PHOTOTOXIC ACTIVITY OF SELECTED THAI RUTACEOUS AND UMBELLIFEROUS PLANT EXTRACTS

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ABSTRACT: Phototoxic activity of ethanol extracts from 25 Rutaceous and Umbelliferous species were assessed. Light mediated antimicrobial activities against gram-nagative bacteria, *Escherichia coli*; gram-positive bacteria, *Staphylococcus aureus*; gram-positive spores forming bacteria, *Bacillus subtilis*; yeast *Candida albicans* and *Saccharomyces cerevisiae* were conducted. Agar diffusion inhibitions potential with and without UVA irradiation at 360 nm were evaluated. Minimum inhibitory concentrations (MIC) were determined. Thirteen species showed UV-induced inhibitory activity against microorganisms. These were from both families as follow: *Aegle marmelos* (L.) Corr., *Atalantia monophylla* DC., *Feroniella lucida* (Scheff.) Swingle., *Hesperethusa crenulata* (Roxb.) Roem., *Murraya koenigii* L., *Triphasia trifolia* (Burm.t.) P. Wils. in Rutaceous plants and *Anethum graveolens* L., *Angelica dahulica* Benth., *Apium graveolens*, *Foeniculum vulgare* Mill., *Heracleum siamicum* Craib, *Petroselinum crispum* (Miller) A.W. Hill, *Pimpinella anisum* L. 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.

Keywords: Phototoxic activity, Rutaceae, Umbelliferae, microorganisms, agar disc diffusion, UV-induced antimicrobial activity

INTRODUCTION

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 exposure to sunlight and contact with some plants containing furocoumarins, frequently the psoralen. Phototoxic reactions resemble hyperpigmentation or sunburn and might also present with irritant, urticaria and allergic, as well as erythema, oedema, blistering and sometime vesiculation [1-5].

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 [6].

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 [7]. 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. In this study, a number of Thai Rutaceous and Umbelliferous plants were selected for screening of phototoxicity against microorganisms. The study was to develop microbiological assay to screen the phototoxic potential of selected Thai Rutaceous and Umbelliferous plant extracts as well.

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MATERIALS AND METHODS

Plant materials

Plant materials from 25 species of selected Thai Rutaceous and Umbelliferous plants were collected from Thai traditional drugstores, local markets and Botanical garden of Pharmaceutical Sciences, Chulalongkorn University, Bangkok. All materials were authenticated by Assoc. Prof. Nijsiri Ruangrungsi, Ph.D. and the voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University. The parts of different plants used in the experiment are given in Table 1.

Extraction

The authenticated plant materials were ground to coarsely powder and macerated with 95% ethanol. The marcs were filtered and re extracted until exhaustion. The filtrates were pooled and evaporated *in vacuo*. All extracts were dissolved in DMSO at various concentrations and employed to the phototoxicity testing.

Table 1 Summarization of phototoxic activity of selected Thai Rutaceous and Umbelliferous plants

NO.	Plant	Family	Part used	1	2	3	4	5
1.	*Aegle marmelos* (L.) Corr.	Rutaceae	Roots[B]	–	+	+	–	–
2.	*Aegle marmelos* (L.) Corr.	Rutaceae	Fruits[B]	–	–	–	–	–
3.	*Atalantia monophylla* DC.	Rutaceae	Leaves[A]	–	–	+	–	–
4.	*Citrus aurantifolia* (Christm) Swing.	Rutaceae	Seeds[B]	–	–	–	–	–
5.	*Citrus reticulata* Blanco	Rutaceae	Seeds[B]	–	–	–	–	–
6.	*Feroniella lucida* (Scheff.) Swingle.	Rutaceae	Leaves[B]	–	–	+	–	–
7.	*Feroniella lucida* (Scheff.) Swingle.	Rutaceae	Stem[B]	–	–	–	–	–
8.	*Glycosmis pentaphylla* (Retz.) DC.	Rutaceae	Leaves[B]	–	–	–	–	–
9.	*Hesperethusa crenulata* (Roxb.) Roem.	Rutaceae	Leaves[B]	–	–	+	–	–
10.	*Hesperethusa crenulata* (Roxb.) Roem.	Rutaceae	Stem[B]	–	–	–	–	–
11.	*Murraya koenigii* L.	Rutaceae	Leaves[A]	–	–	+	–	–
12.	*Murraya koenigii* L.	Rutaceae	Stem[B]	–	–	–	–	–
13.	*Murraya paniculata* L.	Rutaceae	Leaves[A]	–	–	–	–	–
14.	*Triphasia trifolia* (Burm.t.) P.Wils.	Rutaceae	Leaves[A]	–	+	+	–	–
15.	*Zanthoxylum limonella* (Dennst.) Alston.	Rutaceae	Fruits[B]	–	–	–	–	–
16.	*Anethum graveolens* L.	Umbelliferae	Whole plant[A]	–	+	+	–	–
17.	*Anethum graveolens* L.	Umbelliferae	Fruits[B]	–	–	+	–	–
18.	*Angelica dahulica* Benth.	Umbelliferae	Rhizomes[B]	–	+	+	+	+
19.	*Angelica sinensis* (Oliv.) Diels.	Umbelliferae	Roots[B]	–	–	–	–	–
20.	*Apium graveolens* L.	Umbelliferae	Whole plant[A]	–	–	–	–	–
21.	*Apium graveolens* L.	Umbelliferae	Fruits[B]	–	–	–	+	+
22.	*Coriandrum sativum* Vern. Dhania.	Umbelliferae	Whole plant[A]	–	–	–	–	–
23.	*Coriandrum sativum* Vern. Dhania.	Umbelliferae	Fruits[B]	–	–	–	–	–
24.	*Coriandrum sativum* Vern. Dhania.	Umbelliferae	Roots[A]	–	–	–	–	–
25.	*Cuminum cyminum* L.	Umbelliferae	Fruits[B]	–	–	–	–	–
26.	*Daucus carota* L.	Umbelliferae	Fruits[B]	–	–	–	–	–
27.	*Eryngium foetidum* L.	Umbelliferae	Whole plant[A]	–	–	–	–	–
28.	*Ferrula assa-foetida* L.	Umbelliferae	Oleoresin[B]	–	–	–	–	–
29.	*Foeniculum vulgare* Mill.	Umbelliferae	Fruits[B]	–	–	+	–	–
30.	*Heracleum siamicum* Craib	Umbelliferae	Fruits[B]	–	+	+	+	+
31.	*Ligusticum wallichii* Franch.	Umbelliferae	Rhizomes[B]	–	–	–	–	–
32.	*Petroselinum crispum* (Miller) A.W. Hill	Umbelliferae	Fruits[B]	–	–	+	–	–
33.	*Pimpinella anisum* L.	Umbelliferae	Fruits[B]	–	–	+	–	–

NOTE: [A] =fresh, [B]= dried, 1= *E. coli*, 2= *B. subtilis*, 3= *S. aureus*, 4= *C. albicans*, 5= *S. serevisiae*, += Activity, – = No activity

Microorganisms

The tested microorganisms were the gram-positive bacteria *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC 6633; the gram-negative bacteria *Escherichia coli* ATCC 25922; yeast *Candida albicans* ATCC 10230 and *Saccharomyces cerevisiae* ATCC 9763. They were obtained from the Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Culture media

Mueller Hinton agar (MHA) was used for bacteria and Sabouraud dextrose agar (SDA) was used for yeast.

Preparation of inoculum suspensions

All bacteria were cultivated overnight on agar media at 37 °C. 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 of a normal saline solution (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 0.08 to 0.1 which comparable to the turbidity of 0.5 McFarland Standard (approximately 1 to 2 x 10^8 CFU/ml). Two yeast strains were prepared by the same procedure as described for bacteria cell cultures.

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. Each lamp provided a beam of 360 nm wave with 15 W/m^2 at 30 cm.

Phototoxic testing by agar disc diffusion

Microorganism standard strains were grown and the inoculums were adjusted the turbidity to 0.5 McFarland standards. Each inoculum was seeded on MHA plates for bacteria and SDA for yeast. Disc diffusion method according to NCCLS [8-10] including irradiation with and without UVA was applied to investigate the phototoxic potential of plant materials in triplicate. Each of ethanol extracts

Table 2 Activity of selected Rutaceous plants on growth of microorganisms

Plant	Concentration (µg/disc)	Inhibition zone (mm*)									
		Irradiate with UV 360 nm					Without UV				
		E.coli	*B.subtilis*	*S.aureus*	*C.albicans*	*S.cereviseae*	*E.coli*	*B.subtilis*	*S.aureus*	*C.albicans*	*S.cereviseae*
A. marmelos (dried roots)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	6.33 ± 0.29	NA	NA	NA	NA	NA	NA	NA	NA
	500	NA	7.33 ± 0.58	7.00 ± 0.00	NA	NA	NA	NA	NA	NA	NA
	1000	NA	8.17 ± 0.29	8.00 ± 0.00	NA	NA	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	191.1	< 500	-	-	-	-	-	-	-
A. monophylla (fresh leaves)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	500	NA	NA	7.33 ± 0.58	NA	NA	NA	NA	NA	NA	NA
	1000	NA	NA	10.00 ± 1.00	NA	NA	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	-	< 500	-	-	-	-	-	-	-
F. lucida (dried leaves)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	500	NA	NA	6.50 ± 0.00	NA	NA	NA	NA	NA	NA	NA
	1000	NA	NA	7.17 ± 0.29	NA	NA	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	-	< 500	-	-	-	-	-	-	-
H. crenulata (dried leaves)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	NA	7.17 ± 0.29	NA	NA	NA	NA	NA	NA	NA
	500	NA	NA	8.00 ± 0.00	NA	NA	NA	NA	NA	NA	NA
	1000	NA	NA	8.50 ± 0.50	NA	NA	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	-	166.4	-	-	-	-	-	-	-
M. koenigii (fresh leaves)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	NA	6.50 ± 0.00	NA	NA	NA	NA	NA	NA	NA
	500	NA	NA	7.33 ± 0.58	NA	NA	NA	NA	NA	NA	NA
	1000	NA	NA	8.33 ± 0.58	NA	NA	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	-	175.0	-	-	-	-	-	-	-
T. trifolia (fresh leaves)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	500	NA	NA	6.50 ± 0.00	NA	NA	NA	NA	NA	NA	NA
	1000	NA	6.50 ± 0.00	8.17 ± 0.29	NA	NA	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	< 1000	< 500	-	-	-	-	-	-	-

*mean ± SD, NA = no activity
Each experiment was done in triplicate.

was performed in the levels of 100, 50 and 25 mg/ml in DMSO. Paper discs of 6 mm diameter were filled with 10 µl of plant extract and DMSO (negative control disc). Tested plates were exposed to UV lamp in the chamber (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 was calculated [11].

RESULTS AND DISCUSSION

The 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 2. From the study of Shoeb et al. [12], alkaloids and coumarin from roots 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 organisms in presence of exposure to UV radiation. In this study, the extract from *A. marmelos* (dried roots) showed phototoxic activities on microorganisms. According to *Shoeb's res*earch, this might be due to psoralen, xanthotoxin and other furocoumarins.

Phototoxic activity of *A. monophylla* (fresh leaves) showed selected exhibition on *S. aureus* with large clear zone of inhibition at highest concentration. The activity of *F. lucida* (dried leaves) was similar but less potent than the results of *A. monophylla*. *H. crenulata* (dried leaves) exhibition activities on *S. aureus* were in accordance with *M. koenigii* (fresh leaves). *T. trifolia* (fresh leaves) exhibited activity on *B. subtilis* and *S. aureus*. Phototoxic activity of these Rutaceous plant materials showed dose response relationship with 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. The MIC was shown in Table 2.

Selected Umbelliferous plant extracts, except *Apium graveolens* (dried fruits), *H. siamicum* (dried fruits) and *P. crispum* (dried fruits) had no

Table 3 Activity of selected Umbelliferous plants on growth of microorganisms

Plant	Concentration (µg/disc)	Inhibition zone (mm*)									
		Irradiate with UV 360 nm					Without UV				
		E.coli	*B.subtilis*	*S.aureus*	*C.albicans*	*S.cereviseae*	*E.coli*	*B.subtilis*	*S.aureus*	*C.albicans*	*S.cereviseae*
Anethum graveolens (fresh whole plant)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	9.55 ± 0.69	13.00 ± 0.58	NA	NA	NA	NA	NA	NA	NA
	500	NA	13.55 ± 0.84	14.44 ± 0.51	NA	NA	NA	NA	NA	NA	NA
	1000	NA	18.78 ± 0.51	18.11 ± 0.77	NA	NA	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	151.3	41.4	-	-	-	-	-	-	-
Anethum graveolens (dried fruits)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	NA	6.33 ± 0.29	NA	NA	NA	NA	NA	NA	NA
	500	NA	NA	7.67 ± 0.59	NA	NA	NA	NA	NA	NA	NA
	1000	NA	NA	9.67 ± 0.59	NA	NA	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	-	228.2	-	-	-	-	-	-	-
A. dahulica (dried rhizomes)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	7.00 ± 0.00	7.00 ± 0.00	9.67 ± 0.58	7.00 ± 0.00	NA	NA	NA	NA	NA
	500	NA	7.67 ± 0.58	8.67 ± 0.58	11.67 ± 0.58	9.33 ± 0.58	NA	NA	NA	NA	NA
	1000	NA	10.67 ± 0.58	11.33 ± 0.58	13.33 ± 0.58	10.67 ± 0.58	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	198.4	191.4	60.9	161.0	-	-	-	-	-
Apium graveolens (dried fruits)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	NA	NA	NA	7.67 ± 0.58	NA	NA	NA	NA	NA
	500	NA	NA	NA	8.67 ± 0.58	10.33 ± 0.58	NA	NA	NA	8.33 ± 0.58	7.33 ± 0.58
	1000	NA	NA	NA	9.67 ± 0.58	12.67 ± 0.58	NA	NA	NA	9.67 ± 0.58	9.67 ± 0.58
	MIC (µg/disc)	-	-	-	< 500	155.04	-	-	-	-	-
F. vulgare (dried fruits)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	NA	6.67 ± 0.29	NA	NA	NA	NA	NA	NA	NA
	500	NA	NA	7.83 ± 0.76	NA	NA	NA	NA	NA	NA	NA
	1000	NA	NA	9.33 ± 0.58	NA	NA	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	-	182.0	-	-	-	-	-	-	-
H. siamicum (dried fruits)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	11.33 ± 0.58	9.00 ± 0.00	16.33 ± 0.58	14.67 ± 0.58	NA	NA	NA	NA	NA
	500	NA	12.67 ± 0.58	12.33 ± 0.58	17.33 ± 0.58	17.67 ± 0.58	NA	6.50 ± 0.00	NA	NA	NA
	1000	NA	13.67 ± 0.58	13.67 ± 0.58	18.67 ± 0.58	20.33 ± 0.58	NA	7.00 ± 0.00	NA	NA	NA
	MIC (µg/disc)	-	10.3	93.0	1.0	3.0	-	-	-	-	-
P. crispum (dried fruits)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	NA	7.00 ± 0.00	NA	NA	NA	NA	NA	NA	NA
	500	NA	NA	7.33 ± 0.58	NA	NA	NA	NA	NA	NA	NA
	1000	NA	NA	8.67 ± 0.58	NA	NA	NA	NA	7.33 ± 0.58	NA	NA
	MIC (µg/disc)	-	-	125.4	-	-	-	-	-	-	-
P. anisum (dried fruits)	DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	250	NA	NA	6.50 ± 0.50	NA	NA	NA	NA	NA	NA	NA
	500	NA	NA	7.50 ± 0.50	NA	NA	NA	NA	NA	NA	NA
	1000	NA	NA	9.00 ± 0.50	NA	NA	NA	NA	NA	NA	NA
	MIC (µg/disc)	-	-	198.4	-	-	-	-	-	-	-

*mean ± SD, NA = no activity
Each experiment was done in triplicate.

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.

Fresh whole plant of *Anethum graveolens* showed phototoxicity against *B. subtilis* and *S. aureus* while the dried fruits exhibited phototoxic activity against only *S. aureus*. According to Belleinger, *Anethum graveolens* was one of the plants reported to evoke phytophotodermatitis [13]. Phototoxic activitiy of *Angelica dahulica* against *B. subtilis, S. aureus, C. albicans* and *S. cerevisiae* was demonstrated with large zones of inhibition. According to Pathak et al., *Angelica species* were determined and revealed the distribution of furocoumarin [6]. *Apium graveolens* showed not only antimicrobial activity against *S. cerevisiae* but also phototoxic activity. This circumstance was also found for *H. siamicum* against *B. subtilis* and *P. crispum* against *S. aureus* (Table 3). *H. siamicum* showed potent phototoxicity against *B. subtilis, S. aureus C. albicans* and *S. cerevisiae* with large sizes of inhibition zones rather than others. Finally, three extracts as *F. vulgare, P. crispum* and *P. anisum* exhibited phototoxicity only on *S. aureus*. Results were indicated in Table 3 including MIC [13]

Phototoxic properties of furanocoumarins and related compounds have been assayed using fungi [14, 15], green algae [16-18], bacteria [19, 20], laboratory animals [21, 22] and *Artemia salina* [23]. Nowadays cultured human skin systems are available [24, 25]. The methodology in this study is basically similar to those used for testing

antimicrobial properties of the compounds, but further coupled with UV 360 nm irradiation. So this technique is able to quickly screen the possibly phototoxic compounds in plant extracts and calculate MIC from inhibition zone. The first method to measure phototoxicity *in vitro* was a microbiological approach using microorganisms with some modification to test the pure compounds of furocoumarins [14, 26]. Faergemann and Larko tested phototoxic effect of 8-methoxypsoralen and trimethylpsoralen against various microorganisms: *Staphylococcus aureus, S. epidermidis C. albicans* and *Pityrosporum orbiculare*. The results showed phototoxic activities against all microorganisms tested [27]. *S. aureus* and *E. coli* were previously reported as test systems of phototoxicity [28, 29]. *C. albicans* and *S. cerevisiae* have also been tested for phototoxicity study [14, 30, 31]. 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 plants. *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.

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