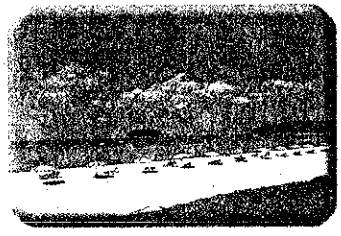
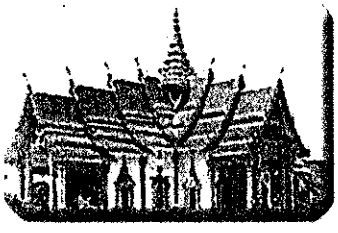
The 10th World Congress on Clinical Nutrition

Nutrition in the Next Decade: Nutraceutical/Functional Food, Product Performance, Health, Disease and Safety

PROGRAMS AND ABSTRACTS





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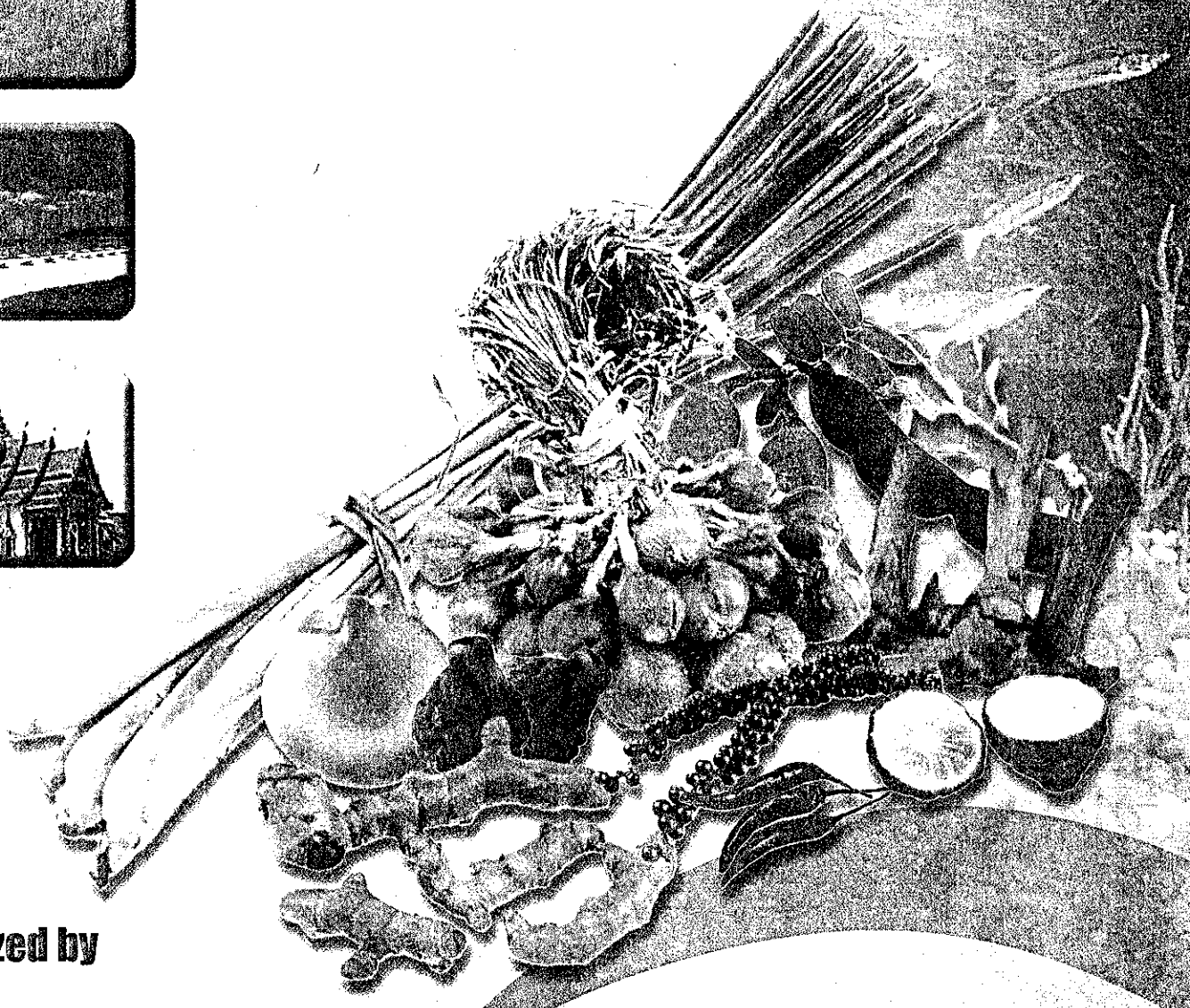

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P2.20. Total phenolic compounds and antioxidant and toxic properties of some plant extracts

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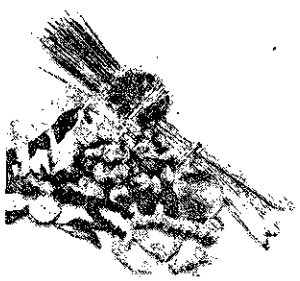
Free radicals have been found to be a significant cause of malfunctions in humans, leading to development of cancer, heart diseases, immune imbalance and other symptoms. Free radicals are atoms or groups of atoms with unpaired number of electrons and can be formed when oxygen interacts with certain molecules. They reactively start a chain reaction, like dominoes, with important cellular components such as DNA, or cell membrane leading to cell death. Some free radicals arise normally during metabolism. Sometimes the cells in the immune system purposely create them to neutralize viruses and bacteria. However, environmental factors such as pollution, radiation, cigarette smoke, pesticides and herbicides can also spawn free radicals. Antioxidants can get rid of free radicals successfully in the body, unless they are in excess. Antioxidants are compounds that can reduce or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing of peroxide. In the present study, the total phenolic compounds of extracts from 15 plants were measured by Folin-Ciocalteau method. Antioxidants/anti-free radicals were analyzed according to Gallic Acid Equivalent (GAEs). Toxicity of plant extracts was determined by Brine Shrimp Lethal Assay using *Artemia salina*. It was found that solvent differences in extraction methods affected the amount of total phenolic compounds. There was no correlation between the amount of total phenolic compounds and antioxidant activity of the plant extracts. The three highest total phenolic compounds are from eucalyptus (152.84 mg/ml), brown salwood (144.57 mg/ml) and Siam weed (122.39 mg/ml), respectively. The three highest antioxidant activities are found in wood apple (26.28), Jew's mallow (51.42) and betel leaf vine (24.04), respectively. The three highest toxic plants are neem (LC_{50} = 0.99 µg/ml), Siam weed (LC50 = 1.30 µg/ml), and hedge flower (LC_{50} = 1.30 µg/ml), respectively. This may be due to the fact that each plant produces different phenolic compounds with differences in antioxidant and toxicity properties.



Total Phenolic Compounds, Antioxidant and Toxicity Properties of Some Plant Extracts

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Abstract

In the present study, the total phenolic compounds of extracts from 15 plants was determined by Folin-Ciocalteau method and antioxidants/anti-free radicals was elucidated as Gallic Acid Equivalent (GAEs). Toxicity of plant extracts was carried out by Microwell Cytotoxicity using *Artemia salina* (Brine Shrimp). It was found that solvents and extraction methods affected to the content of total phenolic compounds in extracts. No relationship among the total phenolic compound, antioxidant property and toxicity in each plant extract was found. This may be due to each plant producers different compounds in different amount. Sugar apple, Neem, Siam weed, Eucalyptus, Hedge flower and Marry gold flower had high toxicity, respectively.

Introduction

Nowadays, increasing utilization of synthetically chemical reagents leads to several diseases and high mortality of mankind, such as, cancer, heart diseases, malfunction of immune system. Plants produce secondary metabolites to protect themselves. These can be antioxidants, which scavenge free radicals, and toxic substances for other organisms. The body has a defense system of antioxidants to get rid of reactive free radicals, if they are not excess. The antioxidant activity of plant phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing of peroxide. It will be beneficial to human. Substances from plants also has toxic property, which can used for biological control of plants and animals.

Experiment

Determination of total phenolic compounds, antioxidant and toxicity properties will be measured in 15 plants as shown in the followings:


ดาวเรือง: African Marigold (*Tagetes erecta* Linn.)


กระถินณรงค์: Wattle (*Acacia auriculiformis* Cunn.)


ใบย่านาง: Yaanaang (*Tiliacora triandra* Diels.)


พญากรอง: Hedge Flower (*Lantana camara* Linn.)


ใบขี้เหล็กอเมริกัน: American Cassia, Golden Wonder (*Cassia spectabilis* DC.)


กระถินเทพา: Brown salwood (*Acacia mangium* Willd)


ใบน้อยหน่า: Sugar Apple Leaf (*Annona squamosa* Linn.)


กระสัง หรือ มะสัง: Wood Apple (*Feronia lucida* Teysm & Binn.)


ปอโมโรเฮยะ: Jew's mallow (*Corchorus olitorius* L.)


ใบชะพลู: Betel Leaf Vine (*Piper betel* Linn.)


สะเดาไทย: Neem Tree (*Azadirachta indica* Juss. var. *siamensis* Valenton)


ใบเตย: Pandanus (*Pandanus amaryllifolius* Roxb.)


ชบา: Chinese Hibicus (*Hibicus rosa-simensis* Linn)


สาบเสือ: Chromolaena odorata (L.), King et Robins. (*Eupatorium odoratum* L.)


ยูคาลิปตัส: Lemon-scented Gum (*Eucalytus citriodora* Hook.)

1. Preparation for plant extracts

Plant samples were collected, and dried at 45°C for 2 days. The dried samples were ground and extracted in water or 95% ethanol by maceration or soxhlet extraction methods. Two grams of sample was extracted in 100 ml solvent for 3 h. Crude extract was dried by lyophilization at -54 ºC and stored at -20 ºC for further studies. The lyophilized extracts were redissolved in 50 ml of its original solvent and kept at 4ºC during study.

2. Determination of total phenolic compounds

The total phenolic compounds were measured using the method of Matthaus [1] and calculated using gallic acid as a standard. Results were expressed as milligram per gram of galic acid equivalents (GAEs).

3. Screening for antioxidant activity by spectrophotometric assay

In this study, 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical was used as a reagent for determining antioxidant property from plant extracts. The antioxidant activity was expressed as % radical scavenger which can be calculated from the following.

$$\% \text{ Radical Scavenger} = \{1 - (A_{SAMPLE} / A_{CONTROL})\} \times 100$$

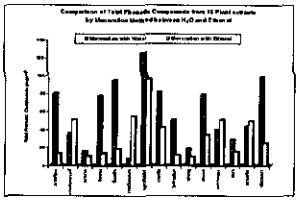
When A_{SAMPLE} = Absorbance of the mixture of plant extract solution and DPPH

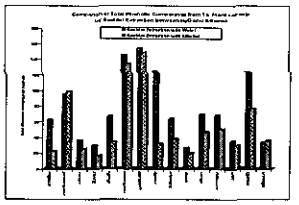
$A_{CONTROL}$ = Absorbance of the mixture of solute and DPPH

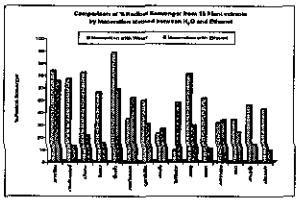
4. Screening for toxicity by Brine Shrimp Lethality Assay (BSLA)

The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity. *Artemia salina* was used since its response to the bioactive agents similar to that of mammalians'[2]. In this study, "microwell cytotoxicity test using *Artemia salina*" [3] was conducted. LC_{50} values will be calculated using "Probit Analysis" [4].

Results and Discussion







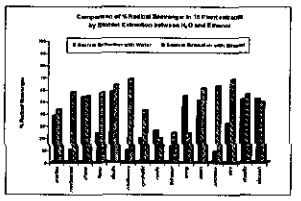


Table 1 Toxicity of plant extracts, by BSLA.

Plants	Water		95% Ethanol	
	Maceration LC_{50} (µL/mL)	Soxhlet Extraction LC_{50} (µL/mL)	Maceration LC_{50} (µL/mL)	Soxhlet Extraction LC_{50} (µL/mL)
ดาวเรือง	296.03 ± 1.36	121.92 ± 6.35	216.03 ± 0.48	98.35 ± 0.45
กระถินณรงค์	885.42 ± 3.80	481.83 ± 1.30	811.52 ± 0.43	347.67 ± 2.02
ย่านาง	8107.60 ± 6.37	7555.57 ± 2.90	6942.07 ± 1.80	6545.63 ± 23.12
ใบเตย	6711.96 ± 0.38	6266.75 ± 5.70	5984.36 ± 0.18	5452.35 ± 0.83
ขี้เหล็ก	4634.93 ± 41.03	4434.75 ± 4.70	3345.75 ± 5.21	3359.75 ± 6.40
กระถินเทพา	2225.24 ± 7.42	529.86 ± 0.52	1728.57 ± 0.55	914.06 ± 0.97
ยูคาลิปตัส	16.50 ± 0.50	15.00 ± 0.50	15.68 ± 0.19	14.24 ± 0.44
กระสัง	3463.94 ± 22.33	4861.10 ± 5.18	2597.92 ± 62.61	2976.92 ± 0.64
โมโรเฮยะ	6597.19 ± 5.44	5597.19 ± 4.80	4845.51 ± 0.81	5197.19 ± 0.79
ชะพลู	5381.98 ± 108.70	5154.97 ± 23.80	5241.73 ± 0.33	5102.59 ± 0.61
สะเดา	2.14 ± 0.42	1.94 ± 0.09	1.72 ± 0.07	2.33 ± 0.12
พญากรอง	69.86 ± 1.03	63.65 ± 1.16	36.33 ± 0.38	51.31 ± 0.40
ชบา	1411.08 ± 5.00	1278.41 ± 0.28	1194.31 ± 0.77	1139.08 ± 0.45
สาบเสือ	6.14 ± 0.54	7.15 ± 0.24	3.20 ± 0.43	3.01 ± 0.12
น้อยหน่า	1.32 ± 0.05	1.64 ± 0.07	0.99 ± 0.49	1.67 ± 0.98

Conclusions

It was found that differences in solvents and extraction methods affected the amount of total phenolic compounds. This can be explained that there was the relationship between the polarity of solvents and the structure of phenolic compounds. However, the efficiency of extraction methods, maceration and soxhlet extraction, cannot be concluded. There was no correlation between the total phenolic compound content and antioxidant activity of the plant extracts. Antioxidant activity mainly depended on the dissociation of hydrogen radical from phenolic compounds to form a stable compound with DPPH radical.

$$\underset{\text{DPPH Radical}}{Ph_2\text{-}N\text{-}N^{\bullet}\text{-}Ph\text{-}(NO_2)_3} \xrightarrow{H^{\circ}\ (\text{Hydrogen radical})} \underset{\text{Stable Compound}}{Ph_2\text{-}N\text{-}NH\text{-}Ph\text{-}(NO_2)_3}$$

The three highest total phenolic compounds were from eucalyptus (152.84 mg/ml), brown salwood (144.57 mg/ml) and Siam weed (122.39 mg/ml), respectively. While the three highest antioxidant activities were found in wood apple, Jew's mallow and betel leaf vine respectively. In addition, the three highest toxic plants were neem (LC_{50} = 0.99 µL/mL), siam weed (LC_{50} = 1.30 µL/mL), and hedge flower (LC_{50} = 1.30 µL/mL), respectively. This may be due to each plant produces different phenolic compounds with differences in antioxidant and toxic properties.

References

[1] Yen G-C., Hsieh, G-L. (1997) Antioxidant Effects on Dopamine and Related Compounds, **Biosci. Biotech.Biochem.** 61: pp 1646- 1649.

[2] Michael A.S., Thompson C.G, Abramovitz, M. (1956). *Artemia salina* as a Test Organism for a Bioassay. **Science.** 123: 464.

[3] Solis N.P., Wright W.C., Anderson M.M. (1993). Microwell Cytotoxicity using *Artemia salina* (brine shrimp). **Planta Med.** 59: 250-253.

[4] Finney D.J. (1971). Probit Analysis, 3rd Edition, U.K. **Cambridge University Press**: 333.