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6 November 2014

Dr. Poppy Hartatie Hardjo
Department of Plant Biotechnology
Faculty of Biotechnology, University of Surabaya
Raya Kalirungkut, Surabaya, Indonesia

Dear Dr. Poppy Hartatie Hardjo,

Invitation to be a Speaker "Joint Symposium on Frontier Research in Biodiversity and Agricultural Resources" 6-7 November 2014

We are pleased to inform you that the Faculty of Science, Mahidol University (MUSC), in collaboration with local universities and regional academic partner institutions will be holding the **"Joint Symposium on Frontier Research in Biodiversity and Agricultural Resources"**, during 6-7 November 2014 at MUSC, Phayathai, Bangkok. The symposium will cover contemporary issues in biodiversity, including biodiversity towards natural resources, biodiversity and sustainable environments as well as frontier research in plants, animals, insects and aqua biotechnology. This symposium will offer a great opportunity for scientists, researchers, lecturers and students to present research findings, discuss, share current thinking and develop productive partnerships in the regions. There will be approximately 70-80 participants from partner universities in Asia together with a number of invited speakers from abroad in attendance.

In this connection, it is our great pleasure to invite you to be a speaker at this upcoming symposium, on the topic entitled **"Micropropagation and antibacterial activity of *Rauvolfia serpentine* (L.) Benth. Ex Kurz"** on 6 November 2014, during 14.30-15.00 hr. at the Stang Mongkolsuk Conference Room.

We would be greatly appreciated and honored if you would consent to be our speaker at the symposium in this November.

Yours sincerely,

Prof. Skorn Mongkolsuk, PhD
Dean, Faculty of Science, Mahidol University

Office of International Cooperation, Faculty of Science, Mahidol University
272 Rama 6 Rd., Phayathai, Ratchathewi, Bangkok 10400 THAILAND
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University of Sussex | UBAYA UNIVERSITAS SURABAYA | Chiba University 千葉大学

Joint Symposium "Frontier Research in Biodiversity and Agricultural Resources"

6-7 November 2014 at the Faculty of Science, Mahidol University (MUSC), Bangkok, Thailand

Time	Thursday 6th November 2014	
08.30 -09.00	Registration : Stang Mongkolsuk Conference Room, Stang Mongkolsuk Building, MUSC (MC & Chair of the session: Dr. Nuttawee Niamsiri)	
09.00-09.15	**Opening Address** **Prof. Skorn Mongkolsuk** Dean, Faculty of Science, Mahidol University	
09.15-09.45	**Prof. Emeritus Pilai Poonswad (Mahidol University, Thailand)** Topic: Hornbills and their role in regenerating and sustaining forest ecosystem	
09.45-10.00	Group photography (invited speakers, guests, MUSC administrators)	
10.00-10.30	***Refreshment (coffee break)***	
10.30-11.00	**Dr. Mika Peck (University of Sussex, UK)** Topic: Using agent-based modeling to focus conservation of the critically endangered brown-headed spider monkey *(Ateles fusciceps fusciceps)* in NW Ecuador	
11.00-11.30	**Assoc. Prof. Bhinyo Panijpan (Mahidol University, Thailand)** Topic: Biodiversity of the Siamese fighting fish	
11.30-12.00	**Dr. Puey Ounjai (Mahidol University, Thailand)** Topic: Structural insights into the biofilm formation of *Chamydomonas reinhardtii*	
12.00-13.00	***Lunch (hosted by MUSC) for guests and registered participants***	
13.00-13.30	**Chair of the session:** Assoc. Prof. Jarunya Narangajavana **Prof. Akikazu Ando (Chiba University, Japan)** Topic: Expression of a bacterial chitosanase in rice plants improves disease resistance to the rice blast fungus *Magnaporthe oryzae*	
13.30-14.00	**Dr. Wisuwat Songnuan (Mahidol University, Thailand)** Topic: Diversity of airborne pollens in Bangkok and relevance to allergy	
14.00-14.30	***Refreshment (coffee break)***	
14.30-15.00	**Dr. Popy Hartatie Hardjo (University of Surabaya, Indonesia)** Topic: Micropropagation and antibacterial activity of *Rauvolfia serpentine* (L.) Benth. Ex Kurz	
15.00-15.30	**Asst. Prof. Sasivimon Swangpol (Mahidol University, Thailand)** Topic: Thailand at the cradle of the bananas (*Musaceae*)	
18.00-20.00	***Reception dinner (for guests & speakers)***	

Update: 29 October 2014 by KS

Time	Friday 7th November 2014	
09.00-09.30	**Chair of the session:** Assoc. Prof. Prayad Pokethitiyook **Prof. Francis Ratnieks** **University of Sussex, UK** Topic: The Sussex plan for honey bee health & wellbeing: Applied research to assist an important beneficial insect in agriculture	
09.30-10.00	**Asst. Prof. Sujinda Thanaphum** **Mahidol University, Thailand** Topic: Genetic studies and genetic control of a key fruit fly pest species complex (*Bactroceradosalis: Diptera: Tephritidae*): from basic research to field operation	
10.00-10.30	**Dr. Alan Stewart** **University of Sussex, UK** Topic: Host plant specificity as the key to understand insect diversity in tropical rainforest ecosystems	
10.30-10.45	***Refreshment (coffee break)***	
10.45-11.15	**Assoc. Prof. Sompoad Srikosamatara** **Mahidol University, Thailand** Topic: Urban ecology	
11.15-11.45	**Dr. Pravech Ajawatanawong** **Mahidol University, Thailand** Topic: Mine the gaps: Evolution of eukaryotic protein indels and their application for testing animal phylogeny	
11.45-12.15	**Dr. Chalita Kongrit** **Mahidol University, Thailand** Topic: Population structure and genetic diversity of wild Asian elephants in Salakphra Wildlife Sanctuary, Thailand	
12.15-13.30	***Lunch (hosted by MUSC) for guests and registered participants***	
13.30-15.30	Poster presentations (students session) Poster presentation award Wrap-up & Closing ceremony Coffee/tea break	
18.00-20.00	**Farewell dinner** (join "MU International Night" at Mahidol University, Salaya campus) (for international speakers, MUSC's van will pick up from hotel lobby on 17.15 hr. to Salaya and send back to hotel or airport after dinner)	

Online registration (no registration fee, limited for 100 seats)
http://www.sc.mahidol.ac.th/register/register06_07112014.php

Update: 29 October 2014 by KS

UNIVERSITAS SURABAYA
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SURAT TUGAS
Nomor: 243/ST/FTb/X/2014

Dekan Fakultas Teknobiologi memberikan tugas kepada:

Nama	:	Dr. Ir. Popy Hartatie Hardjo, M.Si.
N.P.K.	:	195023
Jabatan Fungsional Lokal	:	Lektor Kepala – 400
Jabatan Struktural	:	Kepala Laboratorium Bioteknologi Tanaman Fakultas Teknobiologi
Tugas	:	Menjadi pembicara *Joint Symposium* & Mengikuti *Joint Meeting* di *Faculty of Science, Mahidol University*, dalam rangka pelaksanaan Hibah Penguatan Kelembagaan Kantor Urusan Internasional (PKKUI) DIKTI 2014.
Sumber Dana	:	SPP Fakultas Teknobiologi 2014/2015
Jadwal Pelaksanaan	:	04 November – 07 November 2014
Berangkat dari Surabaya:		
Hari/tanggal	:	Selasa, 04 November 2014
Pukul	:	06.00 WIB
Tempat	:	Faculty of Science, Mahidol University, Bangkok, Thailand
Datang di Surabaya:		
Hari/tanggal	:	Jumat, 07 November 2014
Pukul	:	18.00 WIB
Transportasi	:	Pesawat PP

Harap dilaksanakan dengan sebaik-baiknya dan membuat laporan.

Catatan :
Karya Ilmiah **wajib** diserah simpankan ke Perpustakaan Ubaya melalui mekanisme *Repository* atau diserahkan secara manual paling lambat 2 minggu setelah pelaksanaan kegiatan.

Surabaya, 20 Oktober 2014
Dekan

Mengetahui
Wakil Rektor I

Hendrawan Daniel Putra, Ph.D.

Dr. rer. nat. Maria Goretti M. P.

Tembusan:
1. Wakil Rektor II
2. Wakil Rektor III
3. Direktur Keuangan
4. Direktur SDM
5. Yang bersangkutan

Micropropagation and antibacterial activity of *Rauvolfia serpentina* (L.) Benth. ex Kurz

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Abstract

Due to over exploitation of its bark for medicinal herbs and made worse by problem in conventional breeding, *Rauvolfia serpentina* (L.) Benth ex Kurz, has been considered rare and was currently reported to be an endangered species. Therefore, conservation measure is urgent to be taken by micropropagation. The main objective of present study was to study rapid in vitro multiplication and the antibacterial effect of in vitro leaves from *Rauvolfia serpentina* on *Escherichia coli*. Direct regeneration was recorded best in MS medium with BAP 2.0 mg/l and induction of root with IBA 0.2 mg/l. The antibacterial activity was detected by using agar well diffusion method. Ethanol extract of *Rauvolfia serpentina* effectively inhibited the growth of *E. coli*. The ethanol extract showed antibacterial activity against gram negative organism, which may be due to the presence of alkaloid in the extract.
Key words: *Rauvolfia serpentina*, micropropagation, antibacterial

Introduction

Rauvolfia serpentina (L.) (Apocynaceae) commonly called as 'Pule Pandak' in Indonesian language. It is a medicinal plant that used mostly in treating high blood pressure, sedative, and mental disorders (Ramawat *et al*., 1999). A number of alkaloids as reserpine, serpentine, ajmalin, and isoajmalin could be produced from this plant. Reserpine was used to cure the high blood pressure or hypertension and its complications, stroke, and the diseases related with nervous system (Achmad, 1987)

In this time, Pule Pandak in Indonesia included in groups of plant is endangered. Requirement of raw material of Pule Pandak for the herbal industry and pharmacy progressively mount, where as most raw material (more than 80%) still have to be harvested from natural habitat. To get pule pandak in high amounts of secondary metabolites, the plants have to reach the certain age (years), so that exploitation from nature can menace its species, and also difficult to be done (Ramawat *et al*., 1999).

The propagation of *R. serpentina* through seeds is difficult due to less viability and very low germination percentage. Plant tissue culture technology holds great promise for micropropagation, conservation, and enhancement of the natural levels of valuable secondary plant products and to meet pharmaceutical demands and reduce the in situ harvesting of

natural forest resources. For mass propagation of medicinal plant species in which conventional methods possess limitations, in vitro multiplication provides the way out.

Reserpine is an alkaloid and is important constituent of *Rauwolfia serpentina* which was reported to possess antibacterial activity. Deshwal and Vig (2012) reported that ethanol extract of *Rauvolfia serpentina* have antibacterial activity to *Staphylococcus aureus*, and ethanol extract showed more antibacterial activity as compared to water extract activity.

The objective of the present study was to develop a reproducible and efficient regeneration system for production of uniform and true to the type planlets for large scale in situ and ex situ plantation to conserve this endangered medicinal plant for commercial exploitation. Optimum concentration of BAP for shoot multiplication and study antibacterial activity of ethanol extract from *Rauvolfia serpentine* were investigated.

Material and Methods

The research was conducted in two steps. The first was effort of shoot multiplication and rooting of shoot. The second was evaluated the antibacterial activity of *Rauvolfia serpentina* in vitro shoot.

Micropropagation

Internodes were surface sterilized for 10 min in liquid detergent, soaked in 45% sodium hypochlorite (5.25%) 10 min in sterile aquadest, 5 min in 70% ethanol and then they were washed two times with sterile aquadest in laminar air flow hood. The shoot induction media was composed of MS basal medium supplemented with BAP (Benzyl Amino Purine) at different concentrations ranging from 1.0 to 3.0 mg/l. Rooting was implemented in MS basal medium with IBA concentrations ranging from 0.2 to 1.0 mg/l. Regenerated shoots were excised and transferred to rooting medium.

Preparation of *Rauvolfia serpentina* extracts

Dried samples of leaf powder (3.5 mg) were macerated with 100 ml of ethanol 80% overnight. The residual solvent was removed using rotary evaporator. The resulting organic extracts were further reconstituted to different concentrations (70, 80, and 90 mg/l).

Antibacterial activity

E. coli as the testing bacteria was activated by inoculating a loopful of the strain in nutrient broth (20 ml) and incubated on a rotary shaker. Then 0.1 ml of inoculum (OD_{580nm}= 0.6) was inoculated into Mueller Hinton agar medium and after proper homogenization it was poured into the petridish (Arullappan *et al.,* 2009). Wells were then bored into the agar using

a sterile 3 mm diameter cork borer. The well was filled by 0.1 ml ethanol extract of *Rauvolfia serpentina*, and the petridishes were incubated at 37^{o}C for 24 hours. Control was set up in parallel using the solvent that were used to dissolve the extract. The plate was observed for zone of inhibition that marked with clearly zone without bacterial colony (Satish *et al*., 2002).

Result and Discussion

Supplementing the medium with various concentration of BAP induced multiple shoots from node of internodes explant. The best response was obtained in MS medium, supplemented with BAP 2.0 mg/l. Eventhough the concentration of BAP 3.0 mg/l has the most number of shoot per culture (Table 1), the length of shoot is not normal, in this case curly and shorter than the normal ones. Therefore, BAP 2.0 mg/l was the optimum concentration for multiplication of shoot of *Rauvolfia serpentina*.

Table 1. Multiple shoot induction from node of *R. serpentina* internodes on MS medium supplemented with various concentration of BAP

BAP (mg/l)	% of shoot formation	Mean of shoot per culture	Mean of length of shoot (cm)
1.0	72	2	2.42±0,02
1.5	85	4	2.70±0.05
2.0	100	6	3.45±0.05
2.5	100	8	2.15±0.08
3.0	100	9	1.68±0.03

Values are mean of 10 replicate ± SD

The shoots with multiple leaves were transferred to rooting medium. We found out that root initiation started in all the various concentration of IBA after 10 to 15 days. Normal rooting response of shoots were observed on IBA 0.2 and 0.4 mg/l. From Table 2, we found out that IBA0.2 mg/l is the optimum concentration, meaning that the number root per shoot and length of root were higher than any other concentration. The rooting of shoots at higher concentration of IBA 0.6 to 1.0 mg/l showed declined growth of root, and at IBA 1.0 mg/l was found callus in base of shoot. Therfore, the best result of rooting on MS medium supplemented with IBA 0.2 mg/l.

Table 2. Rooting of the in vitro planlets of *Rauvolfia serpentina* on MS medium supplemented with various concentration of IBA

IBA (mg/l)	% of root	Mean of root per shoot	Mean of length of root
0.2	100	5	5.6±0.12
0.4	100	3	5.4±0.09
0.6	90	2	3.2±0.07
0.8	85	2	3.0±0.15
1.0	70	2	2.0±0.14

Values are mean of 10 replicate ± SD

The antibacterial activity of ethanol extract of *Rauvolfia serpentina* was tested against *E. coli*. The ethanol extract of *Rauvolfia serpentina* is having antibacterial activity against *E. coli* (Table 3 and Fig 1).

Table 3. In vitro antibacterial activity of ethanol extract of *Rauvolfia serpentina* on the growth of *E.coli* by agar diffusion method

Replication	zone of inhibition (cm)			
	Control antibiotic	**E_1(70%)**	**E_2(80%)**	**E_3(90%)**
I	0,740	0,446	0,954	1,085
II	0,712	0,453	0,948	1,061
III	0,709	0,445	0,950	1,112
IV	0,702	0,410	0,904	1,010
V	0,721	0,413	0,899	1,021
Mean	0,717b	0,433 a	0,931 b	1,058c

Mean values are followed by different letters significantly different by LSD test in α=0.05

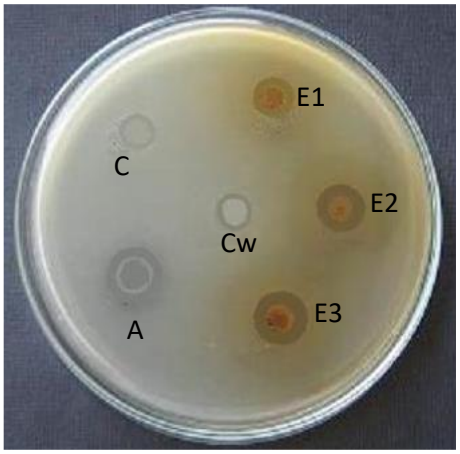

Figure 1. Antibacterial activity of ethanol extract of *Rauvolfia serpentina*
E1: ethanol extract 70% E2: ethanol extract 80%
E3: ethanol extract 90% C: control (ethanol solvent 80%)
Cw: control (water solvent)

Conclusion

Direct regeneration was recorded best in MS medium with BAP 2.0 mg/l and growth of root with IBA 0.2 mg/l.

Ethanol extract of *Rauvolfia serpentina* 90% effectively inhibited the growth of *E. coli*.

References

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Satish, S., Raveesha, K.A. and Janardhana, G.R. Antibacterial activity of plant extracts on phytopatogenic *Xanthomonas campestris* pathovars. Letters in Applied Microbiology 2002, 28: 145-147.

MICROPROPAGATION AND ANTIBACTERIAL ACTIVITY OF *Rauvolfia serpentina* (L.) Benth.

INTRODUCTION

Pule pandak
(*Rauvolfiae serpentina* L.)

Rauvolfiae Radix

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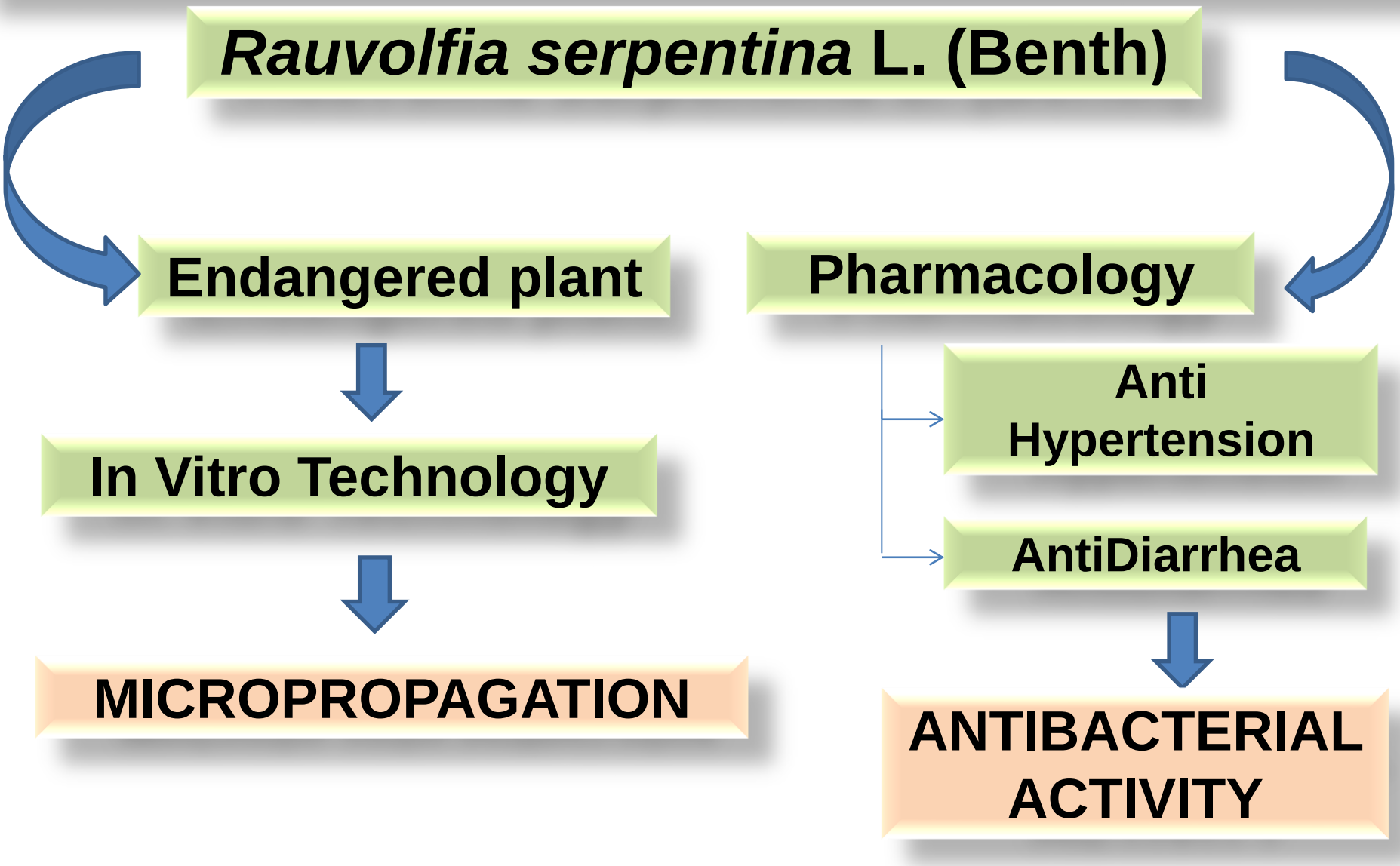
INTRODUCTION



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AIMS AND OBJECTIVE

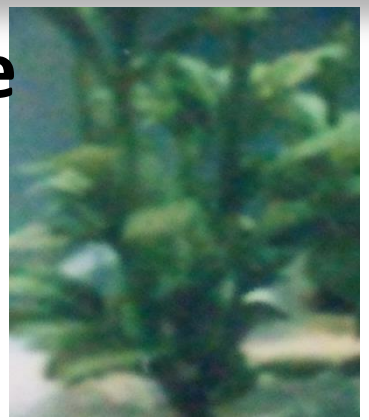
TO FIND OPTIMUM CONCENTRATION OF BAP FOR RAPID MULTIPLICATION OF SHOOTS

TO STUDY ANTIBACTERIAL ACTIVITY OF IN VITRO SHOOT OF *Rauvolfia serpentina*

METHODS

surface sterilize

multiple shoots



root induction

aclimatization

METHODS

Preparation: ETHANOL EXTRACT of *Rauvolfia serpentina* L.

METHODS of EXTRACTION → MACERATION
temperatur 28 °C for 3 days

Organic solvent : ethanol 80%

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RESULTS

Table 1. Multiple shoot induction from node of *R. serpentina* internodes on MS medium supplemented with various concentration of BAP

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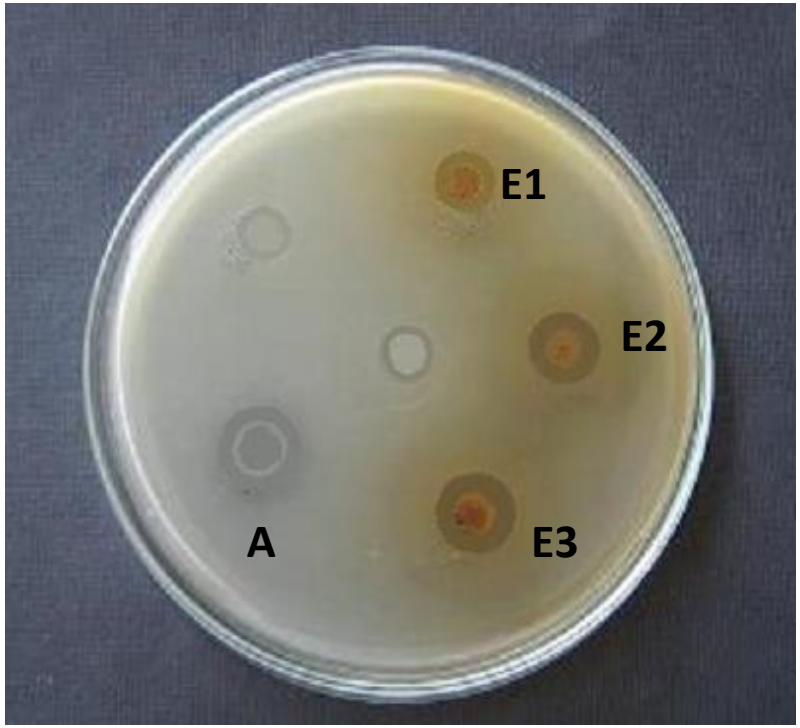
RESULTS



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Mean	0,717b	0,433 a	0,931 b	1,058c

Mean values are followed by different letters significantly different by LSD test in α=0.05

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CONCLUSION

Rauvolfia serpentina L. (Benth):

Shoot multiplication :
MS medium + BAP 2.0 mg/l
Root induction :
MS medium + IBA 0.2 mg/l

Ethanol extract 70-90% have antibacterial activity against *E. coli*

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ACKNOWLEDGEMENT

Thank to:

- **Laboratory of Phytochemistry (Dra. Sajekti Palupi, MS.) for preparing of ethanol extract of *Rauvolfia serpentina* L.**
- **Joint Symposium "Frontier Research in Biodiversity and Agricultural Resources" at the Faculty of Science, Mahidol University, Bangkok, for letting us share this research project.**