ANTIOXIDATIVE AND ANTI-HISTAMINE-RELEASE ACTIVITIES OF *EXCOECARIA AGALLOCHA* L.

Sheikh Julfikar Hossain[1,4,*], Hitoshi Aoshima[1], Magdi El-Sayed[2,5*] and Firoj Ahmed[3]

[1]Applied Molecular Bioscience, Graduate School of Medicine, Yamaguchi University, 1677-1 Yoshida, Yamaguchi-shi 753-8512, Japan; [2]Department of Biological and Environmental Sciences, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi, 753-8515, Japan; [3]Pharmacy Discipline, Khulna University; [4]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh; [5]Botany Department, Aswan faculty of Science, South Valley University, Aswan 81528, Egypt.

Summary

From the dried powder of bark of *Excoecaria agallocha* L. (Euphorbiaceae), hexane (Hex), chloroform (Chl), ethyl acetate (EtA), ethanol (Eth), and water (DW) fractions were prepared to study their polyphenol content, antioxidative and anti-histamine-release activities. DW had the highest total polyphenol content, 348 mg gallic acid equivalent (GAE)/g followed by Eth (160.5 mg GAE/g). Using DPPH free radical scavenging, reducing power, measurement of total antioxidant activity and ionophore A23187-induced histamine-release assays, it was found that DW and Eth had high antioxidative and anti-histamine-release activities compare to other fractions. Thin layer chromatography (TLC) revealed that ellagic acid-like and lupeol-like compounds are found in Eth, and Chl fractions, respectively. Testing standards of lupeol and ellagic acid at 100 μg/mL, lupeol had a little inhibitory effect on histamine release (24.5%) while ellagic acid showed no effect at all. However, our results showed that bark of *E. agallocha*, especially DW and Eth fractions would be considered a potential source of antioxidative and anti-histamine natural products.

Keywords: Antioxidant, Anti-histamine, *Excoecaria agallocha*, Mangrove, Polyphenols

* Authors for correspondence:
Dr. Sheikh Julfikar Hossain
Tel.: 88-041-720171-3 ext. 227
Fax: 88-041-731244
E-mail: sjhossain_ku@yahoo.com
Dr. Magdi El-Sayed
Tel.: 81-50-1537-2965
Fax: 81-83-933-5820
E-mail: magradi2000@yahoo.com

Introduction

Exploration of wild plants as a source of health promoting agents has got much attention. This renewed interest is due to the growing evidence of the numerous healthful activities attributed to wild plants and their components. Many of the health-promoting activities such as anti-cancer, anti-diabetic, and anti-mutagenic activity of natural products, in some terms, may be related to antioxidative activity [1]. Fruits [2] and vegetables [3], as well as natural products such as polyphenols [4], have been reported to show antioxidative activity. Various components of plants such as polyphenols of tea [5], soybean [6] and apple [7]; flavonoids [8], polyunsaturated fatty acids [9], and fruits [2] suppress release of histamine or leukotrienes from basophils or mast cells, which in turn prevent the pathogenesis of type 1 allergy that arises from food proteins or air-borne antigens. For the prevention and treatment of various diseases in human including radical-induced diseases such as cardiovascular disorders, cancer, aging, inflammation and allergy, plant-based medicines are getting importance in current world due to its multiple beneficial effects in combating diseases with least side effects.

Excoecaria agallocha L. (Euphorbiaceae) (local name: Gewa) is a small tree with 6-10 cm alternate, pointed tips leaves, tiny flowers in spike, small-round fruits in clusters, and rough gray bark, and grows among mangrove areas flooded by the sea in the Sundarbans and Chittagong in Bangladesh. The plant is used to treat sores and stings from marine creatures. Smoke from the bark is used to treat leprosy, and the root pounded with ginger is used as an embrocation for swollen hands and feet. The bark and wood of the plant is also used to treat flatulence [10,11]. Reportedly, the plant has anti-HIV and anti-viral properties [12,13,14]. Antioxidative activity of the leaf extract of *E. agallocha*, was investigated and suggested that environmental plants exposed to strong sunlight might have an efficient antioxidant system in order to live [15]. However, no report exists on antioxidative activity of other parts such as bark, and root of the plant. No report, at all, describing anti-allergic activity of the plant. This work is the first reporting antioxidative and anti-allergic activities of the bark extract of *E. agallocha*.

Materials and Methods

Plant material

Excoecaria agallocha L. was collected from the Sundarbans' mangrove forest Bangladesh. The shade-dried powdered bark was extracted with 70% ethanol for 7 days at room temperature. Then, after filtration through filter paper the yield was dried. One gram (1 g) of this extract was taken in an air tight bottle. Hereafter, n-hexane was added to prepare hexane fraction of the sample. After vigorous shaking and filtration through filter paper n-hexane was evaporated and the dried solid was taken as a hexane fraction (Hex). Precipitate on the filter paper was taken in a separate air tight bottle and chloroform was added. Chloroform fraction (Chl) was prepared by evaporation of chloroform after filtration through a filter paper. Subsequently following the same procedure as above, ethyl acetate, then ethanol, and at last distilled water was used to obtain ethyl acetate (EtA), ethanol (Eth), and water fraction (DW). Twenty milligram (20 mg) of the fraction was dissolved in 1 ml ethanol or distilled water for conducting experiments.

Determination of total phenolic compounds (TPH)

The total concentration of phenolic compounds (TPH) in the fractions was determined according to the Folin-Ciocalteu method [16] with gallic acid (GA) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract [17].

DPPH radical scavenging activity

The reaction mixture (total volume, 3 mL), consisting of 0.5 mL of a 0.5 M acetic acid buffer solution at pH 5.5, 1 mL of 0.2 mM DPPH in ethanol, and 1.5 mL of a 50% (v/v) ethanol aqueous solution with the fractions, was shaken vigorously [18,19,20]. After incubation at room temperature for 30 min, the amount of DPPH remaining was determined by measuring absorbance at 517 nm.

Reducing power activity

The reducing power of the fractions was determined according to the method of [21]. Briefly, phosphate buffer (2.5 mL, 0.2 M, pH 6.6) containing different concentrations of the bark fractions were prepared. Then it was added to 2.5 mL of 1% (w/w) potassioum ferricyanide, and mixed. After incubation at 50ºC for 20 min, the mixtures were mixed with 2.5 mL of 10% (w/w) trichloroacetic acid followed by centrifugation at 650 g for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. Then the absorbance of this solution was measured at 700 nm. Mean values were obtained from triplicate experiments. Ascorbic acid (40 µg/mL) served as positive control.

Determination of total antioxidant capacity

The assay was done according to [22]. The tubes containing the fraction and the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 90ºC for 90 min. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

Preparation of rat peritoneal exudate cells (PECs)

Male Wister rats (8~9 weeks old) were purchased from Kyudo Co., Ltd., Tosu, Japan. Tyrode buffer (pH 7.4, consisted of 137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$. $2H_2O$, 1.0 mM $MgCl_2$. $6H_2O$, 11.9 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, and 5.6 mM glucose) containing 0.1% (w/w) BSA (bovine serum albumine) was injected into the peritoneal cavity of rats. After the abdomen was gently massaged for 2 minutes, the cavity was opened, and the fluid containing the PECs was collected with a Pasteur pipette. Cells were gently washed with Tyrode buffer and then centrifuged at 200 x g for 10 min at 4ºC. To remove contaminating erythrocytes by hypotonic lysis, the cell pellets were re-suspended in a modified ammonium chloride buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, and 10 mM EDTA.2Na, p^H 7.4) and incubated for 5 min at 4ºC. The cell suspension was then centrifuged at 200 x g for 5 min at 4ºC and the cells were resuspended in the Tyrode buffer at 2 x 10^6 cells/mL. Cell viability was measured by trypan blue staining and mast cells were identified by toluidine blue staining [6]. The cell viability of this preparation was more than 95% and the proportion of mast cells was 5-10% of all the cells [5].

Measurement of the inhibition of histamine release (%)

Rat peritoneal exudates cells (500 µL, 2 x 10^6) were suspended in 48 µL of 25 mM $CaCl_2$, 12 µL of various concentrations of samples and/or 120 µL of 5 µM A23187 solution, and then the volume was adjusted to 1.2 mL with tyrode buffer, and incubated for 20 min at 37ºC. The reaction was terminated by incubating the mixture for 5 min at 4ºC. The cell suspension was then centrifuged at 300 x *g* for 10 min, and the amount of histamine in the supernatant was measured.

The histamine content was measured by fluorometric assay [23]. To 1 mL of the sample solution, 0.75 g of NaCl and 0.5 mL of 1N NaOH were added. Then, 5 mL of a mixture of *n*-butanol and chloroform (3:2, vol/vol) was also added and mixed for 5 min. After centrifugation at 270 x *g* for 5 min, 4 mL of the organic solvent layer was removed and mixed with 2 mL of *n*-heptane and 1.5 mL of 0.1 N HCl for 5 min. After centrifugation at 270 x g for 5 min, 1 mL of the HCl layer was removed, mixed with 0.15 mL of 1 N NaOH and 0.1 mL of 0.2% *o*-phthalaldehyde, and kept for 5 min at room temperature. The reaction was terminated by adding 0.14 mL of 0.5 N H_2SO_4. Then the fluorescence intensity was measured using a spectrofluorophotometer (Jasco Co., Tokyo) with excitation at 360 nm and emission at 450 nm. The percent inhibition of histamine release was calculated with the following formula: inhibition of histamine release (%) = (histamine release without extract – histamine release with extract) x 100/histamine release without extract. The negative control was the histamine content without stimulation (A23187). The positive control was that after stimulation (A23187). All results were expressed as the mean ± SD of four determinations (n = 4).

***Thin-Layer Chromatography (TLC*)**

TLC was performed on a silica gel plate (20 x 20 cm, silica gel 60 F_{254}, Merk). An aliquot of each fraction (Chl, EtA, and Eth), and reference compounds (ellagic acid, and lupeol) was spotted on the silica gel plate with a solvent system of toluene:ethyl acetate:formic acid:methanol (3:3:0.8:0.2 v/v). The spots were visualized by spraying two spraying solutions on the plates.

Spray solution 1 was a 5% solution of ferric chloride in methanol. Dark blue bands were appreared, indicating the presence of phenolic compounds in the fraction. Ellagic acid, a phenolic compound, produced a dark blue band too.

Spary solution 2 was a 10% H_2SO_4 in methanol. The sprayed plate was heated for 5 min at 100 ^oC. Deep brown bands were appeared, indicating the presence of terpenoids. Lupeol, a triterpene, produced a same colored band too.

Results and Discussion

Antioxidative Activity

The TPH content is highest in DW fraction (348 mg GAE/g) followed by Eth (160.5 mg GAE/g) (Table 1). These two fractions also show very high activities in DPPH free radical scavenging, reducing power, and total antioxidant capacity (Table 1) compare to the other fractions. Figure 1 shows dose-dependent scavenging of DPPH free radical by the fractions. The IC_{50} values for DPPH radical scavenging for DW was 2.1 µg /mL followed by Eth (4.7 µg /mL) and Chl (18.8 µg /mL) fractions. In this study, fraction with the high proportion of polyphenols, displayed the high antioxidative activity, i. e. DW and Eth fractions.

Table 1. Total phenolic content, DPPH radical scavenging activity, reducing power, and total antioxidant activity of the different fractions of bark extract of *Excoecaria agallocha*[a]

Name of fractions	Total phenolic mg GAE/g extract	DPPH radical scavenging activity (%) at 12.5 µg/mL	Reducing power (O.D.) at 0.25	Total antioxidant activity mg/mL µgAAE/mg extract
Hexane	5.0 ± 0.2	Nill	Nill	24.0 ± 2.7
Chloroform	31.7 ± 0.4	38.1 ± 0.8	0.4 ± 0.01	63.6 ± 1.3
Ethyl acetate	37.3 ± 0.3	35.0 ± 0.3	0.3 ± 0.02	48.2 ± 0.4
Ethanol	160.5 ± 1.1	89.7 ± 0.1	1.3 ± 0.04	182.8 ± 0.6
Water	348.0 ± 5.2	91.0 ± 0.2	1.4 ± 0.03	248.2 ± 3.5

$p < 0.01$ by Student's t test for values between the sample and the control in DPPH experiments. GAE: gallic acid equivalent, AAE: ascorbic acid equivalent. [a]Values are the mean of three replicates ± SD.

Antioxidants are believed to intercept the free radical chain reaction and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of lipids [24]. Since phenolic compounds present in the fractions are good sources of electron donors, they show reducing power. Reportedly, the activity of antioxidants is concomitant with the development of reducing power [25]. Owing to the complexity of the oxidation-antioxidation process, no single testing method is capable of providing a comprehensive view of the antioxidative profile of a sample [26]. Therefore, a multi-method approach is necessary to assess antioxidative activity.

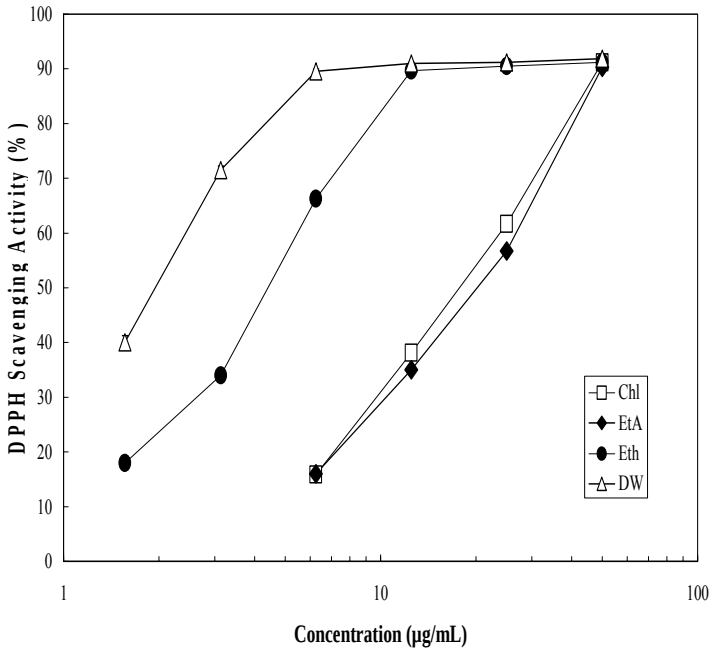

Figure 1. Dose-dependency of the DPPH free radical scavenging activities of the bark fractions of *Excoecaria agallocha*. Data are mean ± SD (bars) values from three experiments. $p < 0.05$ by Student's t test for values between the sample and the control.

Anti-allergic activity

Using the fractions of the extract of bark of *E. agallocha*, we examined their effects on the inhibition of histamine release from rat PECs. The PECs (1 x 10^6/mL) were stimulated with 5 μM A23187 for 20 min in the presence of the fractions. Figure 2 shows effect on the release of histamine from the mast cells by the fractions, and pure compounds- ellagic acid, and lupeol. Mast cells play a crucial role in the pathogenesis of type 1 allergy through the production and release of chemical mediators such as histamine and eicosanoids [5]. Therefore, it is important to inhibit the release of mediators for the prevention and/or alleviation of allergic symptoms. Reportedly, the release of histamine is inhibited by various fruits [2] and polyphenols [5,7,8]. In this study, Eth, and DW fractions strongly inhibited the release of histamine probably due to their larger amount of polyphenols content.

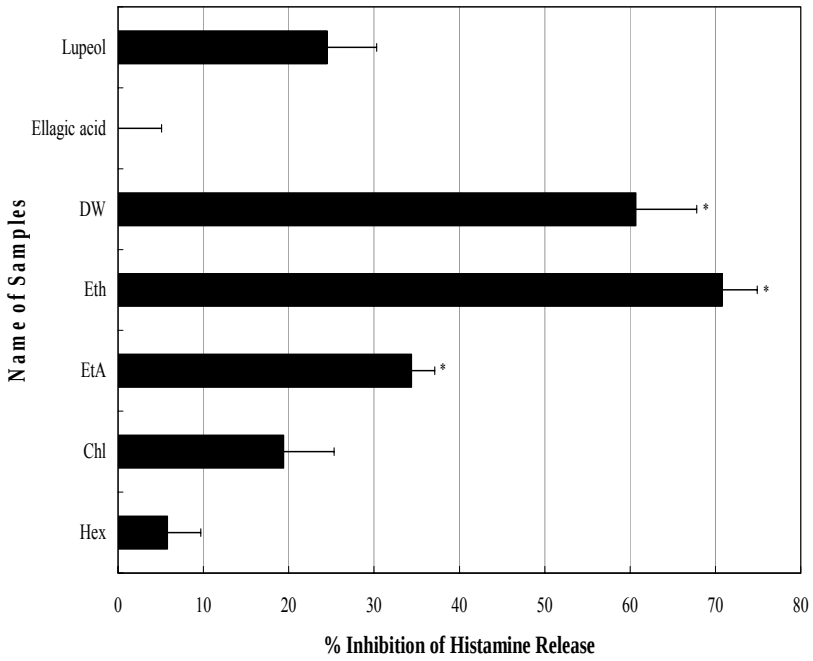

Figure 2. Effect of different samples on the ionophore A23187-induced release of histamine from rat peritoneal exudate cells (PEC). Cells (1 x 10^6) were incubated at 37 ^oC for 20 min in the presence of samples (80 μg/mL: Hex, Chl, EtA, Eth, and DW; 100 μg/mL: ellagic acid, and lupeol). Histamine content of the supernatant was measured by a fluorometric assay. Results are the mean ± SD of four independent experiments. * $p < 0.05$ between the control value and the value in the presence of the sample, by Student's t test.

Figures 3A and 3B show their dose-dependent inhibition of histamine-release. Polyphenols inhibit the binding of specific radio-ligands to various receptors [27]. In the immediate type allergic reaction cascade, IgE sensitizes basophils or mast cells which have a specific Fc receptor for IgE on their surface. It is therefore necessary to elucidate whether these fractions have an effect at a later stage in the sensitization by IgE in the cascade and inhibit histamine release.

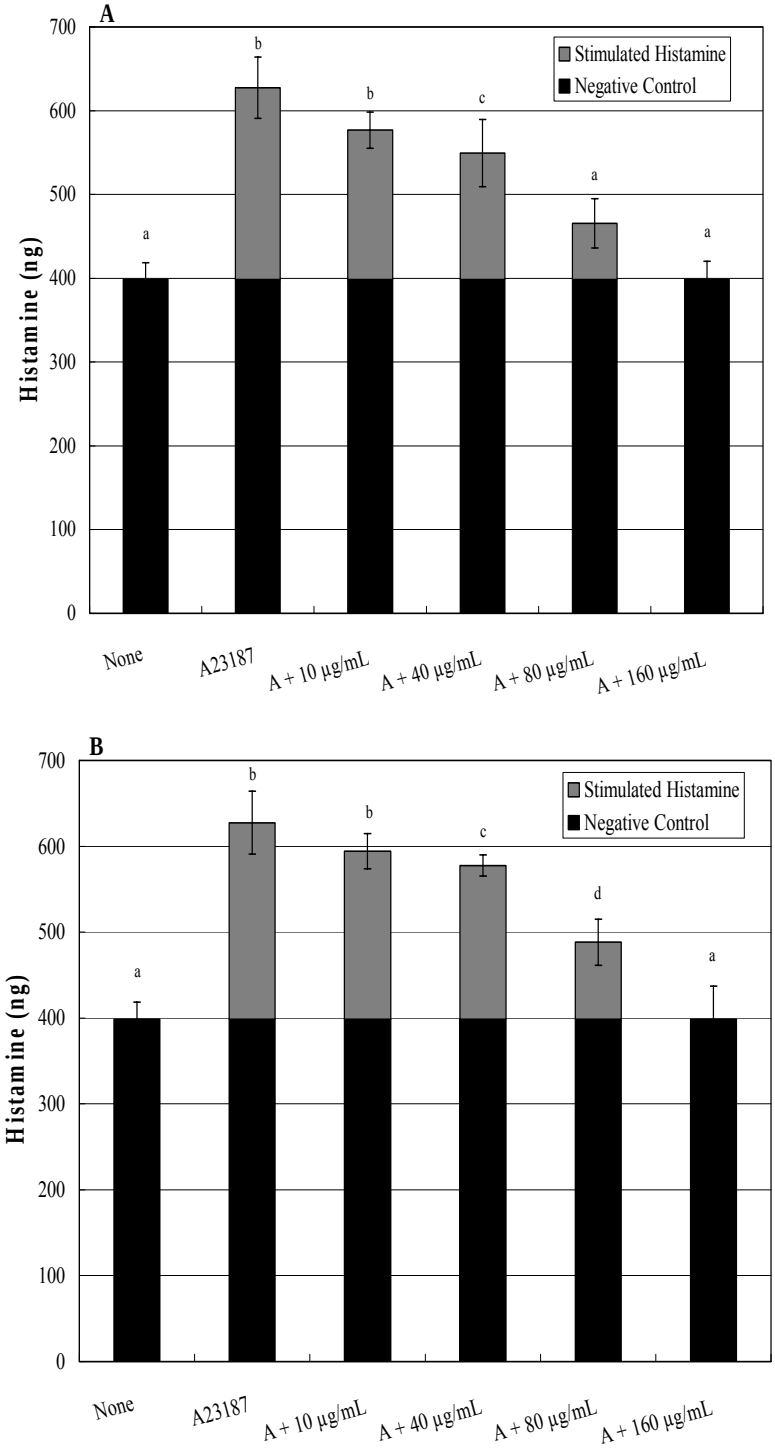

Figure 3. Dose-dependent effect of ethanol (Eth), and water (DW) fraction on histamine release from rat peritoneal exudate cells (PEC). Cells (1 x 10^6) were incubated at 37 ^oC for 20 min in the presence of various concentrations of Eth, and DW. (A): Eth; (B): DW. Results are the mean ± SD of four independent experiments. $^{a-d}$Values not sharing a common letter are significantly different at $p < 0.05$, by Student's t test.

Moreover, histamine is released from intracellular secretory granules with an increase in the intracellular calcium ion concentration and by the activation of signal transduction [28,29]. Polyphenols of *Rubus coreanum* inhibited both the calcium influx into the cells and the uptake of calcium into the cytoplasmic calcium store [30]. Figure 2 shows the strong inhibition of calcium-ionophore induced release of histamine from rat PECs by the Eth, and DW fractions, which consists of high amount of polyphenols. These fractions possibly inhibited the influx of calcium and affected the transduction of signals as well as release of histamine. Bands developed on TLC are shown in Figure 4 that Eth, and Chl fraction of the bark of *E. agallocha* contains "ellagic acid-like" and "lupeol-like" compounds respectively. From the leaves of *E. agallocha,* ellagic acid was isolated [15], and 3-epilupeol was also identified [31]. Reportedly, both ellagic acid and lupeol have various beneficial effects on health including antioxidative, and anti-inflammatory activities. Our experiments fail to show any significant anti-histamine release activity of ellagic acid, and lupeol (Fig. 2). Therefore, possibly other compound(s) in the Eth and DW fractions of *E. agallocha* are involved in signaling pathway(s) to inhibit histamine release of the cells.

Figure 4. Thin layer chromatogram (TLC) of ethanol (Eth), and chloroform (Chl) fractions. Bands for authentic compounds ellagic acid (E) and lupeol (L) along with likely-compounds in the fractions are developed on the TLC.

Conclusions

A relationship between polyphenols and antioxidative and anti-allergic activities has already been reported [32]. The results support the possibility that the plant *E. agallocha* can contribute to health. Further fractionation of these fractions, especially Eth and DW fractions, is essential to know the phenolic and/or nonphenolic compound(s) responsible for the antioxidative and anti-histamine release activities. However, attention should be paid to potential cytotoxic effects since some polyphenols perturb the membrane structure [33,34].

Acknowledgments

This work was supported by grant-in-aid no. 18 • 06189 from the Japan Society for the Promotion of Science (JSPS), Ministry of Education, Science, and Culture of Japan. The author thanks Prof. Matsudomi, N., Faculty of Agriculture, Yamaguchi University for using the fluorescence spectroscope.

References

1. Cheng HY, Lin TC, Yu KH, et al. Antioxidant and free radical scavenging activities of Terminalia chebula. Biol Pharm Bull 2003;26, 1331-1335.
2. Hossain SJ, Tsujiyama I, Takasugi M, et al. Total phenolic contents, antioxidative, antiamylase, antiglucosidase, and antihistamine release activities of Bangladeshi fruits. Food Sci Technol Res 2008;14, 261-268.
3. Hossain SJ, El-Sayed MA, Aoshima H. Anti-oxidative and anti-α-amylase activities of four wild plants consumed by pastoral nomads in Egypt. Orient Pharm Exp Med 2009 (Accepted).
4. Sawa T, Nakao M, Akaike T, et al. Alkylperoxyl radical-scavenging activity of various flavonoids and other polyphenolic compounds: implications for the anti-tumor-promoter effect of vegetables. J Agric Food Chem 1999;47, 397-402.
5. Matsuo N, Yamada K, Mori M, et al. Inhibition by dietary tea polyphenols of chemical mediator release from rat peritoneal exudates cells. Biosci Biotechnol Biochem 2000;64, 1437-1443.
6. Takasugi M, Shimada K, Yamada K. Studies on inhibitory effect of soy isoflavones on release of allergy-related mediators from rat peritoneal exudates cells. Soy Protein Res 2002;5, 99-102.
7. Kanda T, Akiyama H, Yanagida A, et al. Inhibitory effects of apple polyphenol on induced histamine release from RBL-2H3 cells and rat mast cells. Biosci Biotechnol Biochem 1998;62, 1248-1289.
8. Corvazier E, Maclouf J. Interference of some flavonoids and non-steroidal anti-inflammatory drugs with oxidative metabolism of arachidonic acid by human platelet and neutrophils. Biochim Biophys Acta 1985;835, 315-321.
9. Yamada K, Mori M, Matsuo N, et al. Effects of fatty acids on accumulation and secretion of histamine in RBL-2H3 cells and leukotriene release from peritoneal exudates cells isolated from Wistar rats. J Nutr Sci Vitaminol 1996;42, 301-311.
10. Wiriyachitra P, Hajiwangoh H, Boonton P, et al. Investigations of medicinal plants of euphorbiaceae and thymelaeaceae occurring and used in Thailand; II. Criptic irritants of the diterpene ester type from three Excoecaria species. Planta Med 1985;51, 368-371.
11. Karalai C, Wiriyachitra P, Operkuch HJ, et al. Cryptic and free skin irritants of the daphnane and tigliane types in latex of Excoecaria agallocha. Planta Med 1994;60, 351-355.
12. Konishi T, Takasaki M, Tokuda H, et al. Anti-tumor-promoting activity of diterpenes from Excoecaria agallocha. Biol Pharm Bull 1998;21, 993-996.
13. Konoshima T, Konishi T, Takasaki M, et al. Anti-tumor-promoting activity of the diterpene from Excoecaria agallocha. II. Biol Pharm Bull 2001;24, 1440-1442.
14. Singh IP, Bharate SB, Bhutani KK. Anti-HIV natural products. Curr Sci 2005;89, 269-290.
15. Masuda T, Yonemori S, Oyama Y, et al. Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. J Agric Food Chem 1999;47, 1749-1754.

16. Ough CS, Amerine MA. Methods for analysis of musts and wines, Wiley: New York; 1988;pp. 196-221.
17. Aoshima H, Ayabe S. Prevention of the deterioration of polyphenol-rich beverages. Food Chem 2007;100, 350-355.
18. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181, 1199-1200.
19. Hatano T, Kagawa H, Yasuhara T, et al. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem Pharm Bull 1988;36, 2090-2097.
20. Aoshima H, Tsunoue H, Koda H, et al. Aging of whiskey increases 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity. J Agric Food Chem 2004;52, 5240-5244.
21. Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986;44, 307-315.
22. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269, 337-341.
23. Shore PA, Burkhalter A, Cohn VHA. A method for the fluorometric assay of histamine in tissues. J Pharmacol Exp Ther 1959;127, 182-186.
24. Sherwin ER. Oxidation and antioxidants in fat and oil processing. J Am Oil Chem Soc 1978;55, 809-814.
25. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of Harng Tyur (Chrysanthemum morifolium Ramat.). Lebensm Wiss Technol 1999;32, 269-277.
26. Parejo I, Viladomat F, Bastida J, et al. Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. J Agric Food Chem 2002;50, 6882-6890.
27. Zhu M, Phillipson JD, Greengrass PM, et al. Plant polyphenols: biologically active compounds or non-selective binders to protein? Phytochemistry 1997;44, 441-447.

28. Beaven MA, Rogers J, Moore JP, et al. The mechanism of the calcium signal and correlation with histamine. J Biol Chem 1984;259, 7129-7136
29. Penner R. Multiple signaling pathways control stimulus-secretion coupling in rat peritoneal mast cells. Proc Nat Acad Sci USA 1988;85, 9856-9860.
30. Kee YW, Lim DY. Influence of polyphenolic compounds isolated from Rubus coreanum on catecholamine release in the rat adrenal medulla. Arch Pharm Res 2007;10, 1240-1251.
31. Zou JH, Dai J, Chen X, et al. Pentacyclic triterpenoids from leaves of Excoecaria agallocha. Chem Pharm Bull 2006;54, 920-921.
32. Yamada K, Shoji K, Mori M, et al. Structure-activity relationship of polyphenols on inhibition of chemical mediator release from rat peritoneal exudates cells. In Vitro Cell Dev Biol Anim 1999;35,: 169-174.
33. Hossain SJ, Kato H, Aoshima H, et al. Polyphenol-induced inhibition of the response of Na^+/glucose cotransporter expressed in Xenopus oocytes. J Agric Food Chem 2002;50, 5215-5219
34. Aoshima H, Okita Y, Hossain SJ, et al. Effect of 3-o-octanoyl-(+)-catechin on the responses of $GABA_A$ receptors and Na^+/glucose cotransporters expressed in Xenopus oocytes and on the oocyte membrane potential. J Agric Food Chem 2005;53, 1955-1959.