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The Anthocyanin Components and Cytotoxic Activity of *Syzygium cumini* (L.) Fruits Growing in Egypt

Naglaa M. Nazif*

Phytochemistry Dept, National Research Center, El Bohooth St. Dokki, 12311 Cairo, Egypt.

Abstract – Four anthocyanins were isolated from the acidic alcoholic extract of *Syzygium cumini* fruits growing in Egypt: pelargonidin-3-*O*-glucoside, pelargonidin-3,5-*O*-diglucoside, cyanidin-3-*O*-malonyl glucoside, and delphenidin-3-*O*-glucoside. They were identified by the chromatographic, TLC and PC, and spectral analyses, UV, ^{1}H-NMR and FAB/MS. The fruits were found to contain 0.03 gm % anthocyanins calculated on fresh weight basis calculated by spectrophotometric assay. Cytotoxic activity of total alcoholic extract of the fruits was performed against several types of tumor cell lines using the SRB assay. The tested extract exhibited significant cytotoxic activity for MCF7 (breast carcinoma cell line) (IC_{50} = 5.9 μg/mL), while the IC_{50} was > 10 μg/mL for both Hela (Cervix carcinoma cell line), HEPG2 (liver carcinoma cell line), H460 (Lung carcinoma cell line) and U251 (Brain carcinoma cell line).

Keywords – *Syzygium cumini* (L.) fruits, anthocyanin, cytotoxicity.

Introduction

Syzygium cuminii (L.) it is also known (*Eugenia jambolana* Lam.), also called black plum-JAVA plum, Jamun, Jambolan, duhat. Fam. Myrtaceae. It is native to south east Asia and India (British Herbal Pharmacopoeia, 1976).The fruits and seeds of *S.cumini* are used to treat diabetes mellitus for several centuries in folkoloric medicine of south Asia (Bhatia, & Bajaj, 1975). Several authors (Al-Zaid *et al.*, 1991; Teixeira, *et al.*, 1997; Prince, 1998; Grover, *et al.*, 2000; Yarnell, E. 2000) reported that *S.cumini* showed significant hypoglycemic activity but some presented some toxic properties. The seeds are used as astringent and diuretic (Bhatia, & Bajaj, 1975). Craveiro, *et al.*, 1983 studied the essential oils of leaves, stems, and fruits of *S.cumini* and their antibacterial activity was reported by Shafi *et al.*, 2002. In vitro anti-oxidant activity of the fruits of *S. cumini* was proven by several techniques (Banerjee *et al.*, 2005). Anthocyanins are naturally occurring reddish and bluish violet pigments in fruits and vegetables. *Syzygium cumini* L. fruits are rich source of anthocyanins. Athocyanins proved to be anti tumor agents (Lazze, *et al.*, 2004; Ping- Hsiao, *et al.*, 2005). Nowadays great interest was paid to biological and chemical composition of plants containing anthocyanins. No phytochemical or biologigal investigation were carried out on *Syzygium cumini* L. fruits growing in Egypt.

So the present work deals with investigation of the anthocyanins content of *Syzygium cumini* L. fruits growing in Egypt and their biological activity.

Experimental

Plant Material – The fruits of *Syzyguim cumini* L. were collected from the agricultural museum at Dokkie, Cairo, Egypt at July 2006 and kindly identified by professor Dr. Said Farag Khalifa, Botany Department, Ain Shams University Cairo, Egypt To whom the author is deeply indebted.

General – TLC was carried out on precoated microcrystalline cellulose plates (Merck) (Darmstadt, Germany) developed with *n*-butanol-formic acid-H_2O (6 : 1 : 2, 4 : 1 : 5, top layer, 4 : 1 : 2 solvent a1, a2, a3), *n*-butanol -2 M HCl (1 : 1) v/v, the top layer (solvent a4) and EtOAc-H_2O-MeOH-HOAc (13 : 3 : 3 : 4), (solvent b for sugars). PPC was carried out on Whatmann 3 mm eluted by the same solvents. UV detection was carried at 254 and 336 nm. Column chromatography was performed on Sephadex LH-20 (Merck) (Darmstadt, Germany). UV spectra were recorded on a Shimadzu pc-2401 double beam UV-visible spectrophotometer in the region of 200 - 600 nm, using absolute spectroscopic methanol containing 0.1% HCl. FAB/MS were obtained on JEOL JMS-AX500 mass

*Author for correspondence
Fax: 202-3370931; E-mail: maglaanazif@yahoo.com

spectrometer. ^{1}H-NMR was recorded on JEOL GX at 270 MHz using TMS as internal standard. ^{1}H- NMR spectra were recorded in CF_3COOD/DMSO-d_6 1 : 9 v/v.

Preparation of the samples – 50 g of fresh fruits of *S.cumini* was extracted with ethanol till exhaustion .Total ethanolic extract was subjected to evaporation of the solvent in vacuo at 30 °C, the remainder aqueous extract was lyophilized (5.8 g). 1 mg of this lyophilized powder was dissolved in 0.1 ml of DMSO and the volume completed to 1 ml with distilled water and sent to the National Cancer Institute.

The plant extract was screened in vitro using a single tumor (Ehrlich ascites carcinoma cells). The tumor cells were maintained in the laboratory by weekly intra-pritonial transplantation in female albino mice from the animal house of National Cancer Institute. A set of sterile test tubes was used for each test solution, where 2.5×10^6 tumor cells per ml were suspended in phosphate buffer 0.1 ml of different dilutions of each test solution was added separately to the suspension, kept at 37 °C for 2 hours. Trypan blue dye exclusion test (Mclimans, *et al.*, 1957) was then carried out to calculate the percentage of non- viable cells. Using a dose of 100 μg/ml, 50 μg/ml, 25 μg/mL of each extract. Concentrations causing less than 30% non-viable cells in the suspension were considered inactive, while those producing more than 70% non-viable cells were considered active.

Assay method for cytotoxic activity – Potential cytotoxic activity of the total ethanolic extract was performed in the National Cancer Institute using the previously reported method (Skehan *et al.*, 1990). Cells were plated in (10^4 cells/well) for 24hrs before treatment with the isolated compound to allow attachment of cell to the plate. Different concentrations of the compound under test (0, 1, 2.5, 5, 10 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated for 48 hr at 37 °C in atmosphere of 5% CO_2, after 48 hr cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and the drug concentration is plotted fig (1) to get the survival curve of each tumor cell line for the specified compound (Skehan *et al.*, 1990). The effective dose required to inhibit cell growth by 50% (IC_{50} μg/mL) was determined (Skehan *et al.*, 1990). Doxorubicin was used as positive control. The total alcoholic extract was tested for the following human tumor cell lines at concentrations between 1.00 - 10.00 μg/mL using the SRB assay.

1- U251 (brain tumor cell line).
2- HEPG2 (Hepatocellular carcinoma cell line).
3- Hela cell (cervix tumor cell line)
4- H460 (Lung carcinoma cell line)
5- MCF7 (breast carcinoma cell line)

Quantitative determination of anthocyanins – 5 grams of *Syzygium cumini* L. fruits (deprived of Seeds) were exhaustively extracted by methanol containing 0.1% HCl, the extract dried under vacuo at 30 °C the residue transferred to measuring flask 100 ml and, brought to volume by acidic methanol, standard curve of delphinidin 3-o-glucoside was constructed, using 3 mg dissolved in the same solvent, serial dilutions were made, and the absorption was measured at 537 nm for all the diluted samples and the concentration of anthocyanins content was extrapolated from the constructed standard curve. The result was the mean of triplicates.

Isolation of anthocyanins – Anthocyanin pigments were extracted from fresh fruits (3 kg) of *Syzygium cumini* L by methanol-Formic acid-water (MFW, 10 : 1 : 9). 10L, the filtered extracts taken to dryness in vacuo at 30 °C (49 g). 10 g of the residue after dissolution in MFW, was passed through a sephadex LH-20 column (60 g presoaked gel packed in glass column of 80 × 4 cm i.d) in the same solvent. The eluent fractions were again taken to dryness separately and further purified by PPC in the solvents *n*-butanol-HOAc-H_2O (6 : 1 : 2 v/v), and *n*-butanol -HOAc -H_2O (4 : 1 : 5 v/v) the top layer. 4 pure pigments were obtained. Finally each pigment was once more purified on Sephadex LH-20 (column 30 × 1.0 cm i.d.) in MFW.

Results

The fruits were found to contain 0.03 g % anthocyanins calculated on fresh weight basis. It was calculated as delphinidin-3-*O*-glucoside using spectrophotometric method.

Compound (1) – The band appeared as reddish orange spot in visible light, having an R_f (0.44, Pc. 3 mm, solvent a1), R_f (o.34, Pc. 3 mm solvent a4) and R_f (0.46 TLC microcrystalline cellulose, solvent a2) (7 mg) (Terahara *et al.*,1986). The UV/vis spectrum of this pigment showed λmax (MeOH/0.1%HCl) at 510, 431, 339, 272 nm. The absence of bathochromic shift with Aluminium chloride shift reagent indicating the absence of ortho dihydroxy system in B ring. FAB/MS showed molecular mass [M^+] *m/z* at 433 corresponding to the molecular formula ($C_{21}H_{20}O_{10}$), and at *m/z* 271 the molecular weight of the aglycone.^{1}H-NMR Table 1 showed signals were found to

Table 1. ^{1}H-NMR spectral data for *syzygium cumini L.* anthocyanins in $CF_3COOD/DMSO\text{-}d_6$ (1 : 9 v/v)

Carbon no	1	3*	4
4	9.0 (1H, s)	8.84 (1H, s)	8.96 (1H, s)
6	6.75 (1H, d, J = 1.7 Hz)	6.75 (1H, d, J = 2.0 Hz)	6.65 (1H, d, J = 2.0 Hz)
8	7.04 (1H, s)	6.97 (1H, s)	6.87 (1H, dd, J = 2.0, 2.8 Hz)
2'	8.65 (1H, d, J = 9.4 Hz)	8.08 (1H, d, J = 2.0 Hz)	7.76 (1H, s)
3'	7.12 (1H, d, J = 8.8 Hz)	–	–
5'	7.12 (1H, d, J = 8.8 Hz)	7.08 (1H, d, J = 2.0 Hz)	–
6'	8.65 (1H, d, J = 8.8 Hz)	8.15 (1H, dd, J = 8.3, 2.6 Hz)	7.76 (1H, s)
Glucose	(3.2 - 4.0) 6H, m	(3.23 - 4.51) 6H, m	(3.1 - 4.1) 6H, m
1"	5.40 (1H, d, J = 7.8 Hz)	5.44 (1H, d, J = 6.8 Hz)	5.30 (1H, d, J = 7.6 Hz)

* At σ 3.42 (2H, s, malonyl CH_2)

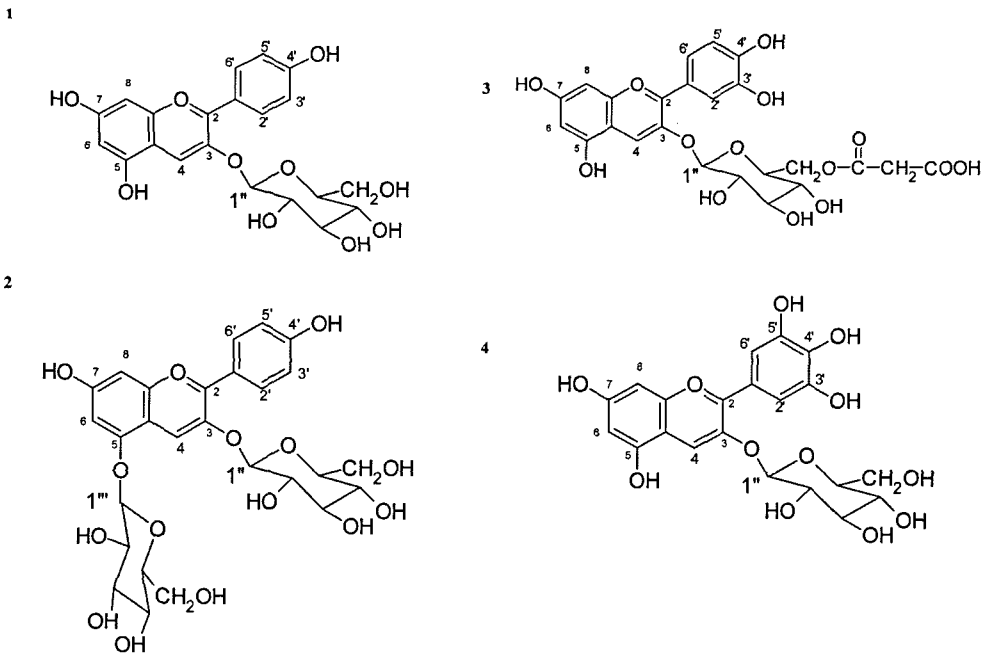

Fig. 1. Isolated anthocyanins

be in agreement with those reported for pelargonidin-3-*O*-glucoside (Harborn & Mabry, 1982).

Compound (2) - The band appeared as reddish orange on the paper chromatogram in visible light and appeared as red fluorescent in the UV light at 366 nm. Having an R_f value of (0.32, Pc.3MM, solvent a2) R_f (0.16 Pc. solvent a3), and R_f (0.39 TLC, solvent a4) (3 mg) (Takeda *et al.,* 1986). The UV/visible. absorption spectrum showed λmax., (MeOH/0.1% HCl) at 512, 429, 330, 270 nm. The FAB/MS of this pigment showed *m/z* at 595 constitutes to molecular formula of $C_{27}H_{30}O_{15}$, and fragment ions at *m/z* 433 (M-glucosyl radical), and *m/z* 271, (M-2-glucosyl radicals). Degradation with H_2O_2 in alkaline medium gave pelargonidin-5-o-glucoside and glucose (Saito *et al.,* 1985) the later was detected by PC eluted by solvent b with authentic samples. Further acid hydrolysis produced pelargonidin and glucose (Takeda *et al.,* 1986). These data were found to be in accordance with that reported for pelargonidin-3, 5-*O*-diglucoside (Cornuz, *et al.,* 1981).

Compound (3) – The pigment has a reddish violet color on paper chromatogram in visible light, of R_f (0.30, Pc. 3 mm, solvent a2), R_f (0.19 on Pc. 3 mm, solvent a3) and R_f 0.35 TLC, solvent a4) (19 mg) (Takeda *et al.,* 1986). The UV/visible absorption spectrum showed λmax (MeOH/0.1% HCl) at 532, 410, 282, 238 nm, it showed bathochromic shift of 18 nm on addition of $AlCl_3$ shift reagent. The FAB/MS showed [M^+] at *m/z* 535 which

constitutes to the molecular formula C_{24} $H_{23}O_{14}$, the malonated anthocyanins exhibited a characteristic fragmentation pattern with an initial loss of 44 mass unit due to decarboxylation of the free carboxylic group (Saito, *et al.*, 1985), followed by further loss of the residual 42 mass unit to give fragment unit at *m/z* 449 (535-86 malonyl radical), and at *m/z* 287 (cyanidin). ^{1}H-NMR Table 1 showed signals were found to be in agreement with those of cyanidin-3-*O*-malonyl glucoside (Bridle, *et al.*, 1984).

Compound (4) – The pigment appeared as mauve colored spot in day light, with R_f (0.2, TLC eluted by solvent a2), R_f (0.03 TLC, eluted by solvent a4), R_f 0.15, Pc. 3 mm, solvent a1) (24 mg), The UV/visible spectrum showed λmax (MeOH/0.1% HCl) 540, 289, 254, 212 nm. FAB/MS showed *m/z* the molecular cation [M^+] at *m/z* 465 in good agreement with the mass calculated for $C_{21}H_{21}O_{12}$ and an ion at *m/z* 303 corresponding to the aglycone delphinidin, which was formed by loss of glucose. ^{1}H-NMR Table 1 was found to be in agreement with that reported for delphinidin-3-*O*-glucoside (Guisti, *et al.*, 1999 & Takeoka, *et al.*, 1997).

Discussion

The total ethanolic extract of *Syzygium cumini* L. showed promising antitumor activity on screening by Ehrlich test since a doses of 100 & 50 μg/mL showed 100% non viable count and that of 25 μg/mL concentration showed 95% non-viable cells. When the total ethanolic extract was subjected to cytotoxic activity on the cell lines available as illustrated before it showed (IC_{50} = 5.9 μg/mL), on MCF7 (breast carcinoma cell line) Fig. 2. This activity may be attributed to the anthocyanins content this postulate potentiated by several recent published researches since they found that the fruits which demonstrated greater antioxidant activity were all rich in anthocyanins, and *S.cumini* showed strong in vitro antioxidant properties (Banerjee, *et al.*, 2005). Phytochemical investigation of *S. cumini* fruits grown in Egypt revealed the isolation of 4 anthocyanins. Two of them are minors (pelargonidin-3-*O*-glucoside and pelargonidin-3,5-*O*-diglucoside), and two majors cyanidin-3-*O*-malonyl glucoside and delphinidin-3-*O*-glucoside.The fast atom bombardment FAB/MS helps in the structural elucidation of the 4 pigments since anthocyanins behave as pre-formed ions and give molecular masses directly as [M^+]. In previous work carried out by Jain and Seshadri, 1975 detected 3 anthocyanins delphinidin, petunidin-3-*O*-gentiobioside, and malvidin-3-*O*-laminaribioside from *E.jambolana* (*S.cumini*) grown in India. Red

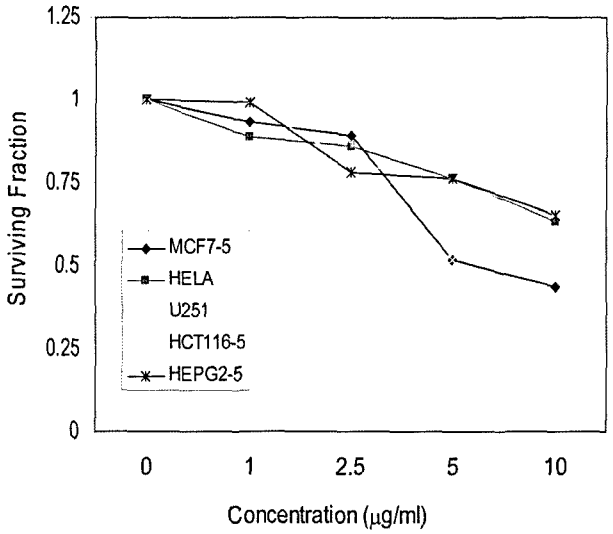

Fig. 2. Cytotoxic activity of total ethanol extract of *Syzygium cumini L.*

wine contains polyphenolics mainly anthocyanins. Its inhibitory effects on human breast cancer have been demonstrated (Hakimuddin, *et al.*, 2004). Cyanidins may also demonstrate antimutagenic activity. Anthocyanidins inhibit prolefiration and induce apoptosis in human gastric adenocarcenoma cells (Ping *et al.*, 2005). Grapfruit juice, a well known source of cyanidins, was able to inhibit colon DNA damage, its anthocyanins protect against DNA damage induced by oxidative agents in rats smooth muscle and hepatoma cells (Miyata *et al.*, 2000). Raspberry extract which is rich in anthocynins demonstrates an ability to inhibit proliferation of HEPG2 human liver cancer cells (Lazze' *et al.* 2003; Meiers *et al.*, 2001). The fruits of four vaccinium species containing cyanidins (bilberry, cranberry, lowbush blue berry and lingonberry) have demonstrated anticarcinogenic properties (Bomser, 2002). Moreover, cyanidins have been shown to inhibit the epidermal growth factor receptor of the human vulva carcinoma cell line A431 (Wang and Mazza 2002), as well as induce production of the tumor necrosis factor (Ghosh, D. McGhie, 2006). Also anthocyanins show inhibitory effects on growth of some cancer cells (Nagase *et al.*, 1998). Delphinidin and cyanidin showed cell cycle progression and induction of apoptosis in human cancer cells (uterine carcinoma and colon adenocarcinoma cells) (Lazze' *et al.*, 2004) Cyanidin and malvidin from *Oryza sativa*, mediate cytotoxicity against human monocytic leukemia cells by arrest of G_2/M phase and induction of apoptosis (Hyun and Chung, 2004). The previous data suggests that consumption of *S. cumini* fruits may supply substantial antioxidants and antitumor activity which may provide health promoting and disease preventing effects.

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References

Al-Zaid, M.M., Hassan, M.A., Badir, N., and Gumma, K.A., Evaluation of blood glucose lowering activity of three plants diet additives, *Int. J. Pharmacog.* **29**(2), 81-88 (1991).

Banerjee, A., Dasgupta, N., and Bratati De, In vitro study of antioxidant activity of *Syzyguim cumini* fruit, *Food Chemistry* **90**, 727-733 (2005).

Bhatia I.S. and Bajaj, K.L., Chemical constituents of the seeds and bark of *Syzyguim cumini, Planta Med.* **28**, 346-352 (1975).

Bomser, J., In vitro anticancer activity of fruit extracts from four Vaccinum spesies. *Planta Med.* **62**, 193-288 (1996).

Bridle, P., Loeffler, R.S.T., Timberlake, C.F., and Roland Self, Cyanidin 3-o-Malonylglucoside In *Cichorium intybus phytochemistry* **23**(12), 2968-2969 (1984).

British Herbal Pharmacopoeia, part 1-ed. pub. British Herbal Medicine Association, Lane House, Cowling, Keighley, West Yprks BD22OLX. 1976, pp. 195.

Cornuz, G., Wyler, H., and Lauterwein, J., Pelargonidin 3-malonylsophoroside from the Red Iceland Poppy, *Papaver nudicaule, phytochemistry* **20**(6) 1461-1462 (1981).

Craveiro, A.A., Andrade, C.H.S., Matos, F.J.A., Alencar, J.W., and Machado, M.I.L., Essential Oil of *Eugenia jambolana, J. Nat. Prod.* **46**, 591-592 (1983).

Ghosh, D. and McGhie, Effects of anthocyanins and other phenolics of boysenberry and black currant as inhibitors of oxidative stress and damage to cellular DNA in SH-SY5Y and HL-60 cells, *J. Sci. of Food and Agric.* **86**(5), 678-686 (2006).

Grover, J.K., Vats, V., and Rathi, S.S., Antihyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism., *J. Ethnopharmcol.* **73**(3), 461-470 (2000).

Guisti, M.M., Rodriguez-Saona, L.E., Griffin, D., and Wrostad, R.E., Electrospray and Tandum Mass Spectroscopy As Tooles for Anthocyanin Characterisation. *J.Agric. Food Chem.* **47**, 4657-4664 (1999).

Hakimuddin, F., Paliyath, G., and Meckling, K., Selective cytotoxicity of a red grape wine flavonoids fraction against MCF-7cells, *J.Breast Cancer Research and treatment* **85**(1), 65-79 (2004).

Harborn J.B. and Mabry, T.J., The Flavonoids: Advances in Research, Chapmann and Hall, London, 1982.

Jain, Mc. and Seshardi, T.R., *Indian J. of Chem.* **13**, 743 (1975) cited in *The flavonoid: Advanced in Research,* (Harborn J. B., Mabry, T. J., Eds.) Chapman & Hall, London, 1982.

Hyun, J.W. and Chung, H.S., Cyanidin and malvidin from *Oryza sativa,* Mediate Cytotoxicity against human monocytic leukemia cells by arrest of G_2/M phase and induction of apoptosis, *J.Agric. Food Chem.* **52**(8), 2213-2217 (2004).

Lazze', M.C., Savio, M., Pizzala, R., Cazzalini, O., Perruca, P., Scovassi, A.I., Stivala, L.A., and Bianchi, L., Anthocyanins induce cell cycle perturbations and apoptosis in different human cell lines, *Carcenogenesis* **25**(8), 1427-1433 (2004).

Lazze', M.C., Pizzala, R., Savio, M., Stivala, L.A., Prosperi, E., and Bianchi, L., Anthocyanins protect against DNA damage induced by tert-butyl-hydroperoxide in rat smooth muscle and hepatoma cells, *Mutat. Res.* **535**, 103-115 (2003).

Mclimans, W.F., Davis, E.V., and Rake, G.W., *Immunology* **79**, 428 (1957).

Meiers, S., Kemeny, Weyand, U., Gastpar, R., Von Angerer, E., and Marko, D., The anthocyanidins cyanidin and delphinidin are potent inhibitors of the epidermal growth factor receptor. *J. Agric. Food Chem.* **49**, 958-62 (2001).

Miyata, M., Suppression of 2-amino-1-methyl-6-phenylimidazol (4, 5-b) pyridine-induced DNA damage in rat colon after grape fruit juice intake. *Cancer Lett.* **183**, 17-22 (2000).

Nagase, H., Sasaki, K., Kito, H., Haga, A., and Sato, T., Inhibitory effect of delphinidin from *Solanum melongana* on human fibrossarcoma HT-1080 invasiveness *in vitro. Planta Med.* **64**, 216-219 (1998).

Ping- Hsiao S., Chi-Tai Y., and Gow-Chin Y., Effects of anthocyanin on the inhibition of proliferation and induction of apoptosis in human gastric adenocarcinoma cells. *Food chem. Toxicol.* **43**(10), 1557-1566 (2005).

Prince, P.S., Menon, V.P., and Pari, L., Hypoglycemic activity of *Syzyguim cumini* seeds: Effect on lipid peroxidation in alloxan diabetic rats, *J. Ethnopharmcol.* **61**(1), 1-7 (1998).

Saito, N., Abe, K., Honda, T., Timberlake, C.F., and Bridle, P., Acylated Delphinidin Glucosides and Flavonols From *Clitoria Ternatea. Phytochemistry* **24**(7), 1583-1586 (1985).

Shafi, P.M., Rosamma, M.K., Kaiser Jamil, P.S., Reddy, Antibacterial activity of *Syzygium cumini* and *Syzyguim travancoricum* leaf essential oils, *Fitoter.* **73**, 414-416 (2002).

Skehan, P., Storeng, R., Scudiero, D., Monks, A., Mc Mahon. J., Vistica, D., Janathan, T.W., Bokesch, H., Kenney, S., and Michael, R.B., New colorimertic cytotoxicity Assay for Anticancer Drug screening, *J. National Cancer Inst.* **82**(13), 1107-1112 (1990).

Takeda, K., Harborne J.B., and Self, R., Identification and distribution of malonated anthocyanins in plants of the compositae. *Phytochemistry* **25**(6), 1337-1342 (1986).

Takeoka, G.R., Dao, L.T., Full, G.H., Wong, R.Y., Harden, L.A., Edwards, R.H., and Berrios J.D., Characterisation of Black Bean (*Phaseolus vulgaris* L.) Anthocyanins. *J. Agric. Food Chem.* **45**, 3395-3400 (1997).

Teixeira, C.C., Pinto, L.P., Kessler, F.H., Knijik, L., and Fuchs, F.D., Effect of *Syzygium cumini* (L.) skeels on postpradial blood glucose levels in nondiabetic rats and rats with streptozotocin induced diabetes mellitus. *J. Ethnopharmcol.* **56**(3), 209-213 (1997).

Terahara, N., Yamaguchi, M.A., Takeda, K., Harborne, J.B., and Self, R., Anthocyanins acylated with malic acid in Dianthus caryophyllus and D. deltoids. *Phytochemistry* **25**(7), 1715-1717 (1986).

Wang J. and Mazza, G., Effect of anthocyanins and other phenolics on the production of tumor necrosis factor alpha in LPS/IFN-gamma activated RAW 264.7 macrophages, *J. Agric. Food Chem.* **50**, 4183-89 (2002).

Yarnell, E., Southwestern and Asian botanical agents for diabetes mellitus, *Alt.Compelementary Ther.* **6**, 7-11 (2000).

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