The International Horticultural Exposition :
Royal Flora Ratchaphruek 2011

ROYAL FLORA RATCHAPHRUEK 2011

THE INTERNATIONAL SYMPOSIUM ON MEDICINAL AND AROMATIC PLANTS

ISHS

HORTICULTURAL SCIENCE SOCIETY OF THAILAND
สมาคมพืชสวนแห่งประเทศไทย

DEPARTMENT OF AGRICULTURE

DECEMBER 15 – 18, 2011
THE EMPRESS HOTEL
CHIANG MAI, THAILAND

THE INTERNATIONAL SYMPOSIUM ON MEDICINAL AND AROMATIC PLANTS

ORGANIZED BY

DEPARTMENT OF AGRICULTURE
HORTICULTURAL SCIENCE SOCIETY OF THAILAND
INTERANTIONALSOCIETY FOR HORTICULTURAL SCIENCE

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SCIENTIFIC PROGRAM

14 December, 2011:

15:00 – 19:00 EARLY REGISTRATION AND POSTER PREPARATION

***15 December 2011* : HALL 1**

08:00 – 12:00 REGISTRATION

09:00 – 09:30 OPENING CEREMONY

- Welcome Address
  by : *Governor of Chiang Mai Province*

- Address from HSST
  by : *Representative of Horticultural Science Society of Thailand*

- Address from ISHS
  by : *Prof. Ian Warrington*
  Representative of International Society of Horticultural Science

- Report from the Organizing Committee
  by : *Mr. Jirakorn Kosaisawe*
  Director General of the Department of Agriculture

- Opening Address
  by : *Ms. Supatra Thanaseniwat*
  Permanent Secretary, Ministry of Agriculture and Cooperatives

09:30 – 10:00 BREAK

SESSION I KEYNOTE AND INVITED PAPERS (PLENARY HALL 1)

Chairperson : Dr. Mantana Milne
Secretary : Ms. Sasitorn Vorapitirangsi

10:00 – 10:30 Heritage of Chiang Mai Declaration on MAPs vs Recent Trends in Medicinal Plant Utilization and Research
A. Mathe (Hungary)

10:30 – 11:00 Curing Incurable Alzheimer's Disease with Thai Medicinal Plants
N. Chomchalow (Thailand)

11:00 – 11:30 Thai Traditional Medicine for Cancer Treatment
A. Itharat (Thailand)

11:30 – 12:00 ASEAN Harmonization in Regulation of Herbal Medicinal Products
S. Wongyai (Thailand)

12:00 – 13:30 LUNCH

SESSION II CONCURRENT CONTRIBUTED PAPER PRESENTATION – HALL 1
NATURAL PRODUCTION AND PROCESSING TECHNOLOGY

Chairperson : Prof. Akos Mathe
Secretary : Dr. Jarun Ditchaiwong

13:30 – 13:45 Traditional Knowledge on Medicinal Plants Used for the Treatment of Skin Diseases in Madurai District, Tamilnadu (India)
Palanichamy Mehalingam and Ponnaiah Gnanadurai Sam Shirley (India)

13:45 – 14:00 Selenium Concentrations of Some Spices Commonly Used in Turkey
Nazim Sekeroglu, Faruk Ozkutlu and Murat Tuncturk (Turkey)

14:00 – 14:15 Selection of Odor from Aromatic Flowers for Relaxing Emotion
S. Promsomboon, S. Korrapetphanee, M. Pothisonothai and P. Promsomboon (Thailand)

14:15 – 14:30 Fractionation of Active Components from *Piper cf. Fragile*. Benth Essential Oil as Aromatherapy for Antiobesity
L.K. Darusman and M.R. Utami (Indonesia)

14:30 – 14:45 BREAK

SESSION III CONCURRENT CONTRIBUTED PAPER PRESENTATION – HALL 1
PHARMACO DYNAMIC OF NATURAL SUBSTANCES

Chairperson : Prof. Arunporn Itharat
Secretary : Ms. Siriporn Pengrang

14:45 – 15:00 Biological Activity of Essential Oil from *Eucalyptus* Species: A Review
D. R. Batish (India)

15:00 – 15:15 Neuroprotective Activity of *Emila sonchifolia* (L.) DC. (*Asteraceae*)
S. Wijaya, K.N Ting, T.J Khoo and C. Wiart (Malaysia)

15:15 – 15:30 Phytochemical and Antimicrobial Studies of Selected Aromatic Plants in the Philippines (*Lantana camara* Linn., *Pandanus amaryllifolius* and *Piper betel*)
M. Patacsil, J. Manuel, L. Laruan, S. Cortez and T. Balangcod (Philippines)

15:30 – 15:45 Effect of Sniffing of *Kaemferia galanga* L. Essential Oils to Rats
I. Batubara, L.D. Assaat, T.T. Irawadi and T. Mitsunaga (Indonesia)

15:45 – 16:00 Effects of Aromatherapy on Horticultural Therapy for Participants' Moods and Emotions
S. Koura, S.M. Snyder, M. Tanaka, T. Oshikawa and N. Ogawa (Japan)

16:00 – 16:15 Influence of Biostress on Accumulation of Secondary Metabolites in *Hypericum* Species
J. Radusiene, Z Stanius, K. Karpaviciene and R. Mackinaite (Lithuania)

SESSION IV CONCURRENT CONTRIBUTED PAPER PRESENTATION – HALL 2 AGRICULTURE AND PRODUCTION

Chairperson : Dr. Narong Chomchalow
Secretary : Ms. Ratchanee Pattaravayo

13:30 – 13:45 Molecular Diversity Assessment of Cumin (*Cuminum cyminum* L.) Using AFLP Markers
Alireza Bahraminejad, Ghasem Mohammadi-Nejad and Mihdzar Abdul Kadir (Malaysia)

13:45 – 14:00 Altered Meiotic Chromosome and Reduced Seed Germination from Micropropagated Plants of Rhubarb (*Rheum rhaponticum* L.)
Yan Zhou, Yipeng Zhao and Zuguo Cai (China)

14:00 – 14:15 Genetic Resource Conservation and Bioprospecting of Medicinal Plants of Western Ghats of India
Ravishankar Rai V (India)

14:15 – 14:30 Effects of Growth Regulators and Explants on Callus Induction and Organogenesis in *Hypericum perforatum* L.
MH. Fotokian, I. Sharifi, SM. Khayyam Nikoei, D. Davoodi, T. Hasanlu, H. Habibi, A. Ghanbari, A. Kordenaeij, AM. Naji, G. Mohammadinejad, B. Nakhoda and S. Ramazani (Iran)

14:30 – 14:45 The Effects of Growth Regulators on Callus Induction and Plant Regeneration of Aromatic Rice (*Oryza sativa* L. cv MRQ 74)
A. Saleh, R. M. Taha and N. A.Hasbullah (Malaysia)

14:45 – 15:00 Effect of BA, NAA and 2,4-D on Micropropagation of Jiaogulan (*Gynostemma pentaphyllum Makino*)
A. Jala and W. Patchpoonporn (Thailand)

15:00 – 15:15 BREAK

SESSION V CONCURRENT CONTRIBUTED PAPER PRESENTATION – HALL 2 AGRICULTURE AND PRODUCTION

Chairperson : Prof. Dr. Surapote Wongyai
Secretary : Ms. Jaruwan maneeraj

15:15 – 15:30 Effect of BA and NAA on Micropropagation of Tea Tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel) *In Vitro*
A. Jala (Thailand)

15:30 – 15:45 Influence of Some Auxins and Cytokinins on Cytodifferentiation and Morphogenesis in *Lavandula officinalis chaix*
Urfi Fatmi, Devi Singh and V.B. Rajwade (India)

15:45 – 16:00 Effects of *Thymus Vulgaris* Essential Oil on Decay Resistance and Quality of Iranian Table Grape
M. Geransayeh, Y. Mostofi, V. Abdossi and M. A. Nejatian (Iran)

16:00 – 16:15 Screening of Biological Activities from *Nostoc* spp. (Cyanophyta)
A. Mahakhant, N. Jaisai and N. Wongsing (Thailand)

16:15 – 16:30 Effect of Clove Size on Rate of Drying and Powder Color
Ali Rezvani Aghdam and Ale Emrani Nejad seyed mohammad hosein (Iran)

16:30 – 16:45 Phytochemical Investigation of Wild *Colchicum szovitsii* Fisch. Growing in Armenia
S.Kh. Mairapetyan, H.M. Galstyan and N. I. Manukyan (Republic of Armenia)

17:00 – 18:30 POSTER SESSION

19:00 – 21:00 RECEPTION

***16 December 2011* :**

08:00 – 09:00 POSTER SESSION

SESSION VI CONCURRENT CONTRIBUTED PAPER PRESENTATION – HALL 1
PHARMACO DYNAMIC OF NATURAL SUBSTANCES

Chairperson : Assoc. Prof. Araya Jatisatienr
Secretary : Dr. Nataya Dumampai

09:00 – 09:15 Antifungal and Antibacterial Properties of Three Medicinal Plants from Malaysia, *Diodia sarmentosa*, *Hydrocotyle sibthorpioides* and *Muehlenbeckia platyclada*
K.S. Kho and S. N. Weng (Malaysia)

09:15 – 09:30 Breeding of German Chamomile (*Matricaria recutita* L.) with High Content of (-)-α- bisabolol
J. Fejer, I. Salamon and T. Alshammari (Slovak Republic)

09:30 – 09:45 Antibacterial and Phytochemical Screening of Selected Medicinal Plants of Bayabas, Sablan, Benguet Province, Cordillera Administrative Region, Luzon, Philippines
Teodora D. Balangcod , Melba Patacsil, Jayjay Manuel, Sonny Cortez, Lianne Marie Victoria A. Laruan, Vilma L. Vallejo, Orlando Apostol and Rosemary M. Gutierrez (Philippines)

09:45 – 10:00 Phytochemical and Antibacterial Study of Lagerstroemia speciosa and its Ethnomedicinal importance to Indigenous Communities of Benguet Province, Philippines
Lianne Marie Victoria A. Laruan, Teodora D. Balangcod, Melba Patacsil, Jayjay Manuel, Sonny Martin Cortez, Vilma L. Vallejo, Orlando Apostol and Rosemary M. Gutierrez (Philippines)

10:00 – 10:15 Some Potential Medicinal Plants Used for Chronic Diseases in Maharashtra, India
C. B. Salunkhe and N. K. Dravid (India)

10:15 – 10:30 BREAK

SESSION VII CONCURRENT CONTRIBUTED PAPER PRESENTATION – HALL 1
AGRICULTURE AND PRODUCTION

Chairperson : Ms. Anchalee Chuphaputti
Secretary : Ms. Jidapa Supapon

10:30 – 10:45 Mahout Community – An Indigenous Knowledge Treasure of Medicinal Plants Used in the Healthcare of Domesticated Elephants of Maharashtra, India
C. B. Salunkhe and S. M. Moholkar (India)

10:45 – 11:00 Composition of the Essential Oils of *Teucrium lamiifolium* subsp. *lamiifolium* and *Teucrium lamiifolium* subsp. *stachyophyllum* from Turkey
Ayla Kaya (Turkey)

11:00 – 11:15 Chemical Composition of Essential Oils Obtained from the Leaves and Stems of *Eryngium caucasicum* Trautv. from Coastal Regions in the North of Iran
Davood Hashemabadi and Behzad Kaviani(Iran)

11:15 – 11:30 Antimicrobial and Antioxidant Activities from *Xylia xylocarpar* (Roxb.) Taub. Wood Extract
P. S. Nakmee and N. Nuengchamnong (Thailand)

11:30 – 11:45 Antinociceptive and Anti-inflammatory Activities of Leaf Extracts of Ethnomedicinal Plant, *Kleinia grandiflora*
Navis Jeyaraj Jeffrey Bose and Palanichamy Mehalingam (India)

11:45 – 12:00 Suppression of Growth and Pathogenecity of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aerugenosa*, and *Salmonella typhimurium* with Plant Extracts of Selected Indigenous Semi-temperate Crops in the *Philippines Cordilleras*
Jay Jay Fernandez Manuel, Balangcod Teodora, Patacsil Melba, Cortez Sonny Martin and Laruan Lianne Marie Victoria (Philippines)

12:00 – 13:00 LUNCH

Neuroprotective Activity of Tassel Flower (*Emila sonchifolia*)

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Keywords: antioxidant, cytoprotective, anti-inflammatory, acetylcholinesterase inhibitor

Abstract

A phytochemical study on the ethanol extract of tassel flower (*Emilia sonchifolia*) led to the isolation of senecionine-N-oxide (1), kaempferol 3-O-rutinoside (2) and protocatechuic acid (3). The structures of these compounds were determined using spectroscopic analyses (UV, NMR and MS), with comparison of their spectral data with previously reported values. These compounds were reported for the first time for this plant. Neuroprotective properties of the ethanol extract and the isolated compounds were examined using antioxidant, cytoprotective, anti-inflammatory and acetylcholin-esterase inhibitory assays. Antioxidant activity was evaluated using ferric reducing antioxidant power (FRAP), β-carotene bleaching and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays. Compounds 2 and 3 displayed profound antioxidant capacities. Compound 2 elicited high cytoprotection with the percentage of protection 56.62% and significant inhibition of 5-lipoxygenase (5-LOX) activities with IC_{50} 44.59 µg ml^{-1}. Compound 2 is also acetylcholinesterase inhibitor.

INTRODUCTION

Approximately 1% of the population over the age of 65 and 4-5% of the population by the age of 85 suffer from the most common form of motor system degeneration and the second most common neurodegenerative disorders, Amyothropic lateral sclerosis and Alzheimer's diseases (Seong et al., 2005; Guido et al., 2008). Neurodegenerative diseases are among the leading causes of death all over the world, with an incidence of about 2/1000 and 8% total death rate. This disease will become the world's second leading cause of death by the middle of the century, overtaking cancer (Menken et al., 2000; Kolominsky-Rabas et al., 1998).

Nature has been a continuous source of pharmacologically active molecules and medicinal herbs have been used by countless human generations (Iriti et al., 2010). Nevertheless, few plant extracts have been demonstrated to be neuroactive (Kelsey et al., 2010) such as epigallocatechin 3-gallate from green tea (*Camellia sinensis* L.), quercetin from apple (*Pyrus malus* L.), curcumin (*Curcuma longa* L.), rosmarinic acid and carsonic acid (*Rosamarinus officinalis* L.), allicin (*Allium sativum* L.) and resveratrol from red grapes (*Vitis vinifera* L.). Among the most promising source of antioxidant and antibacterial principles is the Malaysian flora, which embraces more than one hundred species of medicinal and culinary herbs, including members of the family *Asteraceae* Bercht. & J. Presl (HMRC, 2002).

Tassel flower (*Emilia sonchifolia* L.) (*Asteraceae* Bercht. & J. Presl) is a common tropical weed (Holm, 1997). In India, it is used to treat leprosy, ear disease, fever, asthma and diarrhoea (Holm, 1997; Rajakumar and Shivanna, 2009). In China, it is used to treat diarrhoea and gynaecological diseases (Lee et al., 2008). Young leaves of tassel flower are eaten with rice and in soup in Java and Malaysia (Gao et al., 1993). Preliminary studies reported the presence of sterols and fatty acids in the plant (Muko and Ohiri, 2000) as well as quercetin 3-O-galactoside (Nair and Srinivasan, 1982).

MATERIALS AND METHODS

Plants Collection and Extraction

The aerial part of tassel flower was collected from Semenyih, Selangor, Malaysia in March 2009 and identified by Dr. Christophe Wiart, University of Nottingham Malaysia. Voucher specimens (UNMC48W) were deposited in the herbarium of School of Pharmacy, Faculty of Science, University of Nottingham Malaysia. Air-dried and finely milled samples (300 g) were extracted by hexane, ethyl acetate, ethanol and water sequentially. The extracts were concentrated using rotary evaporator (Buchi, USA) under reduced pressure at 40°C. Dried extracts were kept at -20°C until further tests were carried out.

Antioxidant Assay

1. Ferric Reducing Power Antioxidant (FRAP) Assay. The antioxidant activity of the extracts was estimated by the FRAP method of Benzie and Strain (1996) with slight modifications. The working FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio. Briefly, 180 µl of the FRAP reagent was mixed with 20 µl of the test sample, to obtain a final concentration of 1/10. Readings were taken after 90 min. (λ: 593 nm) using a spectrophotometer (Dynex MRX-Revelation, USA). Ferrous sulfate concentrations in the range 1 µM to 125 µM ($FeSO_4.7H_2O$) were used for calibration. Trolox and quercetin were used as positive controls. FRAP values were calculated as Ferrous Equivalents: the concentration of trolox/quercetin or extracts, which produced an absorbance value equal to 1 mM of $FeSO_4$.

2. β-Carotene Bleaching Assay. The β-carotene bleaching assay was conducted according to Miller (1971) with some modifications. A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform. Two ml of this solution was pipetted into a 100 ml round-bottom flask. After removal of chloroform in vacuo, 40 mg of linoleic acid, 400 mg of Tween 80 and 100 ml of distilled water were added to the flask with vigorous shaking. The zero time absorbance was measured at λ: 490 nm using a spectrophotometer (Dynex MRX-Revelation, USA). Absorbance readings were recorded at 20 min. intervals for 240 min. A blank, devoid of β-carotene, was prepared for background subtraction. Percentage of antioxidant activity (AA) was calculated using the following equation: % AA = ((DR control-DR sample)/DR control) x 100, where DR is degradation rate of sample (DR= ln (initial absorbance at time zero)/(absorbance at 240 min.)/t (time in min.)). The effective concentration values exhibiting 50% of the antioxidant activity of samples (EC_{50}) were calculated from the graph of antioxidant activity percentage against concentration of the extracts.

3. 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay. The DPPH assay was conducted according to the method of Juan-Badaturuge et al. (2011).

Acetylcholinesterase Inhibitory-TLC Bioautography Assay

Acetylcholinesterase (500 U) was dissolved in 150 ml tris-hydrochloric acid buffer (0.05 M, pH=7.8) with 150 mg bovine serum albumin. Naphthyl acetate (125 mg) was dissolved in ethanol and fast blue B salt (400 mg) in distilled water. The spray reagent was prepared fresh by mixing 10 ml of naphthyl acetate and 40 ml of fast blue B salt. Ten µl of samples (10 mg ml^{-1}) were applied to silica thin layer chromatography (TLC) plates and eluted with proper solvent. The TLC plate was dried for complete removal of the solvent. The enzyme was sprayed to the TLC plate and kept in a water bath at 37°C for 20 min. to allow complete enzymatic reaction. The spray reagent was then sprayed onto the TLC plate. Acetylcholinesterase inhibitors showed up as white spots against a purple background (Enz et al., 1993).

Anti-Inflammatory Assay-5-lipoxygenase Assay

The anti-inflammatory activity of tassel flower crude extracts and its isolated compounds were determined using the method of Baylac and Racine (2003) with linoleic acid as the substrate. The crude plant extracts were prepared at the concentration of 50 mg ml^{-1}, meanwhile its isolated compounds were prepared at the concentration of 10 mg ml^{-1}. Five µl of sample was mixed with 970 µl of phosphate buffer (pH 9) and 17 µl of linoleic acid in a 1 ml cuvette maintained at 25°C. The mixture was shaken and 4 µl of the aliquoted enzyme and 4 µl of the phosphate buffer (4°C) were pippeted to initiate enzyme reaction. Absorbance was measured at λ: 234 nm over a period of 10 min. using spectrophotometer (Libra, USA). Absorbance was plotted graphically against the different concentrations used. Nordihydroguaiaretic acid (NDGA) was used as the positive control. The slopes of the straight-line portions of the sample and the control curves were used to determine the activity of the enzyme (Lourens et al., 2004).

Cytoprotection Assay

1. Cell Culture. The human hepatoma cell line (HepG2) were used for the cytoprotection assay. The cells were cultivated at 37°C in a humidified incubator containing 95% air and 5% CO_2, in RPMI-1640 medium containing 10% inactivated fetal bovine serum (FBS) with the addition of 1% antibiotic solution (pen-strep).

2. Antioxidative Cytoprotection Assay. Total 1.5 ml of 10^5 HepG2 cells/ml was inoculated into each well of a 24-well flat-bottom microplate, the 24-well microplate was cultivated for 24 hours at 37°C in an incubator containing 95% air and 5% CO_2. The medium (1.5 ml) was removed from each well and replaced with 1.5 ml of fresh medium containing the plant extracts/pure compounds/standard and incubated for 24 hours at 37°C. Removal of the medium was repeated after 24 hours, 1.5 ml of fresh medium containing 1 mM t-BHP, was added and kept at 37°C under 5% CO_2 for 5 hours. Five hours later, the medium was discarded and neutral red uptake assay was used to measure the viability of the cells. Neutral red solution (400 µl) was added and the plate was kept at 37°C under 5% CO_2 for an hour. Fixative solution (400 µl) was added to stop the reaction and absorbance (540 nm) was measured using a microplate reader. Quercetin was used as the positive control that showed antioxidative effect on cells.

Isolation of Bioactive Compounds

The ethanol extract (6 g) was fractionated by column chromatography on silica gel using a linear gradient from $CHCl_3$-MeOH to yield 91 fractions. The fractions obtained were grouped and coded A (1-32) and B (33-91). Separation on fraction B (1.2 g) on sephadex LH-20 using ethanol 100% and ethanol-water (9:1-7:3, v/v) successively, yielded 51 fractions. Fraction 22-51 were combined and fractionated by HPLC (Varian, Australia) series LC-940 liquid chromatography system with PDA. The separation was achieved on a Pursuit XRs C_{18} column (150×4.6 mm; i.d.: 10 µm) eluted with a linear gradient of methanol-water containing 1% formic acid from 30:70 to 70:30 in 15 min. The flow rate was 0.5 ml/min. and UV detection (PDA) was recorded between 190-400 nm. Structure elucidation of the isolated compounds was employed spectro-scopic techniques of mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. Mass spectrometry was performed on triple quadrupole mass spectrometer, Varian 325-MS with ESI interface, 212-LC pumps (Varian Inc., USA) meanwhile NMR spectrometry was performed on Bruker DRX 500 spectrometer for ^1H proton using CD_3OD solutions.

Statistical Analysis

All data were expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA followed by Tukey test using GraphPad Prism 5 software. A significant difference Aas considered at the level of $P<0.01$.

RESULTS AND DISCUSSION

The antioxidant properties of tassel flower were appreciated using FRAP, DPPH and β-carotene assays. The FRAP assay measures the total antioxidant activity of a sample by the reduction of ferric-tripyridyltriazine to an intense blue colouration ferrous complex, which is measured at λ: 593 nm (Sun et al., 2011). The results are defined as FRAP values: the concentrations of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mmol L^{-1} $FeSO_4.7H_2O$. Low FRAP values indicate high antioxidant activities. The extracts tested in our study displayed reducing abilities in the range of 0.24-18.81 µg ml^{-1} (Table 1). The ethanol extract elicited remarkable reducing abilities, with FRAP values inferior to the values obtained with the standards trolox and quercetin (1.14 and 1.31 µg ml^{-1}, respectively). This was confirmed by the DPPH assay where the same extract gave IC_{50} values equal to 0.15 µg ml^{-1}. The β-carotene bleaching method is based on the fact that linoleic acid produces a free radical, which is reduced by β-carotene. The presence of an antioxidant prevents the reduction of β-carotene, which remains yellowish-orange in colour (Krinsky, 1989). The EC_{50} values represent the concentration at which 50% of β-carotene is reduced. A low EC_{50} value indicates a potent antioxidant activity. The rank order of EC_{50} values were trolox > quercetin > ethanol > ethyl acetate > water > hexane. Overall, ethanol extracts of *E. sonchifolia* elicited profound antioxidant properties.

TLC bioautographic method is a simple and rapid method to determine acetylcholinesterase inhibitors from plant extracts. The test relies on the cleavage by acetylcholinesterase of 1-naphthyl acetate to form 1-naphtol, which reacts with fast blue B salt to give a purple coloured diazonium dye. The presence of acetylcholinesterase inhibitor will give white spot against purple background. This preliminary result showed no acetylcholinesterase inhibitor was present in tassel flower extracts (Table 2).

The mechanisms of inflammation involve a series of events in which the metabolism of arachidonic acid plays an important role. Lipoxygenase is known to catalyse the oxidation of unsaturated fatty acids containing 1-4 diene structures. The hexane extract of tassel flower inhibited the best enzymatic activity of 5-lipoxygenase (5-LOX) with an IC_{50} equal to 26.94 µg ml^{-1} (Table 2). Antioxidants and free radical scavengers have potential to reduce radicals and terminate synthesis of leukotrienes. Therefore, inhibition of the 5-lipoxygenase enzyme can also indirectly reduce free radical production (Ammon et al., 1993). Studies have implicated the oxygen free radicals in the process of inflammation as blocking agent in the cascade process of arachidonic acid metabolism by inhibiting lipoxygenase activity (Trouillas et al., 2003; Sreejayan and Rao, 1996). A combination of anti-inflammatory assay and antioxidant properties (Choi, 2002) constitutes a good indication on the potential anti-inflammatory activity of a drug (Alitonou et al., 2006), where it is believed that inhibition of the lipoxygenases is due to reaction of the inhibitor with free radicals (Takahama, 1985). Surprisingly for this study, preliminary screening of anti-inflammatory properties with the crude extracts of the species did not show positive correlations with antioxidant properties.

In this study, the potential cytoprotective effect of the ethanol extract of tassel flower against *tert*-butyl hydroperoxide (*t*-BHP)-induced oxidative damages in HepG2 cells was assessed. The ethanol extract and quercetin (standard) gave similar cytoprotective effect at dose of 25 and 50 µg ml^{-1}, respectively (Table 2).

Phytochemical analysis of the ethanol extract of tassel flower yielded 3 compounds. Senecionine-N- oxide (1), kaempferol 3-O-rutinoside (2) and protocatechuic acid (3), which were isolated for the first time. The MS and ^1H NMR data were compared with the literature review (Segal, 1985; Bao et al., 2003; Buniyamin et al., 2007). The descriptions of the compounds were listed:

Compound 1: Senecionine-N-oxide (5 mg), brown paste, $C_{18}H_{25}NO_6$; m.p 236°C. EIMS (70 eV, 210°C) m/z: 352 $[M+H]^+$, 336 $[M+H]^+$, 318 $[M+H–18]^+$, 308 $[M+H–28]^+$ and 290 $[M+H–18-28]^+$. ^1H NMR (CD_3OD, 500 MHz): δ 6.25 (1H, *d*), 4.62 (1H, *d*), 4.52 (1H, *d*), 3.99 (1H, *m*), 3.67 (1H, *d*), 2.98 (1H, *m*), 2.46 (1H, *m*), 5.50 (1H, *m*), 4.89 (1H, *m*), 5.53 (1H, *d*), 4.16 (1H, *d*), 1.60 (1H, *m*), 2.14 (1H, *q*), 1.78 (1H, *m*), 1.31 (3H, *s*), 0.90

(3H, *d*), 5.84 (1H, *d*), 1.85 (3H, *dd*).

Compound 2: Kaempferol 3-O-rutinoside (6.5 mg), yellow amorphous powder, $C_{27}H_{30}O_{15}$; m.p 200°C. EIMS (70 eV, 190°C) m/z: 595 $[M+H]^+$, 287 [M+H-rutinose moiety]$^+$. ^{1}H NMR (CD3OD, 500 MHz): δ 6.20 (1H, *br.s*), 6.40 (1H, *br.s*), 7.98 (2H, *d*), 6.88 (2H, *d*), 5.30 (1H, *d*), 4.39 (1H, *br.s*), 3 ~ 4 (16H), 1.18 (3H, *d*).

Compound: Procathecuic acid (5.1 mg), white powder, $C_7H_6O_4$; m.p 152-154°C. EIMS (70 eV, 190°C) m/z: 154 $[M+H]^+$, 137 $[M+H-OH]^+$, 109. ^{1}H NMR (CD3OD, 500 MHz): δ 6.92 (1H, *d*), 7.53 (1H, *d*), 7.5 (1H, *dd*).

Those compounds were tested for their antioxidant, anti-inflammatory, acetyl-cholineesterase inhibitory and the cytoprotective effects. Compounds 2 and 3 displayed profound antioxidant capacities. Compound 2 elicited high cytoprotective effect with a percentage of protection 56.62% (at concentration 25 μg ml^{-1}) and significant inhibition of 5-lipoxygenase (5-LOX) activities with an IC_{50} value of 44.59 μg ml^{-1}. Compound 2 also inhibited the enzymatic activity of acetylcholinesterase.

Considerable efforts have been made in recent decades to find natural products that could help prevent neurodegenerative diseases and even limit the effects of the aging process. tassel flower crude extracts and its constituents are antioxidant, cytoprotective, anti-inflammatory and acetylcholinesterase inhibitors and should be further studied for the development of neuroprotective agent(s).

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Tables

Table 1. Antioxidant properties of crude extracts and isolated compounds of *E. sonchifolia*.

Sample	FRAP assay FRAP value[1] (μg ml^{-1})	DPPH assay IC_{50}[2] (μg ml^{-1})	β-carotene bleaching assay EC_{50}[3] (μg ml^{-1})
Hexane extract	40.33 ± 0.03^{a}	0.74 ± 0.01^{a}	237 ± 0.11^{a}
Ethyl acetate extract	2.37 ± 0.02^{b}	0.17 ± 0.01^{b}	2.65 ± 0.51^{b}
Ethanol extract	0.91 ± 0.04^{c}	0.15 ± 0.01^{b}	1.07 ± 1.49^{c}
Water extract	2.98 ± 0.02^{d}	0.16 ± 0.01^{b}	4.6 ± 1.02^{d}
Kaempferol 3-O-rutinoside	n.d.	0.15 ± 0.02^{b}	2.89 ± 0.01^{b}
Senecionine-N-oxide	99.16 ± 0.02^{e}	n.d.	10.17 ± 0.00^{e}
Procathecuic acid	n.d.	52.84 ± 0.28^{c}	22.44 ± 0.01^{f}
Quercetin	1.31 ± 0.03^{f}	11 x 10^{3} ± 0.00^{d}	0.13 ± 0.17^{c}
Trolox	1.14 ± 0.02^{f}	9 x 10^{3} ± 0.00^{d}	0.05 ± 0.17^{c}

Data were obtained from three independent experiments, each performed in triplicates (n=9) and represented as mean ± SD. Values with the same letter are not significantly different (P<0.01) according to Tukey multiple comparison test.
[1]FRAP value was calculated as Ferrous Equivalents, the concentration of trolox/quercetin or extracts which produced an absorbance value equal to that of 1 mM Fe_2SO_4.
[2]IC_{50} was the concentration of substance that provides 50% inhibition.
[3]EC_{50} represents the effective concentration at 50% of total antioxidant activity.
n.d: not determined.

Table 2. Anti-inflammatory, cytoprotective and acetylcholinesterase inhibitor activities of crude extracts and isolated compounds of *E. sonchifolia* (L.) DC.

Sample	Cytoprotection assay % viability	Anti-inflammatory assay IC_{50} 5-LOX (μg ml^{-1})[1]	Anti acetylcholinesterase assay +/-
Hexane extract	n.d.	26.94 ± 0.02^{a}	-
Ethyl acetate extract	n.d.	133.06 ± 0.01^{b}	-
Ethanol extract	47.69[2]	113.19 ± 0.01^{c}	-
Water extract	n.d.	219.54 ± 0.01^{d}	-
Kaempferol-O-rutinoside	56.62[3]	44.59 ± 0.01^{e}	+
Senecionine-N-oxide	59.93[2]	98.07 ± 0.01^{c}	-
Procathecuic acid	37.96[3]	56.69 ± 0.00^{f}	-
Quercetin	47.87[4]	n.d.	n.d.
NDGA	n.d.	5.33 ± 0.05^{g}	n.d.

Data were obtained from three independent experiments, each performed in triplicates (n=9) and represented as mean ± SD. Values with the same letter are not significantly different (P<0.01) according to Tukey multiple comparison test.
[1]IC_{50} LOX was the concentration of substance that provides 50% inhibition.
[2]% viability of HepG2 cell lines in the level concentration of sample of 25 μg ml^{-1}.
[3]% viability of HepG2 cell lines in the level concentration of sample of 100 μg ml^{-1}.
[4]% viability of HepG2 cell lines in the level concentration of sample of 50 μg ml^{-1}.
n.d: not determined.