Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(5):2483-2489

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antioxidant activity of *Fimbristylis ovata* and its effect on RAGE gene expression in human lung adenocarcinoma epithelial cell line

Suporn Sukjamnong and Rachana Santiyanont*

Center for Excellence in OMICs Nano-Medical Technology Development project, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand

---

ABSTRACT

Fimbristylis ovata (Burm.f.) Kern has long been used in traditional medicine for the treatment of inflammation-associated diseases. However, there has been no scientific data on the active components or the biochemical or molecular mechanism responsible for these therapeutic effects. The aim of this study was to investigate the antioxidant activity of F.ovata and the effect of its extract on the regulation of RAGE gene in human lung adenocarcinoma epithelial cell line (A549). We investigated total phenolic content by Folin-Ciocalteu's phenol assay and evaluated antioxidant activity by DPPH assay and ABTS assay. The effect of F. ovata ethanol extract on cell viability and RAGE gene expression were evaluated on A549 cells by MTT assay and RT-PCR respectively. Total phenolic content of F. ovata extracted by water and ethanol were 161.0 ± 15.2 and 777.7 ± 45.4 μg GAE/g dried F.ovata respectively. Antioxidant capacity of the water and ethanol extract were 34.0 ± 4.4 μg and 970.0 ± 64.7 μg VCEAC/g dried F. ovata respectively. Antioxidant capacity of F. ovata extracted by water and ethanol were 481.1 ± 98.1 and 6,717.0 ± 182.5 μg TEAC/g dried F. ovata respectively. Upon incubation with the ethanol extract at concentration up to 100 μg/ml, cell viability was more than 80%. Interestingly, the ethanol extract could significantly inhibit RAGE gene expression in human lung adenocarcinoma epithelial cell line.

Keywords : *Fimbristylis ovata*, A549, phenolic compound, antioxidant, RAGE gene.

INTRODUCTION

Excessively high level of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually lead to cell death. Oxidative stress occurs when production of oxidants or reactive oxygen species (ROS) exceeds local antioxidant capacity. Oxidative stress plays a major role in the pathogenesis of many degenerative diseases such as cardiovascular disease, stroke, diabetes, cancer, and neurodegeneration [1]. The activation of RAGE in a variety of cells including monocytes was shown to link to generation of ROS by decreasing activity of superoxide dismutase [2]. Both of RAGE activation and oxidative damage have been implicated in chronic degenerative diseases including type 2 diabetes mellitus, cancer, and cardiovascular disease [3, 4].

Numerous pure polyphenolic compounds and natural polyphenolic plant extracts possess antioxidant, anticancer, and anti-inflammatory activities [5-7]. Moreover, polyphenolic compounds in plant have biological activities such as carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis, and inhibition of angiogenesis. In addition, polyphenolic compounds have an antioxidant activity that can prevent damage from reactive oxygen species [8, 9]. Many traditional Thai herbs have been used for therapeutic purpose in traditional medicine [10].

Lagerstroemia speciosa (L) and *Piper sarmentosum Roxb* have an anti-inflammatory effects and anti-oxidant activity [11, 12]. *Boesenbergia rotunda (L.)Mansf* or Kra-chai has anti-inflammatory effect owing to several flavonoid derivatives [13, 14]. *Momordica charantia Linn* or bitter melon can improve immune system in cervical cancer patients by increasing NK cells [15].

Fimbristylis ovata (Burm.f.) Kern has common name as flatspike sedge or "Ya-sae-ma" in Thai, belongs to Family Cyperaceae. [16]. It has been used for treatment of adenitis, scrofula, syphilis, and also in cough, bronchitis and asthma [17, 18]. Plants in Family Cyperaceae has been reported to consists of several components such as phenolic compounds, flavonoids, alkaloid, glycosides proteins, amino acids, tannins and saponins [19,20] . The aim of this study was to determine the antioxidant activity of *Fimbristylis ovata (Burm.f.) Kern* extracts and investigates its effect on the expression of RAGE gene.

EXPERIMENTAL SECTION

1. Plant material

Fimbristylis ovata was collected from a single source in Bangkok, Thailand. The voucher number of 013431(BCU) was identified at the Professor Kasin Suvatabhandhu Herbarium, Department of Botany, Faculty of Science, Chulalongkorn University, Thailand. The fresh plant was cleaned, cut into short pieces, and dried in laboratory drying oven at 45 °C for 5 days and grounded into powder. The plant powder was extracted with ethanol 1:10 (w/v) by maceration in shaking incubator at 37°C for 72 hours, the extract was filtered, the process was repeated twice, then all filtrate was evaporated using rotary evaporator. The plant powder was also extracted with water 1:10 (w/v) by boiling for 2 hours, the extract was filtered and then lyophilized. Crude extracts were dissolved in dimethyl sulphoxide (Merck, Germany) as stock solutions (100 mg/ml), protected from light, and stored at -20°C until used.

2. Cell cultures

Human lung adenocarcinoma epithelial cell line (A549) was cultured in RPMI-1640 medium (Hyclone, USA) supplemented with 10 % fetal bovine serum (Gibco Invitrogen, USA), 100 U/ml penicillin and 100 µg/ml Streptomycin in a humidified atmosphere with 5 % CO_2 at 37 ºC.

3. Total phenolic content

Total phenolic content was determined by Folin-Ciocalteu method. A calibration curve of gallic acid (ranging from 0.39 to 50 µg/ml) was prepared. 20 µl of *F.ovata* extracts or standard gallic acid solution was added to 100 µl Folin-Ciocalteu's reagent and 80 µl of 75 g/L sodium carbonate was added to the solution in the 96-well plate. The plate was incubated for 30 minutes in a dark condition. The absorbance was measured at 765 nm using a Microplate Reader (BioTek, USA). Total phenolic content of *F.ovata* extracts were expressed as µg Gallic acid Equivalent (GAE)/g of dried *F.ovata.*

4. Antioxidant capacity using DPPH assay

Antioxidant capacity was determined by reaction between DPPH (2, 2-Diphenyl-1-picrylhydrazyl) reagent and antioxidant content. A calibration curve of ascorbic acid (ranging from 1.56 to 100 µg/ml) was prepared. 20 µl of *F.ovata* extracts or standard gallic acid solution was added to 180 µl DPPH reagent in the 96-well plate. The plate was incubated for 30 minutes in a dark condition. The absorbance was measured at 517 nm using a Microplate Reader (BioTek, USA). Antioxidant capacity of *F.ovata* extracts were expressed as µg Vitamin C Equivalent Antioxidant Capacity (VCEAC)/g of dried *F.ovata.*

5. Antioxidant capacity using ABTS assay

Antioxidant capacity was determined by reaction between antioxidant and 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) cation radical (ABTS*+). Fresh ABTS*+ solution was prepared for each assay by the reaction between ABTS reagent and potassium persulfate. A calibration curve of Trolox (ranging from 39 to 1250 µM) was prepared. 15 µl of *F.ovata* extracts or standard Trolox solution was added to 1000 µl ABTS working reagent in the 96-well plate. The plate was incubated for 3 minutes in a dark condition at 30 ºC. The absorbance was measured at 734 nm using a Microplate Reader (BioTek, USA). Antioxidant capacity of *F.ovata* extracts were expressed as µg Trolox Equivalent Antioxidant Capacity (TEAC)/ /g of dried *F.ovata.*

6. MTT assay for cell viability

Cell viability was determined by MTT assay, which is the measurement of mitochondrial dehydrogenase enzyme activity that reduces 3-(4,5-dimethyl-triazolyl-2-yl)-2,5-diphenyl tetrazolium bromide (Bio Basic, Canada) to purple formazan. A549 cells were seeded in density of $5x10^3$ cells/well in 96-well plates and incubated in a humidified 5 % CO_2 at 37 ºC for 24 hours. *F. ovata* extracts were added and incubated for 24 hours. Then, 20 µl of 0.5 mg/ml MTT was added and the plates were incubated for 4 hours in a humidified 5 % CO_2 at 37 ºC. Culture medium was removed and 200 µl of dimethylsulfoxide (DMSO) was added to dissolve the formazan crystal. The absorbance was measured at 550 nm using a Microplate Reader (BioTek, USA). The percentage of cell viability was calculated using the following formula:

% cell viability = (absorbance of treatment group – blank) x 100 / absorbance of control group – blank

7. Reverse-transcription polymerase chain reaction (RT-PCR) analysis

Human lung adenocarcinoma epithelial cell line (A549) was seeded in density of $1x10^6$ cells/ml in 6-well plates. *F.ovata* extracts were added and the cells were incubated for 24 hours. Total RNA was extracted from the cells by Tri-RNA Reagent (Favorgen Biotech Corp, Taiwan), and RNA was treated with deoxyribonuclease I (DNase I; Promega, USA). DNase I-treated RNA was reverse transcribed by ImProm-II Reverse Transcription System (Promega, USA) according to the manufacturer's protocol. For PCR amplification of the cDNA, RAGE gene-specific primer pair were 5' GTGGGGACATGTGTGTCAGAGGGAA 3' (Forward primer) and 5' TGAGGAGAGGGCTGGGCAGGGACT 3' (Reverse primer). β-actin gene-specific primer pair composed of Forward primer 5' ACGGGTCACCACACTGTGC 3' and Reverse primer 5' CTAGAAGCATTTGCGGTGGACGATG 3'. The condition for amplification of cDNA using thermocycler (Eppendorf, Germany) for RAGE gene was 35 cycles of 95 °C for 30 s, 65 °C for 30 s, and 72 °C for 30 s , and for β-actin gene was 35 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s. The product was separated on a 2% agarose gel, stained with ethidium bromide, and visualized with gel documentation system (SynGene, UK).

8. Statistical analysis

The data was presented as mean ± SEM of three experiments. Statistical significant differences were determined by one way ANOVA followed a by post hoc Tukey test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Total phenolic content of *F.ovata*

Total phenolic content of *F.ovata* was determined by Folin-Ciocalteu's method, we found that total phenolic content of *F.ovata* extracted by water and ethanol were 161.0 ± 15.2 and 777.7 ± 45.4 µg GAE/g dried *F.ovata* respectively. Water extract had significantly lower total phenolic content than ethanol extract ($p < 0.05$) as shown in Table 1.

Table 1 Total phenolic content of *F.ovata* was determined by Folin-Ciocalteu's method. Data were shown as mean ± SEM of triple determinations (*p<0.05).

***F. ovata* extracts**	**Total phenolic content (µg GAE/g dried *F. ovata*)**
Water extract	161.0 ±15.2*
Ethanol extract	777.7 ±45.4*

Antioxidant capacity using DPPH assay and ABTS assay

Antioxidant capacity of *F.ovata* was determined by DPPH assay. As shown in Table 2, antioxidant capacity of water and ethanol extracts were 34.0 ± 4.4 and 970.0 ± 64.7 µg VCEAC/g dried *F. ovata* respectively. It was noticeable that antioxidant capacity of water extract was remarkedlyly lower than ethanol extract ($p < 0.05$).

Table 2 Antioxidant capacity of *F.ovata* was determined by DPPH assay. Data was shown as mean ± SEM of triple determinations (* p<0.05).

***F. ovata* extracts**	**Antioxidant Capacity by DPPH assay** (µg VCEAC/g dried *F. ovata*)
Water extract	34.0 ±4.4*
Ethanol extract	970.0 ±64.7*

Antioxidant capacity of *F.ovata* was determined by ABTS assay. As shown in Table 3, antioxidant capacity of water and ethanol extracts were 481.1 ± 98.1 and 6,717.0 ± 182.5 µg TEAC/g dried *F. ovate* respectively. Antioxidant capacity of water extract was as well much lower than ethanol extract ($p < 0.05$).

Table 3 Antioxidant capacity of *F.ovata* was determined by ABTS assay. Data was shown as mean ± SEM of triple determinations (* $p<0.05$).

***F. ovata* extracts**	**Antioxidant Capacity by ABTS assay (µg TEAC/g dried *F. ovata*)**
Water extract	481.1 ±98.1*
Ethanol extract	6,717.0 ±182.5*

Effect of *F.ovata* extracts on cell viability of A549

The effect of *F.ovata* extracts on cell viability was investigated by MTT assay. A549 cells were incubated with various concentration of *F.ovata* extracts for 24 hours. The result showed that there was no significant difference in cell viability after treatment of cells with the extracts in the concentration range of 0.78 to 100 µg/ml. Cell viability of A549 cells were over 80% as shown in Figure1.

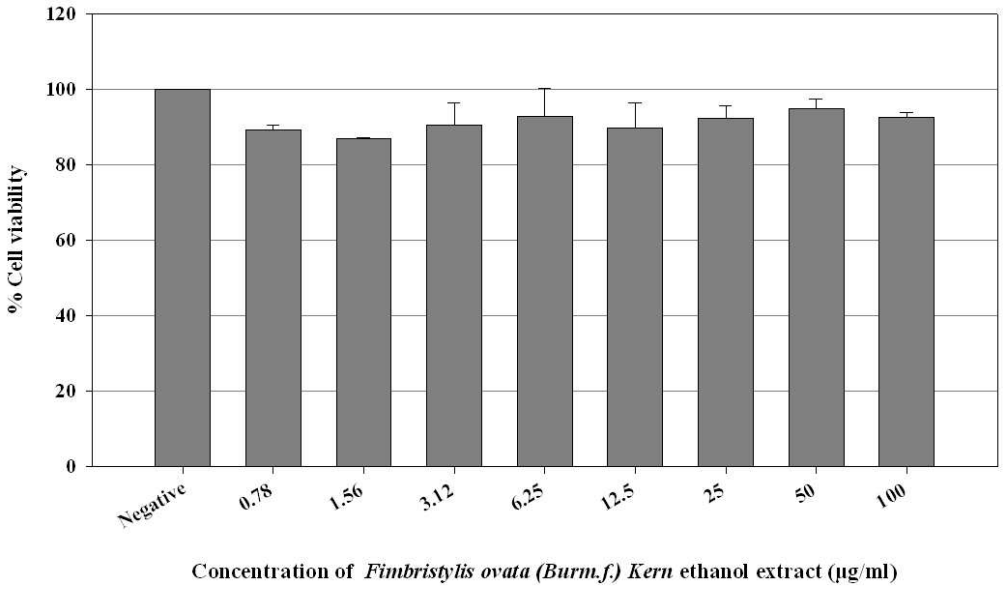
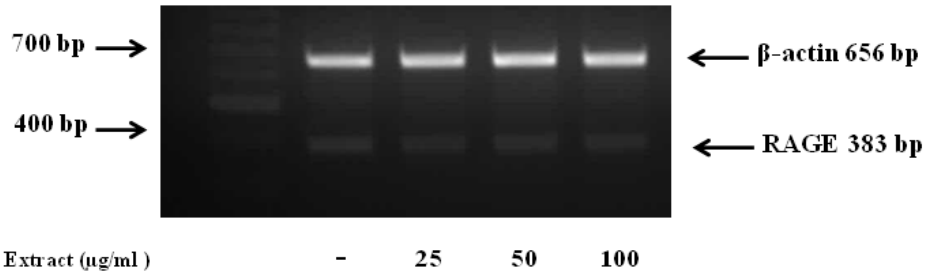


Figure1. Cell viability was determined by MTT assay after incubation of A549 cells with ethanol extract of *F.ovata*. Data was shown as mean±SEM of triple determinations.

***F.ovata* extracts down regulate gene expression of RAGE**

A549 cells were incubated with *F.ovata* extracts in the concentration of 25-100 µg/ml for 24 hours, and then RAGE gene expression was determined using RT-PCR. We found that RAGE gene expression in cells which were incubated with the extracts was significantly decreased ($p<0.05$) when compared with the control cells which had no exposure to the extracts as shown in Figure 2



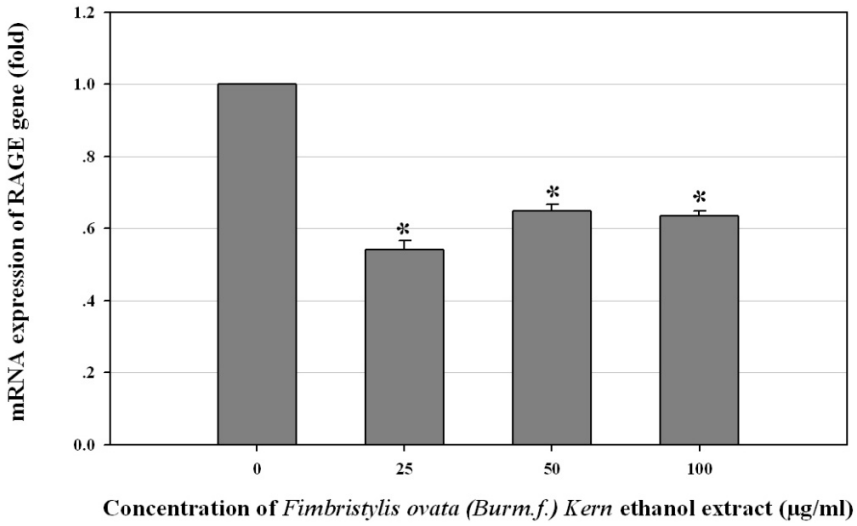


Figure 2. The effect of ethanol extract of *F.ovata* on RAGE mRNA expression in treated cells compared with control cells. β-actin gene expression was used for normalization. (*p<0.05)

DISCUSSION

Since there has never been any study on *Fimbristylis ovata,* this is the first time to report its antioxidant capacity and the possible mechanism of its therapeutic effect. *F.ovata* was extracted with water and ethanol and the total phenolic content can be extracted with ethanol much better than water. The results from this study suggested that variation in the total phenolic content of various extracts, depending on the polarity of solvent used. As well, the result showed that antioxidant capacity from *F.ovata* ethanol extract determined by DPPH assay and ABTS assay was more than that in water extract. According to experimental data, there was correlation between total phenolic content and antioxidant capacity. In addition, the results were in agreement with the previous studies that total phenolic content was positively correlated with scavenging efficiency [21]. Importantly, the result showed that *F. ovata* extract could inhibit RAGE gene expression. Previous studies demonstrated that activation of the receptor for advanced glycation end products (RAGE) by RAGE ligands resulted in the activation of RAGE signaling pathway and increase oxidative stress, which involved in diabetes, inflammatory disorders and tumors [2,22-25]. Therefore, the effect of antioxidant property may be related to its inhibitory action via RAGE signaling pathway. Activation of RAGE transduces the cell surface signals to various intracellular pathways including PI3-kinase/AKT, MAPKs and NF-κB which finally resulted in inflammation [26-28]. More studies will be needed to investigate whether the down regulation of RAGE gene found in this study exerts effect through the regulation of PI 3-kinase/AKT and MAPKs and NF-κB signaling pathways. However, phytochemical other than phenolic compound or antioxidant may as well play role in suppressing RAGE gene expression in this study, the fractionation of active chemicals from *F.ovata* crude extract and further testings will help elucidate this finding.

CONCLUSION

The experimental results showed that *Fimbristylis ovata* extracted by water or ethanol had phenolic compounds and antioxidant activity. The ethanol extract could inhibit RAGE gene expression in human lung adnenocarcinoma epithelial cell line (A549). This suggested that the antioxidant property of *Fimbristylis ovata* may involve in its inflammation-related inhibitory action via RAGE gene signaling pathway.

Acknowledgements
This research had been granted by Chulalongkorn University Centenary Academic Development Project and Faculty of Allied Health Sciences, Chulalongkorn University. Suporn Sukjamnong received tuition fee scholarship and research assistant grant from the Graduate School, Chulalongkorn University. The authors gratefully acknowledge Center for Excellence in Omics-Nano medical technology Development Project and the Innovation Center for Research and Development of Medical Diagnostic Technology Project, Chulalongkorn University for supporting certain laboratory instruments. The technical assistant of Jarunee Ruesopa, Chamaiporn Suwanchairop, Kanokwan Kamkwan, and Natcha Ooppachai were appreciated.

REFERENCES

[1] R Ramasamy; SJ Vannucci; SS Yan; K Herold; SF Yan; AM Schmidt. *Glycobiology*., **2005**, 15(7), 16R-28R.
[2] JM Jiang; Z Wang; DD Li. *Biomedical and Environmental Sciences*., **2004**, 17(1), 79-86.
[3] AL Tan; JM Forbes; ME Cooper. *Seminars in Nephrology*., **2007**, 27(2), 130-43.
[4] R Ross. *New England Journal of Medicine*., **1999**, 340(2), 115-26.
[5] CC Udenigwe; VR Ramprasath; RE Aluko; PJ Jones. *Nutrition reviews*., **2008**, 66(8), 445-54.
[6] B Romier; YJ Schneider; Y Larondelle; A During. *Nutrition reviews*., **2009**, 67(7), 363-78.
[7] L V.B. Hoelz; B A.C.Horta; J Q. Araujo; M G. Albuquerque; R B Alencastro; J F M Silva. *Journal of Chemical and Pharmaceutical Research*., **2010**, 2(5), 291-306.
[8] NR Perron; JL Brumaghim. *Cell Biochemistry and Biophysics*., **2009**, 53(2), 75-100.
[9] S Mandal; S Yadav; S Yadav; R K Nema. *Journal of Chemical and Pharmaceutical Research*., **2009**, 1(1) ,102-104.
[10] B Asha; K.H Krishnamurthy; S Devura. *Journal of Chemical and Pharmaceutical Research*., **2011**, 3(1) ,452-456.
[11] TT Priya; MC Sabu; CI Jolly. *Inflammopharmacology*., **2008**, 16(4), 182-7.
[12] K Hussain; Z Ismail; A Sadikun; P Ibrahim. *Natural Product Research*., **2009**, 23(3), 238-49.
[13] P Tuchinda; V Reutrakul; P Claeson; U Pongprayoon; T Sematong; T Santisuk; WC Taylor. *Phytochemistry*., **2002**, 59(2), 169-73.
[14] AYL Ching; SW Tang; MA Sukari; GEC Lian; M Rahmani; K Khalid. *The Malaysian Journal of Analytical Sciences*., **2007**, 11(1), 154-9.
[15] S Pongnikorn; D Fongmoon; W Kasinrerk; PN Limtrakul. *Journal of the Medical Association of Thailand*., **2003**, 86(1), 61.
[16] T Santisuk. Flora of Thailand: The Forest Herbarium, Royal Forest Department, Bangkok, Thailand, **1998**.
[17] C.P.Khare. Indian Medicinal Plants, Springer, Berlin, **2007**; 269.
[18] HM Burkill. The Useful Plants of West Tropical Africa. Royal Botanic Gardens, **1997**; 969.
[19] NC Chaulya; PK Haldar; A Mukherjee. *International Journal of Pharmacognosy and Phytochemical Research*., **2010**, 3(2), 40-3.
[20] NC Chaulya; PK Haldar; A Mukherjee. *International Journal of Pharmacy and Pharmaceutical Sciences*., **2010**, 3(1), 133-5.
[21] X H Wang; JT Dai. *Journal of Medicinal Plants Research*., **2012**, 6(11), 2210-5.
[22] Y Tomino; S Hagiwara; T Gohda. *Kidney International*., **2011**, 80(2), 132-5.
[23] BW Lee; HY Chae; SJ Kwon; SY Park; J Ihm; SH Ihm. *International Journal of Molecular Medicine*., **2010**, 26(6), 813-8.
[24] SI Yamagishi; K Nakamura; T Matsui; S Ueda; K Fukami; S Okuda. *Expert Opinion on Investigational Drugs*., **2008**, 17(7), 983-96.
[25] S.P. Dholakia; B.N. Suhagia; A.K. Patel; P.P. Kapupara; D.K. Sureja. *Journal of Chemical and Pharmaceutical Research*., **2011**, 3(4), 315-332.
[26] L Lin; S Park; EG Lakatta. *Frontiers in Bioscience*., **2009**, 14, 1403-13.
[27] R Ramasamy; SF Van; AM Schmidt. *Journal of Leukocyte Biology*., **2009**, 86, 505-12.

[28] C.P. Kashyap; B Tikka; S Sharma; S Kumari; P Verma, S Sharma; V Arya. *Journal of Chemical and Pharmaceutical Research*., **2011**, 3(6), 514-520.