Bangladesh Pharmaceutical Journal ***15****(1): 31-37, 2012*

Preliminary Screening of Six Popular Fruits of Bangladesh for *in vitro* IgM Production and Proliferation of Splenocytes

Md. Moklesur Rahman Sarker[1], Ishrat Nimmi[2] and Md. Hassan Kawsar[3]

[1]Department of Immunochemistry, Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Tsushima-naka, Kita-ku, Okayama 700-8530, Japan
[2]BCSIR Laboratories, Bangladesh Council of Scientific and Industrial Research, Dr. Qudrat-E-Khuda Road, Dhaka-1205, Bangladesh
[3]Department of Pharmacy, Dhaka International University, Dhaka, Bangladesh

Abstract

Methanolic extracts of whole fruits or juice of six popular fruits of Bangladesh were evaluated for immunostimulating activity based on immunoglobulin M production and proliferation of murine splenic cells in culture medium. The production of IgM in cultured supernatants was determined by an Enzyme-Linked Immunosorbent Assay (ELISA) and the proliferation of spleen cells was measured by 3-(4,5-dimethylthiazol-2-y)-2,5-diphenylterazolium bromide (MTT) . Of the six crude extracts assayed, methanol extracts of *Spondias dul*cis (L.) and *Dillenia indica* (L.) at 200 µg/ml enhanced polyclonal IgM production by 108.33% and 90.72%, respectively when compared to the control. Treatment of splenocytes with the juice extracts of *Ananas comosus* (L.) Merr. and *Citrus medica* (L.) at 200 µg/ml increased IgM productions by 155% and 103.85%, respectively in comparison to untreated cells. No dose of the methanol extract of *Phyllanthus embelica* (L.) and juice extract of *Citrus grandis* (L.) could increase IgM level in cultured supernatants. The increment of IgM production was observed to be 190% higher than the control which was produced by the juice extract of *Ananas comosus* (L.) Merr. at a dose of 2 mg/ml. None of the extract could stimulate the proliferation of cultured splenocytes. Thus, the present findings suggest that *S. dulcis*, *D. indica*, *A. comosus* and *C. medica* possess moderate immunostimulating activities through the stimulation of B cells for IgM production and hence may be useful for enhancing the immunity of the body. However, comprehensive studies are needed to ascertain their immunopotentiality through both of cellular and humoral arms of immunity. To the best of our knowledge, this is the first report on the immunostimulating activity of the above mentioned fruits of Bangladesh.

Key words: Fruits, Bangladesh, IgM, proliferation, antibody, immunostimulant, splenocytes, mice.

Introduction

Epidemiological studies have shown that an inverse relationship exists between consumption of dietary flavonoids and cardiovascular diseases (Tripoli *et al.*, 2007). Different scientific studies reported that consumption of fruits reduces the risk of cancers (Kris-Etherton *et al.*, 2002), neurogenerative diseases, e.g., Parkinson`s and Alzheimer`s diseases (Di Matteo and Esposito, 2003), inflammation and aging (Ames *at al.*, 1993). Fruits are important sources of macro-and micro-nutrients; they can be used for both the prevention and cure of diseases only if their therapeutic and nutritional values are known. Since plants secondary metabolites are subject to variation in relation to the environment of cultivation, the secondary metabolites in the fruits growing in Bangladesh may not be identical to those of the same fruits grown in other countries (Hossain *et al.*, 2008). Despite the huge consumption of fruits, scientific data regarding their pharmacological and immunological properties are relatively scarce. External immunostimulants are very helpful to induce immune responses against microbes to prevent infections in immune-compromised conditions, such as aging, cancers, sepsis, diabetes, etc. (Goto *et al.*, 2010). Thus, the present investigation was aimed to assess some commonly eaten fruits of Bangladesh for their immunostimulating activities by determining the *in vitro* IgM production and proliferations of murine spleen cells. The traditional use and pharmacological reports of the currently investigating fruits are summarized in Table 1.

Correspondence to: Md. Moklesur Rahman Sarker; E-mail address**:** moklesur2002@yahoo.com

Table 1. Traditional uses and pharmacological activities of some common fruits of Bangladesh

Plant name (Family)	Local name/ common name	Traditional use(s)	Reported phytochemical/ pharmacological activities
Spondias dulcis L. (Anacardiaceae)	Amra/Ambarella	Enhancement of eyesight and treatment of eye infections (Rahmatullah *et al.*, 2009a).	Polysaccharide has been identified from the fruits pulp and it has eliciting activity on peritoneal macrophages (Iacomini *et al.*, 2005).
Citrus grandis L. Osbeck (Rutaceae)	Jambura/grapefruit	Treatment of skin diseases, such as, scabies, eczema, itches, etc. (Rahmatullah *et al.*, 2009b); treatment of fever (Rahmatullah *et al.*, 2010), and as carminative (Rahmatullah *et al.*, 2009b).	Juice has antioxidant and free radical scavenging abilities (Tsai *et al.*, 2007).
Phyllanthus embelica L. (Euphorbiaceae)	Amloki/Aamla	To maintain healthy conditions of body (Rahmatullah *et al.*, 2010), treatment of erectile dysfunction, diabetis (Rahmatullah *et al.*, 2009b).	Fruit extract exhibited anti-diarrheal (Perianayagam *et al.*, 2005), potent antioxidant (sometimes stronger than quercetin and BHA) (Liu *et al.*, 2008; Poltanov *et al.*, 2009; Scartezzini *et al.*, 2006; Rao *et al.*, 2005), anti-tussive (Nosalova *et al.*, 2003), antibacterial (Dhale and Mogle, 2011) and anti-diabetic (Krishnaraju *et al.*, 2005) activities. Phenolics purified from fruits reported to possess antioxidant (Bajpai *et al.*, 2005) and anti-proliferative capacities (Luo *et al.*, 2011; Zhang *et al.*, 2004,).
Dillenia indica L. (Dilleniaceae)	Chalta/Chulta/Ouu	Treatment of dysentery (Rahmatullah *et al.*, 2009c), abdominal pain, regulation of body`s heat, as expectorant in cough mixture, tonic, laxative and astringent (Maniruzzaman, 1993; Shendge *et al.*, 2011). The fruit mixed with sugar and water is used as a cooling beverage for the treatment of fever, to remove fatigue. The fruit juice is used as cardio-tonic (Shome *et al.*, 1980).	Phytochemical studies showed the presence of flavonoids, such as, myricetin, kaempferol, quercetin, isorhamnetin, naringenin; and phenolic materials (Banerji *et al.*, 1975; Pavanasasivam & Suktanbawa, 1975). Fruit extract exhibited antioxidant activity (Abdille *et al.*, 2005).
Ananas comosus (L.) Merr (Bromeliaceae)	Anaras/Pineapple	Treatment of fever, helminthiasis (Rahmatullah *et al.*, 2009a).	Cysteine proteinases (major one identified as bromelain) are present in pineapple and they have anti-edematous, anti-inflammatory, anti-thrombotic and fibrinolytic activities (Larocca *et al.*, 2010).
Citrus medica L. (Rutaceae)	Jamir/Citron	As cardiac tonic in palpitation (Krishnaraju *et al.*, 2006), anti-toxic, pulmonary sedative, appetizer and stomachic (Arias and Ramon-Laca, 2005).	Remedy against febrile illnesses (Ajaiyeoba *et al.* 2003), as anti-cancer (Tian *et al.*, 2001) and anti-oxidant (Jayaprakash and Patil, 2007).

Materials and Methods

Collection of sample and preparation of extract: Raw and green fruits of Amra (*S. dulcis),* Jambura (*C. grandis),* Amloki *(P. embelica),* Chalta (*D. indica),* Anaras (*A. comosus)* and Jamir (*C. medica)* were purchased from commercial stores at Dhaka, Bangladesh. They were authenticated by a Botanist at the Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh, and the voucher specimens were preserved accordingly. Fruits of *S. dulcis, P. embelica* and *D. indica* were sliced into small pieces and extracted with 70% methanol at room temperature with occasional shaking for 14 days. The juices were similarly extracted from *C. grandis, A. comosus* and *C. medica*. The extract was decanted and the solvent was removed with a rotary evaporator followed by freeze-drying to obtain the crude extract.

Chemicals and reagents: RPMI-1640 and Eagle's minimum essential medium (MEM) were purchased from ICN Biomedicals (Irvine, CA, USA) and Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan) respectively; Lipopolysaccharide (LPS) from *Escherichia coli* 055:B5: bovine serum albumin (BSA) (Fraction V), Tween 20, and fetal calf serum (FCS) were purchased from Sigma-Aldrich (Japan). Purified mouse IgM, goat anti-mouse IgM antibody (Ab), and horseradish peroxidase (HRP)-conjugated goat anti-mouse IgM Ab were obtained from Zymed Laboratories Inc (San Francisco, CA, USA), Organon Teknika Corporation (Durham, NC, USA), and Kirkegaad & Perry Laboratories (Gaithersburg, MD, USA), respectively. MTT [3-(4, 5-dimethylthiazol-2-y)-2, 5-diphenylterazolium bromide] reagent was purchased from Sigma Chemical Co. (Japan).

Mice: Female BALB/c mice were purchased from Charles River Japan (Yokohama, Japan). They were maintained under specific pathogen-free conditions in the animal facility of Okayama University and ages varied

between 8 to 12 weeks. All experimental procedures concerned with mice were performed according to the guidelines established by the Ministry of Education, Culture, Sports, Science and Technology of Japan, and to the Guidelines for Animal Experiments at Okayama University. Procedures involved were approved by the Animal Research Control Committee of Okayama University, Japan.

Preparation of murine spleen cells: Spleen cells from BALB/c female mice, void of erythrocytes, were prepared by lysis of erythrocytes with ammonium chloride as described previously (Sarker *et al.*, 2011).Mice were killed and the spleens were collected aseptically and mashed with spatula through the strainer to pass the cells into MEM (pH adjusted to around 7.0 with 1/N NaOH) in a petridish. Cells were suspended by Pasteur pipette and screened by passing through a mesh into the centrifuge tube. The cell suspensions were then centrifuged for 5 min at 4ºC and 2000 rpm. The supernatants were removed and ACK lysis buffer (0.15 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA; pH 7.2) was added to the cells in the centrifuge tube for the lysis of the erythrocytes for 5 min at room temperature. MEM was added to the centrifuge tube, suspended and centrifuged for 5 min at 4ºC and 2000 rpm. The supernatants were removed and the cell pellets were washed twice with MEM. The cells were re-suspended in MEM and passed through a mesh into another centrifuge tube to collect the spleen cells. The viability of the prepared splenocytes was determined by the Trypan-blue exclusion technique and cells having viability higher than 70% were used for the experiments.

Cell culture: Spleen cells which were obtained as described previously were suspended in basal culture medium (RPMI 1640 medium, supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml of penicillin G and 100 µg/ml of streptomycin). The cells (2.5×10^5 cells/100 µl/well) were plated in 96-well U-bottom plates (Nunc, Roskilde, Denmark) and incubated for 30 min at 37ºC in a fully humidified atmosphere containing 5% CO_2. 50 µl of 2-mercaptoethanol (2-ME) (0.2 mM) was added into each well and the plates were incubated for 5 days with or without the addition of different doses of fruits extract and LPS at 37ºC in the CO_2 incubator. The cultural supernatants were then collected and frozen at -30ºC for IgM-ELISA and the cells pellets were used for the investigation in the proliferation of spleen cells.

Cell proliferation study by MTT method: The growth of cultured cells was determined by MTT method as described by Hansen *et al*., 1989. At the end of incubation for 120 hrs, 160 µl supernatants were removed. 60 µl of fresh medium and 25 µl of MTT solution were added in each well and the plates were incubated for 2 hrs. After addition of 100µl stock solution in each well, the plate was incubated overnight in dark at 37ºC .The absorbance was then measured at 570 nm by using a plate reader.

ELISA for the determination of IgM production: The IgM levels produced as a result of immunostimulation by the extracts were measured by a sandwich ELISA as described previously (Sarker *et al.*, 2011; Goto *et al.*, 2010). Each well of 96-well microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) containing 50 µl/well of goat anti-mouse IgM antibody (10 µg/ml) and phosphate buffer saline (PBS),was incubated overnight in the dark at 4 ºC. The plates were washed three times by PBS containing 0.05% Tween 20 (wash-buffer) (200µl/well). The wells were blocked with 200 µl of 1% bovine serum albumin (BSA) in PBS for 2 hours at room temperature. After washing the plates 100 µl/well of cultural supernatants (diluted with 1% BSA-PBS-Tween 20, accordingly) or standard mouse-IgM were added into each well and the plates were incubated for 2 hours at room temperature. The plates were again washed three times by wash buffer (200µl/well). Fifty µl per well of horseradish peroxidase-conjugated goat anti-mouse IgM antibody (0.2 µg/ml) was added into each well and the plates were incubated for 1 h at room temperature. After washing the plates 100 µl/well of 0.1 M citrate buffer (pH 4.0) containing 2.5 mM 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and 0.17% H_2O_2 were added. The plates were incubated for 10 min at room temperature and the optical densities at 405 nm were measured using an automatic plate reader (Bio-Rad Laboratories, USA).

Results and Discussion

Effect of Bangladeshi fruit extracts on the production of polyclonal IgM in vitro: Traditional uses and updated scientific reports six of the fruits under investigation were noted in Table 1. BALB/c female mice spleen cells were sub-cultured with or without the extracts of different fruits

for 5 days and the amount of IgM production in the cultural supernatants were determined by an IgM-ELISA as previously mentioned in the experimental section. LPS was used as a positive control. As shown in the Fig.1, cultured splenocytes treated with 200µg/ml of 70% methanol extract of *S. dulcis* (Amra) and *D. indica* (Chalta) enhanced IgM production by 108.33% (Fig. 1A) and 90.72% (Fig. 1D) in comparison to untreated cells, respectively. Similarly, juice extract of *C. medica* (Jamir) augmented IgM production level by 103.85% when compared to the control (Fig. 1F). Juice extract of *A. comosus* (Anaras) at the doses of 20 µg/ml, 200 µg/ml and 2 mg/ml increased IgM level in cultured supernatants by 123.33%, 155% and 190% respectively (Fig. 1E). On the other hand, juice extract of *C. grandis* (Jambura) and methanol extract of *P. embelica* (Amloki) failed to enhance IgM level regardless to the dose applied (Fig. 1B & C).

Evaluation of six fruit extracts for the proliferation of murine splenocytes: BALB/c female mice whole spleen cells were sub-cultured with or without the indicated doses of the selected fruit extracts for 5 days and the proliferation of cells were measured by MTT assay. The investigations showed that no dose of any extracts could remarkably stimulate the proliferation of cultured cells (Fig. 2). Instead, in some cases, the proliferations of cells were suppressed when treated with extracts (Fig. 2 E & F).

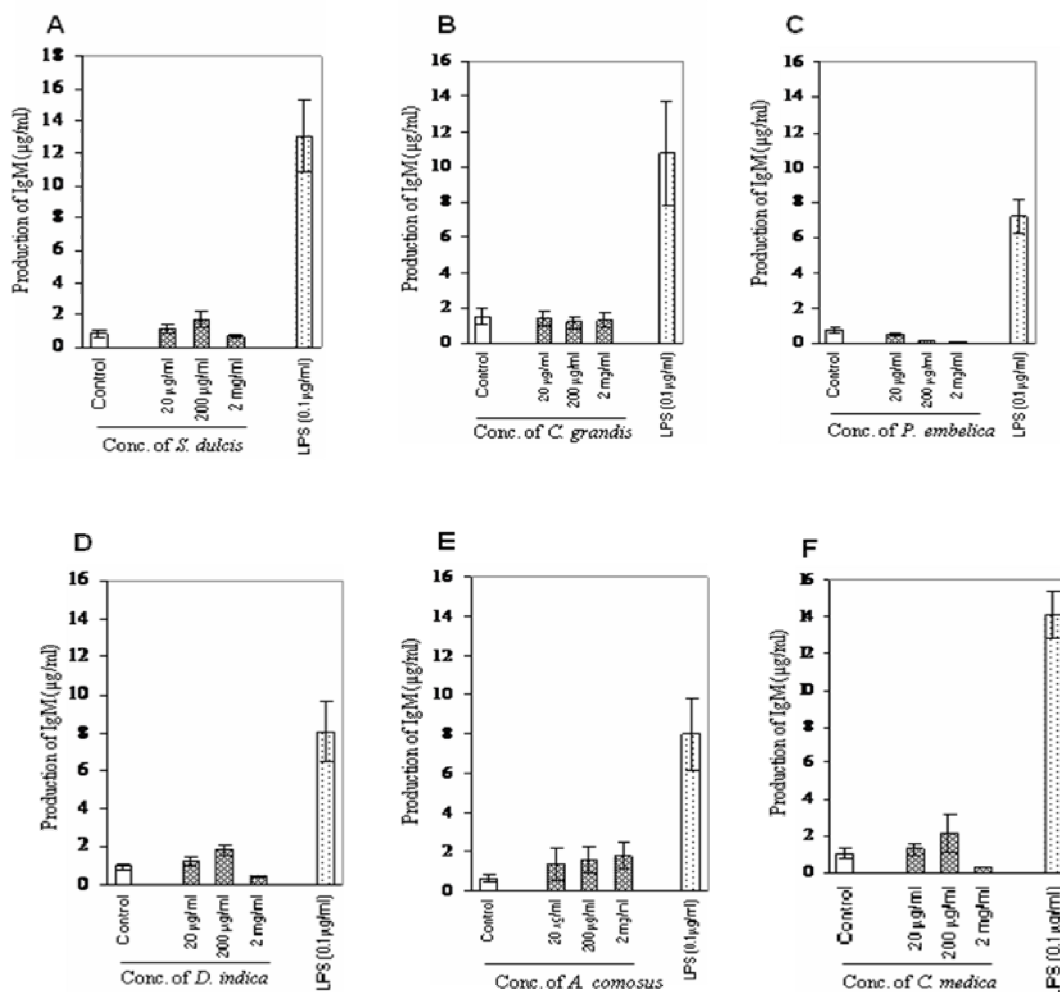

Figure 1. Evaluation of different fruit extracts for the production of polyclonal IgM in cultural supernatants of murine splenocytes. BALB/c mice whole spleen cells (2.5×10^5 cells/well) were incubated with the indicated concentrations of extracts of six Bangladeshi fruits at 37ºC in the 5% CO_2 incubator for 5 days. The IgM levels in the cultured supernatants were determined by an IgM-ELISA. The data are means ± S.D. of triplicate cultures.

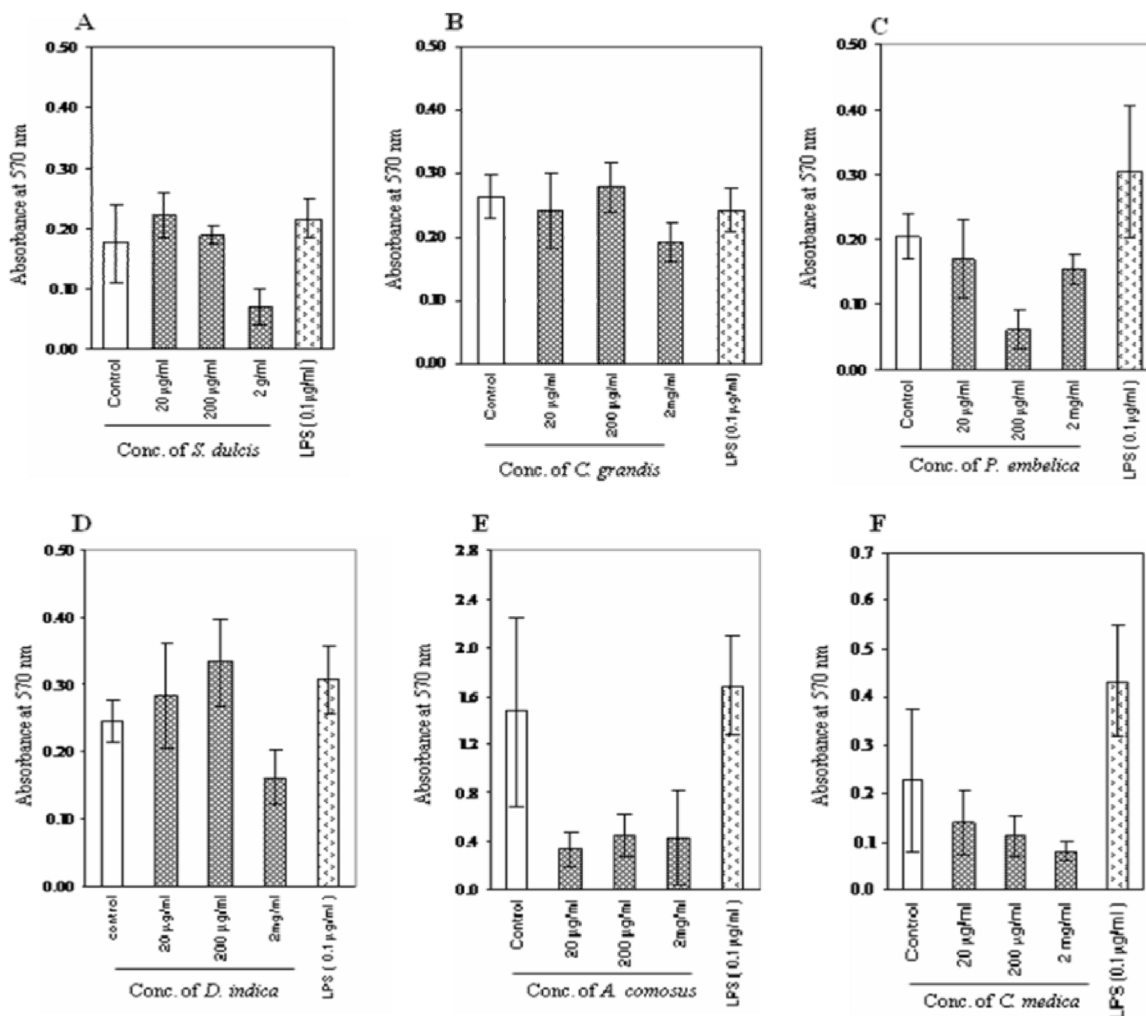

Figure 2. Evaluation of crude extracts of six Bangladeshi fruits for the proliferation of murine splenocytes in culture. BALB/c mice whole spleen cells (2.5×105 cells/well) were incubated with the indicated concentrations of different extracts at 37ºC in the 5% CO_2 incubator for 5 days. The proliferations of cells were measured by MTT method. The data are means ± S.D. of triplicate cultures.

The investigation resulted in moderate immunostimulating properties in four of the six crude extracts of *S. dulcis, D. indica*, *A. comosus* and *C. medica.* Enhanced antibody production demonstrated that the extracts of these fruits promoted the differentiation of B cells to the antibody secreting plasma cells. It is important to note here that although the crude extract of *A. comosus* promoted IgM production (Fig. 1E), it suppressed the proliferation of cultured cells (Fig. 2 E), the exact reason of which is not known.

Lacomini *et al.*, (2005) reported the eliciting activity of *S. dulcis* on peritoneal macrophage. The present report would be an addition to this on the immunomudulatory activities of *S. dulcis* for future investigations. Many reports were published on the antioxidant (Liu *et al.*, 2008; Poltanov *et al.*, 2009) and anti-cancer properties (Luo *et al.*, 2011; Zhang *et al.*, 2004) of *P. embelica*. In the present study, crude methanol extract of this fruit could neither enhance antibody (IgM) production nor stimulate splenocytes proliferation *in vitro*.

It has been reported that cysteine proteinases, which is present in pineapple, has anti-thrombotic, anti-inflammatory and fibrinolytic activities (Larocca *et al.*, 2010). *C. medica* fruit was reported to possess anticancer (Tian *et al.*, 2001) and antioxidant properties (Jayaprakash and Patil, 2007). The present study found that the crude extracts of the two fruits exhibited immunoenhancing capacity through the enhancement of IgM production (Fig. 1E & F). Thus, these fruits can be used for the prevention and treatment of cancer, inflammation and body defense.

Conclusion

This preliminary study reports that four out of six screened fruits possess immunostimulating activities promoting humoral immune response through the enhancement of IgM production. This report may guide for further investigations. This study will help the people to select fruit(s) if they need external immunostimulants through food intake in case of body`s impaired immune system. As this is a preliminary report, additional cellular and humoral scientific investigations are recommended.

Acknowledgements

The authors are indebted to Prof. Dr. Eiichi Gohda, Chairman, Department of Immunochemistry, Faculty of Pharmaceutical Sciences, Okayama University for his valuable suggestions, guidance and for providing the facilities to conduct the experiments in this research. The authors are also grateful to the authorities of Okayama University for their cooperation and for providing the equipment carry out this investigation

References

Abdille, M.H., Sing, R.P., Jayaprakash, G.K. and Jena, B.S. 2005. Antioxidant activity of the extracts from *Dillenia indica* fruits. *Food Chem.* **90**, 891-6.

Ajaiyeoba, E.O., Oladepo, O., Fawole, O.I., Bolaji, O.M., Akinboye, D.O., Ogundahunsi, O.A., Falade, C.O., Gbotosho, G.O., Itiola, O.A., Happi, T.C., Ebong, O.O., Ononiwu, I.M., Osowole, O.S., Oduola, O.O., Ashidi, J.S. and Oduola, A.M. 2003. Cultural categorization of febrile illnesses in correlation with herbal remedies used for treatment in Southwestern Nigeria. *J. Ethnopharmacol.* **85**, 179-185.

Ames, B.N., Shigenaga, M.K. and Hagen, T.M. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Nat. Acad. Sci.* **90**, 7915-7922.

Arias, B.A. and Ramon-Laca, L. 2005. Pharmacological properties of citrus and their ancient and medieval uses in the Mediterranean region. *J. Ethnopharmacol.* **97**, 89-95.

Bajpai, M.A., Pande, A., Tewari, S.K. and Prakash, D. 2005. Phenolic content and antioxidant activity of some food and medicinal plants. *Int. J. Food Sci. Nutr.* **56**, 287-291.

Banerji, N., Majumder, P. and Dutta, N.L. 1975. A new pentacyclic triterpene lactone from *Dillenia indica*. *Phytochemistry.* **12**, 1447-8.

Dhale, D.A. and Mogle, U.P. 2011. Phytochemical Screening and Antibacterial Activity of *Phyllanthus emblica* (L.). *Sci. Res. Rep.* **1**, 138-142.

Di Matteo, V. and Esposito, E. 2003. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer`s disease, Parkinson`s diseases, and amyotrophic lateral sclerosis. *Curr. Drug Target CNS Neurol. Disorder* **2**, 95-107.

Goto, T., Sarker, M.M.R., Zhong, M., Tanaka, S. and Gohda, E. 2010. Enhancement of immunoglobulin production in B cells by the extract of red bell pepper. *J. Health Sci.* **56**, 304-309.

Hansen, M.B., Neilsen, S.E. and Berg, K. 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J. Immunol. Methods* **119**, 203-210.

Haque, M.N., Saha, B.K., Karim, M.R. and Bhuiyan, M.N.H. 2009. Evaluation of Nutritional and Physico-Chemical Properties of Several Selected Fruits in Bangladesh. *Bangladesh J. Sci. Ind. Res.* **44**, 353-358.

Hossain, S.J., Tsujiyama, I., Takasugi, M., Islam, M.A., Biswas, R.S. and Aoshima, H. 2008. Total Phenolic Content, Antioxidative, Anti-amylase, Anti-glucosidase, and Antihistamine Release Activities of Bangladesh Fruits. *Food Sci. Technol. Res.* **14**, 261-268.

Iacomini, M., Serrato, R.V., Sassaki, G.L., Lopes, L., Buchi, D.F. and Gorin, P.A.J. 2005. Isolation and partial characterization of a pectic polysaccharide from the fruit pulp of *Spondias cythera* and its effect on peritoneal macrophage activation. *Fitoterapia* **76**, 676-683.

Jayaprakash, G.K. and Patil, B.S. 2007. *In vitro* evaluation of the antioxidant activities in fruit extracts from citron and blood orange. *Food Chem.* **101,** 410-418.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Caval, S.M., Binkoshi, A.E., Hilpert, K.F., Griet, A.E. and Etherton, T.D. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular diseases and cancers. *Am. J. Med.* **113**, 71S-88S.

Krishnaraju, A.V., Rao, T.V.N., Sundararaju, D., Vanisree, M., Tsay, H-S. and Subbaraju, G.V. 2006. Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test). *Int. J. Appl. Sci. Eng.* **4**, 115-125.

Krishnaraju, A.V.K., Rao, T.V.N., Sundararaju, D., Vanisree, M., Tsay, H-S. and Subbaraju, G.V. 2005. Assessment of Bioactivity of Indian Medicinal Plants Using Brine Shrimp (*Artemia salina*) Lethality Assay. *Int. J. Appl. Sci Engr.* **3**, 125-134.

Larocca, M., Rossano, R., Santamaria, M. and Riccio, P. 2010. Analysis of pineapple [Ananas comosus (L.) Merr.] fruit proteinases by 2-D zymography and direct identification of the major zymographic spots by mass spectrometry. *Food Chem.* **123**, 1334-1342.

Liu, X., Zhao, M., Wang, J., Yang, B. and Jiang, Y. 2008. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions of China. *J. Food Com. Anal.* **21**, 219-228.

Luo, W., Zhao, M., Yang, B., Ren, J., Shen, G. and Rao, G. 2011. Antioxidant and antiproliferative capacities of phenolics purified from *Phyllanthus emblica* L. fruit. *Food Chem.* **126**, 277-282.

Maniruzzaman, F.M. 1993. Udvid Samhita (A compendium of plants in Bangladesh). 1sted., Dhaka, Bangla Academy, p. 270.

Nosalova, G., Mokry, J. and Hassan, K.M.T. 2003. Antitussive activity of the fruit extract of *Emblica officinalis* Gaertn. (Euphorbiaceae). *Phytomedicine.* **10**, 583-589.

Pavanasasivam, G. and Suktanbawa, M.U. 1975. Flavonoids of some Dilleniaceae species. *Phytochemistry.* **14**, 1127-8.

Perianayagam, J.B., Narayanan, S., Gnanasekar, G., Pandurangan, A., Raja, S., Rajagopal, K., Vojayarajkumar, P. and Vijayakumar, S.G. 2005. Evaluation of Antidiarrheal Potential of *Emblica officinalis*. *Pharma. Biol.* **43**, 373-377.

Poltanov, E.A., Shikov, A.N., Dorman, H.J.D., Pozharitskaya, O.N., Makarov, V.G., Tikhonov, V.P. and Hiltunen, R. 2009. Chemical and antioxidant evaluation of Indian gooseberry (*Emblica officinalis* gaertn., syn. *Phyllanthus emblica* L.) supplements. *Phytothera. Res.* **23**, 1309-1315.

Rahmatullah, M., Ferdausi, D., Mollik, M.A.H., Azam, M.N.K., Rahman, M.T.U. and Jahan, R. 2009a. Ethnomedical Survey of Bheramara Area in Kushtia District, Bangladesh. *Am-Eurasian J. Sustain. Agric.* **3**, 534-541.

Rahmatullah, M., Haque, M.R., Islam, S.K., Jamal, F., Bashar, A.B.M.A., Ahmed, R., Ahmed, I., Jahan, R., Ahsan, S. and Chowdhury, M.H. 2010. A survey of the Use of Medicinal Plants by Folk Medicine Practitioners in Three Areas of Pirojpur District, Bangladesh. *Am-Eurasian J. Sustain. Agric.* **4**, 247-259.

Rahmatullah, M., Mukti, I.J., Haque, A.K.M.F., Mollik, M.A.H., Parvin, K., Jahan, R., howdhury, M.H. and Rahman, T. 2009b. An ethnobotanical survey and pharmacological evaluation of medicinal plants used by the Garo tribal community living in Netrokona district, Bangladesh. *Adv. Nat. Appl. Sci.* **3**, 402-418.

Rahmatullah, M., Noman, A., Hossan, M.S., Rashid, M.H.O., Rahman, T., Chowdhury, M.H. and Jahan, R. 2009c. A survey of medicinal plants in two areas of Dinajpur district, Bangladesh, including plants which can be used as functional foods. *Am-Eurasian J. Sustain. Agric.* **3**, 862-876.

Rao, T.P., Sakaguchi, N., Juneja, L.R., Wada, E. and Yokozawa, T. 2005. Amla (*Emblica officinalis* Gaertn.) Extracts Reduce Oxidative Stress in Streptozotocin-Induced Diabetes Rats. *J. Med. Food* **8**, 362-368.

Sarker, M.M.R., Mazumder, M.E.H. and Rashid, M.H.O. 2011. *In Vitro* Enhancement of Polyclonal IgM Production by Ethanolic Extract of *Nigella sativa* L. Seeds in Whole Spleen Cells of Female BALB/c mice. *Bangladesh Pharm. J.* **14**, 73-77.

Scartezzini, P., Antognoni, F., Raggi, M.A., Poli, F. and Sabbioni, C. 2006. Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of *Emblica officinalis* Gaertn. *J. Ethnopharmacol.* **104**, 113-118.

Shendge, P., Patil, L. and Kadam, V. 2011. In vitro evaluation of antioxidant activity of *Dillenia indica* Linn. leaf extract. *Int. J. Pharm. Sci. Res.* **2**, 1814-1818.

Shome, U., Khanna, R.K. and Sharma, H.P. 1980. Pharmacognostic studies on *Dillenia indica* Linn. II-Fruit and Seed. *Proc. Indian Acad. Sci.* **89**, 91-104.

Tian, Q., Miller, E.G., Ahmad, H., Tang, L. and Patil, B.S. 2001. Differential inhibition of human cancer cell proliferation by citrus limonoids. *Nutr. Cancer* **40**, 180-184.

Tripoli, E., Guardia, M.L., Giammanco, S., Majo, D.D. and Giammanco, M. 2007. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chem.* **104,** 466-479.

Tsai, H-L., Chang, S.K.C. and Chang, S-J. 2007. Antioxidant Content and Free Radical Scavenging Ability of Fresh Red Pummelo [*Citrus grandis* (L.) Osbeck] Juice and Freeze-Dried Products. *J. Agric. Food Chem.* **55**, 2867-2872.

Zhang, Y.J., Nagao, T., Tanaka, T., Yang, C.R., Okabe, H. and Kouno, I. 2004. Antiproliferative Activity of the Main Constituents from *Phyllanthus emblica*. *Biol. Pharm. Bull.* **27**, 251-255.