Exploring the antioxidant and anticancer properties of *Punica granatum* L. peels ethanolic extract on Human Cancer Cells

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Abstract
Plant extracts can be used in combination with traditional chemotherapy or used as alternative sources for adjuvant cancer therapy, as some of these extracts have no adverse effects and activate the cells of the immune system. The present study investigated the antioxidant and anticancer activity of PGPEE against HGT-1 cell line. In the present study, 293.2 mg GAE/g DW of total phenol, 126.5 mg CAE/g DW of total flavonoid and 117.6 µg C_3gE/g DW of total anthocyanin were obtained in the PGPEE. Punica granatum peel ethanol extracts were studied by various free radical scavenging assays including DPPH, FRAP and reducing power assays. Among this, FRAP scavenging activity (32.5 µM) showed the potential activity against the various concentration of PGPEE extract.

The present study investigated inhibitory effects of dried and fresh PGPEE extracts on the inhibitory activity of tyrosinase. The fresh PGPEE showed the better inhibition in monophenolase (82.28 ± 10.4^{b} mg/ml) than diphonalse (79.34 ± 6.96^{ab} mg/ml). In the anticancer activity, the minimum cell viability (27.29%) was observed in the 500µg/ml concentration of the PGPEE extract followed by the 400 µg/ml concentration (32.45%). In vivo antiangiogenic effect PGPEE was also tested using CAM assay as an in vivo model at a dose of 72.4 µg/ml. The inhibitory activity for the PGPEE ($65.50\pm3.5\%$) inhibitions was observed.

Keywords: PGPEE, Phenol, FRAP, Anticancer and Cell viability.

Introduction

The plant derived extracts and essential oils are potential source of natural and safer antibacterial, antioxidant, anticarcinogenic, antifungal, analgesic, insecticidal, anticoccidial and hypoglycemic agents[1]. During the growth, plants generate a variety of secondary metabolites for their defense against negative biotic and abiotic environmental factors. Polyphenols, one of the main bioactive secondary metabolites, are natural antioxidant agents which have an important role in human health because of their ability to scavenge free radicals[2] which have been implicated in the development of a number of disorders including cancer, neuro degeneration and inflammation giving rise to studies of antioxidants for the prevention and treatment of diseases[3].

Various extracts of plant parts which are rich in secondary plant metabolites have been used for biosynthesis of AgNPs. *Punica granatum* Linn is a plant of punicaceae family; the tree may grow up to five meters in height. It has glossy, leathery leaves and bears red flowers at the branch tips[4]. The pomegranate is fruit of this plant, locally known as romane variety safferi. The tree is native in Asian countries; it has been cultivated and naturalized over the whole Mediterranean region since ancient times[5]. Pomegranates have prominent medical history and possess remarkable medicinal properties[6] such as anti-inflammation, anti-diabetes, anti diarrhea, treat dental plaque and aphtae and combat intestinal infections and malarial parasites.

Recent studies also revealed the efficacy of the pomegranate fruit against cancer, atherosclerosis, infectious and coronary heart diseases[7]. The pomegranate peels represent 50% of total weight of fruit, they are an important source of bioactive compounds as phenolics, flavonoids, ellagitannins and proanthocyanidin compounds[8], minerals, mainly potassium, nitrogen, phosphorus, magnesium, sodium and calcium and complex polysaccharides. Pomegranate peel extracts have also been used for the synthesis of AgNPs.

Punica granatum fruit peel constitutes about 50% of the total fruit weight[9] and it is often discarded as waste. However, the fruit peel contains higher amounts of polyphenol compounds than the juice and it possesses stronger biological activities[10]. Studies have shown that *Punica granatum* peel extract had markedly higher antioxidant capacity than juice extract in scavenging against superoxide anion, hydroxyl and peroxyl radicals and it inhibited $CuSO_4$ induced LDL oxidation[11]. Besides high antioxidant capacity, *Punica granatum* peel extracts have been reported to possess a wide range of biological actions including anti-cancer activity[12], antimicrobial activity[13], antidiarrheal activity[14], apoptotic and anti-genotoxic properties[15], anti-tyrosinase activity[16], anti-inflammatory and anti-diabetic activities[17].

Punica granatum peel is a source of added-value biologically active compounds for application in food matrixes to increase antioxidant activity and to reduce the risk of pathogenic contamination[18]. The addition of *Punica granatum* peels extracts was successfully tested in yoghurt samples to increase its antioxidant content[19], in meat product

to improve its oxidative stability[20] and in fruits to protect it from mycotoxigenic fungi[21]. The current study investigated the antioxidant and anticancer activity of ethanolic extract of *Punica granatum* peel against human cancer cell line.

Material and Methods

Sample preparation: Fresh *Punica granatum* fruits were collected from a local market of Thanjavur, Tamil Nadu, India. The fruit was thoroughly washed using distilled water and the seeds were subsequently separated to obtain the peel. The isolated peels were dried under shade at the room temperature for 3-5 days. The dried peels were ground into uniform coarse powder using a domestic blender. Approximately 200 g of *Punica granatum* peel powder upon extraction with ethanol (4–5 h, 90 °C) using a Soxhlet apparatus yielded a bright red colour *Punica granatum* peel ethanolic extract (PGPEE). The extract was concentrated under reduced pressure (45–50 °C) using a rotary evaporator to obtain the viscous mass (42 g; 21.0% w/w). The PGPEE was stored at 4 °C in the refrigerator until further use in the experiment.

Qualitative Phytochemical analysis: The preliminary qualitative phytochemical investigation of PGPEE was subjected to phytochemical screening by various qualitative tests for detection of bioactive molecules which included saponins, tannins, flavonoids, terpenoids, cardiac glycosides and phenols as per standard methods[22].

Total phenolic content: The total phenolic content of extracts was measured using the Folin-Ciocalteu method described by the International Organization for Standardization (ISO 14502-1:2005(E)). Aliquots of 1 mL of diluted extracts (2 mg/mL) were mixed with 5 mL Folin-Ciocalteu reagent at 10% (v/v). After 5 minutes, 4 mL of 7.5% sodium carbonate solution was added to the mixture and incubated for 60 minutes at room temperature in the dark. The absorbance was measured at 765 nm against a blank. The total phenolic content was calculated by the regression equation of the calibration curve of gallic acid (ranging from 10 to 50 µg/mL) and the results were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g of DW).

Total Flavonoid Content: Total flavonoids of the samples were measured by the aluminum chloride spectrophotometric assay[23]. Briefly, 0.5 mL of $AlCl_3$ at 2% ethanolic solution was added to 0.5 mL of appropriately diluted extract. After 10 minutes of incubation at room temperature, absorbance was measured at 420 nm. Quercetin was used as a standard for the construction of the calibration curve (ranging from 0.125 to 40µg/mL). The results were expressed in mg equivalent of quercetin per g dry weight (mg QE/g DW).

Anthocyanidin Content: The anthocyanidin content was carried out by the modified vanillin assay[24]. 2.5 mL of each ethanolic solutions of 1:3 (v/v) sulfuric acid and 1% (w/v) of a vanillin solution were mixed with 1mL of appropriately diluted extract in distilled water (1mg/mL). The tubes were incubated at 30°C for 15 minutes, the absorbance was measured using a spectrophotometer (6715 UV / VIS, Jenway) at a wavelength of 500 nm. The tannin content is estimated in mg equivalent of catechin per gram of dry weight (CE/ g) from the calibration curve (ranging from 50 to 400µg/mL).

Antioxidant activity

DPPH scavenging activity: The free radical scavenging capability of *Punica granatum* peel ethanol extracts was determined through DPPH (1, 1-diphenyl-2-picrylhydrazyl) radicals[25]. Briefly, 4 mL of ethanol solution of DPPH (0.1mM) was mixed with 1 mL of methanol extract solution at different concentrations. The reaction mixture was incubated in a dark room for 30 minutes and the free radical scavenging ability was estimated by measuring the absorbance at 515 nm with the spectrophotometer. Ascorbic acid was used as positive control. The reaction was carried out in capped glass test tubes that were tightly wrapped with aluminum foil. The DPPH radical stock solution was freshly prepared every day for the reaction and precautionary measures were taken to reduce the loss of free radical activity during the experiment.

FRAP: The ability to reduce ferric ions was measured using the method[26]. The FRAP (Ferric Reducing Antioxidant Power Assay) reagent was generated by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM (tripyridyl triazine) TPTZ solution and 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Samples at different concentrations (100,200,400,600 and 800 µg/ml) were then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37°C for 30 min. The increase in absorbance at 593 nm was measured. Fresh working solutions of $FeSO_4$ were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as mMol Fe^{+2} g^{-1}.

Reducing power activity: The sample together with ascorbic acid solutions were spiked with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was kept in a 50°C water-bath for 20min. The resulting solution was cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid and centrifuged at 3000rpm for 10 min. The supernatant (5ml) was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride and incubated for 10min. The absorbance was detected at 700nm on spectrophotometer. The extract concentration providing the absorbance was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as standard. Higher absorbance indicates higher reducing power[27].

Tyrosinase inhibition property: Tyrosinase inhibition activity was determined as described by Momtaz et al[26] with L-3,4-dihydroxyphenylalanine (L-DOPA, Sigma) and

tyrosine as substrates. Samples were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 20 mg/ml and further diluted in potassium phosphate buffer (50 mM, pH 6.5) to 600 µg/ml. Assays were carried out in a 96-well micro-titre plate and a Multiskan FC plate reader (Thermo Scientific Technologies, China) was used. All the steps in the assay were conducted at room temperature. In triplicate, each prepared sample (70 µl) was mixed with 30 µl of tyrosinase (333 Units/ml in phosphate buffer, pH 6.5). After 5 min incubation, 110 µl of substrate (2 mM L -tyrosine or 12 mM L-DOPA) was added to the reaction mixtures and incubated further for 30 min.

The final concentration of the extract was between 2.6 – 333.3 µg/ml. Arbutin (1.04 – 133.33 µg/ml) was used as a positive control while a blank test was used as each sample that had all the components except L-tyrosine or L-DOPA. Results were compared with a control consisting of DMSO instead of the test sample. Absorbance values of the wells were then determined at 492 nm. The percentage tyrosinase inhibition was calculated as follows:

% inhibition ¼ __Acontrol–Asample_=Acontrol_ x 100

where A control is the absorbance of DMSO and A sample is the absorbance of the test reaction mixture containing extract or arbutin. The IC50 values of extracts and arbutin were calculated.

***In vitro* cytotoxicity analysis**

Cell culture: The cell line such as HGT-1 was obtained from National Centre for Cell Sciences (NCCS), Pune, India. HGT-1 cells were maintained in RPMI 1640 medium containing 2mM L-glutamine and supplemented with 10% fetal bovine serum (FBS) and 100 100 µg ml^{-1} streptomycin. Cell line was cultured at 37°C in a humidified atmosphere of 5% CO_2.

MTT Assay: Cell viability, after treatment with different concentrations of PGPEE was assessed using MTT assay[29]. This colorimetric test, which is based on the reduction of yellow tetrazolium salt [3-(4,5-Dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide; MTT; Sigma Aldrich, Darmstadt, Germany) to blue formazan product by mitochondrial dehydrogenase in the metabolically active cells, reflects the metabolic rate of treated cells. Cells (1×10^5/well) were plated in 96-well plates and incubated in 37ºC with 5% CO_2. After the cell reaches the confluence, the various concentrations of PGPEE were added and incubated for 24 h.

After incubation, the samples were removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. Then 100µl of 0.5% of MTT (3- 4, 5-dimethyl-2-thiazolyl) -2, 5-diphenyl - tetrazolium bromide) was added individually to each well and incubated for 4h. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV-spectrophotometer using DMSO as the blank. The % cell viability was calculated using the following formula:

Cell viability (%) = A570 of treated cells / A570 of control cells X 100

All the data were from three independent experiments with six wells for each experiment. The half inhibitory concentrations (IC50) of PGPEE were determined using GraphPad Prism 7.0 (GraphPad Software Inc., La Jolla, CA, United States).

Anti angiogenic activity: Anti-angiogenic potential of drug samples was determined by CAM (Chorio allantoic membrane) assay[30]. The fertilized domestic chicken eggs were purchased from poultry trader of thanjavur, Tamil Nadu and were incubated for 3–4 days at 37°C in a humidified incubator and were slowly moved at least three times a day. After the completion of incubation period, the seven day old eggs were observed under flash light to identify and encircle the embryo head. Thereafter, a tiny hole was drilled at the narrow end of the eggs and 0.5–1 ml of albumin was aspirated using eighteen gauge hypodermic needles so that yolk sacs drop away from the shell membrane.

The shell around the embryo air sac was detached via forceps and the shell membrane at the base of air sac was peeled away. On 8th day, Whatmann no.1 filter paper was loaded with 72.4 µg/ml of PGPEE and was carefully placed on the surface of CAM and incubated. After 3 days, the CAM was cut out from eggs and the numbers of vessels were observed. Vessels radially converging in the direction of the center were counted under a microscope. At least twenty eggs were used for each sample dose. The % of increase and inhibition were calculated.

Results and Discussion

Phytochemical screening: Phytochemical screening of hydroalcoholic peel extract of *Punica granatum* was performed. In the present study, preliminary phytochemical constituents of hydroalcoholic pomegranate peel extract were evaluated. The extracts of pomegranate peel revealed the presence of saponins, tannins, flavonoids, terpenoids, cardiac glycosides and phenols. The yield of pomegranate peel extract was 25% (Table 1).

Determination of Phenolic content: Pomegranate fruit components are rich in phenolic compounds which have synergistic and/or additive effects on its pharmacological properties[31]. Phenolic constituents in pomegranate peel have been implicated in bioactivities such as antimicrobial, antioxidant and anti-tyrosinase activities[32].

In the present study, 293.2 mg GAE/g DW of total phenol, 126.5 mg CAE/g DW of total flavonoid and 117.6 µg C_3gE/g DW of total anthocyanin were obtained in the PGPEE (Table 2).

Antioxidant activity: *Punica granatum* peel ethanol extracts was studied by various free radical scavenging assays including DPPH, FRAP and reducing power assays. The strong FRAP radical scavenging activity (32.5 µM) was noted in the 300µg/ml concentration of sample. The strong DPPH value (28.6 %) was also recorded. The more reducing power assay (24.4 µM AAE/g) was observed in the 300 µg/ml concentration followed by 200µg/ml (15.9 µM AAE/g). Among this, FRAP scavenging activity showed the potential activity against the various concentration of PGPEE extract. The value was compared with standard ascorbic acid (Fig. 1).

The FRAP method is based on the capacity of polyphenols to reduce ferric iron Fe^{3+} to ferrous iron Fe^{2+}. The reducing power is one of the anti-oxidative mechanisms. Furthermore, the reducing capacity of a constituent can serve as an important indicator of its antioxidative potential[33]. The reducing activity may be due to polyphenols, such as flavonoids and anthocyanins. Some authors have reported that there is a direct correlation between antioxidizing activities and the ability of plant constituents to reduce ferric iron Fe^{3+} to ferrous iron (Fe^{2+})[34]. In the present study, the PGPEE extract exhibited antioxidant properties as tested in DPPH and FRAP scavenging assays.

Anti-tyrosinase activity: In the present study, inhibitory effects of dried and fresh PGPEE extracts on the inhibitory activity of tyrosinase were studied. The peel extract dried at 50 °C notably showed better inhibition on monophenolase activity. The highest inhibition activity against monophenolase was found to be 62.12 ± 9.7^{b} mg/ml of peel extracts concentration compared to the rest of treatment. Moreover, the extracts of peel dried at 50 °C showed potent inhibitory activity than the arbutin (47.23 ± 4.76^{b} mg/ml). In general, peel extracts showed weaker diphonalase inhibition in all treatments compared to arbutin (control).

However, better inhibitory activity against diphenolase was observed in the peel extracts dried at 50 °C with MIC value of 59.06 ± 3.67^{b} mg/ml. Nevertheless, pomegranate peel contains a mixture of many kinds of secondary metabolism products including phenolics, which vary greatly in their antioxidant capacity and phenolic compounds composition.

Table 1
Phytochemical constituents from PGPEE

S.N.	Phytochemical Constituents	Observation	Result
1	Saponins	Appearance of honeycomb froth	+
2	Tannins	Appearance of Yellowish precipitate	+
3	Flavonoids	Appearance of yellow colour	+
4	Terpenoids	Formation of blue green ring	+
5	Cardiac glycosides	Formation of brown ring	+
6	Phenols	Formation of deep blue colour	+

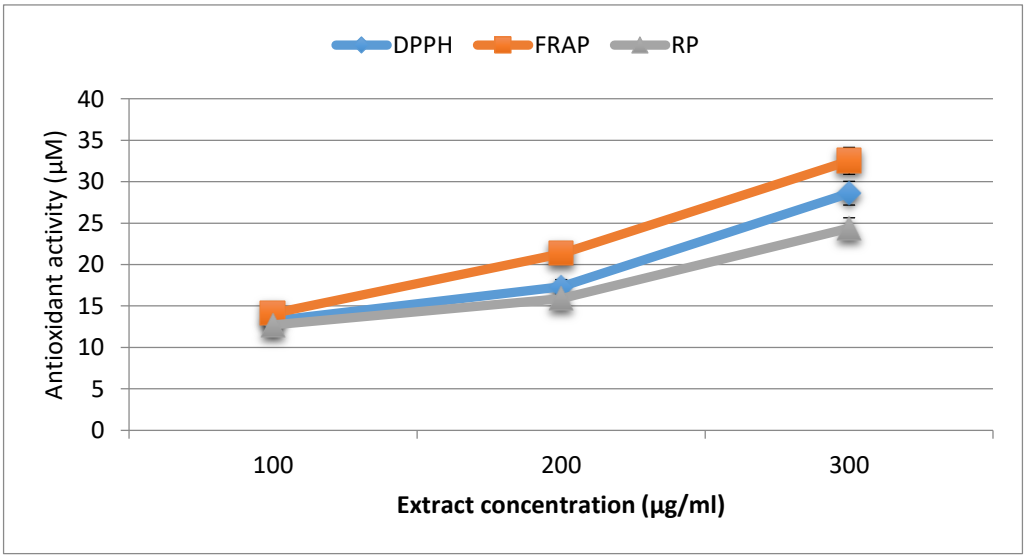

Fig. 1: Antioxidant activity of various concentration of PGPEE

Table 2
Determination of phenolic content from PGPEE

S.N.	Phenolic content	PGPEE
1	Total Phenolics mg GAE/g DW	293.2 ± 21.75^{d}
2	Total Flavonoid mg CAE/g DW	126.5 ± 0.37^{C}
3	Total Anthocyanin µg C_3gE/g DW	117.6 ± 3.57^{b}

The fresh PGPEE showed better inhibition in monophenolase (82.28±10.4[b] mg/ml) than diphonalse (79.34±6.96[ab] mg/ml) (Table 3).

Tyrosinase plays a key role in biosynthesis of melanin which is responsible for pigments of the skin, eyes and hair in mammals as well as in browning of the fruits[35]. Here are two distinct reactions of melanin biosynthesis; the hydroxylation of L-tyrosine (monophenolase activity) and the conversion of L-DOPA (diphenolase activity) to the corresponding monophenolase and diphenolase as the key substrate facilitating the O-quinones[36]. These quinones are highly reactive and tend to polymerize spontaneously to form brown pigments, namely melanin.

***In vitro* cytotoxic activity:** The different concentration of PGPEE was studied in HGT-1 cell lines using MTT assay. The cell line was cultured with PGPEE extract at concentrations in the range of 10µg/ml to 500µg/ml for 24 h and cell viability was determined. The minimum cell viability (27.29%) was observed in the 500µg/ml concentration of the PGPEE extract followed by the 400 µg/ml concentration (32.45%). The maximum cell inhibition showed the increasing concentrations of PPE extract. In a dose depend manner, this extract inhibits the growth of HGT-1 cell. The maximum cell viability (88.24%) was also noted in the 10 µg/ml concentration of PGPEE.

The IC_{50} value of ethanol extract of *Punica granatum* peel was 67.28µg/ml (Fig. 2). There are several cytotoxic assay methods suitable for screening anticancer potency of substances. We used MTT method to measure the cytotoxicity of extract in terms of metabolic inactivity and necrotic death respectively. Generally secondary metabolites of medicinal plant strongly inhibit growth of cancer cell line. In the effective search of anticancer agents, the conception of fruits, vegetables and medicinal plants exhibited reduced cancer risk, which was also scientifically proved[37]. Several studies have demonstrated that extracts from plants have anticancer potential under *in vitro* as well as *in vivo* conditions[38].

Anti -angiogenic activity: *In vivo* antiangiogenic effect PGPEE was also tested using CAM assay as an *in vivo* model at a dose of 72.4 µg/ml. The inhibitory activity for the PGPEE (65.50 ± 3.5%) inhibitions was observed. The number of blood vessels also reduced in treated group when compared with control[13] (Table 4).

Table 3
Anti-tyrosinase activity of PGPEE in different treatment conditions

Treatment	Anti-tyrosinase activity	
	IC_{50} Monophenolase (mg/ml)	IC_{50} Diphenolase (mg/ml)
Fresh PGPEE	82.28±10.4[b]	79.34±6.96[ab]
Dried 50°C PGPEE	62.12±9.7[b]	59.06±3.67[b]
Arbutin (mg/ml)	47.23±4.76[b]	18.02±1.73[c]

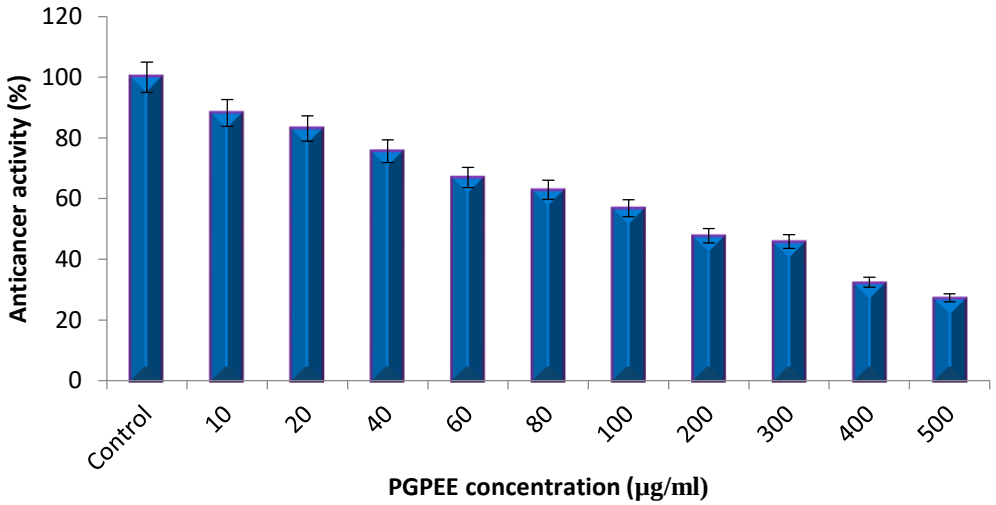

Fig. 2: Anticancer activity of various concentration of PGPEE against HGT-1 cell line

Table 4
Anti-angiogenic activity of PGPEE on CAM assay

S.N.	Sample	No. of blood vessels	Percentage of inhibition (%)
1	Control	13	0
2	Treated with PGPEE	7	65.50

The earlier study stated that the *in vivo* CAM assay illustrated the high impact of *S. triloba* against the newly formed vessel in the chicken embryonic membrane[39]. Angiogenesis is an energetic propagation and differentiation procedure, having need of endothelial propagation, passage and tube development[40]. Tumors with physically powerful angiogenic action are associated with a lesser patient survival speed[41]. *G.lucidium* is a potent ant-angiogenic compound, targeting a number of key proteins in angiogenisis. These results represent a novel biological function for *G.lucidium* as an angiogenic inhibitor[41].

Conclusion

The present studies concluded that the *Punica granatum* peel extract also exhibited antioxidant properties as tested in DPPH, reducing power and FRAP scavenging assays. Our results also indicated that PGPEE is a potent ant-angiogenic compound, targeting a number of key proteins in angiogenisis. These results represent a novel biological function for *Punica granatum* peel as an angiogenic inhibitor and anti tyrosinase activity. More work is needed to determine effects of the extracts on normal primary cells and on animals *in vivo*. PGPEE may be used in future in the various biomedical applications.

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References

1. Ahangari B. and Sargolzaei J., Extraction of pomegranate seed oil using subcritical propane and supercritical carbon dioxide, *Theor Found Chemen*, **46**, 258-265 **(2012)**

2. Alexandre E.M.C., Silva S., Santos S.A.O., Silvestre A.J.D., Duarte M.F., Saraiva J.A. and Pintado M., Antimicrobial activity of pomegranate peels extracts performed by high pressure and enzymatic assisted extraction, *Food Res. Int*., **115**, 167–176 **(2019)**

3. Alotaibi M.A., Tayel A.A., Zidan N.S. and El Rabey H.A., Bioactive coatings from nano-biopolymers/plant extract composites for complete protection from mycotoxigenic fungi in dates, *J. Sci. Food Agric*., **99**, 4338–4343 **(2019)**

4. Al-Said F.A., Opara L.U. and Al-Yahyai R.A., Physico-chemical and textural quality attributes of pomegranate cultivars (*Punica granatum* L.) grown in the Sultanate of Oman, *J Food Eng*, **90**, 129–134 **(2009)**

5. Althunibat O.Y. et al, Protective role of *Punica granatum* L peel extract against oxidative damage in experimental diabetic rats, *Process Biochem*, **45**, 581–585 **(2010)**

6. Benzie I.F.F. and Strain J.J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay, *Anal Biochem*, **239**, 70-6 **(1999)**

7. Chen S.C., Lu M.K., Cheng J.J. and Wang D.L., Antiangiogenic activities of polysaccharides isolated from medicinal fungi, *FEMS Microbiol Lett*., **249**, 247-54 **(2005)**

8. El-Batawy O.I., Ashoush I.S. and Mehanna N.S., Impact of mango and pomegranate peels supplementation on quality characteristics of yoghurt with or without whey powder, *World Journal of Dairy & Food Sciences*, **9**, 57–65 **(2014)**

9. Endo E.H., Cortéz D.A.G., Ueda-Nakamura T., Nakamura C.V. and Filho B.P.D., Potent antifungal activity of extracts and pure compound isolated from pomegranate peels and synergism with fluconazole against *Candida albicans*, *Res Microbiol*, **161**, 534–540 **(2010)**

10. Ferguson L.R., Chronic inflammation and mutagenesis, *Mutat. Res. Fund. Mol*, **690**, 3-11 **(2010)**

11. Ferguson P.J., Kurowska E., Freeman D.J., Chambers A.F. and Koropatnick J., A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cell lines, *J. Nutr*., **134**, 1529-1535 **(2004)**

12. Firuzi M.R., Niakousari M., Eskandari M.H., Keramat M., Gahruie H.H. and Mousavi Khaneghah A., Incorporation of pomegranate juice concentrate and pomegranate rind powder extracts to improve the oxidative stability of frankfurter during refrigerated storage, *LWT*, **102**, 237–245 **(2019)**

13. Fischer U.A., Carle R. and Kammerer D.R., Identification and quantification of phenolic compounds from pomegranate (Punica granatum L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD–ESI/MSn, *Food Chem*., **127**, 807–821 **(2011)**

14. Friedman M., Food browning and its prevention: an overview, *J Agric Food Chem.*, **44**, 631–53 **(1996)**

15. Giatromanolaki A., Koukourakis M., O'Byrne K., Fox S., Whitehouse R., Talbot D.C., Harris A.L. and Gatter K.C., Prognostic value of angiogenesis in operable non-small cell lung cancer, *J Pathol.*, **179**, 80-88 **(1996)**

16. Gözlekçi Ş., Saraçoğlu O., Onursal E. and Özgen M., Total phenolic distribution of juice, peel and seed extracts of four pomegranate cultivars, *Phcog Mag*, **7**, 161–164 **(2011)**

17. Hussain A.I., Anwar F., Hussain S.T. and Przybylski R., Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations, *Food Chemistry*, **108**, 986-995 **(2008)**

18. Khalil E.A., Antidiabetic Effect of an Aqueous Extract of Pomegranate (Punica granatum L.) Peels in Normal and Alloxan Diabetic Rats, *Egy. J. Hosp. Med*, **16**, 92-99 **(2004)**

19. Kowalski I., Samojedny A., Paul M., Pietsz G. and Wilczok T., Effect of kaempferol on the production and gene expression of monocyte chemoattractant protein-1 in J7742 macrophages, *Pharmacol Rep*, **57**, 107–112 **(2005)**

20. Kumazawa S., Hamasaka T. and Nakayama T., Antioxidant activity of propolis of various geographic origins, *Food Chemistry*, **84**, 329-339 **(2004)**

21. Lansky E.P. and Newman R.A., Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer, *Journal of Ethnopharmacology*, **109**, 177-206 **(2007)**

22. Lee S.M., Li M.I., Tse Y.C., Leung S.C., Lee M.M., Fung K.P. and Waye M.M., Paeoniae Radix, a Chinese herbal extract inhibit hepatoma cell growth by inducing apoptosis in a p53 independent pathway, *Life Sciences*, **71**, 2267-2277 **(2002)**

23. Li Y., Guo C., Yang J., Wei J., Xu J. and Cheng S., Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract, *Food Chem*, **96**, 254–260 **(2006)**

24. Lin C.C., Hsu Y.F. and Lin T.C., Effects of punicalagin on arrageenan-induced inflammation in rats, *Am J Chinese Med*, **27**, 371–376 **(1999)**

25. Manivannan C., Vedichi N., Vijayakumar P., Muralikrishanan M. and Anbazhagan V., Host-guest interaction of Nileblue with cyclodextrins: A spectroscopic and computational approach, *Res. J. Chem. Environ.*, **28(12)**, 46-55 **(2024)**

26. Momtaz S., Mapunya B.M., Houghton P.J., Edgerly C., Hussein A., Naidoo S. and Lall N., Tyrosinase inhibition by extracts and constituents of Sideroxylon inerme L Stem bark, used in South Africa for skin lightening, *J Ethnopharmacol*, **119**, 507–512 **(2008)**

27. Mosmann T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol. Methods*, **65**, 55–63 **(1983)**

28. Olapour S., Mousavi E., Sheikhzade M., Hoseininezhad O. and Najafzadeh H., Evaluation anti-diarrheal effects of pomegranate peel extract, *J Iran Chem Soc*, **6**, 115–143 **(2009)**

29. Oyaizu M., Studies on product of browning reaction prepared from glucose amine, *Jpn J Nutr*., **7**, 307-15 **(1986)**

30. Ozturk H., Kolak U. and Meric C., Antioxidant, anticholinesteraseand antibacterial activities of *Jurinea consanguinea* DC, *Rec Nat Prod*, **5**, 43e51 **(2011)**

31. Saeed N., Khan M.R. and Shabbir M., Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla L.* BMC Complement, *Altern. Med*, **12**, 221 **(2012)**

32. Seeram N.P., Adams L.S., Henning S.M., Niu Y., Zhang Y., Nair M.G. and Heber D., *In vitro* anti-proliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice, *J Nutr Biochem*, **16**, 360–367 **(2005)**

33. Seo S.Y., Sharma V.K. and Sharma N., Mushroom tyrosinase: recent prospects, *J Agric Food Chem*., **51**, 2837–53 **(2003)**

34. Shamaki B.U., Geidam Y.A., Abdulrahma F., Ogbe A.O. and Sandabe U.K., Evaluation of phytochemical constituents and in vitro antibacterial activity of organic solvent fractions of *Ganoderma lucidum* methanolic extract, *International Journal of Medicinal Plant Research*, **1(3)**, 026- 031 **(2012)**

35. Sun B., Ricardo da Silva J. and Spranger I., Critical factors of the vanillin assay for catechins and proanthocyanidins, *Journal of Agricultural and Food Chemistry*, **46**, 4267-4274 **(1998b)**

36. Vinotha V. and Rajakumar R., Antioxidant, cytotoxicity and antiangiogenic activities of *Ganoderma lucidum* extracts, *Compliance Engineering Journal*, **11(6)**, 216-229 **(2020)**

37. West D.C. and Burbridge M.F., Three-dimensional in vitro anglogenesis in the rat aortic ring model, *Methods Mol Biol*, **467**, 189–210 **(2009)**

38. Yang J., Guo J. and Yuan J., *In vitro* antioxidant properties of rutin, *LWT*, **41**, 1060-1066 **(2008)**

39. Yildirim A., Mavi A. and Kara A.A., Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts, *J. Agric. Food Chem*., **49**, 4083-4089 **(2001)**

40. Yoshimura M., Watanabe Y., Kasai K., Yamakoshi J. and Koga T., Inhibitory effect of an ellagic acid-rich pomegranate extracts on tyrosinase activity and ultraviolet-induced pigmentation, *Biosci, Biotechnol, Biochem*, **69**, 2368–2373 **(2005)**

41. Zihlif Fatma Afifi et al, The antiangiogenic activities of methanolic crude extracts of four Salvia species, *BMC Complementary and Alternative Medicine*, **13**, 358 **(2013)**.

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