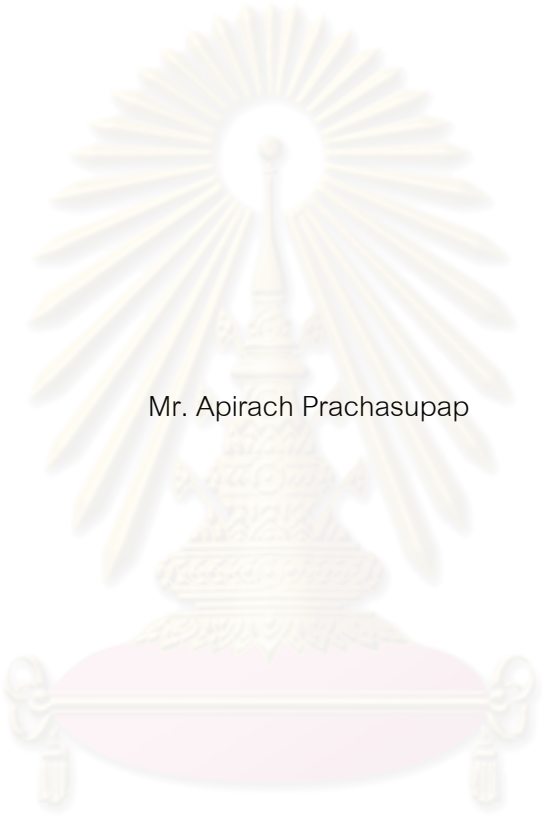
ฤทธิ์ที่ทำให้เซลล์ไวต่อแสงของสิ่งสกัดจากพืชบางชนิดในวงศ์ส้มและวงศ์ผักชี

นายอภิรัช ประชาสุภาพ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์สาธารณสุข
วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2553

PHOTOTOXIC ACTIVITIES OF SELECTED THAI RUTACEOUS AND UMBELLIFEROUS PLANT EXTRACTS

Mr. Apirach Prachasupap



A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Public Health Sciences
College of Public Health Sciences
Chulalongkorn University
Academic Year 2010

Thesis Title	PHOTOTOXIC ACTIVITIES OF SELECTED THAI RUTACEOUS AND UMBELLIFEROUS PLANT EXTRACTS
By	Mr. Apirach Prachasupap
Field of Study	Public Health Sciences
Thesis Advisor	Associate Professor Nijsiri Ruangrungsi, Ph.D.
Thesis Co-advisor	Chanida Palanuvej, Ph.D.

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อภิรัช ประชาสุภาพ : ฤทธิ์ที่ทำให้เซลล์ไวต่อแสงของสิ่งสกัดจากพืชบางชนิดในวงศ์ส้มและวงศ์ผักชี (Phototoxic activities of selected Thai Rutaceous and Umbellifereous plant extracts) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. นิจศิริ เรืองรังษี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ดร. ชนิดา พลานุเวช, 90 หน้า.

ได้ศึกษาในหลอดทดลองเกี่ยวกับฤทธิ์ที่ทำให้เซลล์ไวต่อแสงของสิ่งสกัดจากพืชบางชนิดในวงศ์ส้มและวงศ์ผักชีของไทย ทั้งหมด 25 ชนิด เพื่อค้นหาพืชที่มีฤทธิ์ทำลายเซลล์เมื่อถูกแสงกระตุ้น โดยทำการทดสอบกับเชื้อจุลินทรีย์มาตรฐานสายพันธุ์ต่างๆ ซึ่งให้เชื้อจุลินทรีย์ต่างๆเหล่านี้เป็นตัวแทนลักษณะของเซลล์ ได้แก่ แบคทีเรียแกรมลบ *Escherichia coli* ATCC25922, แบคทีเรียแกรมบวก *Staphylococcus aureus* ATCC6538P และ *Bacillus subtilis* ATCC6633, รายีสต์ *Candida albicans* ATCC10230 และ *Saccharomyces cerevisiae* ATCC9763 จากการทดสอบความไวของเชื้อจุลินทรีย์โดยเทคนิคการแพร่บนอาหารวุ้นร่วมกับการฉายแสงอัลตร้าไวโอเลตเปรียบเทียบกับการไม่ฉายแสงต่อสิ่งสกัดจากพืชทั้งสองวงศ์รวม 25 ชนิด พบว่ามีพืชจำนวน 13 ชนิดที่มีฤทธิ์ทำลายเซลล์หรือฤทธิ์การยับยั้งเชื้อจุลินทรีย์ต่างๆได้เมื่อถูกแสง คือ รากมะตูมแห้ง, ใบมะนาวผีสด, ใบมะสังแห้ง, ใบกระแจะแห้ง, ใบหอมแขกสดและใบมะนาวเทศสด ในวงศ์ส้ม ผักชีลาวทั้งต้นและผลแห้งของผักชีลาวหรือเทียนตาตั๊กแตน, เหง้าโกฐสอแห้ง, ผลคื่นไฉ่แห้ง, เทียนข้าวเปลือกแห้ง, ผลมะแหลบแห้ง, เทียนเยาวพาณีแห้งและเทียนสัตตบุษย์แห้ง ในวงศ์ผักชี ซึ่งการยับยั้งเชื้อจุลินทรีย์ของพืชเหล่านี้เป็นไปในลักษณะเลือกยับยั้งต่อเชื้อได้ในบางสายพันธุ์ และพบว่าสายพันธุ์ *S. aureus* ถูกเลือกในการยับยั้งมากที่สุดในขณะที่ *B. subtilis, C. albicans* และ *S. cerevisiae* ถูกเลือกรองลงมาตามลำดับ ในส่วนของ *E. coli* ถูกปฏิเสธการยับยั้งจากการทดสอบทั้งหมด

สาขาวิชา: วิทยาศาสตร์สาธารณสุข ลายมือชื่อนิสิต .....

ปีการศึกษา: 2553 ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์หลัก .....

ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม .....

5279306853 : MAJOR PUBLIC HEALTH SCIENCES
KEYWORDS : PHOTOTOXIC ACTIVITIES / RUTACEAE / UMBELLIFERAE / FUROCOUMARINS / MICROORGANISM / AGAR DIFFUSION TEST

APIRACH PRACHASUPAP: PHOTOTOXIC ACTIVITIES OF SELECTED THAI RUTACEOUS AND UMBELLIFEREOUS PLANT EXTRACTS. ADVISOR: ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., CO-ADVISOR: CHANIDA PALANUVEJ, Ph.D., 90 pp.

Photoxic activities of selected Thai Rutaceous and Umbelliferous plant extracts were studied *in vitro*. Twenty five species were investigated to find plants which have efficacy in damaging cells when activated by the light. Phototoxicity was performed by evaluating the susceptibility of various microorganisms to plant extracts in combination with ultraviolet light. The standard strains of test microorganisms were represented as the target cells: gram-negative bacteria, *Escherichia coli* ATCC25922; gram-positive bacteria *Staphylococcus aureus* ATCC6538P and *Bacillus subtilis* ATCC6633, fungi or yeast *Candida albicans* ATCC10230 and *Saccharomyces cerevisiae* ATCC9763. The susceptibility test was determined by agar diffusion technique. Comparison of inhibition zones between with and without UV was investigated. Ethanol extracts of 13 species showed UV-induced inhibitory activity against microorganisms. These were from both families as follow: *Aegle marmelos* (L.) Corr. (dried roots), *Atalantia monophylla* DC. (fresh leaves), *Feroniella lucida* (Scheff.) Swingle. (dried leaves), *Hesperethusa crenulata* (Roxb.) Roem. (dried leaves), *Murraya koenigii* L. (fresh leaves), *Triphasia trifolia* (Burm.t.) P.Wils. (fresh leaves) in Rutaceous plants and *Anethum graveolens* L. (fresh whole plants and dried fruits), *Angelica dahulica* Benth. (dried rhizomes), *Apium graveolens* (dried fruits), *Foeniculum vulgare* Mill. (dried fruits), *Heracleum siamicum* Craib (dried fruits), *Petroselinum crispum*(Miller) A.W. Hill (dried fruits), *Pimpinella anisum* L. (dried fruits) in Umbelliferous plants. Furthermore, these extracts exhibited selectively inhibitory effect against the tested microorganisms. *S. aureus* strain was mostly selected, followed by *B. subtilis*, *C. albicans* and *S. cerevisiae* respectively. Whilst *E. coli* showed negative effect of UV induced inhibitory activity.

Field of Study: Public Health Sciences Student's Signature Apirach Prachasupap

Academic Year: 2010 Advisor's Signature Nijsiri Ruangrungsi

Co-advisor's Signature Chanida Palanuvej

ACKNOWLEDGEMENTS

The author wishes to express his hearty gratitude and appreciation to his thesis advisor, Associate Professor Dr. Nijsiri Ruangrungsi, College of Public Health Sciences and Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his continuous guidance, support and encouragement throughout this study.

The author is grateful to his thesis co-advisor, Dr. Chanida Palanuvej, College of Public Health Sciences, Chulalongkorn University, for her valuable suggestion and encouragement throughout this study.

The author is also thankful to Associate Professor Dr. Vimolmas Lipipun, Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for providing microorganisms to test.

It is an honor for the author to thank College of Public Health Sciences, Chulalongkorn University and all the staff members for necessary assistance and instrumental support.

It is a pleasure to thank to teachers and friends, College of Public Health Sciences, Chulalongkorn University, for their contribution and friendship.

Finally, the author will always be thankful to the author's family, especially his parents and all the members for their love, understanding, patience, support and encouragement throughout this study.

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LIST OF ABBREVIATIONS

ATCC	=	American Type Culture Collection, Maryland, USA
°C	=	Degree celsius
CFU	=	Colony forming unit
cm	=	Centimeter
DMSO	=	Dimethyl sulfoxide
g	=	Gram
hr	=	Hour
hrs	=	Hours
kg	=	Kilogram
m^2	=	Square meter
mg	=	Milligram
MHA	=	Mueller hinton agar
MIC	=	Minimal inhibitory concentration
min	=	Minute
ml	=	Milliliter
mm	=	Millimeter
nm	=	Nanometer
NCCLS	=	National Committee for Clinical Laboratory Standard
NSS	=	Normal saline solution
SDA	=	Sabouraud dextrose agar
W	=	Watt
µg	=	Microgram
µl	=	Microliter

CHAPTER I

INTRODUCTION

Background and Significance of the Study

Ultraviolet (UV) radiation is a part of electromagnetic spectrum, which consist of wavelengths from 100 nm to 400 nm. The ultraviolet spectrum can be further divided into three characteristics: long-wave (UVA), medium wave (UVB), and short wave (UVC) which the Earth's ozone layers shield, filter and attenuate the UV radiation. However, the amounts of rays that reach the earth's surface are large enough to cause harmful biological effects on the skin [1-3]. Furthermore, skin disorders precipitated by exposure to sunlight or photosensitive eruption are broadly divided into two types: phototoxic reaction and photoallergic reaction. Both are usually elicited by longer UVA wavelength (>315 nm). Photoallergic reaction are immunological mediated, while phototoxic reaction are non immunological events that inducing toxic cell damage [4].

Additionally, some chemicals cause a skin irritation response only in the presence of light [5]. These types of materials are called phototoxic materials Most substances such as drugs or chemicals as well as cosmetics, vegetables, fruits and food additives which exhibit phototoxic potential are called photosensitizers [6, 7]. Moreover acute skin reactions to photosensitizing compounds may be due to phototoxic or photoallergic. Photosensitivity reaction of the human skin after contact with photosensitizing plants is well known as phytophotodermatitis. It is a classical example of phototoxic reaction which is defined as inflammatory skin reaction caused by exposed to sunlight and contact with some plants containing furocoumarins frequently the psoralen. Phototoxic reactions resemble hyperpigmentation or sunburn and may also present with irritant, urticaria and allergic, as well as erythema, oedema, blistering and sometime vesiculation [8-12].

Furocoumarins as psoralen, 5-methoxypsoralen (5-MOP) and 8-methoxypsoralen (8-MOP) are potent photosensitizers that are activated by near-UV light (300-380 nm). UVA wavelengths between 350 and 365 in the presence of furocoumarins able to induce the maximal phototoxic skin in human [13]. It has been

reported that combination of long-wave UV radiation with some furocoumarins and drugs are toxic to DNA of various microorganism. During the UVA irradiation, furocoumarins form mono- or di- photoadduct with the pyrimidine bases of the DNA, resulting in the cross-linking of two strands of DNA, thereby causing in a partial loss of template activity for RNA synthesis as well as inhibition of DNA replication [14]. From previous studies, furocoumarins have been shown phototoxic to microorganisms such as yeast or bacteria. Therefore, it may be useful to screen the plants of phototoxic activity by microorganisms [15-18].

The identification of chemicals or ingredients and formulation able to elicit a phototoxic reaction is an important step in risk assessment processes. According to the current recommendation, all chemical, ingredients, or cosmetic finished products absorbing UV should be tested for acute phototoxic potential [19, 20]. The phototoxicity screening is important to assess the potential sources of phototoxic chemicals. The phototoxicity testing has been frequently conducted with living animal and human [21].

It is well known that members of Rutaceae and Umbelliferae family are most species containing natural furocoumarins as psoralen, bergapten, xanthoxin and closely related derivatives [22]. In human, exposure with the potent photosensitizing agents can increase sensitivity to sunlight especially UVA wavelength (>315 nm) which causes phototoxic dermatitis of variable intensity [23]. In Thailand, such plants have been consumed for culinary purposes because of the flavor, nutritional values as well as for ingredients of some cosmetics and perfumery which may exhibit phototoxicity. Hence in this study, a number of Thai Rutaceous and Umbelliferous plants were selected as a source of phototoxicity against microorganisms. The bacteria as *Bacillius subtilis, Staphylococcus aureus, Escherichia coli* ; the yeast as *Candida albicans* and *Sacchalomyces cerevisiae* were different cells for evaluating their potential as microorganisms for phototoxicity assay. Therefore, the aim of this study was to develop microbiological assay to screen the phototoxic potential of selected Thai Rutaceous and Umbelliferous plant extracts.

Objectives of the Study

1. To screen a phototoxic activity of selected Thai Rutaceous and Umbelliferous plants.
2. To evaluate an appropriate selection of microorganism to phototoxic reaction.

Scopes of the Study

1. Extraction of Rutaceous and Umbelliferous plants, namely

a.	*Aegle marmelos* (L.) Corr.	Rutaceae
b.	*Atalantia monophylla* DC.	Rutaceae
c.	*Citrus aurantifolia* (Christm) Swing.	Rutaceae
d.	*Citrus reticulata* Blanco.	Rutaceae
e.	*Feroniella lucida* (Scheff.) Swingle.	Rutaceae
f.	*Glycosmis pentaphylla* (Retz.) DC.	Rutaceae
g.	*Hesperethusa crenulata* (Roxb.) Roem.	Rutaceae
h.	*Murraya koenigii* L.	Rutaceae
i.	*Murraya paniculata* L.	Rutaceae
j.	*Triphasia trifolia* (Burm.t.) P.Wils.	Rutaceae
k.	*Zanthoxylum limonella* (Dennst.) Alston.	Rutaceae
l.	*Anethum graveolens* L.	Umbelliferae
m.	*Angelica dahulica* Benth.	Umbelliferae
n.	*Angelica sinensis* (Oliv.) Diels.	Umbelliferae
o.	*Apium graveolens* L.	Umbelliferae
p.	*Coriandrum sativum* Vern. Dhania.	Umbelliferae
q.	*Cuminum cyminum* L.	Umbelliferae
r.	*Daucus carota* L.	Umbelliferae
s.	*Eryngium foetidum* L.	Umbelliferae

t.	*Ferrula assa-foetida* L.	Umbelliferae
u.	*Foeniculum vulgare* Mill.	Umbelliferae
v.	*Heracleum siamicum* Craib	Umbelliferae
w.	*Ligusticum wallichii* Franch.	Umbelliferae
x.	*Petroselinum crispum* (Miller) A.W. Hill	Umbelliferae
y.	*Pimpinella anisum* L.	Umbelliferae

2. *In vitro* studies of the phototoxic activities using susceptibility test with various microorganisms and plant extracts and evaluating MIC value.
3. Comparison of the inhibition zones of each microorganisms against plant extracts among irradiation with and without UV lamp at wavelength 360 nm

Expected Benefits

1. This research contributes the basic information regarding a phototoxic activity of the selected Thai Rutaceous and Umbelliferous plants.
2. This research method can be applied for the screening of photosensitizing property of herbal product especially in skin care purpose.

CHAPTER II

LITERATURE REVIEWS

Photosensitivity

Photosensitivity is broadly divided into two major types, phototoxicity and photoallergy. Both require the agents or chemicals to absorb ultraviolet A (UVA) energy to be caused of activation. The result of Phototoxicity is direct cellular damage caused by phototoxic agent and radiation. Phototoxic disorder can occur in any person who receives enough doses of the agent and who is exposed to sufficient of the activating wavelengths of radiation (UV or visible light). No immunologic mechanisms are involved in phototoxic reaction, which they can exhibit themselves during begin exposed. In contrast, photoallergy refers to immunologically mediated photosensitivity reaction. It is a delayed hypersensitivity response to a molecule that has been modified by absorption of light energy. However, Phototoxicity and Photoallergy have distinguishing features and similarity (Table 1) [24, 25].

Table 1 Differenting features of Phototoxicity and Photoallergy

Feature	Phototoxicity	Photoallergy
Clinical characteristic	Exaggerated sunburn reaction: erythema, edema, vesicales, and bullae; burning, stinging; frequently resolves with hyperimentation	Acute, subacute, or chronic dermatitis: A rash, usually eczemtous lesions and usually pruritic
Onset after exposure	Minutes to hours	24 hr or more
Requirement for immunization	No	Yes
Incidence	High	Low
Dose of agent required	Large	Small

The basic principles of light

Sunlight is Earth's primary source of energy. Solar energy has been essential to the variety of natural and synthetic processes of life on earth. It can produce the so-called photobiological effects on microorganisms, plants, animals and humans. Ultraviolet (UV) irradiances from the sun are defined as the wavelength range of $100 \leq \lambda < 400$ nm, a wavelength shorter than that of visible light, but longer than x-rays. The radiation within the UV spectrum can be further divided by wavelength into three spectral regions: UVA (320-400 nm), UVB (280-320 nm) and UVC (200-290 nm) (Figure 1). Besides, Ultraviolet is classified as follows: [1-4]

Vacuum Ultraviolet (VUV)	(wavelength range of $10 \leq \lambda < 200$ nm)
Extreme Ultraviolet (EUV)	(wavelength range of $10 \leq \lambda < 121$ nm)
Lyman-alpha (Lyman-α)	(wavelength range of $121 \leq \lambda < 122$ nm)
Far Ultraviolet (FUV)	(wavelength range of $122 \leq \lambda < 200$ nm)
Middle Ultraviolet (MUV)	(wavelength range of $200 \leq \lambda < 300$ nm)
Near Ultraviolet (NUV)	(wavelength range of $300 \leq \lambda < 400$ nm)

The Earth's atmosphere (ozone (O_3), dioxygen (O_2) and water vapor (H_2O)) selectively filter out both UVC and UVB radiation. Due to this, UVA makes up about 95% of the UV radiation that reaches the earth (Figure 2). The penetration of UV ray into and through tissue of skin cells has significant consequences (Figure 2) [2, 3]. It can cause damage to the skin such as erythema or sunburn, inflammation, mutagenic, precancerous lesions and skin cancer including melanoma. Formation of singlet oxygen radicals initiated by UV exposure has significance in inducing a quick browning, causing skin aging [4, 26].

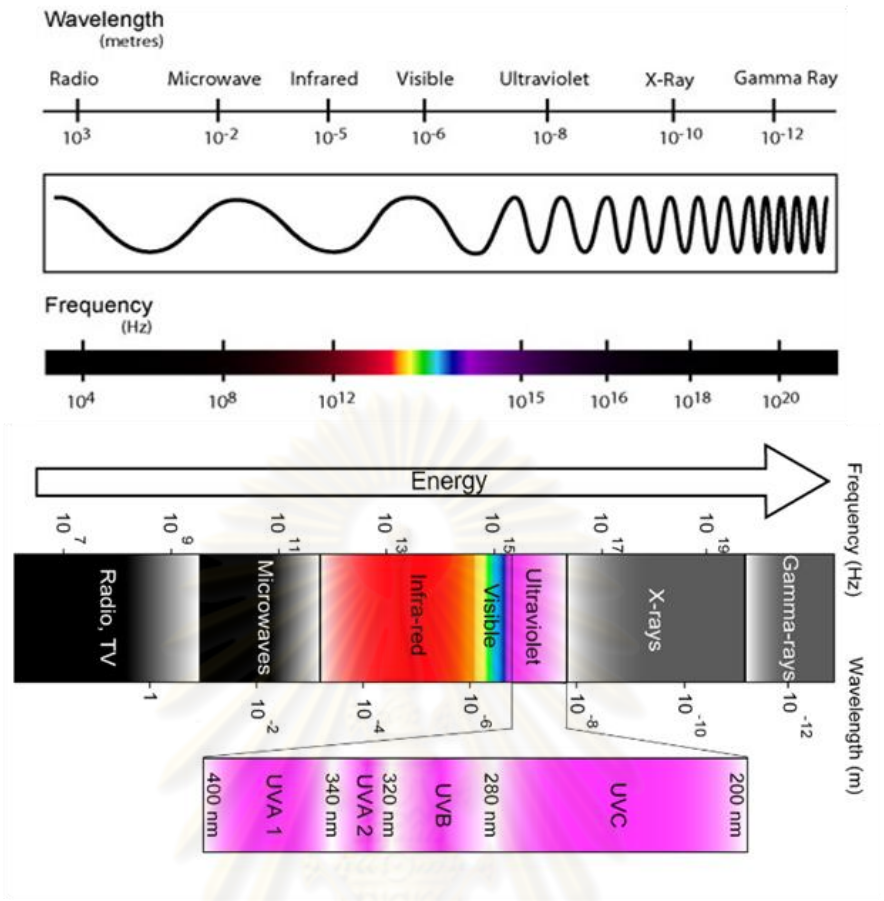


Figure 1 Various wavelength of electromagnetic spectrum

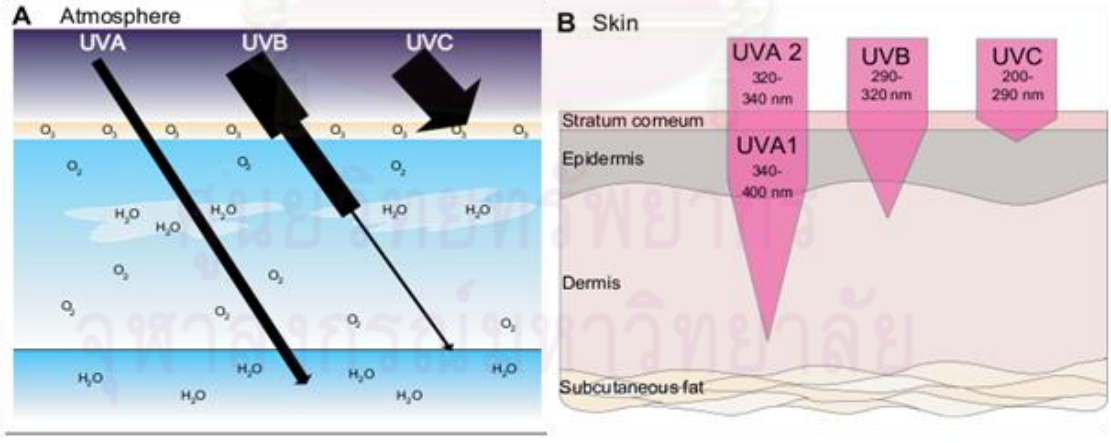


Figure 2

UV region and penetration of light into the skin: (A) Difference of penetration of UV region due to ozone absorption before reaching the surface of the Earth's. (B) Penetration of light of varying wavelength into the skin.

UV absorption of melanin is first defense mechanism when UV radiation penetrates into the skin. This pigment is made in melanocytes and then transferres to keratinocytes *via* long dendritic processes. UV escaping from melanin absorption can induce DNA damage by either creating reactive oxygen species which cause skin aging or by directly inducing chemical reactions within DNA. DNA can absorb the ionizing radiation of ultraviolet light and undergo chemical modifications including the formation of cyclobutane pyrimidine dimers (CPD) or 6-4 photoproducts (6-4PPs) (Figure 3) [27].

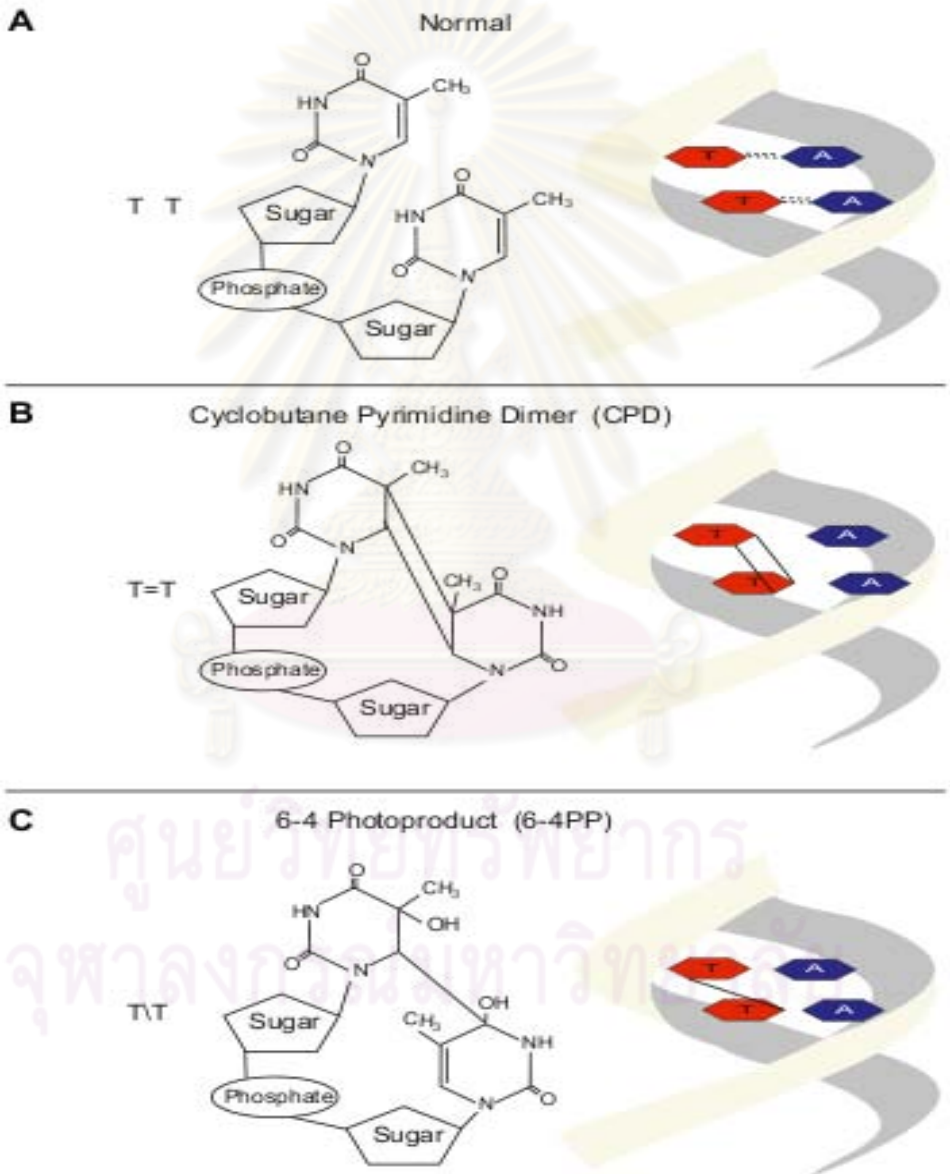


Figure 3

DNA damages: (A) Two normal thymidine residues. (B)&(C) Formation of cyclobutane pyrimidine dimers (CDP) and 6-4 photoproducts (6-4PP)

Furanocoumarins (Furocoumarins)

Furocoumarins are coumarin derivitives with a furan ring attached at the 6, 7- or 7, 8- position coumarin, divided to linear and angular types with substituents at one or both of the remaining benzenoid positions (Figure 4). Furocoumarins occur mainly in the Rutaceae and Umbelliferae and are of toxicological importance because of their photosensitizing properties. After percutaneous and also oral absorption, effect of light (UV radiation energy) is to bring about injury to the skin cell with erythema and blistering, swelling and increased pigmentation (phytophotodermatitis, PPD) [27]. Furocoumarins naturally are in the leaves, roots and fruits of plants which have been used for centuries in India, Egypt and other oriental countries for treatment of vitiligo. Linear furanocoumarin, xanthotoxin purified from *Ammi majus* was first introduced in the treatment of pigmentation defects as vitiligo long time ago [27, 28]. Most of the compounds implicated are liner furocoumarins: psoralen, bergapten (5-methoxypsoralen), xanthotoxin (8-methoxypsoralen). Some angular furocoumarins are also phototoxic: pimpinellin and the weaker toxin angelicin and sphondin (6-methoxyangelicin). It is known that linear furocoumarins can undergo cycloadditions at the 3, 4- and/or 4', 5'- positions onto the pyrimidine bases of DNA, yielding, in the presence of light, mono- or bi-functional adducts. The latter can then cross-link the macromolecule. This property explains that mutagenic activity and cell mortality, but it does not account for the resulting photosensitivity and hyperpigmentation (Figure 5) [28].

However, Klaber R.E. [29] repoted in the term of Phytophotodermatitis, emphasising the need for both plant which containing derivative isomers of furocoumarins and light to cause the reaction, sunburn and widespread blistering lesions or damage to epidermal cell. Furthermore, Solis R.R. *et. al.* [11] reported a phytophotodermatitis due to preparing margaritas by squeezing limes with hands, subsequently, sun exposure throughout the day. The next day, erythema affecting of fingers was occurred. Two days after the sun exposure, vesicles developed over the eryhematous areas (Figure 6). According to Weber I.C. *et. al.* finding on case reported, a patient squeezed limes and put them in the beverages. Result was shown phototoxic eruptions, the parallel streaks on the patient's thigh apparently developed after wiping excess lime juice from her fingers (Figure 7) [9].

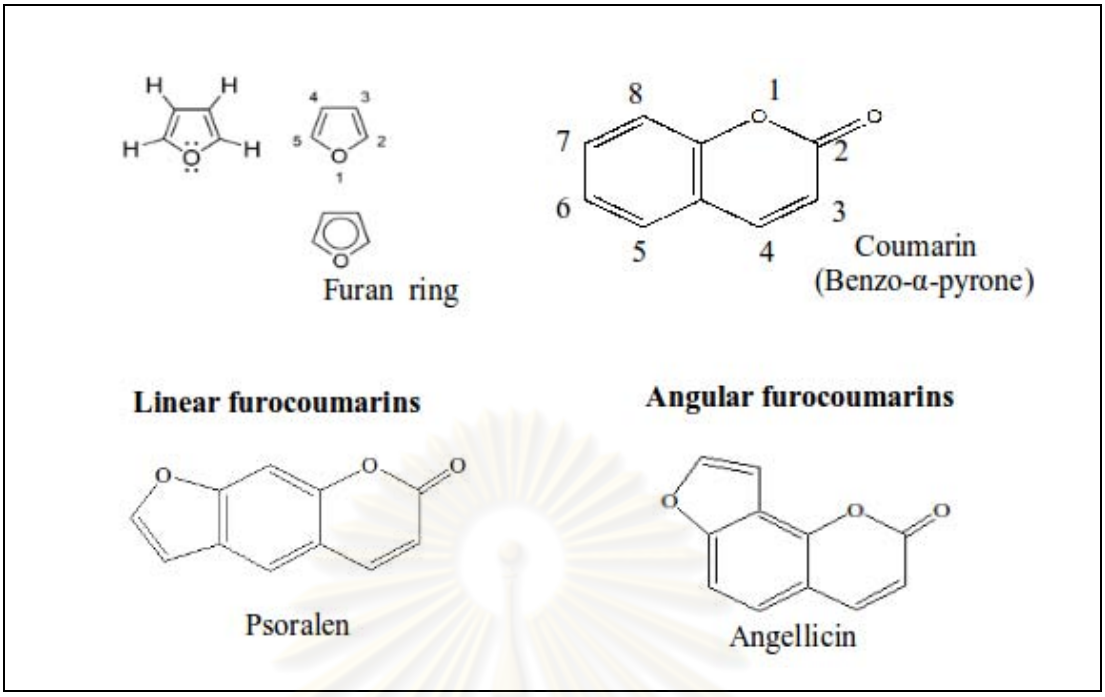


Figure 4

The chemical structure of furanocoumarins consists of a furan ring fused with coumarin

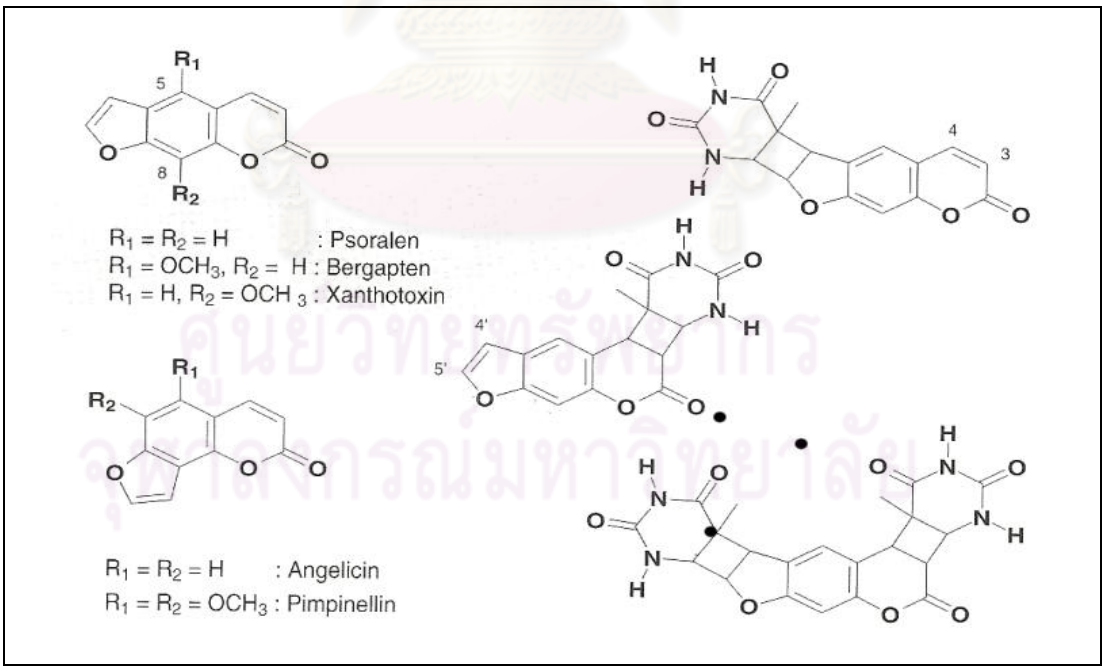


Figure 5 Linear or angular furocoumarins with pirimidine bases of DNA

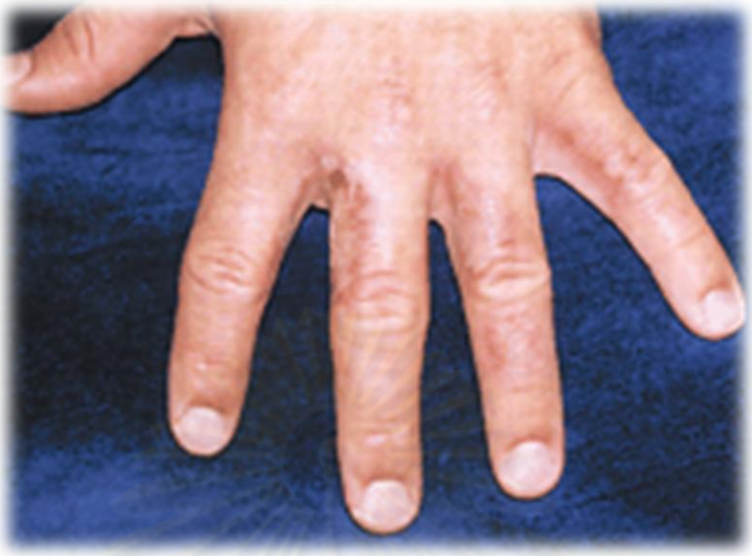


Figure 6 Vesicles distributed in erythematous areas of the fingers

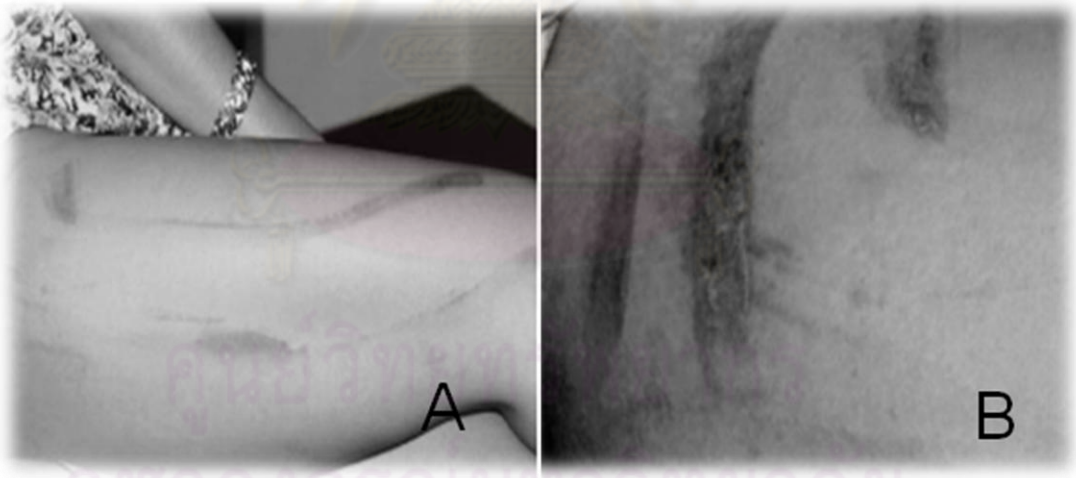


Figure 7

Phototoxic eruptions: (A) Patient's lower extremity showing hyperpigmented parallel linear streaking on the right lateral thigh. The streaks are a uniform hue. (B) Close-up of the patient's lower extremity

Nevertheless, 7 patients from 11 presenting to phytophotodermatitis, showed a variable degree of skin involvement in a parsnip picker at the local farm but one had severe bullous eruptions of the fingers (Figure 8) [10]. In addition, Kadde S. *et. al.* [30] studied the oil of bergamot, an extract from the rind of bergamot orenge (*Citrus aurantium* ssp. *bergamia*) which has been used as an ingredient in cosmetics and popularity in aromatherapy. The results demonstrated as photosensitive and melanogenic properties because of the presence of furocoumarins, primarily bergapten (5-methoxypsoralen, 5-MOP), which provided evidence that commercially available bergamot aromatherapy oil might cause serious bullous phototoxic reactions.

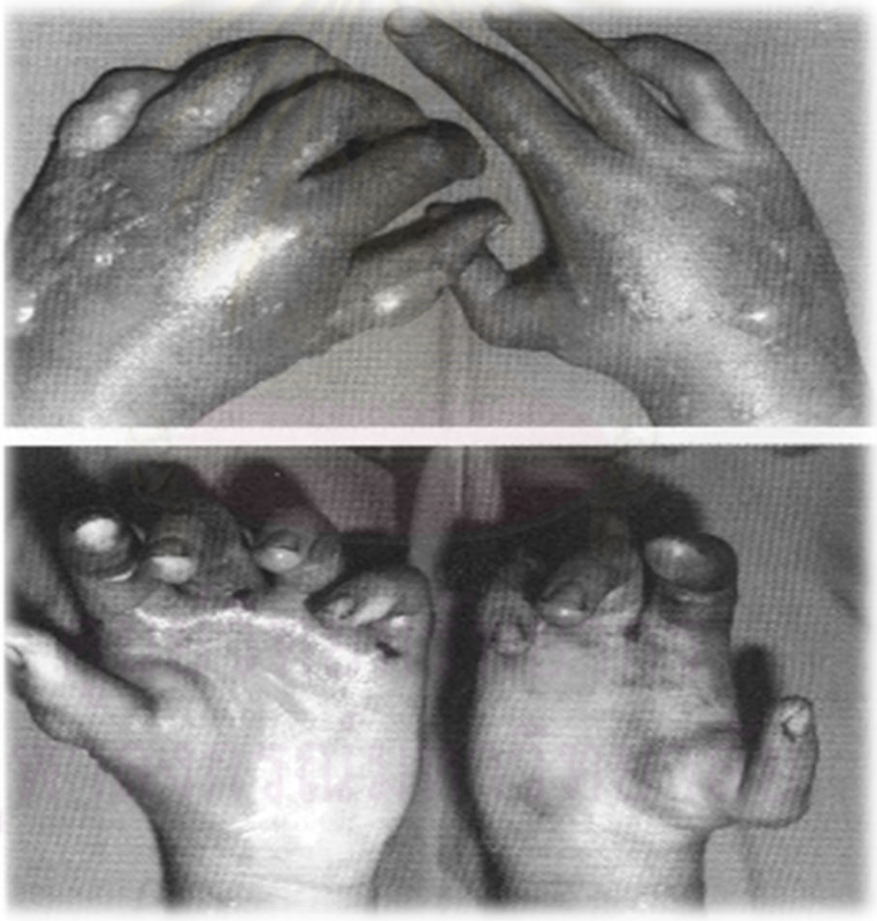


Figure 8 Severe hand involvements in a parsnip picker

However, the survey of the literatures has been made in an attempt to determine how widespread the distribution of furocoumarins (psoralen) in plants. The Umbelliferae and Rutaceae have been found to contain most furocoumarins than other families (Table 2). On the other hand, various investigators have studied the photosensitizing action of many naturally occurring furocoumarins and synthetically prepared derivatives in human skin, guinea pig skin and bacteria. Not all of naturally occurring furocoumarins tested were found to produce photosensitization [22]. The reported member of several plants to causing photosensitization was shown in Table 3. Major of them were Umbelliferous and Rutaceous plants. Other families associated with photosensitization were Convolvulaceae, Compositae, Cruciferae, Rosaceae and Ranunculaceae.

Table 2 Distributions of Furocoumarins in Nature [22, 31-36]

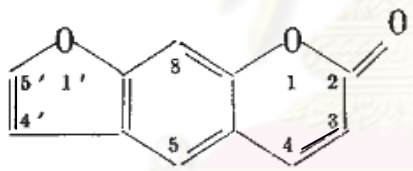
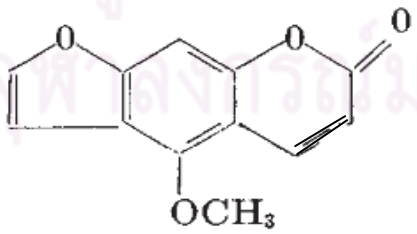
No.	Compound and structure	Natural sources	Family
1.	Psoralen (Ficisin) 	*Psoralea corylifolia* *Ficus carica* *Coronilla glauca* *Phebalium argenteum* *Xanthoxylum flavum*	Leguminosae Moraceae Leguminosae Rutaceae Rutaceae
2.	5-Methoxypsoralen (Bergapten, Majudin, Heraclin) 	*Ficus carica* *Fagara xanthoxyloides* *Skimmia laureola* *Citrus bergamia* (Risso) *Ruta graveolens* *Citrus limonum* *Citrus acida* *Faraca schinofolia* *Ligustucum acutifolium*	Moraceae Rutaceae Rutaceae Rutaceae Rutaceae Rutaceae Rutaceae Rutaceae Umbelliferae

Table 2 Distributions of Furocoumarins in Nature (Continue)

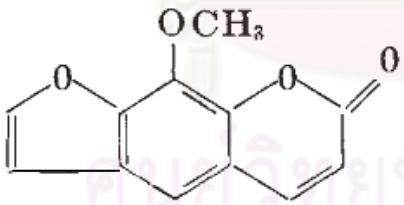
No.	Compound and structure	Natural sources	Family
		Ligustucum acutifobum	Umbelliferae
		Heracleum sphondylium	Umbelliferae
		Heracleum gigantum	Umbelliferae
		Ammi majus	Umbelliferae
		Heracleum nepalense	Umbelliferae
		Seseli indicum	Umbelliferae
		Pastinaca sativa	Umbelliferae
		Heracleum lanatum	Umbelliferae
		Angelica archangelica	Umbelliferae
		Pimpinella magna	Umbelliferae
		Pimpinella saxifrage	Umbelliferae
3.	8-Methoxypsoralen (Xanthotoxin or Methoxalen or Ammoidin) 	*Ammi majus*	Umbelliferae
		Angelica archangelica	Umbelliferae
		Pastinaca sativa	Umbelliferae
		Ficus carica	Moraceae
		Ruta chalepensis	Rutaceae
		Fagara xanthoxyloides	Rutaceae
		Ruta Montana	Rutaceae
		Aegle marmelos	Rutaceae
		Ruta graveolens	Rutaceae
		Luvanga scandens	Rutaceae
		Xanthoxylum flavum	Rutaceae

Table 2 Distributions of Furocoumarins in Nature (Continue)

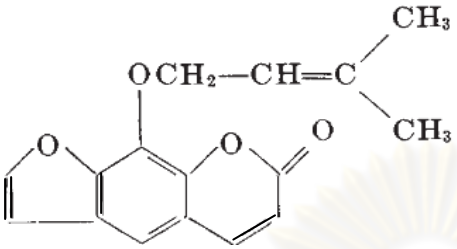
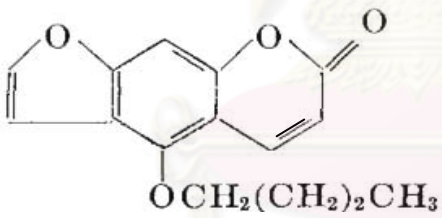
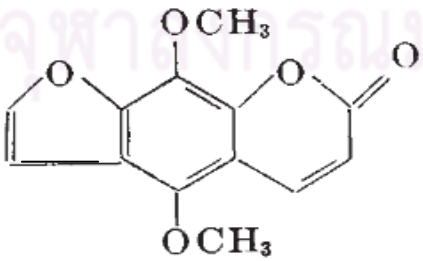
No.	Compound and structure	Natural sources	Family
4.	8-Isopentenyloxypsoralen (Imperatorin or Ammidin) 	*Ruta bracteosa* *Imparatoria ostruthium* *Angelica glabra* *Angelica archangelica* *Ammi majus* *Peucedanum ostruthium* *Pastinaca sativa* *Prangos pabularia* *Aegle marmelos* *Ruta chalepensis*	Rutaceae Umbelliferae Umbelliferae Umbelliferae Umbelliferae Umbelliferae Umbelliferae Umbelliferae Rutaceae Rutaceae
5.	Prangenine 	*Prangos pabularia*	Umbelliferae
6.	5,8-Dimethoxypsoralen (Isopimpinellin) 	*Pimpinella saxifrage* *Heracleum sphondylium* *Seseli indicum* *Skimmia laureola* *Citrus aurantifolia* *Luvanga scandens* *Thamnosma Montana* *Heracleum lanatum (var. nipponicum)* *Citrus acida*	Umbelliferae Umbelliferae Umbelliferae Rutaceae Rutaceae Rutaceae Rutaceae Rutaceae Rutaceae

Table 2 Distributions of Furocoumarins in Nature (Continue)

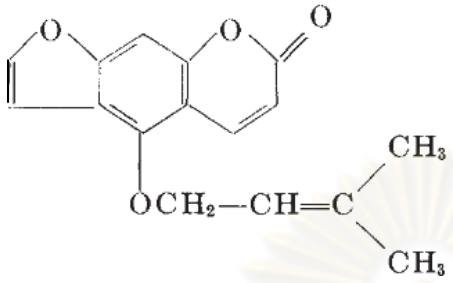
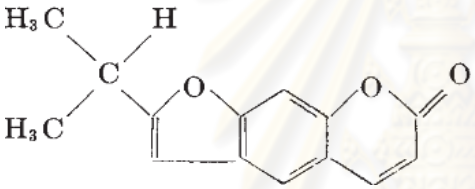
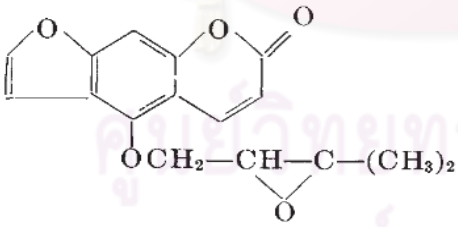
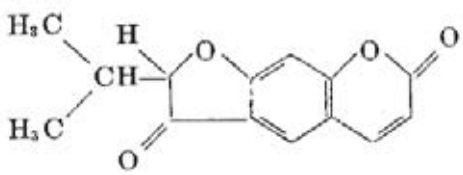
No	Compound and structure	Natural sources	Family
7.	5-Isopentenyloxypsoralen (Isoimperatorin) 	*Peucedanum ostruthium* *Imperatoria ostruthium* *Pastinaca sativa*	Umbelliferae Umbelliferae Umbelliferae
8.	4′-Methoxy, 5′-Isopropylpsoralen (Peucedanin) 	*Peucedanum officinale* *Prangos pabularia*	Umbelliferae Umbelliferae
9.	5-Epoxy isopentenyloxypsoralen (Oxypeucedanin) 	*Peucedanum ostruthium* *Peucedanum ostruthium* *Prangos pabularia* *Imperatoria ostruthium*	Umbelliferae Umbelliferae Umbelliferae Umbelliferae
10.	Oreoselone 	*Peucedanum officinale* *Peucedanum oreoselinum*	Umbelliferae Umbelliferae

Table 2 Distributions of Furocoumarins in Nature (Continue)

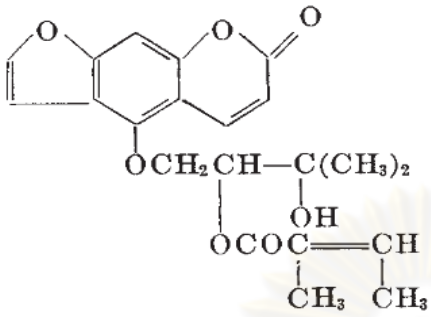
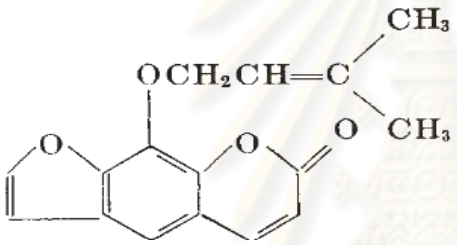
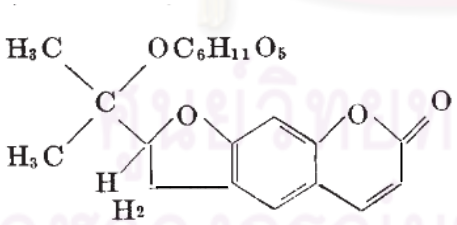
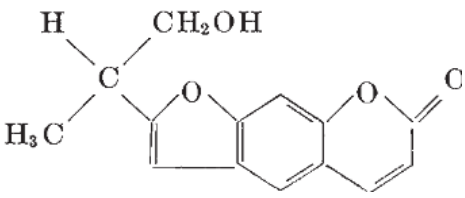
No	Compound and structure	Natural sources	Family
11.	Ostruthol 	*Peucedanum oreoselinum*	Umbelliferae
12.	5-Methoxy, 8-Isopentenyloxypsoralen (Phellaptorin) 	*Angelica glabra* *Phellopterus littoralis*	Umbelliferae Umbelliferae
13.	4′,5-dihydro, 5′(-1-glucosoxy-isopropyl) psoralen (Nodakenin) 	*Peucedanum decursivum*	Umbelliferae
14.	Aglucone of nodakenin (Nodakenetin) 	*Peucedanum decursivum*	Umbelliferae

Table 2 Distributions of Furocoumarins in Nature (Continue)

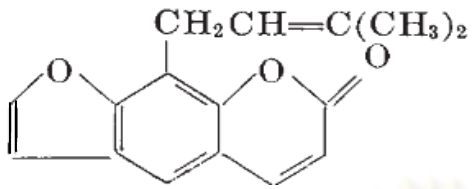
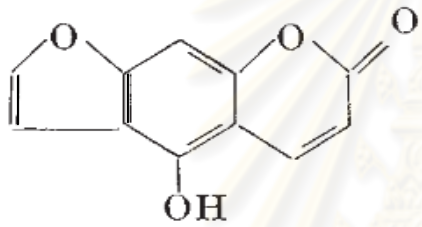
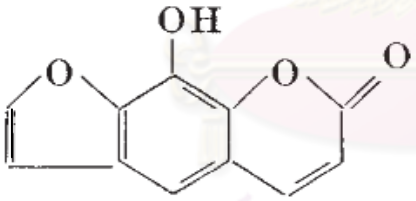
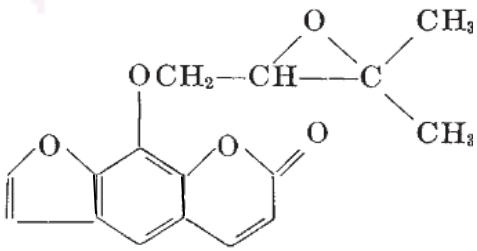
No	Compound and structure	Natural sources	Family
15.	Psoralidin 	*Psoralea corylifolia*	Leguminosae
16.	5-Hydroxypsoralen (Bergaptol) 	*Citrus bergamia* (Risso) *Citrus aurantifolia*	Rutaceae Rutaceae
17.	8-Hydroxypsoralen (Xanthotoxol) 	*Angelica archangelica*	Umbelliferae
18.	5-Methoxy-8-epoxy isopentenyloxypsoralen (Byak anglicol) 	*Angelica glabra*	Umbelliferae

Table 2 Distributions of Furocoumarins in Nature (Continue)

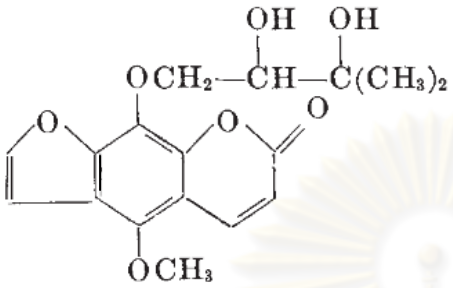
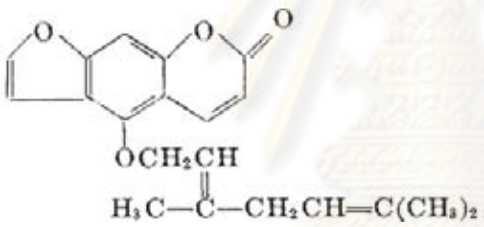
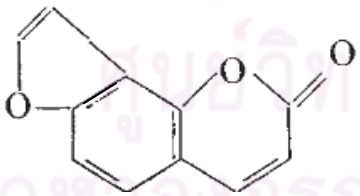
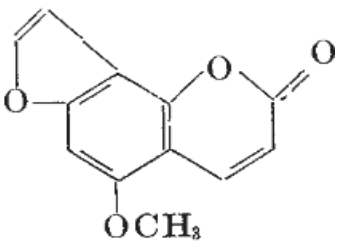
No	Compound and structure	Natural sources	Family
19.	5-Methoxy-8-(2,3-dihydro) isopentenyloxypsoralen (Byak anglicin) 	*Angelica glabra*	Umbelliferae
20.	5-Geranyloxypsoralen (Bergamotin) 	*Citrus aurantifolia*	Rutaceae
21.	Isopsoralen (Angelcin) 	*Psoralea corylifolia* *Angelica glabra*	Leguminosae Umbelliferae
22.	5-Methoxyisopsoralen (Isobergapten) 	*Pimpinella saxifrage* *Heracleum sphondylium* *Heracleum lanatum* *Pimpinella magna*	Umbelliferae Umbelliferae Umbelliferae Umbelliferae

Table 2 Distributions of Furocoumarins in Nature (Continue)

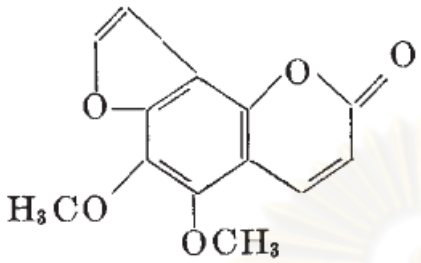
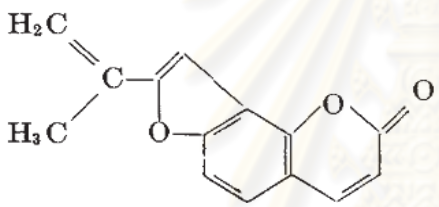
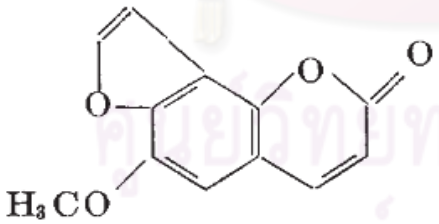
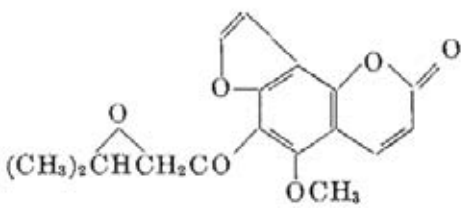
No	Compound and structure	Natural sources	Family
23.	5,6-Dimethoxy Isopentenyloxypsoralen (Pimpinellin) 	*Pimpinella saxifrage* *Heracleum sphondylium* *Heracleum lanatum* *Pimpinella magna*	Umbelliferae Umbelliferae Umbelliferae Umbelliferae
24.	Oroselon 	*Peucedanum oreoselinum*	Umbelliferae
25.	6-Methoxyisopsoralen (Sphondin) 	*Pimpinella saxifrage* *Heracleum sphondylium* *Thamnosma Montana* *Heracleum lanatum*	Umbelliferae Umbelliferae Rutaceae Umbelliferae
26.	Thamnosmin 	*Thamnosma montana*	Rutaceae

Table 2 Distributions of Furocoumarins in Nature (Continue)

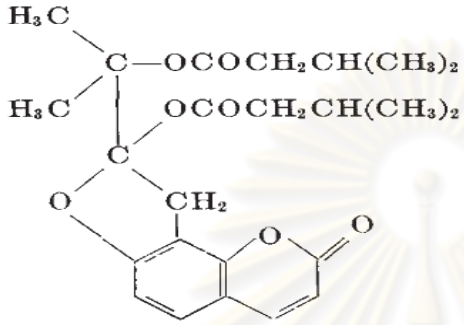
No	Compound and structure	Natural sources	Family
27.	4′,5′- dihydro -5′- (1- hydroxyisopropyl), 4′- hydroxydiisovaleryl ester (Athamentin) 	*Athamanta oreoselinum* *Peucedanum oreoselinum*	Umbelliferae Umbelliferae

Table 3 Plants reported to evoke phytophotodermatitis

Common Name	Botanical Name	Family	References
Fig	*Ficus carica*	Moraeeae	[37-40]
Parsnip	*Pastinaca sativa*	Umbelliferae	[44]
Cow parsnip	*Heracleum sphondylium*	Umbelliferae	[42, 44]
Garden parsnip	*Heracleum gigantum*	Umbelliferae	[22]
Wild parsnip	*Heraeleum mantegazzianum*	Umbelliferae	[42]
Fennel	*Foeniculum vulgare*	Umbelliferae	[44]
Dill	*Anethum graveolens*	Umbelliferae	[41]
Parsley	*Peucedanum oreoselium*	Umbelliferae	[41]
	Petroselinum crispum	Umbelliferae	[44]
Wild carrot	*Daucus carota*	Umbelliferae	[41, 44]
Garden carrot	*Daueus sativa*	Umbelliferae	[22]
Masterwort	*Peucedanum ostruthium*	Umbelliferae	[22]
Celery	*Apium graveolens*	Umbelliferae	[42, 44]

Table 3 Plants reported to evoke phytophotodermatitis (Continue)

Common Name	Botanical Name	Family	References
Atrillal	*Ammi majus*	Umbelliferae	[44]
Angelica	*Angelica species*	Umbelliferae	[22]
Common rue	*Ruta graveolens*	Rutaeeae	[42, 43]
Gas plant	*Dictamus albus*	Rutaeeae	[42]
Lime bergamot	*Citrus bergamia*	Rutaeeae	[30, 41, 42]
	Dictamnus fraxinella	Rutaeeae	[42]
Lime	*Citrus aurantiom*	Rutaeeae	[9, 11, 22]
	Citrus aurantifolia	Rutaeeae	[9, 11, 22]
Buttercup	*Renuneulus species*	Ranunculaceac	[22]
Mustard	*Brassiea species*	Cruciferae	[41]
	Sinapsis arevensis		[22]
Blind weed	*Convolvulus arevensis*	Convolvulaceac	[41]
Agrimony	*Agrimony eupatoria*	Rosaeeae	[22]
Yarrow (mill oil)	*Achilleae millefolium*	Compositae	[22]
Goose foot	*Chenopodium species*	Chenopodiaeeae	[41]
Bavaehi	*Psoralea coryilolia*	Leguminosae	[22]
St. John's wort	*Hypericum perforatum*	Hypericaceae	[22]

Plant Description of Family Rutaceae [45]

General Description: shrub or trees (rarely herb), aromatic; sometimes thorny with bitter compounds.

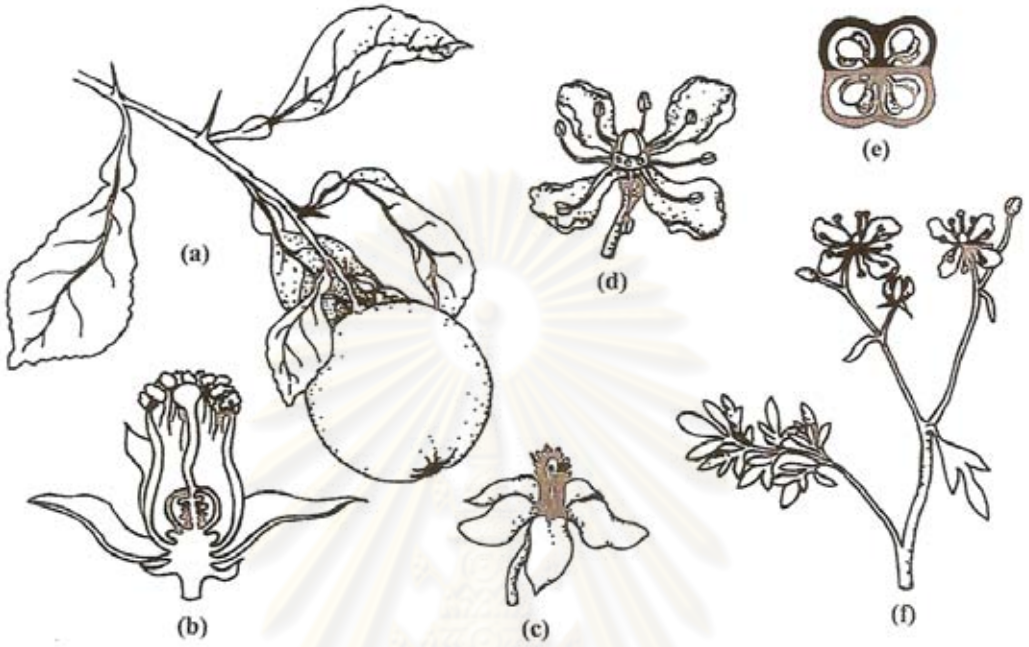


Figure 9 ***Citrus*****: (a) leafy, thorny branch with fruit; (b) longitudinal section through flower; (c) flower.** ***Ruta*****: (d) flower; (e) cross section through ovary; (f) leafy branched stem with flowers.**

Leaves: alternate (rarely opposite), simple or pinnately compound; usually with resin or oil glands or dots on the leaves, commonly giving off a strong aroma; no stipules.

Flowers: greenish-yellow, regular (rarely irregular), perfect (rarely unisexual); hypogynous or perigynous; inflorescence of a solitary flower or flowers borne in cymes or racemes. *Sepals:* 4-5, distinct or connate. *Petals:* 4-5 (rarely 0), alternate the sepals, distinct or connate at base. *Stamens:* 4-10 (rarely many), filaments distinct or connate toward the base; anthers opening by longitudinal slits and gland-tipped; nectary disk present. *Pistill:* compound of 2-5 (rarely 1 or 6-many) united carpels; locules 2-5 (rarely 1 or 6-many); ovules 1-several per locule and attached to axile or parietal placentas; ovary superior and lobed; style 1, slender, stigma small.

Fruit: a berry, drupe, hesperidum, or schizocarp.

Seed: with embryo curved or straight; oily endosperm may be absent

Economic Value: very important, with the genus *Citrus* (16 species) the most significant for its fruits. Cultivated species include *C. aurantium,* Seville orange *C. aurantifolia*, lime; *C. limon*, lemon; *C. medica*, citron; *C. paradisi*, grapefruit; *C. reticulata*, mandarins and tangerines; and *C. sinensis*, sweet orange. Other species are used as ornamentals, such as *Ruta graveolens*, the ruta.

Plant Description of Family Umbelliferae [45]

General description: herbs (rarely woody), with hollow internodes; commonly aromatic and poisonous.

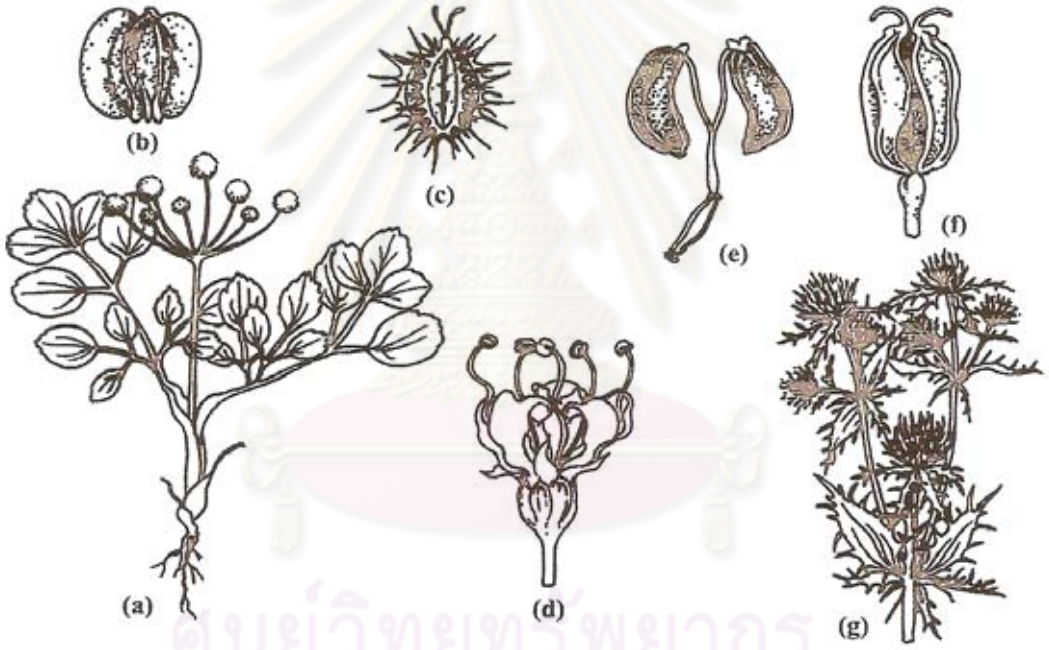


Figure 10 ***Lomatium:*** **(a) leafy plant with compound umbel inflorescence; (b) winged fruit; (c) spiny fruit; (d) flower; (e) splis schizocarp of two mericarps on carpophore; (f) pistil.** ***Eryngium*****: (g) stem apex with head inflorescence and involucral bract.**

Leave: alternate (rarely opposite) or basal, simple, more commonly pinnately or palmately lobed, compound or dissected; petioles with sheathing base; no stipules.

Flowers: small, regular (rarely irregular), perfect (rarely unisexual); epigynous; inflorescence usually a compound umbel, occasionally in heads or simple umbel, often subtended by an involucre of bracts. *Sepals:* 5, distinct, small, or absent. *Petals:* 5 (rarely 0), distinct. *Stamens:* 5, filaments distinct, attached to the epigynous nectary disk; anthers opening by longitudinal slits. *Pistil:* compound of 2 united carpels; locules 2; ovules 1 per locule and borne on apical-axile placentas; ovary inferior; styles 2, often subtended by an enlarged stylopodium.

Fruit: schizocarp of 2 mericarps, attached by a common stalk (carpophore); ribbed, winged, or covered with bump or prickles.

Seed: with a small embryo; endosperm present.

Economic Value: many species grown for food and spices. *Daucus carota*, the carrot, and *Pastinaca sativa*, parsnip, are root crops. *Anthriscus cereifolium*, chervil; *Anethum graveolens*, dill; *Apium graveolens*, celery; *Carum carvi*, caraway; *Petroselinum crispum*, parsley; and *Pimpinella anisum*, anise are used as flavorings, spices, or vegetables. Some poisonous species are *Aethusa, Cicuta* (*C. maculata*, said to be most poisonous of all north temperate plants), *Conium* (*C. maculatum*, poison hemlock, said to have killed Socrates) and *Oenanthe*.

CHAPTER III

MATERIALS AND METHODOLOGY

Chemicals

1. Dimethyl sulfoxide (Merck, Germany)
2. Mueller Hinton Agar (Merck, Germany)
3. Sabouraud Dextrose Agar (Merck, Germany)
4. Sodium chloride (Mallinckrodt, USA)

Equipments

1. Autoclave (ALP Co., Ltd., Japan)
2. Rotary evaporation (Buchi R210, Switzerland)
3. Hot air oven (WTB binder No.4940006, Germany)
4. Spectrophotometer (T60 Visible Spectrophotometer, Moscow)
5. UV chamber with two lamp 15 watt (Tokiva, Japan, wavelength 360 nm)

Plant Materials

Plant materials from 25 species of selected families Rutaceae and Umbelliferae were studied. Samples were collected from botanical gardens, the local markets and Thai Traditional drug stores. All materials were authenticated by Associate Prof. Nijsiri Ruangrungsi, Ph.D. and voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University. Rutaceous and Umbelliferous plants were studied as follows:

1. ***Aegle marmelos*** **(L.) Corr. (มะตูม)**

Family: Rutaceae

Collected place: Thai traditional drugstore, Bangkok

Part used: dried fruits and roots

2. ***Atalantia monophylla*** **DC. (มะนาวผี)**

Family: Rutaceae

Collected place: Botanical garden, Faculty of Pharmaceutical Sciences, University, Bangkok

Part used: fresh leaves

3. ***Citrus aurantifolia*** **(Christm) Swing. (มะนาว)**

Family: Rutaceae

Collected place: Thai traditional drugstore, Bangkok

Part used: dried seeds

4. ***Citrus reticulata*** **Blanco (ส้ม)**

Family: Rutaceae

Collected place: Thai traditional drugstore, Bangkok

Part used: dried seeds

5. ***Feroniella lucida*** **(Scheff.) Swingle. (มะสัง)**

Family: Rutaceae

Collected place: Botanical garden, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok

Part used: dried leaves and stem branches

6. ***Glycosmis pentaphylla*** **(Retz.) DC. (เขยตายแม่ยายชักปรก)**

Family: Rutaceae

Collected place: Botanical garden, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok

Part used: dried leaves and stem branches

7. ***Hesperethusa crenulata*** **(Roxb.) Roem. (กระแจะ)**

Family: Rutaceae

Collected place: Botanical garden, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok

Part used: dried leaves and stem branches

8. ***Murraya koenigii*** **L. (หอมแขก)**

Family: Rutaceae

Collected place: Botanical garden, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok

Part used: fresh leaves and dried stem branches

9. ***Murraya paniculata*** **L. (แก้ว)**

Family: Rutaceae

Collected place: Botanical garden, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok

Part used: fresh leaves

10. ***Triphasia trifolia*** **(Burm.t.) P.Wils. (มะนาวเทศ)**

Family: Rutaceae

Collected place: Botanical garden, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok

Part used: fresh leaves

11. ***Zanthoxylum limonella*** **(Dennst.) Alston. (มะแขว่น)**

Family: Rutaceae

Collected place: local market, Nan, Thailand

Part used: dried fruits

12. ***Anethum graveolens*** **L. (ผักชีลาว หรือ เทียนตาตั๊กแตน)**

Family: Umbelliferae

Collected place: local market and Thai traditional drugstore, Bangkok

Part used: fresh whole plants and dried fruits

13. ***Angelica dahulica*** **Benth. (โกฐสอ)**

Family: Umbelliferae

Collected place: Thai traditional drugstore, Bangkok

Part used: dried rhizomes

14. ***Angelica sinensis*** **(Oliv.) Diels. (โกฐเชียง)**

Family: Umbelliferae

Collected place: Thai traditional drugstore, Bangkok

Part used: dried roots

15. ***Apium graveolens*** **L. (คื่นไฉ่)**

Family: Umbelliferae

Collected place: local market, Bangkok

Part used: fresh whole plants and dried fruits

16. ***Coriandrum sativum*** **Vern. Dhania. (ผักชี)**

Family: Umbelliferae

Collected place: local market, Bangkok

Part used: fresh whole plants, dried fruits and fresh roots

17. ***Cuminum cyminum*** **L. (เทียนขาว)**

Family: Umbelliferae

Collected place: Thai traditional drugstore, Bangkok

Part used: dried fruits

18. ***Daucus carota*** **L. (แครอท)**

Family: Umbelliferae

Collected place: local market, Bangkok

Part used: dried fruits

19. ***Eryngium foetidum*** **L. (ผักชีฝรั่ง)**

Family: Umbelliferae

Collected place: local market, Bangkok

Part used: fresh whole plants

20. ***Ferrula assa-foetida*** **L. (มหาหิงค์)**

Family: Umbelliferae

Collected place: Thai traditional drugstore, Bangkok

Part used: oleoresin

21. ***Foeniculum vulgare*** **Mill. (เทียนข้าวเปลือก)**

Family: Umbelliferae

Collected place: Thai traditional drugstore, Bangkok

Part used: dried fruits

22. ***Heracleum siamicum*** **Craib (มะแหลบ)**

Family: Umbelliferae

Collected place: local market, Nan, Thailand

Part used: dried fruits

23. ***Ligusticum wallichii*** **Franch. (โกฐหัวบัว)**

Family: Umbelliferae

Collected place: Thai traditional drugstore, Bangkok

Part used: dried rhizome

24. ***Petroselinum crispum*** **(Miller) A.W. Hill (เทียนเยาวพาณี)**

Family: Umbelliferae

Collected place: Thai traditional drugstore, Bangkok

Part used: dried fruits

25. ***Pimpinella anisum*** **L. (เทียนสัตตบุษย์)**

Family: Umbelliferae

Collected place: Thai traditional drugstore, Bangkok

Part used: dried fruits

Extraction

The samples weighed 10 to 30 g were extracted by grinding to corse powder and maceration with 95% ethanol. The marcs were filtered and reextracted until exhaustion at room temperature. The ethanol filtrate were pooled and evaporated *in vacuo*. The extracts yield were weighed, recorded and stored at 4 °C to avoid degradation of active constituents. All extracts were dissolved in DMSO at various concentrations and were employed to the phototoxicity testing.

Microorganisms

Microorganism (standard strains) were obtained from the Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University. Selected microorganisms were studied as follows:

1. Gram negative bacteria *Escherichia coli* ATCC25922
2. Gram positive bacteria *Staphylococcus aureus* ATCC6538P
3. Gram positive spore forming bacteria *Bacillus subtilis* ATCC6633
4. Pathogenic yeast: *Candida albicans* ATCC10230
5. Non pathogenic yeast: *Saccharomyces cerevisiae* ATCC9763

Preparation of agar media

All agar media were dispensed in water and sterilized in autoclave for 15 min at 15 pounds pressure (121 °C). MHA of 38 grams was suspended in 1000 ml while SDA of 65 grams was suspended in 1000 ml. Immediately after autoclaving, allowed it to cool in a 45 to 50°C water bath. Poured the freshly prepared and cooled medium into flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm (approximately 25 to 30 ml). The plates contained agar medium allowed to cool to room temperature**.** Agar media of each batch of plates was examined for sterility by incubating at 30 to 35°C for 24 hrs before test. Plates were used for agar disc diffusion susceptibility test within seven days after preparation**.** All bacteria were test on MHA. SDA were used as test media on two yeast strains.

Preparation of inoculums suspensions

All bacteria were cultivated overnight (18-24 hrs) on MHA at 37 °C. Three to five well-isolated colonies of the same morphological type were selected from an agar plate to avoid testing mixed cultures. The top of seed selected colony was touched with a loop and transferred into a tube containing about 5 ml of a NSS.

The turbidity of bacterial culture in NSS was verified using a spectrophotometer with a 1-cm light path cuvette. The absorbance at 625 nm was 0.008 to 0.100 which comparable to the turbidity of 0.5 McFarland standard (approximately 1 to 2 x 10^8 CFU/ml).

Two yeast strains, *Candida albicans* ATCC10230 and *Saccharomyces cerevisiae* ATCC9763 were used and cultivated on Sabouraud Dextrose Agar (SDA). The yeast suspension was prepared by the same procedure as described for bacteria cell cultures.

Preparation of dried filter paper discs

Whatman AA discs size 6 mm in diameter was used. The discs were placed in a Petri dish and sterilized in a hot air oven.

Preparation of UV chamber

The chamber for incubation of organisms with UV lamp was made. The chamber size 60 x 50 x 30 cm was installed with two UV lamps (Figure 11, 12). Each lamp provided a beam of 360 nm wave with 15 W/m^2 at 30 cm.

Figure 11 UV lamp

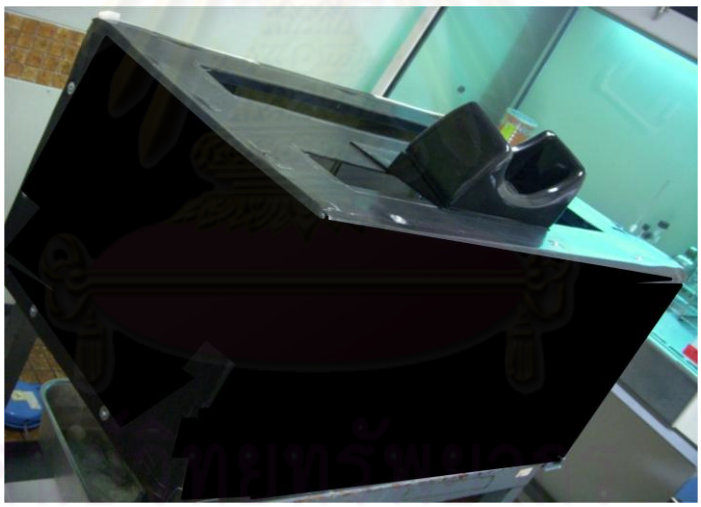


Figure 12 UV chamber

Phototoxic testing by disc diffusion method

Microorganism standard strains were grown and the inoculums were adjusted the turbidity to 0.5 McFarland standards. Each inoculums were seeded on MHA plates for bacteria and SDA for yeast. Disc diffusion method according to NCCLS [46-48] including irradiation with UVA was applied to investigate the phototoxic potential of plant materials in triplicate. Each of Rutaceous and Umbelliferous ethanol extract were performed in the levels of 100, 50 and 25 mg/ml in DMSO. Paper discs of 6 mm diameter were filled with 10µl of plant extract and DMSO (negative control disc). Test plates were exposed to UV lamp in the chamber (apart 30 cm above the surface agar) for 24 hr whilst the control was kept without UV lamp. The inhibition zones were determined and MIC of the extracts were calculated [49].

Interpretation and data analysis

Comparing the effect of phototoxic activity on plates after incubation with exposure to UV lamp. Inhibition zones against each microorganism were measured, among irradiation with and without UV. The extracts of plants which caused a inhibit zone under UV light and not in the dark were phototoxic. Those plants which cause area of inhibition in both light and dark were antibiotic. In some cases the areas of inhibition on irradiated plates were much larger in diameter than the zone of inhibition on control plates. Those were both antibiotic and phototoxic and the effect might be synergistic. All data were represented by mean and standard deviation (n=3). MIC was determined as the zero intercept of linear fitting of the squared radius (diameter) of the inhibition zones to the natural logarithm of concentration of the tested extract [49, 50].

CHAPTER IV

RESULTS AND DISCUSSION

Rutaceous ethanol extraction

Fifteen samples of selected Thai Rutaceous plants were studied. Rutaceous ethanol extracts yielded range from 4.70 % to 51.85 % as results shown in Table 4.

Table 4 Rutaceous extraction from selected plants

Plants	Used parts	Yield (% w/w)
Aegle marmelos (L.) Corr.	Roots	8.53 (dry weight)
Aegle marmelos (L.) Corr.	Fruits	19.90 (dry weight)
Atalantia monophylla DC.	Leaves	10.23 (fresh weight)
Citrus aurantifolia (Christm) Swing.	Seeds	49.91 (dry weight)
Citrus reticulata Blanco	Seeds	51.85 (dry weight)
Feroniella lucida (Scheff.) Swingle.	Leaves	18.40 (dry weight)
Feroniella lucida (Scheff.) Swingle.	Stem branches	6.23 (dry weight)
Glycosmis pentaphylla (Retz.) DC.	Leaves	22.22 (dry weight)
Hesperethusa crenulata (Roxb.) Roem.	Leaves	18.96 (dry weight)
Hesperethusa crenulata (Roxb.) Roem.	Stem branches	4.70 (dry weight)
Murraya koenigii L.	Leaves	8.34 (fresh weight)
Murraya koenigii L.	Stem branches	4.70 (dry weight)
Murraya paniculata L.	Leaves	10.17 (fresh weight)
Triphasia trifolia (Burm.t.) P. Wils.	Leaves	8.45 (fresh weight)
Zanthoxylum limonella (Dennst.) Alston.	Fruits	19.51 (dry weight)

Umbelliferous ethanol extraction

Eighteen samples of selected Thai Umbelliferous plants were studied. Umbelliferous ethanol extracts yielded range from 3.19% to 33.83% as results shown in Table 5.

Table 5 Umbellifrous extraction from selected plants

Plants	Used parts	Yield (% w/w)
Anethum graveolens L.	Whole plants	3.19 (fresh weight)
Anethum graveolens L.	Fruits	6.76 (dry weight)
Angelica dahulica Benth.	Rhizomes	7.80 (dry weight)
Angelica sinensis (Oliv.) Diels.	Roots	29.56 (dry weight)
Apium graveolens L.	Whole plants	3.78 (fresh weight)
Apium graveolens L.	Fruits	6.05 (dry weight)
Coriandrum sativum Vern. Dhania.	Whole plants	4.11 (fresh weight)
Coriandrum sativum Vern. Dhania.	Fruits	10.96 (dry weight)
Coriandrum sativum Vern. Dhania.	Roots	5.66 (fresh weight)
Cuminum cyminum L.	Fruits	13.26 (dry weight)
Daucus carota L.	Fruits	8.66 (dry weight)
Eryngium foetidum L.	Whole plants	4.06 (fresh weight)
Ferrula assa-foetida L.	Oleoresin	4.30 (dry weight)
Foeniculum vulgare Mill.	Fruits	13.82 (dry weight)
Heracleum siamicum Craib	Fruits	11.78 (dry weight)
Ligusticum wallichii Franch.	Rhizomes	33.83 (dry weight)
Petroselinum crispum (Miller) A.W. Hill	Fruits	21.44 (dry weight)
Pimpinella anisum L.	Fruits	13.93 (dry weight)

Phototoxic susceptibility

The *in vitro* activity of phototoxicity in selected Thai Rutaceous and Umblliferous plant extracts was determined against microorganisms, bacteria and yeast strains with unexposed and exposed to UV at wavelength 360 nm overnight. The strain of gram-positive bacteria, *S. aureus* is the cause of skin infection and one of spore forming bacteria, *B. subtilis* can be found in skin as normal flora and in environment. The strains of gram-negative bacteria, *E. coli* can be found in gastrointestinal tract as normal flora. Two yeast strains, *S. cerevisiae* can be found in environment, whereas *C. albicans* can cause infection in healthy individuals [51, 52]. Six extracts of Rutaceous and eight extracts of Umbelliferous plants showed selectively inhibitory activity against the studied microorganisms except *E. coli* by agar diffusion test with UVA irradiation.

The inhibition zones of both selected Rutaceous and Umbeliferous plant extracts were observed on agar media with concentration at 0, 250, 500 and 1000 μg/ml respectively. Inhibition zones of sharp and clear margin were obtained. An increment of inhibition zones diameter were found with respect to increasing concentration of extract and estimation of average MIC were investigated from inhibited zone of each concentration.

Selected Rutaceous plant extracts under exposure to UVA selectively exhibited inhibition zones against the tested microorganisms as shown in the extract of *A. marmelos* (dried roots), *A. monophylla* (fresh leaves), *F. lucida* (dried leaves), *H. crenulata* (dried leaves), *M. koenigii* (fresh leaves) and *T. trifolia* (fresh leaves). Results were indicated in Table 6, 8, 10, 12, 14, 16. All of those were interpreted for diameter of zone of inhibition as being low activity.

From the study of Shoeb, A. *et. al*. [53], alkaloids and coumarin from root of *A. marmelos* as psoralen, xanthotoxin, 6, 7-dimethoxycoumarin and other constituent isolate were reported. In the literature, evidences have been provided that the distribution of furocoumarin and their metabolites in nature can be phototoxic to live organism in presence of exposure to UV radiation. In this studied, the extract from *A. marmelos* (dried root) showed phototoxic activities on microorganism (Table 6). According to Shoeb, A. *et. al.,* this was due to psoralen, xanthotoxin and other furocoumarins. Phototoxic activity of *A. marmelos* (dried root) showed dose response

relationship against gram positive bacteria: *B. subtilis* and *S. aureus*. Whilst gram negative bacteria as *E. coli* and two strains of test yeast, *C. albicans* and *S. cerevisiae*, appeared no inhibition zones. All strains on control group (without UV) were not inhibited by this extract according to this assay. Results were indicated in Table 6. The estimation of average MIC were shown in Table 7.

Phototoxic activity of *A. monophylla* (fresh leaves) showed selected exhibition on *S. aureus* with large clear zone of inhibition at highest concentration (Table 8). The MIC was shown in Table 9. The activity of *F. lucida* (dried leaves) was similar but less potent than the results of *A. monophylla*. The result of inhibition and MIC were shown in Table 9 and Table 10. *H. crenulata* (dried leaves) exhibition activities on *S. aureus* in accordance with *M. koenigii* (fresh leaves) (Table 12 and Table 14) and their MIC were shown in Table 13 and Table 15. For Rutaceous plant extracts, *T. trifolia* (fresh leaves) exhibited activity on *B. subtilis* and *S. aureus*. The lowest activity even at high level of concentration was shown on *B. subtilis* (Table 16). Results of MIC were indicated in Table 17.

Selected Umbelliferous plant extracts, except *Apium graveolens* (dried fruits), *H. siamicum* (dried fruits) and *P. crispum* (dried fruits) had no antimicrobial activity against all tested microorganisms on the control agar plates. Under exposure to UVA, the extracts of *Anethum graveolens* (fresh whole plant and dried fruits), *A. dahulica* (dried rhizomes), *Apium graveolens* (dried fruits), *F. vulgare* (dried fruits), *H. siamicum*, (dried fruits), *P. crispum* (dried fruits) and *P. anisum* (dried fruits) selectively exhibited inhibition zones against the tested microorganisms.

On the plate of irradiation with UVA, the inhibitory activity were from *Anethum graveolens* (fresh whole plant) against *B. subtilis* and *S. aureus* (Table 18) with MIC of 151.3 and 41.4 µg/disc respectively while the dried fruits exhibited activity against *S. aureus* with MIC of 228.2 µg/disc. According to Belleinger, H. E, *Anethum graveolens* was one of the plants reported to evoke phytophotodermatitis [41].

Phototoxic activity of *A. dahulica* against *B. subtilis, S. aureus*, *C. albicans* and *S. cerevisiae* were shown in Table 22 with large zones of inhibition. Results of MIC were indicated in Table 23. According to Pathak, M.A. *et. al.*, *Angelica species* were determined and revealed the distribution of furocoumarin [22].

Activity of *Apium graveolens* was shown phototoxic on *S. cerevisiae* which the effect might be synergistic. The results with MIC were indicated in Table 24 and 25. *H. siamicum* against *B. subtilis*, *S. aureus C. albicans* and *S. cerevisiae* had high activity as large sizes of inhibition zones rather than other. Results were shown in Table 28. The MIC of this extract exhibited high activity as indicated in Table 29. Finally, three extracts as *F. vulgare, P. crispum* and *P. anisum* exhibited phototoxicity only on *S. aureus* with MIC of 182.0, 125.4 and 198.4 µg/disc respectively. The results were indicated in Table 26, 27 and 30-33.

The phototoxic properties of furanocoumarins and related compounds have been assayed using fungi [15, 54], green algae [55-57], bacteria [58, 59], laboratory animals [60, 61] and *Artemia salina* [62]. Nowadays cultured human skin systems were available [63, 64]. The methodology in this study was basically similar to those used for testing the antimicrobial properties of compounds, but further coupled with UV 360 nm irradiation. So this technique was able to quickly screen the possibly phototoxic compounds in plant extracts and calculate MIC from inhibition zone.

The first effort to measure phototoxicity *in vitro* was a microbiological approach as the test organism [15] with some modification [65]. Pure compound of furocoumarin were previously studied. Faergemann, J and Larko, O. tested phototoxic effect of eight methoxypsoralen (8-MOP) and trimethylpsoralen (TMP) against various microorganisms: *Staphylococcus aureus, S. epidermidis C. albicans* and *Pityrosporum orbiculare*. The results showed phototoxic activities against all microorganisms tested [66]. *S. aureus* and *E. coli* were previously reported as test systems of phototoxicity [16, 17]. *C. albicans* and *S. cerevisiae* have also been tested for phototoxicity study [15, 18, 67]. In this studied, crude extracts of selected Thai Rutaceae and Umbelliferae plant were tested on five microorganisms. On the contrary of the previous studies, the phototoxicity showed selectivity among the tested microorganisms. *S. aureus* was more sensitive than others. *E. coli* showed no effect from these selected Rutaceous and Umbelliferous plant. *B. subtilis*, *C. albicans* and *S. cerevisiae* were sensitive for some of the studied species as well. The microbiological test for phototoxicity screening should be performed by using a variety of microorganisms for more reliability. The summarization of phototoxic activity were indicated in Table 34 and 35.

Table 6 Activity of *A. marmelos* (dried root) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
A. marmelos	DMSO	NA	NA	NA	NA	NA
	250	NA	**6.33 ± 0.29**	NA	NA	NA
	500	NA	**7.33 ± 0.58**	**7.00 ± 0.00**	NA	NA
	1000	NA	**8.17 ± 0.29**	**8.00 ± 0.00**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 7 Estimation of average MIC in *A. marmelos* (dried root) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)	
	B. subtilis	*S. aureus*
A. marmelos	**191.1**	**250 < MIC < 500**

Table 8 Activity of *A. monophylla* (fresh leaves) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
A. monophylla	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	**7.33 ± 0.58**	NA	NA
	1000	NA	NA	**10.00 ± 1.00**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 9 Estimation of average MIC in *A. monophylla* (fresh leaves) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)
	S. aureus
A. monophylla	**250 < MIC < 500**

Table 10 Activity of *F. lucida* (dried leaves) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
F. lucida	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	**6.50 ± 0.00**	NA	NA
	1000	NA	NA	**7.17 ± 0.29**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 11 Estimation of average MIC in *F. lucida* (dried leaves) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)
	S. aureus
F. lucida	**250 < MIC < 500**

Table 12 Activity of *H. crenulata* (dried leaves) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
H. crenulata	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	**7.17 ± 0.29**	NA	NA
	500	NA	NA	**8.00 ± 0.00**	NA	NA
	1000	NA	NA	**8.50 ± 0.50**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 13 Estimation of average MIC in *H. crenulata* (dried leaves) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)
	S. aureus
H. crenulata	**166.4**

Table 14 Activity of *M. koenigii* (fresh leaves) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
M. koenigii	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	**6.50 ± 0.00**	NA	NA
	500	NA	NA	**7.33 ± 0.58**	NA	NA
	1000	NA	NA	**8.33 ± 0.58**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 15 Estimation of average MIC in *M. koenigii* (fresh leaves) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)
	S. aureus
M. koenigii	**175.0**

Table 16 Activity of *T. trifolia* (fresh leaves) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
T. trifolia	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	**6.50 ± 0.00**	NA	NA
	1000	NA	**6.50 ± 0.00**	**8.17 ± 0.29**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 17 Estimation of average MIC in *T. trifolia* (fresh leaves) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)	
	B. subtilis	*S. aureus*
T. trifolia	**500 < MIC < 1000**	**250 < MIC < 500**

Table 18 Activity of *Anethum graveolens* (fresh whole plants) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
A. graveolens	DMSO	NA	NA	NA	NA	NA
	250	NA	**9.55 ± 0.69**	**13.00 ± 0.58**	NA	NA
	500	NA	**13.55 ± 0.84**	**14.44 ± 0.51**	NA	NA
	1000	NA	**18.78 ± 0.51**	**18.11 ± 0.77**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 19 Estimation of average MIC in *Anethum graveolens* (fresh whole plants) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)	
	B. subtilis	*S. aureus*
A. graveolens	**151.3**	**41.4**

Table 20 Activity of *Anethum graveolens* (dried fruit) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
A. graveolens	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	**6.33 ± 0.29**	NA	NA
	500	NA	NA	**7.67 ± 0.59**	NA	NA
	1000	NA	NA	**9.67 ± 0.59**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 21 Estimation of average MIC in *Anethum graveolens* (dried fruit) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)
	S. aureus
A. graveolens	**228.2**

Table 22 Activity of *A. dahulica* (dried rhizomes) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
A. dahulica	DMSO	NA	NA	NA	NA	NA
	250	NA	**7.00 ± 0.00**	**7.00 ± 0.00**	**9.67 ± 0.58**	**7.00 ± 0.00**
	500	NA	**7.67 ± 0.58**	**8.67 ± 0.58**	**11.67 ± 0.58**	**9.33 ± 0.58**
	1000	NA	**10.67 ± 0.58**	**11.33 ± 0.58**	**13.33 ± 0.58**	**10.67 ± 0.58**
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 23 Estimation of average MIC in *A. dahulica* (dried rhizomes) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)			
	B. subtilis	*S. aureus*	*C. albicans*	*S. cerevisiae*
A. dahulica	**198.4**	**191.4**	**60.9**	**161.0**

Table 24 Activity of *Apium graveolens* (dried fruit) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
A. graveolens	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	**7.67 ± 0.58**
	500	NA	NA	NA	**8.67 ± 0.58**	**10.33 ± 0.58**
	1000	NA	NA	NA	**9.67 ± 0.58**	**12.67 ± 0.58**
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	**8.33 ± 0.58**	**7.33 ± 0.58**
	1000	NA	NA	NA	**9.67 ± 0.58**	**9.67 ± 0.58**

*mean ± SD, n = 3, NA = no activity

Table 25 Estimation of average MIC in *Apium graveolens* (dried fruit) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)
	S. cerevisiae
A. graveolens	**155.04**

Table 26 Activity of *F. vulgare* (dried fruits) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
F. vulgare	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	**6.67 ± 0.29**	NA	NA
	500	NA	NA	**7.83 ± 0.76**	NA	NA
	1000	NA	NA	**9.33 ± 0.58**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 27 Estimation of average MIC in *F. vulgare* (dried fruits) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)
	S. aureus
F. vulgare	**182.0**

Table 28 Activity of *H. siamicum* (dried fruits) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
H. siamicum	DMSO	NA	NA	NA	NA	NA
	250	NA	**11.33 ± 0.58**	**9.00 ± 0.00**	**16.33 ± 0.58**	**14.67 ± 0.58**
	500	NA	**12.67 ± 0.58**	**12.33 ± 0.58**	**17.33 ± 0.58**	**17.67 ± 0.58**
	1000	NA	**13.67 ± 0.58**	**13.67 ± 0.58**	**18.67 ± 0.58**	**20.33 ± 0.58**
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	**6.50 ± 0.00**	NA	NA	NA
	1000	NA	**7.00 ± 0.00**	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 29 Estimation of average MIC in *H. siamicum* (dried fruits) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)			
	B. subtilis	*S. aureus*	*C. albicans*	*S. cerevisiae*
H. siamicum	**10.3**	**93.0**	**1.0**	**3.0**

Table 30 Activity of *P. crispum* (dried fruit) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
P. crispum	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	**7.00 ± 0.00**	NA	NA
	500	NA	NA	**7.33 ± 0.58**	NA	NA
	1000	NA	NA	**8.67 ± 0.58**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	**7.33 ± 0.58**	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 31 Estimation of average MIC in *P. crispum* (dried fruit) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)
	S. aureus
P. crispum	**125.4**

Table 32 Activity of *P. anisum* (dried fruit) on growth of microorganisms by agar disc diffusion

Plant	Concentration (µg/disc)	Inhibition zone (mm*)				
		Irradiated with UV lamp 360 nm				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
P. anisum	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	**6.50 ± 0.50**	NA	NA
	500	NA	NA	**7.50 ± 0.50**	NA	NA
	1000	NA	NA	**9.00 ± 0.50**	NA	NA
	Concentration (µg/disc)	Without UV lamp				
		E. coli	***B. subtilis***	***S. aureus***	***C. albicans***	***S. cerevisiae***
	DMSO	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA
	500	NA	NA	NA	NA	NA
	1000	NA	NA	NA	NA	NA

*mean ± SD, n = 3, NA = no activity

Table 33 Estimation of average MIC in *P. anisum* (dried fruit) extracts against microorganism with irradiated with UVA

Plant	MIC (µg/disc)
	S. aureus
P. anisum	**198.4**

Table 34 Summarization of the phototoxic activity of selected Thai Rutaceous plant extracts

Plants	Used parts	1	2	3	4	5
A. marmelos	Roots	−	+	+	−	−
A. marmelos	Fruits	−	−	−	−	−
A. monophylla	Leaves	−	−	+	−	−
C. aurantifolia	Seeds	−	−	−	−	−
C. reticulata	Seeds	−	−	−	−	−
F. lucida	Leaves	−	−	+	−	−
F. lucida	Stem branch	−	−	−	−	−
G. pentaphylla	Leaves	−	−	−	−	−
H. crenulata	Leaves	−	−	+	−	−
H. crenulata	Stem branch	−	−	−	−	−
M. koenigii	Leaves	−	−	+	−	−
M. koenigii	Stem branch	−	−	−	−	−
M. paniculata	Leaves	−	−	−	−	−
T. trifolia	Leaves	−	+	+	−	−
Z. limonella	Fruits	−	−	−	−	−

NOTE: **1**= *E. coli*, **2**= *B. subtilis*, **3**= *S. aureus*, **4**= *C. albicans*, **5**= *S. cerevisiae*

+ = Activity, − = No activity

Table 35 Summarization of the phototoxic activity of selected Thai Umbelliferous plant extracts

Plants	Used parts	1	2	3	4	5
Anethum graveolens	Whole plants	−	+	+	−	−
Anethum graveolens	Fruits	−	−	+	−	−
A. dahulica	Rhizomes	−	+	+	+	+
A. sinensis	Roots	−	−	−	−	−
Apium graveolens	Whole plants	−	−	−	−	−
Apium graveolens	Fruits	−	−	−	+	+
C. sativum	Whole plants	−	−	−	−	−
C. sativum	Fruits	−	−	−	−	−
C. sativum	Roots	−	−	−	−	−
C. cyminum	Fruits	−	−	−	−	−
D. carota	Fruits	−	−	−	−	−
E. foetidum	Whole plants	−	−	−	−	−
F. assa-foetida	Oleoresin	−	−	−	−	−
F. vulgare	Fruits	−	−	+	−	−
H. siamicum	Fruits	−	+	+	+	+
L. wallichii	Rhizomes	−	−	−	−	−
P. crispum	Fruits	−	−	+	−	−
P. anisum	Fruits	−	−	+	−	−

NOTE: **1**= *E. coli,* **2**= *B. subtilis,* **3**= *S. aureus,* **4**= *C. albicans,* **5**= *S. cerevisiae*

+ = Activity, − = No activity

CHAPTER V

CONCLUSION

Screening of the phototoxic activity among twenty-five species on various microorganisms showed that six ethanol extracts of Rutaceous plant and eight ethanol extracts of Umbelliferous plant selectively exhibited inhibition zones against the tested microorganisms. The most sensitive microorganism was *S. aureus.* The strains of *B. subtilis*, *C. albicans* and *S. cerevisiae* were sensitive for some of the studied species as well. Whilst *E. coli* was not susceptible to this phototoxic testing. Thus, the microbiological test for phototoxicity screening should be performed by using a variety of microorganisms for more reliability. In view of the rapid, easiness, sensitivity and low cost of microorganism test. *S. aureus* was suitable to investigate UVA-radiation assisted phototoxicity of plant extract. Therefore *S. aureus* might be used as one of the alternate *in vitro* test for phototoxic potential of plant extract. This method was simple and able to be used as a presumptive test for the presence of photosensitizing in plant materials. The interpretation was based upon correlation with the distribution of the compounds in nature, in addition to the pure specific chemical compounds.

The microorganisms test system could be very useful to provide phototoxicity potential information of plant materials. Using the products containing these plants should beware to avoid the sunlight exposure. This method can be used as screening tool for the presumptive identification of plants causing phytophotodermatitis.

REFERENCES

[1] ISO 21348 INTERNATIONAL STANDRAD. Space environment (natural and artificial) — Process for determining solar irradiances. 1st edition. Published in Switzerland, 2007.

[2] Diffey, B.L. Sources and measurement of ultraviolet radiation. Methods 28 (2002): 4–13.

[3] Madronich, S., McKenzie, R.L., Bjorn, L.O., and Caldwell, M.M. Changes in biologically active ultraviolet radiation reaching the Earth's surface. J. Photochem. Photobiol. B: Biol. 46 (1998): 5-19.

[4] Jhappan, C., Noonan, F.P., and Merlino, G. Ultraviolet radiation and cutaneous malignant melanoma. Oncogene. 22 (2003): 3099–3112.

[5] Chobot, V., Vytlačilová, J., and Jahodář L. Phototoxic activity and the possibilities of its testing. Cent. Eur. J. Publ. Health 12 (2004): S31–S33.

[6] Placzek, M., Frömel, W., Eberlein, B., Gilbertz, K.P., and Przybilla, B. Evaluation of Phototoxic properties of Fragrances. Acta. Derm. Venereol. 87 (2007): 312-316.

[7] Hans, R.K., *et al.* Assessment of the phototoxic potential of cosmetic products. Food Chem. Toxicol. 46 (2008): 1653–1658.

[8] Wolf, R., and Oumeish, Y. Photodermatoses. Clinics in Dermatology. 16 (1998): 41-57.

[9] Weber, I.C., Davis, C.P., and Greeson, D.M. Phytophotodermatitis: The other ~~l~~ime" disease. The Journal of Emergency Medicine. 17 (1999): 235–23.

[10] Lutchman, L., Inyang, V., and Hodgkinson, D. Phytophotodermatitis associated with parsnip picking. J. Accid. Emerg. Med. 16 (1999): 453-454.

[11] Solis, R.R., Dotson, D.A., and Trizna, Z. Phytophotodermatitis. Arch. Fam. Med. 9 (2000): 1195-1196.

[12] Koh, D., and Ong, C-N. Phytophotodermatitis due to the application of *Citrus hystrix* as a folk remedy. British Journal of Dermatology. 140 (1999): 737-738.

[13] Schlatter, J., Zimmerli, B., Dick, R., Panizzon, R., and Schlatter, Ch. Dietary intake and risk assessment of Phototoxic furocoumarins in humans. Food Chem. Toxicol. 29 (8) (1991): 523-530.

[14] Herber, L.C., and Baer, R.L. Pathogenic mechanisms of drug- induced photosensitivity. J. Invest. Derm. 58 (1972): 327-342.

[15] Daniels, F.J. A simple microbiological method for demonstrating phototoxic compounds. J. invest. Derm. 44 (4) (1965): 259-263.

[16] Coutinho, H.D.M., Costa, J.G.M., Lima, E.O., and Siqueira-Junior, J.P. In vitro phototoxic activity of *Eugenia jambolana* L. and *Hyptis martiusii* Benth. J. Photochem. Photobiol. B: Biol. 93 (1) (2009): 63-65.

[17] Verma, K., Agrawal, N., Misra, R.B., Farooq, M., and Hans, R.K. Phototoxicity assessment of drugs and cosmetic products using *E. coli*. Toxicology *in Vitro*. 22 (1) (2008): 249-253.

[18] Sukiyama, M., Itagaki, H., and Kato, S. In vitro Assay to Predict Phototoxicity of Chemicals: (II) Yeast Growth Inhibition Assay and Battery System. AATEX. 2 (1994): 193-202.

[19] Liebsch, M., *et al.* UV-induced effects. Alternatives to Laboratory Animals. 33 (1) (2005): 131-146.

[20] Mitsui, T. safety of cosmetics. New cosmetic science 1st ed. pp. 209-217 Published by Elsevier Science B.V., 1997.

[21] Maurer, TH. Phototoxicity testing – *in vivo* and *in vitro.* Food Chem. Toxicol. 25 (5) (1987): 407-414.

[22] Pathak, M.A., Daniels, F.J. and Fitzpatrick, T.B. The presently known distribution of furocoumarins (psoralens) in plants. J. Invest. Derm. 24 (1961): 226-239.

[23] Kaier, K., Schmitt-Landgraf, R., and Siegemund, B. Development of an *in vitro* test system with human skin cells for evaluation of phototoxicity. Toxic. *in vitro*. 5(1991): 457-461.

[24] Lim, H.W. Abnormal responses to ultraviolet radiation: photosensitivity induced by exogenous agent. In: Freedberg, I.M., Eisen, A.Z., Wolff, K., Austin, A.F., Goldsmith, L.A., Katz, S.I. (Eds.). Dermatology in General Medicine. 6th ed. pp.1298-1307. New York: McGraw-Hill, 2003.

[25] Gould, J.W., Mercurio, M.G., and Elmets, C.A. Cutaneous photosensitivity diseases induced by exogenous agents. J. Am. Acad. Dermatol. 33 (1995): 551-573.

[26] Wang, S.Q., *et al*. Ultraviolet A and melanoma: A review J. Am. Acad. Dermatol. 44 (2001): 837- 846.

[27] Frohne, D., and Pfänder, H.J. Poisonous Plants. 2nd ed. English edition Translated by Alford I. Appendix: McKinney, P. and Cumpston, K. pp. 15-16, 23-24, 39, 346. Albuquerque, New Mexico, USA: Manson press, 2005.

[28] Brueneton, J. Toxic Plants Dangerous to Humans and Animals. by Hatton, C.K. pp. 115-117, 441-444. France: Lavoisier press, 1999.

[29] Klaber, R.E. Phytophotodermatitis. Arch. Dis. Child. 91 (2006): 385.

[30] Kaddu, S., Kerl, H., and Wolf, P. Accidental bullous phototoxic reactions to bergamot aromatherapy oil. J. Am. Acad. Dermatol. 45(3) (2001): 458-461.

[31] Beier, R.C., Ivie, G.W., and Oertli, E.H. Liner furanocoumarins and gravelone from the common herb parley. Phytochemistry. 36(4) (1994): 869-872.

[32] Scott, B.R., Pathak, M.A., and Mohn, G.R. Molecular and genetic basis of furocoumarin reactions. Mutation Research. 39 (1976): 29-74.

[33] Wagstaff, D.J. Dietary Exposure to Furocoumarins. Regulatory Toxicology and Pharmacology. 14 (1991): 26l-272.

[34] Ceska, O., Chaudhary, S. K., Warrington, P. J., and Ashwood-Smith, M. J. Photoactive furocoumarin in fruit of some Umbellifers. Phytochemistry. 26(1) (1987): 165-169.

[35] Nigg, H.N., *et al.* Phototoxic coumarins in limes. Food Chem. Toxicol. 31(5) (1993): 331-335.

[36] Jarvis, M.W. The Photosensitizing furanocoumarins of *Phebalium argenteum* (Blister bush). Aust. J. Chem. 21 (1968): 537-538.

[37] Bollero, D., *et al.* Fig leaf tanning lotion and sun-related burns: case reports. Burns 27 (2001): 777–779.

[38] Derraik, J.G.B., and Rademaker, M. Phytophotodermatitis caused by contact with a fig tree (*Ficus carica*). NZMJ. 120(1259) (2007).

[39] Khachemoune, A., Khachemoune, K. and Blanc, D. Assessing phytophotodermatitis: boy with erythema and blisters on both hands. Dermatol Nurs. 18 (2006): 153–154.

[40] Dechamp, C., Bessot, J.C., Pauli, G., and Deviller, P. First report of anaphylactic reaction after fig (*Ficus carica*) ingestion. Allergy. 50 (1995): 514–516.

[41] Belleinger, H.E. Phyto-photo-dermatitis. Brit. Med. J. 1 (1949): 984-986.

[42] Carlsen, K., and Weismann, K. Phytophotodermatitis in 19 children admitted to hospital and their differential diagnoses: Child abuse and herpes simplex virus infection. J. Am. Acad. Dermatol. 57(5) (2007): s88-s91.

[43] Murray, V.S.G. Toxic plants (excluding fungi) Chapter 31 in J. Descotes (Ed.). Toxic plants Human Toxicology. Elsevier Science B.V. All rights reserved, 1996.

[44] Lenković, M., *et al*. Phytophotodermatitis. Coll. Antropol. 32 (2008) Suppl. 2: 203–205

[45] Woodland, D.W. Contemporary plants systematic 3rd ed. pp. 267,275. USA, Andrews University press, 2000.

[46] Lorian,V. Antibiotics in laboratory medicine. 4th ed., Baltimore, London: Williams&Wilkins, 1996.

[47] Bauer, A.W., Kirby, W.M., Sherris, J.C., and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45(4) (1966): 493–496.

[48] Traub, W.H., Geipel, U., Leonhard, B. and Bauer, D. Antibiotic Susceptibility Testing (Agar Disk Diffusion and Agar Dilution) of Clinical Isolates of *Corynebacterium jeikeium.* Chemotherapy; 44(1998): 230-237.

[49] Bonev, B., Hooper, J., and Parisot, J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. J. Antimic. Chemoth. 61 (2008): 1295-1301.

[50] Barry, L. An Overview of the Clinical and Laboratory Standards Institute (CLSI) and Its Impact on Antimicrobial Susceptibility Tests Chapter 1 Schwalbe, R., Steele-Moore, L. and Goodwin, A.C. (ed.), Antimicrobial Susceptibility Testing Protocols. pp 1-6. New York: CRC press.

[51] Tortora, G.J., Funk, B.R., and Case, C.L. Microbiology an introduction, 5th ed., The Benjamin: Cummings Publishing, 1995.

[52] Nantawan nantawanit. Antimicrobial property of polysaccharide gel from durian fruit-hulls. Master's Thesis, Department of Biochemistry Faculty of Sciences Chulalongkorn University, 2001.

[53] Shoeb, A., Kapil, R.S. and Popli, S.P. Coumarins and alkaloids of *Aegle marmelos* Photochemistry 12 (1973): 2071-2072.

[54] Asthana, A., Mccloud, E.S., Berenbaum, M.R. and Tuveson, R.W. Phototoxicity of *Citrus jambhiri* to fungi under enhanced UVB radiaton: role of furanocoumarins. Journal of Chemical Ecology. 19(12) (1993): 2813- 2830.

[55] Gala, W.R. and Giesy, J.P. Using the carotenoid biosynthesis inhibiting herbicide, Fluridone, to investigate the ability of carotenoid pigments to protect algae from the photoinduced toxicity of anthracene. Aquatic Toxicology. 27 (1993): 61-70.

[56] Cody, T.E., Radike, M.J. and Warshawsky, D. The phototoxicity of benzo[*a*]pyrene in the green alga *Selenastrum capricornutum*. Environmental Research. 35(1) (1984): 122-132.

[57] Schimmer, O. and Kűhed, I. Mutagenic compounds in an extract from Rutae Herba *(Ruta graveolens* L.) II. UV-A mediated mutagenicity in the green alga *Chlamydomonas reinhardtii* by furoquinoline alkaloids and furocoumarins present in a commercial tincture from Rutae Herba. Mutation Research. 243 (1990): 57-62.

[58] Proksch, P. and Proksch, M. Phototoxic and insecticidal activity of chromenes and benzofurans from *encelza*. Journal of Natural Products. 46 (1983): 331-334.

[59] Phoenix, D.A., Sayed, Z., Hussain, S., Harris, F. and Wainwright, M. The phototoxicity of phenothiazinium derivatives against *Escherichia coli* and *Staphylococcus aureus*. FEMS Immunology and Medical Microbiology. 39 (2003): 17-22.

[60] Lambert, L.A., Warner, W.G. and Kornhauser, A. Animal models for phototoxicity testing. Toxicology Methods. 6(2) (1996): 99-114.

[61] Owen, K. Comparative grepafloxacin phototoxicity in mouse skin. J. Antimicrob. Chemo. 42 (1998): 261-264.

[62] Ojala, T., Vuorela, P., Kiviranta, J., Vuorela, H. and Hiltunen, R. A bioassay using *Artemia salina* for detecting phototoxicity of plant coumarins. Planta Med. 65(8) (1999): 715-8.

[63] Trisciuoglio, D., *et al.* Phototoxic effect of fluoroquinolones on two human cell lines. Toxicology in Vitro. 16 (2002): 449-456.

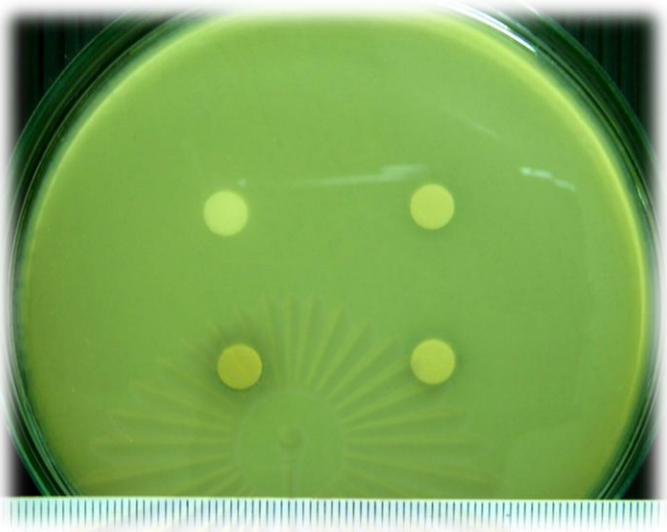
[64] Kejlová, K., *et al.* Phototoxicity of bergamot oil assessed by in vitro techniques in combination with human patch tests. Toxicology in Vitro. 21 (2007) 1298-1303.

[65] Knudsen, E.A. The *Candida* phototoxicity test. The sensitivity of different strains and species of *Candida*, standardization attempts and analysis of dose-response curves of 5- and 8- methoxypsoralen. Photodermatology. 2 (1985): 80-85.

[66] Faergemann, J. and Larko, O. Phototoxicity of skin microorganisms tested with a new model. Arch. Dermatol. Res. 280 (1988): 168-170.

[67] Marrot, L., Labarussiat, A., Perez, P. and Meunier, J.R. Use of the yeast *Saccharomyces cerevisiae* as a pre-screening approach for assessment of chemical-induced phototoxicity. Toxicology *in Vitro*. 20 (2006): 1040-1050.

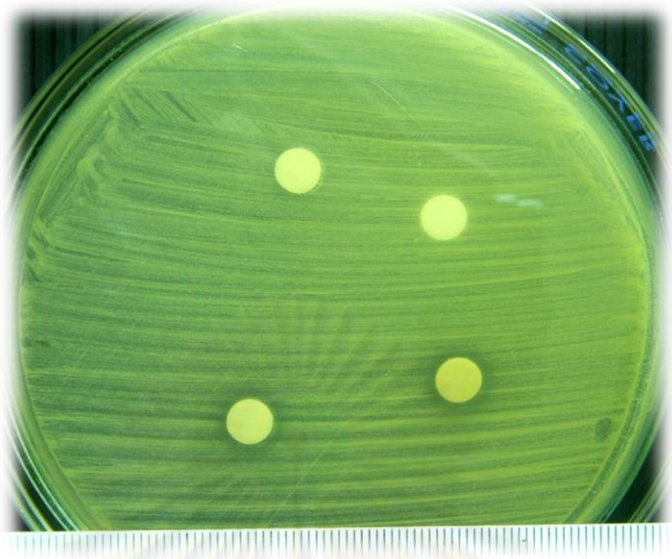
APPENDIX



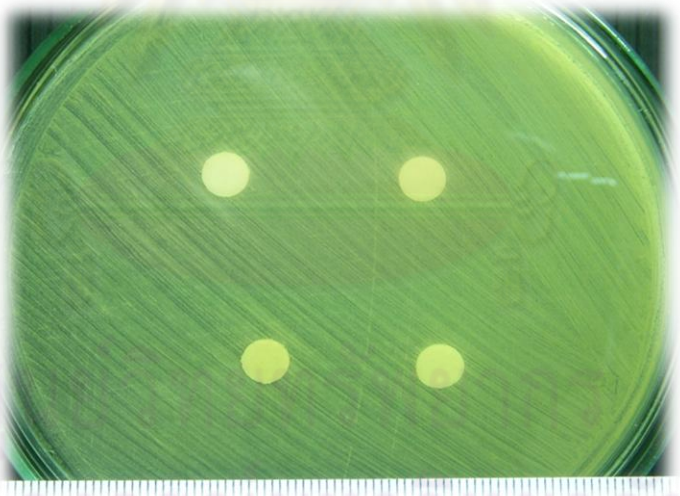
Irradiation with UV lamp 360 nm

Without UV lamp

Figure 13 ***A. marmelos*** **(dried root) against** ***B. subtilis*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**



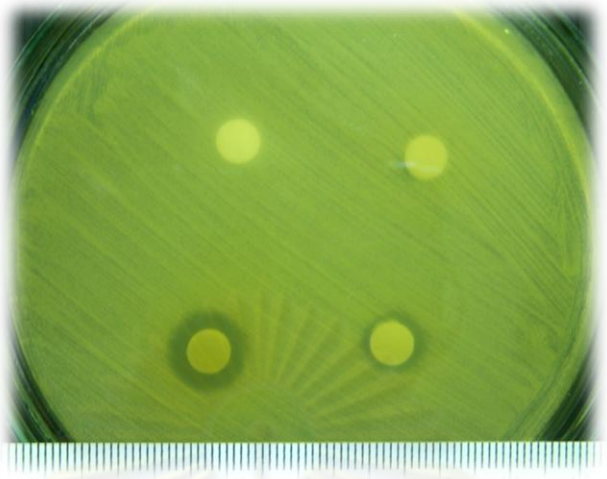
Irradiation with UV lamp 360 nm



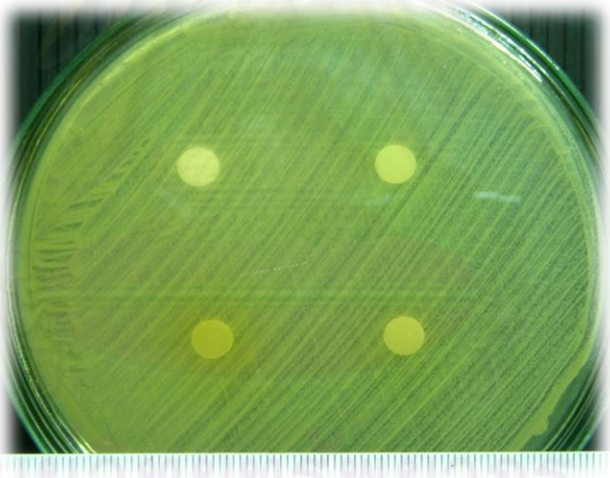
Without UV lamp

Figure 14

***A. marmelos* (dried root) against *S. aureus*. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

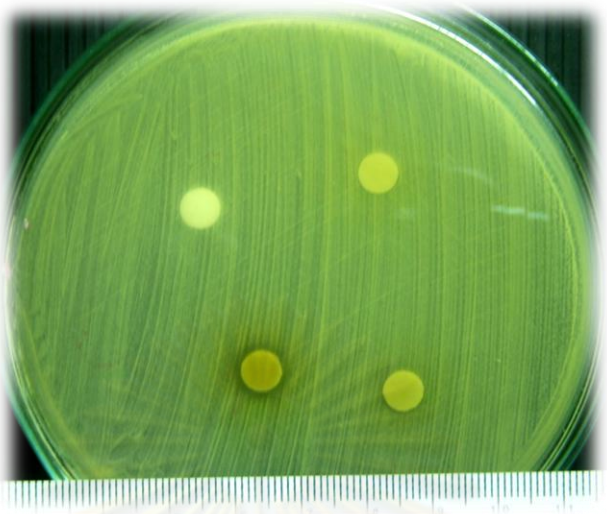


Irradiation with UV lamp 360 nm

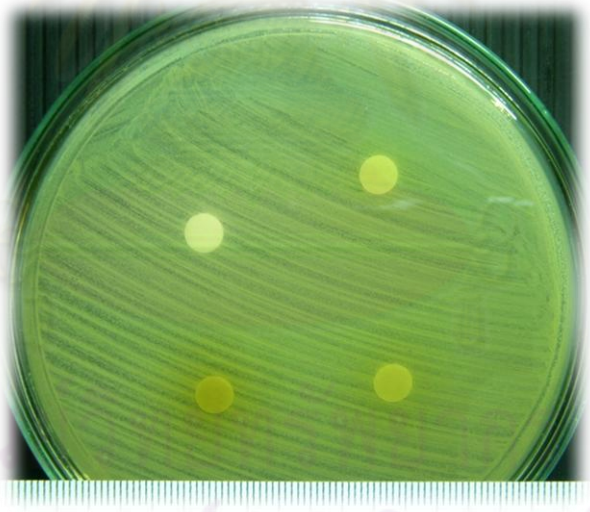


Without UV lamp

Figure 15 ***A. monothylla*** **(fresh leaves) against** ***S. aureus*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**



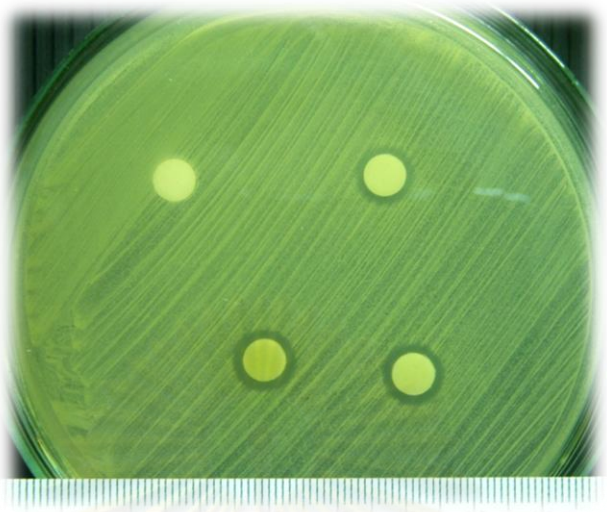
Irradiation with UV lamp 360 nm



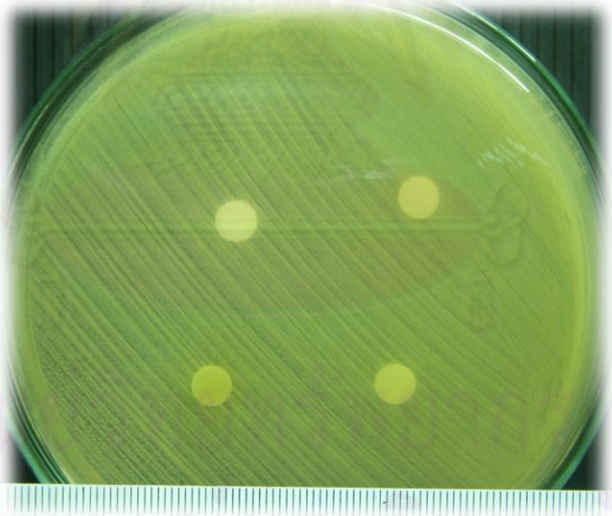
Without UV lamp

Figure 16

***F. lucida* (dried leaves) against *S. aureus.* The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**



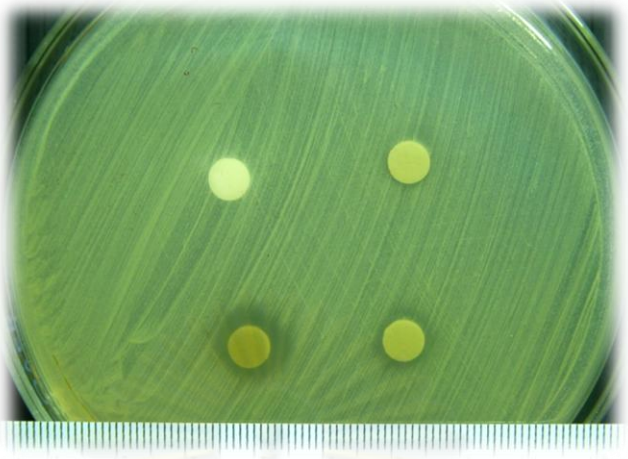
Irradiation with UV lamp 360 nm



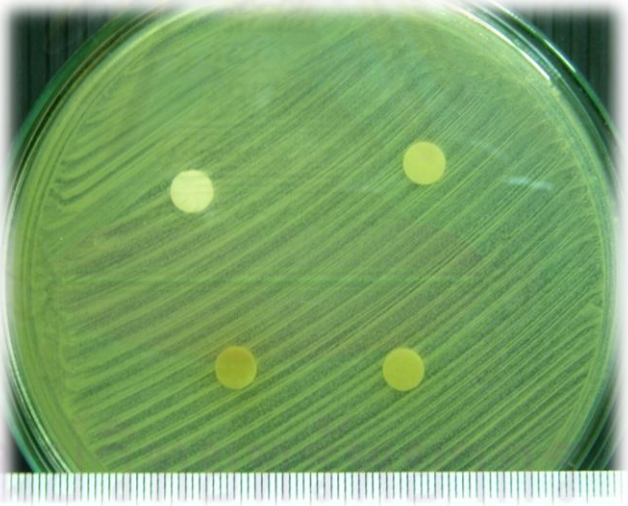
Without UV lamp

Figure 17

H. crenulata **(dried leaves) against** ***S. aureus.*** **The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

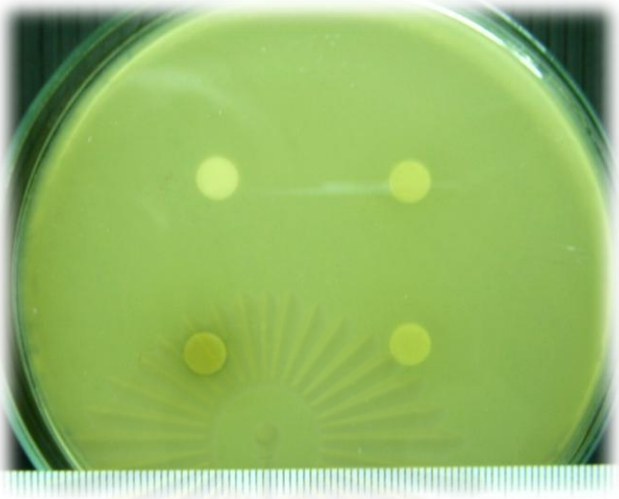


Irradiation with UV lamp 360 nm



Without UV lamp

Figure 18 ***M. Koenigii*** **(fresh leaves) against** ***S. aureus.*** **The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

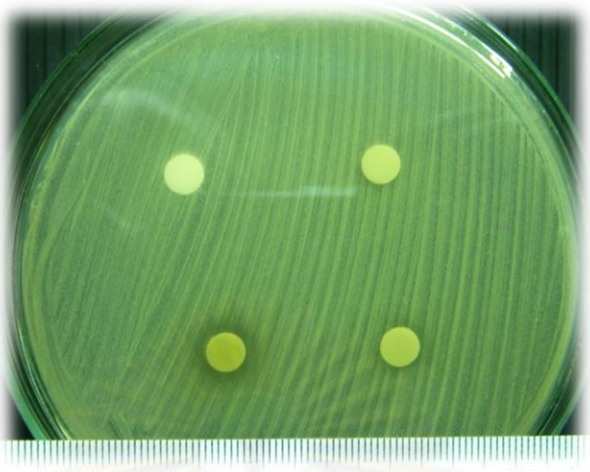


Irradiation with UV lamp 360 nm

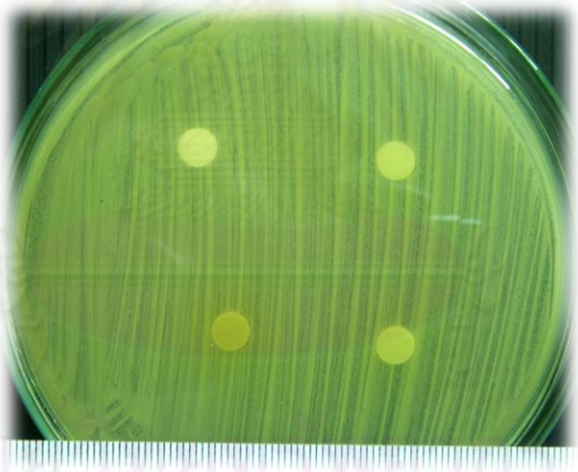
Without UV lamp

Figure 19

T. trifolia **(fresh leaves) against** ***B. subtilis.*** **The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

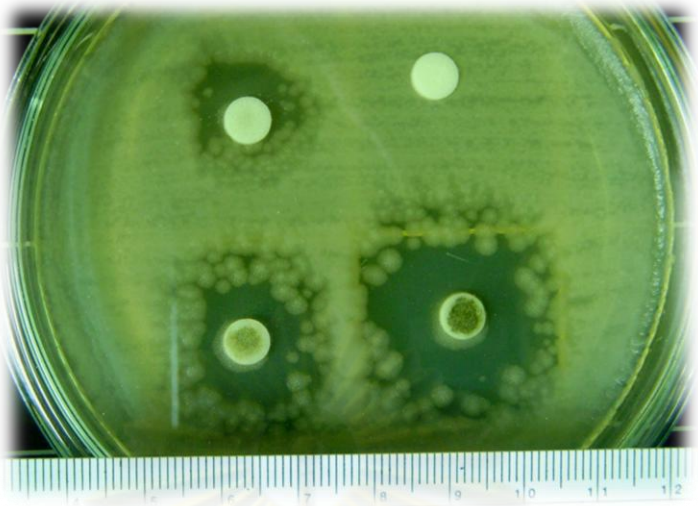


Irradiation with UV lamp 360 nm

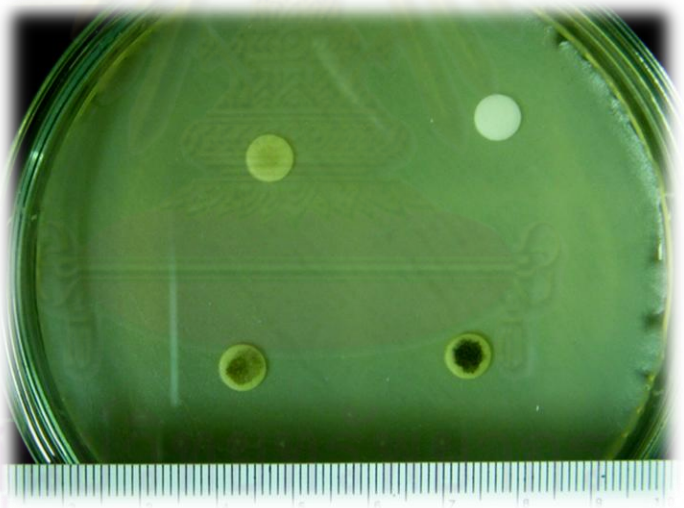


Without UV lamp

Figure 20 ***T. trifolia*** **(fresh leaves) against** ***S. aureus.*** **The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**



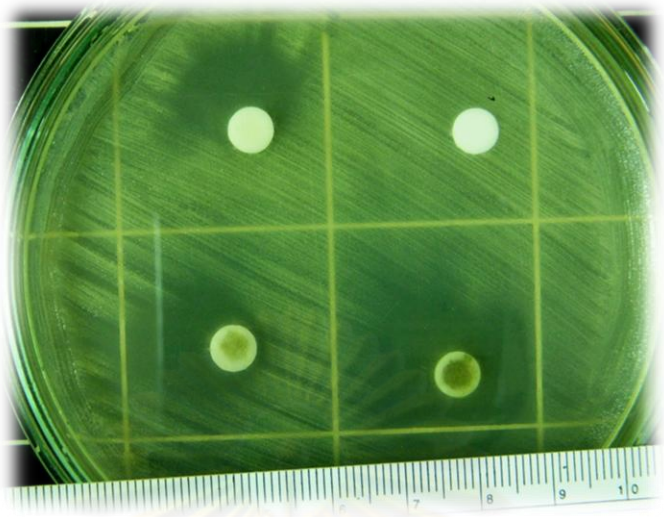
Irradiation with UV lamp 360 nm



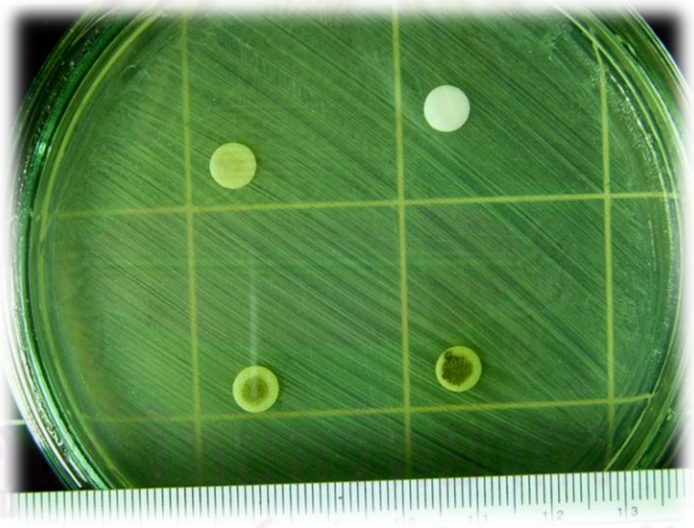
Without UV lamp

Figure 21

Anethum graveolens **(fresh whole plants) against** ***B. subtilis*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**



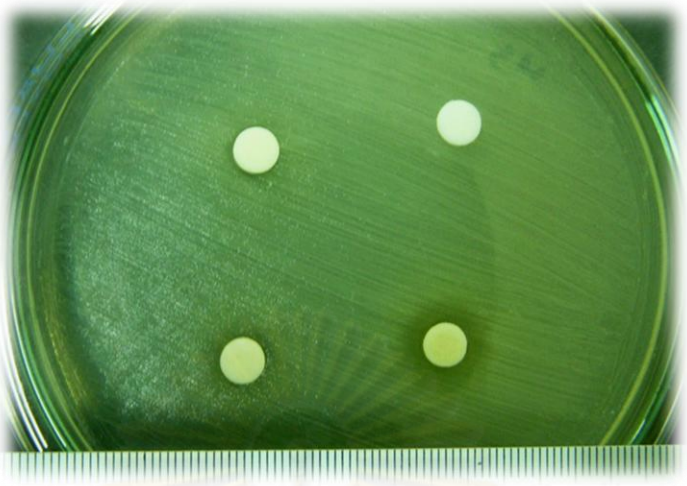
Irradiation with UV lamp 360 nm



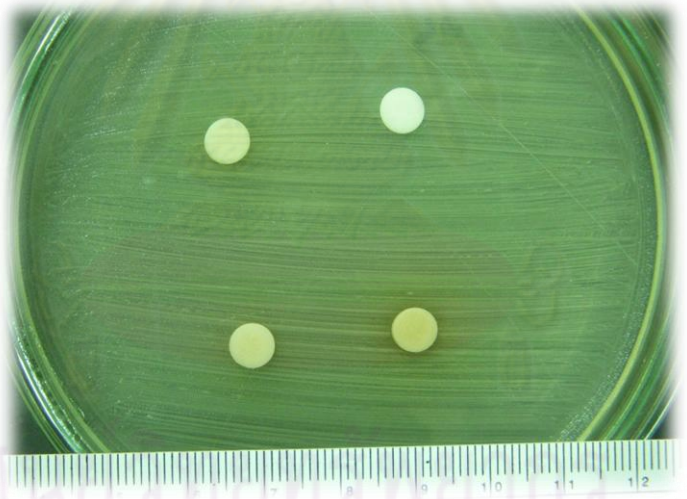
Without UV lamp

Figure 22

Anethum graveolens **(fresh whole plants) against** ***S. aureus*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

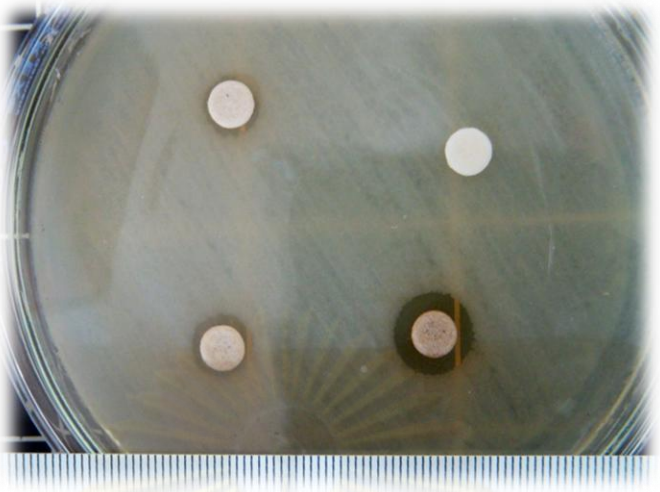


Irradiation with UV lamp 360 nm

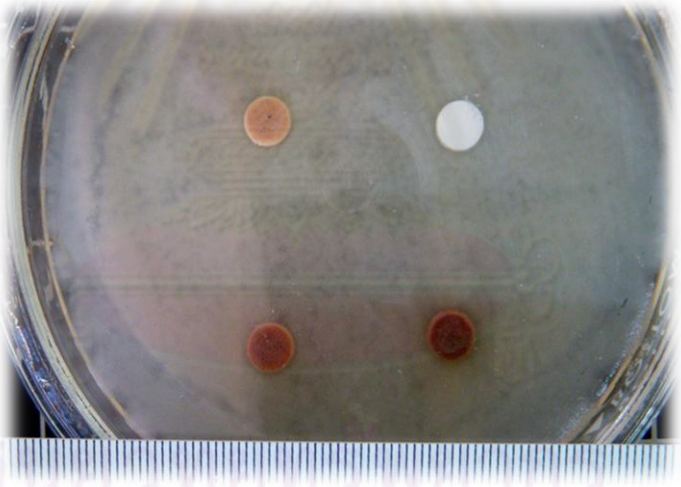


Without UV lamp

Figure 23 ***Anethum graveolens*** **(dried fruits) against** ***S. aureus.*** **The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

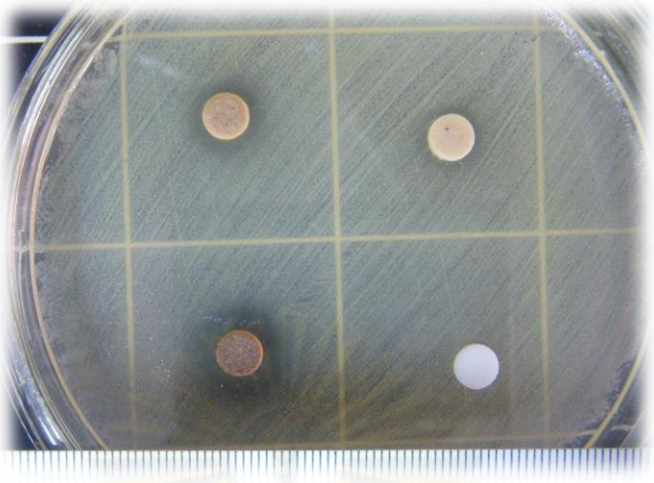


Irradiation with UV lamp 360 nm

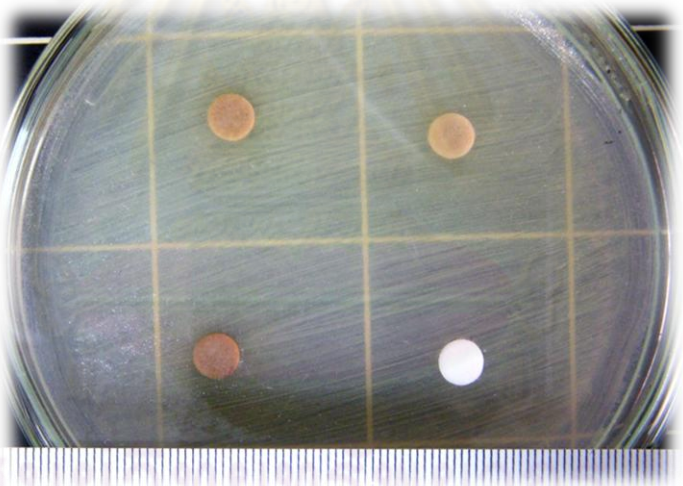


Without UV lamp

Figure 24 ***A. dahulica*** **(dried rhizomes) against** ***B. subtilis*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

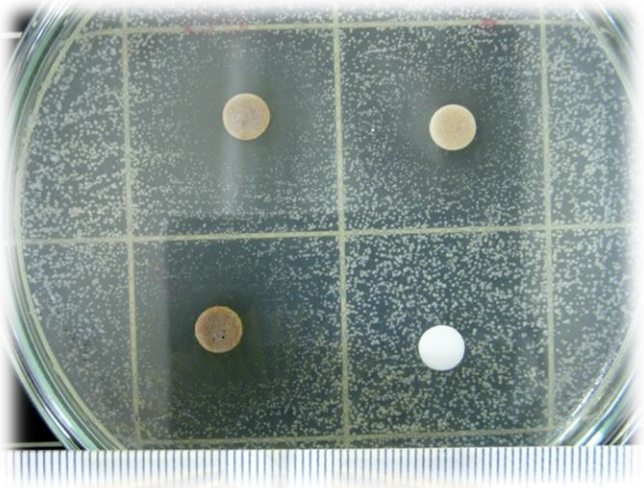


Irradiation with UV lamp 360 nm

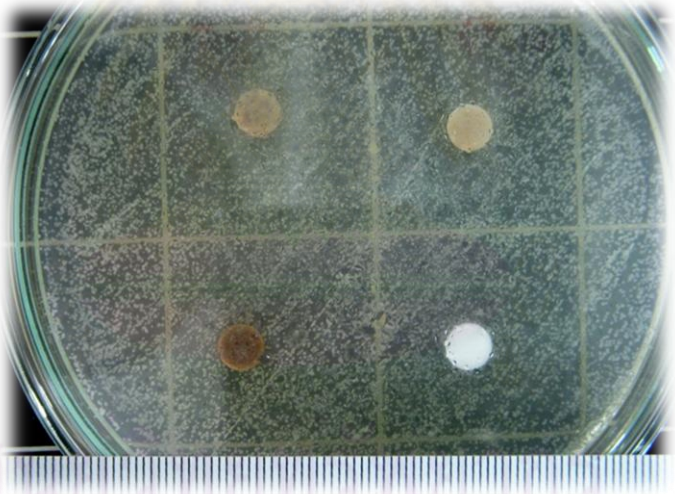


Without UV lamp

Figure 25 ***A. dahulica*** **(dried rhizomes) against** ***S. aureus*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

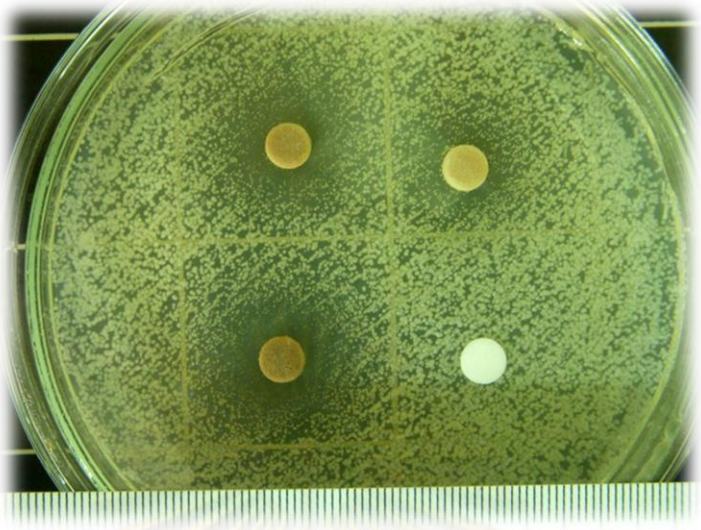


Irradiation with UV lamp 360 nm

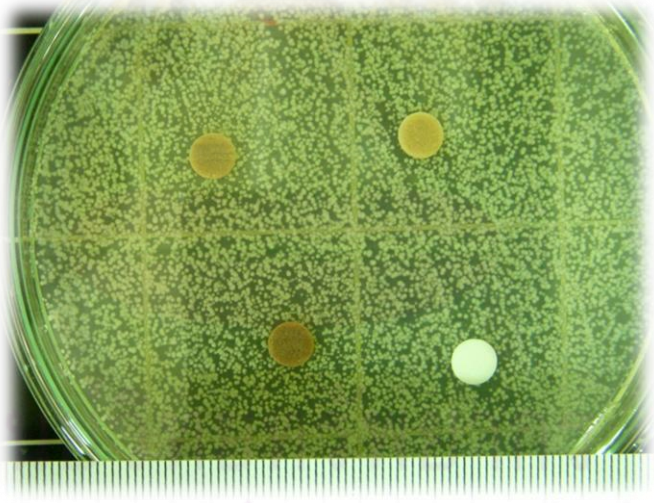


Without UV lamp

Figure 26 ***A. dahulica*** **(dried rhizomes) against** ***C. albicans*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**



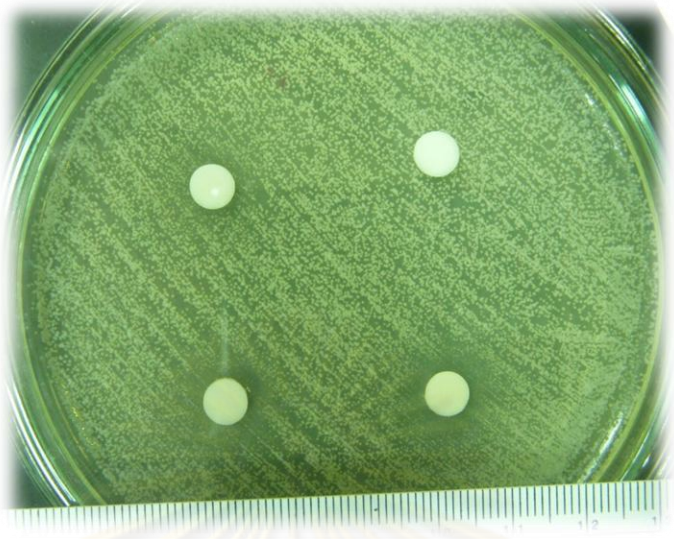
Irradiation with UV lamp 360 nm



Without UV lamp

Figure 27

A. dahulica **(dried rhizomes) against** ***S. cerevisiae*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

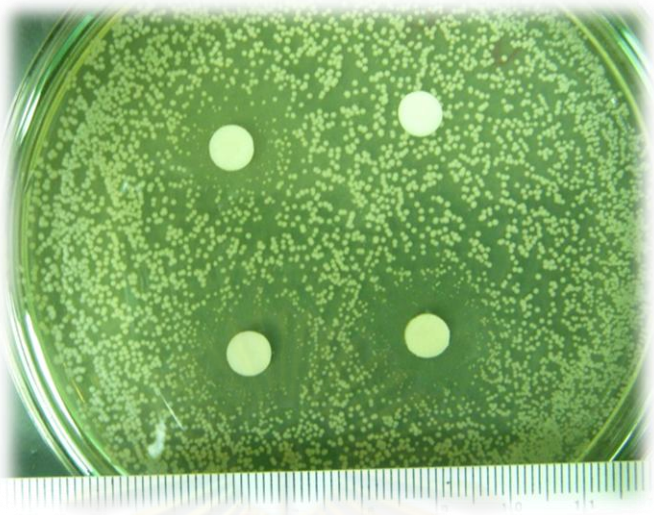


Irradiation with UV lamp 360 nm

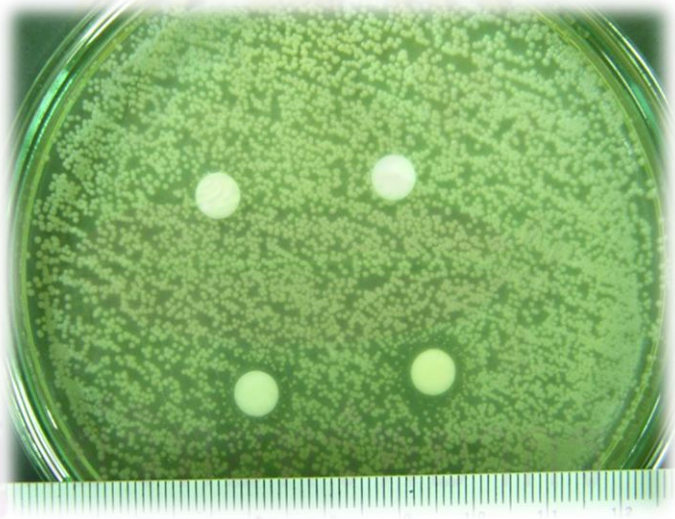
Without UV lamp

Figure 28

***Apium. graveolens* (dried fruits) against *C. albicans*. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**



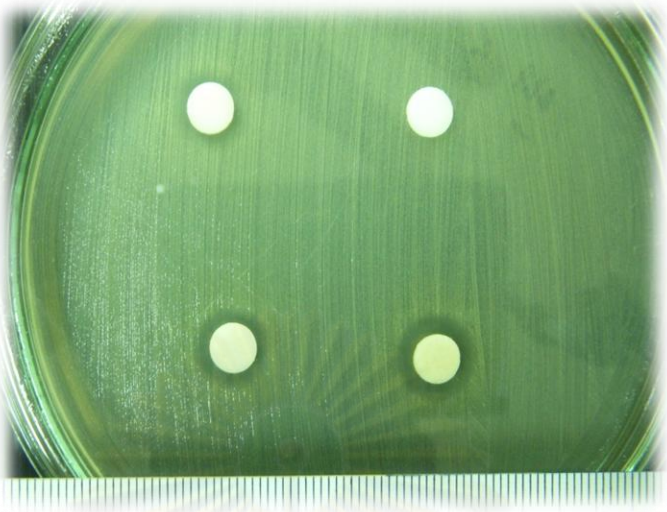
Irradiation with UV lamp 360 nm



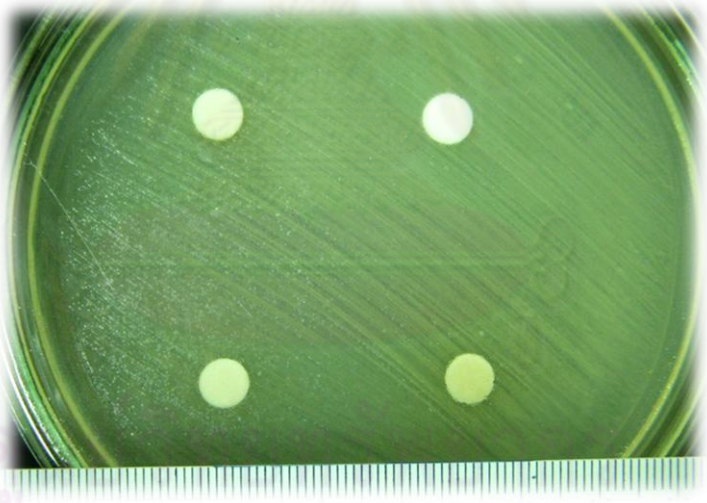
Without UV lamp

Figure 29

***Apium graveolens* (dried fruits) against *S. cerevisiae*. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

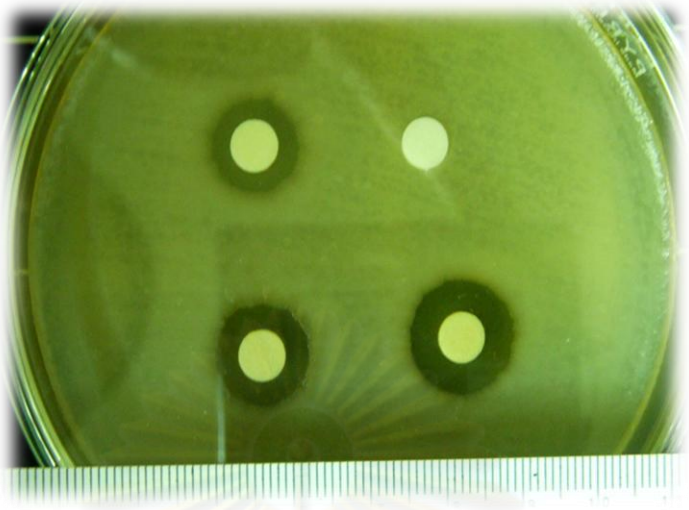


Irradiation with UV lamp 360 nm

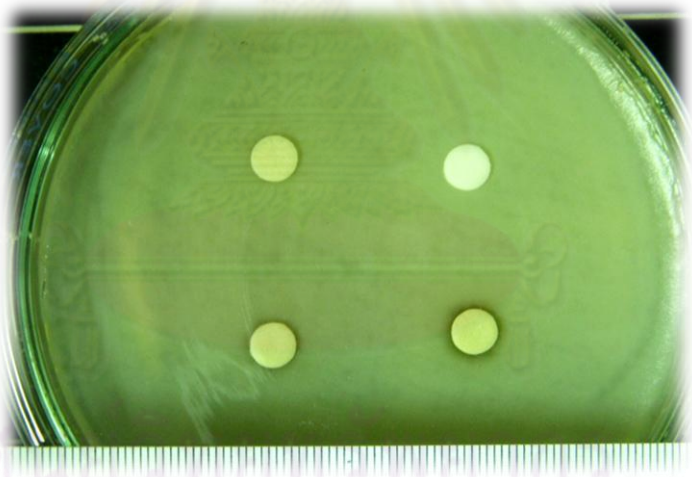


Without UV lamp

Figure 30 ***F. vulgare*** **(dried fruits) against** ***S. aureus*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

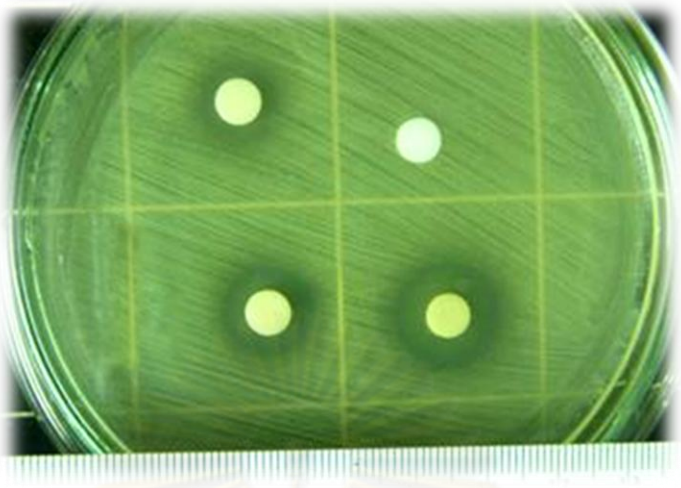


Irradiation with UV lamp 360 nm

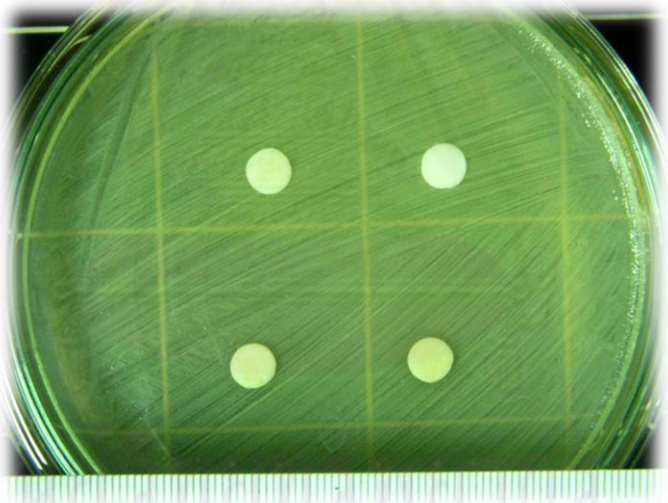


Without UV lamp

Figure 31 ***H. siamicum*** **(dried fruits) against** ***B. subtilis*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

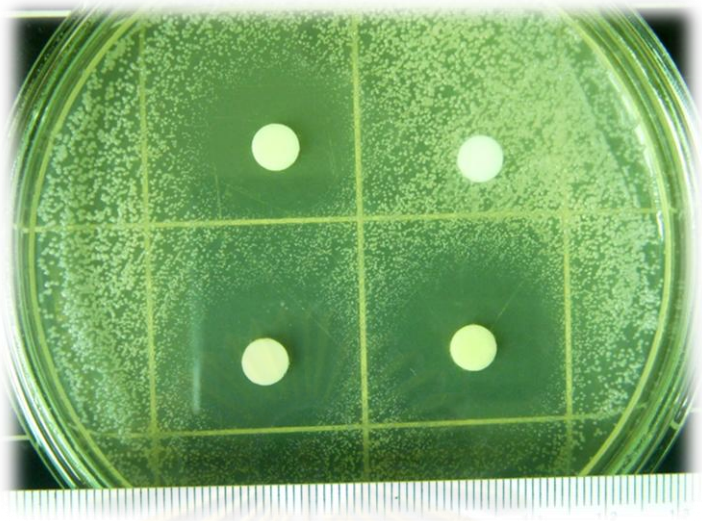


Irradiation with UV lamp 360 nm



Without UV lamp

Figure 32 ***H. siamicum*** **(dried fruits) against** ***S. aureus*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

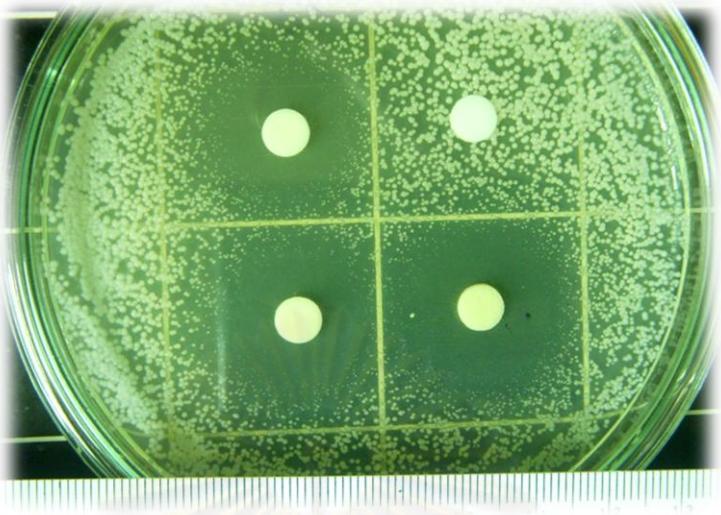


Irradiation with UV lamp 360 nm

Without UV lamp

Figure 33

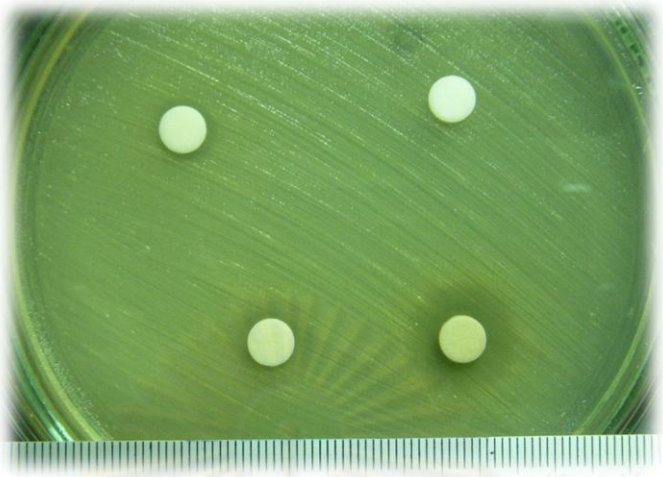
H. siamicum **(dried fruits) against** ***C. albicans*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**



Irradiation with UV lamp 360 nm

Without UV lamp

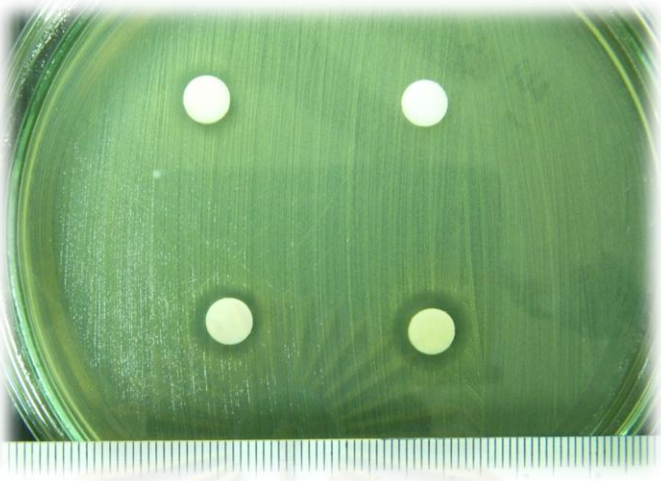
Figure 34 ***H. siamicum*** **(dried fruits) against** ***S. cerevisiae*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**



Irradiation with UV lamp 360 nm

Without UV lamp

Figure 35 ***P. crispum*** **(dried fruit) against** ***S. aureus*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**



Irradiation with UV lamp 360 nm

Without UV lamp

Figure 36

P. anisum **(dried fruit) against** ***S. aureus*****. The extract concentrations were 10 µl/disc of 0, 250, 500 and 1000 µg/ml.**

VITA

Mister Apirach Prachasupap was born on August 19, 1985, Bangkok, Thailand. He got Bachelor's degree of Science (Oriental Medicine) from Faculty of Oriental Medicine, Rangsit University, Pathumthani, Thailand in 2007.

PROCEEDING:

Prachasupap A., Palanuvej C., Ruangrungsi N. "Phototoxic activity of selected Thai Umbelliferous plants" The 9th Joint Seminar of JSPS-NRCT Core University Program on Natural Medicine in Pharmaceutical Sciences, December 8-9, 2010, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.