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Full Length Research Paper

Phytochemical screening, antioxidant and antimutagenic activities of selected Thai edible plant extracts

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Antioxidant and antimutagenic activities of plant extracts possess great potential as functional foods for cancer prevention. The aims of the current study were to evaluate antioxidant and antimutagenic activities and to study the chemical constituents of five Thai edible plant extracts. Antioxidant activity was expressed as the ability of each extract to scavenge the free radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH). Antimutagenic activity was evaluated with the Ames test using *Salmonella typhimurium* strains TA 98, and TA 100. The results showed that *Oroxylum indicum* (Linn.) Kurg. and *Tiliacora triandra* Diels. extracts had high antioxidant activity with the EC_{50} values at concentrations of 12.69 ± 1.02 and 14.51 ± 0.67 μg/ml, respectively, whereas *Basella alba* Linn. extract had the strongest antimutagenicity with both strains of *S. typhimurium* with percentage of inhibition values ranging from 54.06 ± 1.52 to 86.08 ± 2.78 %. In the present study, flavonoids and phenolic compounds from the herbal extracts are proposed to be antioxidant and antimutagenic agents, respectively. The apparent antioxidant and antimutagenic activities of Thai edible plants further suggests their potential usefulness in cancer prevention.

Key words: Phytochemical screening, antioxidant, antimutagenic.

INTRODUCTION

Several studies provide strong evidence that vegetables, fruits and phytochemicals protect against some cancers (Lako et al., 2007; Geoffrey et al., 2010). Many antioxidative agents and antimutagens have been identified as anticarcinogens (Ames, 1983). Therefore, the regular intake of plants possessing antimutagenic or antioxidative agents can reduce genotoxic effects of mutagenic and carcinogenic factors (Ikken et al., 1999). Thai people consume many plants as part of their diet. Therefore, it is of interest to know whether traditional edible plants have antioxidant and antimutagenic activities. If they did then they could be useful in dietary supplement development with the herbal extracts as agents for cancer prevention. The aims of this investigation were to test the antioxidant and anti mutagenic activities and to screen the chemical constituents of selected Thai edible plant extracts.

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EXPERIMENTAL METHOD

Plant materials

Oroxylum indicum (Linn.) Kurg (fruits), *Tiliacora triandra* Diels. (leaves), *Morinda citrifolia* Linn. (leaves), *Basella alba* Linn.(entire plant) and *Sauropus androgyrus* (L.) Merrill (leaves) obtained from Maha Sarakham province and identified by one of the authors (Dr. Phadungkit). Voucher specimens have been deposited in the Herbarium at the Faculty of Pharmacy, Mahasarakham University, Thailand. Each plant was extracted with 95% ethanol by the maceration method. The macerates were evaporated to dryness in a rotary evaporator and each crude herbal extract was kept at 4°C.

Phytochemical screening

The solutions of each plant extract were freshly prepared from the crude extract described previously. They were analyzed for the

Table 1. Phytochemical screening of the herbal extracts.

Sciencetific name	Alkaloids	Condensed tannins	Phenolic compounds	Triterpenes	Steroids	Flavonoids	Saponins	Antraquinones
Oroxylum indicum	+	-	+	-	+	+	-	-
Tiliacora triandra	-	+	-	+	-	+	+	-
Morinda citrifolia	-	-	+	-	+	+	-	-
Basella alba	-	-	+	-	+	-	-	-
Sauropus androgyrus	-	-	+	-	+	-	-	-

Key: - absent; + present.

presence of alkaloids, condensed tannins, phenolics, triterpenes, steroids, saponins and anthraquinones according to the methods described by Trease and Evans (1989).

Antioxidant activity assay

Antioxidant activity of each sample was determined based on its ability to react with the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical (Yamasaki et al., 1994). An aliquot (750 µl) of the extract (50 to 1000 µg/ml in absolute ethanol) was added to 750 µl of 152 µM DPPH in absolute ethanol. After incubation at room temperature for 20 min, the absorbance of each solution was determined at 520 nm. Percentage of inhibition and the concentration of sample required for 50% scavenging of the DPPH free radical (EC_{50}) were determined. Ascorbic acid was used as the reference standard.

Mutagenicity activity

Salmonella typhimurium test strains TA 98 and TA 100 provided by Dr. Wannee Kusamran (National Cancer Institute, Ministry of Public Health, Thailand) were used throughout this study. The test strains were manipulated as suggested by Maron and Ames (1983). An overnight culture of bacteria was prepared by inoculating 10 mL of Oxoid nutrient broth No. 2 from a frozen stock culture (after thawing at room temperature) and incubating at 37°C. This was used for the mutagenesis assay. 1-Aminopyrene (1-AP) treated with nitrite in acid solution was used as the positive mutagen (Kangsadalampai et al., 1996). The pre-incubation method suggested by Yahagi et al. (1975) was used to determine the mutagenicity of the positive standard and each sample in the Ames test throughout this study.

Antimutagenicity activity

The antimutagenic effect of the plant extracts on the mutagenicity of nitrite treated 1-AP was studied. Briefly, 0.1 ml quantities of DMSO containing 3.75 to 30 mg of each crude extract was mixed with 0.7 ml volumes of sodium phosphate buffer (0.2 M, pH 7.4) and 0.1 ml of overnight culture of *S. typhimurium*. Then, 0.1 ml quantities of DMSO were prepared both with and without 0.1 µg of the positive standard mutagen.

The entire mixtures were then separately pre-incubated in 20 mL of Oxoid nutrient broth No. 2 at 37°C for 20 min before 2.0 ml agar containing NaCl (5.0 g/l), L-histidine (0.025 mM), biotin (0.025 mM) and agar (6.0 g/l) was added. The mixture was then poured onto a minimal glucose agar plate. The histidine revertant colonies were counted after incubation at 37°C for 48 h. Each sample was assayed using triplicate plates. The inhibitory effect of each of the herbal extracts on the mutagenicity of the standard direct mutagen was determined as a percentage of inhibition as described subsequently:

Percentage of inhibition = (A-B)/(A-C) x 100

Where A is the number of revertants per plate induced by the positive mutagen; B is the number of revertants per plate induced by the positive mutagen in the presence of each extract; and C is the number of spontaneous revertants per plate. The inhibition of each herbal extract was considered according to Calomme et al. (1996) as being strong, moderate or weak when the value was higher than 60, 40 to 60 or 20 to 40%, respectively. The values of less than 20% were considered negligible.

RESULTS AND DISCUSSION

Phytochemical screening

The screening for phytochemical constituents showed the presence of some bioactive compounds in the herbal extracts (Table 1). These results indicated phenolic compounds and steroids in the herbal extracts. However, anthraquinones were not found in any herbal extract. It is well understood that plants are a major source of phenolic compounds, which are synthesized as secondary metabolites during normal development in response to stress conditions, such as wounding and UV radiation among others (Stahl and Sies, 2003). Plants may contain simple phenolics, phenolic acids, coumarins, flavonoids and stilbene (Naczk and Shahidi, 2006). The information found in this study suggests that phenolic compounds are the major component in the herbal extracts except those of *T. triandra*.

Antioxidant activity assay

The antioxidant activity of the herbal extracts is summarized in Table 2. All herbal extracts possessed lower antioxidant activity than that of the standard ascorbic acid in terms of EC_{50} values. The two strongest antioxidant scavengers were the herbal extracts of *O. indicum* and *T. triandra*. Their EC_{50} values were 12.69 ± 1.02 and 14.51 ± 0.67 and µg/ml for *O. indicum* and *T. triandra*, respectively. Furthermore, most reports indicated that the protective effect against oxidative

Table 2. DPPH scavenging activity of the herbal extracts.

Family name	Sciencetific name	50% DPPH scavenging activity (EC50 µg/ml)
Bignoniaceae	*Oroxylum indicum*	12.69 ± 1.02
Menispermaceae	*Tiliacora triandra*	14.51 ± 0.67
Rubiaceae	*Morinda citrifolia*	36.27 ± 1.08
Basellaceae	*Basella alba*	102.99 ± 4.37
Euphorbiaceae	*Sauropus androgyrus*	179.11 ± 15.11
Ascorbic acid		5.61 ± 0.37

damage of any samples or compounds was attributable to phenolic compounds (Robbins, 2003). In addition, Tenpe et al. (2009) concluded that the *O. indicum* leaf extract was a good antioxidant and expressed its hepatoprotective activity. This activity was probably due to the presence of polar phenolic compound such as flavonoids, tannins etc. According to the result shown in Table 2, herbal extracts, which had strong antioxidant activity, had phenolic and flavonoid contents. Most of the antioxidant substances in plants are phenolic compounds and phenolic substances serve as oxidation terminators by scavenging radicals to form resonance stabilized radicals (Rice-Evans et al., 1997) and the finding of flavonoids in the present investigation may suggest its antioxidant role previously reported by Cook and Samman (1996). The high antioxidant activity of the extract of *O. indicum* was similar to the result of Kalaivani and Lazar (2009).

Mutagenicity activity

The concentrations of herbal extracts used to evaluate the mutagenic activity were neither toxic nor mutagenic, since the numbers of revertant colonies were not higher than that of the negative control. The range of revertant colonies of most samples was 10 to 22 for TA 98 and 96 to 168 for TA 100 which were less than the spontaneous revertants. Yen et al. (2001) evaluated the mutagenic effect of new leafy vegetables in Taiwan and found that *B. alba* extract was not mutagenic on TA 98 or TA 100 cultures either with or without the activating system. The range of herbal extracts on revertant colonies was similar to those reported previously by several authors (Maron and Ames, 1983; Gonzalez de Mejia et al., 1999; Cardador-Martnez et al., 2002).

Antimutagenicity activity

The main product of nitrite treated 1-aminopyrene that did not require metabolic activation before expressing its mutagenicity was determined to be 1-nitropyrene (Kato et al., 1991). The percentage of inhibition on strains TA 98 and TA 100 revertants had values ranging from 77 to 86 and 54 to 63, respectively (Table 3) indicating that *Basella alba* extract (3.75 to 30 mg/plate) was strongly antimutagenic against nitrite treated 1-aminopyrene. Yen et al. (2001). reported that the extract from *B. alba* was a moderate inhibitor of 2-amino-3-methyl-imidazo [4,5-f]quinoline (IQ) in *S. typhimurium* TA 98 and TA 100.

The high antimutagenic activity of *B. alba* extracts may be due to the presence of phenolic compounds as suggested by Jayaprakasha et al. (2007). The correlation of antimutagenicity of natural compounds of plant origin was positive with polarity of flavonols; also phenolic compounds were classified as potent antimutagens (Edenharder et al., 1997). Phenolic compounds presented in plants were considered to be responsible for their antimutagenic activity (Romina and Luis, 2006). Rocha et al. (2007) also suggested that phenolic compounds present in the acetone extracts from beans were potent antimutagens against the direct mutagen 1-nitropyrene in *S. typhimurium* TA 98.

B. alba extract expressed high antimutagenic activity. The possible mechanisms may be classified as follows. Firstly, *B. alba* extracts might include the blocking of the mutagen transfer into the cytosol by phenolic binding or insertion into the transporters of the outer membrane of the cell (Hour et al., 1999). Elvira et al. (1999) suggested that phenolic compounds could interact directly and non-enzymatically with the proximate and/or ultimate mutagen; or form a complex with known mutagens e.g. Benzo[*a*]pyrene (B[*a*]P), thereby reducing the bioavailability of mutagen. Secondly, *B. alba* extracts modified the permeability for mutagens across bacterial membranes. Edenharder and Tang, (1997) reported that 1-nitropyrene were in general more effectively antagonized by potent antimutagenic flavonoids. They suggested that different mechanism of antimutagenesis were at work. Antimutagenic flavonoids might modulate the mutagenic response of the nitroarenes, which have 1-nitropyrene as a member, tested by modification of the permeability of bacterial membranes.

Antimutagens and/or anticarcinogens are found in all categories of foods, but mainly in fruits and vegetables (Nakamura et al., 1998). Five herbal extracts in the present study, especially from the *B. alba*, that are generally consumed as vegetables or herbs showed antimutagenic activity in the Ames test. These data

Table 3. Antimutagenic activity of the herbal extracts.

Family name	Scientific name	Concentration (mg/plate)	%Inhibition	
			TA 98	TA 100
Bignoniaceae	*Oroxylum indicum*	3.75	75.43 ± 3.83	-13.16 ± 2.16
		7.5	84.38 ± 1.77	-14.43 ± 3.89
		15	89.91 ± 2.07	21.39 ± 1.03
		30	97.95 ± 3.76	35.06 ± 1.53
Menispermaceae	*Tiliacora triandra*	3.75	50.09 ± 2.56	43.26 ± 3.46
		7.5	51.37 ± 5.73	47.74 ± 1.08
		15	47.65 ± 4.10	48.63 ± 1.77
		30	44.84 ± 8.98	58.84 ± 10.81
Rubiaceae	*Morinda citrifolia*	3.75	37.33 ± 2.13	27.63 ± 1.19
		7.5	8.09 ± 1.94	3.75 ± 0.75
		15	51.52 ± 0.11	57.59 ± 10.33
		30	74.30 ± 15.57	78.49 ± 3.10
Basellaceae	*Basella alba*	3.75	77.48 ± 1.41	54.06 ± 1.52
		7.5	82.71± 5.33	59.77 ± 2.64
		15	83.84 ± 2.28	63.02 ± 6.83
		30	86.08 ± 2.78	63.11 ± 2.54
Euphorbiaceae	*Sauropus androgyrus*	3.75	83.55 ± 13.92	14.49 ± 1.48
		7.5	88.72 ± 16.07	42.52 ± 2.09
		15	89.25 ± 4.01	43.64 ± 2.83
		30	94.42 ± 5.79	44.58 ± 6.25

further indicated that *B. alba* may also be a health-promoting food.

Conclusion

The present study indicates that the herbal extracts of *O. indicum* and *T. triandra*, which posses high antioxidant activity, and the herbal extracts of *B. alba*, which posses potent antimutagenic activity, have high potential to be further developed as functional foods for cancer prevention. Increased consumption of these plants would seem to be of great benefit to most consumers since theses plants are cheap and plentiful. Phenolic compounds are suggested to be responsible for the antioxidant and antimutagenic activity exhibited in this study. Thus total phenolic content may be used to predict the ability of the extracts to scavenge free radicals and to decrease the mutagenicity induced by environmental toxicants.

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REFERENCES

Ames BN (1983). Dietary carcinogens and anticarcinogens. Sci., 221: 1256-1263.

Calomme K, Pieters L, Vlietinck A, Berghe V (1996). Inhibition of bacterial mutagenesis by *Citrus* flavonoids. Planta Med., 62: 222-226.

Cardador MA, Castano TE, Loarca PG (2002). Antimutagenic activity of natural phenolic compounds present in the common bean (*Phaseolus vulgaris*) against aflatoxin B-1. Food Add. Cont., 19: 62-69.

Cook NC, Samman S (1996). Flavonoids- chemistry, metabolism, cardioprotective effects, and dietary sources. J. Nutr. Biochem., 7: 66-76.

Edenharder R, Tang X (1997). Inhibition of the mutagenicity of 2-nitrofluorene, 3 nitrofluoranthene and 1-nitrtopyrene by flavonoids, coumarin, quinones and other phenolic compounds. Food Chem. Toxicol., 35: 357-372.

Elvira GM, Eduardo CT, Guadalupe LP (1999). Antimutagenic effects of natural phenolic compounds in beans. Mutat. Res./Genet. Toxicol. Environ. Mutagen., 1: 1-9.

Geoffrey C, Kabat YP, Albert R, Hollenbeck R, Arthur S, Thomas ER (2010). Intake of fruits and vegetables, and risk of endometrial cancer in the NIH-AARP Diet and Health Study. Cancer Epidemiol., 34: 568-573.

Gonzalez ME, Castafio TE, Loarca PG (1999). Antimutagenic effects of natural phenolic compounds in beans. Mutat. Res., 441: 1-9.

Hour T, Liang Y, Chu I, Lin J (1999). Inhibition of eleven mutagens by various tea extracts, (-)-epigallocatechin-3-gallate, gallic acid and caffeine. Food Chem. Toxicol., 37: 569-579.

Ikken Y, Morales P, Martinez A, Marin ML, Haza AI, Cambero MI (1999). Antimutagenig effects of fruit and vegetables. J. Agric. Food Chem., 47: 3257-3264.

Jayaprakasha GK, Negi PS, Jena BS, Rao JM (2007). Antioxidant and Antimutagenic Activities of *Cinnamomum zeylanicum Fruit Extracts*. J. Food Compos. Anal., 20: 330-336.

Kalaivani T, Lazar M (2009). Phytochemistry and Free radical scavenging activities of *Oroxylum indicum*. Environ. We. Int. Sci. Tech., 4: 45-52.

Kangsadalampai K, Butryee C, Manoonphol K (1996). Direct mutagenicity of the polycyclic aromatic hydrocarbon-containing fraction of smoked and charcoal-broiled foods treated with nitrite in acid solution, Food Chem. Toxicol., 35: 213–218.

Kato T, Tadokoro N, Tsutsui M, Kikugawa K (1991). Transformation of arylamines into the direct-acting mutagens by reaction with nitrite. Mutat. Res., 249: 243-254.

Lako J, Trenerry C, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S, Premier R (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chem., 101: 1727-1714.

Maron DM, Ames BN (1983). Revised methods for the Salmonella mutagenicity test. Mutat. Res., 113: 173-215.

Naczk M, Shahidi F (2006). Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. J. Pharmaceu. Biomed. Anal., 41: 1523-1542.

Rice-Evans C, Miller N, Paganga G (1997). Antioxidant properties of phenolic compounds. Trends Plant Sci., 2: 152-159.

Robbins RJ (2003). Phenolic acids in foods: An overview of analytical methodology. J. Agric. Food Chem., 51: 2866-2887.

Rocha-Guzmána NE, Herzogb A, González-Laredoa RF, Ibarra-Pérezc FJ, Zambrano-Galvána G, Gallegos-Infantea JA (2007). Antioxidant and antimutagenic activity of phenolic compounds in three different colour groups of common bean cultivars (*Phaseolus vulgaris*) Food Chem., 103: 521-527.

Romina P, Luis CZ (2006). Antimutagenic and antioxidant properties of phenolic fractions from andean purple corn (*Zea mays* L.). J. Agric. Food Chem., 54: 4557-4567.

Stahl W, Sies H (2003). Antioxidant activity of carotenoids. Mol. Aspects Med., 24: 345-351.

Tenpe CR, Upaganlawar A, Burle S, Yeole P (2009). *In vitro* antioxidant and preliminary hepatoprotective activity of *Oroxylum indicum* leaf extracts. Pharmacol. Online, 1: 35-43.

Trease GE, Evans WC (1978). A Textbook of Pharmacognosy. 11th ed. Bailliere Tindall Ltd London, p. 784.

Yahagi T, Degawa, M, Seino Y, Matsushima T, Nagao M, Sugimura T, Hashimoto Y (1975). Mutagenicity of Carcinogen Azo Dyes and their Derivatives. Cancer Lettr., 1: 91-96.

Yen G, Chen H, Peng H (2001). Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of emerging edible plants. Food Chem. Toxicol., 39: 1045-1053.