ANTIOXIDANT PROPERTY AND MTT ASSAY SCREENING OF LOCAL AND IMPORTED PUNICA GRANATUM

Aqilah Salim[a], Shamima Abdul Rahman[b]*, Shafii Khamis[c], Hazlina Ahmad Masalli[d], Mohamed Zafar Ali Mohamed Amiroudine[e], Daryl Jesus Arapoc[f]

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Abstract: Pomegranate or *Punica granatum* is a fruit that is known for its various pharmacological activities such as antioxidant and cytotoxic activities. Commercial *Punica granatum* in Malaysia are mostly imported but there are pomegranates that are grown locally. Many studies and research have been done on imported *Punica granatum* but very little is done on locally grown *Punica granatum*. This study aims to evaluate and compare the antioxidant and cytotoxic activities of methanolic pulp and peel extract of both local and imported *Punica granatum* through in vitro studies. The antioxidant study was done by evaluating the Total Phenolic Content (TPC) and 2, 2- diphenyl-1-picrylhydrazyl (DPPH) assay. TPC was done using Folin-Ciocalteu assay and DPPH assay was done using 2, 2- diphenyl-1-picrylhydrazyl. The standards used in these assays were gallic acid, quercetin and ascorbic acid respectively. Cytotoxic study was conducted through MTT (3 (4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay against MCF-7 and MDA-MB 231 breast cancer cell lines, head and neck tumour HTB-143 cell line and 3T3 normal cell line. This study revealed that peel extracts have a higher content of phenol and the anticancer analysis shows that the peel extract of local *Punica granatum* has cytotoxic activity against all cell lines including 3T3 normal cell line.

[a]. Department of Pharmaceutical Science, Faculty of Pharmacy, University of Cyberjaya, 63000 Cyberjaya, Malaysia.
[b]. Department of Pharmaceutical Science, Faculty of Pharmacy, University of Cyberjaya, 63000 Cyberjaya, Malaysia.
[c]. Medical Technology Division, Malaysian Nuclear Agency, Bangi, Malaysia.
[d]. Medical Technology Division, Malaysian Nuclear Agency, Bangi, Malaysia.
[e]. Medical Technology Division, Malaysian Nuclear Agency, Bangi, Malaysia.
[f]. Medical Technology Division, Malaysian Nuclear Agency, Bangi, Malaysia.

***Corresponding Author**

E-mail: shamima@cyberjaya.edu.my

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INTRODUCTION

Oxidation occurs when there is a transfer of electrons from one atom to another and it is a crucial part that represents aerobic life and the body's metabolism(Pietta, 2000). This redox reaction produces free radicals as a by-product. The production of free radical is considered normal when the levels of pro-oxidants and antioxidants are balanced. However, oxidative stress can occur when there is an imbalance between the antioxidants and pro-oxidants level. Various studies have shown that increases of oxidative stress contributes to the progression of various diseases such as cancer, diabetes, cardiovascular and pulmonary diseases (Bekir et al., 2013). Antioxidants may accept or donate electrons to the unpaired electron of the free radical as to relieve it of its free radical status (Lü et al., 2009).

Cancer is a disease caused by abnormally rapid proliferation of cells without control. There are three types of genes that help in the inhibition of cancerous cell growth which are the tumour suppressor genes, proto-oncogenes and DNA repair genes Schneider (2001). The several causes for carcinogenesis stated by Tsuda et al.,(2004), are oxidative stress, inflammation, hormone-dependant growth action, immune activity and modulation, xenobiotic metabolism and enzyme induction, molecular association with carcinogen, angiogenesis and signal transduction pathways and their regulation.

Punica granatum has been mentioned in the Holy Quran, it was stated that the pomegranate grew in gardens of paradise and it was an example of God's good creation (Rahimi et al., 2012). The pomegranate is originally found in the Himalayas in Northern India to Iran. However, since ancient times, it has been cultivated in the Mediterranean region (Jurenka, 2008). Many plant-derived medicines are used in traditional healthcare system (Sadik, & Asker, 2014). The pomegranate was considered as "a pharmacy unto itself" in Ayurvedic medicine. This is because it was believed that the bark and root have antihelminthic and vermifuge properties, and the peel of the fruit is a powerful astringent and antidiarrhoea cure (Chaturvedula et al., 2011).

METHODS

Preparation of Extract

5 local and imported *Punica granatum* (PG*)* weighing of a range between 90g to 300g were washed, cut into small pieces and separated into pulp and peel. Then, pulp and peel are weighed separately. The drying process is conducted using

liquid nitrogen and was later grinded using mortar and pestle. Methanol solution is added to sample with a ratio of 1:1 to obtain the extract. Next, mixture is placed in an incubator shaker of 100rph for an hour. The extracts were then filtered using a vacuum pump and using rotary evaporator, the filtrate was evaporated for 8 hours at a temperature of 41°C. The supernatant collected was stored in a vacuum oven at a pressure of 50mmHg (Atikah M., 2013).

Phytochemical Screening: The individual extracts were subjected to various qualitative phytochemical screening test for the presence of phytochemical constituents. Tests were performed for terpenoid, flavonoid, phenol, saponin, tannins, alkaloid, glycoside, stero, lipid, reducing sugar, coumarin, and anthocyanin.

Terpenoid: 0.5 g of the PG extract was mixed with 2ml of chloroform before 95% sulphuric acid was carefully added to form a layer. The presence of terpenoid is indicated by a reddish-brown colouration of the interface (Tiwari et al., 2011).

Flavonoid: PG extract was heated with 10ml of ethyl acetate for 3 minutes. The mixture was then filtered and the filtrate was shaken with 1ml of dilute ammonia solution. The presence of flavonoid was indicated with a yellow colouration (Kujur et al., 2010).

Phenols: 2ml of PG extract was added to 5% ferric chloride solution. Then, few drops of ferric chloride-potassium ferricyanide was added to the mixture. Presence of dark green precipitate indicate the presence of phenol in the extract (Kujur et al. 2010).

Saponin: 0.5 g of PG extract was shaken with 2ml of water. The presence of saponins is indicated when the foam produced persists for 10 min (Tiwari et al. 2011).

Tannin: 0.5 g of PG extract was added to 0.1% ferric chloride. A blue black colouration indicated the presence of gallic tannin whereas the turning colour to brownish or green black colouration indicate the presence of cathecholic tannin (Ayoola et al. 2008).

Alkaloid: One drop of extract was placed on a filter paper and was left to dry. Then, Dragendorff's reagent was placed near the crude extract and was left to move towards the extract. The changing of clolour from yellow to orange indicates the presence of alkaloid (Tiwari et al. 2011).

Glycoside: Glacial acetic acid was added to the extract. Then, a few drops of 5% ferric chloride and concentrated sulphuric acid were added. The formation of a reddish brown colouration at the junction of two layers and a bluish green colour in the upper layer indicates the presence of glycosides (Ahirrao 2013).

Sterol: 2ml of sulphuric acid was added to PG extract. The formation of purple ring at the upper surface indicate the presence of sterols (Igbinosa et al. 2009).

Lipid: 2 drops of extract were placed on a filter paper. The present of lipid is indicated by the presence of oil (Mamta & Jyoti 2012).

Reducing Sugar: Extract was dissolved in 5ml of distilled water and was filtered. The filtrate was boiled with 6-9 drops of Fehling's solution A and B for a few minutes. The presence of reducing sugars is indicate by a presence of orange red or brick red precipitation form (Tiwari et al. (2011).

Coumarin: 3ml of 10% sodium Hydroxide was added to 2ml of extract. The presence of coumarins is indicated when the formation of yellow colour extract (Linga Rao & Savithramma (2011).

Anthocyanin: 2ml of extract was added to 2ml of 2N hydrochloric caid and ammonia. The appearance of pink-red which turns to blue-violet indicates the presence of anthocyanins (Sawant & Godghate (2013).

Total Phenolic Content (TPC): 0.1 ml of 1mg/ml sample of each extract diluted with ethanol were diluted with 4.5ml of distilled water. Then, 0.1ml of 2N Folin-Ciocalteu reagent was added to the solution and shaken for 3 minutes. Next, 0.2ml of 2% sodium carbonate solution was added to the solution and was incubated for 3 hours at room temperature. After incubation, 0.2ml of each sample was transferred into a 96-wells plate. The well plate was read at absorbance of 760nm against blank. Gallic acid was used as a standard and the phenolic content was expressed as µg gallic acid equivalence (GAE)/mg extract (Yang et al., 2011).

DPPH Assay: 0.1ml of each extract diluted in DMSO:Ethanol (5:95) with a strength of 1mg/ml (0-1mg/ml) was added to 0.1ml of 0.2mM DPPH dissolved in ethanol. Then, the solution was incubated at room temperature in the dark for 15 minutes. After incubation, the solution was read at 517nm absorbance. The standards used were ascorbic acid (0-0.1mg/ml) and quercetin (0 – 0.5mg/ml). The results obtained were read as IC50 values. This method is adapted from Bekir et al. (2013).

MTT Assay: This method is adapted from Sineh Sepehr et al. (2012). In MTT colorimetric assay, 1 x 10^4 cells were seeded in 96 well plates and incubated for 48 hours. The cells are then treated with *Punica granatum* extracts of different concentrations for another 48 hours. MTT 50µG/mL is added to each well and cells are incubated for 4 hours. The supernatant in each well is removed and 100µl of DMSO (dimethyl sulfoxide) is added. Next, using an ELISA plate reader, the amount of formazan crystal is determined at an absorbance of 595nm. Lastly, cell death and cell viability will be calculated using the formula below:

$$Cell\ death\ (\%) = ((control\ OD - sample\ OD)) / (cotrol\ OD) \times 100$$

$$Cell\ viability\ (\%) = 100 - (\%\ cell\ death)$$

RESULTS

Phytochemical Screening

Table 1: Phytochemical screening of local and imported *Punica granatum* pulp and peel methanol extracts.

Phytochemical test *	LPGPe	LPGPu	IPGPe	IPGPu
Terpenoid	+++	+++	+++	+++
Flavonoid	+++	---	+++	---
Phenol	+++	+++	+++	+++
Saponin	+++	+++	+++	+++
Tannin	+++	+	+++	---
Alkaloid	---	---	---	---
Glycoside	---	---	---	---
Steroid	---	---	---	---
Lipid	---	+++	+++	+++
Reducing sugar	---	+++	+++	+++
Coumarin	---	+++	---	+++
Anthocyanin	---	---	---	---

* Three replicate are maintained for each test.
- = absent
+ = present

Total Phenolic Content (TPC)

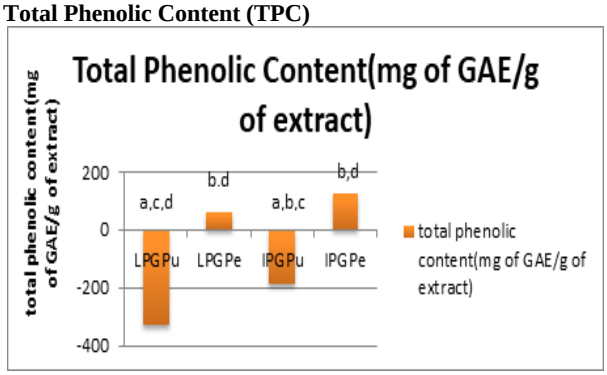

Figure 1: The results of total phenolic content of LPGPe, LPGPu, IPGPe and IPGPu

From the results obtained, IPGPe has the highest TPC which was 125.67 mg of GAE/g of extract followed by LPGPe which was 62.3mg of GAE/g of extract. Both LPGPu and IPGPu show no significant total phenolic content when compared to LPGPe and IPGPe.

DPPH Assay

Table 2. The results for DPPH assay done show that LPGPe, LPGPu, IPGPe and IPGPu has very little radical scavenging activity.

Extract	IC50 (µg/ml)
LPGPe	~0.01303
LPGPu	~1.729 e+ 007
IPGPe	4268
IPGPu	4294

MTT Assay

Table 3. IC50 of methanolic extract of local and imported Punica granatum pulp and peel extracts against various cancer cell lines.

Extracts	Cell lines (IC50, µg/ml)[m]			
	MCF-7	**MDA-MB 231**	**HTB**	**3T3**
LPGPe	29.46 µg/ml[bcde]	131.88 µg/ml[bcde]	>500 µg/ml[ce]	71.74 µg/ml[bde]
LPGPu	>500 µg/ml[acde]	>500 µg/ml[ace]	>500 µg/ml[ce]	>500 µg/ml[acde]
IPGPe	36.12 µg/ml[abde]	307.06 µg/ml[abde]	356.67 µg/ml[abde]	70.20 µg/ml[bde]
IPGPu	>500 µg/ml[abce]	>500 µg/ml[ace]	>500 µg/ml[ce]	277.0 µg/ml[abce]
Tamoxifen*	32.29 µg/ml[abcd]	42.08 µg/ml[abcd]	28.23 µg/ml[abcd]	41.56 µg/ml[abcd]

*Tamoxifen is used as the positive control; m: data are mean of triple replication

DISCUSSION

Phytochemical Screening: his study revealed that there is a presence of terpenoid, flavonoid, phenol, saponin, tannin and reducing sugar in the methanolic peel extracts of both local and imported *Punica granatum.* This study also shows that both methanolic pulp extracts of local and imported *Punica granatum* shows an absence of flavonoid, sterol, alkaloid and anthocyanin. A review done by Chaturvedula et al. (2011) on bioactive chemical constituent of Pomegranate peel, seed and juice reported that there is a presence of tannins, flavonoid, phenols in pomegranate peel.

There is a possible reason behind the differences of some of the results from this study compared to the previous studies. A study done by Wang et al. (2009) on the influence of light and maturity on flavonoid content and fruit quality of red raspberries reported that maturity stage plays an important role in phytonutrient level. It reported that immature berries have lower content of sugar and acids than ripe berries, and harvested berries at 100% maturity had higher anthocyanin content and stronger antioxidant activities. In the case of local *Punica granatum*, it was harvested before it was ripe. Another study by Wysocki (2012) on factors affecting the strawberry fruits' chemical composition reported that the cultivation condition such as irrigation, fertilization as well as health condition of the plant has an effect on the chemical constituents of strawberries.

Total Phenolic Content (TPC)

In this study, the peel extracts of both local and imported pomegranate have higher phenolic content compared to the pulp extracts. The results obtained from this study are similar to those done by other researchers although the phenolic content obtained is far lesser than those done in other studies. The phenolic content of imported pomegranate peel extract is far more than that of the local pomegranate peel extract. Results also show that both pulp extracts of local and imported *Punica granatum* have no phenolic content since the values obtained are of negative values.

Based on the study by Li et al. (2006) which assessed the antioxidant properties of pomegranate pulp extract and pomegranate peel extract, it reveals that pomegranate peel extract has a phenolic content possess higher than that of the pulp extract at 10-fold. Physical properties of a fruit or plant can affect its antioxidant properties. It was reported that the darker the colour of the honey, the higher its total phenolic content(Liza A-Rahaman et al., 2013). This can support this research' findings since the imported *Punica granatum* peel has a darker and redder colour whereas the local peel of *Punica granatum* was a greenish yellow colour.

There have been several studies that explain the reason behind peel extract of fruits having a higher phenolic content than the flesh or the pulp extract. The phenolic substances generally accumulate in the peel of peaches due to their protective function in against ultraviolet radiation by the sun (Manzoor et al., 2012). These findings correlate with the results obtained by this study.

DPPH Assay

In this study, it was revealed that all extracts used in this study has very minute radical scavenging activity against DPPH radical. Eventhough, the IC50 value is minute; it shows that the peel extracts do have to a certain degree, radical scavenging

activity. A study done by Abdel Motaal (2011) reported that the peel extract of *Punica granatum* has a more pronounced antioxidant activity compared to the whole pulp and fruit extracts.

A study done by Garcia-Salas et al. (2010) reported that there is a significant correlation between its DPPH radical scavenging activity with its total flavonoid and total phenolic content. The different type of solvent used and possible interferences from non-antioxidant compounds can affect the results of antioxidant assays. According to Pérez-Jiménez et al. (2008) it was reported that sample preparation in terms of drying of sample and extraction of sample can affect the result of antioxidant assay. The losses of antioxidant compound can be reduced through freeze-drying and if freeze drying is not available, the second option is by drying under vacuum provided that the temperature does not exceed 50-60°C.

MTT Assay

Based on the results obtained, the peel extract of both local and imported *Punica granatum* are most effective against MCF-7 breast cancer cell line and to a lesser extent to oestrogen negative MDA-MB 231. This is supported by findings from Toi et al. (2003) which states that peel extract of *Punica granatum* is most effective against MCF-7, less pronounced in MDA-MB 231. From the results gained, there is a slight anti-proliferative activity on HTB head and neck tumour cell line when IPGPe was used against it. However, to this date, there have been no studies done regarding anticancer activities of *Punica granatum* against HTB cancer cell line.

Cytotoxic activity of the extract or plant can be caused by the phytochemical constituent present in the plant (Akter et al., 2014). According to Jurenka (2008) phenol compound found in pomegranate exerts potent chemopreventative properties. It was also reported that ellagic acid exhibits powerful anticarcinogenic and antioxidant properties. The presence of flavones (flavanoids) and phenolic contents, contribute as natural antioxidants which in turn reveals the anticancerous activity of fruit peel extract against Liver and Breast cancer cell lines (Rather et al., 2010).

From this study too, it was revealed that peel extract of local pomegranate exert cytotoxic activity against normal cell line 3T3. This finding is supported by a study done by Okonogi et al. (2007) in the comparison of antioxidant capacities and cytotoxicities of certain fruit peels. It was reported that the peel extract of mangosteen and pomegranate exhibit cytotoxic effect against normal cell line and if it were to be used for treatment, it should be used with caution.

CONCLUSION

In conclusion, this study has revealed that the total phenolic content is higher in peel extract than in the pulp extract and in comparison between the local and imported Punica granatum, the imported Punica granatum has a higher phenolic content than the local Punica granatum. However, this study has shown that the Punica granatum samples used in this study shows very little DPPH radical scavenging activity.

This study has also shown that the peel extracts particularly LPGPe exert a more pronounce anticancer activity against MCF-7 and MDA-MB 231 breast cancer cell lines than the pulp extracts, and does not exhibit any selective cytotoxicity activity.

REFERENCES

i. Abdel Motaal, A. (2011). Anticancer and Antioxidant Activities of Standardized Whole Fruit, Pulp, and Peel Extracts of Egyptian Pomegranate. *The Open Conference Proceedings Journal*, *5*(October 2008), 41–45. doi:10.2174/2210289201102010041

ii. Ahirrao S.D., S. S. P. (2013). Phytochemical Screening and Antimicrobial Activities of Medicinally Important Plant Punica Granatum L. Rind Against Various Microorganisms, *3*(3), 330–335.

iii. Akter, S., Sarker, A., Mondal, M., Bappy, A. H., & Hossen, S. (2014). INTERNATIONAL JOURNAL OF ADVANCES IN Antioxidant , antimicrobial and cytotoxic activities of rind of Punica granatum, *3*(3), 807–814.

iv. Atikah M. (2013) Protective Effect of *Punica granatum* (Buah Delima) on Gentamicin-induced Nephrotoxixity.

v. Ayoola, G., Coker, H., Adesegun, S., Adepoju-Bello, A., Obaweya, K., Ezennia, E., & Atangbayila, T. (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, *7*(September), 1019–1024. doi:10.4314/tjpr.v7i3.14686

vi. Bekir, J., Mars, M., Souchard, J. P., & Bouajila, J. (2013a). Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of pomegranate (Punica granatum) leaves. *Food and Chemical Toxicology*, *55*, 470–475. doi:10.1016/j.fct.2013.01.036

vii. Garcia-Salas, P., Morales-Soto, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Phenolic-Compound-Extraction Systems for Fruit and Vegetable Samples. *Molecules*, *15*, 8813–8826. doi:10.3390/molecules15128813

viii. Igbinosa, O. O., Igbinosa, E. O., & Aiyegoro, O. a. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn). *African Journal of Pharmacy and Pharmacology*, *3*, 58–62.

ix. July, V. I., Venkata, C., Prakash, S., & Prakash, I. (2011). Bioactive Chemical Constituents from Pomegranate (Punica granatum) Juice , Seed and Peel-A Review. *International Journal of Research in Chemistry and Environment*, *1*, 1–18.

x. Jurenka, J. S. (2008). Therapeutic applications of pomegranate (Punica granatum L.): a review. *Alternative Medicine Review : A Journal of Clinical Therapeutic*, *13*, 128–144.

xi. Kujur, R. S., Singh, V., Ram, M., Yadava, H. N., Singh, K. K., Kumari, S., & Roy, B. K. (2010). Antidiabetic activity and phytochemical screening of crude extract of Stevia rebaudiana in alloxan-induced diabetic rats. *Pharmacognosy Research*, *2*, 258–263. doi:10.4103/0974-8490.69128

xii. Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., & Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with

pomegranate pulp extract. *Food Chemistry*, *96*, 254–260. doi:10.1016/j.foodchem.2005.02.033

xiii. Linga Rao, M., & Savithramma, N. (2011). Phytochemical studies of Svensonia hyderobadensis (walp.) Mold: A rare medicinal plant. *Der Pharmacia Lettre*, *3*, 51–55. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-80052834762&partnerID=40&md5=1b8829e0882a5f95f28357c8054741f4

xiv. Liza A-Rahaman, N., Suan Chua, L., Roji Sarmidi, M., & Aziz, R. (2013). Physicochemical and radical scavenging activities of honey samples from Malaysia. *Agricultural Sciences*, *04*, 46–51. doi:10.4236/as.2013.45B009

xv. Lü, J.-M., Lin, P. H., Yao, Q., & Chen, C. (2010). Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *Journal of Cellular and Molecular Medicine*, *14*(4), 840–860. doi:10.1111/j.1582-4934.2009.00897.x

xvi. Mamta, S., & Jyoti, S. (2012). Phytochemical Screening of Acorus Calamus and Lantana Camara. *International Research Journal of Pharmacy*, *3*, 324–326.

xvii. Manzoor, M., Anwar, F., Mahmood, Z., Rashid, U., & Ashraf, M. (2012). Variation in minerals, phenolics and antioxidant activity of peel and pulp of different varieties of peach (Prunus persica L.) fruit from Pakistan. *Molecules (Basel, Switzerland)*, *17*, 6491–506. doi:10.3390/molecules17066491

xviii. Okonogi, S., Duangrat, C., Anuchpreeda, S., Tachakittirungrod, S., & Chowwanapoonpohn, S. (2007). Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. *Food Chemistry*, *103*, 839–846. doi:10.1016/j.foodchem.2006.09.034

xix. Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz-Rubio, M. E., Serrano, J., Goñi, I., & Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International*, *41*, 274–285. doi:10.1016/j.foodres.2007.12.004

xx. Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products*. doi:10.1021/np9904509

xxi. Print, I., Online, I., Sadik, M. S., & Asker, M. M. S. (2014). World Journal of Pharmaceutical Sciences Antioxidant and antitumor activities of Pomegranate (Punica granatum) peel extracts, *2*.

xxii. Rahimi, H. R., Arastoo, M., & Ostad, S. N. (2012). A comprehensive review of Punica granatum (Pomegranate) properties in toxicological, pharmacological, cellular and molecular biology researches. *Iranian Journal of Pharmaceutical Research*.

xxiii. Rather, R. A., Swetha, C., & Rajagopal, K. (2010). Screening of Peel Extracts as Antioxidants , Anticancer Agents and Antimicrobials. *Advances In Bioresearch*, *1*(June), 29–33.

xxiv. Sawant, R. S., & Godghate, A. G. (2013). PRELIMINARY PHYTOCHEMICAL ANALYSIS OF LEAVES OF TRIDAX PROCUMBENS LINN. *International Journal of Science, Environment and Technology*, *2*(3), 388–394.

xxv. Schneider, K. (2001). Cell Biology and Cancer. *Counseling about Cancer: Strategies for Genetic Counseling*, 1–17.

xxvi. Sineh Sepehr, K., Baradaran, B., Mazandarani, M., Khori, V., & Shahneh, F. Z. (2012). Studies on the Cytotoxic Activities of Punica granatum L. var. spinosa (Apple Punice) Extract on Prostate Cell Line by Induction of Apoptosis. *ISRN Pharmaceutics*, *2012*, 547942. doi:10.5402/2012/547942

xxvii. Tiwari, P., Kumar, B., Mandeep, K., Kaur, G., & Kaur, H. (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, *1*, 98–106.

xxviii. Toi, M., Bando, H., Ramachandran, C., Melnick, S. J., Imai, A., Fife, R. S., … Lansky, E. P. (2003). Preliminary studies on the anti-angiogenic potential of pomegranate fractions in vitro and in vivo. *Angiogenesis*, *6*, 121–128. doi:10.1023/B:AGEN.0000011802.81320.e4

xxix. Tsuda, H., Ohshima, Y., Nomoto, H., Fujita, K., Matsuda, E., Iigo, M., … Moore, M. A. (2004). Cancer prevention by natural compounds. *Drug Metabolism and Pharmacokinetics*, *19*, 245–263. doi:10.2133/dmpk.19.245

xxx. Wang, S. Y., Chen, C. T., & Wang, C. Y. (2009). The influence of light and maturity on fruit quality and flavonoid content of red raspberries. *Food Chemistry*, *112*, 676–684. doi:10.1016/j.foodchem.2008.06.032

xxxi. Wysocki, K. (2012). Factors affecting the chemical composition of strawberry fruits. *Polish Journal Of Natural Sciences*, *27*(1), 5–13. doi:10.2307/3954338

xxxii. Yang, H., Dong, Y., Du, H., Shi, H., Peng, Y., & Li, X. (2011). Antioxidant compounds from propolis collected in Anhui, China. *Molecules*, *16*, 3444–3455. doi:10.3390/molecules16043444