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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIMUTAGENIC ACTIVITIES OF SELECT ED THAI EDIBLE PLANT EXTRACTS

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Abstract

Studies showed that 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 in 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 concentration of 12.69 ±1.02 μg/ml, whereas *Basella alba* Linn. extract had the most strongest antimutagenicity in 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 were proposed to be antioxidant and antimutagenic agents, respectively. In conclusion, the apparent antioxidant and antimutagenic activities of Thai edible plants further suggests their potential usefulness in cancer prevention.

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1. INTRODUCTION

Several researches provide strong evidences that vegetables, fruits and phytochemicals protect against some cancers. Many antioxidative agents and antiumtagens have been identified as anticarcinogens (Ames, 1983; Stavric,1994). Therefore, the regular intake of plants with 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. They could be useful in dietary supplement development comprised of the herbal extracts as agents for cancer prevention. Moreover, the finding of the current study could be an evidence-based practice to promote people for consuming more edible plants. The aims of this investigation were to test the antioxidant and antimutagenic activities and to screen the chemical constituents of selected Thai edible plant extracts.

2. EXPERIMENTAL METHOD

2.1 Plant materials

Oroxylum indicum (Linn.) Kurg (fruits) *Tiliacora triandra* Diels. (leaves), *Morinda citrfolia* Linn. (leaves), *Basella alba* Linn.(entire plant) and *Sauropus androgyrus* (L.) Merrill (leaves) obtained from Mahasarakham province and identified and identified by one of the authors (Dr. Phadungkit). Avoucher specimen has 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 solvent was evaporated to dryness in a rotary evaporator and each crude herbal extract was kept at 4 °C.

2.2 Phytochemical screening

The solution of each plant extract was freshly prepared from the crude extract described above. They were analyzed for the presence of alkaloids, condensed tannins, phenolics, triterpenes, steroids, saponins and anthraquinones according to the methods described by Trease and Evans (1989).

2.3 Antioxidant Activity assay

Antioxidant activity of each sample was determined based on its ability to react with the stable DPPH (1,1-diphenyl-2-picryl hydrazyl) 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.

2.4 Mutagenicity activity

Salmonella typhimurium tester 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 tester strains were manipulated as suggested by Maron and Ames (1983). Overnight culture of bacteria inoculated from frozen stock culture in Oxoid nutrient broth No.2 at 37°C was used for 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.

2.5 Antimutagenicity activity

Antimutagenic effect of the plant extracts on the mutagenicity of nitrite treated 1-AP was studied. Briefly, 0.1 ml DMSO containing 3.75–30 mg of each crude extract was added with 0.7 ml of sodium phosphate buffer (0.2 M, pH 7.4) and 0.1 ml of each overnight culture of *Salmonella typhimurium*. Then, 0.1 ml DMSO was added both with and without 0.1 μg the positive standard mutagen. The entire mixture was then pre-incubated at 37 °C for 20 min before 2.0 ml top 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 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 herbal extracts on mutagenicity of the standard direct mutagen was determined as percentage of inhibition described below:

$$\text{Percentage of inhibition} = (A-B)/(A-C) \times 100$$

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 accordingly to Calomme *et al.* (1996) as being strong, moderate or weak when the value is higher than 60%, 40-60% or 20-40%, respectively. The value less than 20% is considered negligible.

3. RESULTS AND DISCUSSION

3.1 *Phytochemical screening*

The screening for phytochemical showed the presence of some bioactive compounds in the herbal extracts (Table 1). There result was almost found phenolic compounds and steroids in the herbal extracts. However, antraquinones were not found in any herbal extracts. 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; Close *et al.*, 2005). 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 herbal extracts except that of *T. triandra.*

3.2 *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 EC50 values. The two strongest antioxidant scavengers were the herbal extracts of *O. indicum* and *T. triandra.* Their EC_{50} values ranged between 12.71 ± 0.28 and 12.69 ± 1.02 µg/ml. *O. indicum* and *T. triandra* contained antiradical activity. Furthermore, most reports indicated that the protective effect against oxidative damage of any samples or compounds was attributable to phenolic compounds (Robbins, 2003). In addition, Jiwajinda et al. (2002) revealed the antioxidant activity of *O. Indicum* and Tenpe *et al.* (2009) concluded that the *Oroxylum indicum* leaf extract was a good antioxidant and expressed its hepatoprotective activity. This activity was probably due to presence of polar phenolic compound flavonoid, tannin etc. According to the result shown in Table 2, herbal extract, 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 flavonoid in the present investigation may suggest its antioxidant role previously reported by Cook and Samman (1996).

3.3 *Mutagenicity activity*

The concentrations of herbal extract used to evaluate the mutagenic activity were neither toxic nor mutagenic, since the number of revertant colonies were not higher than that of negative control. The range of revertant colonies of most samples was 10 to 22 for TA 98 and 96 to 168 for TA 100 with were less than the spontaneous revertants (data not shown). 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 either with or without the activating system. The range of herbal extract 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).

3.3 *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 at the values ranging from 77 to 86 and 54 to 63, respectively (Table 3) indicated that *Basella alba* extract (3.75 - 30 mg/plate) was strong antimutagenic against nitrite treated 1-aminopyrene. Yen *et al.* (2001) reported that the extract from *B. alba* was 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 present in herbal juices were considered to be responsible for the

antimutagenic activity (Stich et al., 1984; Steele and Lalies, 1985; Mitscher *et al.*, 1992). Rocha et al. (2007) also suggested that phenolic compounds presented 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 with possible mechanisms may be classified as following. 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 forming 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 of mutagen 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 further indicated that *B. alba* may also be health-promoting foods.

5. CONCLUSION

The present study indicates that the herbal extracts of *O. indicum* and *T. triandra* which posses high antioxidant activity and the herbal extract of *B. alba* which posses potent

antimutagenic activity have high potential to be further developed as functional food for cancer prevention. Increase consumption of these plants seems to be of great benefit to most consumer since theses plants are cheap. Phenolic compounds were suggested to take their responsibility 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 radical and to decrease the mutagenicity induced by environmental toxicants.

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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 citrfolia	-	-	+	-	+	+	-	-
Basella alba	-	-	+	-	+	-	-	-
Sauropus androgyrus	-	-	+	-	+	-	-	-

Key: - absent ; + present

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 citrfolia*	36.27 ± 1.08
Basellaceae	*Basella alba*	102.99 ± 4.37
Euphorbiaceae	*Sauropus androgyrus*	179.11 ± 15.11
Ascorbic acid		5.61 ± 0.37

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*	3.75	83.55 ± 13.92	14.49 ± 1.48
	androgyrus	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

Antioxidant, Antibacterial and Antimutagenic Activities of *Polygonum odoratum* Extract

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Abstract

The aims of the current study were to evaluate antioxidant, antibacterial and antimutagenic activities of *Polygonum odoratum* extract. Antioxidant, antibacterial and antimutagenic activities were determined using the DPPH scavenging method, the disk diffusion method and the Ames test, respectively. The results showed that *the* herbal extract had high antioxidant activity with the EC_{50} value at concentration of 12.71 ± 0.28 μg/ml. The extract had moderate antibacterial activity against *Staphyloccus aureus* and had strong antimutagenic activity in *S. typhimurium* TA 100.

Key words : Antioxidant, Antibacterial, Antimutagenic, *Polygonum odoratum*

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INTRODUCTION

Polygonum odoratum Lour. (Polygonaceae) is an edible plant commonly grown in subtropical and Southeast Asia. The plant is widely used in Southeast Asian cuisines. The plant has a strong coriander leaf, lemony, green type of smell (Starkenmann, 2006). In Thailand, the fresh leaves are eaten with traditional pork, chicken or fish salad, as well as in some traditional soups. The plant is used in traditional Thai medicine to treat cough,

joint pain and dyspesia (Subcharoen. 2004). Phytochemical study in its oil revealed many organic compounds such as (Z)-3-hexenal, (Z)-3-hexenol, decanal, undecanal, and dodecanal (Starkenmann, 2006). Despite the phytochemical studies, there has been minimal investigation of its biological activity of the aerial part. The aims of the current investigation were to test the antioxidant, antibacterial and antimutagenic activities.

MATERIALS AND METHODS

Plant material

The plant sample (aerial part) of *Polygonum odoratum* was collected from the Mahasarakham province of Thailand and identified by the authors. A voucher specimen has been deposited in the Herbarium at the Faculty of Pharmacy, Mahasarakham University, Thailand.

Preparation of plant extracts

The sample was washed, dried in a hot air oven (50°C) and then pulverized. The powdered drug was exhaustively extracted with 95 % ethanol in a soxhlet extraction apparatus. The solvent was evaporated to dryness by a rotary evaporator yielding the herbal extract.

Antioxidant testing by DPPH Free radical scavenging assay

The test was carried out using the DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging assay. The free radical scavenging activity of the extract and standard ascorbic acid solution in absolute ethanol were determined based on their ability to react with the stable DPPH free radical (Yamasaki *et al.*, 1994). A 750 µL aliquot of the

extract (50 to 1000 μg/ml, dissolved in) was added to 750 μL of DPPH in absolute ethanol (152 μM). After incubation at room temperature in the dark for 20 minutes, the absorbance of each solution was determined at 520 nm using a UV spectrophotometer. The results were expressed as percentage inhibition, %inhibition = $[(A_{control} - A_{sample})/A_{control}] \times 100$. The effective concentration of sample required to scavenge DPPH radical by 50% (EC_{50} value) was obtained by linear regression analysis of a dose-response curve plotting %inhibition versus concentration.

Antibacterial activity assay

The disk diffusion method was used to determine the antibacterial activity of the herbal extract against *Staphylococcus aureus* ATTC 25923, Escherichia *coli* ATTC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Testing was performed in accordance with NCCLS guidelines but with some modification (NCCLS, 2000). The antibacterial activity was evaluated by measuring the zone of inhibition (in mm) against the test organisms. Amoxycillin (10 μg /disk) was used as a positive reference standard.

Antimutagenic activity assay

Antimutagenic effects of the herbal extract on the mutagenicity of nitrite treated 1-aminopyrene in gastric-like condition, were studied using the Ames test with some application in *Salmonella typhimurium* TA98 and TA100 strains (Yahgi.1975). Briefly, 0.1 ml DMSO containing 3.75–30 mg of sample was added with 0.5 ml of sodium phosphate buffer (0.2M, pH 7.4) and 0.1 ml of the overnight activated culture of *S. typhimurium*. Then, 0.1 ml DMSO was combined both with and without 0.1 μg the mutagen. The entire mixture was then pre-incubated at 37 °C for 20 min before 2.0 ml molten top agar containing L-histidine (0.025 mM) ,biotin 0.025 mM and agar (6.0 g/L)

was added. The mixture was poured onto a minimal glucose agar plate. The His + revertant colonies were counted after incubation at 37 °C for 48 h. Each sample was assayed using triplicate plates. The antimutagenic activity was expressed as a percentage of mutagenic inhibition which was calculated as the following formula: Percentage of inhibition = (A-B)/ (A-C) × 100. Where A is the number of histidine revertants induced by nitrite-treated 1-aminopyrene, B is the number of histidine revertants induced by nitrite-treated 1-aminopyrene in the presence of the herbal extract and C is the number of histidine spontaneous revertants induced in the presence of dimethylsulfoxide alone (negative control). The inhibition is considered either strong, moderate or weak when its effect is higher than 60%, 40-60% or 20-40%, respectively, When the inhibiton is less than 20 %, no inhibition effect is pronounced (Caomme *et al.*, 1996).

RESULTS AND DISCUSSION

Antioxidant activity

The ethanol extract of *P. odoratum* showed potent antioxidant activity (EC50 = 12.71± 0.28 μg/ml) when compared to the standard ascorbic acid (EC50 = 5.61± 0.37 μg/ml) . The high antioxidant activity of the extract of *P. odoratum* was similar to the result of the previous study (Pannangpetch *et al.,* 2002). Antioxidants are believed to be able to aid in the prevention of diseases including cardiovascular diseases, stroke and cancer (Enayat *et al.*, 2009). Therefore, *P. odoratum may* warrant further investigation for its ability to promote good health.

Antibacterial activity

The antibacterial activity of the herbal extract was active only in the gram positive bacterium *S. aureus* when compared to the standard amoxicillin (the inhibition zones were 8.6 ± 0.5 and 10.7 ± 0.6 mm, respectively) but inactive in *E .coli* or *Ps. aeruginosa*. The antibacterial activity against *S. aureus* was observed in the same genus of *Polygonum multiflorum* (Zuo *et al.*, 2008).

Antimutagenic activity

The antmutagenic activity of the herbal extract is summarized in Table 1. The herbal extract from *P. odoratum* exhibited strong antimutagenic activity in the base-substitued tester strain of *S. typhimurium* TA100 with percentage inhibition values ranged between 64.48 ± 11.57 and 76.59 ± 12.76 % . Only high concentration of the herbal extracts (30mg/plate) showed strong antimutagenic activity in the frame-shift tester strain of *S. typhimurium* TA 98. Studies have shown that antimutagens play an important role in prevention of cancer (Vaidya *et al.*, 2008). Therefore, the ethanol extract of *P. odoratum* can serve as a good candidate for further evaluation of cancer chemopreventive effects.

CONCLUSION

The present study discovered that the ethanolic extract posseses potent antioxidant activity, antimutagenicity activity in *S. typhimurium* TA 100 and posseses moderate antibacterial activity against *S. aureus.* In conclusion, *P. odoratum* warrants further investigation for its ability to protect against cancer and other diseases.

ACKNOWLEDGMENT

The authors would like to thank The Thailand Research Fund for financial support.

Table 1 : Antimutagenic activity of the herbal extract in *S. typhimurium* TA 98 and TA 100

Concentration (mg/plate)	%Inhibition	
	TA 98	TA100
3.75	28.92 ± 4.76	64.48 ± 11.57
7.5	29.69 ± 7.93	67.12 ± 1.55
15	48.15 ± 3.51	78.78 ± 2.43
30	70.49 ± 8.77	76.59 ± 12.76

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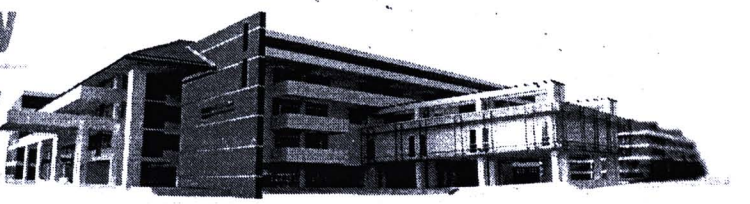

Antioxidant and Antimutagenicity of Selected Thai Edible Plants

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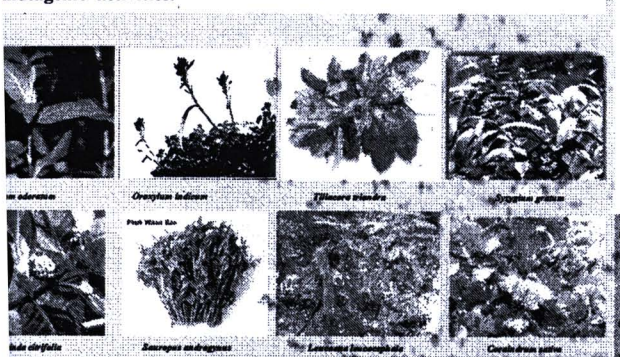
BSTRACT

The aim of the current study was to evaluate antioxidant and ıtimutagenic activities of selected Thai edible plant extracts. Antioxidant tivity was determined by the ability of each extract to scavenge the free dicals 1,1-diphenyl-2-picrylhydrazyl (DPPH). Antimutagenic activity was aluated using the Ames test in *Salmonella typhimurium* (TA 98,TA 100 ains). The results showed that *Oroxylum indicum* (Linn.) Kurg., lygonum *odoratum* Lour and *Tiliacora triandra* Diels showed strong tioxidant activity, respectively whereas only *Piper sarmentosum* Roxb.ex ınter. showed strong antimutagenicity in both strains of *Salmonella himurium*. The apparent antioxidant and antimutagenic activities of Thai

TRODUCTION

Several researches provide strong evidence that vegetables, fruits and ytochemicals protect against some cancers. Many antioxidative agents l antiumtagens have been identified as anticarcinogens (1,) . Therefore, regular intake of plants with antimutagenic or antioxidative agents reduce genotoxic effects of mutagenic and carcinogenic factors (2). In ailand, people consume many kinds of plants as part of their diet. It is of erest to know whether traditional edible plant have antioxidant and imutagenic activities. They could be useful in dietary supplement elopment comprised of the herbal extracts as agents for cancer vention. Moreover, the finding of the current study could be an lence-based practice to promote people for consuming more edible its. The aims of this investigation were to test the antioxidant and mutagenic activities.



Acknowledgement

The authors would like to thank The Thailand Research Fund for financial support.

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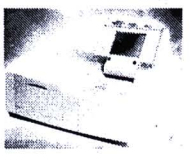
EXPERIMENTAL METHOD

Antioxodamt activity was carried out using *DPPH free radical scavenging activity* testing.

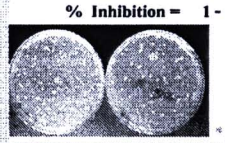


% Radical scavenging activity = (Control OD- sample OD) x 100 / (Control OD)

EC 50

Antimutagenic effects of the plant extracts on the mutagenicity were studied in *Salmonella typhimurium* strains TA98 and TA100 using Ames test with pre-incubation technique. The antimutagenic activity was expressed as a percentage of mutagenic inhibition which was calculated as the following formular:

% Inhibition = 1 - (mutagen induced revertant/plate (in the presence of extract) / mutagen induced revertant/plate (in the absence of extract)) x 100



RESULTS AND DISCUSSION

The three strongest anti-oxidant scavenging activities were exhibited by the herbal extracts of *Oroxylum indicum, Polygonum odoratum* and *Tiliacola triandra*, respectively (Table 1). The herbal extract of *Basella alba* at the concentration ranged form 3.75 to 50 mg/plate exhibited the strongest anti-mutagenic with the percentage of inhibition in TA98 and TA100 strains (Table 2).

Table 1 Antioxidant activity of herbal extracts

Scientific name	50% DPPH scavenging activity (EC_{50} µg/ml)
Oroxylum indicum	12.68 ± 1.02
Tiliacora triandra	14.71 ± 0.67
Morinda citrifolia	36.27 ± 1.68
Basella alba	102.96 ± 4.37
Sauropus androgynus	31.91 ± 2.20
Leucosceptrum canum	19.44 ± 0.20
Centella asiatica	111.25 ± 12.35
Piper sarmentosum	58.87 ± 3.80
Polygonum odoratum	12.71 ± 0.28
Ascorbic acid	5.61 ± 0.37

Table 2 Antimutagenic activity of *Piper sarmentosum* extract

Piper sarmentosum	Concentration (mg/plate)	%Inhibition TA 98	%Inhibition TA100
	3.75	87.67 ± 2.61	79.66 ± 1.51
	7.5	88.46 ± 9.82	75.72 ± 9.76
	15	91.02 ± 2.15	82.35 ± 11.48
	30	97.24 ± 9.44	91.33 ± 2.42

CONCLUSION

The present study indicates that the herbal extracts of *O. indicum, Polygonum odoratum* and *T. triandra* which posses high antioxidant activity and the herbal extract of *B. alba* which posses potent antimutagenic activity have high potential to further developed as functional food for cancer prevention. Moreover, more consumption of these plants has been proposed as a way to prevent some kinds of cancers.

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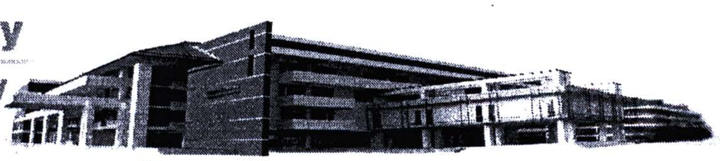

Phytochemical Screening, Antioxidant and Antimutagenicity Activities of *Polygonum odoratum* Extracts

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\BSTRACT

Polygonum odoratum Lour. (Polygonaceae) is an edible plant commonly grown in ıbtropical and Southeast Asia. The aims of the current study were to evaluate antioxidant and ntimutagenic activities and to screen its chemical constituents of *P. odoratum* extract. Antioxidant ıd Antimutagenic activities were determined using the DPPH scavenging method, and the Ames test, ıspectively. Phytochemical screening was performed using standard procedures. The results showed ıat *the* herbal extract had high antioxidant activity with the EC_{50} value at concentration of 12.71 ± 28 μg/ml. The extract had strong antimutagenic activity in *S. typhimurium* TA 100 but showed eak activity in *S. typhimurium* TA 98. Phytochemical screening of the extracts showed the ·esence of phenolic compounds, flavonoids and saponins which could be responsible for the ological activities.

\TRODUCTION

Polygonum odoratum Lour. (Polygonaceae) is an edible plant commonly grown in ıbtropical and Southeast Asia and widely used in Southeast Asian cuisines. The plant is a perennial ·rb with a distinctive dark purple marking in the center of the leaves. The plant has a strong riander leaf, lemony, green type of smell. In Thailand, the fresh leaves are eaten with traditional ·rk, chicken or fish salad, as well as in some traditional soups. The plant is used in traditional Thai ·dicine to treat cough, joint pain and dyspesia. Phytochemical study in its oil revealed many organic ·mpounds such as (Z)-3-hexenal, (Z)-3-hexenol, decanal, undecanal, and dodecanal (Starkenmann, 06). Saponins were isolated in its rhizomes (Qin *et al*, 2003). Despite the phytochemical studies in oil and rhizomes, there has been minimal investigation of its biological activity and chemical ·mponents in the aerial part. The aims of the current investigation were to test the antioxidant and ·imutagenic activities and screen the chemical constituents of ethanol extract from this herb.

\PERIMENTAL METHOD

·paration of plant extracts

The sample was washed, dried in a hot air oven (50°C) and then pulverized. The powdered g was exhaustively extracted with 95 % ethanol in a soxhlet extraction apparatus. The solvent was porated to dryness by a rotary evaporator yielding the herbal extract.

ioxidant testing by DPPH Free radical scavenging assay

The test was carried out using the DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical ·enging assay. The free radical scavenging activity of the extract and standard ascorbic acid tion in absolute ethanol were determined based on their ability to react with the stable DPPH free cal (Yamasaki *et al*., 1994). A 750 μL aliquot of the extract (50 to 1000 μg/ml, dissolved in) added to 750 μL of DPPH in absolute ethanol (152 μM). After incubation at room temperature e dark for 20 minutes, the absorbance of each solution was determined at 520 nm using a UV trophotometer. The results were expressed as percentage inhibition, %inhibition Acontrol - Asample)/Acontrol] x 100. The effective concentration of sample required to scavenge H radical by 50% (EC50 value) was obtained by linear regression analysis of a dose-response ː plotting %inhibition versus concentration.

Antimutagenic activity assay

Antimutagenic effects of the herbal extract on the mutagenicity of nitrite treated 1-aminopyrene in gastric-like condition, were studied using the Ames test with some application in *Salmonella typhimurium* TA98 and TA100 strains (Yahgi *et al*.,1975). Briefly, 0.1 ml DMSO containing 3.75–30 mg of sample was added with 0.5 ml of sodium phosphate buffer (0.2M, pH 7.4) and 0.1 ml of the overnight activated culture of *S. typhimurium*. Then, 0.1 ml DMSO was combined both with and without 0.1 μg the mutagen. The entire mixture was then pre-incubated at 37 °C for 20 min before 2.0 ml molten top agar containing L-histidine (0.025 mM) ,biotin 0.025 mM and agar (6.0 g/L) was added. The mixture was poured onto a minimal glucose agar plate. The His + revertant colonies were counted after incubation at 37 °C for 48 h. Each sample was assayed using triplicate plates. The antimutagenic activity was expressed as a percentage of mutagenic inhibition which was calculated as the following formula: Percentage of inhibition = (A-B)/(A-C) × 100. Where A is the number of histidine revertants induced by nitrite-treated 1-aminopyrene, B is the number of histidine revertants induced by nitrite-treated 1-aminopyrene in the presence of the herbal extract and C is the number of histidine spontaneous revertants induced in the presence of dimethylsulfoxide alone (negative control). The inhibition is considered either strong, moderate or weak when its effect is higher than 60%, 40-60% or 20-40%, respectively. When the inhibiton is less than 20 %, no inhibition effect is pronounced.

Phytochemical screening

The ethanol extract were freshly prepared and divided into different test tubes. Their various chemical constituents were determined according to methods described by Trease and Evans (Trease *et al*.,1989). Classes of compound tested for phytochemical constituents included alkaloids, tannins, phenolics, triterpenes, steroids, flavonoids, saponins, cardiac glycosides and anthraquinones.

RESULTS AND DISCUSSION

The ethanol extract of *P. odoratum* showed potent antioxidant activity (EC50 = 12.71± 0.28 μg/ml) when compared to the standard ascorbic acid (EC50 = 5.61± 0.37 μg/ml) . Antioxidants are believed to be able to aid in the prevention of diseases including cardiovascular diseases, stroke and cancer (Enayat *et al*., 2009). Therefore, *P. odoratum may* warrant further investigation for its ability to promote good health.

The antmutagenic activity of the herbal extract is summarized in Table 1. The herbal extract from *P. odoratum* exhibited strong antimutagenic activity in the base-substitued tester strain of *S. typhimurium* TA100 with percentage inhibition values ranged between 64.48 ± 11.57 and 76.59 ± 12.76 %. Only high concentration of the herbal extracts (30mg/plate) showed strong antimutagenic activity in the frame-shift tester strain of *S. typhimurium* TA 98. Studies have shown that antimutagens play an important role in prevention of cancer (Vaidya *et al*., 2008). Therefore, the ethanol extract of *P. odoratum* can serve as a good candidate for further evaluation of cancer chemopreventive effects.

In the phytochemical screening, the ethanol extract from the aerial part showed the presence phenolic compounds, flavonoids and saponins but absence of tannins, steroids, cardiac glycoside and anthraquinones. The detected compounds could be responsible for the biological activities. The presence of saponins was similar to the results of previous study (Qin *et al*., 2003) but from the different part (aerial and rhizome, respectively).

Table 1 : Antimutagenic activity of the herbal extract in *S. typhimurium* TA 98 and TA 100

Concentration (mg/plate)	%Inhibition	
	TA 98	TA100
3.75	28.92 ± 4.76	64.48 ± 11.57
7.5	29.69 ± 7.93	67.12 ± 1.55
15	48.15 ± 3.51	78.78 ± 2.43
30	70.49 ± 8.77	76.59 ± 12.76

CONCLUSION

The present study discovered that the ethanolic extract posseses potent antioxidant activity and antimutagenicity activity in *S. typhimurium* TA 100. The phytochemical screening demonstrated the presence of different types of compounds including phenolic compounds, flavoniods and saponins which could be responsible for the biological activities. In conclusion, *P. odoratum* warrants further investigation for its ability to protect against cancer and other diseases.

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Phytochemical Screening, Flavonoid Content and Antioxidant Activity of *Tiliacora Triandra* Leaf Extracts

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Abstract

Tiliacora triandra (Menispermaceae) is used in traditional medicine as antipyretic and anti-cancer agents. The current study sought to evaluate the antioxidant activity, to screen their chemical constituents and to investigate the flavonoid content of the leaf extracts. Antioxidant were determined by the DPPH scavenging and the ferric reducing antioxidant power (FRAP) methods. Phytochemical screening was performed using standard procedures. Flavonoid content was measured by the aluminum chloride colorimetric assay. The results showed that the methanol extract had the highest antioxidant activity in both methods when compared to the other extracts. Phytochemical screening of the methanol extract showed the presence of alkaloid, flavonoid, tannin and saponins. Flavonoid content was found to be 18.67±0.28 mg quercetin equivalent/ g of extract. In conclusion, the apparent antioxidant activitiy suggest its potential usefulness in the prevention of some diseases.

Key words: *Tiliacora Triandra*, Phytochemical Screening, Flavonoid Content, Antioxidant

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Introduction

Several research provide strong evidence that plant is a source of inspiration for novel plant compounds, as plant derived compound have made large contributions to human health as well being. Antioxidants derived from fruits, vegetables, herbs, and beverages has been increasing interest (Pourmorad, 2006) for prevented of some disease such as coronary heart disease (Hertog, 1997 and Giugliano, 2000), stoke (Keli,1996), and cancer (Hollman, 1999). In Thailand, the people consume edible plants as part of their diet. *Tiliacora triandra* Diels,known in Thai as Ya-nang, belongs to Menispermaceae family. In traditional Thai medicine, its root has been used as antipyretic agent for all kind of fever. The leaf juice has been used traditionally for anticancer and immunomodilator[8]. Previous phytochemical studies in its root revealed some bisbenzylisoquinoline alkaloids including tiliacorine and tiliacorinine (Chalerm, 2003 and Katchrinnee, 2006) but there has been minimal investigation of its chemical constituents in its leaves. The current study sought to evaluate the antioxidant activity, to screen their chemical constituents and to investigate the flavonoid content of the *Tiliacora triandra*'s leaf extracts.

Methods and Materials

Plant material and extraction

The plant sample (leaves) of *T. triandra* was collected from the Mahasarakham province of Thailand and identified by the authors. The sample was extracted with petroleum ether, dichloromethane, ethyl acetate, methanol in a soxhlet apparatus, respectively. Another sample was macerated with distilled water. The organic solvents were evaporated by a rotary evaporator while the water extract was lyophilized.

Phytochemical screening

Phytochemical screening were perfomed using standard procedures (Ayoola, 2008)

Test for anthraquinones

Zero point five g of extract was boiled with 10 ml of sulfuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for color changes.

Test for terpenoids (Salkowski test)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test of flavonoids (Shinoda test)
To 2-3 ml of each fraction, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. A pink red or red coloration of the solution indicate the present of flavonoids.

Test for saponins
To 0.5 g of each fractions were added 5 ml of distilled water in test tube. The solution was shaken vigourously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously after which it was observed for the formation of an emultion.

Test for tannin
About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drop of 0.1 % ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for alkaloids
Zero point five g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloids base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream(with Mayer's reagent) or reddish brown precipitate(with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (keller-Killiani test)
To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicate the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Total flavonoid (Jia, 1999)
Flavonoid content was measured by the aluminum chloride colorimetric assay. Briefly, an aliquot(1ml) of extract or standard solution of quercetin (20, 40, 60, 80, and 100 mg/l) was added to 10 ml volumetric flask containing 4 mg of distilled water. To the flask was added 0.3 ml 5%$NaNO_2$, after 5 min, 0.3 ml 10% $AlCl_3$ was added. At 6th min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepare reagent blank at 510 nm. Total flavonoids content of extracts was expressed as mg quercetins (QE)/g extract. Samples were analysed in duplicates.

DPPH method (Pongtip, 2005)
Their fractionations were assessed for antioxidant activities in vitro using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay. The free radical scavenging activity of extract and the standard ascorbic acid solutions in absolute ethanol were determined base on their ability to react with the stable DPPH radical. A 750 µl aliquot of the extract (50-1000 ppm, dissolved in absolute ethanol) was added to 750 µl of DPPH in absolute ethanol (152 µM). After incubation at room temperature for 20 minutes, the absorbance of each solution was determined at 520 nm using a UV spectrophotometer. Percentage inhibition and the concentration of sample required for 50 % scavenging of the DPPH free radical (EC_{50}) were determined. Ascorbic acid was determine the same method as the reference standard.

FRAP method (Penpun, 2008)
Preparation of Frap reagent
This solution was dissolve in distilled water. FRAP reagent prepared by mixing 30 mL of 0.3 mol/L sodium acetate buffer pH 3.6, 3 mL of 0.01 mol/L TPTZ in 0.04 mol/L HCl and 3 mL of 0.02 mol/L $FeCl_3 \cdot 6H_2O$.

Calibration curves of $FeSO_4$
Ferrous sulfate was used for preparing calibration curve in concentration 0.1, 0.5, 1, 5,10, 15, 20 and 25 µmol/10 mL in ethanol.the experiments were done in hexaplicate in each concentration.

For calibration, mixing 3,000 µL FRAP reagent, and 100 µL of each concentration of standard solutions. Absorbance was moniterd at 593 nm for 30 mins. Blank was prepared by mixing 3,000 µL FRAP reagent and 100 µL ethanol.

Calculation of antioxidant activity
Calibration curve was plotted between concentration of Ascorbic acid and the average of absorbances of each concentration at 30 mins. the coeffient of determination(r^2)was calculated from the graph. All experiments were done in hexaplicate by mixing 3 mL FRAP reagent, and 100 µL(0.1, 0.5, 1, 5 and

10 mg/ml) in each fractions and ascorbic acid was determined by the same method as the reference standard. Absorbance was moniterd for 593 nm at 30 min.

Statistical analysis

The statistical analysis of the data was carried out by analysis of the variance (ANOVA) and Scheffe's test to show measurements which can be considered statistically different. The criterion for statistical signiticance was *p*-value less than 0.05.

Results

Phytochemical screening of the methanol extract showed the presence of alkaloid, flavonoid, tannin and saponins. The antioxidant activity of the both method and flavonoid content of each extract are summarized as shown in Table 1.

Discussion and Conclusion

The antioxidant activity of the herbal extracts was assessed by the DPPH scavenging and FRAP assay. The DPPH assay results are known to correlate well with the lipid peroxidation inhibitory capacity of a test compound (Rajbir, 2007). The FRAP assay was performed to estimate the capacity of the extract to reduce Fe 3+/Fe 2+. In the current study,the highest antioxidant activity in both assays was found in methanol extract followed by ethyl acetate extract. Antioxidants play a role in numberous diseases and also thought to protect against coronary heart diseases, skin aging, diabetes and Alzheimer's disease (Cornelli, 2009)

Since the highest activity was found in the methanol extract in this study, the phytochemical screening was performed in the extract. The methanol extract showed the presence of the presence of alkaloid, flavonoid, phenolics and saponins. The detected compounds could be responsible for the antioxidant activity. The current investigation also suggested that content of flavonoids in the herbal extracts is highly related to their antioxidant capacity.

In conclusion, *Tiliacora triandra* warrants further investigation for its ability to protect against diseases.

Table 1 The antioxidant activities and flavonoids content of *Tiliacora triandra* Diels extracts

The herbal extracts	EC_{50} of DPPH assay* (ppm)	Fe II equivalent (mmol/mg extract) of FRAP assay	Quecertin equivalent (mmol/mg extract)
Petroleum ether	113.81 ± 0.8542^{D}	0.49139 ± 0.0018^{D}	7.2150 ± 0.0790^{D}
Dichloromethane	75.57 ± 1.6791^{C}	0.3709 ± 0.0023^{E}	17.0367 ± 0.0590^{B}
Ethyl acetate	15.02 ± 0.4654^{B}	0.5762 ± 0.0008^{C}	14.7100 ± 0.0760^{C}
Methanol	9.63 ± 0.5628^{A}	0.7343 ± 0.1275^{B}	18.6767 ± 0.2795^{A}
Water	16.19 ± 0.4523^{B}	0.151 ± 0.0012^{F}	2.0100 ± 0.0730^{E}
Ascorbic acid	5.26 ± 0.5739^{A}	1.3752 ± 0.2383^{A}	-

* Means with different letters within a row show statistical differences ($p<0.05$) between lines.

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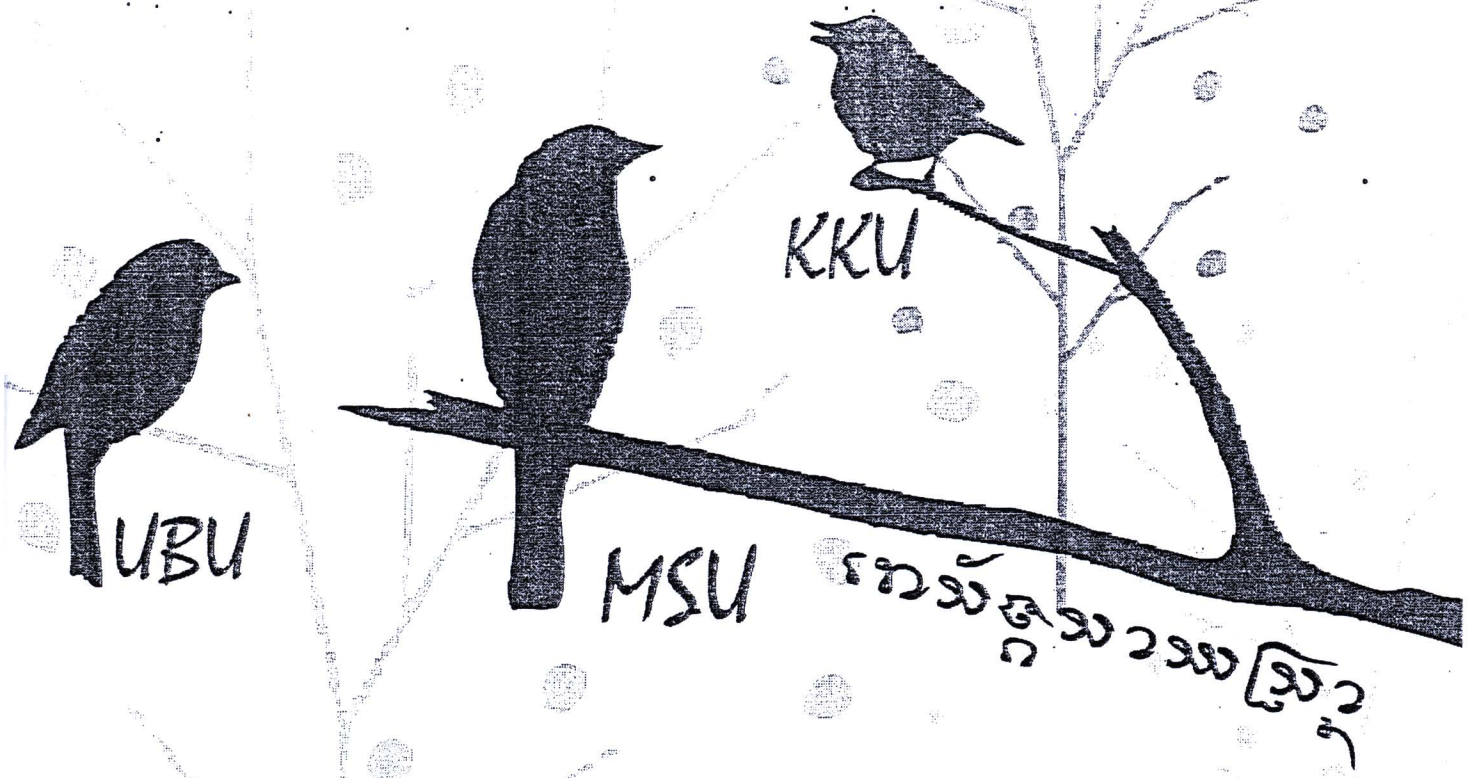

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