Industrial Crops & Products 124 (2018) 548–554

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Biological and neuroprotective activity of Thai edible plant extracts

Pimmada Junsathian[a], Kanokwan Yordtong[a], Henry M. Corpuz[b], Shigeru Katayama[b], Soichiro Nakamura[b], Saroat Rawdkuen[a,c,*]

[a] Program of Food Science and Technology, School of Agro-Industry, Mae Fah Luang University, Muang, Chiang Rai, 57100, Thailand
[b] Department of Bioscience and Biotechnology, Faculty of Agriculture, Shinshu University, 8304 minamiminowa, Ina, Nagano 399-4598, Japan
[c] Unit of Innovative Food Packaging and Biomaterials, School of Agro-Industry, Mae Fah Luang University, Muang, Chiang Rai, 57100, Thailand

ARTICLE INFO

Keywords:
Alzheimer's disease
Amyloid-beta (Aβ)
Antioxidant capacity
Neuroprotective activity
Thai edible plant

ABSTRACT

Polyphenols present in plant-diets as natural antioxidant substances play important roles in prevention of Alzheimer's disease associated with deposits of amyloid beta (Aβ) peptide. Aβ is peptide pathogenesis which increases reactive oxygen species production in neuron, resulting cell death. This study aimed to quantify various biological and neuroprotective activities in some varieties of Thai edible plants. Total phenolic content (TPC), flavonoid content (TFC), antioxidants, and other neuroprotective activities work against Aβ aggregation in human neuroblastoma cells (SK-N-SH) are all investigated. Cytotoxicity was also analyzed. The highest TPC and TFC were observed in an extract from *Anacardium occidentale* L. leaves. The highest antioxidant activity by DPPH radical scavenging and Ferric reducing antioxidant power assays was found in the extracts from leave and flower of *Careya sphaerica* Roxb. In the SK-N-SH cell, the high protective effect of *Anacardium occidentale* L. significantly ameliorated cytotoxic effects induced by H_2O_2. Leaves of *Anacardium occidentale* L. demonstrated to be the most active extract against Aβ fibril induced by Aβ peptide aggregation. The results conclude that *Anacardium occidentale* L. contains some antioxidant compounds that can potentially be used to treat neurodegenerative diseases as well as other plants.

1. Introduction

Alzheimer's disease (AD) is well-known as a neurological disorder in humans. The major symptomatic elements of AD is characterized by neurofibrillary tangles (NFTs), deposition of senile plaques that are composed of amyloid beta (Aβ) peptide, and synapse loss (Badshah et al., 2015; Thummayot et al., 2014). As of now, the main cause of AD still remains unclear; however, many studies have suggested that Aβ plays a significant role. Aβ peptide consists of 40–42 amino acids. The brain of an Alzheimer's patient has more amyloid fibrils than a healthy brain does (Mao and Readdy, 2011; Thummayot et al., 2014). The aggregation of Aβ peptides formed in the β-sheet structure (cross-β conformation), as well as the accumulation of Aβ, are neurotoxic. This is because Aβ aggregation can induce oxidative stress in cells (Mao and Readdy, 2011; Thummayot et al., 2014). There are studies have demonstrated that oxidative stress is one of the prime causes for cell damage and death, especially in brain cells that associate with the pathologies and development of AD (Christen, 2000; Huang et al., 2016). Recent studies revealed that oligomeric Aβ42 could induce reactive oxygen species (ROS) production (Mao and Readdy, 2011; Thummayot et al., 2014). Normally, small traces of ROS may not be toxic, but excessive amounts may lead to oxidative stress, which is the primary factor in neurodegenerative diseases (Birben et al., 2012). Interestingly, antioxidants are classified as a remover of free radicals, or a scavenger of ROS generation, which works against oxidative stress (Mao and Readdy, 2011; Thummayot et al., 2014; Shivapriya et al., 2015). Antioxidants can reduce ROS production, tissue damage, and improve cell survival. Chemical compounds in plants known as phytochemical compounds can contain phenolic and flavonoid groups, which have potential health benefiting properties such as protection against oxidative damage to the cell, prevention of AD, as well as many types of cancer (Kumari and Jain, 2012; Custódio et al., 2015; Shivapriya et al., 2015). Plants (fruits, vegetables, or medicinal plants) may contain a wide variety of phenolic compounds such as phenolic acids and flavonoids that are rich in antioxidant activity. It has been reported that water curcumin and water-soluble polyphenol like tannic acid could inhibit Aβ fibrils formation (Ono et al., 2004).

This study draws attention to extracts from Thai edible plants (TEPs). TEPs used are *Azadirachta indica* (Neem leaves and flowers), *Careya sphaerica* Roxb. (Kradon leaves and flowers), *Glochidion*

* Corresponding author at: Program of Food Science and Technology, School of Agro-Industry, Mae Fah Luang University, Muang, Chiang Rai, 57100, Thailand.
E-mail address: saroat@mfu.ac.th (S. Rawdkuen).

https://doi.org/10.1016/j.indcrop.2018.08.008
Received 10 April 2018; Received in revised form 11 July 2018; Accepted 5 August 2018

Perakense Hook.f (Monpu leaves), *Anacardium occidentale* L. (Cashew leaves), *Polygonum odoratum* Lour. (Pak-Pai), and *Caesalpinia mimosoides* Lamk. (Pak Pu-Ya). These varieties are all cultivated widely in northern and southern Thailand. All of them have been used as a folk medicine against general injuries and diseases. However, there is no study reporting the neuroprotective activity of these plants and little is known about their antioxidant activity. The objectives of this study were to (1) determine phenolic content, flavonoid content and antioxidant activity of TEP; (2) investigate the inhibitory effects on amyloid aggregation by observing the structural morphology of Aβ fibrils; and (3) evaluate the cytotoxicity and protective effects of some Thai edible plant extracts on human neuroblastoma (SK-N-SH) cells.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteu's phenol reagent, Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), Gallic acid, and quercetin were all purchased from SIGMA-Aldrich (St. Louis, MO, USA). Synthetic Aβ1-42 was purchased from Peptide Institute Inc. (Osaka, Japan). MEMα culture medium, ThT solution, and 30% H_2O_2 were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). SK-N-SH human neuroblastoma cells line was contributed by the food chemistry laboratory at Shinshu University (Nagano, Japan). MTT was purchased from SIGMA-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical reagent grade.

2.2. Preparation of Thai edible plant crude extracts

Samples investigated were *Azadirachta indica* (Neem leaves and flowers), *Careya sphaerica* Roxb. (Kradon leaves and flowers), *Glochidion Perakense* Hook.f (Monpu leaves), *Anacardium occidentale* L. (Cashew leaves), *Polygonum odoratum* Lour. (Pak-Pai), and *Caesalpinia mimosoides* Lamk. (Pak Pu-Ya). The samples were purchased from local fresh markets (Chiang-rai, Thailand) and collected from southern part of Thailand. All of samples are classify as edible plant species, which mostly use for Thai cuisine as well as some parts are used as folk medicine for treating injuries and diseases. Preparation of the plant extracts was performed according to the method described in Farid Dahmoune (2015) with some modifications. About 1 g of sample was stirred with 32 ml of distilled water for 30 s and heated using a household microwave (LG Electronics, Thailand) at 500 W for 62 s. The suspended solution was filtered through a filter paper (Whatman No.4). The filtrate was collected and freeze dried (FD 8–55, Australia). The crude extract powder was collected and stored in a freezer until further use. The samples were re-dissolved using distilled water.

2.3. Preliminary screening of Thai edible plant crude extracts

The Thai edible plant water-extracts were evaluated screening for the presence of phytochemicals constituents (tannins, flavonoids, steroids, and terpenoids) according to different methods (Matar et al., 2013) with some modifications. The extracts that observed the result following test method were selected for next study.

2.3.1. Test for tannins

About 2 ml of diluted extract was mixed with ferric chloride ($FeCl_3$) 1 ml. Blue coloration resulting from the addition of ferric chloride reagent indicated the presence of tannins in the extract.

2.3.2. Test for flavonoids

About 2 ml of diluted extract was mixed with 2 ml of methanol and then heated. A few drops of concentrated hydrogen chloride (HCl) were added and the occurrence of a red or orange color was indicative of the presence of flavonoids.

2.3.3. Test for steroids and terpenoids

About 1 ml of diluted extract was mixed with 3 ml of acetic anhydride. Concentrated H_2SO_4 was carefully added to the filtrate to promote the phase's separation. Blue or green color formation indicated the presence of steroids. Terpenoids were identified using the Salkowski test. 3 ml of crude extract was mixed in 2 ml of chloroform and 3 ml of concentrated Sulfuric acid (H_2SO_4). A red-dish brown color at the interface indicated the presence of terperoid.

2.4. Quantification of chemical constituents

2.4.1. Total phenolic content (TPC)

Total phenolic content was determined by the Folin–Ciocalteu assay (Deetae et al., 2012) with some modifications. The reaction was performed using a 96-well microplate. The sample extracts (30 µl) were transferred into a 96-well plate of 120 µl Folin–Ciocalteau's reagent (10-time dilution) and 150 µl 7.5% w/v sodium carbonate (Na_2CO_3). The mixture was then left to stand in the dark for 30 min at room temperature. The absorbance was measured at 765 nm using a microplate readerSpectrophotometer microplate reader (Thermo Scientific/ Multiskan GO, USA). The results express as milligram Gallic acid equivalents (GAE) per gram of crude extract. All samples were tested in triplicate.

2.4.2. Total flavonoid content (TFC)

Flavonoid content was determined by colorimetric analysis (Mohammed and Manan, 2015) with some modifications. The reaction was performed using a 96-well microplate. Briefly, 45 µl extract and 36 µl of a 5% w/v sodium nitrite ($NaNO_2$) were mixed and incubated for 6 min at room temperature. Then 36 µl of 10% w/v aluminium chloride hexahydrate ($AlCl_3{\cdot}6H_2O$) was added and incubated for 6 min at room temperature. Lastly, 180 µl of 10% w/v NaOH solution was added and incubated for 15 min. For the blank sample, distilled water was used to replace the sample. Absorbance was measured at 510 nm using a microplate reader (Thermo Scientific/ Multiskan GO, USA). Results were expressed as mg Quercetin equivalent (QE) per gram of crude extract. All samples were tested in triplicate.

2.5. Evaluation of antioxidant activity

2.5.1. Free radical DPPH scavenging activity

The antioxidant activities were measured by a DPPH method using the free radical 2,2-diphyenyl-picrylhydrazyl (Wang et al., 2011) with some modifications. Diluted extract 5 µl was added to 195 µl of 0.1 M DPPH solution, and the absorbance of the DPPH solution was measured at 515 nm after 30 min of incubation at room temperature using a microplate reader (Thermo Scientific/ Multiskan GO, USA). Ethanol 95% solution of Trolox in a range of 0–200 µM was used for calibration to compare the antioxidants. The antioxidant capacity of the sample was expressed as µM Trolox equivalents/100 g of crude extract. All samples were tested in triplicate.

2.5.2. Ferric reducing antioxidant power activity (FRAP)

The antioxidant power was measured using the Ferric Reducing Antioxidant Power method as described in Benzie and Strain (1996) with some modifications. 10 µl of the sample was added to 190 µl of FRAP solutions, and the absorbance was determined at 593 nm after 30 min of incubation at room temperature using a microplate reader (Thermo Scientific/ Multiskan GO, USA). The ferric reducing antioxidant power was expressed as µM $FeSO_4$/100 g of crude extract. All samples were tested in triplicate.

2.6. Evaluation of neuroprotective activity

2.6.1. Preparation of Amyloid beta (Aβ) fibrils (1 mM)

The content of Amyloid beta (Aβ) in the vial (0.54 mg) was