BAHAGIAN PENYELIDIKAN & PEMBANGUNAN
CANSELORI
UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1) Nama Penyelidik: DR. AMIRIN SADIKUN

Nama Penyelidik-Penyelidik Lain (Jika berkaitan): PN. PAZILAH IBRAHIM

2) Pusat Pengajian/Bahagian/Unit: SAINS FARMASI

3) Tajuk Projek: Sintesis Sebatian-sebatian Terbitan Etil-p-Etil-p-Metoksisinamat dan Penskrinan Sifat Antibakterianya.

4) (a) Penemuan Projek/Abstrak

(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

Kajian awal pada ekstrak mentah petroleum-eter (60 - 80°) *Kaempferia galanga* bersama-sama dua sebatian utama yang dipencilkan daripadanya iaitu etil-p-metoksisinamat dan etil sinamat menunjukkan setiap satunya boleh merencat pertumbuhan 6 mikroorganisma (*Staphylococcus aureus, Escherichia coli, Candida albicans, Salmonella typhi, Enterobacter aerogenes* dan *Klebsiella aerogenes*) daripada 8 mikroorganisma yang diuji (*Pseudomonas aeruginosa* dan *Bacilus subtilis* memberikan keputusan yang negatif.

Memandangkan etil-p-metoksisinamat menunjukkan keaktifan yang tertinggi dan wujud sebagai komponen utama ekstrak petroleum eter (54%), maka beberapa sebatian terbitan telah disintesiskan dan diuji keaktifan antibakterianya menggunakan kaedah pembauran agar-agar dengan sistem pelarut DMSO 50%. Sebatian yang diuji ialah asid p-metoksisinamik, metil-p-metoksisinamat, propil-p-metoksisinamat, p-metoksisinamil alkohol, terbitan amida asid p-metoksisinamik dan termasuk juga etil-p-metoksisinamat.

Hasil kajian daripada 3 bentuk ester iaitu metil, etil dan propil-p-metoksisinamat; etil-p-metoksisinamat menunjukkan keaktifan tertinggi merencat pertumbuhan semua mikroorganisma kecuali *Pseudomonas aeruginosa* sehingga ke kepekatan 0.0975 mg/ml. Manakala daripada terbitan asid, alkohol dan amida; p-metoksisinamil alkohol memberikan zon perencatan yang besar pada *Candida albicans, Bacillus subtilis* dan *Salmonella typhi.*

Etil-p-metoksisinamat dan p-metoksisinamil alkohol juga mempunyai potensi yang tinggi sebagai agen antiyis (antiyeast) kerana kebolehannya merencat pertumbuhan *Candida albicans* terutama p-metoksisinamil alkohol sehingga ke kepekatan 0.0975 mg/ml. Kedua sebatian ini juga menunjukkan sifat antitumor dengan IC_{50}: 50 μg/ml (etil-p-metoksisinamat) dan 12.5 μg/ml (p-metoksisinamil alkohol).

(b) Senaraikan Kata Kunci yang digunakan di dalam abstrak:

Bahasa Malaysia	Bahasa Inggeris
Kaempferia galanga	Kaempferia galanga
etil-p-metoksisinamat	ethyl-p-methoxycinnamate
p-metoksisinamil alkohol	p-methoxycinnamyl alcohol
antibakteria	antibacteria
antitumor	antitumor
Candida albicans	Candida albicans
Kaedah pembauran agar-agar	agar diffusion method
zon perencatan	zone of inhibition
mikroorganisma	microorganism

5) Output Dan Faedah Projek

(a) Penerbitan *(termasuk laporan/kertas seminar)*
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

(i) Antimicrobial Studies on Ethyl-p-methoxycinnamate and Ethyl cinnamate from Kaempferia galanga.

P. Ibrahim and A. Sadikun

School of Pharmaceutical Sciences, USM, 11800 Minden, Penang

Proceedings of 7th National Seminar on Natural Products, USM, 27-28 June 1990, p. 174-178

(ii) Antimicrobial Activity of Ethyl-p-Methoxycinnamate (from Kaempferia galanga) Linn and its Derivatives.

P. Ibrahim, A. Sadikun, M.H. Nik Mohamaed

School of Pharmaceutical Sciences, USM, 11800 Minden, Penang
Poster Presented at Third Malaysian International Conference On Essential Oils & Flavour Chemicals (TMIC - EOFC '92)
20 -23 July 1992, Langkawi, Kedah.

(iii) Antitumor Activity of Ethyl-p-Methoxycinnamate (from Kaempferia galanga Linn) and its alcohol derivative, p-methoxycinnamyl alcohol
P. Ibrahim, A. Sadikun and M.J. Cardosa
School of Pharmaceutical Sciences, USM, 11800 Minden, Penang.
Poster presented at Seminar Kebangsaan ke 9 Kimia Sebatian Semulajadi, 21-22 Oktober 1992, Universiti Pertanian Malaysia

(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila gunakan kertas berasingan)

Tumbuhan cekur senang ditanam dan memerlukan jagaan yang ringkas. Ia mempunyai kandungan sebatian etil-p-metoksisinamat yang tinggi dan agak mudah untuk dipencilkan. Dari kajian didapati sebatian etil-p-metoksisinamat dan terbitan alkoholnya iaitu p-metoksisinamil alkohol mempunyai potensi untuk dikembangkan sebagai agen antiyis dan antitumor.

(c) Latihan Gunatenaga Manusia

i) Pelajar Siswazah .....

ii) Pelajar Prasiswazah: Seorang. Beliau melakukan sebahagian projek ini untuk Disertasi bagi kursus FEL 302.6 di bawah tajuk: Penskrinan Aktiviti Antimikrob Etil-p-metoksisinamat dari Kaempferia galanga dan terbitan-terbitannya.

iii) Lain-Lain: 2 orang pelajar sambilan semasa cuti panjang dan cuti antara semester.

6. Peralatan Yang Telah Dibeli:

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UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

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Antimicrobial Studies on Ethyl-p-methoxycinnamate and Ethyl cinnamate from *Kaempferia galanga*.

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Abstrak

Etil-p-metoksisinamat dan etil sinamat merupakan dua komponen utama yang terdapat dalam ekstrak petroleum eter ringan *Kaempferia galanga*. Kedua-dua komponen ini bersama dengan ekstrak mentah, masing-masing diuji keaktifan antimikrobnya terhadap lapan organisma terpilih. Ujian dilakukan dengan menggunakan kaedah resapan agar-agar. Kesemua organisma, kecuali *Pseudomonas aeruginosa* dan *Bacillus subtilis* telah direncat pertumbuhannya oleh ekstrak mentah, etil-p-metoksisinamat dan etil sinamat.

Abstract

Ethyl-p-methoxycinnamate and ethyl cinnamate are two major components found in the light petroleum ether extract of *Kaempferia galanga*. These two components together with the crude extract were tested respectively for their antimicrobial activity against eight selected organisms. Tests were performed using the agar diffusion method. Except for *Pseudomonas aeruginosa* and *Bacillus subtilis*, the rest of the organisms were shown to be inhibited by the crude extract, ethyl-p-methoxycinnamate and ethyl cinnamate.

Introduction

Kaempferia galanga or better known locally as 'cekur' is a kind of herb purported to be of medicinal value. Among its medicinal values include the use of the juice as expectorant and carminative. The leaves serve for making lotions and poultices for almost all kinds of ailments. Burkhill (1966) mentioned them as use for sore throats, fevers, swellings and even rheumatism. The rhizomes are also found to contain a monoamine oxidase (MAO) inhibitor which could be used for the treatment of depression (Noro et al, 1983); and are also highly toxic to He La cells (Kosuge et al, 1985). The aqueous extract of the Kaempferia galanga rhizome has also been proven to be anti-asthmatic (Sadikun et al, 1988). The essential oil of Kaempferia galanga has also been shown to contain a number of components (Din et al, 1988) of which two of the main components are ethyl-p-methoxy-cinnamate and ethyl cinnamate.

Documentation of the various uses of this plant has thus prompted this investigation into its antimicrobial activities, in particular, on ethyl-p-methoxycinnamate and ethyl cinnamate, the two major components found in the light petroleum ether extracts of Kaempferia galanga.

Materials and Methods

Extraction and Separation

Dried powdered rhizome of Kaempferia galanga (500 g) was heated in light petroleum ether at 80°C for 2 days. Filtration followed by evaporation of excess solvent under reduced pressure afforded a crude extract (28.60 g, 5.72%) in the form of semi-crystalline material.

25.0 g of the crude light petroleum ether extract was subjected to silica gel column chromatography and eluted with a mixture of 1:9 ethyl acetate -- light petroleum ether to afford a colourless oil of ethyl cinnamate (1.75 g, 7%) and a solid material of ethyl-p-methoxycinnamate (13.5 g, 54%) which after recrystallisation from hexane -- ethyl acetate afforded a colourless crystal, m.p. 48-49°C.

The structure of ethyl cinnamate was confirmed on the basis of spectral (ir and nmr) comparisons with standard ethyl cinnamate obtained from Aldrich Co. Ltd. In the case of ethyl-p-methoxy-cinnamate, the structure was identical to ethyl-p-methoxy-cinnamate, as reported by Noro, et al, 1983 on the basis of m.p., ir and nmr spectral comparisons.

A known weight of the crude extract and the pure compound was taken up in a known volume of Tween 80 : Water mixture to yield the following concentrations:- 100, 75, 50 and 25 mg/ml for antimicrobial studies.

Media

All media were prepared according to the instructions given by the manufacturer and sterilised by autoclaving at 121°C for 15 minutes.

Microorganisms

The following strains were used as test organisms: Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 25922), Escherichia coli (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), Candida albicans (ATCC 10235), and laboratory cultures of Salmonella typhi, Enterobacter aerogenes and Klebsiella

aerogenes. The test organisms were maintained on Nutrient agar slants and were recovered for testing by growth in Nutrient Broth (Oxoid CMI) overnight.

Antimicrobial Activity

The crude extract and the two compounds were tested for antimicrobial activity using the agar-diffusion method (Verpoote, et al., 1982). The overnight cultures were diluted with nutrient agar to give a suspension of about 10^5 organisms/ml. 20 ml portions of this bulk seeded agar was poured into sterile Petri dishes and allowed to set. In all test plates holes of 8 mm in diameter were made with a sterilised cork borer, into each of which 0.1 ml of the test solution was pipetted. The holes were filled such that the solutions and their respective controls were on the same plate. All determinations were made at least in duplicate. After holding the plates for 1 hour at room temperature and incubation for 18-24 hours at 37°C, zones of inhibition were observed and recorded.

Results and Discussion

The results of the antimicrobial study are summarized in Table 1. Except for Pseudomonas aeruginosa and Bacillus subtilis, the rest of the organisms were shown to be inhibited by the crude extract, ethyl-p-methoxycinnamate and ethyl cinnamate. The inhibition is expressed as an absolute inhibition of microbial growth around the holes. Of the two compounds tested, ethyl-p-methoxycinnamate proved to be more promising as it was shown to have activity at all the concentrations tested.

As a follow up to the above study, attempts are now being made to synthesize various derivatives of ethyl-p-methoxycinnamate and to screen for their antibacterial activities.

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Acknowledgement:

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Table I: Antimicrobial Effect of *Kaempferia galanga*

	Antimicrobial Activity Against							
	E.coli	*E.aerogenes*	*S.aureus*	*Kl.aerogenes*	*S.typhi*	*Ps.aeruginosa*	*C.albicans*	*B.subtilis*
Crude extract	++++	+++	+++	+++	++++	-	++++	-
Ethyl cinnamate	+++	+++	+++	+++	+++	-	+++	-
Ethyl-p-methoxycinnamate	++++	++++	++++	++++	++++	-	++++	-

Grading of the results:

- \- no zone of inhibition
- +++ zone of inhibition at 100, 75 and 50 mg/ml
- ++++ zone of inhibition at 100, 75, 50 and 25 mg/ml

ANTIMICROBIAL ACTIVITY OF ETHYL-*P*-METHOXYCINNAMATE (FROM *KAEMPFERIA GALANGA* LINN) AND ITS DERIVATIVES

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Poster Presented at Third Malayasian International Conference
On Essential Oils & Flavour Chemicals (TMIC - EOFC 92)
20 - 23 July 1992, Langkawi, Kedah, Malaysia

INTRODUCTION

Kaempferia galanga or locally known as `cekur' is a kind of herb purported to be of medicinal value. The leaves can be used to make lotions and poultices for various ailments while the juice is used as expectorant and carminative (Burkhill, 1966). The rhizomes were found to contain a mono-amine oxidase (MAO) inhibitor which could be used for the treatment of depression (Noro, *et al*, 1983), and are toxic to He La cells (Kosuge *et al*,1985). The essential oil of *Kaempferia galanga*

has been shown to contain a number of components (Din *et al*, 1988) of which two of the main components are ethyl-*p*-methoxycinnamate and ethyl cinnamate. Ethyl-*p*-methoxycinnamate was shown to be more active in inhibiting the growth of several microorganisms as compared to ethyl cinnamate (Pazilah *et al*, 1990).

Based on this finding, futher work was carried out to synthesize various derivatives of ethyl-*p*-methoxycinnamate, namely, methyl-*p*-methoxycinnamate, propyl-*p*-methoxycinnamate, *p*-methoxycinnamic acid, *p*-methoxycinnamyl alcohol and amide of *p*-methoxycinnamic acid. These were then screened for their antimicrobial activities. Of the derivatives tested, only *p*-methoxycinnamyl alcohol was shown to be potentially active.

MATERIALS AND METHODS

(i) ethyl-*p*-methoxycinnamate

Dried powdered rhizome of *Kaempferia galanga* (500 g) was heated in light petroleum ether at 80°C for 2 days. Filtration followed by evaporation of excess solvent under reduced pressure afforded a crude extract (28.60 g, 5.72 %) in the form of semi-crystalline material.

25.0 g of the crude light petroleum ether extract was subjected to silica gel column chromatography and eluted with a mixture of 1:9 ethyl acetate - light petroleum ether to afford a colourless oil of ethyl cinnamate (1.75 g, 7%) and a solid material of ethyl-*p*-methoxycinnamate (13.5 g, 54%) which after recystallisation from hexane - ethyl acetate afforded a colourless crystal, m.p. 48-49°C.

The structure of ethyl-*p*-methoxycinnamate was identical to ethyl-*p*-methoxycinnamate as reported by Noro, *et al*, 1983 on the basis of m.p., ir and nmr spectral comparisons.

(ii) *p*-methoxycinnamyl alcohol

To a solution of lithium aluminium hydride (2.2 g) in anhydrous ether (50 ml) was added absolute alcohol (0.3 g) in ether (10 ml). The solution was then made up to 100 ml with ether. A portion (2 ml) of this reagent was added to a stirred solution of ethyl-*p*-methoxycinnamate (2.0 g) in anhydrous ether (50 ml). After 1 hour, a further portion (2 ml) of the reagent was added, and this process was continued until a total of 15 ml lithium aluminium hydride solution had been used. The reaction was stirred for a further 1 hour and followed by dropwise addition of saturated ammonium chloride until a granular precipitate was formed. The precipitate was removed by filtration, washed with chloroform (100 ml), and the combined filtrate dried (Na_2SO_4 anhydrous), filtered and solvent evaporated to afford *p*-methoxycinnamyl alcohol (1.2 g). The alcohol was then recrystallised with hexane - ethyl acetate to afford white crystals, m.p. 74-76°C.

(iii) ***p*-methoxycinnamic acid**

Ethyl-*p*-methoxycinnamate (6.0 g) was hydrolysed with 2 M sodium hydroxide and afforded 4.50 g of acid (m.p. 170-172°C).

(iv) **methyl-*p*-methoxycinnamate**

p-methoxycinnamic acid (1.0 g) was esterified with methanol in the presence of concentrated sulphuric acid and afforded 0.8 g of methyl ester (m.p. 80-82°C).

(v) **propyl-*p*-methoxycinnamate**

p-methoxycinnamic acid (1.0 g) was esterified with propyl alcohol in the presence of concentrated sulphuric acid and afforded a viscous liquid (1.05 g).

(vi) **amide of *p*-methoxycinnamic acid**

p-methoxycinnamic acid (1.5 g) was treated with thionyl chloride in tetrahydrofuran followed with addition of aqueous ammonium to afford the amide (0.5 g), m.p. 187-188°C.

Microorganisms

The following strains were used as test organisms: *Staphylococcus aureus* (ATCC 25922), *Escherichia coli* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and laboratory cultures of *Candida albicans, Salmonella p-typhi, Enterobacter aerogenes, Klebsiella pneumoniae* and *Bacilus subtilis*. The test organisms were maintained on Nutrient agar slants and were recovered for testing by growth in Nutrient Broth overnight.

Antimicrobial activity

The test for antimicrobial activity was carried out using the agar diffusion method. Test organisms were cultured overnight at 37°C in Nutrient Broth. After incubation, the inoculum was adjusted so that its turbidity matched that of a No. 0.5 McFarland barium sulfate standard (to give a suspension of ≈ 10^8 organisms/ml). 0.15 ml of this suspension was pipetted into an empty sterile petri dish, after which 15 ml of nutrient agar was poured to give a final concentration of 10^6 organisms/ml. In all test plates, holes of 8 mm in diameter were made, into each of which 0.1 ml of the test solution was pipetted. The holes were filled such that the test solutions and their respective controls were on the same plate. After incubation at 37°C for 18-24 hours, inhibition zones were observed and measured.

RESULTS

The results obtained showed that ethyl-*p*-methoxycinnamate inhibited the growth of all the organisms tested except *Pseudomonas aeruginosa*. Of its 5 derivatives tested, *p*-methoxycinnamyl alcohol was shown to be particularly active, having produced large zones of inhibition towards *Candida albicans, Bacillus subtilis* and *Salmonella p-typhi*. Methyl-*p*-methoxycinnamate, propyl-*p*-methoxycinnamate, *p*-methoxycinnamate acid and in particular, the amide of *p*-methoxycinnamic acid did not show any significant antibacterial activity. However, the overall results obtained showed that all of the 6 compounds tested (particularly ethyl-*p*-methoxycinnamate and *p*-methoxycinnamyl alcohol) were active towards *Candida albicans* (Table I - VI).

Average zone diameters (mm) produced by ethyl-*p*-methoxycinnamate and its derivatives

Table I. Ethyl-*p*-methoxycinnamate

Organism	[ ] mg/ml 6.25	3.125	1.56	0.78	0.39	0.195	0.0975
Escherichia coli	11.91	12.45	9.72	9.05	9.60	9.22	9.64
Enterobacter aerogenes	9.75	10.04	9.50	9.68	9.60	9.31	9.42
**Klebsiella pneumoniae*	10.88	10.45	9.85	10.40	10.79	10.80	10.88
Salmonella p-typhi	9.58	9.38	9.38	9.31	-	-	-
Bacillus subtilis	11.35	11.10	10.55	10.05	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Staphylococcus aureus	10.81	11.16	10.55	-	-	-	-
Candida albicans	21.78	21.72	20.30	20.52	14.72	-	-
Control (DMSO 50%)	No Zone of Inhibition Observed						

Table II. *p*-methoxycinnamyl alcohol

Organism	[ ] mg/ml 6.25	3.125	1.56	0.78	0.39	0.195	0.0975
Escherichia coli	10.98	9.51	-	-	-	-	-
Enterobacter aerogenes	-	-	-	-	-	-	-
Klebsiella pneumoniae	11.49	9.86	9.76	-	-	-	-
Salmonella p-typhi	16.25	9.55	-	-	-	-	-
Bacillus subtilis	23.66	16.25	9.10	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-
Candida albicans	24.60	11.92	10.58	10.78	11.08	11.35	11.02
Control (DMSO 50%)	No Zone of Inhibition Observed						

* Zone of inhibition was surrounded by a ring of partial inhibition.

Table III. Methyl-*p*-methoxycinnamate

[ ] mg/ml							
Organism	6.25	3.125	1.56	0.78	0.39	0.195	0.0975
Escherichia coli	-	-	-	-	-	-	-
Enterobacter aerogenes	10.30	10.55	10.30	10.35	10.35	10.55	10.42
**Klebsiella pneumoniae*	11.10	11.42	11.02	10.75	10.55	10.50	10.32
Salmonella p-typhi	10.15	9.85	9.65	9.65	9.32	9.00	9.50
Bacillus subtilis	10.06	10.00	9.80	9.98	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-
Candida albicans	10.41	9.79	9.62	-	-	-	-
Control (DMSO 50%)	No Zone of Inhibition Observed						

* Partial inhibition

Table IV. Propyl-*p*-methoxycinnamate

[ ] mg/ml							
Organism	6.25	3.125	1.56	0.78	0.39	0.195	0.0975
Escherichia coli	-	-	-	-	-	-	-
Enterobacter aerogenes	-	-	-	-	-	-	-
Klebsiella pneumoniae	10.66	10.49	10.79	10.52	10.85	10.62	10.40
Salmonella p-typhi	-	-	-	-	-	-	-
Bacillus subtilis	11.00	11.00	10.22	9.41	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-
Candida albicans	13.70	14.10	13.10	16.30	13.18	11.65	11.52
Control (DMSO 50%)	No Zone of Inhibition Observed						

Table V. *p*-methoxycinnamic acid

[ ] mg/ml							
Organism	6.25	3.125	1.56	0.78	0.39	0.195	0.0975
Escherichia coli	-	-	-	-	-	-	-
Enterobacter aerogenes	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-	-	-
Salmonella p-typhi	9.62	9.46	-	-	-	-	-
Bacillus subtilis	9.50	9.65	9.68	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-
Candida albicans	12.32	12.11	11.48	11.25	10.78	10.32	10.58
Control (DMSO 50%)	No Zone of Inhibition Observed						

Table VI. Amide of *p*-methoxycinnamic acid

[ ] mg/ml							
Organism	6.25	3.125	1.56	0.78	0.39	0.195	0.0975
Escherichia coli	9.75	-	-	-	-	-	-
Enterobacter aerogenes	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-	-	-
Salmonella p-typhi	9.07	9.67	9.17	9.37	9.02	9.17	9.24
Bacillus subtilis	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-
Candida albicans	10.52	10.25	9.11	9.08	-	-	-
Control (DMSO 50%)	No Zone of Inhibition Observed						

CONCLUSION

Although ethyl-*p*-methoxycinnamate and its derivatives were able to inhibit certain test organisms, its activity as an antibacterial agent, however, can only be considered as moderate. The zones of inhibition produced were not dose responsive, and this could possibly reflect on the solubility of the compounds. The above findings may also suggest that the mechanism of action of the above compounds was bacteriostatic in nature. This deduction was made on the basis of the occasional presence of an inner ring of partial growth within the zones of inhibition, and that some of the zones of inhibition produced were observed to become smaller as the incubation continued.

ANITITUMOR ACTIVITY OF ETHYL-*P*-METHOXYCINNAMATE (FROM *KAEMPFERIA GALANGA* LINN) AND ITS ALCOHOL DERIVATIVE, *P*-METHOXYCINNAMYL ALCOHOL

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Poster presented at Seminar Kebangsaan ke 9 Kimia Sebatian Semulajadi,
21 - 22 Oktober 1992, Universiti Pertanian Malaysia

INTRODUCTION

Ethyl-p-methoxycinnamate which is the major constituent of *Kaempferia galanga* was isolated from the petroleum ether extract of the dried rhizome. Previous investigations have shown that the compound and its alcohol derivative, *p*-methoxycinnamyl alcohol exhibited antimicrobial activity against selected test organisms. (Pazilah et al, 1992). As an extension to the above finding, the two compounds were tested for antitumour activity using mouse tumour cell line P388 D1. Both ethyl-*p*-methoxycinnamate and its derivative, *p*-methoxycinnamyl alcohol showed cytotoxic activity with $1C_{50}$ of 50 and 12.5 μg/ml respectively.

MATERIALS AND METHODS

(i) ethyl-*p*-methoxycinnamate

Dried powdered rhizome of *Kaempferia galanga* (500 g) was heated in light petroleum ether at 80°C for 2 days. Filtration followed by evaporation of excess solvent under reduced pressure afforded a crude extract (28.60 g, 5.72 %) in the form of semi-crystalline material.

25.0 g of the crude light petroleum ether extract was subjected to silica gel column chromatography and eluted with a mixture of 1:9 ethyl acetate - light petroleum ether to afford a colourless oil of ethyl cinnamate (1.75 g, 7%) and a solid material of ethyl-*p*-methoxycinnamate (13.5 g, 54%) which after recystallisation from hexane - ethyl acetate afforded a colourless crystal, m.p. 48-49°C.

The structure of ethyl-*p*-methoxycinnamate was identical to ethyl-*p*-methoxycinnamate as reported by Noro, *et al*, 1983 on the basis of m.p., ir and nmr spectral comparisons.

(ii) *p*-methoxycinnamyl alcohol

To a solution of lithium aluminium hydride (2.2 g) in anhydrous ether (50 ml) was added absolute alcohol (0.3 g) in ether (10 ml). The solution was then made up to 100 ml with ether. A portion (2 ml) of this reagent was added to a stirred solution of ethyl-*p*-methoxycinnamate (2.0 g) in anhydrous ether (50 ml). After 1 hour, a further portion (2 ml) of the reagent was added, and this process was continued until a total of 15 ml lithium aluminium hydride solution had been used. The reaction was stirred for a further 1 hour and followed by dropwise addition of saturated ammonium chloride until a granular precipitate was formed. The precipitate was removed by filtration, washed with chloroform (100 ml), and the combined filtrate dried (Na_2SO_4 anhydrous), filtered and solvent evaporated to afford *p*-methoxycinnamyl alcohol (1.2 g). The alcohol was then recrystallised with hexane - ethyl acetate to afford white crystals, m.p. 74-76°C.

Antitumour Activity

Antitumour activity of the compounds was assayed on the methylchloranthene induced mouse tumour cell line P388 D1. Cells were grown in 24 well cluster dishes in Leibovitz (L15) medium supplemented with 3% heat inactivated foetal bovine serum at a density of 2.5×10^5 cells/well. Monolayers were then treated with various concentrations of the compounds or diluent controls and incubated at 37°C for 24 hours and observed for evidence of cytotoxicity.

Sample Preparation

Sample was weighed and dissolved in 95% ethanol to give a stock solution of 100 mg/ml. Further dilutions were prepared in L15 medium. In the highest concentration of the sample, the final concentration of ethanol was 0.95%. Equivalent controls without sample were also included at all times.

RESULTS

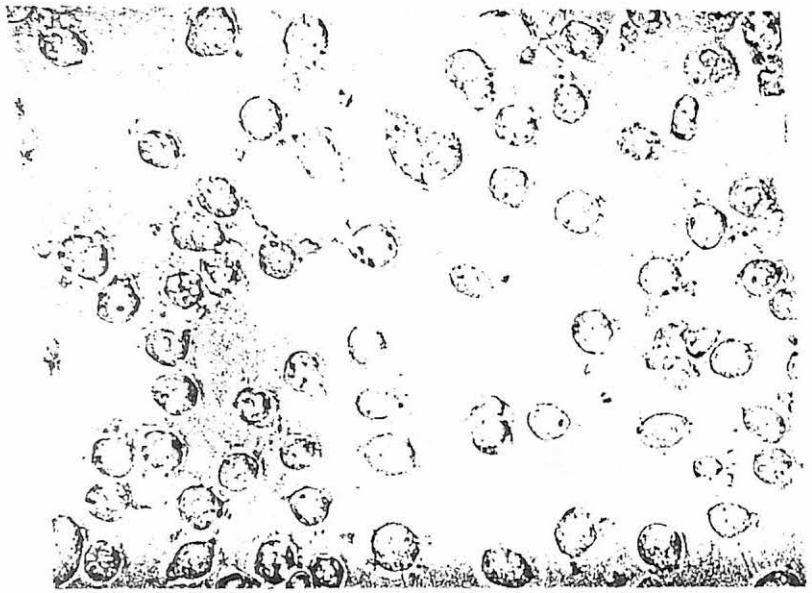
Cultures were scored for level of cytotoxicity as follows:

100 % cells dead	+ + + +
75 % cells dead	+ + +
50 % cells dead	+ +
<50 % cells dead	+
none of the cells dead	-

Table 1: Antitumour activity of ethyl-*p*-methoxycinnamate and its alcohol derivative, *p*-methoxycinnamyl alcohol.

Sample Final [ ]/ml	Ethyl-*p*-methoxycinnamate	*p*-methoxycinnamyl alcohol
Set I		
1000.0 μg	++++	++++
500.0 μg	++++	++++
250.0 μg	++++	++++
125.0 μg	+++	++++
62.5 μg	+	++++
31.3 μg	-	+++
Set II		
100.0 μg	+++	++++
50.0 μg	++	++++
25.0 μg	-	+++
12.5 μg	-	++
6.3 μg	-	-
3.1 μg	-	-
CONTROL	-	-
IC_{50}	≈ 50 μg/ml	≈ 12.5 μg/ml

Effects of ethyl-*p*-methoxycinnamate on P388 D1 cells



←

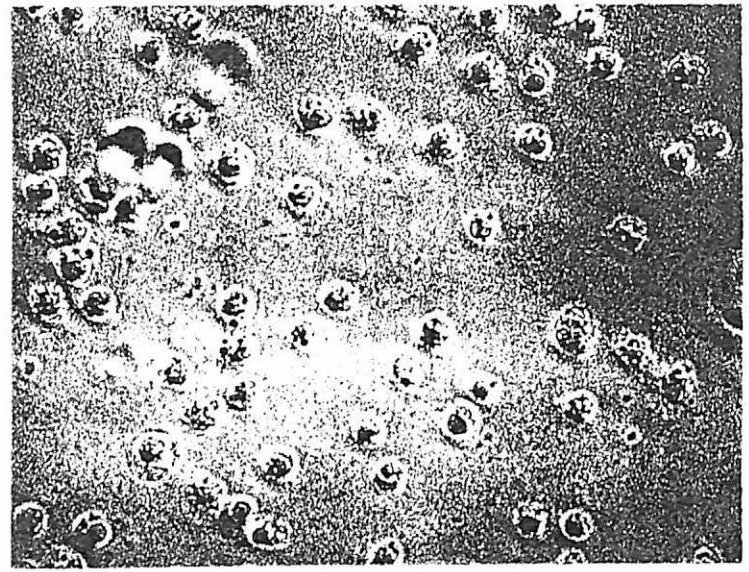
Control:

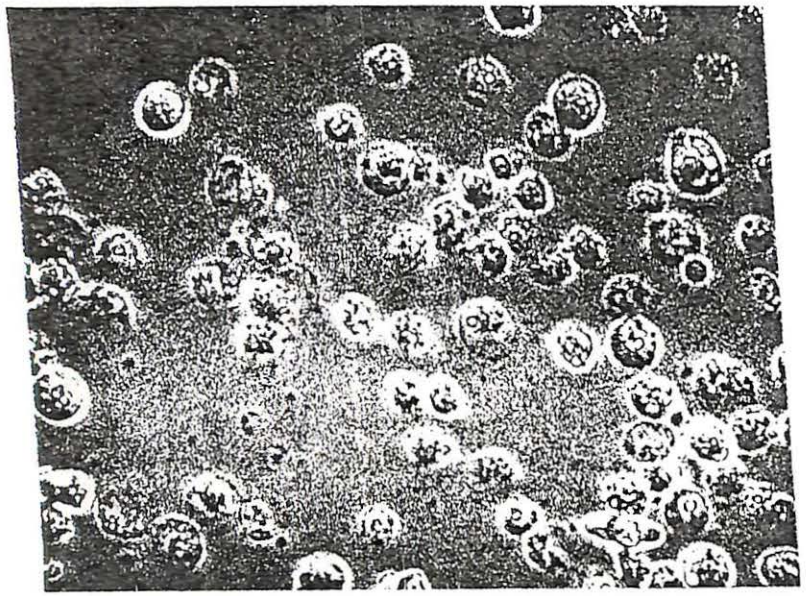
adherent;
many processes

Concentration: 500 μg/ml

→

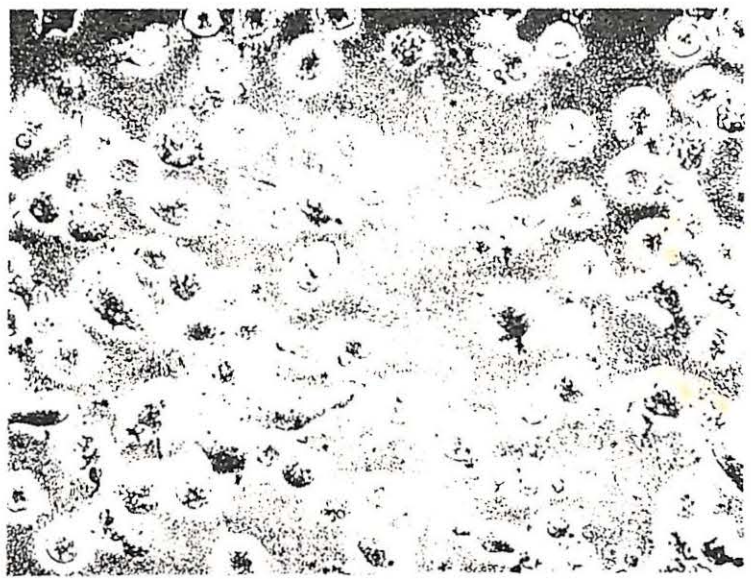
All cells dead





Concentration: 125 μg/ml

Most cells dead



Concentration: 62.5 μg/ml

some cells dead
some living cells are rounded
and some have normal
morphology