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Antioxidant Activities of Some Edible Thai Local Plant Extracts for Oxidative Stability in Low Fat Creamy Salad Dressing

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ABSTRACT: The aim of this research was to study the potential of three edible Thai local plant extracts on retardation of lipid oxidation. Those plants including *Glochidio sphaerogymum*, *Piper sarmentosum* and *Limacia triandra* were extracted by distilled water and 80% ethanol. All plant extracts contained mostly flavonoids but saponins was found in small amount. The *G. sphaerogymum* extracted with 80% ethanol showed the highest polyphenol content (990.24 µg GAE/ml) and DPPH radical scavenging activity (92.52%) while *P. sarmentosum* extracted with distilled water showed the highest inhibition of lipid peroxidation in thiobarbituric acid reactive substances (TBARS) (61.60%). Thus, two plant extracts were chosen for adding into low fat creamy salad dressing. Two concentrations including 200 and 500 µg/ml were used for analysis of sensory characteristics and lipid oxidation in creamy salad. It was found that *P. sarmentosum* extract at 200 µg/ml had the highest overall acceptability score of 7.50. Finally, all tested samples were kept at 35°C for 35 days for oxidative stability measurement by determination of change of PV every week comparing with control (no adding extract). This results indicated that *G. sphaerogymum* and *P. sarmentosum* extracted with 80% ethanol and distilled water had a high potential as natural antioxidant in low fat creamy salad dressing.

Keywords: *Glochidion sphaerogymum*, *Piper sarmentosum*, *Limacia triandra*, antioxidant, creamy salad dressing

INTRODUCTION

Lipid oxidation is one of the most problem in many foods. Oil is the major ingredient containing in emulsion products. Oil-in-water emulsion such as milk, salad dressing, mayonnaise, sauce, cream, etc. are susceptible to oxidation due to the large contact surface between lipid hydroperoxides in emulsion droplets and water-soluble prooxidants resulting in the propagation of oxidation reactions. These reaction causes the rancid odors and flavors, modifies texture and color, and decreases shelf life (Alamed *et al.*, 2009). To avoid this problem, synthetic food additives such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary buthyl hydro quinone (TBHQ), are widely used to act as chain-breaking antioxidants (Reische *et al*, 1998). However, there are more consumer s' tendencies toward the safety of food ingredients on their own health.

Plant extracts rich in phenolic compounds may be a good alternative to replace synthetic antioxidants to prevent lipid

oxidation. Many researches have been reported about effect of plant extracts for retarding lipid oxidation. Skowyra *et al.*, (2014) revealed that leaves and stalks of *Perilla frutescens* at 320 ppm was as effective as butylated hydroxyanisole (BHA) at 20 ppm in slowing down the lipid oxidation as measured by peroxide value, thiobarbituric acid reactive substances and hexanal content in oil-in-water system. Putthawan and Areekul (2013) reported that *G. sphaerogymum* and *Cratoxylum formosum* extracts have the most potential for oxidative stability in oil-in-water system. *Eugenia pollicina* leaf extract was also found to protect emulsions of soybean oil and extra virgin olive oil against lipid auto-oxidation (Ramful *et al.*, 2011).

In recent years, consumers have focus on processed foods rich in nutritional and functional properties such as more natural, low fat and free of synthetic food additives. In Thailand, many plants growing in the north of Thailand. *G. sphaerogymum, P. sarmentosum* and *L. triandra* are edible local plants consumed either cooked or raw. Therefore, they were selected to investigate the phytochemical and antioxidant properties with different solvents (distilled water and 80% ethanol) on oxidative stability in low fat creamy salad dressing. Also, analysis of sensory characteristic for development as a source of natural antioxidant to prolong quality and stability.

MATERIAL AND METHODS

Plant materials and reagents

All plants were collected from Chiang Rai province. Soybean oil was bought from a market in Chiang Rai, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), 2,2-diphenyl-l-picrylhydrazyl; DPPH, and gallic acid were purchased from Sigma (USA). Folin-Ciocalteu reagent was purchased from Carlo, Italy. Tween 80 was purchased from Merck (Germany). Chloroform and acetic acid were purchased from Lab Scan (Ireland).

Preparation of plant extracts

G. sphaerogymum, P. sarmentosum and *L. triandra* were washed, blanched (80 °C for 15 s) and dried at 50°C in a tray dryer until moisture content below to 10%. After size reduction, the dried samples were then powdered in blender. Sample powder was extracted with a ratio of 1:5 (25 g was blended with 125 ml of 80% aqueous ethanol and distilled water) at room temperature for 24 h and then filtered using a 0.45 µm filter. Then, the filtrate was concentrated by a rotary evaporator for 80% aqueous ethanol extract and by freeze dryer for distilled water extract. Dried crude extract of 0.2 g was dissolved in 10 ml of the same solvent to give a final concentration of 20 mg/ml and kept at -20°C until analyses.

Preparation of low fat creamy salad dressing

Low fat creamy salad dressing (30:70 w/w) was prepared by mixing soybean oil (30%) with other ingredients (70%) including egg yolk, sugar, sweetened condensed milk, lemonade, vinegar and salt. Plant extracts were added into the mixture to obtain the final concentration of 200 and 500µg/ml. The mixture was then homogenized at 11,000 rpm for 15 min.

Phytochemical screening

Flavonoids were determined by the method of Wadood *et al.* (2013). About 0.5 g of each selected plant extract were added in a test tube and 10 ml of distill water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract, followed by addition of 1 ml concentrated H_2SO_4. Indication of yellow

color shows the presence of flavonoid in each extract.

Terpenoids were determined by the method of Ayoola *et al* . (2008). About 0.5 g each of the extract was added with 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids

Tannins were determined by the method of Ayoola *et al.* (2008). About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Saponins were determined by the method of Ayoola *et al.* (2008). About 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Determination of Total Phenolic Content (TPC)

TPC was determined using Folin-Ciocalteu method (Singleton *et al.*, 1999). Briefly, each extract was diluted with distilled water. Each extract (0.5 ml) was added in test tube and adjusted volume to 10 ml with distilled water. Afterwards, 0.5 ml of Folin–Ciocalteu reagent was added and incubated at room temperature for 5 min. Finally, 2 ml of 10% sodium carbonate was added. The extracts were then placed in the dark for 10 min and the absorbance was measured at 760 nm. The results were shown as microgram of gallic acid equivalence (GAE)/ml of extracts.

Determination of Antioxidant Activities

DPPH free radical-scavenging assay

The method of Brand-Williams *et al.* (1995) was used for evaluating the DPPH free radical scavenging. Briefly, each extract was diluted with distilled water. Each extract (1 ml) was mixed with 3 ml of 0.2 mM DPPH in test tube and the extracts were kept in the dark at room temperature for 30 min. The blank was used as 1 ml of ethanol with 3 ml of DPPH. The absorbance was measured at 517 nm. The percentage of inhibition in DPPH radical of each plant extract was calculated as the following equation.

$$\% \text{ (DPPH radical scavenging)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Thiobarbituric acid reactive substances (TBARS)

TBARS described by McDonald and Hultin (1987) was used with some modifications. Briefly, each extract was diluted with distilled water. 0.2 ml of each extracts was added into test tube. After that, 0.8 ml of linoleic emulsion (1%) was added. Each tube was incubated in water bath at 50°C for 18 h. Then, 2 ml of TBARS reagent was added and boiled for 15 min. Then, the samples were cooled down with cold water and the absorbance was measured at 532 nm. The percentage of inhibition of each plant extracts was calculated as the following equation.

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

Sensory analysis

All samples were prepared with 5 formulations including creamy salad dressing added with 200 and 500 µg/ ml of two extracts as following; 0.4 g of extracts was soluble in 10 ml of distilled water. Then, 2.5 ml and 6.25 ml of solution were added into 500 g of creamy salad dressing to given the final concentration 200 and 500 µg/ ml of extracts respectively. Control is