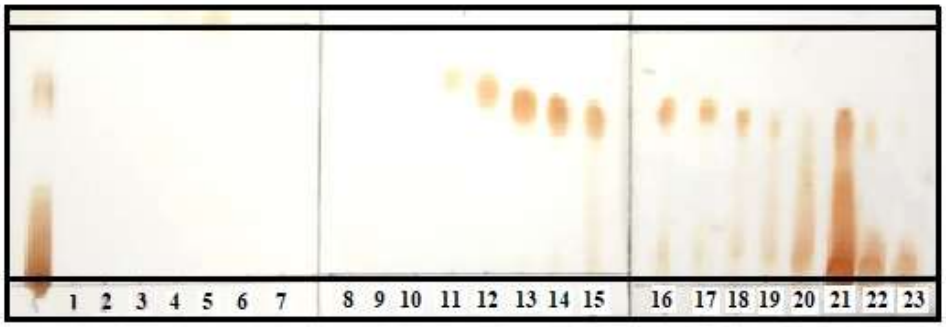


Figure 2. TLC chromatogram of EtOAc extract fractionation by VLC

Tabel 1. The MIC's values and optical density (OD) means of all fractions tested against *E. coli* and *B. subtilis*

No	Fraction code	OD mean against *E. coli*	OD mean against *B. subtilis*	MIC (µg/mL) against *E. coli*	MIC (µg/mL) against *B. subtilis*
1	$E_2$1	1.16363	1.02819	50	-
2	$E_2$2	1.49338	1.54488	25	25
3	$E_2$3	1.25269	1.74956	25	25
4	$E_2$7	1.39525	2.34931	25	6,25
5	Positive control	2.16563	2.28425	0,78	0,78
6	Negative control	0.86413	0.60838	-	-

Noted: (+) control: chloramphenicol (*B. subtilis*) and amoxicillin (*E. coli*); (-) control: MeOH 12.5%

Three fractions exhibited antibacterial activity against both bacterial strains with quite similar MIC's values, therefore fraction $E_2$2 (439.5 mg) was subjected to further fractionate due to the simplest chemical profile on its TLC. Fraction $E_2$2 was redissolved in acetone and then purified with CC on silica gel G-60 (35-70 Mesh) using *n* – hexane / isopropyle alcohol with the ratio volumes of 70/30, 60/40, 50/50,

and 40/60, generating 6 major subfractions, $E_2$2a (132.0 mg); $E_2$2b (8.4 mg); $E_2$2c (9.0 mg); $E_2$2d (47.0 mg); $E_2$2e (2.0 mg); and $E_2$2f (50.3 mg). All subfractions were screened against both bacterial strains tested using microdilution methods. The MIC's values and optical density (OD) means of bioactive subfractions were described on Table 2. Among them, two subfractions ($E_2$2e and $E_2$2f) performed the most antibacterial activity against *B. subtilis* and *E. coli* with the MIC's values ranging of 12.5-25 µg/mL. Only subfraction $E_2$2f was selected to be analyzed further by ^{1}H-NMR spectroscopy as having sufficient quantity.

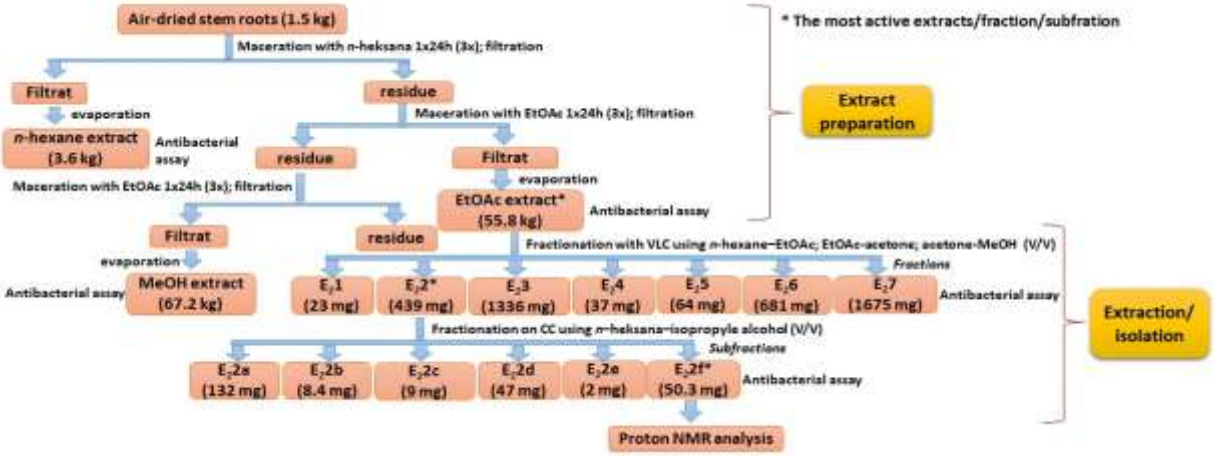


Figure 3. Scheme of bioassay guided separation of EtOAc extract

Phytochemical screening

The phytochemical screening was performed for triterpenes/steroids, alkaloids, flavonoids and saponins by using the standard procedures[8]. The formation of precipitate or the colour intensity was applied for analytical response of screening results.

Evaluation of antibacterial activity

For the testing antibacterial activity, the microbial strains employed in the biological assay are Gram positive bacteria: *Bacillus subtilis* ; Gram negative bacteria: *Escherichia coli* has been obtained from stock culture Hospital Abdul Muluk, Bandar Lampung, Indonesia. Original culture are further stored at low temperature in the

refrigerator to maintain stock culture. Fresh cultures are used for testing antibacterial activity using disc diffusion assay and dillution methods.

Disc diffusion assay

The antibacterial activity of the stem roots extract (*n*-hexane, ethyl acetate and methanol) were tested by disc diffusion method[9] against pathogenic bacteria gram negative (*Escherichia coli*) and gram positive (*Bacillus subtilis*). In this method, freshly prepared agar media is dispensed into the sterilized Petri-dish. The agar is allowed to solidify and 100µl of bacterial suspension poured over the agar media and spread by a spreader or a rod. Ampicillin and chloramfenicol (30µg/dish) is used as positive control, while methanol is used as a negative control. In the each culture medium petri-dish four dish were used, one disk of antibiotics, two discs separately for (*n*-hexane, ethyl acetate and methanol) extracts, one disk used as a control (methanol). The plates are sealed and incubated overnight at 37ºC in the incubator. Antibacterial activity is assigned by measuring the inhibition zone formed around the discs. The diameter of zone of inhibition (mean of three replicates SD) as indicated by clear area was measured to determine the antibacterial activity. The experiment is replicated three times to confirm the reproducible.

Determination of MIC via Resazurin assay

Resazurin assay was carried out in 96 well titration plates with some minor modification[10]. In complete nutrient broth two fold dilutions of plant extracts and antibiotics are prepared in the test wells. The final concentration (20µL of each bacterial suspension) was added to 180 µl of antibiotics and plant extracts (30-0.02 mg/mL in sequence) contained in culture medium as well as the antibiotics concentrations of 0.06 mg/mL and 0.12 mg/mL for amphicilin and chloramphenicol, respectively. For comparative study control plates were prepared only with culture medium and bacterial suspension. The plates were sealed and incubated for 12 hours at 37 C for additional 5 hour. At the intervals of 1 hour plates were observed for colour change blue to pink and pink to colorless in live bacterial strains containing wells. Preliminary micro titre plate assay reveal that the fast decolonization of resazurin extract doesn't possessed antibacterial potential. The bioactivity of extracts were screened which shows that the extracts inhibit the dye reduction.

Results and discussion

The plant material was macerated using gradient polarity of solvent and afforded *n*-hexane (3.6 kg), ethyl acetate (55.8 kg), and methanol (67.2 kg) extracts. EtOAc extract performed the most bioactive extract against two bacterial strains, *Escherichia coli* and *Bacillus subtilis* using agar disc diffusion and microdilution methods.

2. Conclusions

The ethyl acetate extract of jengkol (*Archidendron jiringa* (Jack) I. C. Nielsen) root bark has antibacterial activity against *E. coli* and *B. subtilis* bacteria which is better than *n*-hexane extract. E13 fraction and E28 fraction have better antibacterial activity against *E. coli* and *B. subtilis* bacteria compared to other fractions from the same column. E13 fraction and E28 fraction have better antibacterial activity against *E. coli* and *B. subtilis* compared to E13 subfaction and E28 fraction as a result of its purification. Based on the ^{1}H-NMR result data the compounds detected in the $E_2 8_1 6$ subfraction is phenolic compound type. This study is the first reported from this plant and could be potential to further investigated for the invention of new promising antibacterial agent from plants.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors thank the Directorate of Research and Community Services, The Ministry of Research, Technology and Higher Education, Republic of Indonesia through LPPM University of Lampung for providing funds: PKSI Grant (No.3093/UN26.21/PN/2019). We acknowledge the support of Universiti Sains Malaysia for the facility and research collaboration.

REFERENCE

1. Salni., H. Marisa, dan R.W. Mukti. 2011. Isolasi Senyawa Antibakteri dari Daun Jengkol (*Pithecolobium lobatum* Benth) dan Penentuan Nilai KHM-nya. *J. Pen. Sains*. 14: 1-2.

2. Siti Juariah, Shofriyanti Oktaviyani. The Activity Test Of Ethanol Extract Jengkol Skin (*Pithecellobium jiringa*) To Inhibit of Fungus Growth *Candida albicans*. *Prosiding*, 1-Sep 2016 1th Celscitech-UMRI 2016.

3. Susilowati, D.N., R.D. Hastuti, dan E. Yuniarti. 2007. Isolasi dan Karakterisasi Aktinomisetes Penghasil Antibakteri Enteropatogen *Escherichia coli* K1.1, *Pseudomonas pseudomallei* 02 05, dan *Listeria monocytogenes* 5407. *J. AgroBiogen*. 3 (1):15-23.

4. Maryati., R.S., Fauzia, dan T. Rahayu. 2007. Antibacteria Activity Test of *Ocimum basilicum* L. Toward *Staphylococcus aureus* and *Escherechia coli*. *J.Pen. Sains Tek.*. 8 (1): 30 – 38.

5. Rizal, M., Yusransyah, dan S.N., Stiani. 2016. Uji Aktivitas Antidiare Ekstrak Etanol 70% Kulit Buah Jengkol (*Archidendron paucifiorum* (Benth.) I.C.Nielsen) terhadap Mencit Jantan yang Diinduksi Oleum Ricini. *Jurnal Ilmiah Manuntung*. 2 (2): 131-136.

6. Refdenita., R. Maksum., A. Nurgani, dan P. Endang. 2004. Pola Kepekaan Kuman Terhadap Antibiotika di Ruang Rawat Intensif Rumah Sakit Fatmawati Jakarta Tahun 2001 – 2002. *MAKARA Kesehatan*. 8 (2). 41-48.

7. Nurussakinah. 2010. Skrining Fitokimia dan Uji Aktivitas Antibakteri Ekstrak Kulit Buah Tanaman Jengkol (*Pithecellobium jiringa* (Jack) Prain.) terhadap Bakteri *Streptococcus mutans, Staphylococcus aureus* dan *Escherichia coli. (Skripsi)*. Universitas Sumatra Utara. Sumatra Utara.

8. Gul, R., Syed, Umer Jan, Syed Faridullah, Samiullah Sherani, and Nusrat Jahan. 2017. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra intermedia* Indigenous to Balochistan. *Sci. World.J.* 7 pg. https://doi.org/10.1155/2017/5873648

9. Bauer, A.W., Kirby, W.M.M, Sheriss. J.C et al., 1966. Antibiotic susceptibility testing by a standardized single disk method. *Ame. J. Clin. Pathol*.45: 493496.

10. Karuppusamy, S., and Rajasekaran, K.M. 2000. High Throughput Antibacterial Screening of Plant Extracts by Resazurin Redox with Special Reference to Medicinal Plants of Western Ghats Global. *J. Pharmacol*.3 (2): 63-68

APPENDIX 2. ATTENDING INTERNATIONAL CONFERENCE

1. ABSTRACT

Isolation and Separation Bioactive Secondary Metabolites from Jengkol Plant (*Archidendron jiringa* (Jack) I. C. Nielsen) Through *Bioassay Guided Fractination* Approach

Noviany[1,*], Dicky Sialdian[1], Andi Setiawan[1], Sutopo Hadi[1], Rizky Fijaryani[1], Bambang Irawan[2], and Mohamad Nurul Azmi Mohamad Taib [3]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, 35145, Indonesia*

[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, 35145, Indonesia*

[3]*School of Chemical Sciences, Universiti Sains Malaysia, 11800, Penang, Malaysia.*

*Corresponding author: noviany@fmipa.unila.ac.id

Infectious diseases caused by bacteria has become the global health issues especially antibacterial drug resistance. The most serious concern with antibacterial resistance is that some bacteria have become resistant to almost all antibacterial drugs and making them less effective. Plants are the one of the most important natural resources because they are relatively less side effects and cheap. One of the potential plants that has antibacterial properties is the jengkol plant (*Archidendron jiringa* (Jack) I. C. Nielsen). Jengkol plant is the one of Lampung's natural resources that has not been optimally used, particularly in the field of technology development and drug health. In current research, isolation and separation secondary metabolites of the roots bark of *A. jiringa* have been conducted through *Bioassay Guided Fractination* Approach. Bioactive secondary metabolites from jengkol plant roots has been carried out using general isolation procedures including extraction with maceration method, fractionation and purification by various chromatographic techniques such as vacuum chromatography (VLC), column chromatography (CC) and medium performance liquid chromatography (MPLC). The preliminary identification of bioactive metabolites were recognized by TLC monitoring and ^{1}H-NMR spectroscopy analysis, and its displayed a good antibacterial activity against resistant bacteria, *S. aureus* and

E. coli with the same MIC value of 25 □g / mL Based on the ^{1}H-NMR spectroscopy analysis, the major compound identified on the bioactive metabolites was predicted as a phenolic compound. However, the purification of these bioactive metabolites is still in progress and will be reported in the future.

Keywords: *Archidendron jiringa*; jengkol plant; bioassay guided fractionation; antibacterial activity; *Staphylococcus aureus*; *Escherichia coli*;

2. ACCEPTANCE LETTER

Click here for printer friendly version

No: 004/08/2019 18th August 2019

LETTER OF ACCEPTANCE

Dear Mrs. Dr Noviany Noviany S.Si., M.Si,

On behalf of the organizing committee, I would like to inform you that the **The 14th Joint Conference on Chemistry (14th JCC)** will be held on 10-11 September 2019 at Solo Paragon Hotel and Residence, Surakarta, Indonesia.

We have accepted your abstract entitled: "Isolation and Separation Bioactive Secondary Metabolites from Jengkol Plant (Archidendron jiringa (Jack) I. C. Nielsen) Through Bioassay Guided Fractination Approach"

for Oral_Presentation**.**

For presenter(s) who interesting to submit their full article, please submit your

full-paper manuscript on (https:/jcc.uns.ac.id) and sign in to your dashboard, by August 14th, 2019. All submitted paper will be peer reviewed. Selected paper will be published in AIP Conference proceeding, which thus author should prepare their paper according the AIP template (https://aip.scitation.org/apc/authors/preppapers). In case the Scientific Committee suggested paper to be publish in others publisher/journal and the author(s) agree to do so, the author(s) should reformat their paper according to its respective template (https://jcc.uns.ac.id/index.php/abstract-and-paper-submission/). The author may have to pay additional cost for submission to and/or publication in journal.

If you have any questions feel free to contact the conference committee at (https://jcc.uns.ac.id/index.php/contact-us/) or jcc14@mail.uns.ac.id.

Regards,
Organizing Committee

Dr. rer. nat. Atmanto Heru Wibowo, M.Si