

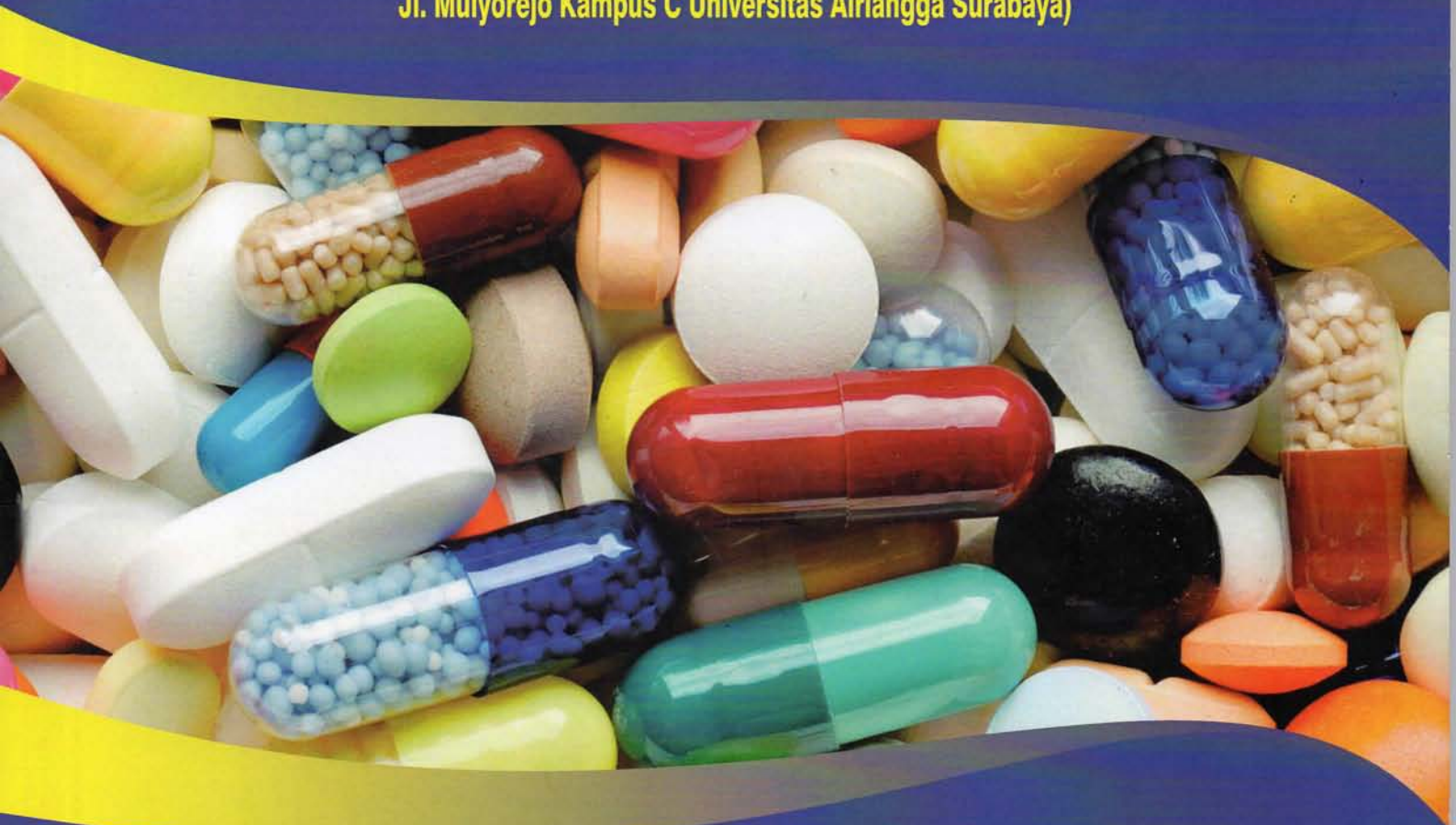


PROSIDING/PROCEEDING

**MUSYAWARAH NASIONAL KE III
ASOSIASI FARMAKOLOGI DAN FARMASI VETERINER INDONESIA**

**7-8 Oktober 2017 di Fakultas Kedokteran Hewan Universitas Airlangga
Jl. Mulyorejo Kampus C Universitas Airlangga Surabaya**

**(3rd National Conference of Indonesia Veterinary Pharmacy and Pharmacology Association,
October 7th-8th, 2017 In The Faculty of Veterinary Medicine Airlangga University,
Jl. Mulyorejo Kampus C Universitas Airlangga Surabaya)**



EDITOR:
Mochamad Lazuardi
Rinidar
Ietje Wientarsih

The Indonesia Veterinary Pharmacy and Pharmacology Association at www.affaveti.org

Bekerja sama dengan

**Pusat Penelitian Pengkajian Penerapan Ilmu Farmasi Veteriner Indonesia (P4IFVI)
Indonesia Research Center Analysis and Apply Veterinary Pharmacy Science (IRCAAVPS)
Fakultas Kedokteran Hewan Universitas Airlangga**



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SAMBUTAN DIREKTUR KESEHATAN HEWAN DIREKTORAT JENDERAL PETERNAKAN DAN KESEHATAN HEWAN

Puji syukur kita panjatkan kepada Tuhan Yang Maha Esa atas berkah-Nya, sehingga Prosiding Pertemuan Ilmiah Nasional ke III Asosiasi Farmakologi dan Farmasi Veteriner Indonesia (AFFAVETI) dapat diterbitkan, yang berkaitan dengan kegiatan Musyawarah Nasional AFFAVETI pada tanggal 7-8 Oktober 2017

Prosiding ini merupakan ekstrapolasi pemikiran anggota dan pengurus AFFAVETI periode 2013-2017 yang menekankan arti pentingnya penanganan dan penggunaan obat hewan dengan benar. Pemikiran tersebut sangat tepat mengingat obat hewan yang diaplikasikan ke ternak pada akhirnya dapat berpengaruh langsung terhadap kesehatan manusia dan habitat hewan. Oleh sebab itu upaya pemikiran arti pentingnya penggunaan obat hewan dengan bijak, pada akhirnya akan membawa kemaslahatan kehidupan manusia, hewan dan lingkungan.

Hal tersebut sejalan dengan Peraturan Menteri Pertanian Nomor : 14/Permentan/PK.350/5/2017 tentang Klasifikasi Obat Hewan Pasal 15 bahwa dilakukan pelarangan penggunaan obat hewan pada ternak yang produknya untuk konsumsi manusia, yang antara lain : untuk mencegah terjadinya residu obat hewan pada ternak, untuk mencegah gangguan kesehatan manusia yang mengkonsumsi produk ternak, karena sulit didegradasi dari tubuh hewan target, karena menyebabkan efek hipersensitif, karsinogenik, mutagenik dan teratogenik pada hewan dan/atau manusia, untuk mencegah timbulnya resistensi mikroba patogen, dan/atau karena tidak ramah lingkungan. Disamping itu, dalam rangka pengawasan terhadap keamanan penggunaan obat, maka terhadap obat keras dipersyaratkan hanya dapat diperoleh dengan resep dokter hewan, dan pemakaian obat keras wajib dilakukan oleh dokter hewan atau tenaga kesehatan hewan di bawah pengawasan dokter hewan.

Pada kesempatan ini, kami menyampaikan terima kasih yang tak terhingga kepada anggota dan pimpinan AFFAVETI periode 2013-2017, yang telah merintis dan memikirkan arti pentingnya penggunaan obat hewan dan alat kesehatan hewan. Tentunya program-program AFFAVETI ke depan selalu seiring dan mampu menjadi penguat terhadap seluruh komponen di peternakan dan kesehatan Hewan. Ucapan terimakasih juga kami tuju kepada semua institusi di bawah Direktorat Jenderal Peternakan dan Kesehatan Hewan yang mengikuti secara aktif kegiatan ilmiah AFFAVETI selama ini. Kehadiran prosiding ini diharapkan dapat dijadikan acuan ilmiah di masa-masa mendatang.

Akhir kata semoga kegiatan-kegiatan AFFAVETI di masa-masa mendatang makin berkembang dan tetap membawa nama harum bangsa dan negara Republik Indonesia.

Jakarta. 17 Januari 2018

SAMBUTAN KETUA MUNAS ASOSIASI FARMAKOLOGI DAN FARMASI VETERINER INDONESIA

Assalamu'alaikum warahmatullahi wabarokatuh

Alhamdulillah selalu kita semua kumandangkan atas limpah dan berkah-Nya, pada akhirnya Prosiding Pertemuan Ilmiah Nasional (PITNAS) ke III dalam rangkaian Musyawarah nasional (MUNAS) ke III Asosiasi Farmakologi dan Farmasi Veteriner Indonesia (AFFAVETI) tanggal 7-8 Oktober 2017, pada akhirnya terselesaikan. Banyak hal yang sudah Panitia Munas III lakukan hingga berakhirnya dengan terbitnya Prosiding. Kegiatan PITNAS pada prinsipnya merupakan manifestasi dari kegiatan yang dilakukan setiap acara MUNAS yang diawali di Denpasar (Munas I) dan di Surabaya, PUSVETMA (Munas II). Dalam kegiatan MUNAS III, ini terlihat mutu karya ilmiah dari peserta makin meningkat dibanding munas-munas sebelumnya. Namun demikian prinsip Novelties dan Anti-plagiat selalu dipegang teguh. Dengan demikian di tahun-tahun mendatang pada akhirnya karya ilmiah dalam prosiding-prosiding ke depan harus makin tinggi keterbacaannya dan disitasi oleh masyarakat ilmiah dunia.

Pada kesempatan yang baik ini Saya sebagai Ketua Panitia Munas III AFFAVETI, mengucapkan terima kasih atas bantuan banyak pihak yang membantu secara langsung maupun tak langsung sehingga pada akhirnya Prosiding tersebut dapat terbit sesuai rencana. Namun demikian terdapat pepatah TIDAK ADA GADING YANG TAK RETAK, oleh karena itu segala kekurangan pada pembuatan Prosiding hingga penerbitan Prosiding, secara kangsung saya mohon maaf atas ketidak nyamanan tersebut.

Terima kasih.

Wassalamu'alaikum warahmatullahi wabarokatuh

Surabaya, 18 Desember 2017
Ketua panitia MUNAS III AFFAVETI

Professor Mochamad Lazuardi

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TECHNIQUE SEPARATION PHYTO-HORMONES OF PROGESTERONE ON CRUDE EXTRACT BENALU DUKU LEAF BY ANALYTICAL COLUMN OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

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ABSTRACT

The information about Benalu duku (*Dendrophthoe pentandra* L., Miq) containing phyto androgen hormone was known at last of 2013 referred to research report from Indonesia. Beneficially part of the trees containing phyto-hormones was predicted in the leaf especially active compound dissolved in methanol. The new technique for separated phyto-hormones from matrix from crude extract benalu duku leaf was researching. The study aims was to prove the suitable technique separation of the pure active compounds from irrelevant compounds in matrix crude material. 5 g of crude extract methanol benalu duku leaf as a sample were analysis to separate analyte between irrelevant matrix compounds. The active compound will be separate was progesterone like-effect as follows; progesterone, medroxy progesterone acetat, megestrol acetat, dydrogesterone. The samples were added with 5 ml of methanol and shaking hard until dissolved to methanol. A 40 µL was injected to HPLC isocratic system by eluent mobile phase methanol: water (70%:30%) at wavelength 254 nm. Result research showed that progesterone was obtained at a retention time 4.22 min to 6.488 min. By LC-ESI MS showed that accumulation of the chromatogram at retention time at above was progesterone ($p < 0.05$). Conclusion the separated technique by analytical column was suitable for separated for obtained pure analyte.

Keywords: LC ESI-MS, analytical column, percolation, chromatogram samples, preparative technique

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INTRODUCTION

The *Dendrophthoe pentandra* L. Miq., (Benalu duku) was known as herbal medicine since early of 1990 referred to Nuraini *et al.*, (2000). First scientific report showed that benalu duku had been beneficially to eliminated the developed of cell cancer (Ratna *et al.*, 2011). The new report research at early of 2013, some researcher reported that benalu duku were predicted many beneficially to achieved phyto hormonal. These phenomenon were finding by Bambang, Lazuardi (2013), from experiment in female rate. Specifically, potency to treat clinical case about fertilized hormone. By background research described at above, we are try to advance explore of extract benalu duku for obtained super specific fertilize hormone. The objective of the research aim was to determined clearly of the progesterone-like effect compound in crude extract methanol of benalu duku leaf using technique separate by analytical column.

METHODS

Dendrophthoe pentandra L. Miq was obtained from Muara Enim District South of Sumatera and growth up in *Lancium domesticum* at up to 5 years old. The benalu duku was clarified of Systematic of herbal in Pusat Penelitian Biologi-LIPI Cibinong Science Center, Jl. Raya Jakarta-Bogor, Km. The analysis of bioactive of Progesterone-like effect was using HPLC reverse phase Shimadzu with specification pump LC-6AD, degasser DGU-20A5, communication module (CBM)-20A, DAD SPD-M20A, Fraction collector FRC-10A. The column was Solid Phase Extraction SOLATM HRP from Thermo scientific corp. The research location was operating in Institute of Tropical Diseases Airlangga University at start January 2017 until August 2017. The standard of progesterone-like effect was using from standard pure from Sigma corp and from Europa Pharmacopoeia as follows; progesterone, medroxy progesterone acetat, megestrol acetat, dydrogesteron.

The protocol of the research was described as follows: (1) pulverizing of the leaf of benalu duku, (2) percolation by moving method, (3) separate using analytical column (4) clarified by LC-ESI MS. LC-ESI MS was adjusted similar as described by Lazuardi, Bambang (2016).

Pulverizing technique and percolation by moving method were describe at follows; Leafs from muara enim district at about 50 kg were cleaned twice by aquadem than following to dry at room temperature during the 48 h. Pulverizing were using by electric blander than following filtration by filter at diameters hole No. 000. Pulverize were weighing and following percolate using separated glassware 1 L of 43 g of pulverize benalu duku leaf dissolve 1 L of methanol p.a. Separated glassware was shaking well each 3 minutes until 48 h. Filtrate were keeping in one bottle than following to dry by nitrogen gas at water bath adjusted 40 °C. Crude extract was weighing and keep on special sample tube.

Separated was doing by early step with optimization and validation of HPLC System as described by Lazuardi and Bambang (2017). The retention time (RT) peak of standard were noted well than following injected of the sample after preparation. The preparation sample were using protocol as follows; (1) crude extract added 1 ml of methanol pro HPLC shacking well until dissolve, (2) filter with nylon membrane filters 0.2 µm, (3) injected to HPLC system. A 40 µL was injected to HPLC isocratic system by eluent mobile phase methanol: water (70%:30%) at wavelength 254 nm. Peak of the chromatogram were directly to keep of the waste products. The waste product was predicted containing phyto-hormones. Accumulate of the waste product were vacuum drying by nitrogen gas and ready to use for clarified LC-ESI MS to determine progesterone, medroxy progesterone acetat, megestrol acetat, dydrogesteron.compound. Statistic analysis was using MINITAB 17.0 for analysis comparative each observation data at 95 % significantly.

DISCUSSION

Standard of progesterone-like effect were obtained at RT at about 6.44 min as medroxy progesterone acetate, than 8.801 min as dydrogesterone, and 8.348 min as a megestrol acetate, and some tail find out in 11 to 12 min as progesterone. The chromatograms sample were find at three peak of RT that are at RT 3 min to 9.7 min, than 13 min to 16.141 than 17 to 20 min (Table 1). Three group chromatograms peaks have a characterization moving fastest or moving to latest at about ± 2 or 3 min. Figure 1 at below represented sample chromatogram with three chromatograms peak.

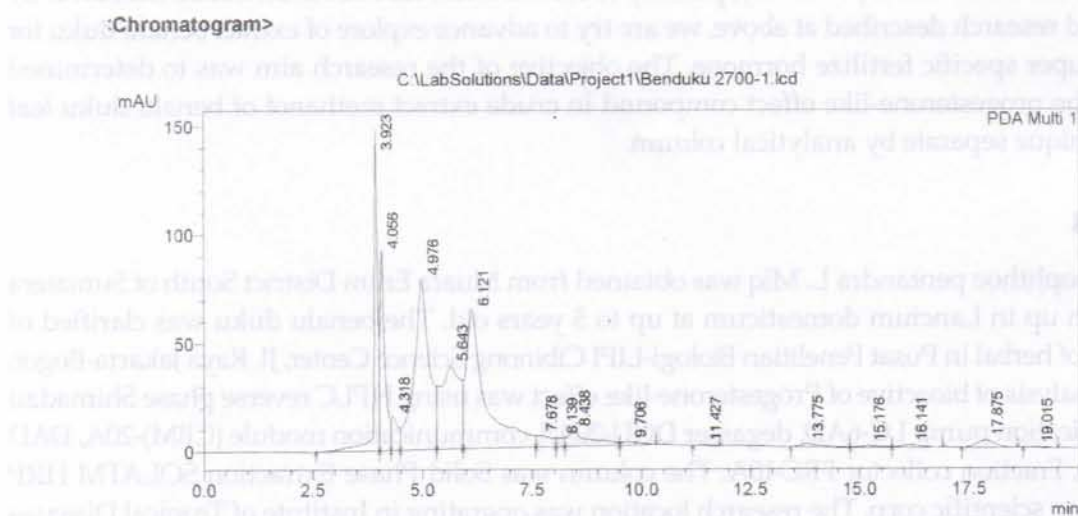


Figure 1. Chromatogram of crude extract benalu duku leaf eluent mobile phase methanol: water (70%:30%) at wavelength 254 nm. The analytical column was using octa desil xylane and accumulate peak at RT 3 min to 9.7 min, than 13 min to 16.141 than 17 to 20 min.

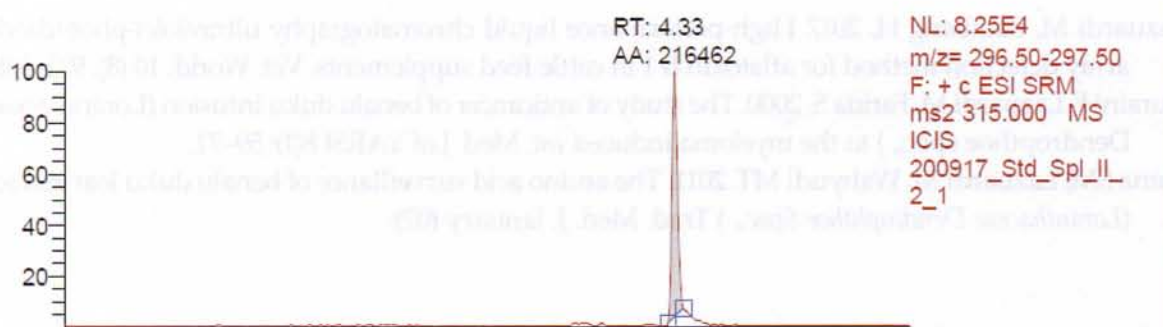


Figure 2. m/z mass of progesterone in 296.50 to 297.50 in accumulate peak of HPLC by analytical column at min 3.923 to 9.706 min.

From the figure 1, at above showed the RT of min 3.923 to 9.706 min containing progesterone, medroxyprogesteron, megesterol acetat and dydrogesterone were noted well. From LC-ESI MS was performed RT (min) at time of 3.923 to 9.706 containing progesterone (Figure 2).

Table 1. The Retention time (min) peak chromatogram of crude extract benalu duku

Sample (N)	Retention time of peak chromatogram crude extract benalu duku (min)			
Sample-1 ^a	4.244-4.924	5.697-6.488	-	17.500-20.00
Sample-2 ^b	3.110-5.358	7.300-7.500	10.39-10.755	13.145-20.00
Sample-3 ^c	3.716-7.727	10.728-11.400	12.004-12.54	18.00-20.00
Sample-4 ^d	3.901-9.087	10.327-10.913	16.122-16.573	16.573-20.00
Sample-5 ^e	3.878-6.719	8.050-10.349	12.018-12.672	16.868-20.00

Mean of superscript a,b,c,d,e by one-way anova were different ($p < 0.05$)

CONCLUSION

The new technique for separate analyte in matrix biology was suitable for using separate pure analyte to matrix biology. From statistic obtained that RT (min) at 3 min to 9.7 min, than 13 min to 16.141 than 17 to 20 min were stable and predicted containing progesterone-like effect compound ($p < 0.05$). The recommended of this research are needed advances explored about sample preparation.

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Figure 1. HPLC chromatogram of aflatoxin B1 in cattle feed supplements. The peak at 17.50 min is identified as aflatoxin B1.

from the figure 1 it shows showed the RT of aflatoxin B1 is 17.50 min. The peak at 17.50 min is identified as aflatoxin B1. The peak at 17.50 min is identified as aflatoxin B1. The peak at 17.50 min is identified as aflatoxin B1.

Table 1. The Retention time (min) peak chromatogram of crude extract benalu duku

Sample ID	Retention time of peak chromatogram crude extract benalu duku (min)
Sample 1	17.50-17.50
Sample 2	17.50-17.50
Sample 3	17.50-17.50
Sample 4	17.50-17.50
Sample 5	17.50-17.50

Mean of retention time (min) peak chromatogram of crude extract benalu duku = 17.50

CONCLUSION

The new technique for aflatoxin B1 analysis in matrix biology was suitable for using HPLC. The results of this research are needed to be used as a reference for aflatoxin B1 analysis in matrix biology. The results of this research are needed to be used as a reference for aflatoxin B1 analysis in matrix biology.

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