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Short Communication

Antimicrobial activity of Thai medicinal plants against beverage spoilage microorganisms and their potential in retarding Alzheimer's disease progression

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ABSTRACT: Thai medicinal plants (34 crude methanolic extracts) were tested for their antimicrobial activities, anti-acetylcholinesterase and antioxidant activities, total phenolic and flavonoid contents. *Acorus calamus*, *Cassia alata*, *Crocus sativus*, *Nymphaea lotus* and *Phyllanthus emblica* extracts showed strong antimicrobial activity against spoilage yeasts, lactic acid bacteria and acetic acid bacteria tested. Most susceptible yeast strains were *Rhodotorula glutinis* and *Schizosaccharomyces pombe*. *Kaempferia parviflora*, *Nelumbo nucifera*, *Rauvolfia serpentina* and *Centella asiatica* extracts exhibited strong acetylcholinesterase inhibitory activity (more than 70 % inhibition). *Terminalia chebula*, *Cinnamomum bejolghota*, *Uncaria gambir* and *P. emblica* extracts had the strongest antioxidant activity, EC_{50} of 387.23 – 490.47 μg extract/mg 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by DPPH radical scavenging activity method and reducing capacity of 656.19 – 774.41 mmol Fe(II)/g by ferric reducing antioxidant power (FRAP) method. *U. gambir* extract had the highest total phenolic and flavonoid contents (771.59 mg gallic acid equivalents (GAE)/g and 2,292.43 mg quercetin equivalents (QE)/g, respectively).

KEYWORDS: anti-acetylcholinesterase activity, antioxidant activity, phenolics, flavonoids, medicinal plants.

INTRODUCTION

Yeasts, lactic acid bacteria and acetic acid bacteria play an important role in spoilage of beverages such as wine, carbonated soft drinks, fruit and vegetable juices. Beverages spoiled by yeasts undergo changes in their physical and sensory characteristics. The metabolic activity of yeast is characterized by abundant gas production in acid containing beverages with or without sugar added. This causes deforming of the packages, cloudiness and sediment in the product, pellicle formation as well as formation of off-flavour dominated by off-taste and fermentation odor. Spoilage of beverages by lactic acid bacteria (*Lactobacillus*) and acetic acid bacteria (*Acetobacter*) also results in formation of compounds providing off-flavour[1]. These microorganisms have recently gained an increasing importance in beverage industries as they are responsible for significant economic losses. Nowadays, consumers demand increasingly for more natural food products with microbiological safety. Using natural antimicrobials from plants can be the effective way to prevent spoilage of beverages. However, the reports on antimicrobial activity of Thai medicinal plants against these spoilage microorganisms are still rare. There are still many species of Thai medicinal plants that have not been investigated on their antimicrobial activities and other phytochemical properties such as anti-acetylcholinesterase and antioxidant activities.

Alzheimer's disease (AD), a progressive, neurodegenerative disease is the most common cause of age-related dementia in the elderly. One in every eight persons over 65 years of age is estimated to suffer by AD. This disease has become a major health problem in society around the world. AD is characterized by progressive decline in memory with impairment of multiple cognitive func-

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tion. The symptoms of AD usually start with short-term loss of memory and continue with cognitive and emotional dysfunction, and language deficits, depression and behavioral disturbances in more advanced stage of the disease[2, 3]. Prevention of this disease is urgently needed to minimize its ill effects. Several evidences have indicated that the brains of patients with AD had decreased levels of acetylcholine, one of the main neurotransmitters in the central nervous system serving to increase attention and facilitate learning[4]. In AD patients, brain deficits in the cholinergic system were commonly reported. Thus, many treatments for AD have aimed at enhancing cholinergic transmission. Inhibitors of acetylcholinesterase, an enzyme responsible for hydrolysis of acetylcholine at the cholinergic synapse are used for symptomatic treatment of AD. They delay the breakdown of acetylcholine, thereby prolonging the cholinergic neurotransmission[3].

Numerous plants currently used for medical purposes are believed to be safer than synthetic drugs currently used for treating AD patients such as tacrine, donepezil, rivastigmine and rivastigmine which resulted in various adverse effect. Some of herbal drugs are used in traditional medicine to alleviate AD symptoms by enhancing cognitive function. Some of the alkaloid containing plants used traditionally as inhibitors of acetylcholine include physostigmine isolated from *Physostigma venenosom*, galantamine isolated from *Galanthus nivalis* and Huperzine A isolated from *Huperzia serrate*[2]. Although some of the herbal drugs have promised significant health benefits, they have been found to be either effective or ineffective, and some time showed adverse effects. These suggest the need to find new herbal drugs. Some researchers have recently studied the acetylcholinesterase inhibitory activity in plants from some countries[5, 6].

In recent year, some evidences have indicated that reactive oxygen species causing oxidative stress are associated with the pathology of AD. Oxidative stress is an important factor related to development and progression of AD and other forms of dementia[7]. A number of active compounds isolated from many plants have previously reported to possess both anti-acetylcholinesterase and antioxidant activities. These compounds included phenolic glycosides and flavonol glycosides in rhizome of black galingale (*Kaempferia parviflora*)[8], and flavones derivatives, sesquiterpenes and triterpenic acids in leaves of Asiatic Pennywort (*Centella asiatica*)[9]. Therefore, it is interesting to screen for the medicinal plants with multifunctional activities such as antimicrobial, anti-acetylcholinesterase and antioxidant activities for prevention of AD, aging and other diseases as a result of oxidative stress.

MATERIALS AND METHODS

Microorganisms

Twelve microbial strains (3 species of lactic acid bacteria (LAB), 1 species of acetic acid bacteria and 8 species of yeasts) were used in this study. *Lactobacillus casei* ssp. *casei* BCC 4308 was obtained from Biotec Culture Collection, Bangkok, Thailand. Three bacterial species, *Acetobacter aceti* TISTR 102, *Leuconostoc mesenteroides* TISTR 453 and *Lactobacillus plantarum* TISTR 050, and eight species of yeasts, *Candida lipolytica* TISTR 5655, *Debaryomyces hansenii* TISTR 5155, *Pichia membranaefaciens* TISTR 5093, *Rhodotorula glutinis* TISTR 5159, *Zygosaccharomyces rouxii* TISTR 5044, *Hanseniaspora uvarum* TISTR 5153, *Schizosaccharomyces pombe* TISTR 5205 and *Saccharomyces cerevisiae* TISTR 5019 were obtained from the Microbiological Resources Centre for Southeast Asian Region (Bangkok MIRCEN), Thailand.

CULTURE PREPARATION

The LAB cultures (*L. mesenteroides*, *L. plantarum*, and *L. casei* ssp. *casei*) were subcultured twice onto de Man Rogosa Sharpe (MRS) agar (Difco Laboratories, USA) and incubated for 24 h at 37 °C while *A. aceti* was subcultured onto Glucose Yeast Extract Agar (GYEA) and incubated at 30 °C for 24 h. All yeast cultures were subcultured twice onto Yeast Extract Malt Extract Agar (YMA) and incubated at 30 °C for 72 h. Then, a loopful of *A. aceti*, and each strain of LAB and yeasts was inoculated into 5 mL of GYE broth, MRS broth and YM broth, respectively. After incubation, cells were collected by centrifugation at 3000 ´g for 15 min, washed twice and resuspended in 0.1% peptone water. Turbidity was adjusted to match the turbidity of 0.5 McFarland standard for bacteria and 5 McFarland standard for yeasts to obtain an inoculum concentration of 10^7 and 10^8 CFU/ml, respectively.

Extraction of Plant Materials

Thirty three species of Thai medicinal plants (Table 1) were used this study. These plant materials were dried, cut into small pieces and ground to a fine powder. Then, 20 g of each were soaked in 200 ml methanol, and shaken at 200 rpm for 72 hours at ambient temperature. The mixtures were filtered. The filtrates were evaporated using vacuum rotary evaporator and air dried

Table 1: Thai medicinal plants used in this study

Botanical name	Common name/ *Thai name*	Family	Plant part
Acorus calamus L.	Mytle Grass / *Wan Num*	Araceae	Stem
Angiopteris evecta Hoffm.	Giant Fern / Wan Gieb Rad	Marattlaceae	Stem
Ardisia polycephala Wall	Philang kasa fruits	Myrsinaceae	Fruit
Ardisia polycephala Wall	Philang kasa leaves	Myrsinaceae	Leaves
Atractylodes lancea (Thunb.) DC.	Atractylodes rhizome /Goch kamow	Compositae	Rhizome
Cassia alata (L.) Roxb.	Ringworm Bush / Chumhedted	Caesalpiniaceae	Stem bark
Centella asiatica (L.) Urban.	Asiatic Pennywort / Buabok	Umbelliferae	Leaves
Cinnamomum bejolghota (Ham.) Sweet	Cinnamon/ Samunlaveang	Lauraceae	Stem bark
Clitoria ternatea L.	Blue pea / Aunchan	Leguminosae	Flower
Crocus sativus L.	Saffron / Yafaran	Lridaceae	Pollen
Dendrobium Sonia	Dendrobium orchids / Grouymai	Orchidaceae	Flower
Derris scandens Benth.	Hog Creeper / Tauwanpreang	Papilioneae	Stem
Foeniculum vulgare Mill.	Fennel / Tienkaupeaug	Umbelliferae	Fruit
Globba malaccensis Ridl.	Pudkon / Wanrontong	Zingiberaceae	Rhizome
Ginkgo biloba L.	Maidenhair tree / Gingko	Ginkgoaceae	Leaves
Holarrhena curtisii King & Gamble.	Phut Thung / Hatsakhunted	Apocynaceae	Stem
Imperata cylindrica Beauv.	Cogongrass / Rarkyaka	Poaceae	Root
Jasminum sambac (L.) Aiton.	Jasmine / Mali	Oleaceae	Flower
Kaempferia galanga Linn.	Aromatic ginger / Prokhom	Zingiberaceae	Rhizome
Kaempferia parviflora Wall. Ex Baker.	Black galingale / Krachaidam	Zingiberaceae	Rhizome
Lepisanthes fruticosa (Roxb.) Leenh.	Luna nut / Pumreang	Sapindaceae	Stem
Melodorum fruticosum Lour.	Lumduan	Annonaceae	Flower
Nelumbo nucifera Gaertn.	Sacred Lotus or Roseum plenum / Buasatabongkot	Nelumbonaceae	Petal
Nymphaea lotus Linn.	White lotus / Bualuangdeang	Nymphaeaceae	Petal
Orthosiphon aristatus (Blume) Mig.	Cat's Whisker / Yanuangmeaw	Lamiaceae	Whole plant
Phyllanthus emblica L.	Indian gooseberry / Makampom	Euphorbiaceae	Fruit
Polygonum odoratum Lour.	Vietnamese coriander / Pakpeaw	Polygonaceae	Leaves
Psophocarpus tetragonolobus Linn.	Winged bean/ Tuapu	Zingiberaceae	Fruit
Rauvolfia serpentina (L.) Benth.	Serpentine root / Rakrayom	Apocynaceae	Root
Saussurea lappa Clark	Costus / Gochkaduk	Compositae	Root
Terminalia chebula Retz.	Myrabolan wood/ Samoathai	Combretaceae	Fruit
Uncaria gambir (Hunter) Roxb.	Gambir/ Siseandted	Rubiaceae	Stem
Zingiber cassumunar Roxb.	Cassumunar ginger / Plai	Zingiberaceae	Rhizome
Zingiber officinale Roscoe.	Ginger /Kieng	Zingiberaceae	Rhizome

at 40 °C. Stock solutions of crude methanolic extracts were prepared by diluting the dried extracts with 10% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 200 mg/ml.

Antimicrobial Susceptibility Testing

Antimicrobial activity of all methanolic extracts of plants listed in Table 1 were tested against 12 microorganisms by disk diffusion assay and minimum inhibitory concentration determination.

Disk diffusion assay

The disk diffusion test was performed using the standard procedure as described by Jorgensen *et al*[10]. The inoculum suspension (100 ml) of each microbial strain was added and swabbed onto the surface of YMA for yeasts, MRS agar for LAB, and GYEA for acetic acid bacteria. Sterile 6-mm filter paper discs (What man, GE Healthcare UK Limited, Buckinghamshire, UK) were aseptically placed on YMA, MRS agar and GYEA surfaces. Crude methanolic extract (15 ml) was immediately added to the

paper disc. A 15-ml aliquot of 10% DMSO was added to the paper disc as a negative control while 15 ml of 100 units/ml penicillin G and 2.5 mg/ml amphotericin B were added to the paper discs as positive controls. The plates were incubated at 37 °C for 24 h for LAB, except for *A. aceti* which was incubated at 30 °C for 24 h, and for yeasts which were incubated at 30 °C for 48 h. Antimicrobial activity was evaluated by measuring inhibition zone diameters. The experiment was done in triplicate.

Determination of the minimum inhibitory concentrations

The minimum inhibitory concentrations (MICs) of all plant extracts against 8 yeast species and 4 bacterial species were determined using an agar dilution method[11]. Each plant extract at the final concentrations of 0.64 - 10 mg/ml was examined for the MIC. Negative control was performed using distilled water. Penicillin G (at the final concentration of 31.25 - 4000 units/ml) and amphotericin B (at the final concentration of 0.005 - 0.1 mg/ml) were tested as positive controls. After surface drying, a loopful of each microbial suspension was streaked onto the surface of each agar slant. After incubation at appropriate time and temperature, the growth of each microbial strain at different concentrations of plant extract was recorded. The lowest concentration of the plant extract that completely inhibited visible growth of each microbial strain was recorded as the MIC.

Acetylcholinesterase inhibitory activity assay

The anti-acetylcholinesterase activity of all plant extracts was determined according to the method previously reported by Ellman *et al*[12]. and Sancheti *et a* [13]. with slightly modification. Acetylcholinesterase from electric eel (E.C. 3.1.1.7, Sigma, Sigma-Aldrich, USA), acetylcholine iodide (ATCI, Fluka, Sigma-Aldrich, United Kingdom), 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB, Sigma, Sigma-Aldrich, USA) were employed. Galanthamine hydrobromide from *Lycoris* sp. (Sigma, Sigma-Aldrich, USA) was used as the standard drug. In this method, 240 ml acetylcholinesterase solution (0.025 U/ml), 120 ml sample (0.1 and 1 mg/ml of the plant extract in 30% methanol), 2,160 ml Tris-HCl buffer (50 mM Tris-HCl, pH 8) were mixed and incubated at 4 °C for 30 min. Then, 240 ml DTNB (0.3 mM) and 240 ml ATCI (1.8 mM) were added. The reaction mixture was incubated at 37 °C for 20 min. Then, the absorbance was measured at 412 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). The blank was prepared for correcting the background absorbance, in which the acetylcholinesterase enzyme was replaced by buffer. Control was performed in the same manner by replacing the sample with 30% methanol. The percentage of inhibition was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times (A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}$$

where $A_{Control}$ and A_{Sample} are the absorbance values of the control and the sample, respectively.

Antioxidant activity assays

Antioxidant activity of 34 methanolic extracts of plants was determined by two different methods including DPPH radical scavenging activity assay and ferric reducing antioxidant power assay.

DPPH radical scavenging activity assay

The free radical scavenging activity of the plant extracts was measured according to the method of Brand-Williams *et al*[14]. Each stock solution of extracts and a–tocopherol (a positive control) were prepared and diluted to the concentrations of 1 - 1,000 mg/ml in methanol. Each diluted extract (75 µl) at five concentrations was added to 2.925 ml of a 0.025 g/l 2, 2-diphenyl-1-picrylhydrazyl (DPPH, Aldrich, Sigma-Aldrich, Germany) solution in methanol. The reaction mixtures were then incubated in the dark for 30 min. The absorbance at 515 nm was measured at 0 and 30 min of incubation using the UV-Visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). To prepare a standard curve of DPPH, the absorbance of DPPH at different concentrations (0.025 - 0.0008 g/l) was measured at 515 nm. The remaining $DPPH^{\times}$ concentration in the reaction mixture was calculated from the DPPH standard curve, and the percentage of the remaining $DPPH^{\times}$ was calculated using the following equation:

$$\% \text{ DPPH}^{\times}_{\text{REM}} = [\text{DPPH}^{\times}]_T / [\text{DPPH}^{\times}]_{T=0}$$

Where $[DPPH^{\times}]_T$ and $[DPPH^{\times}]_{T=0}$ were the concentration of $DPPH^{\times}$ at steady state (30 min) and zero time, respectively. The percentage of the remaining $DPPH^{\times}$ in each reaction mixture of five different concentrations of all extracts was then plotted against µg of extract / mg of $DPPH^{\times}$ to obtain the amount of antioxidant or extract necessary to decrease the initial $DPPH^{\times}$ by 50% (EC_{50}). The EC_{50} values of all extracts were calculated by the following linear regression of plots, and the antiradical efficiency ($AE=1/EC_{50}$) values were also calculated.

$$[\% \text{ DPPH}^{\times}_{\text{REM}}] = b\,[\mu\text{g antioxidant/ mg DPPH}^{\times}] + a.$$

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity of the plant extracts by the FRAP method was analyzed according to the procedure as pre-

viously described by Lado *et al*[15]. To do FRAP assay, 1 mg/ml extract (100 µl) was mixed with 3.0 ml FRAP reagent (25 ml of 300 mm acetate buffer, 2.5 ml of 10 mm 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ, Fluka, Sigma-Aldrich, Switzerland) in 40 mm hcl and 2.5 ml of 20 mm $fecl_3.6H_2O$), and incubated at 37 °C for 5 min. The absorbance was measured at 594 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia) against blank (FRAP reagent without the sample). The concentration of Fe^{2+}-TPTZ (reducing capacity) was calculated by comparing the absorbance at 594 nm with the standard curve of the Fe (II) standard solutions (ferrous sulfate heptahydrate) at the concentration of 6–0.0469 mm. Alpha-tocopherol was used as a positive control. The reducing power of each extract was expressed as mmol Fe(II)/g extract.

Determination of total phenolic content

Total phenolic content of plant extracts was determined according to the method as described by Singleton *et al*[16]. The sample (100 µl of 1 mg/ml plant extract) was transferred to a volumetric flask containing 6 ml ultra-pure water. Folin-Ciocalteu's phenol reagent (VWR international S. A. S, France) (500 µl) was added, shaken thoroughly, and allowed to stand for 1 min. Then, 1.5 ml of 20% Na_2CO_3 and 1.9 ml ultra-pure water were added and mixed. After incubation for 30 min at 25 °C, the absorbance was measured at 760 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). A standard curve of gallic acid (10–1,000 mg/ml, Fluka, Sigma-Aldrich, Spain) was prepared using a similar procedure. The results were expressed as mg gallic acid equivalents (GAE) /g extract.

Determination of total flavonoid content

Total flavonoid content of plant extracts was determined according to the method as described by Kathirvel and Sujatha[17]. Briefly, 250 µl plant extract (1 mg/ml), 1.2 ml distilled water, 75 µl of 5% $NaNO_2$ solution were mixed together. The mixture was allowed to stand for 5 min at ambient temperature. Then, 150 µl of 10% $AlCl_3$ solution was added and the mixture was allowed to stand for 6 min before adding with 500 µl of 1M sodium hydroxide and 275 µl distilled water. After mixing well, the absorbance was measured at 510 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). Standard curve of quercetin (Sigma-Aldrich, Germany) at the concentration of 12.5–1000 µg/ml was prepared using similar procedures. The results were expressed as µg quercetin equivalents (QE) /g extract.

RESULTS AND DISCUSSION

Antimicrobial activity

Extracts of *C. sativus* (saffron) exhitbited the broadest inhibitory action against all yeast strains tested by disk diffusion test, followed by the extracts of *K. galanga* (aromatic ginger) and *A. calamus* (mytle grass) and *C. alata* (ringworm bush) (Table 2). *S. pombe* and *R. glutinis* were sensitive to most of the plant extracts. The extract of *N. lotus* (white lotus) effectively inhibited the growth of *R. glutinis* with the MIC of 0.64 mg/ml whereas the extracts of mytle grass, ringworm bush and white lotus showed the best inhibitory effect against *S. pombe* (0.64 mg/ml MIC) (Table 3).

Some plant extracts showed antibacterial activity against *A. aceti*. Among all plant extracts, the extract of saffron exhibited the widest inhibition zone diameter against *A. aceti* (15.56 mm) and 5.12 mg/ml MIC (Table 2 and 3), and ringworm bush extract showed the widest inhibition zone diameter against *L. casei* (16.67 mm) and *L. plantarum* (19.11 mm) by disk diffusion test (Table 2). Similarly, the extract of *R. serpentina* (serpentine root) was most effective at inhibiting *L. mesenteroides* (15.5 mm). *L. casei* was susceptible to ringworm bush extract (2.56 mg/ml MIC). Mytle grass extract could inhitbit the growth of *L. plantarum* at 5.12 mg/ml MIC, but ringworm bush extract was able to inhibit the growth of *L. mesenteroides* at 7 mg/ml MIC (Table 3). The positive control, a pure compound of penicillin G could effectively inhibit the growth of all bacterial strains tested (31.25–250 units/ml).

The antimicrobial action of saffron and mytle grass extracts may relate to their active components. Safranal and crocin, the main components in saffron were reported to inhibit the growth of several bacteria including *E. coli*, *S. aureus* and some serotypes of *Salmonella*[18]. Similarly, Rajput and Karuppayil[19] found that hexane extract of mytle grass rhizome and β-asarone isolated from this extract could inhibit the growth of *C. albicans* at the MIC of 10 mg/ml and 0.5 mg/ml, respectively.

The extract of ringworm bush, white lotus and Indian gooseberry had antifungal and antibacterial activities. Antimicrobial action of ringworm bush (*C. alata*) and white lotus (*N. lotus*) may relate to their active components. Rahman *et al*[20]. reported that 2,5,7,4´-tetrahydroxy isoflavone and 3,5,7,4´- tetrahydroxy isoflavone isolated from leaves of *C. alata* could inhibit the growth of several strains of human, animal and plant pathogenic fungi. Akinjogunla *et al*[21]. found that *N. lotus* leaves extract contained high concentration of anthraquinones, cardiac

Table 2: Antimicrobial activity of Thai local fruit extracts using disk diffusion test

Microorganism	Diameter of inhibition zone (mm) ± SD								
	A. calamus	***A. evecta***	***C. alata***	***C. asiatica***	***C. sativus***	D. sonia	K. galanga	L. fruticosa	N. lotus
Yeast									
Candida lipolytica	8.11 ± 0.69	-	-	6.67 ± 0.33	6.99 ± 0.02	-	7.78 ± 1.95	-	12.67 ± 2.03
Debaryomyces hansenii	-	-	-	-	12.67± 0.58	-	7.78 ± 0.19	-	-
Hanseniaspora uvarum	-	-	-	-	14.78 ± 0.84	-	-	-	-
Pichia membranaefaciens	7.00 ± 0.00	-	-	-	6.33 ± 0.38	-	-	-	-
Rhodotorula glutinis	9.89 ± 1.07	8.56 ± 0.19	24.56 ± 0.19	-	7.00 ± 0.00	13.11 ± 0.69	7.44 ± 0.19	7.89 ± 1.02	10.89 ± 1.17
Saccharomyces cerevisiae	7.67 ± 0.00	-	-	-	8.89 ± 0.19	-	7.89 ± 0.51	8.22 ± 0.96	-
Schizosaccharomyces pombe	8 .56 ± 0.51	13.33 ± 0.58	23.22 ± 0.69	7.00 ± 0.00	7.00 ± 0.00	11.11 ± 0.19	7.42 ± 0.63	9.22 ± 0.38	20.83 ± 2.93
Zygosaccharomyces rouxii	-	12.33 ± 0.33	6.94 ± 0.35	-	7.94 ± 0.35	-	7.67 ± 1.00	-	-
Bacteria									
Acetobacter aceti	-	-	-	9.61 ± 0.35	15.56 ± 0.19	9.39 ± 0.35	7.22 ± 0.19	8.06 ± 0.92	-
Lactobacillus casei	9.50 ± 0.17	-	16.67 ± 0.29	-	8.56 ± 0.39	-	-	-	-
Lactobacillus plantarum	9.78 ± 2.04	-	19.11 ± 0.19	-	7.50 ± 0.17	-	-	-	-
Leuconostoc mesenteroides	-	-	13.00 ± 0.00	-	-	-	-	-	-
Yeast									
Candida lipolytica	7.37 ± 0.84	-	-	-	9.78 ± 0.19	-	6.98 ± 0.04	12.33 ± 0.58	-
Debaryomyces hansenii	-	-	-	-	-	-	-	10.00 ± 0.00	-
Hanseniaspora uvarum	-	-	9.44 ± 0.84	-	-	-	-	12.33 ± 0.58	-
Pichia membranaefaciens	-	-	-	-	-	-	-	10.33 ± 0.58	-
Rhodotorula glutinis	7.44 ± 0.19	14.78 ± 0.51	7.22 ± 0.19	9.11 ± 2.27	10.44 ± 0.38	13.44 ± 0.51	7.00 ± 0.00	14.00 ± 0.00	11.00 ± 0.00
Saccharomyces cerevisiae	-	-	-	-	-	-	-	10.00 ± 0.00	-
Schizosaccharomyces pombe	20.44 ± 3.34	11.83 ± 0.76	10.18 ± 0.13	8.75 ± 0.58	17.00 ± 1.00	17.11 ± 0.19	7.00 ± 1.00	10.00 ± 0.00	7.00 ± 0.00
Zygosaccharomyces rouxii	-	-	-	7.97 ± 0.35	7.89 ± 0.69	-	-	14.33 ± 1.15	-
Bacteria									
Acetobacter aceti	-	8.00 ± 0.00	-	7.00 ± 0.00	-	-	9.61 ± 0.10	9.00 ± 0.00	-
Lactobacillus casei	-	-	-	-	-	-	-	10.00 ± 0.00	-
Lactobacillus plantarum	-	-	9.61 ± 0.09	-	-	-	-	9.00 ± 0.00	14.00 ± 0.00
Leuconostoc mesenteroides	-	-	15.50 ± 0.17	-	-	10.56 ± 0.19	-	8.00 ± 0.00	18.00 ± 0.00

Data are mean of three replications.

- indicates that no inhibition was observed.

Note: All plant extracts at 200 mg/ml, Amphotericin B at 2.5 mg/ml and Penicillin G at 100 units/ml were used to test the antimicrobial activity.

Table 3: Minimum inhibitory concentrations of Thai medicinal plant extracts using agar dilution method

Microorganism	Minimum inhibitory concentration (MIC, mg/ml)												
	A. calamus	*C. alata*	*C. asiatica*	*C. sativus*	*N. lotus*	*P. emblica*	*P.odoratum*	*R. serpentina*	*T. chebula*	*U. gambir*	*Z. officinale*	Amphotericin B	Penicillin G
Yeast													
Candida lipolytica	1.28	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.04	> 4000
Debaryomyces hansenii	5.12	10	>10	10	>10	>10	>10	>10	>10	>10	>10	0.005	> 4000
Hanseniaspora uvarum	7	10	>10	2.56	10	10	>10	>10	>10	10	>10	0.005	> 4000
Pichia membranaefaciens	0.64	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	0.01	> 4000
Rhodotorula glutinis	1.28	1.28	>10	>10	0.64	1.28	7	>10	10	>10	>10	0.04	> 4000
Saccharomyces cerevisiae	0.64	>10	>10	>10	10	10	10	10	>10	>10	>10	0.02	> 4000
Schizosaccharomyces pombe	0.64	0.64	>10	>10	0.64	1.28	>10	>10	>10	5.12	10	0.04	> 4000
Zygosaccharomyces rouxii	5.12	>10	>10	7	10	>10	>10	>10	>10	>10	>10	0.005	> 4000
Bacteria													
Acetobacter aceti	>10	>10	>10	5.12	10	>10	>10	>10	>10	>10	>10	>0.1	250
Lactobacillus casei	10	2.56	>10	>10	>10	>10	>10	10	>10	>10	>10	>0.1	31.25
Lactobacillus plantarum	5.12	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>0.1	31.25
Leuconostoc mesenteroides	>10	7	>10	>10	>10	>10	>10	>10	>10	>10	>10	>0.1	31.25

Units/ml for penicillin G

glycosides and terpenes, moderate concentration of alkaloids, flavonoids and phenolics, and low concentration of tannins and saponins. In addition, the antimicrobial activity and active components of Indian gooseberry fruit extract have been reported by some researchers[22,23]. The fruit extract of Indian gooseberry contained kaempferol 3-β-D-glucopyranoside, kaempferol, qu ercetin, isocorilagin and geraniin as well as its volatile compounds such as β-bourbonene, teracosane, palmitic acid, thymol, β-caryophyllene and undecane[22].

Anti-acetylcholinesterase and antioxidant activities

The plant extracts possessing strong acetylcholinesterase inhibitory activity (more than 70 % inhibition) were *K. parviflora* (black galingale), *N. nucifera* (sacred lotus), *R. serpentina* (serpentine root) and *C. asiatica* (asiatic pennywort) extracts at 1 mg/ml with 89.35, 75.25, 74.50 and 72.15 % inhibition, respectively followed by *C. bejolghota* (cinnamon) extract with 61.41 % inhibition. The extracts possessing moderate inhibitory activities (more than 40 % inhibition) were the extracts of *L. fruticosa* (luna nut), ringworm bush, winged bean, Indian gooseberry, aromatic ginger, *A. polycephala* (philang kasa fruit), white lotus and gambir (53.85–43.18 % inhibition at 1 mg/ml). The positive control, a pure compound of galantamine (1 mg/ml) had high inhibitory activity with 78.54% inhibition (Table 4).

The extracts of *T. chebula* (fruit of myrabolan wood), *C. bejolghota* (cinnamon), gambir, Indian gooseberry had strong antioxidant activity with the EC_{50} of 387.23–490.47 mg extract/mg DPPH (Table 4). The extracts with relatively strong DPPH scavenging activity were the extracts of lumduan, sacred lotus, *P. odoratum* (Vietnamese coriander), philang kasa fruits, white lotus, philang kasa leaves, ginger, *Foeniculum vulgare* (fennel), asiatic pennywort and luna nut with the EC_{50} of 895.29–1,935.39 mg extract/mg DPPH. Similarly, the plant extracts with the highest antioxidant power by FRAP method were the extracts of gambir, Indian gooseberry, cinnamon and fruit of myrabolan wood with reducing capability of 774.41 to 656.19 mmol Fe(II)/g extract. The plant extract with relative high reducing capability was white lotus extract (636.83 mmol Fe(II)/g extract) while the extracts of *O. aristatus* (cat's whisker), ginger, Vietnamese coriander, lumduan, philang kasa leaves, luna nut and sacred lotus had lower reducing capability of 365.49 to 189.58 mmol Fe(II)/g extract.

Total phenolic and flavonoid contents

The extract of gambir had the highest amount of total phenolics (771.59 mg GAE/g extract), followed by the extracts of cinnamon, Indian gooseberry, and white lotus (501.25, 405.06 and 312.42 mg GAE/g extract, respectively). The extracts with relatively high phenolic content were the extracts of myrabolan wood, lumduan and Vietnamese coriander (163.58, 117.22 and 108.33 mg GAE/g extract, respectively). Gambir extract had the highest flavonoid content of 2,292.43 mg QE/g extract, followed by the extracts of cinnamon, lumduan, ringworm bush, phut thung and Vietnamese coriander (1,317.61, 323.48, 242.41, 225.70 and 220.87 mg QE/g extract, respectively), and the other extracts with moderate to low amount of total flavonoids (Table 4). These results indicated that high phenolic and flavonoid contents of gambir, cinnamon and Indian gooseberry extracts related to their high antioxidant activities. Plant polyphenolics have been shown to be highly effective scavengers of most oxidizing molecules. Their high redox potentials allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Therefore, consumption of these plants may have been associated with lowered risks of diseases such as cardiovascular diseases, cancers, neurodegenerative diseases, diabetes and other diseases. These chronic diseases are considered to be caused by oxidative processes, especially those involving excess free radicals and reactive oxygen species[24].

In the current study, the extract of black galingale (*K. parviflora*) rhizome had the strongest anti-acetylcholinesterase activity (more than 70%). The analytical studies have shown that *K. parviflora* rhizome contained flavonoids[25], phenolic glycosides and flavonol glycosides[8]. For the extract of sacred lotus, Ingkaninan *et al*[6]. reported that 0.1 mg/ml stamen extract of this plant had 23.11 % inhibition of acetylcholinesterase activity. These results indicated that this plant extract may be used to improve memory. Yang *et al*[26]. reported that the methanolic extract of sacred lotus rhizome displayed significant improvements of memory functions and neurogenesis in the dentate gyrus of the hippocampus in the Wistar rat. The activity of this plant extract may be concluded mainly to be due to its phytoconstituents. Several bioactive compounds from these plant parts have been isolated and identified as belonging to different chemical groups such as alkaloids, flavonoids, glycosides, triterpenoids, vitamins and other compounds[27].

The extract of serpentine root exhibited strong anti-acetylcholinesterase activity, but moderate antioxidant activity. Serpentine roots are a rich source of several indole-type alkaloids. Wachsmuth and Matusch[28] isolated five anhydronium bases from a methanolic extract of *R. serpentina* roots. One of these anhydronium bases was the

Table 4: Phytochemical properties of Thai medicinal plant extracts

Thai medicinal plant extract	Common name	Acetylcholinesterase inhibition (%) ± SD		Antioxidant activity			Total Phenolic content (mg GAE/ g extract) ± SD	Total Flavonoid content (mg QE/ g extract) ± SD
		1 mg/ml	0.1 mg/ml	DPPH assay		FRAP assay (mmol Fe(II)/ g extract) ± SD		
				EC_{50} (µg extract/mg DPPH) ± SD	AE ($\times 10^{-3}$) ± SD			
Acorus calamus	Mytle Grass	23.17[a] ± 0.72	17.55 ± 0.96	42,218.57 ± 0.57	0.02 ± 0.61	123.07 ± 2.42	20.90 ± 2.62	37.45 ± 0.24
Angiopteris evecta	Giant Fern	26.12 ± 1.47	18.95 ± 1.88	4,581.61 ± 0.59	0.22 ± 0.63	96.44 ± 2.36	42.03 ± 0.45	93.29 ± 0.36
Ardisia polycephala	Philang kasa fruits	46.26 ± 0.46	22.51 ± 2.09	1,501.64 ± 1.63	0.67 ± 1.61	163.13 ± 1.71	38.22 ± 1.77	72.69 ± 0.35
Ardisia polycephala	Philang kasa leaves	35.37 ± 1.12	32.54 ± 0.48	1,680.61 ± 1.09	0.60 ± 1.01	224.18 ± 2.69	82.29 ± 2.60	115.83 ± 0.94
Atractylodes lancea	Atractylodes rhizome	27.70 ± 0.16	22.66 ± 2.37	10,361.36 ± 1.01	0.10 ± 1.04	51.95 ± 2.81	29.83 ± 2.46	32.92 ± 0.25
Cassia alata	Ringworm Bush	49.92 ± 1.85	25.43 ± 0.48	3,934.82 ± 0.52	0.25 ± 0.51	109.27 ± 2.75	80.40 ± 1.79	242.41 ± 0.81
Centella asiatica	Asiatic Pennywort	72.15 ± 2.08	34.65 ± 0.48	1,926.68 ± 0.22	0.52 ± 0.21	76.04 ± 2.83	46.08 ± 0.85	151.85 ± 0.61
Cinnamomum bejolghota	Cinnamon	61.41 ± 2.07	15.50 ± 2.67	457.82 ± 0.63	2.18 ± 0.67	657.69 ± 1.81	501.25 ± 2.59	1,317.61 ± 0.66
Clitoria ternatea	Blue pea	25.55 ± 0.72	23.79 ± 1.85	29,874.11 ± 1.78	0.03 ± 1.67	8.67 ± 2.90	24.91 ± 2.74	24.80 ± 0.24
Crocus sativus	Saffron	29.21 ± 1.94	21.64 ± 0.97	62,125.45 ± 1.05	0.02 ± 1.07	14.06 ± 1.69	8.14 ± 0.25	74.41 ± 0.34
Dendrobium Sonia	Dendrobium orchids	22.32 ± 0.48	3.90 ± 1.89	18,549.01 ± 1.63	0.05 ± 1.62	55.12 ± 1.28	27.13 ± 1.28	41.55 ± 0.14
Derris scandens	Hog Creeper	11.11 ± 0.43	6.82 ± 0.73	5,456.02 ± 2.25	0.18 ± 2.21	52.49 ± 2.23	85.23 ± 1.23	30.97 ± 0.20
Foeniculum vulgare	Fennel	30.51 ± 1.44	25.56 ± 1.54	1,731.79 ± 0.96	0.58 ± 0.98	156.03 ± 2.06	75.28 ± 3.13	168.98 ± 0.51
Globba malaccensis	Pudkon	22.10 ± 0.18	3.92 ± 0.92	36,564.12 ± 0.46	0.03 ± 0.54	14.65 ± 2.82	4.64 ± 0.42	25.38 ± 0.31
Ginkgo biloba	Maidenhair tree	32.73 ± 1.35	17.68 ± 1.26	6,124.09 ± 0.91	0.16 ± 0.92	64.86 ± 2.83	49.63 ± 2.35	50.32 ± 0.17
Holarrhena curtisii	Phut Thung	35.98 ± 5.47	25.08 ± 1.50	2,731.75 ± 0.33	0.37 ± 0.31	128.30 ± 1.91	75.78 ± 2.13	225.70 ± 0.73
Imperata cylindrica	Cogongrass	25.30 ± 2.15	19.87 ± 0.92	19,913.66 ± 1.10	0.05 ± 1.11	45.10 ± 2.39	22.66 ± 2.54	5.53 ± 0.16
Jasminum sambac	Jasmine	29.60 ± 1.46	7.84 ± 2.47	3,736.93 ± 1.55	0.27 ± 1.64	68.51 ± 2.08	57.26 ± 3.40	75.81 ± 0.62
Kaempferia galanga	Aromatic ginger	46.77[a] ± 2.04	38.33 ± 2.88	5,245.92 ± 2.31	0.19 ± 2.41	37.14 ± 1.87	5.30 ± 0.90	9.87 ± 0.02
Kaempferia parviflora	Black galingale	89.35 ± 0.49	49.92 ± 2.85	10,005.01 ± 1.20	0.10 ± 1.21	126.47 ± 2.72	24.62 ± 2.79	17.20 ± 0.18
Lepisanthes fruticosa	Luna nut	53.85 ± 0.14	23.00 ± 2.89	1,935.39 ± 0.01	0.52 ± 0.04	214.83 ± 2.56	79.50 ± 3.02	196.70 ± 0.81
Melodorum fruticosum	Lumduan	28.96 ± 1.61	24.70 ± 1.44	895.29 ± 2.38	1.12 ± 2.31	244.37 ± 2.96	117.22 ± 1.87	323.48 ± 0.31
Nelumbo nucifera	Secred Lotus	75.25 ± 2.60	43.79 ± 2.41	1,109.99 ± 0.57	0.90 ± 0.60	189.58 ± 2.87	93.01 ± 3.04	114.41 ± 0.31
Nymphaea lotus	White lotus	45.58 ± 1.41	30.16 ± 2.33	1,591.40 ± 0.80	0.63 ± 0.81	636.83 ± 2.64	312.42 ± 3.38	125.16 ± 0.17
Orthosiphon aristatus	Cat's Whisker	21.12 ± 2.37	13.06 ± 1.46	2,215.89 ± 1.56	0.45 ± 1.64	365.49 ± 2.60	94.12 ± 2.97	90.72 ± 0.19
Phyllanthus emblica	Indian gooseberry	47.97 ± 0.35	21.47 ± 1.74	490.47 ± 0.42	2.04 ± 0.41	740.28 ± 2.12	405.06 ± 3.22	119.00 ± 0.15
Polygonum odoratum	Vietnamese coriander	22.42 ± 0.13	19.76 ± 1.68	1,223.80 ± 2.71	0.82 ± 2.75	262.00 ± 1.56	108.33 ± 0.95	220.87 ± 0.88
Psophocarpus tetragonolobus	Winged bean	49.51 ± 0.45	18.52 ± 1.05	10,315.36 ± 1.27	0.10 ± 1.21	20.74 ± 2.36	17.50 ± 3.05	64.79 ± 0.24
Rauvolfia serpentina	Serpentine root	74.50 ± 0.33	40.47 ± 0.79	4,969.19 ± 0.65	0.20 ± 0.69	149.54 ± 1.62	75.78 ± 1.35	49.90 ± 0.32
Saussurea lappa	Costus	23.02 ± 1.89	3.58 ± 1.44	19,522.59 ± 0.53	0.05 ± 0.57	64.00 ± 1.69	24.71 ± 1.61	61.64 ± 0.54
Terminalia chebula	Myrabolan wood	27.68 ± 2.01	22.15 ± 1.44	387.23 ± 0.83	2.58 ± 0.81	656.19 ± 2.11	163.58 ± 2.45	64.79 ± 0.35
Uncaria gambir	Gambir	43.18 ± 1.63	36.15 ± 0.92	478.71 ± 0.51	2.09 ± 0.61	774.41 ± 1.86	771.59 ± 2.21	2,292.43 ± 2.00
Zingiber cassumunar	Cassumunar ginger	34.48 ± 0.23	25.73 ± 0.92	11,649.64 ± 0.87	0.09 ± 0.82	87.01 ± 1.61	20.53 ± 1.90	45.97 ± 0.32
Zingiber officinale	Ginger	30.64 ± 0.49	12.84 ± 0.40	1,715.44 ± 1.15	0.58 ± 1.65	266.59 ± 2.82	74.34 ± 2.37	147.75 ± 0.51
Galanthamine	-	78.54 ± 1.33	47.71 ± 1.70	-	-	-	-	-
α – Tocopherol	-	-	-	479.57 ± 0.87	2.09 ± 0.81	713.77 ± 2.48	136.95 ± 1.89	1,015.84 ± 4.63

Data are mean of three replications.

well-known serpentine. This compound had anticancer and antimalarial properties.

C. asiatica leaf extract has previously been found to possess anti-acetylcholinesterase and antioxidant activities, and has been used in folk medicine for strengthening nervous function and memory[5]. Several groups of compounds were found in *C. asiatica* including essential oil (0.1%), flavone derivatives, sesquiterpenes, triterpenic steroids, triterpenic acids (asiatic acid, 6-hydroxy asiatic acid and betulinic acid), triterpenic acid sugar esters (asiaticoside, braminoside and other compounds)[9]. However, the extract of aromatic ginger (*K. galanga*) rhizome showed moderate anti-acetylcholinesterase activity. This may be due to the presence of active compounds in its rhizome. Surveswaran *et al*[29]. identified the major phenolic compounds in Indian *K. galanga* rhizome and found to be phenolic volatile oils, flavonols (kaempferol) and phenolic acids (hydroxybenzoic acids).

Gambir and cinnamon extracts were the plant extracts with very strong antioxidant activity and high total phenolics and flavonoids. Kassim *et al*[30]. also found that methanolic extract of *U. gambir* at 50 ppm concentration had high DPPH radical scavenging activity of 85.98%, and contained total phenolics and flavonoids of 99.25 mg GAE/ g extract and 70.94 mg catechin equivalent/ g extract, respectively. The major flavonoids in this gambir extract were identified as catechin, (-)-gallocatechin and (-)-epigallocatechin. For the cinnamon (stem bark of *C. bejolghota*) extract, both antioxidant and anti-acetylcholinesterase activities were found to be relatively strong. These may relate to its high amount of total phenolics and flavonoids.

Fruit extract of *T. chebula* was found to possess very strong antioxidant activity. These findings are in agreement with other reports. Surveswaran *et al*[29]. reported that among all plant extracts tested the methanolic extract of *T. chebula* fruits from India had the highest antioxidant activity (679.69 mmol Trolox equivalents / 100 g dry weight by DPPH method, and 85.6 μmol Trolox equivalents/g dry weight by FRAP method). This *T. chebula* fruit extract had total phenolic content of 35.63 g GAE /100 g dry weight, and its major phenolic compounds were ellagitannins, gallotannins (punicalagin, chebulanin, corilagin, chebulagic acid, di/tri-galloyl-glucoses), ellagic acid, chebulic acid and gallic acid. These active components may be responsible for its antioxidant activity.

P. emblica (Indian gooseberry) fruit extract contained a high amount of phenolic compounds (290 mg/g extract) and ascorbic acid (11.21%)[23]. Liu *et al*[31]. reported that Indian gooseberry extract contained geraniin, quercetin 3-β-D-glucopyranoside, kaempferol 3-β-D-glucopyranoside, isocorilagin, quercetin and kaempferol. These purified compounds exhibited strong antioxidant activity. Therefore, this study suggested that 12 plant extracts had remarkable phytochemical properties. They are the extracts of *K. parviflora*, *N. nucifera*, *R. serpentina* and *C. asiatica* with strong acetylcholinesterase inhibitory activity, the extracts of *T. chebula*, *C. bejolghota*, *U. gambir* and *P. emblica* with strong antioxidant activity, and the extracts of *A. calamus*, *C. alata*, *C. sativus*, *N. lotus* and *P. emblica* with strong antimicrobial activity. These plant extracts may be used in combination as natural ingredients for production of long shelf-life food and beverage products rich in anti-acetylcholinesterase, antioxidant and antimicrobial phytochemicals providing health benefits for consumers.

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REFERENCES

1. Lawlor KA, Schuman JD, Simpson PG, Taormina PJ. Microbiological spoilage of beverages. In: Srer WH, Doyle MP, Editors. Compendium Microbiological Spoilage of Food and Beverages. New York: Springer Science Business media LLC. 2009; p. 245–284.
2. Howes MJ, Perry NS, Houghton PJ. Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. Phytother Res. 2003; 17: 1–18.
3. Heinrich M, Lee Teoh H. Galantamine from snowdrop-the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. J Ethnopharmacol. 2004; 92: 147–62.
4. White P, Hiley CR, Goodhardt MJ, Carrasco LH, Keet JP, Williams IEI, et al. Neocortical cholinergic neurons in elderly people. Lancet. 1977; 309: 668–71.
5. Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratili D, Padmaja R, et al. Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. J Ethnopharmacol. 2007; 109: 359–63.
6. Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J Ethnopharmacol. 2003; 89: 261–64.
7. Zhu X, Raina AK, Lee HG, Casadesus G, Smith MA, Perry G. Oxidative stress signaling in Alzheimer's disease. Brain Res. 2004; 1000: 32–9.
8. Azuma T, Tanaka Y, Kikuzaki H. Phenolic glycosides from *Kaempferia parviflora*. Phytochemistry. 2008; 69: 2743–48.
9. Brinkhaus B, Lindner M, Schuppan D, Hahn EG. Chemical, pharmacological and clinical profile of the East Asian medicinal plant *Centella asiatica*. Phytomedicine. 2000; 7: 427–48.
10. Jorgensen JH, Turnidge JD, Washington JA. Antibacterial susceptibility test: dilution and disk diffusion methods. In: Murray PR, Barron ER, Praller MA, Tenover FC, Yolken RH, Editors. Manual of clinical microbiology. Washington, DC: ASM Press. 1999; 1526–62;
11. Collin CH, Lyne PM, Grange JM. Collin and Lyne's microbiology methods. New York: Oxford University Press Inc. 2001.

12. Ellman GL, Courtney KD, Andres jr. V, Featherstone RM. A new rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7: 88–95.
13. Sancheti S, Sancheti S, Um B-H, Seo S-Y. 1,2,3,4,6-penta-O-galloyl-b-D-glucose: A cholinesterase inhibitor from *Terminalia chebula*. S Afr J Bot. 2010; 76: 285–8.
14. Brand-Williams W, Cuvelier M, Bersert EC. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995; 28: 25–30.
15. Lado C, Then M, Varga I, Szóke E, Szentmihályi K. Antioxidant property of volatile oils determined by the ferric reducing ability. Biosciences. 2004; 59: 354–8.
16. Singleton VL, Orthofer R, Lamuela-Raventor RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocateu reagent. Methods Enzymol. 1999; 299: 152–78.
17. Kathirvel A, Sujatha V. In vitro assessment of antioxidant and antibacterial properties of *Terminalia chebula* Retz. leaves. Asian Pac J Tropical Biomed. 2012; 2: 788–95.
18. Pintado C, de Miguel A, Acevedo O, Nozal L, Novella JL, Rotger R. Bactericidal effect of saffron (*Crocus sativus* L.) on *Salmonella enterica* during storage. Food Control. 2011; 22: 638–42.
19. Rajput SB, Karuppayil SM. β-asarone, an active principle of *Acorus calamus* rhizome, inhibits morphogenesis, biofilm formation and ergosterol biosynthesis in *Candida albicans*. Phytomedicine. 2013; 20: 139–42.
20. Rahman MS, Ali MY, Ali MU. *In vitro* screening of two flavonoid compounds isolated from *Cassia alata* L. leaves for fungicidal activities. J Bio-Sci. 2008; 16: 139–42.
21. Akinjogunla OJ, Yah CS, Eghafona NO, Ogbemudia FO. Antibacterial activity of leave extracts of *Nymphaea lotus* (Nymphaeaceae) on Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Staphylococcus aureus* (VRSA) isolated from clinical samples. Ann Biol Res. 2010; 1: 174–84.
22. Liu X, Zhao M, Wang J, Luo W. Antimicrobial and antioxidant activity of *Emblica* extracts obtained by supercritical carbon dioxide extraction and methanol extraction. J Food Biochem. 2009; 33: 307–30.
23. Mayachiew P, Devahastin S. Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts. LWT-Food Sci Technol. 2008; 41: 1153–9.
24. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al. Antioxidant activity of plant extract containing phenolic compounds. J Agri Food Chem. 1999; 47: 3954–62.
25. Tewtrakul S, Subhadhirasakul S, Karalai C, Ponglimanont C, Cheenpracha S. Anti-inflammatory effects of compounds from *Kaempferia parviflora* and *Boesenbergia pandurata*. Food Chem. 2009; 115: 534–8.
26. Yang WM, Shim KJ, Choi MJ, Park SY, Choi B-J, Chang MS, Park SK. Novel effects of *Nelumbo nucifera* rhizome extract on memory and neurogenesis in the dentate gyrus of the rat hippocampus. Neurosci Lett. 2008; 443: 104–7.
27. Mukherjee PK, Mukherjee D, Maji AK, Rai S, Heinrich M. The sacred lotus (*Nelumbo nucifera*)-phytochemical and therapeutic profile. J Pharm Pharmacol. 2008; 61: 407–22.
28. Wachsmuth O, Matusch R. Anhydronium bases from *Rauvolfia serpentina*. Phytochemistry. 2002; 61: 705–9.
29. Surveswaran S, Cai Y-Z, Corke H, Sun M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem. 2007; 102: 938–53.
30. Kassim MJ, Hussin MH, Achmad A, Dahon NH, Suan TK, Hamdan HS. Determination of total phenol, condensed tannin and flavonoid contents and antioxidant activity of *Uncaria gambir* extracts. Majalah Farmasi Indonesia. 2011; 22: 50–9.
31. Liu X, Cui C, Zhao M, Wang J, Luo W, Yang B, et al. Identification of phenolics in the fruit of emblica (*Phyllanthus emblica* L.) and their antioxidant activities. Food Chem. 2008; 109: 909–15.