SCREENING OF THAI MEDICINAL PLANTS FOR FREE RADICAL SCAVENGING AND DNA PROTECTIVE PROPERTIES

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ABSTRACT: Twenty-one methanolic extracts prepared from 20 plant species were examined for their free radical scavenging activity and DNA protective property. Assays for free radical scavenging activity were conducted using two chemical species, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and superoxide anion. Evaluation for DNA protective activity was performed on pBR322 plasmid DNA in the presence of photosensitized riboflavin. Trolox was used as the positive control in all experiments. At the concentration of 100 μg/mL, ten plant extracts showed higher than 50 % inhibition against DPPH and superoxide radicals, with the bark extract of *Peltophorum dasyrachis* being the most potent sample. When these 10 plant extracts were further tested for their inhibitory activity against DNA strand breakage, nine exhibited greater than 70% inhibition, and were then selected for IC_{50} determination. The extract prepared from *Artocarpus gomezianus* leaves exhibited the most promising DNA protective activity, with a potency about 14-fold greater than that of Trolox. The fruit extract of *Cochlospermum religiosum* and the bark extract of *Peltophorum dasyrachis* were also interesting, showing strong inhibitory effects on DNA breakage.

Keywords: antioxidant, DNA damage, DNA protective property, DPPH, NBT

INTRODUCTION

Free radicals are reactive chemical species (RCS) known to be able to cause oxidative reactions of biomolecules, including lipids, proteins and DNA [1, 2]. Some radicals are endogenously generated along multiple physiological processes in living cells. Exogenous radicals, which are produced by irradiation, environment, pollutions and xenobiotics, can be introduced into living organisms at ease. Radicals from either endogenous or exogenous route participate in the oxidation of biomolecules in living organisms, leading to numerous pathological consequences. Recently, research interests on defective human health have been focused on how to protect DNA from the deteriorative effects induced by oxidative reactions. There are a number of studies reporting DNA impairment as one of the contributors to cell death, which, as a consequence, results in apoptosis, aging and several degenerative diseases [3-5].

Phytochemicals widely present in nature are sources of new bioactive compounds. Several investigations have reported natural substances from various plants for their antioxidative and DNA protective activities [6-10]. It is likely to find new bioactive compounds with free radical scavenging and/or DNA protective properties from Thai plants, due to the high level of biological diversity in Thailand. In this investigation, we studied twenty-one plant extracts for their free radical scavenging activity using the DPPH and NBT assays. In addition, the ability of these plant extracts to prevent the damage on pBR322 plasmid DNA induced by photosensitized riboflavin was examined.

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MATERIALS AND METHODS

Plant material and chemicals

Plant materials were purchased from Chatuchak plant market or collected from the natural habitat (Table 1). Voucher specimens are deposited at the Pharmacognosy and Pharmaceutical Botany Department, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. Trolox (Sigma-Aldrich,USA), pBR322 plasmid DNA (New England Biolabs, USA) and riboflavin (Fluka analytica) were commercially obtained. All other chemicals and solvents were of analytical grade.

Preparation of medicinal plant extracts for bioactivity assays

Plant materials were dried at the temperature not higher than 60°C. Each plant material was powdered and then extracted with methanol to give a methanol extract after removal of the organic solvent. For the DPPH radical assay, each plant extract was dissolved in methanol to give a sample solution with the concentration of 1 mg/mL. For the superoxide radical assay and analysis of DNA damage, a sample solution (0.5 mg/mL in methanol) was

Table 1 List of Thai medicinal plants evaluated for free radical scavenging activity and DNA protective property

Sample No.	Scientific name	Thai name	Family	Part used	Collection site (cs) or location of market (lm)
1	*Abutilon indicum* Sweet	Khrop chak krawan	Malvaceae	heartwood	cs: Phuket
2	Artocarpus gomezianus Wall. ex Trec.	Hat nun	Moraceae	leaf	cs: Trang
3	*Cajanus cajan* Millsp.	Ma hae	Leguminosae	bark	cs: Phrae
4	*Cardiospermum halicacabum* Linn.	Khok kra om	Sapindaceae	whole plant	cs: Suphanburi
5	*Cochlospermum religiosum* Alston	Fai kham	Bixaceae	fruit	cs: Chachoengsao
6	*Dalbergia cultrata* Grah. ex Benth.	Kra phi khao khwai	Leguminosae	bark	cs: Phrae
7	*Dalbergia oliveri* Gamble	Chingchan	Leguminosae	heartwood	cs: Phrae
8	*Dendrobium aphyllum* C.E.C. Fischer	Ueang sai nok kra chip	Orchidaceae	leaf	lm: Bangkok
9	*Dendrobium crepidatum* Lindl. & Paxt.	Ueang sai nam khiao	Orchidaceae	whole plant	lm: Bangkok
10	*Dendrobium densiflorum* Wall.	Ueang mon khai	Orchidaceae	stem	lm: Bangkok
11	*Dendrobium findlayanum* Par. & Reichb. f.	Phuang yok	Orchidaceae	stem	lm: Bangkok
12	*Dendrobium pendulum* Roxb.	Ueang mai thao ruesi	Orchidaceae	stem	lm: Bangkok
13	*Hura crepitans* Linn.	Pho farang	Euphorbiaceae	heartwood	cs: Bangkok
14	*Macaranga tanarius* Muell. Arg.	Lo bai leun	Euphorbiaceae	leaf	cs: Phuket
15	*Michelia figo* Spreng.	Champi khaek	Magnoliaceae	leaf	cs: Bangkok
16	*Millettia erythrocalyx* Gagnep.	Chan	Leguminosae	heartwood	cs: Phetchaburi
17	*Millettia erythrocalyx* Gagnep.	Chan	Leguminosae	pod	cs: Phetchaburi
18	*Peltophorum dasyrachis* Kurz	A rang	Leguminosae	bark	cs: Phrae
19	*Schleichera oleosa* Merr.	Ta khro	Sapindaceae	leaf	cs: Bangkok
20	*Solanum torvum* Sw.	Ma khuea phuang	Solanaceae	leaf	cs: Suphanburi
21	*Tribulus cistoides* Linn.	Khok krasun	Zygophyllaceae	whole plant	cs: Suphanburi

DPPH radical assay

In each reaction, 20 μL of the sample solution was mixed with 180 μL of 50 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) methanolic solution at room temperature for 30 minutes. Thus, the final concentration of the plant extract in the reaction mixture was 100 μg/mL. Methanol was used as a control. The reduction of the DPPH, a purple-colored stable free radical, was measured by reading the absorbance at 510 nm (Victor^3 multilabel counter, Perkin Elmer). DPPH is reduced to the yellow-colored diphenylpicrylhydrazine when antioxidants are present. Trolox was used as a positive control. The inhibition ratio (percent) was calculated by the equation:

$$\% \text{ inhibition} = [A - (B - C)] \times 100 / A,$$

where *A* is the absorbance of the control reaction; *B* is the absorbance of the sample reaction; *C* is the absorbance of the sample control reaction (without DPPH solution).

Superoxide radical assay

The superoxide anion radical scavenging activity was evaluated using a previously developed method [11]. In brief, superoxide radicals were generated in the mixture (200 μL) of 50 mM potassium phosphate buffer (20 μL) 266 μM riboflavin (100 μL), 1 mM EDTA (20 μL), 750 μM NBT (20 μL) and sample solution (40 μL). Thus, the final concentration of the plant extract in the reaction mixture was 100 μg/mL. The production of blue formazan from the color reaction of superoxide radicals and NBT was measured at 570 nm after a 10-min fluorescent lamp exposure. A closed box lined with aluminium foil was used for the entire reaction. A similar reaction mixture was kept in the dark and served as the blank. Trolox was used as a positive control. The inhibition ratio (percent) was calculated by the equation:

$$\% \text{ inhibition} = [(A - B) - (C - D)] \times 100 / (A - B),$$

where *A* is absorbance of the control reaction in light condition; *B* is absorbance of the control reaction in dark condition; *C* is absorbance of the sample reaction in light condition; *D* is absorbance of the sample control in dark condition.

Generation of DNA damage by photosensitized riboflavin and analysis of DNA damage

The experiment was performed according to an established method [11]. Each sample solution was prepared by dissolving the test substance in a solution of 30% MeOH in potassium phosphate buffer to give a sample solution with the initial concentration of 500 μg/mL. For IC_{50} determination, the serial dilution method was employed to give a set of sample solutions with appropriate concentrations. Each reaction mixture (10 μl) contained 2 μl of the sample solution, mixed with 1 μl of 50 mM potassium phosphate buffer, 5 μl of 265.6 μM riboflavin, 1 μl of 1 mM EDTA and 1 μl of 100 ng/μL pBR322 plasmid DNA. The mixture was then irradiated with a fluorescent lamp in a box lined with aluminium foil. An identical reaction mixture was kept in the dark as blank. After 30 min, the incubated mixture was treated with 2 μl of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose in water), and 12 μl of the reaction mixture was then loaded onto a 0.7% agarose gel. Gel electrophoresis was performed at 100 V in a Tris-acetic acid-EDTA buffer. After electrophoresis, the gel was stained with ethidium bromide (0.5 μg/ml in deionized distilled water). Images of the gels were taken using MiniBIS Gel Documentation under ultraviolet light and analyzed using Gel Quant Analysis (DNR BioImaging Systems, Jerusalem, Israel). All experiments were run in triplicate. Trolox was used as a positive control. The inhibitory effect of each sample was calculated by the following equation:

$$\% \text{ inhibition} = [A - (B - C)] \times 100 / A,$$

where *A* is intensity of the SC fraction of the control in dark condition; *B* is intensity of the SC fraction of the sample in dark condition; *C* is intensity of the SC fraction of the sample in light condition; IC_{50} represented the concentration of a sample where 50% inhibition was observed.

RESULTS

Free radical scavenging activity

In this study, 21 plant extracts prepared from 20 plant species (Table 1) were investigated for their free radical scavenging potential. Each of the extracts was tested at the concentration of 100 μg/mL against DPPH and superoxide radicals, and the results are shown in Table 2. For the DPPH radical, a wide range of activity (26-97 % inhibition) was observed for these plant extracts. Similar results were also obtained for their inhibitory activity against the superoxide radical, with 18 – 96 % inhibition. Among the samples tested, the extract of the bark of *Peltophorum dasyrachis* exhibited the most potent inhibitory activity, showing more than 95 % inhibition against both types of free radical, whereas the positive control Trolox showed approximately 96 and 71 % inhibition against DPPH and superoxide at the concentration of 25.03 and 62.57 μg/ml, respectively.

DNA protective effect

Each of the 21 plant extracts was subjected to DNA protective effect evaluation using a recently reported DNA nicking assay [11]. The assay was based on the DNA strand breakage generated by the photosensitized chemical reaction of riboflavin. The electrophoregram (Figure 1) shows the DNA protective effect of Trolox and plant extracts on DNA strand scission. DNA was subjected to the damage, and exhibited in open circular form by

Table 2 Free radical scavenging activity and DNA protective property of selected Thai medicinal plants

Sample No.	Scientific name	% Inhibition of DPPH free radical[a]	% Inhibition of superoxide[a]	% Inhibition of DNA strand breakage[a]	IC_{50} for DNA strand breakage (µg/mL)
1	*Abutilon indicum*	41.68 ± 3.29	20.81 ± 3.04	54.77 ± 1.05	ND[e]
2	*Artocarpus gomezianus*	56.44 ± 0.40	64.35 ± 2.16	87.17 ± 0.46	7.31 ± 0.03
3	*Cajanus cajan*	38.32 ± 2.41	15.64 ± 3.41	47.33 ± 0.74	ND
4	*Cardiospermum halicacabum*	33.91 ± 1.62	13.70 ± 4.66	30.02 ± 1.52	ND
5	*Cochlospermum religiosum*	96.02 ± 0.60	69.07 ± 5.52	76.69 ± 1.67	9.47 ±1.06
6	*Dalbergia cultrata*	91.95 ± 2.51	80.23 ± 3.07	90.47 ± 1.22	11.37 ± 0.18
7	*Dalbergia oliveri*	77.13 ± 1.31	56.40 ± 3.19	81.72 ± 1.36	47.07 ± 1.97
8	*Dendrobium aphyllum*	75.04 ± 2.65	50.13 ± 4.58	69.51 ± 0.84	48.52 ± 0.80
9	*Dendrobium crepidatum*	52.47 ± 1.35	21.70 ± 1.98	2.27 ± 1.55	ND
10	*Dendrobium densiflorum*	58.00 ± 1.12	52.73 ± 3.20	70.17 ± 1.71	67.74 ± 1.73
11	*Dendrobium findlayanum*	42.59 ± 2.55	41.25 ± 3.08	19.40 ± 0.49	ND
12	*Dendrobium pendulum*	42.33 ± 1.69	32.97 ± 1.39	59.08 ± 1.85	ND
13	*Hura crepitans*	25.77 ± 1.11	17.76 ± 5.90	61.63 ± 2.00	ND
14	*Macaranga tanarius*	96.46 ± 2.72	89.26 ± 1.93	91.48 ± 2.43	20.90 ± 0.59
15	*Michelia figo*	94.65 ± 1.02	80.10 ± 4.68	84.58 ± 0.98	12.11 ± 0.19
16	*Millettia erythrocalyx*	47.05 ± 6.13	37.17 ± 1.69	65.95 ± 0.89	ND
17	*Millettia erythrocalyx*	36.30 ± 1.61	15.86 ± 3.78	35.26 ± 0.99	ND
18	*Peltophorum dasyrachis*	96.65 ± 1.41	95.50 ± 2.44	90.91 ± 3.29	10.37 ± 0.39
19	*Schleichera oleosa*	75.54 ± 4.68	36.28 ± 4.26	88.12 ± 2.22	ND
20	*Solanum torvum*	80.20 ± 3.86	20.97 ± 4.70	66.04 ± 4.24	ND
21	*Tribulus cistoides*	61.74 ± 1.49	57.07 ± 2.70	36.21 ± 1.78	ND
	Trolox	96.42 ± 0.72[b]	71.48 ± 5.51[c]	84.93 ± 1.29[d]	105.54 ± 1.16

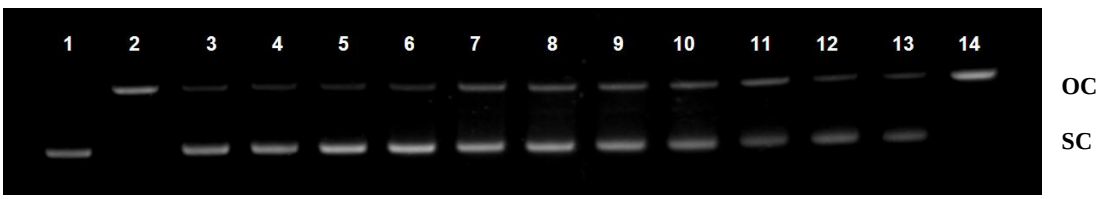
[a]At concentration of 100 µg/mL; [b]at concentration of 25.03 µg/ mL; [c]at concentration of 62.57 µg/ mL; [d]at concentration of 150 µg/mL; [e]ND = not determined

Figure legend

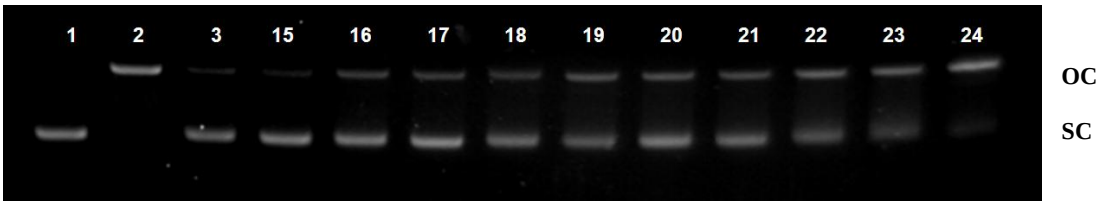
Figure 1 DNA protective effect of methanolic extracts against plasmid DNA breakage induced by superoxide from photosensitized riboflavin. (A. and B.) pBR322 DNA (100 ng) was incubated with the superoxide generation reaction for 30 min with the following: Lane 1, DNA alone under dark condition; Lane 2, DNA alone under light condition; Lane 3, 150 µg/mL Trolox; Lane 4-24, 100 µg/mL methanolic plant extracts as follows: Lane 4, *Macaranga tanarius* (No. 14); Lane 5, *Dalbergia cultrate* (No. 6); Lane 6, *Artocarpus gomezianus* (No. 2); Lane 7, *Dalbergia oliveri* (No. 7); Lane 8, *Dendrobium densiflorum* (No. 10); Lane 9, *Solanum torvum* (No. 20); Lane 10, *Hura crepitans* (No. 13); Lane 11, *Abutilon indicum* (No. 1); Lane 12, *Tribulus cistoides* (No. 21); Lane 13, *Cardiospermum halicacabum* (No. 4); Lane 14, *Dendrobium crepidatum* (No. 8); Lane 15, *Peltophorum dasyrachis* (No. 18); Lane 16, *Dendrobium aphyllum* (No. 19); Lane 17, *Michelia figo* (No. 15); Lane 18, *Cochlospermum religiosum* (No. 5); Lane 19, *Schleichera oleosa* (No. 19); Lane 20, *Millettia erythrocalyx* (No. 16); Lane 21, *Dendrobium pendulum* (No. 12); Lane 22, *Cajanus cajan* (No. 3); Lane 23, *Millettia erythrocalyx* (No. 17); Lane 24, *Dendrobium findlayanum* (No. 11)**.** OC stands for open circular form or nicked DNA. SC stands for close circular or supercoiled DNA.

Figure 1

A.



B.



photosensitized riboflavin under light condition whereas it was intact and displayed in close circular form under dark condition (Lanes 1 and 2). In the presence of 150 μg/mL Trolox (Lane 3), DNA nicking was found to be inhibited by 84.93 ± 1.29 % (Table 2). Lanes 4 – 24 were obtained from the 21 plant extracts. It appears that the leaf extract of *Macaranga tanarius* possessed potent inhibitory activity on DNA strand breakage, with about 91 % inhibition. The positive control Trolox at the concentration of 150 μg/mL exhibited relatively high inhibitory activity on DNA strand breakage, with 84.93 ± 1.29 % inhibition.

DNA protective potency of the examined Thai plants

Plant extracts exhibiting greater than 70 %inhibition on DNA strand breakage and greater than 50 % inhibition on either DPPH free radical or superoxide anion were selected for subsequent investigation for their DNA protective potency (Table 2). The leaf extract of *Artocarpus gomezianus* was found to be the most potent sample (IC_{50} 7.31 ± 0.03 μg/mL), followed by the fruit extract of *Cochlospermum religiosum* (IC_{50} 9.47 ± 1.06 μg/mL) and the bark extract of *Peltophorum dasyrachis* (IC_{50} 10.37 ± 0.39 μg/mL). The positive control Trolox was not effective in this assay, showing an IC_{50} value of 105.54 ± 1.16 μg/mL.

DISCUSSION

In this study, screening for antioxidative activity of some plant extracts was carried out using DPPH and superoxide radicals as the substrates. Most of the samples showed parallel results. Unexpectedly, the leaf extract of *Solanum torvum* seemed to have much higher activity against DPPH than superoxide. This finding implies that constituents of this plant extract scavenged radicals mostly through a direct electron-transfer reaction. Previous studies reported the presence of alkaloids, steroid sapogenins, flvanoids and long-chain hydrocarbons in the leaves and aerial parts of *S. torvum* [12-14]. It is likely that the saponins and flavonoids from this plant may contribute to the direct free radical scavenging reaction since results from several research groups demonstrated their anti-DPPH activity [15-18].

There is much evidence illustrating that DNA damage is caused by the presence of various free radical species. In our study, a positive correlation was observed between the DNA protective property and anti-radical activity. For the plant extracts showing 70% inhibition on DNA scission and 50% inhibition on either DPPH or superoxide radical, we further analyzed their inhibitory potency on DNA strand breakage in terms of IC_{50} values. Our results showed that each of these plant extracts inhibited the DNA strand breakage in a dose-dependent manner.

From Table 2, it should be noted that the leaf extract of *Artocarpus gomezianus* showed potent DNA protective activity (IC_{50} 7.31 μg/mL) despite its moderate inhibitory efffects on the free radicals (56.44 and 64.35 % inhibition against DPPH and superoxide anion, respectively). Flavonoids and stilbenes were reported from the heartwood, bark and roots of *A. gomezianus* [19-21], whereas triterpenoids were present in the leaves [22]. Based on these data, it might be hypothesized that the triterpenoids in the leaf extract of *A. gomezianus* could prevent the DNA scission possibly through the shielding effect, but not through the antioxidative activity.

Some plant extracts appeared to show their DNA protective potential through their radial scavenging properties. For example, the bark extract of *Peltophorum dasyrachis*, the bark extract of *Dalbergia cultrata*, the leaf extract of *Michelia figo* and the leaf extract of *Macaranga tanarius*, each at the concentration of 100 μg/mL, exhibited high levels of inhibitory activity against DNA breakage (IC_{50} of 10.37, 11.37, 12.11 and 20.90 μg/mL, respectively), in accordance with their potent scavenging effects on DPPH and superoxide free radicals. A number of biological activities were reported for these plants [23-33]. It appears that the flavonoids from *P. dasyrachis* bark [24] and tannins from *M. tanrius* leaves [33] might be responsible for the free radial scavenging activity. Nonetheless, the DNA protective potential of these plants and their chemical constituents has not yet been reported.

In conclusion, the screening of 21 methanolic extracts prepared from 20 Thai plants *in vitro* was conducted aiming to discover extracts with potential anti-free radical activities as well as DNA protective activity. The leaf extract of *Artocarpus gomezianus*, the fruit extract of *Cochlospermum religiosum* and the bark extract of *Peltophorum dasyrachis* are recommended for more detailed examination. Further investigations should be carried out to identify the active constituent(s).

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REFERENCES

1. Sies H. Oxidative Stress: Oxidants and Antioxidants. New York: Academic Press; 1991.
2. Martinez GR, Loureiro APM, Marques SA, Miyamoto S, Yamaguchi LF, Onuki J, et al. Oxidative and alkylating damage in DNA. Mutat Res. 2003; 544: 115-27.
3. Wang JYJ. DNA damage and apoptosis. Cell Death Differ. 2001; 8: 1047-8.
4. Roos WP, Kaina B. DNA damage-induced cell death by apoptosis. Trends Mol Med. 2006; 12(9): 440-50.
5. Helbock HJ, Beckman KB, Ames BN. 8-Hydroxydeoxyguanosine and 8-hydroxyguanine, as a marker odd cellular oxidative stress during carcinogenesis. Meth Enzymol. 1999; 300: 156-66.
6. Gandhi NM, Maruya DK, Salvi V, Kapoor S, Mukherjee T, Nair CKK. Radioprotection of DNA by glycyrrhizic acid through scavenging free radicals. J Radiat Res. 2004; 45: 461-8.
7. Bhattacharya S, Subramanian M, Roychowdhury S, Bauri AK, Kamat JP, Chattopadhyay S, Bandyopadhyay SK. Radioprotective property of the ethanolic extract of *Piper betel* leaf. J Radiat Res. 2005; 46: 165-71.
8. Yamaguchi L, Vassão DG, Kato MJ, Mascio PD. Bioflavonoids from Brazilian pine *Araucaria angustifolia* as potentials protective agents against DNA damage and lipoperoxidation. Phytochemistry. 2005; 66: 2238-47.
9. Fuentes JL, Vernhe M, Cuetara EB, Sănchez-Lamar A, Santana JL, Llagostera M. Tannins from barks of *Pinus caribaea* protect *Escherichia coli* cells against DNA damage by γ-rays. Fitoterapia. 2006; 77: 116-20.
10. Lodovici M, Menichetti S, Viglianisi C, Caldini S, Giuliani E. Polyhydroxylated 4-thiaflavans as multipotent antioxidants: protective effect on oxidative DNA damage in vitro. Bioorg Med Chem Letters. 2006; 16: 1957-60.
11. Chatsumpun M, Chuanasa T, Sritularak B, Likhitwitayawuid K. Oxyresveratrol protects against DNA damage induced by photosensitized riboflavin. Nat Prod Commun. 2010 Nov; 6(1): 41-4.
12. Mahmood U, Shukla YN, Thakur RS. Non-alkaloidal constituents from *Solanum torvum* leaves. Phytochemistry. 1983; 22(1): 167-9.
13. Lu Y, Luo J, Huang X, Kong L. Four new steroidal glycosides from *Solanum torvum* and their cytotoxic activities. Steroids. 2009; 74: 95-101.
14. Yuan-Yuan L, Jian-Guang L, Ling-Yi1 K. Chemical constituents from *Solanum torvum*. Chinese J Nat Med. 2011; 9(1): 30-2.
15. Bigoniya P, Rana AC. Radioprotective and *in-vitro* cytotoxic sapogenin from *Euphorbia neriifolia* (Euphorbiaceae) leaf. Trop J Pharm Res. 2009 Dec; 8(6): 521-30.
16. Woźniak D, Lamer-Zarawska E, Matkowski A. Antimutagenic and antiradical properties of flavones from the roots of *Scutellaria baicalensis* Georgi. Nahrung. 2004 Feb; 48(1): 9-12.
17. Saija A, Scalese M, Lanza M, Marzullo D, Bonina F, Castellif. Flavonoids as antioxidant agents: Importance of their interaction with biomembranes. Free Radical Bio Med. 1995; 19(4): 481-6.
18. Bochoráková H, Paulová H, Slanina J, Musil P, Táborská E. Main flavonoids in the root of *Scutellaria baicalensis* cultivated in Europe and their comparative antiradical properties. Phytother Res. 2003 Jun; 17(6): 640-4.
19. Likhitwitayawuid K, Sritularak B. A new dimeric stilbene with tyrosinase inhibitiory activity from *Artocarpus gomezianus*. J Nat Prod. 2001 Nov; 64(11):1457-9.
20. Likhitwitayawuid K, Chaiwiriya S, Sritularak B, Lipipun V. Antiherpetic flavones from the heartwood of *Artocarpus gomezianus*. Chem Biodivers. 2006; 3: 1138-43.
21. Sritularak B, Tantituvanont A, Chanvorachote P, Meksawan K,Miyamoto T, Kohno Y, Likhitwitayawuid K. Flavonoids with free radical scavenging activity and nitric oxide inhibitory effect from the stem bark of *Artocarpus gomezianus*. J Med Plants Res. 2010 Mar; 4(5): 387-92.
22. Kingroungpet K, Phadungcharoen T, Ruangrungsi N, Sekine T. Constituents of *Artocarpus gomezianus* leaves. Thai J. Pharm. Sci. 1996; 20(1): 59-62.
23. Fujiwara M, Yago N, Miyazawa M. Acetylcholinesterase inhibitory activity of volatile oil from *Peltophorum dasyrachis* Kurz ex Bakar (yellow batai) and bisabolane-type sesquiterpenoids. J Agri Food Chem. 2010; 58: 2824-9.
24. Fujiwara M, Yagi N, Miyazawa M. Tyrosinase inhibitory constituents from the bark of *Peltophorum dasyrachis* (yellow batai). Nat Prod Res. 2011; DOI: 10.1080/14786410903313106.
25. Chihiro Ito C, Itoigawa M, Kanematsu T, Ruangrungsi N, Higashihara H, Tokuda H, Nishino H, Furukawa H. New cinnamylphenols from *Dalbergia* Species with cancer Chemopreventive activity. J Nat Prod. 2003; 66: 1574-7.
26. Takahashi M, Fuchino H, Satake M, Gatsuma Y, Sekita S. *In vitro* screening of leishmanicidal activity in Myanmar timber extracts. Biol Pharm Bull. 2004; 27(6): 921-5.
27. Phrutivorapongkul A, Ichino C, Ruangrungsi N, Ishiyama A, Sekiguchi H, Namatame M, et al. Anti-plasmodial activity of bisbenzylisoquinoline alkaloids from *Michelia figo* leaves. Thai J Health Res. 2006; 20(2): 121-9.
28. Tucker SC. Wound regeneration in the lamina of magnoliaceous leaves. Can J Bot. 1975; 53: 1352-64.
29. Chericoni S, Testai L, Campeol E, Calderone V, Morelli I, Martinotti E. Vasodilator activity of *Michelia figo* Spreng. (Magnoliaceae) by *in vitro* functional study. J Ethnopharmacol. 2004; 91: 263-6.
30. Kawakami S, Harinantenaina L, Matsunami K, Otsuka H, Shinzato T, Takeda Y. Macaflavanones A-G, prenylated flavanones from the leaves of *Macaranga tanarius*. J Nat Prod. 2008, 71: 1872-6.
31. Phommart S, Sutthivaiyakit P, Chimnoi N, Ruchirawat S, Sutthivaiyakit S. Constituents of the leaves of *Macaranga tanarius*. J Nat Prod. 2005; 68: 927-30.
32. Tseng M, Chou C, Chen Y, Kuo Y. Allelopathic prenylflavanones from the fallen leaves of Macaranga tanarius. J Nat Prod. 2001; 64: 827-88.
33. Gunawan-Puteri MDPT, Kawabata J. Novel α-glucosidase inhibitors from *Macaranga tanarius* leaves. Food Chem. 2010; 123: 384-9.