Antimutagenic Potentials of Hydroalcoholic Herbal Extracts towards UV-Induced Mutation

Ponnaree Kittiwannachot, Panida Borisut, Pimphaka Wanasawas, Lakkhana Ponpanich, Orathai Rattanasuk and Malyn Chulasiri*

Research & Development Division, SJI, Bangkok, Thailand.

ABSTRACT

Twelve antimutagenic herbs reported elsewhere towards chemical mutagens were selected for the study. Their used parts which had been dried and ground were extracted with hydroalcohol. These extracts were evaluated for the antimutagenicity towards UVA- and UVB-induced mutation using modified Ames test having *Escherichia coli* B/r WP2 (*trpE65*) as the tester strain. Results revealed the hydroalcoholic extracts from damask rose (*Rosa damascena* Mill) flower, emblic (*Phyllanthus emblica* Linn) fruit pulp, guava (*Psidium guajava* Linn) leaf and myrobalan (*Terminalia chebula* Retz) fruit pulp were rather promising. Their IC_{50} towards both UVA- and UVB-induced mutation was less than 4 mg/plate. These extracts were then separately incorporated into SPF 8 sunscreen lotion. It was revealed that the damask rose and myrobalan sunscreen lotions were more appreciable than the guava and emblic sunscreen lotions. The IC_{50} of lotions incorporated with damask rose and myrobalan extracts was 4.3 and 2.3% w/w respectively towards UVA-induced mutation, and was 2.5 and 4.2 9% w/w respectively towards UVB-induced mutation. Whereas the IC_{50} of lotions incorporated with emblic and guava extracts was more than 7% w/w and was significantly higher than the IC_{50} shown by the unformulated extracts ($p < 0.05$). The sunscreen lotions in the presence and absence of these extracts were eventually evaluated for SPF. All lotions with the extracts showed higher values than the non-herbal one.

Keywords: Hydroalcoholic herbal extracts, antimutagenicity, Ames test, UV-induced mutation

INTRODUCTION

Two main types of solar ultraviolet radiation, UVA and UVB, reach the earth. Both induce a number of pathological changes initiated in human skin, including erythema, edema, hyperplasia, sunburn cell formation, immune suppression, and skin cancer.[1] Among them, skin cancer is of the greatest concern as it is the most prevalent of cancers and its rates have been steadily increasing.[2,3] In view of the added care about ozone depletion, the use of sunscreens to prevent skin cancer has long been recommended.[4] These products help attenuate the amount of the UV reaching viable cells in the skin. No sunscreen protects total UV or prevents photodamage. It has been demonstrated that suberythemal doses of UV cause a variety of molecular changes including DNA damage in UV exposed cells.[5] Therefore, the UV escaping from sunscreen is likely to pene-

* Corresponding author: Malyn Chulasiri
Research & Development Division, SJI,
115 Narathiwasrajnakarin Road (Soi 10)
Sathon Dist, Bangkok 10120
E-mail: malyn@snjinter.com

trate into the skin cells and the accumulation from the excessive exposure length of sunlight tends to reduce the protection potential of sunscreen and would lead to the harmful effect afterwards. There is now an increasing body of evidence that the use of sunscreen is not entirely safe for sunscreen protection.[6,7]

UV is highly genotoxic and many studies agreed that the mutations generated in critical growth-control genes by the UV component of sunlight, is the first stage in skin cancer development.[8,9] To prevent such DNA damage induced by UV that penetrates into skin cells, the antimutagens having the potentials against UV is likely to be considered. The present study was carried out by examining the antimutagenic herbs revealed elsewhere[10-14] towards both UVA- and UVB-induced mutations. The appreciable extracts from these herbs were selected and incorporated into sunscreen lotions and evaluated for the protection towards UV-induced mutation. In addition, these herbal lotions were assayed for SPF.

MATERIALS AND METHODS

Plants and part use: Asiatic pennywort (*Centella asiatica* Linn) plant (whole), cassia (*Cinnamomum cassia* Blume) leaf, damask rose (*Rosa damascena* Mill) flower, emblic (*Phyllanthus emblica* Linn) fruit pulp, guava (*Psidium guajava* Linn) leaf, ink nut (*Terminalia bellerica* Roxb) fruit pulp, lotus (*Nelumbo nucifera* Gaertn) stem, myrobalan (*Terminalia chebula* Retz) fruit pulp, orange (*Citrus aurantium* Linn) peel, roselle (*Hisbiscus sabdariffa* Linn) flower, tea (*Camellia sinensis* Linn) leaf and Tumeric (*Curcuma longa* Linn) root were used.

Extraction and preparation: The aforementioned plant parts were dried and ground. The ground materials were each extracted by macerating in 80% ethanol except damask rose which was macerated in 50% ethanol. After extraction, these extracts were filtered and the filtrates were completely dried. The percentage yield was calculated from the dry weight of the extract towards the dry weight of its original ground herb. Before use, each dried extract was dissolved and diluted in dimethylsulfoxide (DMSO).

Sunscreen lotion: The formulation consisted of 2% cetyl alcohol, 0.5% glyceryl stearate SE, 2.5% glyceryl stearate/PEG-100 stearate, 0.5% stearic acid, 1.25% polysorbate 60, 0.5% sorbitan stearate, 1% olive oil, 1% squalane, 1% benzophenone, 6% octyl methoxycinnamate, 4% octyl salicylate, 0.65% preservative, 0.03% sodium hydroxide and water to 100%. The selected antimutagenic herbal extracts at various doses were each additionally incorporated in this lotion.

Antimutagenic evaluation: Both the herbal extracts and antimutagenic herbal sunscreen lotions were evaluated for the antimutagenicity towards UVA- and UVB-induced mutation by the modified Ames test.[15] *Escherichia coli* B/r WP2 (*trpE65*) was used as the tester strain. This bacterial strain was cultured in Oxoid Nutrient Broth No. 2 in a shaking water-bath at 37^{o}C for 13 h. The growth culture was then centrifuged, washed twice and suspended in cold 1/15 M phosphate buffer saline (PBS). The cell suspension was put in the opened sterile plate and irradiated by UVA (365 nm) and UVB (275 nm) from UV lamp source (model UVGL-55, UVP Inc., Uplan, CA, USA) of 21 J/m^2 and fixed at the height of 40 cm. The irradiated cell suspension (10^9 cells/ml) was diluted 10 and 10^6 folds with cold PBS for revertant and survivor detection, respectively. Fifty µl of the sample was mixed well with 200 µl of UV- irradiated cells, 500 µl of PBS and 2 ml of molten top agar (50^{o}C), and poured onto semi-enriched minimal agar. The hardened agar plates were incubated at 37^{o}C for 48 h. DMSO in place of the herbal extract and sunscreen lotion in place of the herbal sunscreen lotion were served as the negative controls.

The number of revertants and survivors was counted. Each experiment was done twice and with the duplicate plates.

The antimutagenicity is expressed as the relative mutagenic activity (RMA, % of control) using the following formula.

$$\text{RMA, \% of control} = [(r/s)/(R/S)] \times 100$$

r = averaged revertants in presence of test sample, s = averaged survivors in presence of test sample, R = averaged revertants in absence of test sample and S = averaged survivors in absence of test sample

IC_{50} (the inhibitory concentration needed to achieve an RMA of 50%) and its 95% confidence interval were calculated from a linear regression line derived from points taken from 5 concentrations. An inactive sample was defined when its RMA was not lower than 50% at the highest tested dose or the dose of that observed of 50% survivors. Two experiments with duplicate plates were done for each sample. Statistical analysis was performed using the unpaired student's t-test.

Sun protection factor (SPF) evaluation: The selected herbal sunscreen lotions with the volume of 110 µl were each spread evenly on 7.5 cm X 7.4 cm Transpore$^{C@}$ tape, and detected under SPF-290S spectrophotometer at 290 – 400 nm.

RESULTS

Table 1 showed the yield of the hydroalcoholic extracts from 12 studied herbs. The highest was from ink nut and the lowest was from lotus. Their obtained yields were 32.3 and 4.4%, respectively. The antimutagenic potentials of these extracts towards UVA- and UVB-induced mutations are shown in Figure 1. The appreciable extracts were those from damask rose, emblic, guava and myrobalan. Their IC_{50} shown in Table 2 was 2.6, 2.9, 3.4 and 0.7 mg/plate, respectively towards UVA-induced mutation, and was 2.0, 2.4, 4.0 and 2.3 mg/plate respectively towards UVB-induced mutation. These promising extracts were then each incorporated into sunscreen lotion (SPF 8) and studied for their antimutagenicity towards UV radiations. Table 3 showed the damask rose and myrobalan sunscreen lotions were more appreciable than emblic and guava sunscreen lotions. The IC_{50} of damask rose lotion was 4.3 and 2.5% w/w, and myrobalan lotion was 2.3 and 4.2% w/w towards UVA- and UVB-induced mutations, respectively. These values were not different from their original extracts. The IC_{50} of emblic sunscreen lotion was 7.3 and 7.7% w/w, and guava sunscreen lotion was 18.9 and 13.3 % towards the above two UV-induced mutations respectively. These values were significantly higher than their original extracts ($p < 0.05$). In addition to the antimutagenic potentials, the aforementioned extracts showed the SPF boosting effects. Table 4 revealed the damask rose, emblic, guava and myrobalan sunscreen lotions had higher SPF in a dose response manner than non-herbal sunscreen lotion.

DISCUSSION

Although UV radiation has some benefits, but its negative impact to human health is much more. Skin cancer is one of the serious results. UV is a strong physical mutagen. Its penetration into skin cells is likely to cause gene mutation and this is believed to be the first stage in skin cancer development.[16,17] To cut down this occurrence, one possible way is by antimutagens that have the activity towards UV-induced mutation. A number of herbs and their components have been found to have the antimutagenic potentials with their proposed mechanisms of action.[11-14,18-21] Most of them were studied upon chemical mutagens. The present study aimed to determine the antimutage-

Table1 Appearance and yield of hydroalcoholic extracts from studied herbs.

Herb	Part used	Appearance	Yield (%)*
Asiatic pennywort	Whole plant	Brown	8.37
Cassia	Leaf	Dark green	10.17
Damask rose	Flower	Dark brown	23.94
Emblic	Fruit pulp	Pale green	15.78
Guava	Leaf	Dark brown	13.23
Ink nut	Fruit pulp	Dark red	32.31
Lotus	Stem	Brown	4.39
Myrobalan wood	Fruit pulp	Dark brown	29.75
Orange	Peel	Brown	20.31
Roselle	Flower	Red	20.82
Tea	Leaf	Dark brown	21.50
Tumeric	Root	Dark yellow	22.53

*Yield of dry extract calculated towards ground dried herbs before extraction

nic potentials of herbs towards UV-induced mutagenicity, and the appreciable results were from the hydroalcoholic extracts of damask rose, emblic, guava and myrobalan. There is no report showing the antimutagenic potentials of these extract towards UV-induced mutations.

The potential extracts from this study were then incorporated into sunscreen lotions to see whether they still showed the antimutagencitiy while formulated. The damask rose and myrobalan

Table 2 IC_{50} of herbal extracts towards UVA- and UVB-induced mutations in *E. coli* B/rWP2

Herbal extract	IC50 (mg/plate) towards*	
	UVA	UVB
Asiatic pennywort	3.1	> 6.0
Cassia	2.5	> 6.0
Damask rose	2.6	2.0
Emblic	2.9	2.4
Guava	3.4	4.0
Ink nut	> 6.0	5.2
Lotus	> 6.0	> 6.0
Myrobalan	0.7	2.3
Nutmeg	4.0	> 6.0
Orange	> 6.0	> 6.0
Roselle	> 6.0	> 6.0
Tea	2.7	4.7
Turmeric	4.4	> 6.0

* Survival rate of the tested *E. coli* was over 50% at all tested doses

Table 3 IC_{50} of sunscreen lotions containing antimutagenic herbal extracts towards UVA- and UVB-induced mutations in *E. coli* B/r WP2

Product	IC50 (% w/w) towards*	
	UVA	UVB
Damask rose extract	3.9	3.6
Damask rose sunscreen lotion	4.3	2.5
Emblic extract	4.7**	4.0**
Emblic sunscreen lotion	7.3**	7.7**
Guava extract	6.0**	7.6**
Guava sunscreen lotion	18.9**	13.3**
Myrobalan extract	1.0	3.2
Myrobalan sunscreen lotion	2.3	4.2

* Survival rate of the tested *E. coli* was over 50% at all tested doses
** Significantly different ($p < 0.05$)

lotions revealed the inhibitory effect upon UVA- and UVB-induced mutations similarity with the original extracts. The emblic and guava lotions also showed such inhibitory effects but were found to be significantly lower than their original extracts ($p < 0.05$). Since the sunscreen products with PA and SPF for protection of UVA and UVB, respectively cannot assess the complete protection of UV penetration into skin cells,[5] it is, therefore, more helpful if these products are additionally incorporated with the antimutagenic substances. The damask rose and myrobalan extracts are likely to be the candidates. Besides the antimutagenicity study, the damask rose, emblic, guava and myrobalan lotions were also evaluated for SPF. Interestingly, all these lotions showed higher SPF than those without and in a dose response manner. This would be another benefit of

Figure 1 Antimutagenicity of the hydroalcoholic herbal extracts towards UVA-(●) and UVB-(▲) induced mutations

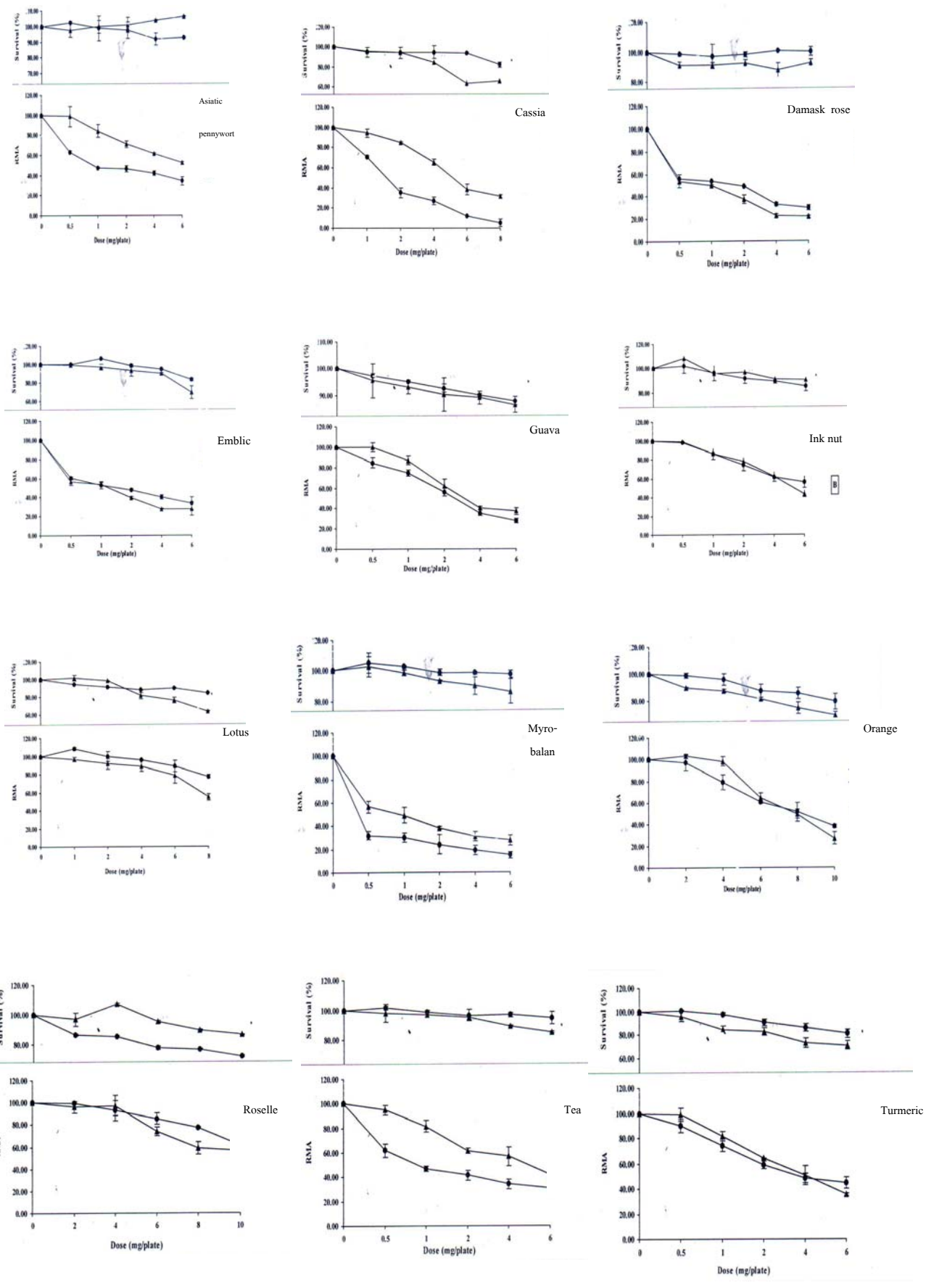


Table 4 SPF values of herbal sunscreen lotions

Sunscreen lotion	Herbal dose (%)	SPF
Non-herbal	-	8.1±0.4
Damask rose	1.25	10.6±0.5
	2.5	11.2±0.5
	5	13.8±0.7
	10	14.5±0.6
Emblic	1.25	9.5±0.4
	2.5	10.3±0.3
	5	12.2±0.7
	10	15.9±0.9
Guava	1.25	10.3±0.8
	2.5	11.3±0.5
	5	12.2±1.2
	10	13.8±0.2
Myrobalan	1.25	10.7±0.4
	2.5	11.3±0.6
	5	13.2±0.5
	10	14.3±0.5

the investigated extracts because of their SPF boosting activity. Our further studies will be aimed to their antioxidant activity which is also important in UV protection. Nevertheless, the related investigations as well as the studies on the physicochemical properties of the extracts for the purpose of use in sunscreen products are unavoidably carried out.

ACKNOWEDGEMENTS

This work was supported by SJI research grant. Thanks go to Ms Wannapa Boonrueng for the technical assistance and Ms Umaporn Somnak for the office work.

REFERENCES

1. Ichihashi M, Ueda M, Budiyanto A, *et al*. UV-induced skin damage. *Toxicology* 2003; 189: 21-39.
2. F'guyer AF, Mukhtar H. Photochemoprevention of skin cancer by botanical agents. *Photodermatol Photoimmunol Photomed* 2003; 19: 56-72.
3. NCI. Common Cancer Types. National Cancer Institute (2007). Available at: http://www.cancer.gov/cancertopics/commoncancers, accessed 2007 Jul 21.
4. Thompson SC, Jolley D, Marks R. Reduction of solar keratoses by regular sunscreen use. *N Engl J Med* 1993; 16: 1147-51.
5. Gasparro FP. Sunscreens, skin photobiology, and skin cancer: the need for UVA protection and evaluation of efficacy. *Environ Health Perspect* 2000; 108 (Suppl)1: 71-8.
6. Vainio H, Bianchini F. Cancer-preventive effects of sunscreens are uncertain. *Scan J Work Environ and Health* 2000; 26:529-31.
7. Westerdahl J, Ingvar C, Masback A, *et al*. Sunscreen use and malignant melanoma. *Int J Cancer* 2000; 87: 145-50.
8. Ananthaswamy HN, Fourtanier A, Evans RL, *et al*. p53 Mutations in hairless 39SKH-hr1 mouse skin tumors induced by a solar simulator. *Photochem Photobiol* 1998; 67: 227-32.
9. deGruijl FR. P53 mutations as a marker of skin cancer risk: comparison of UVA and UVB effects. *Exp Dermatol* 2002; 11(S1): 37-9.
10. Chun KS, Keum YS, Han SS, *et al*. Curcumin inhibits phorbol ester-induced expression of cyclooxygenase-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-kappa B activation. *Carcinogenesis* 2003; 24: 1515-24.
11. Grover IS, Bala S. Studies on antimutagenic effects of guava (*Psidium guajava*) in *Salmonella typhimurium*. *Mutat Res* 1993; 300:1-3.
12. Kaur S, Grover IS, Singh M, *et al.* Antimutagenicity of hydrozable tannins from *Terminalia chebula* in *Salmonella typhimurium*. *Mutat Res* 1998; 419: 169-79.

13. Lin JK, Liang YC. Cancer chemoprevention by tea polyphenols. *Proc Natl Sci Counc* 2000; 24: 1-13.
14. Miyazawa M, Okuno Y, Fukuyama M, *et al.* Antimutagenic activity of polymethoxyflavanoids from *Citrus aurantium*. *J Agric Food Chem* 1999; 47: 5239-44.
15. Shimoi K, Nakamura Y, Tomita I, *et al*. Bioantimutagenic in the homogenates of *Cruciferae* and *Liliaceae* vegetables. *Biosci Biotechnol Biochem* 1996; 60: 1439-43.
16. Ichihashi M, Ueda M, Budiyanto A, *et al*. UV-induced skin damage. Toxicology 1993; 89: 21-39.
17. Matsumura Y, Ananthaswamy HN. Toxic effects of ultraviolet radiation on the skin. *Toxicol Appl Pharmacol* 2004; 195: 298-308.
18. Kada T, Shimon K. Desmutagens and bioantimutagens - their modes of action. *Bioassays* 1987; 7:113-6.
19. Kaur S, Arora S, Kaur K, *et al.* The in vitro antimutagenic activity of Triphala - an Indian herbal drug. *Food Chem Toxicol* 2002; 40: 527-34.
20. Kinae N, Masuda S. Studies on antimutagenesis (a review). *Environ Mutagen Res* 2002; 129-44.
21. Matsuo T, Hanamura N, Shimoi K, *et al.* Identification of (+) – gallocatechin as a bioantimutagenic compound in *Psidium guava* leaves. *Phytochemistry* 1994; 36:1027-9.

ศักยภาพของสารสกัดสมุนไพรในการต้านการก่อกลายพันธุ์ที่เหนี่ยวนำโดยรังสียูวี

พรนรี กิตติวรรณโชติ, พนิดา บริสุทธิ์, พิมผกา วนสวัสดิ์, ลักขณา ผลพานิชย์, อรทัย รัตนสุข และมาลิน จุลศิริ*

ส่วนวิจัยและพัฒนา, บมจ. เอส แอนด์ เจ อินเตอร์เนชั่นแนล เอนเตอร์ไพรส์, กรุงเทพมหานคร

บทคัดย่อ

พืชสมุนไพรจำนวน 12 ชนิดที่มีศักยภาพต้านสารก่อกลายพันธุ์เคมี ได้รับการคัดเลือกสำหรับการศึกษา โดยนำส่วนของพืชสมุนไพรดังกล่าวมาทำแห้ง บด และสกัดด้วยน้ำ-แอลกอฮอล์ จากนั้น นำสารสกัดที่ได้ทดสอบฤทธิ์ต้านการก่อกลายพันธุ์ที่เกิดจากการเหนี่ยวนำของรังสียูวีเอและยูวีบี โดยใช้วิธี Ames ที่ปรับปรุงเล็กน้อย และมี *Escherichia coli* B/r WP2 (*trpE65*) เป็นเชื้อทดสอบ ผลปรากฏว่าดอกกุหลาบมอญ (*Rosa damascena* Mill) ผลมะขามป้อม (*Phyllanthus emblica* Linn) ใบฝรั่ง (*Psidium guajava* Linn) และผลสมอไทย (*Terminalia chebula* Retz) ให้ฤทธิ์ที่ค่อนข้างน่าสนใจ โดยค่า IC_{50} ต่อการเหนี่ยวนำของทั้งรังสียูวีเอ และยูวีบี น้อยกว่า 4 มก./plate หลังจากนั้น ได้นำสารสกัดเหล่านี้แยกกันใส่ในโลชั่นกันแดดที่มี SPF 8 ซึ่งพบว่าสารกันแดดกุหลาบมอญ และสมอไทย ให้ผลต้านการก่อกลายพันธุ์ดีกว่าสารกันแดดมะขามป้อม และใบฝรั่ง ค่า IC_{50} โลชั่นที่มีสารสกัดกุหลาบมอญและสมอไทยอยู่ที่ 4.3% และ 2.3% น้ำหนัก/น้ำหนัก ตามลำดับ ต่อการเหนี่ยวนำการก่อกลายพันธุ์ของรังสียูวีเอ, และ 2.5% and 4.2 9% น้ำหนัก/น้ำหนัก ตามลำดับ ต่อการเหนี่ยวนำการก่อกลายพันธุ์ของรังสียูวีบี ขณะที่ค่า IC_{50} ของโลชั่นที่มีสารสกัดมะขามป้อมและใบฝรั่งมากกว่า 7% น้ำหนัก/น้ำหนัก ทั้งมีความแตกต่างโดยสูงกว่าค่า IC_{50} ของสารสกัด เดี่ยว ($p < 0.05$) ท้ายที่สุด ได้นำโลชั่นกันแดดที่มีและไม่มีสารสกัดเหล่านี้มาวัดค่า SPF พบว่าโลชั่นที่มีสารสกัดทั้งหมด มีค่า SPF สูงขึ้น (SPF > 8)

คำสำคัญ: กุหลาบมอญ, ผลมะขามป้อม,ใบฝรั่ง, ผลสมอไทย, ฤทธิ์ต้านการ ก่อกลายพันธุ์

* Corresponding author: มาลิน จุลศิริ
บมจ. เอส แอนด์ เจ อินเตอร์เนชั่นแนล เอ็นเตอร์ไพรส์
115 ถ.นราธิวาสราชนครินทร์ (ซ. 10)
เขตสาธร กรุงเทพฯ 10120
E-mail: malyn@snjinter.com